ADVANCES
in
TROPICAL AQUACULTURE

Workshop at Tahiti, French Polynesia
February 20 - March 4, 1989

Actes de Colloques nº 9 - 1990

IFREMER

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Photo couverture

Le centre océanologique du Pacifique, principal établissement de recherche de l'IFREMER pour l'aquaculture en milieu tropical.
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Le colloque « *Advances in tropical aquaculture* » qui s'est tenu à Tahiti, en 1989, a été l'occasion de faire le point sur l'aquaculture marine dans les pays du Pacifique Sud : Australie, Fiji, Hawaï, Nouvelle-Calédonie, Nouvelle-Zélande et Polynésie française; ainsi qu'une synthèse des connaissances et des recherches dans trois domaines :

— pathologie des élevages : crevettes, mollusques et poissons tropicaux,
— nutrition des crustacés : pénéides tropicales,
— sélection et les essais d'élevage des poissons tropicaux.

Outre les communications présentées lors de ce colloque, le lecteur trouvera les bibliographies qui font référence dans ces matières.

The workshop « Advances in tropical aquaculture » held in Tahiti, in 1989, was the opportunity to review the marine aquaculture activities in the South Pacific countries : Australia, Fiji, Hawaii, New Caledonia, New Zealand and French Polynesia; as well as a synthesis of the knowledge and research in three fields :

— *pathology of shrimps, molluscs and finfishes,*
— *nutrition of crustaceans,*
— *evaluation of candidate species for fish farming.*

Beside the communications presented during this workshop, the reader will find the bibliographical references.

ADVANCES IN TROPICAL AQUACULTURE

Workshop held in TAHITI, French Polynesia

February 20 - March 4, 1989

Presently the Aquaculture development in tropical countries is largely the result of a pragmatic approach of improvements of the old traditionnal practises. Specific research programs devoted to the tropical species and tropical conditions are quite recent and the applications of their results are just entering the production sector.

Every one is now conscious of the urgent need to improve our research effort in such fields as pathology, nutrition and also to select the best species for culture within some groups like marine fishes where the choice is wide.

The aim of these working groups organised by IFREMER and funded by the French Ministry of research and technology is to open a large discussion between scientists working in tropical and temperature aquaculture.

to share their expertises and their experience,
to make a review of our present knowledge or lack of knowledge,
to define the priority programs to be conducted in the next years,
to improve the control of the culture systems.

Our goal is to provide the scientific community with a synthesis of the more recent findings and perspectives in these fields of tropical aquaculture.

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ACRONYMES ET SIGLES

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Reseachers and technicians of the Aquaculture Team working in the Centre océanologique du Pacifique

AQUAPAC
Société d'aquaculture du Pacifique

IFREMER
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LPGIM
Laboratoire de pathologie et de génétique des Invertébrés marins

AIMS
Australian Institute of Marine Science

NMFS
National Marine Fisheries Service

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Alain MICHEL
Directeur du Centre Océanologique du Pacifique (COP)

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Conseiller-Maire de Taïarapu-Ouest

Jacques DROLLET
Ministre de la Santé, de l'Environnement et de la Recherche Scientifique de la Polynésie Française

Jean MONTPEZAT
Haut-Commissaire de la République en Polynésie Française

Alain MICHEL

Directeur du Centre océanologique du Pacifique.

Monsieur le Haut-Commissaire,
Monsieur le Ministre de la Recherche,
Messieurs les Maires de Taïarapu-Est et Ouest,
Monsieur l'Administrateur des Iles du Vent,
Mesdames,
Messieurs.

L'**IFREMER** et, plus particulièrement, le personnel du Centre océanologique du Pacifique (COP) de Vairao sont très honorés de votre présence et de votre participation à cette séance inaugurale de nos groupes de travail sur l'aquaculture tropicale. Cette réunion est patronnée par le ministère de la Recherche et de la Technologie et va dans le droit fil des recommandations faites par Monsieur Curien, ministre de la Recherche lors des Assises Territoriales d'octobre 1988, à savoir coopération internationale et diffusion des résultats. Nous avons décidé de l'organiser dans le cadre superbe de la presqu'île de Tahiti, ce qui est bien normal puisque, depuis 16 ans, le COP est installé à Vairao. La recherche montre la voie en se décentralisant.

Je voudrais tout d'abord souhaiter la bienvenue à nos collègues scientifiques métropolitains et étrangers qui ont accepté de venir à Tahiti pour ce qui, je l'espère, sera de fructueux échanges de vues. Ils représentent le Pacifique au sens large du terme et viennent, pour certains, de plus loin encore. L'Australie, l'Italie, Israël, les îles Fidji, Hawaii, la Martinique, la Métropole, la Nouvelle-Calédonie, la Nouvelle-Zélande, les Philippines, Singapour, la Suisse, Taïwan, la Thaïlande, les Etats-Unis et le Japon sont ou seront présents aujourd'hui ou dans les prochains jours.

Vous me permettrez donc, quoique cette séance se déroule en français, de leur dire quelques mots en anglais, qui est maintenant la langue le plus utilisée dans le domaine scientifique :

It is a great honor for me to welcome all of you coming from different foreign countries some very far from here to participate to our working groups and to share with us our interest on aquaculture. This opening session is in French to be able to inform the french speaking participants and the people of French Polynesia of our common work, but don't be afraid, the working groups will be in English.

Je remercie aussi tous ceux qui nous ont aidé ou appuyé pour l'organisation de cette réunion, en particulier la Société UTA, l'Agence de voyage Gondrand et l'Huilerie de Tahiti. Vous me permettrez aussi d'associer à mes remerciements l'Hotel Puunui, qui fait que le séjour de nos hôtes se déroule le mieux possible et tout le personnel du Centre océanologique du Pacifique qui a oeuvré depuis plusieurs mois à la réalisation de ce projet.

Pourquoi cette réunion sur l'aquaculture marine tropicale ?

Les origines de cette activité sont très anciennes (1000 AC en Chine), mais ce n'est que depuis les années 60 que l'aquaculture a connu un développement spectaculaire pour les productions en eau de mer et en eau saumâtre, qui sont venues prendre le relais des productions plus traditionnelles en eau douce. La première estimation effectuée en **1975** créditait de **5 MT** la production aquacole; on a atteint près de **11 MT** en **1985**, et les prévisions pour l'an **2000** font état de **20 MT** face à la production des pêcheries qui serait alors de l'ordre de **80** à **90 MT.**

Les exemples les plus récents de ce développement très rapide sont l'élevage des sérioles au Japon qui dépasse 100 000 tonnes, l'élevage des saumons en Norvège qui est passé de quelques milliers de tonnes dans les années 70 à près de 70 000 tonnes en 1988, l'élevage des crevettes de mer qui est passé de 10 à 20 000 tonnes en 1970 à plus de 310 000 tonnes en 1988 (500 000 tonnes prévues en l'an 2000).Les retombées économiques et sociales de ce développement sont importantes. Il s'agit le plus souvent de produits vendus à l'exportation et donc générateurs de devises fortes. On estime que l'élevage des Salmonidés a créé 200 000 emplois dans le monde et celui des crevettes 150 000 emplois rien qu'en Equateur. Le développement est aussi d'importance dans le secteur des algues, des mollusques et, plus récemment, des poissons. En Polynésie, on peut citer le développement très rapide de la perliculture, forme particulière d'aquaculture, qui est devenue la première activité exportatrice du Territoire et présente, en outre, un intérêt social évident, car elle permet de maintenir des activités rémunératrices dans les îles Tuamotu.

Les pays en développement de la ceinture tropicale sont particulièrement intéressés à cette progression de l'aquaculture car, bénéficiant de températures élevées et souvent de grandes zones littorales, ils peuvent prétendre atteindre un double objectif : exporter des produits dont la demande est forte sur les grands marchés de consommation; augmenter les quantités de protéines disponibles pour leur populations.

Pour les scientifiques que nous sommes, engagés dans la recherche-développement en aquaculture, plusieurs questions se posent.

— Est-ce que la recherche a joué un grand rôle dans le développement des années 60 à 80 ?

— Comment se situe-t-elle actuellement face aux demandes du secteur productif ?

— Qu'est-ce que l'on attend d'elle dans la prochaine décennie ?

Je ne prétends pas, dans cet exposé, faire le tour de ces questions, mais, je voudrais simplement, à travers l'expérience de l'équipe **AQUA-**

COP, plus particulièrement dans l'élevage des crustacés, apporter quelques éléments de réponse.

Le développement des années 60-80 a été souvent le fait d'une approche pragmatique liée à des situations et des contextes particuliers, où la recherche n'est intervenue qu'en deuxième ligne. Le Japon a trouvé là un moyen de valoriser les sous-produits d'une activité de pêche très importante. Les Norvégiens, qui pratiquaient l'élevage de la truite en eau douce, ont trouvé dans leurs fjords et avec les saumons une solution à leur problème de manque de sites terrestres. Les Equatoriens, ayant constaté que des juvéniles de crevettes entrés dans des bassins littoraux naturels grossissaient très rapidement, se sont mis à bâtir des digues artificielles et, en 15 ans, ont développé 100 000 hectares de bassins pour répondre à une très forte demande du marché nord-américain avec des cours en augmentation constante de 70 cts US/kg en 1970 à 6,40 US $/kg en 1980. Tout cela s'est souvent fait empiriquement avec l'utilisation maximale des ressources naturelles : juvéniles capturés dans les eaux côtières et utilisation de la productivité endogène pour soutenir des productions de quelques centaines de kilogrammes à l'hectare. Cette étape a été rapidement suivie d'un désir d'intensification qui a très vite fait apparaître les limites des techniques mises en place spontanément : plus assez de juvéniles dans le milieu naturel, productivité naturelle insuffisante pour soutenir la croissance des animaux, produits du métabolisme dépassant les capacités d'épuration du milieu, etc. C'est alors que de nombreuses questions ont été posées aux scientifiques, dès cette prise de conscience d'un dépassement des capacités du milieu à supporter les élevages.

Si les questions ont été nombreuses, les réponses n'ont pas été évidentes et rapides en raison d'une méconnaissance tant des espèces elles-mêmes que des mécanismes mis en jeu par les systèmes de production. Les interrogations nous ont révèlé l'étendue de notre ignorance et l'essentiel du travail de recherche, effectué par des équipes naissantes entre 70 et 80, a surtout été de constituer les outils d'expérimentation, de former le personnel et de développer les méthodologies d'étude, tout en commençant à maîtriser la reproduction en captivité : le manque de juvéniles apparaissant comme le facteur limitant au développement de la plupart des élevages.

Ce n'est donc que dans les années 80, au moins pour les crustacés, que la recherche a commencé à intervenir de façon constante et à avoir un impact significatif dans les processus de développement. Dans le cas du C.O.P., deux actions très différentes ont été menées selon qu'il s'agissait des DOM-TOM ou des pays étrangers.

L'action, dans les DOM-TOM, a été très volontariste de la part des pouvoirs publics puisque l'objectif était de créer de nouvelles activités et de nouveaux emplois en zone rurale. Ce sont donc les résultats et les acteurs de la recherche qui se sont trouvés en première ligne des opérations de développement. Ne pouvant s'appuyer sur aucune activité traditionnelle, il a fallu tout créer au même moment : mettre au point et fabriquer des aliments, produire des juvéniles en écloserie, créer les premières fermes et former les premiers producteurs. Il y a donc eu une interaction très forte recherche-développement. Le résultat en est

l'émergence d'un secteur productif avec 450 tonnes de crustacés commercialisés en 1988, dont pour la première fois une part significative à l'exportation de 125 tonnes.

L'action vis-à-vis des pays étrangers a été, bien sûr, très différente et a consisté à répondre à des demandes ponctuelles permettant de lever des facteurs limitants du développement. L'atout du C.O.P. a été d'avoir, à ce moment, maîtrisé la production de masse de petites crevettes à partir de stocks en captivité et de pouvoir proposer la construction de grandes écloseries permettant de pallier les déficiences d'approvisionnement à partir du milieu naturel. De là toute une série d'actions menées par l'intermédiaire de la filiale France-Aquaculture, tant en Amérique du Sud (Equateur, Colombie) que dans le Sud-Est asiatique (Indonésie, Malaisie, Philippines, Sri Lanka, Inde). Les retombées ont été intéressantes car l'implication, même ponctuelle, dans les systèmes de production a permis d'identifier les points de blocage essentiels et de les transcrire en terme de programme de recherche afin d'être en phase avec les demandes à venir.

On constate, en effet, une évolution dans les demandes formulées à la recherche. Après cette période pionnière de mise en place d'une activité correspondant à une demande forte du marché et à des prix attractifs laissant une marge bénéficiaire importante, le contexte change et l'on rentre progressivement dans la période où la fiabilité des résultats et la diminution des coûts de production deviennent les objectifs principaux pour que, face à une production en constante augmentation, l'activité reste rémunératrice.

Cette nouvelle étape demande des réponses plus pointues et une approche beaucoup plus thématique sur différents aspects comme le choix des souches à élever, la nutrition, le contrôle du milieu, le contrôle des maladies, la gestion des systèmes d'élevage, etc.

Cette meilleure connaissance des animaux que l'on élève et de leurs réactions vis-à-vis des systèmes d'élevage ne peut résulter que d'une approche conjointe des différentes équipes de recherche fondamentale ou finalisée travaillant sur ces domaines dans différentes parties du monde. De là l'organisation de ces groupes de travail pour faire le point sur l'état actuel de nos connaissances sur les problèmes à résoudre en priorité et donc sur les axes de recherche à privilégier.

Le thème **Pathologie** est, bien sûr, au centre de tous les élevages qui suivent le processus d'intensification : distinguer ce qu'est un animal en bonne santé d'un animal stressé, maintenir les équilibres bactériens des milieux d'élevage, savoir identifier et diagnostiquer virus, parasites et bactéries susceptibles de causer des ravages d'autant plus rapides que nous travaillons en température élevée, prévenir par une bonne prophylaxie et un bon environnement, guérir s'il le faut. Tels sont les sujets qui sans nul doute, feront l'objet des débats du premier groupe de travail.

Le thème **Nutrition des Crustacés** est aussi particulièrement important : d'une bonne alimentation résulte une bonne croissance et donc de bons rendements. Les besoins nutritionnels des animaux marins ne sont pas encore complètement connus et l'importance de facteurs de croissance a été récemment mis en évidence : Comment

avancer dans la connaissance des contraintes de la nutrition ? Comment conduire les expérimentations ? Comment répondre au moindre coût, aux exigences alimentaires des crevettes ? Comment fabriquer les aliments pour les rendre le plus accessible, le plus attractif et donc le plus efficace ? Comment distribuer ces aliments ? seront à l'ordre du jour du deuxième groupe.

Enfin, le thème du troisième groupe « **Choix des espèces tropicales de poissons les plus aptes à l'élevage** » indique à l'évidence que, parmi de nombreuses espèces candidates, la priorité reste à établir ou à confirmer sur des critères tels que la capacité de croissance rapide sur des aliments composés, la facilité d'obtenir la reproduction, la résistance aux stress d'élevage et aux maladies et, bien sûr, l'acceptation du produit par le consommateur.

Vous voyez que le champ encore ouvert à la recherche est vaste. L'ensemble des équipes scientifiques impliquées dans ce domaine a un impact de plus en plus important sur les progrès du développement. Je souhaite donc, pour ma part, que ces groupes de travail apportent quelques pierres de plus à l'édifice aquaculture en pleine construction, tant pour le bénéfice de la Polynésie que pour l'ensemble des pays de la ceinture tropicale.

Roger DOOM
Conseiller-Maire de Taïarapu-Ouest

Monsieur le Haut-Commissaire,
Monsieur l'Administrateur,
Monsieur le Directeur du COP,
Mesdames et Messieurs les Scientifiques Français et Etrangers.

Je voudrais tout simplement et du fond du cœur, au nom du Conseil Municipal et au mien, vous souhaiter la bienvenue à Taîarapu-Ouest.

Ce 20 février sera une date qui marquera un plus puisque, pour la première fois, les deux atouts majeurs de cette Commune se réunissent en un seul lieu.

Je veux parler tout d'abord, du Centre océanologique du Pacifique — établissement scientifique de recherches appliquées de renommée internationale —, puis de la station touristique de Puunui — hôtel de qualité —.

Je vous souhaite un très bon travail, et un agréable séjour à Vairao.

Jacques DROLLET
Ministre de la Santé, de l'Environnement et de la Recherche Scientifique de Polynésie Française

Monsieur le Haut-Commissaire,
Monsieur le Ministre,
Monsieur le Maire,
Monsieur le Directeur,
Mesdames,
Messieurs.

Je suis honoré d'être parmi vous aujourd'hui et je remercie Monsieur le Directeur du Centre océanologique du Pacifique d'avoir bien voulu m'inviter à la cérémonie d'ouverture du premier Colloque international sur l'Aquaculture Tropicale.

Il y a quelques mois, les premières Assises Territoriales de la Recherche, organisées à l'initiative du Gouvernement de la Polynésie, ont donné pour la première fois l'occasion à l'ensemble des chercheurs de la Communauté scientifique du territoire, de définir leurs priorités afin que la recherche se mette au service du développement de la Polynésie.

A cet égard, votre colloque se place tout à fait dans le prolongement des Assises, puisque le groupe « Océan et Ressources marines », dans un parfait consensus, a recommandé qu'en ce domaine l'effort du Territoire soit porté sur le développement de l'Aquaculture et, en particulier, de la perliculture.

Cet effort n'est pas neuf puisque, depuis 15 ans, les organismes territoriaux et nationaux ont déja consacré la plus grande partie de leur énergie à l'étude du domaine marin. toutes ces recherches sur le développement de l'aquaculture en Polynésie ont permis d'aboutir en 1988 à la production de 60 tonnes de crustacés, de 5,5 tonnes de mollusques et surtout à la création de la première industrie d'exportation du Territoire : l'aquaculture perlière.

Pourtant, aujourd'hui et plus qu'avant, j'invite et j'engage fortement les chercheurs à se concerter et à accorder une priorité à la coopération territoriale, nationale et internationale en matière de recherche et de développement de l'aquaculture et notamment de la perliculture. A ce titre, je rappelle que le Conseil des Ministres a abondé sans réserve dans le sens des recommandations soulignées par les chercheurs lors des assises, en retenant l'étude de la nacre comme l'un des thèmes de recherche prioritaires.

En effet, si les thèmes que vous avez choisis de débattre durant ce colloque sur l'aquaculture tropicale concernent des aspects fondamentaux dont dépend la réussite des fermiers de la mer, je souhaiterais également que les experts puissent se pencher sur les problèmes particuliers de la perliculture en Polynésie. Car si la recherche a précédé le développement aquacole de toutes les espèces d'élevage introduites, qu'il s'agisse de crustacés, de poissons ou de mollusques, il n'en n'est pas de même pour cette grande activité d'élevage marin du Territoire que constitue l'industrie nacrière et perlière.

En effet, il faut bien reconnaître que, dans ce secteur, la recherche est à la traîne d'un processus de développement démarré en 1960 qui a provoqué, malgré toutes les mesures prises, la surexploitation des stocks naturels de nacre, dont on constate que certains sont déja épuisés.

Bien sûr, les techniques employées, tant pour le collectage et l'élevage des naissains que pour le greffage et l'élevage des nacres greffées, ont permis à l'activité perlière de connaître une réussite à ce jour spectaculaire, puisqu'elle intéresse plus de 300 exploitants et représente 2,5 milliards de revenus d'exportation pour l'année 1988. Et ce, contrairement à d'autres activités aquacoles qui ne représentent qu'une partie très faible de la production totale commercialisée sur le Territoire.

Malheureusement, les vagues de mortalité anormale de la nacre qui sont apparues dès 1985 ont démontré que nous ne pouvions pas nous satisfaire de ces résultats. Si nous voulons préserver et gérer correctement cette ressource fondamentale des atolls polynésiens, il faut bien se rendre à l'évidence que les critères de performance économique, tant en volume de stocks vendus qu'en masse de revenus perçus, ne suffisent pas toujours à valider définitivement l'utilisation de telle ou telle technique d'exploitation.

Les hypothèses sont posées : le développement à outrance de l'élevage de naissains et la prolifération des coopératives et sociétés perlières concourent-ils à participer voire à aggraver les phénomènes de mortalité nacrière ?

J'attache donc une grande importance à ce que vous puissiez, dans vos séances de travail, apporter une attention toute particulière à cette activité, d'autant que notre connaissance de la biologie de l'espèce et notamment son éthologie alimentaire reste très embryonnaire. Les services territoriaux auront d'ailleurs l'occasion de vous exposer leurs programmes durant ce colloque.

Je vous souhaite un excellent séjour en Polynésie et je vous fais confiance pour qu'à l'issue de vos échanges, de vos confrontations et de vos réflexions, l'aquaculture tropicale progresse et que les résultats puissent être profitables et applicables dans tous les pays de la région du Pacifique sud ainsi que dans les pays ayant des caractéristiques voisines des nôtres.

Jean MONTPEZAT
Haut-Commissaire de la République en Polynésie Française

Monsieur le Ministre,
Monsieur le Maire,
Mesdames,
Messieurs,

Je suis très honoré d'avoir été invité à cette séance inaugurale du Colloque sur l'Aquaculture Tropicale et me rejouis que cette initiative de l'IFREMER ait suscité une participation aussi brillante de scientifiques français et étrangers.

Vous me permettrez, tout d'abord, de saluer, au nom du Gouvernement de la République, tous les chercheurs étrangers qui ont répondu à l'invitation du Centre océanologique du Pacifique et vont, durant ces douze jours à Puunui, apporter leur savoir et échanger leurs connaissances et observations pour travailler à l'amélioration de l'aquaculture en milieu tropical.

Leur seule présence, venant du Pacifique, certes, mais également d'Asie, d'Amérique et d'Europe, témoigne de l'importance de ce colloque.

La France porte un grand intérêt à de tels échanges internationaux en matière scientifique, manifestant ainsi sa volonté de participer activement au progrès et à l'intérêt général, au-delà des frontières.

Les travaux qui s'ouvrent aujourd'hui sont la poursuite d'une action engagée depuis plusieurs annees par le ministère de la Recherche et de la Technologie. Ils s'inscrivent dans la ligne de la réunion des organismes scientifiques français présents dans le Pacifique, qui s'était tenue à Nouméa en 1986, à l'initiative du Conseil du Pacifique sud.

Je me dois, également, de souligner les efforts du Gouvernement du Territoire pour que la Polynésie Française soit un grand centre dans la zone Pacifique en matière de recherche. En octobre dernier, se sont tenues les premières Assises Territoriales de la Recherche auxquelles M. Hubert Curien, ministre de la Recherche et de la Technologie a assisté, manifestant ainsi sa volonté de développement et de collaboration.

Aujourd'hui, ce colloque international d'importance témoigne à son tour du rôle de la Polynésie dans la zone Pacifique.

Je veux également rendre hommage au Centre océanologique du Pacifique de Vairao qui laborieusement, depuis de nombreuses années, a mis au point des techniques désormais fiables et participe ainsi non seulement au développement de la Polynésie Française mais exporte également ses connaissances et son savoir-faire dans les pays de la Ceinture Tropicale, par le biais de « France-Aquaculture », filiale de l'IFREMER. C'est ainsi que plusieurs scientifiques français coopèrent actuellement dans les pays de la zone dont un technicien polynésien du centre de Vairao qui travaille en Equateur.

Bien sûr, et les intervenants précédents l'ont souligné, des difficultés importantes subsistent. Les élevages connaissent des mortalités qui peuvent mettre en danger le développement d'actions naissantes et prometteuses telles que la perliculture en Polynésie Française et il convient de rechercher leurs causes. Le mode de nutrition des crustacés mérite d'être affiné, des progrès considérables sont encore à faire dans le développement des poissons d'élevage.

Tous ces problèmes qui subsistent ou qui surgissent, toutes ces questions qui se posent, toutes ces investigations qui demeurent à entreprendre nécessitent, à l'évidence, une coopération internationale. Sont disséminés dans le Pacifique des pays qui ne sont pas au même stade de développement. Un progrès harmonieux des territoires de la zone exige donc une mise en commun des résultats des travaux effectués à ce jour, pour la construction de théories nouvelles qui devront être soumises à des vérifications pratiques.

Travail laborieux, je le disais tout à l'heure, action persévérante et volontaire. Ce colloque qui s'ouvre aujourd'hui est une étape.

Je n'ai pas qualité pour apprécier l'organisation de ces journées et me garderai d'émettre un jugement, mais j'observe une réelle volonté d'efficacité qu'il me plait de souligner.

En vous proposant, Mesdames et Messieurs, de vous réunir en trois ateliers plutôt que de tenir un grand amphithéâtre, vous voulez aborder concrètement les sujets qui vous préoccupent, cerner au plus près les difficultés rencontrées, rechercher les solutions les plus appropriées.

Je souhaite que la rencontre de vos idées soit largement productive et que le résultat des expériences d'hier, mises en commun aujourd'hui, soit oeuvre de progrès pour demain.

Je vous renouvelle, à toutes et à tous, mes souhaits de bienvenue à Puunui et mes remerciements pour votre présence, et forme des voeux pour que le labeur auquel vous vous attachez soit fructueux.

CHAPTER I

AQUACULTURE IN SOUTH PACIFIC ISLANDS STATE OF THE ART

ADVANCES IN TROPICAL AQUACULTURE. Tahiti, Feb. 20 - March 4, 1989
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1

The state of the art of IFREMER in tropical aquaculture

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Abstract — *IFREMER, a french governmental agency, is engaged in research on tropical aquaculture since 1971, with the aims to develop new activities in French overseas territories and to export French technology to foreign countries. With 80 searchers and technicians the « Centre Océanologique du Pacifique » is the main IFREMER's centre involved in tropical aquaculture.*

The research has been focused on the complete control of the whole cycle : constitution of broodstock, matuuration and reproduction, larval rearing, grow-out. The results are :

*— Edible molluscs : **Saccostrea echinata, Crassostrea gigas, Crassostrea rhizophorae, Perna viridis**.* The reproduction in captivity, the larval rearing, the settlement and the pregrowing are under control. The main problem is the poor productivity of the water in tropical islands.

*— Freshwater prawn : **Macrobrachium rosenbergii**. Larviculture : the C.O.P. has defined an intensive technique in recirculated water. The production is 80-100 post-larvae per litre. Grow-out : two techniques are used continuous grow-out : 1.5 to 2.5 t/ha/yr. Discontinuous grow-out : 1 to 2 t/ha/yr. In 1988, 188 t have been produced in the French overseas territories.*

*— Seawater shrimp : four species have been selected : **P.monodon, P.indicus, P.vannamei, P.stylirostris**. Constitution of broodstock, maturation and spawning : the C.O.P. is now working on a routine basis with animals of the 10th to the 13th generation in captivity. Larviculture : production of 80 to 100 post-larvae per litre in closed (or open) system with algae artificial feeds and artemia. Grow-out : semi-intensive : 2 to 4 tonnes/ha/yr. Super-intensive : 15 to 30 tonnes/ha/yr. AQUACOP has formulated different artificial feeds for the 4 species. In 1988, 262 tonnes of shrimps have been produced in the French overseas territories. The transfer of technology has been made in different tropical countries (Asia, Africa, South-America).*

*— Finfish : At experimental scale the selection of species is under progress. The most suitable for tropical aquaculture seem to be **Lates calcarifer** and **Sciaenops ocellatus**. In 1988, the first spawning of captive broodstock of **Lates calcarifer** has been obtained. The commercial size (500 g) is reached by one year, in floating cages (15-30 kg/m3) with an artificial food formulated by AQUACOP. Shrimp and prawn technologies have yet reached the commercial scale. In the next future, finfish culture should reach the pilot scale in French overseas territories.*

RATIONALE AND GOALS

IFREMER is engaged in research on tropical aquaculture since 1972 with aims to develop new activities in French overseas territories and to export French technology to foreign countries (AQUACOP, 1974).

THE WORKING TOOLS

With 80 people the « Centre Océanologique du Pacifique » is IFREMER's main centre involved in tropical aquaculture. Different experimental or predevelopment stations in French overseas territories (New-Caledonia, French Guayana, F. West Indies) allow the adaptation of the techniques to the local conditions and the transfer to the private sector.

THE STRATEGY

The work strategy on molluscs, crustaceans and fishes is to obtain the complete control of the biological cycle. This approach is the only one which opens the way to genetic improvement possibilities. These ones will without any doubt bring repercussions as important as those recorded in terrestrial rearing productivity. On the other hand the control of the whole life cycle allows the most performing species spreading for culture in the whole intertropical zone, and the regular supplying of juveniles, base of any profitable enterprise management.

The main steps of the rearing are :

the constitution and the maintenance of the broodstocks,

the induction of the maturation and spawning in captivity,

the larval rearing to produce spats, post-larvae or fry,

the pregrowing to provide good quality juveniles,

the gow-out to reach the commercial size,

the fixing and transfer of the different techniques follow four different steps :

- the experimental step in small tanks and ponds to precise the zootechnical standards;
- the pilot step in the C.O.P. or in the different transfer stations to prove the technical feasibility and to fix the production cost scales;
- the demonstration step occuring in production units conceived for thid special purpose, having a participation of private and public funds side by side; this step must lead to prove the economical feasibility of the project;
- the development step which carries along the increase of production units; the C.O.P. or transfer stations will not be involved any more, except by helping to face new difficulties.In foreign countries the transfer of technology is done through France-Aquaculture, subsidiary company of IFREMER.

MOLLUSC REARING

Species : Green mussel from the Philippines : *Perna viridis*
Rock oyster : *Saccostrea echinata*
Japanese oyster : *Crassostrea gigas*
Mangrove oyster : *Crassostrea rhizophorae*
Clam : *Ruditapes philippinarum*

The species selected are : *P. viridis, C. rhizophorae* and *S. echinata.*

Concerning the others species, different tests were not successful : the Japanese oysters showed particularly important mortalities at high temperatures.

Are now well controlled :

— the constitution and maintenance of broodstocks,

— the maturation and spawning (thermic shock),

— the larval rearing (AQUACOP, 1977, 1979),

— the nursing to furnish 10 mm spats.

The growing is done in bays or lagoons (AQUACOP and de Gaillande, 1979). But there are few suitable sites in tropical islands due to the very low productivity of the seawater. Concerning the pearl oyster *Pinctada margaritifera*, the C.O.P. has essentially worked on reproduction in hatchery. Some results were obtained at the experimental scale : maturation and spawning but the larval rearing realized only produced some spat.

FRESHWATER PRAWN REARING

The C.O.P. has worked on two species : *Macrobrachium lar*, the Tahitian species and *M. rosenbergii. M. lar* has not been chosen because of its inclination to escape from the ponds. *M. rosenbergii* has been selected.

Are now well controlled :

— the constitution of the broodstock,

— the mass production of post-larvae (AQUACOP, 1975, 1977, 1987); a new technique has been developed by the C.O.P., this technique in recirculated water allows high reliable production of post-larvae with 70 to 80 % survival, 80 to 100 PL/litre; this technique has been adapted to large production hatcheries.

Two grow-out systems are used (AQUACOP, 1983, Lacroix D., J.M. Griessinger, J.C. Falguière and Th. Pollet, 1988) :

— continuous method : the yields are 1,5 to 2,5 t/ha/year,

— discontinuous : 1 to 2 t/ha/year.

These two systems of production correspond to different sites, water quality and marketing conditions. The results are still submitted to changes and important management programmes of the ponds are under exami-

nation : water exchange, aeration. The harvesting technique remains to be improved.

The C.O.P. has formulated different artificial feeds for larvae, juveniles and adults and the production has been transferred to a local feed mill (AQUACOP, 1983).

Presently, *Macrobrachium* culture is at the commercial scale in the French overseas territories. The goals are to satisfy the different local markets and to reach through improvements of the techniques the world market. The small sized farms up to 3 ha in a secondary activity state have proved their profitability. The larger sized farms running in single activity must prove their ability to maintain a stable production. AQUAPAC in Polynesia is the prototype of this sort of farm.

In 1988 the French overseas territories have produced 190 tonnes of freshwater prawn.

PENAEID SHRIMP CULTURE

A selection done over more than 10 species allowed to select four of them as the more suitable for tropical aquaculture (AQUACOP, 1984)

Penaeus monodon and *P. indicus* are originated from Asia, *P. stylirostris* and *P. vannamei* from Latino-America.

These four species require different temperatures, rearing and feeding conditions and each species gives a specific product in size and color. So it is possible to make a choice according to the environmental conditions and market.

Are now well controlled :

— the constitution of captive broodstock,

— the induction of maturation and spawning in captivity (and the artificial insemination) (AQUACOP, 1975, 1979, 1983),

— the mass production of post-larvae (AQUACOP, 1983, 1987),

— according to the different sites two growout systems are used :

- semi-intensive in large surfaced sites at reasonable cost (New Caledonia). The yields are 2 to 4 t/ha/year (Goxe et *al*, 1987).
- intensive growout in small surfaced area at high cost (F. Polynesia).The yields are 15 to 30 t/ha/year (AQUACOP, Patrois J., Barret J. and Mazurié J., 1987); the feeding formulae have been set up for the different species and stages, from available products and by products in each zone; the formulation has been transferred to a commercial feed mill.

Presently the penaeid culture is at a commercial scale in French overseas territories. In Tahiti three intensive farms (1 ha each) are in production and the local governmental hatchery is under construction. The goal is to satisfy the local market. In New Caledonia 175 ha of semi-intensive ponds are in production. In 1988 Tahiti and New Caledonia have produced 260 tonnes of shrimps.

The transfer of technology concerning *Macrobrachium* and penaeid cultures has been made in different tropical countries through the subsidiary France Aquaculture. Asia : India, Skri Lanka, Vietnam, Philippines, Malaysia, Indonesia. Fiji in the South Pacific. America : Ecuador, Colombia, and the French overseas territories of. F.W. Indies and Guayana. Africa : Senegal.

FINFISH CULTURE

Presently the work is focused on the selection of species.

The criteria chosen are :

— ability to reproduce in captivity,

— possibility of mass larval rearing,

— good growth rate with artificial food,

— ability of high density growth in floating cages.

After tests realized on the local species as Carangidae (AQUACOP, 1975), Lutjanidae, Siganidae in French Polynesia and Martinique no species revealed favourable selection criteria.

So foreign species have been selected : *Lates calcarifer* in Tahiti and *Sciaenops ocellata* in Martinique (Soletchnick P. Thouard E., Goyard E., Baisnee D., 1987; Soletchnick P., Thouard E., Goyard E., Yvon C., Baker P., 1987). But other species are under study :

- in Polynesia, the grouper *Epinephelus microdon* and the dolphin fish *Coryphaena hippurus.*
- in Martinique, the red tilapia (*Oreochromis* sp.).

The sea bass *Lates calcarifer* is hermaphrodite protandric. The broodstock was constituted from fry imported in 1984 from the P.P.D. of Singapore. In 1987 the first sexual reversal was observed and 20 spawnings were obtained in 1988. The larval rearing is under progress. The chief problem being mortality after 12 days of rearing.

This species is well adapted to net pen culture : at biomass of 20 to 30 kg/m^3 the commercial size (500 g) is obtained after 12 months. Artificial feeds are used in the different stages : larvae, juveniles, adults (AQUACOP and Fuchs J., 1986).

In Martinique, the species *Scianops ocellata* from the U.S. seems to be well adapted to intensive net pen culture. The commercial size 500-700 g is reached on 12 months.

The potential of net pen culture development in tropical islands seems good for several reasons :

— lagoons are sheltered sites where it is easy to install floating cages,

— an interesting local market exists,

— the cultured fishes will be free from ciguatera.

The following years should see the implementation of pilot production units.

CONCLUSION

Now, we just emerge from the pure zootechnical phases, which allowed us :

— to select different species suitable for culture,

— to constitute and train teams which have now acquired experience,

— to construct the work tools,

— to start the first production projects.

We come now in the phases where the supporting of fundamental research teams is necessary.

To improve the productivity and to decrease the production costs we need better knowledge in various fields.

— Physiology : the hormonal control of crustaceans and fish reproduction, cryopreservation of gametes and embryos.

— Genetic : polyploids, incorporation of genes.

— Pathology : a better knowledge of the specific bacteria populations linked to the rearing conditions; the setting up of immunodiagnostics.

— Nutrition : the setting up of artificial food to replace the live prey during the larval rearing; the precise nutritional needs for the animals. A better knowledge of the growth factors.

I hope these workshops will bring fruitful answers to these questions.

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2

The Australian mariculture industry : finfish, molluscs and crustaceans.

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Abstract — *Australia's interest in mariculture has been comparatively recent when compared with the developments in many other Pacific countries. Two exceptions are native oysters, cultivated for food since the late 1880's, and pearl oysters which have been cultured, particularly in the northern part of Australia, following the initial work of Saville-Kent.*

The additional organisms of current interest are penaeid prawns (shrimps), mud crabs, scallops, abalone, mussels, clams, brine shrimp, halophilic microalgae and several fish of temperate and tropical waters. Australian mariculture is also directed towards the production of fine chemicals and the replenishement of wild stocks.

Australia offers great opportunities for mariculture. Its coastal area is vast, a wide range of water temperature regimes is available and its coastal waters are of high quality.

Disadvantages include distance from major markets and consequent high shipping and labour costs, and a relatively inexperienced labour force.

This paper describes recent developments in mariculture in Australia and the progressive input of science to the art.

THE AUSTRALIAN SETTING, HISTORY AND GENERAL DEVELOPMENTS

Physiographic details of Australia's coast are given in figure 1.

Australia contrast with other countries in the Pacific by its latitudinal extent, its continental age and the consequent characteristics of the majority of its river systems.

Australian settlement by Europeans occured just over 200 years ago. The Aboriginal populations which have lived in Australia for more than 40,000 years do not appear to have practised extensive mariculture operations. However, evidence from southern Australia illustrates that aborigines were practising aquaculture in fresh water systems from about 4,000 years ago (Coutts *et al.*, 1978; Flood, 1983). There is no clear evidence that Aboriginal people used mariculture products in trade.

Commercial mariculture has developed in Australia only in the past 10 years. Apart from the efforts in oysters and to some extent mussels in Victoria, the greatest emphasis has been paid to penaeid prawns in the tropical regions and to Atlantic salmon and ocean trout cage culture in Tasmania.

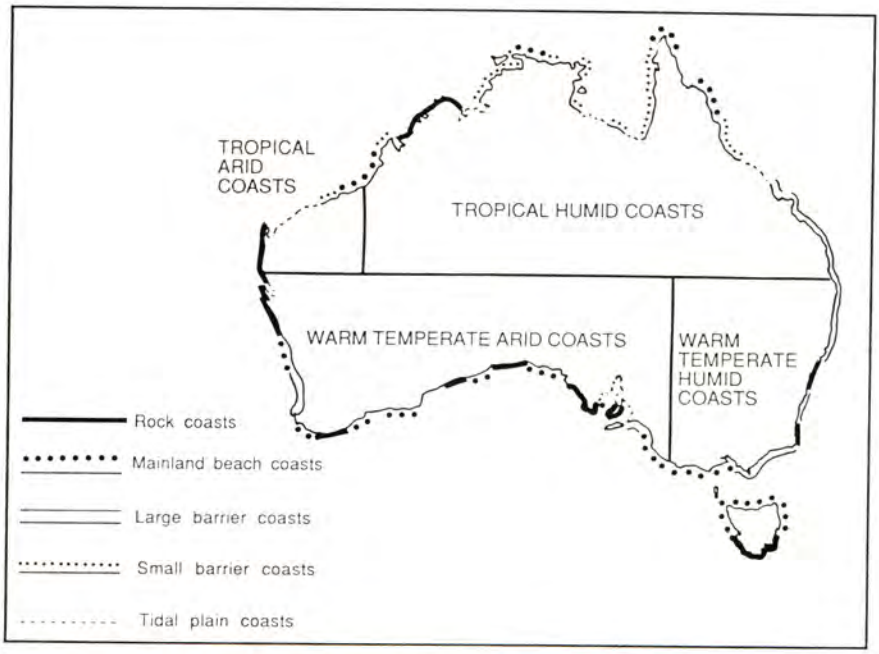

Fig. 1. — Coastal types of Australia based on Davies 1977

Penaeid prawn culture is now well established in the area from northern New South Wales and throughout Queensland as far north as Cooktown. Over more than 2,000 kilometres of coastline the principal pocket of activity are : northern New South Wale, Mackay, Townsville, Innisfail, Cairns, Port Douglas/Mossman, Cooktown and Darwin.

Oysters have been grown in Australia since the 1880's with the industry centred in New South Wales. In the past oyster farming concentrated on one principal species. *Saccostrea commercialis*, making the Sydney rock a regular treat in Australia. Recent developments have seen new methods of farming of different species of oysters.

The pearl oyster industry of northern Australia, particularly around Broome and Thursday Island, may not be regarded as a conventional mariculture industry but it was a very carefully managed resource.

During some 190 years Australians have neglected the potential of farming the sea whilst significantly modifying the land for agriculture. Australia now stands poised to redress this imbalance and impart significant effort to commercial mariculture. Education, training and research are the keys to ensure the success of the industry.

THE ADVANTAGES AND DISADVANTAGES OF AUSTRALIA FOR MARICULTURE DEVELOPMENT

a. Advantages

The country has a very wide range of coastal features and water temperature regimes suitable for different types of marine animals and plants.

Because of its relatively low population density most of Australia's coastline has very long stretches little influenced or unaffected by human pollutants.

Large areas of coastal land are avalaible at relatively low prices. This characteristic has been somewhat reduced over the past few years with significant tourist and residential developments which compete for areas of land suitable for mariculture.

In spite of some over-exploitation of commercial species most of Australia's naturally occuring marine animals and plants can still provide healthy broodstock, comparatively free of disease.

The law level of pollutants and generally high quality of Australian waters give the opportunity to produce mariculture products acceptable to the most discerning world markets.

Australia has regular reliable airlinks to major markets of Southeast Asia, Japan, Europe and the U.S.A.

Australia's stable political environment encourages major investment from overseas countries.

b. Disadvantages

Australia's disadvantages with regard to mariculture relate predominantly to the country's late entry into the field and its very high labour costs.

Although it does have airlinks to overseas markets, the distances involved are greater and the services fewer than for many competitor countries.

Fresh water supplies are limited and seasonal in many of the regions of Australia which would otherwise have ideal conditions for mariculture.

The high costs of wages and services in Australia require the development of methods which are not labour-intensive and which maximize efficiency.

A long term advantage, but a short term perceived disadvantage, is the concern of all Governments of the Australian Commonwealth for environmental quality and stability. The import of species from overseas is regulated strictly, requiring breeding through several generations has completely enclosed quarantined systems prior mariculture production. These regulations, **which are defended as absolutely essential,** increase initial costs but offer the prospects of long term sustainable disease-free productivity, and minimize the likelihood of infection of natural stocks.

INCENTIVE

Most states in Australia have given very few incentives to mariculture. This places the developing industry at a comparative disadvantage with many other countries (such as Taiwan) where signifiant taxation benefits apply during the first five to seven years of operation. In contrast, in many States of Australia license fees and the fees for discharge of water from mariculture ponds may be regarded as high for a new industry. There are some tax incentives for industries which wish to undertake research or to support research in mariculture. Perhaps the most attractive is a 150 % tax saving on monies invested in approved research whether conducted within a company or contracted to a research institution.

ENVIRONMENTAL CONTROL

The Australian Government and the different State Governments have been very cautious in their attitudes towards the development of mariculture and its possible effects on the environment. Strict regulations control water quality and standards define the conditions under which different types of nutrient and sediment load from mariculture can be discharged into open waters. This is clear recognition that the growth of

Tab. 1. — Lists those species presently cultured in Australia as well as those thought to have future potential, adapted from Finney, A.J.F. and Garland, C.D. (unpublished data)

Commercial	Experimental (Pilot Scale)	Potential
	FINFISH	
Salmo salar	*Sillago ciliata*	*Plectropomus maculatus*
Salmo gairdneri	*Coryphaena hippurus*	*Ltjanus argentimaculatus*
Lates calcarifer	*Lutjanus johni*	*Epinephelus suillis*
	Epinephelus tauvina	*Epinephelus malabaricus*
		Acanthopagrus australis
		Chrysophys auratus
		Seriola lalandi
		Seriola hippos
		Argyrosomus hololepidatus
		Latris lineata
		Rhomboslea spp.
		Aquarium spp.
	MOLLUSCS	
Saccostrea commercialis	*Haliotis rubra*	
Crassostrea gigas	*Haliotis laevigata*	*Amusium balloti*
Ostrea angasi	*Tridacna gigas*	
Saccostrea echinata	*Pecten fumatus*	
Mytilus edulis planulatus		
	CRUSTACEA	
Penaeus monodon	*Penaeus esculentus*	*Panulirus cygnus*
Metapenaeus macleayi	Various others *Penaeus* spp.	
Artemia sp.	*Scylla serrata*	

many species under high density situations is likely be accompanied by the development of diseases and there is a major concern that any disease occuring under farming conditions must not be transmissed to the open waters where it may affect the very large commercial industry based on open-range harvesting.

Most States of Australia have pollution control commissions or environmental agencies which check very carefully on all types of coastal development. Most mariculture operations are developed either in coastal ponds where the waters are discharged back into the rivers or to the ocean, or in cages which are suspended at different depths either in channels, river mouths or in the open sea. Raft and rack culture techniques are preferred for commercial oysters production, loose-weave rope culture for mussels, and Scallops have been grown on plastic strips.

The selection of land for a proposed mariculture operation is very carefully screened. In some areas ponds are prohibited because of concern about aquaculture discharges into creeks or rivers which are believed to be important for convential commercial fisheries.

The levels of pollutants which are allowable in discharged waters are very carefully defined. An example is shown in Appendix 1 (State Pollution Control Commission N.S.W. 1988).

FINFISH

Atlantic Salmon and Ocean Trout

The sea cage culture of introduced Atlantic Salmon and ocean trout in Tasmania is undoubtedly the most successful Australian finfish farming activity. Production figures are given in Table 2.

Tab. 2. — Salmon and Trout production in Tasmania. Data from Purser (1988) and Purser J.

	Atlantic Salmon *Salmo salar* tonnes	Rainbow Trout *Salmo gairdneri* tonnes
1986/87	50	300
1987/88	250	1200
1988/89	500	1 200
1989/90	2 500	1 500

The higher market value of salmon (75 % greater than trout) makes this the preferred farm species as more smolt become available. Currently the production of smolt is controlled by Salmon Enterprises of Tasmania Pty Ltd (SALTAS); a joint venture between the Tasmania Government, a Norwegian aquaculture company and commercial growers. The company produces smolts in its freshwater hatchery in the upper Derwent Valley and has a monopoly on their production until 1995.

The hatchery production of rainbow trout comes from two commercial hatcheries which together are capable of supplying the anticipated demand.

In october 1987, the Tasmanian Government declared a moratorium on applications for more permits. This has now been further extended to allow an accurate assessment of the industry's development. There has been some local protest from recreational groups who claim that the farms are impeding protected waterways.

During the cage culture period the fish is entirely on a formulated diet. Food conversion rates (1.5-2) : 1 have been achieved using locally produced pellets. Nets are changed every 2-3 weeks as fouling is a problem. Predators such as seals have been reported from some areas.

Gjovik (1987) in his overview of salmon and trout farming lists the key factors responsible for the successful establishment of the industry in Tasmania as follows :

1. close to ideal climatic conditions and sites suitable for net pen culture,

2. the disease-free status of the fish stocks, maintained by Tasmania's isolation from any other stocks of salmonids,

3. the captive market provided by quarantine legislation,

4. government support, and early establishment of suitable legislation,

5. industry organization through the joint development company SALTAS,

6. early development of support industry (fish feed, equipment),

7. creation of infrastructural support (advice, research, fish pathology, etc).

Research priorities for this industry include : the development of triploid stock, feed cost reduction (i.e. need to reduce the dependence on fish meal), optimization of feeding rates and several disease problems (e.g. amoebic gill disease and *Streptococcal* infection) normally associated with fish being exposed to high water temperatures ($>20^{o}C$) for long periods (Maguire. G. and Munday, B. pers. comm.).

Lates calcarifer - **Barramundi**

The recent appearance of plate-sized (400-500 g) barramundi on the Australian market signals the emergence of a new tropical finfish mariculture industry for Australia. Sea Hatcheries Limited of Queensland produced 150,000 such fish using cage culture methods during 1988 and already forecast production of up to 600,000 during the 1989 season (Heasman 1988).

The Queensland Department of Primary Industries is also actively researching barramundi culture and Mr McKinnon of that Department will present an overview of recent advances during this workshop.

Coryphaena hippurus - **Dolphin Fish (mahimahi)**

Southern Sea Farms Ltd. of Western Australia have been actively engaged in research directed toward the commercial culture of mahimahi. Already broodstok conditioning and spawning techniques have been established and problem areas during the larval rearing process identified. The company list some of its recent achievements as including the development of live food enrichment techniques for mahimahi larval

culture and the formulation of specific inert pelletized food suitable for weaning juveniles (Southern Sea Farms 1988). The hatchery at Yanchep. Western Australia, has already been upgraded and now has the capacity to supply enough juveniles to stock a 50 tonnes/annum grow-out farm. Grow-out trials are now in progress at a number of locations in Western Australia (Nel. S. pers. comm.).

OTHER FINFISH SPECIES

Although there are no other commercial mariculture ventures in Australia relying on finfish, other than those already mentioned, several other species are considered to have culture potential.

One of these, the summer whiting *Sillago ciliata*, has been reared in low densities at a commercial oyster hatchery in NSW. Researchers in Southern Queensland have also developed reliable sperm storage and spawning techniques for this species (Goodall, A. pers. comm). Similar work is about to be carried out on the yellow finned bream *Acanthopagrus australis.*

Further to the North Sea Hatcheries Limited have recently acquired broodstock of the estuary cods *Epinephelus tauvina*, *E. suillis* and *E. malabaricus*, the golden snapper *Lutjanus johni* and the mangrove jack L. *argentimaculatus* (Heasman, 1988a). Initial spawning work on E. *tauvina* and L. *johni* using techniques similar to those developed for L. *calcarifer* has proven successful (Heasman, 1988b). Spawning trials on the coral trout *Plectopomus maculatus* have been carried out by the Queensland Department of Primary Industries in Cairns (Rimmer, M. pers. comm.).

Southern Sea Farms of Western Australia intends to investigate the potential of the snapper *Crysophysis auratus*, yellowtail kingfish *Seriola lolandi*, the Western Australian jewfish *Glaucosoma hebraicum*, sampson fish *Seriola hiopps* and the mulloway *Argyrosomus hololepidotus.*

Garland (1988) has included the Tasmanian trumpeter *Latris lineata*, some aquarium spp. and the flounders *Rhomboslea* spp. as having potential.

MOLLUSCS

Saccostrea commercialis — Sydney rock oyster

Cultivation of the Sydney rock oyster is based in New South Wales and has an annual production value of A $ 35 million. Despite the impressive track record of this well established mariculture industry a recent decline in production has occured (Maguire *et al.*, 1988). Some of the factors contributing to this decline are : rising labour costs, disruption to conventional production methods (especially regulations to control the spread of *Crassostrea gigas*) declines in growth rates, disease problems (e.g. winter mortality in southern NSW and the QX haplosporidian parasite (*Marteilia sydneyi*) in northern NSW) and toxicity problems associated with the use of tributyltin antifouling paints.

The NSW industry is also under competition from *C. gigas*, both in terms of biological effects e.g. overcatch of faster growing *C. gigas* (Holliday and Nell, 1985; Nell, 1988), and marketing factors, especially the supply of cheaper *C. gigas* from Tasmania and New Zealand (Holliday *et al.*, 1988).

Following an extensive oyster research programme at the Brackish Water Fish Culture Centre in NSW methods such as subtidal culture and single seed production have been proposed to re-stimulate *S. commercialis* production within the industry.

Some of the advantages listed by those authors for subtidal culture over conventional methods include :

- quicker growth rates (Wisely *et al.*, 1979);
- utilization of deep water areas;
- increased production through expansion of growing areas and higher yields per unit water surface area;
- reduced production costs (Green, unpublished report, 1983)
- reduced risk from heat kill during intertidal exposure (Poter and Hill 1982);
- the ability to work the crop regardless of tidal influences.

Holliday *et al.* (1988) state that the main advantage of single seed culture over conventional methods is the virtual elimination of the laborious task of culling oysters. The single seed spat are also more uniform in shape and this allows the use of grading and packaging machines which are used widely overseas to reduce production costs. Single seed culture in hatcheries potentially allows the genetic selection and improvement of faster growing or triploid oysters (Mason, 1986; Griffiths *et al.*, 1988).

Crassostrea gigas — Pacific oyster

The Pacific oyster is a fast growing introduced species now widely cultured in Tasmania. In 1988, 1,5 million dozen oysters valued at A$4 million were produced in this state (Crawford, C. pers. comm.). Three large hatcheries are presently supplying growers with spat. Suitable sites for culture have also been identified in South Australia (Grove Jones, 1988). The culture of this species is banned in Victoria, and in NSW regional boundaries have been imposed to limit the transfer of oysters between river systems in an attempt to control the natural spread of *C. gigas* populations.

Ostrea angasi — Flat oyster

Research into hatchery production, nursery techniques, growth and meat yields of the flat oyster by the Victorian Marine Science Laboratories has demontrated that a commercial crop can be grown within two years (Hickman *et al.* 1988; O'Meley 1988). In response Government has approved 21 hectares of lease site and the pilot scale hatchery is to be upgraded to enable the production of 5 million seed by 1988/89 (Kirner, 1988).

Crassostrea echinata — Tropical blacklip oyster

The tropical blacklip oyster *C. echinata* is cultivated on a small scale in north Queensland.

Pinctada maxima — Pearl oyster

The development of culture techniques to produce hatchery reared pearl oyster spat for pearl culture is well underway in Western Australia. This research is regarded as vital to ensure the long term viability of the industry in that State, worth A$55 million annually.

Each year 450,000 shells are collected in Western Australian waters by the 13 licensed pearling companies and transferred to their lease sites for culture (Scoones, 1988). During the early 1980's high mortalities from *Vibrio* infections during these transfers were of paramount concern. Handling methods were later improved after the infections were linked to :

— cold water temperatures during the transfer period,

— crowding of oysters during transport,

— inadequate water circulation in carrier tanks (Pass, 1988).

Attempts to collect wild spat for reseeding purposes have been largely unsuccessful as there are several spawning peaks during the season with no specific periodicity (O'Sullivan and Munro, 1987).

The current hatchery programme in Broome, WA relies on local broodstock, the transport of which to southern research centres often hindered previous rearing experimentation. Larvae are now reared to the settlement stage in 2-3 weeks then ongrown for a further 12-15 months to a size suitable for nucleus implantation (Dybdahl *et al.*, 1988).

The Queensland pearl culture industry had 16 farm licences in 1988 (Curtis, 1988). One operation at the Escape River includes a small hatchery which has successfullly produced spat but is suffering significant juvenile mortalities.

Declining shell production in some of the traditional grounds around the Torres Strait is also of concern to the Queensland industry (Colgan, 1988).

Mytilus edulis planulatus — Blue Mussel

Production of the blue mussel *Mytilus edulis planulatus* in Port Phillip Bay, Victoria has increased from 30 tonnes in 1984/85 (O'Sullivan, 1987) to over 1,000 tonnes in 1988 (Hickman *et al.*, 1988). This increase has been due largely to research into the culture of this species at the Marine Science Laboratories at Queenscliff, Victoria.

Mussels are rope cultured and breeding studies have indicated that the industry could rely on the collection of wild spat during their spring settlement. Prior immersion of mussel ropes for two months to allow a dense growth of filamentous hydroids increased spat catches. An optimum spat density of 250/m of rope was determined and found to produce 30 % more meat/mussel than those grown at 750/m of rope (Hickman *et al*,. 1988).

Haliotis ruber, Haliotis laevigata — Abalone

Abalone are a high priced shell fish with annual production from the wild fishery in excess of A $ 100 million. Abalone culture in Australia is underdeveloped and untried on a commercial scale (McShane, 1988a). Successful culture techniques have been developed overseas (e.g. Japan, China and the USA) and are now being tested on Australian native species. *Haliotis ruber* and H. laevigata appear to have the greatest potential (McShane, 1988b).

By far the largest commercial investment today is that of Tasmanian Univalve Pty. Ltd. who, together with a joint venture Japanese partner, have begun to establish a A $ 5 million land based farm on the east coast of Tasmania. At full capacity they plan to export 1,000,000 abalone annually (McShane, 1988c).

McShane (1988b) predicts a bright future for abalone culture in Australia and has indicated that :

— land based culture to a marketable size of 6-8 cm shell length is preferable.

— a suitable artificial food needs to be developed because the natural harvesting of their preferred food (red seaweeds) is not practicable on a large scale.

— developments in selective breeding, including hybridization, should provide a mean of tailoring the cultured product to meet market demand.

Tridacna gigas — Giant Clams

The largest and fastest growing of the giant clams (*T. gigas)* is now considered to have commercial mariculture potential. Research into this species by a team from the James Cook University of North Queensland commenced in Australia in 1985 as part of an international collaborative project funded by the Australian Centre for International Agricultural Research. Additional funding to assess the potential of clam mariculture in northern Australia was provided by the Fishing Industry Research Trust Account (FIRTA) (Lucas, 1988).

The culture of *T. gigas* can be divided into five phases (Crawford, Lucas and Munro, 1987).

1. **Spawning** : Recent advances have included the development of gonad biopsy techniques and the use of serotonin to induce spawnings (Braley, 1985; Crawford *et al*,. 1986).

2. **Hatchery Phase** : During this phase the free swimming veliger larvae are fed unicellular algae (*Pavlova salina, Isochrysis galbana* and *Tetraselmis chuii*) at concentrations of 10,000 to 20,000 cells per ml (Barker, J. pers. comm.). It is envisaged that a microencapsulated diet will be formulated for this phase (Southgate, 1988).

3. **Nursery** : Late stage pediveliger larvae are transferred to raceway systems and soon after settlement are inoculated with zooxanthellae. This essential symbiont is either cultured or extracted from the mantle tissue of other clams (Crawford *et al.*, 1986). High mortalities post-metamor-

phosis and the fouling of juvenile clams by benthic algae are the main problems during the phase.

4. **Open Nursery Phase** : The juvenile clams of >20 mm are then reared for 1-2 years in mesh cages to exclude predators. During this stage maximum growth and high survival were obtained in the interdinal zone when compared with subtidal cultures, especially when cages were located on protected fringing reefs (Lucas, 1988).

5. **Grow Out Phase** : *T. gigas* of two years and older are about 200 mm in shell length and have virtually no predators. They are grown on the intertidal zone without protection (Lucas, 1988).

James Cook University is now seeking a joint venture partner to translate the experimental results into commercial production. The identification of appropriate markets will be critical to this development. Preliminary trials have indicated that meat from 2-3 years old clams is potentially a high priced « sashimi » suitable for Japanese cuisine (Cowan, 1988).

Two commercial clam growers are in operation in Australia although only small numbers of juvenile have been produced so far.

Pecten fumatus — Scallops

In 1987 the Tasmanian Government signed a Memorandum of Agreement with the Overseas Fisheries Co-operation Foundation of Japan for three years project to examine the potential for scallop cultivation — both reseeding and hanging cage culture — in Tasmania (Crawford, 1987).

Already the results from this project indicate that overseas scallop growing technology can be readily applied to one of the Tasmanian species, *Pecten fumatus.* Under culture conditions this species exhibited excellent growth rates, much higher than those reported from the wild (Friend. R. pers. comm.).

The major problem facing researchers is the limited availability of spat. Attempts to capture wild spat have been largely unsuccessful due to a low density of natural broodstock. Efforts to increase this density by maintaining scallops in hanging cage cultures may increase future spat catches in localized areas. Hanging cage culture is preferred to reseeding at this stage because of limited spat availability and the higher mortality rate associated with reseeding (Friend. R. pers. comm.).

Limited availability of natural spat has prompted further hatchery research. Two commercial oyster hatcheries were recently contracted to supply spat for this research but few were produced.

The economics of culturing this species have yet to be demonstrated.

Trochus niloticus — Trochus Shell

There is presently some interest in the reseeding of depleted trochus stocks, particularly in the Torres Strait region. This has been prompted by the high value of trochus shell : currently A $ 4.000/tonne.Trochus eggs are lecithotropic (the developing larvae feed on their yolk reserves) which makes the larvae easy to rear to settlement stage. Nash (1988) obtained almost 100 % survival in some experimental trials, however, considerable

mortalities occured after settlement, due to predation. He considers that until geographic areas of the low juvenile mortality are identified, reseeding programmes would not be successful.

CRUTACEANS

Penaeid Prawns

The pond rearing of penaeid prawns, although very much a new industry in Australia, has developed rapidly during the last few years. Development has been concentrated primarily in Queensland and northern NSW. Farms have also been constructed in the Northern Territory and certain areas in north western Western Australia have been identified as having future potential.

Today 200 ha of ponds have been constructed in NSW with another 300 ha being approved (Maguire and Allan 1988; Maguire *et al.*, 1988). Pondage area in Queensland is about 180 ha (20 farms) (Curtis, 1988). However, recent developments in the Cardwell area will increase this total. Farms range in size from 1 ha to >50 ha and are owned largely by cane farmers diversifying their activities or investment companies (Robertson 1988). The average size of newly constructed ponds is c. 1 ha.

Production estimates for Queensland and NSW during the 1987/88 summer season were 200 tonnes and >40 tonnes respectively (Curtis, 1988; Maguire, 1988). These figures are misleading as a significant number of grow-out ponds were not stocked effectively. Yields of around 3T/ha per crop have been the highest achieved so far but these are expected to increase during the 1988/89 harvest. One particular farmer in southern Queensland is confidently predicting a summer crop in excess of 7T/ha from certain ponds (Moreton Bay Prawn Farms pers. comm.).

Penaeus monodon is the main species cultured. However, the industry is actively investigating alternative species especially *P. esculentus.* Small quantities of *Metapenaeus macleayi* are grown in NSW where the capture of wild juvenile seed is currently allowed. Trial crops of *P. plebejus, P. merguiensis* and *P. semisulcatus* have also been produced.

Twelve hatcheries are now producing postlarvae for stocking ponds. These range from small backyard operation to larger complexes with production capacities in excess of 3 million postlarvae/month (these numbers are rarely achieved).

Pond management techniques are continually improving but the lack of a regular supply of healthy robust postlarvae is the biggest single constraint to the development of the industry. Irregular supply, low numbers and variable larval quality have meant that farmers are faced with poor initial stockings.

Intermittent hatchery production often results from the irregular supply of wild caught broodstock. To overcome this dependence, several maturation units are in operation. However, this industry generally questions the quality of the larvae from these units.

Disease problems, usually associated with poor water quality or inadequate nutrition have arisen, often during the hatchery phase.

Drs Lester and Owens will present results of their research on these diseases during this workshop.

In an attempt to overcome of the constraints imposed by temperature regimes upon grow-out a number of farmers are presently trailing heated nursery systems in an attempt to "head start" prawns held through the winter for a spring stocking. Apart from some obvious advantages, especially in assessing postlarval feeding and mortality, these systems will be necessary in the southern farming region if two crops/year are to be achieved.

Another top priority is to produce local feeds comparable in quality to the Taiwanese pellets on which the industry currently relies (Maguire *et al.*, 1988). Several companies are actively involved and trials are being conducted.

Research will play a critical role in the future development of this industry. Maguire (1988) summarizes some of the projects already in progress.

Scylla serrata — Mud crab

Although seven licences have been granted in Queensland for the production of mud crabs (Curtis, 1988), no commercial industry exists for the culturing of this species in Australia. The establishment of the industry awaits the development of sound larval rearing techniques as the collection of wild seed stock is illegal in this country. This contrasts with the Asian situation where seed used are taken from the wild.

Preliminary hatchery research by the Queensland Department of Primary Industries (DPI) at Deception Bay has identified the moult from zoea 5 to megalopa 1 as a particularly critical period which usually results in high mortalities. Inadequate larval nutrition is suspected as the cause. The DPI researchers have been successful in reducing mortalities associated with the first three days following hatching from $>90\,\%$ to $<50\,\%$ and have also produced 1st stage (C1) crabs (Potter, M. pers. comm.).

Low larval survival to the C1 crab stage (1-2 %) has also been experienced by a private company, Sea Hatcheries Ltd. They also consider it important to identify the essential nutrients during the rate larval stages as a guide towards the development of a synthetic larval food. This is necessary to streamline production by reducing dependence on live foods (Fielder. S. pers. comm.).

Artemia sp — Brine Shrimp

Although the demand for brine shrimp products is escalating because of the recent increases in finfish and crustacean larviculture there are currently no commercial quantities of Australian produced cysts on the market. In Western Australia, where large tracts of land appear suitable, there is interest by several companies.

A small pilot scale farm has been recently established on a salt pan area near Townsville, Queensland. Presently the owners are still assessing the viability of producing either cysts or live *Artemia* products. In Victoria, a private company, Victorian Brine Shrimp Growers, produces up to 40 kgs of live adult brine shrimp per week using intensive culture methods. These

are marketed in pet shops throughout Australia as live or fresh frozen products (O'Sullivan, 1988).

Paulirus cygnus — Western rock lobster

Given the value of the Australian rock lobster catch (A$220 million in 86/87° it comes as no surprise to learn that there is tremendous enthusiasm to develop commercial mariculture for this crustacean. Culture difficulties are immediately apparent because of its long oceanic larval phases of 9-11 months (Phillips, 1985).

One suggestion was to collect wild puerulus larvae (the final planktonic stage) and grow these to a marketable size. However, this would invariably lead to conflict with established fishermen (Phillips, 1988).

CSIRO in Western Australia is investigating *Panulirus cygnus* and consider that the fundamental step required is to rear this species through all of its larval stages in the laboratory. This group is presently seeking a commercial partner to undertake a research programme aimed at developing a technological base to establish a mariculture industry for this species in Australia.

ECONOMIC ASPECTS

Initially, effort in the mariculture industry has concentrated on production but very careful economic analysis of the viability of each specific production is required.

Ruello (1986), commenting upon the fresh water species *Macrobrachium rosenbergii*, stressed the importance of analysis market potential and identifying the most likely receivers of production well before production actually starts.

For prawn mariculture in Australia, very careful product analyses have been conducted, and it is clear that a well managed penaeid prawn farm can yield a very high economic return. Tisdell (1987) analysed the potential of giant clam farming in Australian waters, and concluded that further market analysis was essential, particularly for the mantle meat, and for a wider market for adductor muscle.

THE EXTENT OF SUPPORT THROUGH EDUCATION PROGRAMMES

Weir and Garland (1988) estimated that the total farm gate value of mariculture products in Australia for the financial year 1987/88 was A$105.17 million. The principal species were molluscs, crustaceans, fish, macro-algae; molluscs, crustaceans and fish accounting approximately A $ 102 million. By contrast the current value of the wild fishery of Australia was estimated at A $ 720 million per annum in the same fiscal year.

The authors estimate that the mariculture industry income of Australia will increase at approximately 33 % in the financial year 1988/89

and by a further 43.5 % above that level in 1989/90. Their analysis reveals that Queensland is the principal State as far as crustacean mariculture is concerned, New South Wales the principal State for molluscs mariculture and that fish mariculture is concentrated in Tasmania.

ROLE OF RESEARCH

If Australia is to compete successfully in the international markets for marine products based on mariculture it will have to develop effective and efficient intensive methods of culture because our wage structure will not allow us to compete using methods of production which are labour-intensive.

Many of the techniques originally used in Australia for prawn mariculture were imported from overseas countries, notably Taiwan. While these procedures are very effective in their countries of origin they are sometimes unsuited to Australian conditions and prospectively are not competitive in international export markets or even in local markets. Territorial agriculture in Australia is competitive internationally largely through improved stock performance by adapting existing breeds to Australian conditions. As yet there have been no such developments in the mariculture industry and genetic research directed to the improvement of stock performance and productivity should be a major research emphasis in Australia.

Several institutions in Australia are now involved in mariculture research or have initiated plans to undertake such research.

One of the Commonwealth authorities, the Australian Institute of Marine Science (AIMS), has begun research into the genetics of prawns relative to mariculture. We believe that this is the most important fundamental research that should be undertaken for the prawn industry. Additional research must be undertaken towards better understanding the life cycle and improving survival, particularly at the larval stages. Such understanding is fundamental to further genetic studies.

Our research will also cover a careful analysis of hatchery water conditions because AIMS technical support is excellent in this area.

Research in nutrition in general and in food supplies is not being emphasized within our institutions, but we are anxious to collaborate with institutions and companies on this and other aspects of research.

COLLABORATION AND COMMUNICATION

It is our firm belief that communication among research organisations and between research organisations and growers is essential to the success of the mariculture industry.

CSIRO. which is Australia's largest research organisation has only limited research activity associated with prawn mariculture at this stage. However, it does have extensive research facilities at the Cleveland Laboratories in Brisbane and at the Fisheries Laboratories located in

Hobart and in Western Australia. One of the key features of the CSIRO Laboratories in Hobart is their algal culture collection which has been the basis for the supply of algal foods for hatcheries in Australia as the mariculture industry develops.

The different State Government Departments have been involved in research into those species which are of most interest to their local production. In Tasmania the emphasis has concentrated on salmon, scallops and oysters. In Victoria research has concentrated on mussels and oysters, and in New South Wales on oysters, prawns and certain species of fish. The Queensland Government has recently constructed a research facility at Bribie Island near Moreton Bay and has been particularly active in barramundi farming in its north Queensland operations near Cairns.

The Northern Territory has stimulated investors to develop prawn farming in the Territory. That Government has a small support operation and has supported the industry without directly undertaking research.

South Australia, early involved in a joint venture with I.C.I. in oyster mariculture (particularly in development of hatchery techniques), has completed a new research facility without as yet making public the particular species on which research will concentrate.

In Western Australia the principal emphasis has been on the halophilic microalga *Dunalialla salina* and also on the brine shrimp *Artemia salina.* These activities have been largely conducted by commercial operations with a backup service provided by the State. That State has commited its efforts to better understanding its lucrative crayfish open water fishery.

During the last two to three years most universities of Australia have been giving attention for the first time to research supporting mariculture and aquaculture in general. This has been stimulated by the availability of special grants through the Fishing Industry Research Development Committee. In North Queensland, James Cook University of North Queensland is very active in research on giant clam mariculture and has coordinated a major ACIAR project over the last four years. The university also has a smaller research activity in prawn farming, particularly looking at aspects of desease and nutritional requirements in microalgal food sources.

The University of Queensland is also involved in crustacean research, but with an emphasis on the freshwater species. However, that University has indicated clearly that it wishes to be more involved in general mariculture farming.

EDUCATION

Mariculture, a relative new industry in Australia, is presently faced with a shortage of skilled personnal. The future long term success of the industry depends on training technicians and researchers well versed in aquacultural methods. Several institutions already have included aquacultural components in existing science courses. Specialist courses (undergraduate and postgraduate) are offered by the Tasmanian State Institute

of Technology (since 1983) which has been identified by the Federal Government as Australia's key centre for teaching and research in aquaculture (Forteath, 1988).

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APPENDIX 1. STATE POLLUTION CONTROL COMMISSION OF NEW SOUTH WALES 1988

Licence conditions :

1. Volume of wastes
   a) the volume of wastes discharged from the « grow-out ponds » shall not exceed..... kilolitres per day under dry weather conditions (except for during the harvesting period).
   b) The maximum volume of wastes discharged during a haversting period shall not exceed a rate of..... kilolitres over a period of four days (under dry weather conditions).

2. The wastes shall not :
   a) cause a pH less than 6,8 or greater then 8.9.
   b) contain a dissolved oxygen concentration less than 4 milligrams per litre.
   c) cause more than 20 milligrams per litre of biochemical oxygen demand
   d) contain more than 30 milligrams per litre of non-filtrable residues.

3. The following monotoring shall be carried out on wastes discharged :
   a) total volume (kilolitres) of wastes discharged daily
   b) pH of the final effluent determined on a daily basis
   c) dissolved oxygen content of the final effluent determined on a daily basis
   d) biochemical oxygen demand (milligrams per litres), of a composite sample (comprising of samples taken from each pond contributing to the final effluent being discharged at the time of sampling) determined on a monthly basis
   e) non-filtrable residues (milligrams per litres), of a composite sample (comprising of samples taken from each pond contributing to the final effluent being discharged at the time of sampling) determined on a monthly basis
   f) total nitrogen content (milligrams per litres), of a composite sample (comprising of samples taken from each pond contributing to the final effluent being discharged at the time of sampling) determined on a monthly basis
   g) total phosphorus content (milligrams per litres), of a composite sample (comprising of samples taken from each pond contributing to the final effluent being discharged at the time of sampling) determined on a monthly basis
   h) chlorophyll (milligrams per litres), of a composite sample (comprising of samples taken from each pond contributing to the final effluent being discharged at the time of sampling) determined on a monthly basis

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3

Aquaculture activities in Fiji

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Abstract — *Fiji has no aquaculture tradition, but the Government is engaged in aquaculture production since 1969, with the help of other countries and international organization like F.A.O. Some species have been tested :*

— *Fishes : Grass carp (**Ctenopharyngodon idellus**)* and ***Tilapia;***

— *Molluscs : Oyster (**Crassostrea echinata**)* and Mussel (***Mytilus viridis***);

— *Crustacean : freshwater prawn (**Macrobrachium rosenbergii**) and shrimps (**Penaeus monodon, P. indicus, P. stylirostris**).*

Fiji's expertise in aquaculture is very limited and because there is no history in aquaculture the idea of private enterprise is not easily developed in a community based on social structure, which means that a lot of ground work has to be laid first.

FINFISH

In 1974, the Freshwater Fish Farm at Naduruloulou was established in order to propagate the chinese carp species called Grass carp (*Ctenopharyngodon idellus)* which is used for the control of aquatic weeds that infest rivers, lakes and canals. In the early 1960's it was observed that the largest river in Fiji (Rewa River) was heavily infested with the noxious submerged weed *Hydrilla verticillata* and *Potamogeton crispus* which then formed extensive beds especially in the lower reaches. The biomass of these weeds increased the siltation problem, prevented the free flow of water and blocked the water ways of the local people. The growth of these weeds has increased rapidly due to the stopage of the major dredging works and the major river traffic in the 1960's. Grass carp (*Ctenopharyngodon idellus)* has been popular throughout the world in its ability to utilize and effectively control water weeds.

It is a voracious feeder, feeding on the soft and tender leaves of plants or grass thus converting into fish flesh. The fisheries Division made several attempts to import frys of grass carp from Malacca in 1969, Taiwan in 1970-1973, India in 1974, New Zealand in 1979 and Japan in 1984. The frys were raised to about 50 to 100 g size and then released into the rivers.

By 1974, the Fiji Government realized the need to establish a hatchery so that frys could be produced locally.

Hormone injection experiments were carried out but successful spawning was achieved in 1985 utilising silver carp pituitary gland.

Here at Naduruloulou the males are observed to mature in the second year and females in the 3rd year.

Selected ripe brooders are injected with hormone (lst injection at midday) dose 0.5-0.8 mg/Kg body weight of female fish. The second injection 8 hours later- dosage 1.01-1.6 mg/Kg body weight of female fish and 0.5-0.8 mg/Kg body weight of male fish. The eggs are released into the spawning tank during early hours of the morning in an egg collection case which would then be transferred to the hatching tanks.

Eggs would hatch out after 20 hours at a water temperature range of 26-28°C.

Feeding would start after all eggs hatch, hence at NRS after 3 or 4 days, hard boiled egg yolk would be blended and fed - (18-20 g) per day/10,000 Frys. Feeding done 4 times daily for 8-10 days.

The hatching would then be transferred to knookdown tanks and reared for at least a month before being transferred into nursery ponds.

In the nursery ponds, the frys are fed artificial food a mixture of Fish meal + Rice pollard in a 50 : 50 % ratio. The frys remain in the nursery ponds until they reach size of 50-100 g.

Silver carp (*Hypophalmyths molitrix)* and big head carp *(Aristichthys nobilis)* have been maintained for stocking into freshwater impoundments and for pituitary donors. Polyculture trials of the 3 species are also being carried out.

FRESHWATER PRAWN *(Macrobrachium rosenbergii)*

Successful rearing of *Macrobrachium rosenbergii* larvae to post-larvae under local condition has been accomplished at Naduruloulou Fisheries Station. The clear water technique has been adopted. Live *Artemia salina nauplii*, fish flesh, freshwater bivalve Kai (*Batissa violacea*), Kalkoso or ark shell (*Anadara cornea*), egg custard and ox-liver have been used as larvae feed. The present hatchery technique has produced an average of 60 post-larvae per litre. Production cost was calculated at $ 7.00 per 1,000 post-larvae which is lower than that obtained from any other *Macrobrachium* hatchery.

Pond culture trials have been carried out to establish a suitable culture method in Fiji. Fiji has no aquaculture tradition. Initially high stocking densities of 15 to 20 prawns per sq. meter were utilized. Results showed that 70 to 80 % of prawns were under market size after 6 to 8 months rearing. This was due to lack of proper management skills and the inavailability of a suitable feed. Some later trials were disturbed by the occasional flooding at the farm. At present lower stocking densities and mixed culture with grass carp (*Ctenopharyngodon idella*) and tilapia (*Oreochromix niloticus*) are being examined.

Stocking prawns at 1/sq. meter with grass carp has shown that prawns can grow above 40 grams within 4 months.

OREOCHROMIS *(Tilapia niloticus)*

In 1982, saw the initiation of the Rural Aquaculture programme (RAP) which is being assisted by the US Peace Corp Volunteer Scheme. The main objective is to supply seeds of *Tilapia nilotica* to farmers in rural areas where protein supply was not easily available and at the same time generate income for the farmers. Seedling production of the species is going on very well at the station and as recorded last year, a total of over 50,000 frys were supplied to farmers from the hatchery. To date there are more than 50 farmers and about 70 to 80 ponds with an average pond area of 300 m2.

The average culture period is around 150-180 days after which the fish would be about 140-180 g in body weight. Feed applied is 25 % fish or meat meal, 40 % copra meat + 35 % mill mix and fed at 3 % of the total body weight.

Last year the highest production obtained in six months was above 4,000 Kg/ha/year.

The programme is slowly extending to other parts of the main islands and at present have 6 Peace Corp Volunteers, 5 of whom are based in outer stations, at the hatchery who is assisting in the seedling production and also liaising with the Fisheries Division and the other PCV.

OYSTERS AND MUSSEL

The culture of oysters and mussels was initiated in 1969. A consultant on mollusc was in Fiji under the UNDP/FAO regional project to do a shellfish survey.

The project continued till 1976 under another FAO expert. Extensive grow-out trials and spat collection were carried out.

Till 1979, the project continued but on a small scale due to limited success in cultivation, the occassional disturbance by natural disasters and unsuitable sites.

During 1981, grow-out experiments on oysters were carried out in two new sites : Seeds of *Crassostrea echinata* were obtained from CNEXO, Tahiti with the objective of determining the growth and survival of the species under local conditions.

Factors like salinity, oyster density, depth and turbidity were examined for their effects on growth and survival.

Results showed that the growth rates were very poor, growth increment of many individuals was about 6 mm in 7 months. The growth surfaces were heavily infested with brown algae and a layer of silt, which resulted in high mortality.

The project was later suspended at the end of the year.

A small private company was assisted in starting a pilot mussel and oyster culture farm with seeds donated again by CNEXO, Tahiti. The initial growth rate of green mussel, *Mytilus viridis* and the rock oyster *Crassostrea echinata* was reported promising small shipments of CNEXO bred green mussel were received to replace the old stock.

The culture of oysters and mussels was again continued during the arrival of a japanese expert in 1982 under the JAPAN-FIJI agreement. Results were not very promising and so the project has been somewhat suspended.

SHRIMP

The Government of Fiji and France Aquaculture joint shrimp Culture Project at Raviravi was established in 1981.

The main objective was :

(i) to investigate the economic feasibility of *Penaeus monodon* culture in Fiji and to establish a commercial joint venture between FIJI GOVT., and French Aquaculture.

(ii) to design and implement further development of the prawn farm to full commercial production.

(iii) to investigate local and overseas prawn markets and to establish and adopt optimum marketing procedures for the shrimp.

The 1st Phase of Development — (1981-1983)

During the 26 months period, the main activity was on grow-out of shrimps.

Target was set on 1 tonne/ha/year.A hatchery was to be built up to allow the Project to produce its own post larvae.It was also necessary to investigate the possibility of manufacturing local feed pellets.

At this stage, the Fiji Government was to provide the sites and ponds, labour and technical staff plus other operational costs.

France Aquaculture was to provide for post larvae supply, a biologist, feed supply and a hatchery set-up.

Results

Some problems were identified during the grow-out of post larvae obtained from Tahiti and New Caledonia.

1. Water was too acidic in some ponds.
2. Pond sides were leaking.
3. Predation by birds and milkfish; even some were lost by poaching.
4. Growth of toxic mould on feed pellets during humid conditions.
5. Some post larvae were lost during transportation.

Anyhow, a total production of 1.7 tonnes/ha/year was obtained and that was higher than the target.

The 2nd phase from July 1984 — July 1986

Because of the results obtained during the 1st phase, the aim at this stage was to try and solve the domestic demands for prawns. The farm was then planned to be expanded from 7 ha to 20 hectares.

During November 1985, the Raviravi Prawn Farm had changed its name to Prawns Fiji Ltd.

This came about due to the transfer of the project from Fiji Government to the Fiji Development Bank nominees. The technical expertise was provided by France Aquaculture, this time the contract was to try and develop the farm into a commercial level. The species cultured are *Penaeus monodon, P. indicus* and *P. stylorostris.*

The shrimp farm at Raviravi is constructed on mangrove reclaimed land and does not allow a good and clean water supply to the hatchery : secondly the pond soil has a high ph value of 3-4.

During the year 1986, the project aimed at producing 7.2 million of post larvae but managed to get only 1.8 million. The post larvae were not of good quality because of bacterial infection. At this stage it was necessary to look into the possibility of modifying the various stage of the hatchery operation plus the sanitary problems to be carefully considered.

After some modification and various treatments the larvae production was perfected.

In pond culture a lot of problems is still being experienced due to the high acidity of the pond soils, predation and pond leakaged etc. An average production of 2.7 tonnes/ha/year was achieved.

In the following year, it was aimed that the production was to reach 3.2 tonnes/ha/year.

The problems mentioned earlier need to be solved to perfect productions.

OTHER SPECIES

Studie have also begun on Giant clams in 1986, especially on *Tridacna gigas* species. A common species in Fiji is *T. derasa* but mostly found in clean oceanic waters.

Experiments are on going on production of seeds and is being funded by Australian Aid.

Seawed *Eucheuma alverzii* tambalang is on-going as from 1986, with funding from the Commonwealth Fund for Technical Co-operation (CFTC). Grow-out trials of the species was started by the New Zealand Company Coastal Biological. The farming of this seaweed is increasing slowly and proves profitable.

In 1986, 171.3 tonnes of dried *Eucheuma* was exported to New Zealand.

To conclude, Fiji has a lot of potential on Aquaculture but the main draw-back be the little knowledge we have on aquaculture. The concept

of aquaculture has to be introduced to a large number of people and hopefully to instill some tradition into the field.

	1975	1980	1985	1990
Fishes				
Grass carp *(ctenopharyngodon idellus)*	Introduction	Experimental	Development	
Big head carp *(Aristichthys nobilis)*			Introduction	Experimental
Silver carp *(Hypophalmyths molitrix)*			Introduction	Experimental
Tilapia *(Oreochromis niloticus)*		Introduction	Development	
Molluscs				
Oysters *(Crassostrea echinata)*		Introduction	No Development	
Mussel *(Perna viridis)*		Introduction	No Development	
Crustacean				
Fresh water prawn *(Macrobranchium rosenbergii)*		Introduction	Experimental	Development
Shrimps				
Peneus monodon *Peneus stylirostris*		Introduction	Development	

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4

Aquaculture in Hawaii : past, present and future

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Abstract — *Hawaii's aquaculture industry has a long and colorful history. When Cook arrived in Hawaii in 1778, over 350 fishponds were in operation. Native Hawaiians had practised aquaculture for 400 years before Western contact. In 1903, the Hawaiian ponds produced 310,000 kg of fish from over 2,000 ha of ponds. Today, less than a handful are in use.*

Modern aquaculture in Hawaii began in the early 1960's, with studies of mullet culture at Oceanic Institute and mass rearing of freshwater prawns at the State's Anuenue Research Center. State sponsored industry development efforts focused on the freshwater prawn, Macrobrachium rosenbergii. By 1976, 14 prawn farms produced 19,500 kg of product worth $152,000. In the 1980's, industry effort has turned to marine species including shrimp, abalone, and seaweeds. In 1986, crop value reached $3.6 million with the majority contributed by marine shrimp. The largest shrimp farm in Hawaii, Amorient Aquafarm, Inc. produced 259,000 kg of shrimp from 56 ha of ponds in 1987.

In addition to commercial production, several international consulting companies operate out of Hawaii with annual revenues exceeding $2 million. Both University of Hawaii and Oceanic Institute conduct aquaculture research which brings over $7 million per year into the State.

Future prospects for Hawaii's industry are bright. Expanded R + D activities have the greatest growth potential with technology transfer through international consulting and training likely. Commercial activities will focus on intensive culture of shrimp, finfish and seaweeds.

INTRODUCTION

From prehistoric times (before 1778), when Hawaiian fishponds produced fish for the kings, to present marine shrimp farms, the Hawaii aquaculture industry has gone through many transformations. This paper reviews the highlights of that colorful history. It is divided into three sections : Past (14th century to mid 1960's); Present (mid 1960's to 1988); and Future, and describes the key elements and characteristics of the industry during those periods.

THE PAST

When Capt. James Cook discovered the Hawaiian Islands in 1778, he found a self-sufficient Polynesian society of 300,000 people with sophisticated resource management and food production practises. It has been estimated that perhaps 360 fishponds were actively managed for production of a variety of aquatic species of plants and animals for food (Kikuchi, 1976).

Kikuchi (1973) documented that fishponds were commonly mentioned in oral histories attributed to the 14th through the 19th centuries and concluded that fishponds appeared in Hawaiian Islands sometime prior to the 14th century. He further documented in surveys throughout Oceania. That prehistoric fishponds were unique to Hawaii, not seen in other cultural areas of Oceania. He proposed that coastal fishponds in Hawaii evolved from irrigated agricultural plots and that the fishpond was an independent Hawaiian innovation.

Apparently, Hawaiians utilized practically all sizeable bodies of water for construction of fishponds. From inland freshwater taro ponds where fish were secondary crops, to large coastal ponds where rock walls enclosed reef flats, fishponds were common throughout the islands. Fishponds were symbols of the chiefly right to conspicuous consumption and to ownership of the land and its resources. They symbolized the chief's political power and the extent of his resources. The ponds were not managed to maximize production, but provided food for the chiefs when they travelled from district to district, surveying their holdings and collecting taxes. Most of the fish from the ponds were forbidden (kapu) to commoners to be consumed only by the ruling class.

Kikuchi estimated that of the 360 ponds, area is known for 304 ponds totalling 2,240 ha (7.4 ha/pond). therefore total fishpond area in precontact times could have been 7.4 x 360 = 2,652 ha.

By the turn of the 20th century, Western influences had radically changed Hawaiian culture and fishponds had become commercial fish farms selling their products in the local seafood market. Total fish and shrimp production from ponds was documented in 1900 and 1903 by J.N. Cobb who was a fisheries biologist from the United States Fish Commission studying the commercial fisheries in the islands. A summary of his detailed reports are listed in Table 1. It should be remembered that these data represent commercial pond production for fish that was sold in the market place. The relative consistency in production comparing the two years indicates that the commercial aquaculture industry in Hawaii was well established and significant in size and value. Using current wholesale value of $6.60/kg for pond-raised mullet and milkfish in Hawaii, the fishpond industry at the turn of century was worth more than $2 million (current $) in annual production.

While previous authors writing about Hawaiian ponds have attempted to use Cobb's data to estimate pond production in Hawaiian times, it is unlikely that pond production in Hawaiian times was similar to that at the turn of the century. Most of the ponds at the turn of the century were operated by Chinese immigrants. Because of their commer-

cial interests, fertilization of ponds with animal manures was probably common, a practise that was forbidden by religious sanctions in Hawaiian times (Kikuchi, 1976). Therefore, pond production in prehistoric times was probably substantially lower than at the time of Cobb's studies.

Tab. 1. — Total fish production and value in Hawaiian fishponds in 1900 and 1903

Species	1900		1903	
	Amount (kg)	Value ($)	Amount (kg)	Value ($)
Mullet	220,696	119,902	195,507	87,706
Milkfish	88,260	47,526	101,964	22,662
Other	1,255	313	8,417	953
TOTAL	310,211	167,741	305,888	111,321

THE PRESENT

Modern aquaculture in Hawaii began in the mid-1960's at two institutions. Oceanic Institute began studies of mullet reproduction and larval rearing in 1964. Substantial basic information about mullet reproduction was developed during the 60's and 70's (reviewed in Nash and Shehadeh, 1980). In 1965, the State's Anuenue Fisheries Research Center (AFRC) began efforts to develop mass culture techniques for larvae and juveniles of the freshwater prawn (*Macrobrachium rosenbergii*). By the mid-1970's, AFRC had developed mass rearing techniques and was distributing prawn postlarvae to prawn farmers (Fujimura and Okamoto, 1970). Farmers were given free stocking material and extension services for a three-year period in exchange for production and water quality data.

By 1976, 14 prawn farms were operating and the modern commercial industry had begun. The methods used at the farms have come to be called the Anuenue method and have been described by Corbin et al (1983) and Malecha (1983). The technology was a semi-continuous system using modest stocking densities, an inexpensive feed, 0.4 ha earthen ponds, and monthly cull-harvesting. While the market in Hawaii paid a premium price for large prawns, low production levels (1000-1500 kg/ha/year) could not offset high production costs. Today, only a few prawn farms remain in operation. Recent efforts to intensify prawn production using higher stocking densities, improved feeds and mechanical aeration have resulted in production up to 4,500 kg/ha/yr on a small farm.

By the late 1970's, several large investment projects in aquaculture were started. In time, most of these failed. Analysis of the cause of these failures was compiled by an advisory committee (GAIDC, 1985). These projects failed due to a combination of poor management, poor initial citing, inappropriate marketing strategy, weak financial commitment and uneconomical production technology.

The largest farm in Hawaii is Amorient Aquafarm, Inc. Founded in 1978 as a freshwater prawn farm, the privately owned farm now raises

shrimp, prawns and a variety of fishes in 143 0.4-ha earthen ponds, 34 0.1-ha square ponds, and a shrimp/prawn hatchery. About 50 ha are devoted to marine shrimp culture (*P. vannamei* and *P.monodon*) using semi-intensive methods and about 15 ha use freshwater to produce prawns and fish polyculture (Chinese catfish, *Clarius sp.*, channel catfish and tilapia). In 1987, Amorient produced about 259,000 kg of shrimp which were mostly marketed fresh chilled whole in the local ethnic markets or as shrimp cocktail in their roadside stand (Rosenberry, 1988).

Tab. 2. — Growth of the Commercial Production Sector of the Aquaculture Industry in Hawaii 1976-1982.

	1976	1977	1978	1979	1980	1981	1982	1983	1985	1986	1987	1988
Total Area(ha)	94	98	128	197	230	219	257	203	175	175		
Prawns	10	13	43	110	124	104	122	102				
Other	84	85	85	87	87	115	135	101				
Total Production (1,000 kg)	43	56	82	112	145	154	250	192				
Prawns	20	25	50	93	136	109	144	122				
Other	23	31	32	19	9	45	106	70				
Total Value ($ 1,000)	210	281	525	1,531	1,655	1,868	2,625	1,614	2,700	3,600	6,300	5,500
Prawns	152	206	420	787	1,125	1,031	1,553	1,390	1,600	800		
Other	58	75	105	745	530	837	1,072	224	1,100	2,800		

Four or five other shrimp farms are in various stages of start-up but to date are severely limited by lack of available shrimp seed. Kahuku Prawn Co. is the largest freshwater prawn farm in the state. They produced about 16,000 kg of prawns in their 8.8 ha of ponds in 1987. Total statewide freshwater prawn production in 1988 was about 45,000 kg.

In 1981, Grace and Co. moved its superintensive shrimp company, Marine Culture Enterprises to Hawaii. Utilizing a greenhouse raceway controlled environment culture system, MCE had achieved harvests up to 100,000 kg/ha/yr in their pilot system. In 1986, MCE opened its phase I production facility consisting of 26 greenhouses with total production area of 1 ha. In 1987, when shrimp production had reached 4000 kg/wk, disaster struck. An outbreak of IHHN virus caused mass-mortality in the sensitive *P. stylirostris* and the facility had to be depopulated. The company was subsequently sold to a Norwegian firm and has still not resumed commercial activities by the end of 1988.

In 1986, an extensive survey of the commercial aquaculture industry was conducted (Main and Deupree, 1987a). They found 36 producing farms mostly located on Oahu and more than 200 people employed by the industry. Limitations on production often identified by producers were lack of capital for expansion, high production costs, disease, predation, and feed costs. Limited capital, though, was reported to be the major limiting factor. The industry was dominated by cottage-industry farms with limited financial resources. The authors recommended that the State loan programme be expanded to increase capital resources needed for industy expansion.

On the Big Island of Hawaii, several high tech aquaculture ventures have made major investments in the 1980's. Hawaii Abalone Farms pumps deep ocean water on land to produce kelp which is fed to abalone. Cyantech is a marine biotechnology company specializing in culture of microalgae, Spirulina and Dunaliella. Cyanotech uses algal raceway technology to produce their high priced algal products.

The information sector of Hawaii's aquaculture industry significantly exceeds the production sector in total revenues. This sector involves research, training and technology transfer and generates about $10 million in revenues each year (Table 3). Major contributors to this sector include Federal research grants (about 50 %), State research (about 10 %), private research companies and consulting companies (40 %). A bibliography of aquaculture research in Hawaii was compiled in 1987 (Main and Deupree, 1987b). It is anticipated that the information sector could expand to over $20 million annual revenues in the next few years. A national applied aquaculture center is being planned for construction at Oceanic Institute in 1989.

Tab. 3. — Growth of Research, Training and Technology Transfer Sector of the Aquaculture Industry in Hawaii 1980-1986.

Year	*Project Value (millions)*
1980	$ 2.4
1981	$ 3.8
1982	$ 3.6
1983	$ 5.6
1985	$ 10.0
1986	$ 9.7
1988	$ 13.0

Aquatic Farms, Ltd. is the largest international consulting firm in Hawaii and reported 1988 revenues at about $2.2 million. Most of their projects focus on tropical shrimp culture with an emphasis on *P. monodon* hatchery technology in Southeast Asia. Other consulting groups include Aquaculture Concepts, Hawaii Aquaculture Company, Inc., and OI Consultants. Each of these companies have consulting projects throughout the tropics with an emphasis on penaeid shrimp technology.

The Hawaii aquaculture industry had a combined total value of $13.3 million in 1986 and reached $18.8 million in 1988. Total full-time and part-time employment was approximately 423 persons.

THE FUTURE

The future of Hawaii's aquaculture industry will continue to be a mixture of activities. Economic analyses predict that for commercial production to be profitable, systems will have to be intensive (Wyban et al., 1987). Since marine shrimp is one of the highest priced, highest demand seafood items in the world, it is likely that commercial shrimp production

in Hawaii will be the cornerstone of the production sector as well as the focus of the information sector.

Recent work at the Oceanic Institute has developed an intensive round pond production system suited to Hawaii's rigorous financial requirements. Capable of reliably producing 45 MT/ha/year of high quality shrimp, this system has the best chance of commercial success of systems that are currently being tested or considered.

The system was first developed in 1985 and consists of a round, self-cleaning pond with paddlewheel aeration and uses disease-free seed and high quality feed. Work at both experimental (0.03 ha) and commercial (0.2 ha) scale over the last several years have demonstrated the production capacity of the system (Wyban and Sweeney, 1988; Wyban et al., 1988).

Recently, three grow-out trials were completed in the commercial scale pond. A total of 9,000 kg of premium quality *P.vannamei* were sent to market from the 0.2 ha pond in 46 weeks from stocking of trial 1 to harvest of trial three (Table 4). Ongoing work in the round pond focuses on producing larger shrimp to capitalize on the premium created by world market conditions.

Tab. 4. — Mean shrimp production and growth in Oceanic Institute. Commercial scale (0.2 ha) round pond in three 1988 trials.

Parameter	*Mean*
Stocking weight (g)	.62
Density (shrimp/m^2)	107
Harvest weight (g)	15.7
Harvest size (count/kg)	64
Duration (d)	94
Survival (%)	90
Feed conversion	2.15
Weekly biomass (g/m^2/wk)	110
Growth (g/wk)	1.15
Mean production (kg/ha)	14,971
Total production (kg/ha/yr)	44,913

Another area in commercial development with interesting potential in the future is revitalization of Hawaiian fishponds. Ideal resources for fish and crustacean culture, a number of Hawaiian ponds will likely be restored for commercial culture of mullet, milkfish, threadfin and seaweeds. Fish production in these ponds could be integrated with visitor activities such as fee fishing or visitor centers.

The information sector probably has the greatest growth potential. Because of its tropical locale, Hawaii will expand on its leadership role as an international center of tropical and subtropical aquaculture research and development and training. By the turn of the 21st century, annual revenues from aquaculture research, training and international consulting could reach $ 25 million in 1988 dollars.

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5

Shrimp Aquaculture in New Caledonia

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Abstract — *In New-Caledonia, there is no indigeneous shrimp species for commercial aquaculture and it is necessary to control the complete cycle in captivity. Since 1973 and the creation of the Station d'Aquaculture de Saint-Vincent (S.A.S.V.), a joint project between IFREMER,* ***France-Aquaculture*** *and the* ***territory of New Caledonia****, nine species have been tested and for one of these,* ***P. stylirostris****, (introduced in 1980) we are now in a commercial scale production with the tenth generation obtained in captivity.*

A semi-intensive grow-out technique, with pelleted feed, in 8 to 10 ha earthen pond, at a stocking density of 15 to 20 P10/m², give commercial and effective yields of 2 to 3 tonnes/ha/year with two crops per year. On the experimental scale yields rise to 5 or 6 tonnes/ha/year.

Over and above the experimental S.A.S.V. (breeders, maturation facilities and larval rearing for 20 millions of post-larvae/year + 10 ha of pond) there are now in New Caledonia :

— 165 ha of pond in production.
+ 16 ha in construction.
+ 120 ha in project.

— a production hatchery for 50 millions of post-larvae/year with its stocks of breeders and maturation facilities,

— a manufacture of feed (IFREMER/N.R.M. feeds/SICA NC),

— a processing plant for IQF whole frozen prawn (raw of cooked).

An inventory of the estimated 3 000 ha available for the development of aquaculture is conducted by IFREMER with the SPOT satellite.

To increase the yields and decrease the cost price we are now working on :

— an intensification of the rearing with stocking densities of 25 to 30 Pls/m² and oxygenation,

— an improvement of the pellet,

— a reliability of the hatchery (already 45 millions of Pls in 1988).

New Caledonia is a small French territory (400 km long, 50 km wide) located between Australia and New Zealand.

In New Caledonia, the aquaculture is directed by the « Station d'Aquaculture de Saint-Vincent (S.A.S.V.) » located about 70 kms from Nouméa, main town of the country.

This experimental station was created in 1972 on the initiative of South Pacific Commission and with the aid of the F.A.O.. Since 1978, it is managed by **France Aquaculture**, subsidiary of **IFREMER**, and financed jointly by the Territory of New Caledonia and **IFREMER**.

It works in collaboration with **AQUACOP** on :

— Molluscs :

- culture of the green mussel (*Perna Viridis*);
- culture and reproduction of the indigeneous trochus (*Trochus niloticus*);

— Crustaceans :

- *Macrobrachium rosenbergii*, at a small scale because of the few sites available;
- marine shrimps, the main activity, as there is in New Caledonia :
  - ⋆ favourable climatic conditions allowing year round production,
  - ⋆ an abundance of suitable sites, on the western and North coast, just behind the mangrove coastline, very good for big earthen pond. An inventory of these estimated 3000 ha available for the development of aquaculture is now conducted by IFREMER with the SPOT satellite.

For the marine shrimp aquaculture, in the seventies the first species studied were the indigeneous ones : *P. merguiensis, P. monodon, P. semisulcatus, M. ensis.*Other species have been imported : *P.monodon* from Fiji and from Malaysia, *P. japonicus, P. orientalis, P. stilirostris* from Panama and from Mexico.

P. stylirostris (mexican strain), imported in 1980 is really appropriate to the caledonian context with two very different seasons (summer temperature of the pond 32/33° C., winter temperature 18/20° C.).This species is now used on a commercial scale production with the 12th generation obtained in captivity.

1. SITUATION IN FEBRUARY 1989

Over and above the « S.A.S.V. » there are now a local pellet manufacture (SICA/NRM feeds) and 4 private marine shrimp farms or 171 ha of ponds :

— **SODACAL** : located at MOINDOU, 130 kms far from Noumea. It is an integrated farm with :

- its own hatchery (capacity of 50 million PLs/year, 30 million produced in 1988 for its first whole year production).
- 132 ha of ponds
- its own processing plant in Nouméa (capacity 5T/24 hours).

This farm, **IFREMER** is the main shareholder, must prove that the big farms can technically and economically produce and export prawns

outside New Caledonia. In 1988 it produced about 200 tonnes of shrimps, 145 tonnes have been harvested, 70 tonnes exported. The mean cost price was about 1000 CFP (8.7 US $) per kilo.

— **AQUAMON** : 25 ha of ponds, 50 kms far from Noumea, which harvested 57 tonnes in 1988, with a mean yield of 2.6 T/ha/year. These shrimps have been sold on the local market.

— **CHEVALIER** : 6 ha of ponds (9 ponds of 0.2 to 3 ha).

— **DUMBEA'S BAY** : 1 pond of 10 ha.

On the last six years Caledonian shrimp aquaculture produced :

1983 : 19 tonnes
1984 : 54 tonnes
1985 : 95 tonnes
1986 : 65 tonnes
1987 : 87 tonnes
1988 : 240 tonnes

In 1989, a production of 350 tonnes of shrimps, with the same farms as in 1988, is expected. 210 tonnes will be exported.

20 ha of new ponds are under construction and about 120 ha planned.And this year **AQUAMON** will begin the construction of a big production hatchery which could induce the construction of new ponds.The goals of the « **S.A.S.V.** », in 1989, in this development process, remain :

— to improve the techniques in the local context in collaboration with **AQUACOP**,

— to transfer these techniques to production,

— to train the present and the future producers,

— to give technical assistance to the producers,

— to maintain stocks of breeders to secure the production.

2. THE TECHNIQUES

The shrimp aquaculture in New Caledonia is based on a reproduction in captivity, larval rearing in controlled conditions and a semi intensive grow-out.

2.1. The reproduction in captivity

The breeders are reared, at low density (5/m2 till 20 grs and less than 1/m^2 after), in small earthen ponds (0.1 to 0.3 ha). In 8 months 60 to 70 grs females and 45 to 50 grs males can be obtained.At the harvest of the pond breeders are placed in 7 m^2 maturation tanks in a closed maturation room. The maturation technique is the **AQUACOP**'s technique with :

— ablation of the females,

— artificial photoperiod; daytime 12 to 14 hours,

— separate stocking of males and females : 10 animals/m^2,

- adjusted temperature of the water : 29°C + − 1°C for the females and less than 28°C for the males,
- mixed food 3 times a day : pelleted feed and fresh food (squid, mussel, shrimp),
- 50 % of water renewal,
- artificial insemination of the females ready to spawn,
- common spawning tanks (5 to 6 females).

Before each exploitation the males are checked, the spermatophores are observed under an epifluorescent light microscope after an acridine coloration. If the sperm seems inappropriate, all the males are ejaculated and used only after a ten days regeneration period.

By this way, steady results can be expected :

- more than 3 spawns/female/month,
- about 400 000 egges/spawn,
- 20 to 30 % of fertilization,
- 70 to 80 % of hatching.

2.2. The larval rearings

They are conducted till P3 stage in 5 or 10 m3 fiber tanks at an initial density of 100 Nii/litre. at 29°C + − 1°C.

The normal sea water (treated with EDTA 5 grs/m3) is filtered over 1 µm. The water renewal is low at the zoea stages but important (50 to 100 %/day) at the mysis and post larva stages.

The larva are fed algae (Z1), algae + microparticule diets (Z2, Z3), algae + artemias + microparticule diets (M1), artemias + microparticule diets (after M1).

Antibiotics (furazolidone, chloramphenicol) and antifungol (triflua-rine) are used at preventive or curative doses.The survival Nii/P3 is of 50 to 80 %.

After P3 (15 days of larval rearing) the post larva are gradually acclimatized (temperature, filtration of the sea water) to the outside conditions and P5 are transferred to a nursery 7 days phase :

- 1.5 million PLS in an outer cement tank,
- 50 % of daily water renewal, sea water filtered in 50 then 300 ñ,
- artemias and microparticule diets,
- survival between 70 and 95 %.

2.3. The semi intensive grow-out

It occurs in 8 to 10 ha ponds filled by pumping.

2 or 3 days after the filling up of the pond, 15 to 20 PLS/m2 are stocked.

The daily water change raises from 5 % during the first month to 25 % on the last month.

The grawns are fed 3 times a day with the local pellet which cost about 0.9 USD/kg. The daily feeding rate increases till 5 grs (4 %) and then decreases : 3 % at 10 grs, 1.5 % at 20 grs.

The pH varies between 25 to 41 %. without problems.

Every week the growth and the sanitary aspect of the shrimps are controlled by catching about 400 shrimps with a cast net.After 5 to 6 months rearings we obtain the following results.

— 20 to 22 grs animals,

— 2 tonnes of shrimps/ha (extrapoled yields 4T/ha/year),

— food conversion ratio : 2 to 2.5,

— survival 50 to 60 %.

After each rearing, the pond is dried out, the bottom is ploughed and the competitors are eliminated with rotenone.

NB : extrapoled yields of 6T/ha/year have already been obtained on small 1000 m^2 pond at the experimental station.

3. CONCLUSION, PERSPECTIVES

Now it is very profitable to product shrimps and to sell it on the caledonian market at the price 1500 CFP (13 US$)/kg. But this local market can only absorb about 150 tonnes/year.

It is much difficult to make some profit on the export market were the best price obtained is 9.3 US $ (CAF price).

The cost price must be lowered and that is now the goal of most of our works, specially on :

— the intensification of the rearings, with stocking densities of 25 to 30 PLS/m2, which needs an oxygenation of the ponds. AIRE O2 acration systems are used, 30 to 40 HP/10ha. The first trials are very good and, in such conditions, mean yields of 5 T/ha/year can be expected (on 1 ha pond, a current trial will produce more than 4 T/5 months),

— the improvement of the pellet (better growths can be obtained),

— the increase of the reliability of the hatcheries. The first larval rearings on biological filter (without water renewal) are now realized.

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6

Aquaculture in New Zealand

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Abstract — *Aquaculture is a relatively new activity in New Zealand. The indigenous rock oyster (**Saccostrea glomerata**) was the first target for aquaculture in the 1930's although it was not intensively cultured until the 1960's. The faster growing Pacific oyster (**Crassostrea gigas**) appeared in 1969/70 and now comprises virtually all the farmed oysters in New Zealand.*

*New Zealand's most valuable aquaculture industry is the culture of green-shelled mussels (**Perna canaliculus**) which are grown by suspended culture from longlines, earning NZ $ 30 to $ 35 million in export and local sales.*

*Natural stocks of New Zealand scallops (**Pectin novaezelandiae**) are dredged to their maximum limit but a joint New Zealand/japanese enhancement program over the past 5 years has successfully shown scallop farming to be a viable proposition in New Zealand and production could increase by 500 percent in the next 10 years.*

*Three years ago, salmon farming was also at a very early stage of development but sea cage and pond rearing of salmon is now well established (mostly quinnat **Onchorynchus tshawytscha** and a small quantity of sockeye **O.nerka**).*

Other species being cultured include a native abalone, the New Zealand freshwater crayfish or koura, Australian marron, grass carp and giant freshwater prawns. Species being cultured on an experimental basis include seaweeds, the New Zealand rock lobster, native flat oysters, channel catfish, silver carp and scampi.

Control of marine and freshwater farming is vested in several different government agencies many of which are operating under outdated legislation so obtaining aquaculture permits can be expensive and time consuming. This, combined with the limited availability of venture capital has slowed the development of aquaculture in New Zealand in past years.

New Zealand's economy has traditionally been based on meat and dairy products — not surprising in a land of only 3.5 million people but 60 million sheep ! But we also have a fishing tradition which goes back a very long way. New Zealand comprises 3 main islands — the North Island, the South Island and a smaller one to the south, Stewart Island. The Maori in New Zealand, who, it is believed, originally came from the island of Huahine here in Tahiti, have a legend about a god called Maui who went fishing. He was so intent on catching a big one, that he punched himself on the nose and smeared the blood on his hook, and in so doing, he caught the biggest fish of all — the North Island.

Unfortunately, this early acknowledgement of the sea and its resources has only very recently been translated into economic prosperity for New Zealand. We have established a large Exclusive Economic Zone extending 200 miles out to sea and as a result seafood has now become one of New Zealand's leading cash crops. But, as with all world fisheries, production is rapidly approaching its maximal projected level, and attention is turning increasingly to aquaculture to fulfil market demands for seafood.

New Zealand's location and geography lead themselves well to aquaculture. We are an island nation consisting of 3 main islands and several smaller ones. We are approximately the same size as Japan or the British Isles (268,000 km^2) and within that area we have approx. 13,000 km of coastline and approx. 3000 km^2 of lakes and rivers. So there is no shortage of clean, sheltered water.

During the ice age, many coastal valleys were formed in New Zealand and since then, the sea level has risen and turned them into extensive estuaries. (That is the geologists' view point but the real reason is that Maui's fish fought and thrashed about so much when he caught it, that the edges were damaged !).

In the north, where the climate is sub-tropical, the estuaries support dense groves of low mangroves and a rich fauna including our native rock oysters (*Saccostrea glomerata*). Further south, the climate becomes temperate and there are extensive rush and sedge wetlands which provide habitats and spawning grounds for species such as whitebait (*Galaxias* spp.), freshwater crayfish (*Paranephrops* spp.) and eels (*Anguilla* spp.).

What are New Zealand's aquaculture industries ?

* Green shelled mussels.
* Salmon.
* Rock oysters.
* Scallops.

Paua (New Zealand abalone).

Prawns :	— *Penaeus orientalis*
	— *Macrobrachium* sp.
	— *Metanephrops* sp.
Crayfish	— New Zealand Koura
	— Australian marron
Carps	— Grass
	— Silver
Flat oysters	
Seaweeds	— *Porphyra* spp.
	— *Gracillaria* spp.

Rock lobsters.

This appears to be a wide variety of species for such a small country just getting established in aquaculture, but only those marked with * are large established industries. The rest are either very small (eg. 1 or 2 operators) or are still in experimental or development phases. I will concentrate my talk on the established industries.

The indigenous rock oyster (*Saccostrea glomerata*) was the first target

for aquaculture in New Zealand. Hard substrata were placed in appropriate foreshore areas in order to increase settlement surfaces for oysters during the 1930's. Methods for collecting spat and rearing oysters on intertidal racks were not developed until the 1960's, and there are now approx. 300 hectares of fully developed farms. Wild spat is caught on bundles of sticks, although we can supply hatchery-reared spat if necessary. As the oysters grow, the sticks are separated and laid out on intertidal racks to grow to maturity. Culchless oysters are also grown by some farmers, and some deepwater farming is also carried out, mostly to gain condition and to delay spawning. Growth is regulated so crops are sold from 12 months to 2 years after settlement.

The faster growing Pacific oyster (*Crassostrea gigas*) appeared in the 1970's and now comprises virtually all the farmed oysters in New Zealand. The industry is quite small by world standards, producing approx. 4000 tonnes/yr (whole shell weight), 50 % of which are exported, mostly to Australia, Asia and the Pacific Islands.

The industry is very lucky in that it does not seem to suffer from many of the oyster pests found elsewhere eg. MSX, QX, winter or summer mortality, paralytic shellfish poisoning, predation nor major industrial or domestic pollution. We have a good shellfish sanitation programme which allows us to export to such particular markets as the U.S.A.. The only problem that the oyster industry seems to suffer from is *Polydora* sp. mudworm infestation.

Culture of the green-shelled mussel *Perna canaliculus* began after the collapse of the dredge fisheries for mussels. Developing technology and increased numbers of farm licences have resulted in an exponential increase in production of mussels since 1978. Mussels are now our 6th most valuable export « fishery » in New Zealand and certainly the largest aquaculture industry earning approx. N.Z. $ 35 million in 1988.

The mussels are grown by suspended culture from long-lines using technology based on Japanese techniques. The major growing area is the Marlborough Sounds which is at the northern end of the South Island. The industry has 2 sources of spat. Either it is caught within the Marlborough Sounds growing area by hanging « hairy » catching ropes in the water during summer and autumn. Or it is obtained by gathering spat-covered drift seaweed which is washed ashore on to beaches in the far north. The weed is wrapped around the culture rope and held in place by a cotton stocking. Within a few days, the mussels attach themselves to the culture rope, and within 1-2 weeks, the seaweed and stocking have rotted away.

When the mussels have grown to 10-20 mm, they are stripped off the ropes and reseeded back on to culture ropes at a much thinner density to allow room for rapid and uniform growth. The mussels are harvested mechanically approx. 18 months after settlement.

Like the rock oyster industry, the mussel industry suffers no apparent disease problems, and the water in the growing areas is clean and very productive. The industry does have some problems though -namely predation by fish which eat thousands of dollars worth of mussels each year, and an unreliable spat supply.

Three years ago, salmon fishing was at a very early stage of development in New Zealand but sea cage and pond rearing of salmon is

now well established and growing very rapidly. Three species, Chinook, Sockeye and Atlantic, were introduced into New Zealand around the turn of the century. The Chinook established self-sustaining sea-run populations in our rivers, but the sockeye and Atlantic did not and have become voluntary lake-limited, completing their entire life cycle in freshwater. Chinook has proved the most suitable for aquaculture in New Zealand and present production is almost all Chinook.

We have in the order of 12 fish hatcheries in New Zealand producing salmon and/or other freshwater species. Juvenile Chinook from the hatcheries are transferred to sea cages for on-growing to market size. We are world leaders in the technology of large scale cage culture of salmon with cages capable of holding 35,000 adult salmon and able to withstand gale force winds and 10 foot breaking seas.

Peak harvesting occurs in the summer (Dec.-March). This is done by herding the fish into special plastic-lined pens where they are stunned with carbon dioxide and bled immediately. They are then packed in an ice-slurry and taken to shore for dressing and packing. We can have them in Los Angeles restaurants 48 hours after harvest.

At present we are the world's largest producer of Chinook, over 90 % of the production coming from 11 sea cage farms. We also have 11 freshwater pond farms rearing salmon -mostly up to a pan size of about 600 g for the domestic restaurant and hotel trade.

Ocean ranching is also being tried using both upriver and coastal release and recapture sites. Flesh quality of fish returning to upriver sites tends to be poor however so these hatcheries are really just enhancing the recreational fishery and generating broodstock for other farms. Four coastal release sites have now been developed but to date, fish returns have not been substantial enough to make them economic.

So, at least in the short term, our salmon future seems to be in large-scale, sea cage culture of Chinook which is not farmed on a large scale anywhere else and which is readily acceptable on world markets. As little as 6 % of the world farmed salmon is Chinook. Our southern hemisphere location also provides a « window » into northern hemisphere markets as we provide fresh salmon at a time when northern producers can only supply frozen product.

In New Zealand, we have a delicious scallop, *Pecten novaezealandiae*, but unfortunately natural stocks are dredged to their maximum limit, as with most scallop fisheries around the world, are subject to large fluctuations in landings. So 5 years ago, New Zealand embarked on a joint programme with the Japanese Overseas Fisheries Co-operation Foundation to attempt to enhance the fishery by seeding.

We have found that there is no need to hatchery-rear scallops as we have plenty of natural larvae in our waters. What they lack are suitable settlement surfaces for the early juvenile stage when they need to be attached to something. The lack of settlement surfaces is partly due to the method of dredging used at harvest which destroys benthic epifauna and distributes a layer of silt. The method we have developed is to sink to 2-3 metres from the bottom a whole long-line of the « hairy » mussel ropes I talked about earlier. The ropes are left for approx. 1 month during which

time the scallop larvae settle, grow to 5-10 mm and then detach themselves. In some areas the ropes will have a good by-catch of mussels remaining after the scallops have dropped off which are sold to the mussel industry.

If we wish to seed an area that does not normally contain scallop larvae, we use the same submerged longline technique, but instead of mussel rope, we use lengths of plastic mesh stuffed inside finer mesh bags. The inner mesh provides the settlement sites for the scallop larvae. The finer outer mesh prevents the juveniles from escaping when they grow to 5-10 mm and are ready to detach and move on. The technique also gives a boost to their survival stakes because they are relatively protected from predators on the sea floor.

Approx. 3 months from settlement, the juvenile scallops are emptied out of the bags and seeded out into the new growing areas, simply by tipping them over the side of a boat. Two years later they become part of the natural scallop harvest. Last year was the first year of commercial harvest from the reseeding programme and it was very successful. If it continues in this vein, then we will be able to increase our production by 500 % in the next 10 years and we will have a $ 24 million industry.

I would like to briefly mention abalone before I finish, because we at the Fisheries Research Centre have been largely responsible for establishing aquaculture of the species. We have 3 species- *Haliotis iris, H. australis* and *H. virginica* and have an established fishery for both the meat and the shell of *H. iris.* But the natural fishery is fully developed and even over-fished in some areas. We have successfully developed techniques for rearing *H. iris* in our hatchery for 8 months of the year. There are now two farms established on-growing seed stock obtained from our hatchery but as yet they have not had their first harvest.

We have also done some successful research into reseeding the wild fishery and the first commercial trials of that are about to begin. We will use hatchery-reared juveniles to outplant into appropriate rocky habitats for these trials, but we are also experimenting with out-planting larvae directly into the fishery.

This has been just a small taste of some of the aquaculture industries in New Zealand. It is an exciting time to be involved as it is starting to establish and grow. But there are still some impediments to that development. The current economic climate in New Zealand means that investment capital for aquaculture is difficult to obtain and research funding is short.

We also have some problems with our legislation relating to aquaculture which is very out-of-date and limited. It does not have the flexibility to cope with the range of species now being considered for aquaculture. Because many of the regulations have been designed to conserve the wild fisheries, they actually impede the development of aquaculture, e.g. by making it illegal to possess small abalone or rock lobster, even in a hatchery. So we are currently reviewing our legislation as rapidly as possible in an effort to assist people into aquaculture rather than to deter them as at present.

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Problems and perspectives of the pearl oyster aquaculture in French Polynesia

P. CABRAL
Antenne de Rangiroa, E.V.A.A.M. B.P. 11, Avatoru, RANGIROA. French Polynesia.

Abstract — *The first attempt to obtain artificials pearls from the black-lip pearl oyster* ***Pinctada margaritifera*** *in French Polynesia dates back 1963. The pearl oyster culture has grown quickly especially since the eighties. This industry is now the first exportation in value of French Polynesia. 19 atolls of the Tuamotu islands have at this time one or more farms and this activity become more and more attractiveness. The rise of this industry has consequently a lot of socio-ecomicals repercussions on these atolls. However, as a young industry, the technics and the development are not always controlled and a lot of problems appeared these last years :*

+ *overfishing and depletion of broodstocks in some lagoons,*
+ *mass mortalities of cultured oysters,*
+ *large occupation of the lagoons by long lines and galvanised structures,*

...

It is now time for the management of the resource and the environment. Actually, some measures have been applied :

+ *the development of spat collection technics,*
+ *the control of the farms,*
+ *the estimation of the natural stocks in some exploited lagoons,*
+ *experiments on the artificial reproduction and culture of spat in hatchery.*

As the management of the lagoons might be very complex, a multidisciplinary research program will started next year to provide datas on the diseases and the impact of the pearl oyster culture on the ecosystem.

INTRODUCTION

Unlike most of the aquaculture production concerning the Pacific region, the Pearl oyster culture distinguishes itself by the magic of the final product, the pearl.

Dressed up in so many and marvelous term for centuries, the mystery and charm which exist around pearls have contributed, to some degree, to the development of this activity.

But it is mainly the high value of these uncommon black pearls that lead so many Polynesians to work on this field as the present situation might be compared with a kind of « gold rush ».

However, despite and because of the rise of this aquaculture in so few years, some problems appeared recently and we have to be extremely careful for the future.

A BRIEF HISTORICAL REVIEW

In French Polynesia, the fishing and use of mother of pearl as an ornament was a traditional activity which was followed by the commercial fishing and diving of shells for ornaments, studs and natural pearls. (Reed, 1973). Nevertheless, this fishery was very fluctuating and, with the exhaustion of some lagoons and the massive use of plastics, has resulted in the decline of this industry in the fifties/sixties (Intes, 1984).

It is approximately at the same time, under the incitement of the local Fishery Service, that some attempt were done to graft black-lip pearl oysters by Japanese technicians, according to their techniques in Japan.

Despite the harvest of some beautiful black pearls, it was necessary to wait 10 years before some private companies tried again to obtain round pearls in order to complete the production of half pearl, the so-called « mabe », for which the commercial market was increasing rapidly. During all those years, the first pearls produced were not sold for a long time and consequently slowed down the attractiveness of this new activity, and, above all, it seems that no Polynesian was able to master the grafting technique.

In the seventies, from a few pioneer farms, the situation has quickly evolved. Japanese technicians have decided to try the experience with some privates and the Fishery Service. They took a great part in the establisment and improvement of the first techniques to culture and graft black-lip in the lagoons of the Tuamotu atolls.

Because of the marketable value of the produced pearls, the local government has simultaneously encouraged the creation of Cooperatives in the Tuamotu islands where the broodstock was still important.

THE PEARL OYSTER CULTURE IN FRENCH POLYNESIA

Oyster supply

The supply of oysters for the graft operation has evolved in the past few years. Originally, all the oysters were fished by diving. They were usually big and old shells but, as some lagoons were exhausted (Intes and Coeroli, 1985), the lack of oysters came a great problem for the growing farms.

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INTRODUCTION

Unlike most of the aquaculture production concerning the Pacific region, the Pearl oyster culture distinguishes itself by the magic of the final product, the pearl.

the best shells are kept alive for a new operation, a few months after the harvest.

THE ACTUAL SITUATION

Over 15 years, the rise of this activity has been exceptional for the local economy (Coeroli, 1983). From 1975 to 1987, the number of cooperatives has grown from 7 to 103 and, in 1985, a new type of company was created called « family company ». They were constituted by a few members of the same family and due to their flexibility, these companies have had immediate great success and their numbers have risen immediately (Fig. 1).

Fig. 1. — Evolution of Cooperatives and family societies (1975-1987).

All these companies are managed by the G.I.E. Poe Rava Nui which is an organization that takes care, with the help of EVAAM of their loans, contacts and chooses the graft technicians, harvests and expertises their pearls and organizes the sales of the pearls and shells.

The number of private companies has also quickly grown to 24 big farms and numerous small scale enterprises. They sell their product directly in Tahiti or export it. Some of them run also a local jeweller in Tahiti. The size of the farms varies from 5 000 cultured oysters to nearly one million oysters composed of juveniles and grafted oysters.

The number of atolls exploited is growing every year. Today, more than 37 atolls have a pearl oyster culture activity, eg. spat collection, rearing of juveniles or grafted oysters. 25 atolls dispersed in all the Tuamotu. For their produce pearls. they are chosen natural broodstocks available, communication with Tahiti and above all, local inhabitants who are the first concerned and able to manage this activity so far from any urban center.

Although experiments to collect pearl oyster spat have been done in many countries for a long time (Cabral et *al.*, 1985; Victor and *al.*,1987), the improvement and success of the spat collection techniques in some lagoons by EVAAM have been realized since 1977 (Coeroli et *al.*, 1984). The collectors, attached on long lines immersed at 3 metres depth, were first made of natural bushes or coconut train but now almost everybody use artificial polypropylene ribbons. Of course, the results differ greatly between islands and over the years hovewer several millions of spats are collected every year. The main problem is the high mortality among the young oysters less than six months old.

To protect the broodstocks, the fishing of shells is now forbidden except in some cases; so the fishery is strictly controlled. Now the majority of grafted shells come from spat collection as they are also prefered by the farmers because the shells are younger and seem to produce more beautiful pearls.

Rearing techniques

The culture areas are only in the lagoons, never offshore, often near the villages. Scuba diving is necessary to build the growing structures, the spat collectors stations or check the oysters as most of the farms have underwater structures, between 3 and 15 metres.

The young shells often grow directly on the spat collectors. Some harvest them when they are 6-8 months old. There are several hanging methods, similar to those used in Japan (Kafuku and Ikenoue, 1983) : the pocket baskets, the boxes made of plastic or wood, the lantern baskets and the ear hang method. Galvanized rafts and long lines.are mostly used as hanging structures.

At the opposite of Japanese and Indian techniques, (Cahn, 1949; Chellam et *al.*,1987), surface rafts are rarely used, only in shallow water or to prepare the shells before the operation near the graftlab.

Graft operation and harvest

Two to three years are needed to have ready-to-graft oysters. The grafting technique is always monopolized in great part by Japanese technicians but a few locals have good results too, since a few years. However, because of the characteristic of the black-lip pearl oyster, experiments are done to improve the pearl quality (Coeroli and Mizuno, 1985).

Usually, the number of pearls produced is between 20 and 30 % of operated oysters but many factors might influence these results such as the experience of the graft technician, the health condition of the oysters but also the type of nucleus, the part of the mantle introduced with the nucleus and the high mortalities in some lagoons.

Less than two years are necessary to obtain a good size pearl and sufficient mother of pearl layer around the nucleus. The harvested shells are usually killed to take the pearl out of the gonad, but since a few years,

The success of the pearl oyster culture in the Tuamotu with its consequences, large income, new jobs and a pause in the rural depopulation lead other archipelagos of French Polynesia and particularly the Society islands (Tahaa, Bora Bora, Huahine...) to try the experience too. The lack of numerous oysters in these islands however slowed down these projects.

Presently, the number of pearl oysters licences is very important (table 1), and each year, several licence requests are rejected in the areas where they are already too numerous. In fact, even if these licences are controlled and compulsory to establish a culture ground, there are also numerous of unknown or under-estimated farms, so it is quite difficult to appreciate exactly the number of farms and their importance in all these isolated atolls.

Tab. 1. — Distribution of Pearl oyster licences in the Tuamotu in 1989.

Authorized Licences for 1988 in 28 Islands		
SPAT COLLECTIONS 105	JUVENILES 90	GRAFTED OYSTERS 35

THE ECONOMICAL CONSEQUENCES

For these last years, the pearls have become the first exportation in value in French Polynesia, well over the coprah. The main part is due to the private farms and particularly one or two of them. The part of the cooperatives varies between 10 to 20 %.

Tab. 2. — Export statistics and mean price of the plack pearls in French Polynesia.

Years	Exported value Pacific Francs × 1 000	Mean price of black pearls Pacific Francs
1972	300	214
1973	2 000	2 517
1974	13 400	3 454
1975	8 900	569
1976	14 700	2 413
1977	18 200	2 975
1978	128 700	2 575
1979	158 100	1 836
1980	101 800	1 836
1981	404 800	4 678
1982	98 700	3 055
1983	711 700	5 088
1984	441 300	3 933
1985	1 392 500	6 744
1986	997 800	9 584
1987	2 251 500	5 523
1988	2 514 000	5 901

For 1988, the export value was 25 million U.S. $. This is mainly the result of the increase of the production, as the mean price of the pearl has greatly fluctuated over the years (Table 2).

All these results have involved a lot of socio-economic changes in these isolated islands. The main consequence is the return of local people to their islands and the development of a new micro-economy with sometimes good standard of living. About 3000 people work directly in the pearl oyster industry in the Tuamotu atolls.

Of course, even if the Pearl oyster culture seems in good health, there are some problems occurring in many fields of this industry. The present results do not have to hide that. For the cooperatives for example, according to the number of operated oysters each year, the results might be about twice the actual production (Table 3).

Tab. 3. — Production results of the cooperatives.

Years	Grafted Oysters	Harvested Oysters
1979	48 118	—
1980	59 920	—
1981	97 787	33 012
1982	97 211	36 034
1983	116 837	67 356
1984	156 208	64 809
1985	186 357	76 603
1986	156 982	72 052
1987	—	63 998
1988	—	61 341

THE MAIN PROBLEMS

— The mass mortalities which appeared in 1985

In some lagoons, 50 to 80 % of cultured oysters died and no explanation was found. Grafted oysters as spats and juveniles have been attacked.

The general symptoms of the disease seemed quite similar in many islands with mantle lesions, absence of growth and hypersecretion of mucus. None infectious pathogens were observed on the diseased oysters but more studies are necessary before any conclusion today.

Today, the mortality is ever high in some islands, particularly on the grafted oysters, and this situation really compromises the future of many farms.

The overdensity of cultured shells and some inadequate culture techniques might be greatly involved in the mortalities. In this way, much stress occurs during the four or five years of cultivation such as bad rearing techniques, repeated washing of shells against high biofouling, as grafting operation.

the moment, the larvae culture is not really mastered but some spat have been obtained; the production of artificially produced spat to restock some exhausted lagoons is hoped for the next years.

- a pearl oyster culture school will open soon in the atoll of Rangiroa; this will allow young Polynesians to learn many aspects of the culture techniques, as spat collection and graft operation, but also the management of the farms and the commercial aspect of this industry.
- recent efforts for the promotion of black pearls all over the world and the creation of a technical international laboratory to expertise the black pearls might contribute to the development of an international market.

Other points need to start quickly such as a research programme on the mortalities, the biology of the oysters and better understanding of the relationship between pearl oyster culture grounds and lagoons.

CONCLUSION

Pearl oyster culture has risen very quickly and sometimes with anarchical tendency in French Polynesia. It was a gold mine due to the quite simple techniques : everything was happening in the natural conditions, the oysters seemed to be numerous and to support the cultivation techniques.

With the multiplication of farms, of cultured areas, of high mortalities and the problems of pearl quality, the pearl oyster culture enters a second phase.

Now is the time to manage the activity if we want to succeed in this aquaculture experience. We have to remember that after the gold rush, many ghost cities appeared.

Acknowledgement. *I would like to thank the S.M.A. (Service de la Mer et de l'Aquaculture) for the statistics on the pearl oyster farm licences and the GIE Poe Rava Nui for the data on the cooperatives.*

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— The oyster supply

In some lagoons, the spat collection is not sufficient to supply ready-to-graft oysters, and farms in these areas need oysters from other islands. But, to avoid any propagation of an enigmatic infectious agent, transfers of oysters were forbidden in all the lagoons, since early as 1985. The main consequence was a lack of ready-to-graft oysters these last years and a tendency to fish natural stocks. In 1988, numerous of transfers were authorized as the mortalities seemed less important. However we have to be extremely careful about these practises as most of the time, there is no selection on the transferred oysters.

— The large occupation of lagoons

There are farms which often use much more area than allowed by their licenses. In some lagoons, there are sometimes too many farms with extensive cultured areas. Small houses built on the lagoons, primarily as graft labs but often used as domestic houses also contribute to water pollution around the cultured areas.

Presently, the rules to define the cultivation areas are not restrictive enough, and very often, the situation is very confused. With the development of tourism, it will soon be necessary to plan the distribution of cultured areas in order to protect both activities.

— The lack of knowledge on basic biology of *Pinctada margaritifera*

To master the farming of such a species, we need a lot of information on several parts of its biology. However, except old works (Seurat, 1904; Ranson, 1952 and 1955), studies on the biology of Pinctada margaritifera in French Polynesia focus only on some aspects of the natural stocks (Intes, 1982; Intes et *al.*, 1986) and on the genetics of this species (Blanc, 1983; Blanc et *al.*, 1985). Elsewhere, studies have been made on the reproduction of this species in Australia (Tranter, 1958) but these data cannot be easily reliable to the particular Polynesian lagoonal ecosystem.

— The organization of the farms, especially the cooperatives

These companies are greatly helped every year by interesting loans but only a few of them have good results. It seems that these loans are more or less like subsidies for some of them and this situation does not encourage these farms to be competitive.

— The monopoly of Japanese graft technicians

The dependency over Japanese technicians for most of the farms contributes to rise the cost of the operation. Today, the grafting is the most expensive cost for the farms, and only enough competent local technicians would change this situation.

— The market, still very restricted

The monopoly of Japanese jeweller's in the black-lip pearl oyster market has to be broken to avoid great surprise in the future. Today, more than 60 % of the market is under their control with consequences on the prices. As a matter of fact, the development of the black pearl market supposes an international audience for this high price product (Table 4).

Tab. 4. — % of Black Pearl export in value by countries.

Countries	% exported Pearls in value
W. GERMANY	0,6
N. CALEDONIA	0,3
AUSTRALIA	0,1
SPAIN	0,01
FRANCE	0,8
HONG KONG	1,2
SWITZERLAND	7,0
U.S.A.	20,0
JAPAN	69,2

THE FUTURE

In this near future, it seems very important to develop the following points :

- to improve the general cultivation techniques and discover the reasons of the high mortalities,
- to improve the control of the farms to avoid heavy problems in and with the ecosystem,
- to improve our knowledges on the relationship between cultivation grounds and evolution of the environment of the lagoons, and consequently to improve our knowledge on the general biology of this species,
- to manage the stocks by the limitation of broodstock harvest, by the improvement of spat collectors and artificial breeding techniques in hatchery to restock exhausted lagoons,
- to develop the Pearl oyster culture in other sites, other lagoons but respect the other activities like fishing and tourism,
- to improve the quality of pearls instead of the quantity,
- to develop the market in countries other than Japan and U.S., to promote the product and maintain actual prices.

Some of these points are already implemented :

- artificial breeding experiments have been done by EVAAM in the atoll of Rangiroa where a hatchery was built two years ago; for

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Aquaculture of edible species in French Polynesia

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Abstract — *In French Polynesia, the research on rearing edible species has began in 1971. The aims were : to develop the local production of sea foods to satisfy the domestic market and to create new activities and employments in rural areas.*

The institutions involved in this development are : ***E.V.A.A.M.*** *(Etablissement pour la Valorisation des Activités Aquacoles et Maritimes),* ***S.M.A.*** *(Service de la Mer et de l'Aquaculture) and* ***I.F.R.E.M.E.R.*** *(Institut Français de Recherche pour l'Exploitation de la Mer).* ***E.V.A.A.M.*** *and* ***S.M.A.*** *are French Polynesian territorial agencies.* ***I.F.R.E.M.E.R.****, is a French metropolitan Government agency.*

The first step in the research /development process had been to select the more suitable species. After a few years of screening, nine candidates were selected :

— *Mollusc :*	***Saccostrea echinata, Crassostrea rhizophorae, Perna viridis.***
— *Freshwater prawn :*	***Macrobrachium rosenbergii.***
— *Seawater Shrimp :*	***Penaeus monodon, P. indicus, P. stylirostris, P. vannamei.***
— *Finfish :*	***Lates calcarifer.***

The second step has been to control, on a routine basis and at a pilot-scale the different phases of the rearing : maturation, larval rearing and grow-out. The last was to transfer the technology to the private sector.

Nowadays, edible mollusc and finfish cultures are still experimental, but three intensive shrimp farms (1ha each) and three Macrobrachium farms (10 ha, 2,5 ha, 2,8 ha) are producing at a commercial scale. In 1988, 40 tonnes of shrimps and 20 tons of fresh water prawns have been harvested; the pilot hatchery of the **C.O.P.** *produces the seeds and a local feed mill the food. The territory of French Polynesia is now constructing a commercial hatchery that will allow the development of new farms.*

So the future of aquaculture in Polynesia must be an increase in the crustacean production, but also in the development of net-pen for fishes.

The aquaculture of edible species in French Polynesia is still a new activity, without any real tradition. It really started during the 1970's when the French state agency was in charge with marine resources management,

CNEXO (**C.O.P.**) to develop aquaculture technics for the tropical and subtropical countries. The French Polynesian Territory and its fishery agency (**E.V.A.A.M. - S.M.A.**) were associated, from the beginning, to the different research programmes aimed at the selection of the best candidate species, their propagation and the different culture methods.

Most of the experimentations and pilot scale studies were conducted in Tahiti and since no species of commercial interest were found in Polynesian waters, all species had to be imported from countries all around the Pacific region and raised in captivity. The Territory profited by these activities and one can now really speak of a polynesian aquaculture, adapted to local conditions and the socio-economical context. If the quantities of fresh water prawns, shrimps, molluscs and finfish produced may look as insignificant compared to world production, the technologies used are modern and compared very advantageously to others. An increasing part of the local market is being satisfied and the initial promoting role played by the **IFREMER** is rapidly being taken over by the **E.V.A.A.M. - S.M.A.** and the private sector.

1. THE INSTITUTIONS

They are the backline of all the aquaculture activities and help directly or indirectly to the development of the productive sector by specific actions.

— The Oceanological Center of the Pacific (**C.O.P.**)

- Selection of adapted species and culture methods
- Constitution and management of the brood stocks needed
- Pilot scale experimentation
- Studies an feed formulation and processing technics
- Technical assistance
- Training of **E.V.A.A.M.** Technicians
- Evaluation of the problems encountered by the existing farms, cultures and other related activities.

— The agency for the valorization of aquacultural and marine activities (E.V.A.A.M.-S.M.A.).

- Technical assistance to grow-out farms
- Management of demonstration farms and cultures
- Production of juveniles of all cultured species in the **COP** facilities and, by the end of 1989, in their own facilities
- Promotion of aquacultural technics and enforcement of the Territorial politic for aquaculture (legal and economical aspects, land and lagoon uses).

— The « Huilerie de Tahiti »

This semi-private feed mill, in addition to coprah oil production and the manufacture of cattle and poultry feeds, produces fish and shrimp feeds with the **COP** technical assistance.

2. THE PRODUCTION

— Freshwater prawns

- Selected species : *Macrobrachium rosenbergii*
- Evolution

YEAR	POSTLARVAE PRODUCED (X10^6)	CULTURE AREA (HA)	YIELDS (MT)
1976	1	3	2
1980	3	5	6
1982	5	15	13
1988	5	15	20

- The farms : AQUAPAC 10 hectares
  LAGARDE 2.5 hectares
  E.V.A.M.M. 2.5 hectares.

- The slight increase of the production is mainly due to a better management of the farms and an optimization of discontinuous culture system. Production will not increase much more until more postlarvae can be produced by the Territorial hatchery which is built-up. New farms can also be constructed.

- Sale price of postlarvae 10 US $/1000
- Sale price of prawns 15-20 US $/kg

— Shrimps

- Selected species *Penaeus Vannamei*
  P. stylirostris
  P. monodon
- Evolution

YEAR	POSTLARVAE (X 10^6)	CULTURE AREA (HA)	YIELDS (MT)
77	1.8	0.7	1.5
80	1.2	3.0	3.0
83	3.8	3.0	4.5
86	3.8	3.0	10.0
88	11.6	6.0	40.0

The farms :

SOPOMER	1.0 ha
TAIARAPU Aq.	1.0 ha
AQUAPAC	1.0 ha
E.V.A.A.M.	2.3 ha
COP	0.7 ha

- Many reasons for the increase of the production :
  - increase of the grow-out ponds total area,
  - intensification of the cultures,
  - regular supply of postlarvae of the difficult species,
  - producers master the technics
- Sale price of postlarvae : 9 US $/1000
- Sale price of shrimps : 15-20 US $/kg

— Bivalves

- Evolution

YEAR	SPAT PRODUCED ($X 10^6$)	YIELDS (MT)
79	3.0	4.0
81	1.5	5.5
83	5.2	4.0
85	2.5	18.0
88	2.8	6.0

- The production is carried out in the very few propicious protected bays and shallow coastal lagunas of the Society Islands under the supervision of **E.V.A.A.M.**
- The production is very irregular due to the low productivity of the waters and the very variable hydrological conditions, mainly salinity and temperature.
- Sale price of spat : 10 US $/1000
- Sale price of mussels : 4 US $/Kg

— Finfish

- Selected species : *Lates calcarifer*
- Since 1987, experimental productions in pen-nets at the **COP** : 1.5 MT/year

— Feeds

- The production follows the demand to reach 200 MT in 1988
- The quality of the pellet is improving regularly
- Sale price : 0.9-12 US $/Kg.

3. WHAT FUTURE FOR THE POLYNESIAN AQUACULTURE ?

— Increase of the production to meet the demand of the local market which is far from being satisfied.

	PRODUCTION 88 (HT)	IMPORTS 88 (MT)
Fresh water prawn	20	50
Shrimp	40	90
Mussel	6	60
Fish	>4 000	2 000

For prawn and shrimp, this goal should be reached within a few years by :

- increasing the yields of the existing farms,
- building new farms according to the demand.For mussels, the rational use of the few propicious sites should allow a 50 MT production.

For finfish, now that the production of juveniles is possible in French Polynesia, the economic feasibility should be confirmed by a pilot-scale project in collaboration with **E.V.A.A.M.**

Regular and sufficient supply of juveniles of the different species.

This production is now realized in the **COP** facilities but it is limited by the size of the installations and the other tanks assigned to the personnel. The construction of the Territorial hatchery is underway; It will be managed by **E.V.A.A.M.** with the technical assistance of **COP** and will also include all the facilities to maintain the different broodstocks. This will enable the **COP** to reorient its activities foward new subjects and the improvement of the actual technics.

— Conditions for a successful Polynesian aquaculture.

Different measures should be planned :

- a middle and long term plan for the development of aquaculture,
- an easier access to potential sites,
- territorial support for the supply of juveniles,
- financial incentives for the potential investors.

Now that the production is increasing, the technics are mastered and the local market is conditionned to the aquaculture products, the private sector still hesitates to enter this new activity due to the limited local market and the large investments required. The exportation of aquaculture products might be the solution but the production costs are still high and make the products incompetitive.

CHAPTER II

PATHOLOGY

II. PATHOLOGY

II.1. PATHOLOGY OF CRUSTACEANS

ADVANCES IN TROPICAL AQUACULTURE Tahiti, Feb. 20 - March 4, 1989
AQUACOP IFREMER Actes de Colloque 9 pp. 75-87

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Disease problems in farmed penaeids in Italy

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Abstract — *The industrial farming of penaeid shrimps began in Italy in the early eighties with the introduction of* ***Penaeus japonicus***, that replaced ***Penaeus kerathurus***.

In Italy there are no farms for producting eggs and fry on a commercial basis, the restocking material is usually imported from abroad at the stage of post-larval 22 - 25.

The growing normally takes place from May to October in earth ponds at shrimp concentrations ranging from 1 to 30/m².

Various pathologies were found that appeared to be connected with husbandry techniques, such as overfeeding with low water exchange, overcrowding and poor water quality.

Our laboratory diagnosed diseases caused by :

*1. — **VIRUS.** Infectious pancreactic necrosis (IPN) virus was isolated on EPC - RTG2 cells from spawners without apparent mortality. Biological tests were carried out placing post larvae into infecting baths, with 7 × 10⁴ cfu for 60 min. The infected individuals presented locomotor ataxy without mortality; histological exmination showed cell vacuolization of the tubules of the hepatopancreas.*

*2. — **BACTERIA.** The most frequently isolated bacterium was **Vibrio anguillarum**, on TSA, marine agar and TCBS. Vaccination trials were carried out utilizing a commercial vaccine, with infection tests after 30 days. Post-larvae were bathed for 60 min. in an infected solution of 66 × 10⁷ cells. The vaccine seems to give a good protection.*

*3. — **PARASITES.** Serious infections of the gills were seen caused by zootamnium, a peritrichous ciliated protozoan that was satisfactorily controlled with permanent baths of formalin at a concentration of 20 ppm.*

*4. — **FUNGI.** Very few infections by **Fusarium solani**, causing lesions in the eye, were diagnosed.*

INTRODUCTION

A great interest in sea water crustacean rearing has developed in Italy in the last few years. After some experiences on *Penaeus kerathurus* the

interest has now shifted to *Penaeus japonicus* and *Penaeus monodon* because they appear to be more suitable for our climatic and environmental conditions. In fact, *P. japonicus* in manured earth ponds without artificial feeding can grow up to 28-30 g in 4 months (from June to September), *P. monodon* 10-20 g more in the same time.

Since in our country it is not possible to produce restocking material for production purposes, we depend totally on foreign countries. In 1988 we have imported *P. japonicus* and *P. monodon* postlarvae especially from France, Spain and China. The sanitary control at the customs is non-existent practically based on the health certificate of the country of origin. This involves the danger of importing any kind of diseases. We know that baculovirus disease has caused serious problems in some foreign areas of production and therefore our attention must always be directed to importation of juvenile stages only from surely disease free areas or farms.

PURPOSE OF THE STUDY

The main purpose of this study has been to know the actual sanitary situation as regards shrimp culture in order to programme a suitable plan of prophylaxis. In the activity of our laboratory we have tried to locate the pathological conditions which occur in any kind of shrimp farming. Where possible, we have tried to programme a study of virus and bacteria pathogenicity.

In particular we have studied :

1. the pathogenicity of infectious pancreatic necrosis virus (IPN) isolated from spawners on juvenile stage;
2. the pathogenicity of *Vibrio anguillarum*, the most widely encountered bacterium in sea water fish and shrimp farms; we have also tried to plan an active immunization (vaccination) against this agent; we must recognize that there is little experience on the resistance mechanisms of the invertebrata; our trial had no scientific object but only a practical one to assist shrimp farmers in their problems.

STUDY ON KNOWLEDGE OF SANITARY SITUATION : MATERIAL AND METHODS

We planned a series of visits to 11 shrimp farms located throughout Italy in the period 1986 to 1988. Farming is carried out in earth ponds, manured or not, almost always without artificial feeding, at shrimp density varying from 0.5 to 3-4 for m^2; water height is 80-120 cm, water exchange very poor and depending on the tide. Some trials were also tried at higher density, up to 30 shrimps for mó in small tanks but were unsuccessful.

During each visit to the farms we tried to spot weak animals; anyway, at least 20 shrimps were sampled and subjected to necroscopic tests in order to find type and location of possible external lesions. Water samples were analysed to determine the main parameters (temperature, dissolved oxygen, BOD5, salinity, phosphates, nitrites, turbidity, etc...) to find

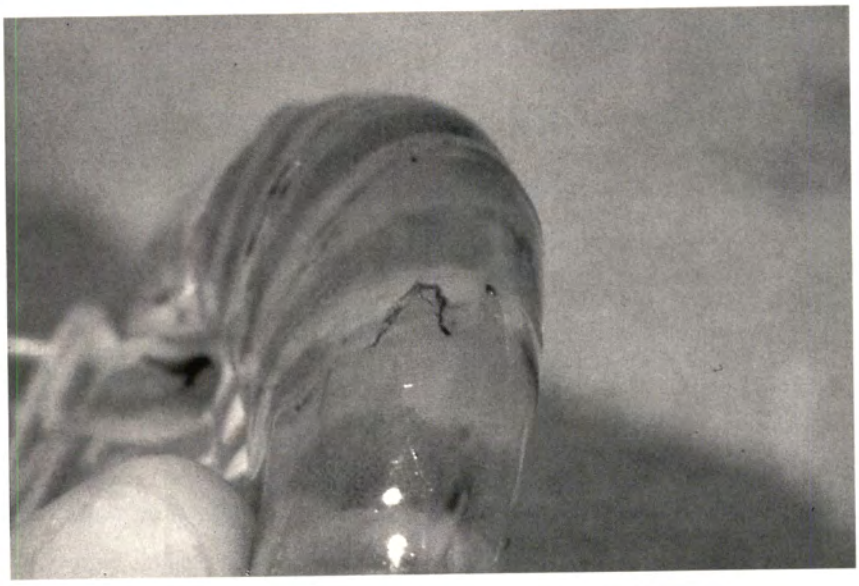

Photo 1. — Lesions by chitinolitic bacteria : dorsal area.

Photo 2. — Lesions by chitinolitic bacteria : marked erosion on branchial area.

possible relationships between environmental conditions and pathological aspects.

The sampled shrimps were immediately placed into thermic containers to bring them still alive to the Fish Disease Laboratory, where

microscopic tests were carried out on gills for parasites and cultural tests were carried out from hepatopancreas for the isolation and identification of bacteria and virus.

Microbiological tests for bacteria were carried out on the following media :

TSA
TSA NaCl 3,5 %
TSA NaCl 7 %
TCBS
Ordal
Mac Conchey
Marine Agar
Pseudomonas Agar Base
Vibrio Selective Agar
Antibiotic Sulphonamide Sensitivity Test Agar
O-F Basal Medium

Microbiological tests for mycetes were carried out on the following media :

Sabouraud glucose 4 %
Selective Agar for mycetes
Mycosel
Mycosel Agar
Littman Agar

Virus isolation was carried out on RTG2 and EPC cells, virus identification through indirect immunofluorescence.

Results

The following pathological forms were found.

1. *Chitinolitic bacteria* were present almost in all samples with external lesions, with prevalence of *Vibrio* sp. and *Alcaligenes* sp.. The lesions caused by these bacteria can be of different seriousness, and are normally restricted to modest area of erosion in the abdominal part of the exoskeleton.

These lesions were almost constantly reported during the whole period of the study, but usually they were not serious. We did not think it was necessary to treat this pathology, because of the low percentage of affected animals and also the low number of individuals per unit volume.

2. *Ciliate protozoa* were responsible for heavy losses in the farms at higher stocking density (up to 30 shrimps per m^3) and where water exchange was almost inexistent, depending only on the tide. Microscopic test on gills of examined individuals showed numerous colonies of *Zoothamnium* sp.. This is a peritric ciliate protozoan with branched colonies, which interferes in gaseous exchanges.

Shrimp behaviour was the main sign of the condition; many individuals swam on the surface of the water during the day or remained still near the edges of the pond during the night. In this pathology we found that the water had high pH and BOD values and there was notable presence of nitrogen and phosphorus.

Photo 3. — Lesions by chitinolitic bacteria : deep cephalic ulceration.

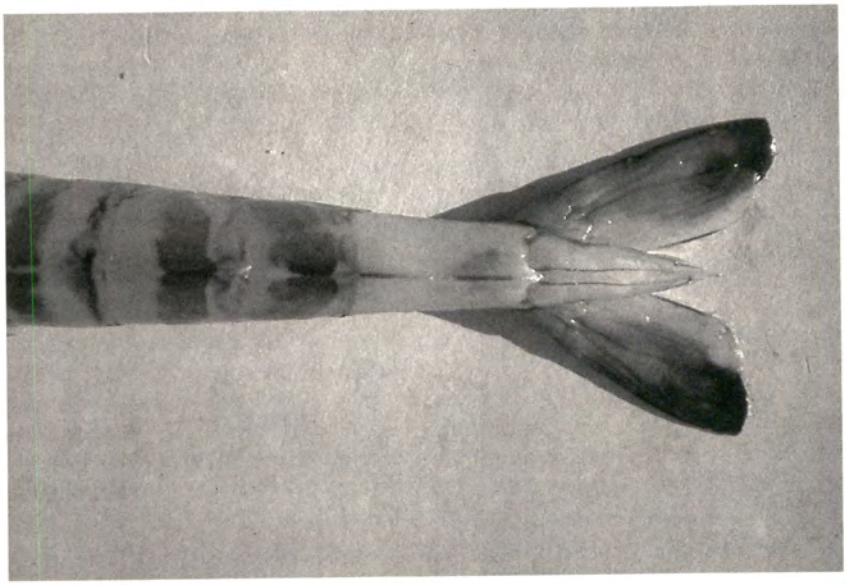

Photo 4. — Reddening of tail in *Vibrio* infection.

We also carried out disinfections with formalin (20 ppm for permanent baths) which proved effective to eliminate these parasites.

3. *Vibrio* sp. was more frequently isolated from stressed shrimps, sometimes in association with other bacteria, such as *Aeromonas* sp., *Alcaligenes* sp., and other opportunistic bacteria.

The lesions consisted of reddened areas on the caudal part of animal; blackening areas were also sometimes present on the gills.

4. *Virus* : IPN virus was sometimes isolated from adult individuals; the presence of *Baculovirus* was never detected.

Environmental factors were of remarkable importance in epizoology; in fact in some cases excessive salinity (more than 40 ‰) and high number of individuals (more than 30 shrimps per m^3) greatly impaired shrimp growth.

Still more important was the oxygen level which, in some particularly eutrophyzed basins, reached levels of 1-2 ppm in critical hours. This caused survival problems and favoured occurance of *ciliate protozoa* and *chitinolitic bacteria* on shrimps.

Discussion

Tests carried out during the study period have not showed big generalized pathological problems. Although at the moment these problems can be controlled with a suitable environmental management, we can foresee an increase of their incidence with the spreading of intensive culture methods and therefore with higher stocking density per unit volume.

BIOLOGICAL TESTS

Pathogenicity test of IPN virus on shrimp larvae

IPN virus isolated from *Penaeus japonicus* spawners was used. This virus is frequently encountered in aquatic environments and is of remarkable importance in salmonid juvenile stages.

Material and methods

We have used 2 000 postlarvae PL30 provided by the laboratory of the Italian National Research Council (NRC) and acclimatized in tanks of the Fish Disease Laboratory : salinity 25 ‰, temperature 25°C, recycle with Eheim filters, feeding an experimental feed B 1000 μ supplied by the Italian NRC.

The shrimps were divided into two groups :

1) the *control group* was placed for 60 min. into a container with 1 l of water to which 100 ml of Eagle's MEM medium for virus growth were added.

2) the *infected group* was placed 60 min. into a container with 1 l of water to which 100 ml of Eagle's MEM medium containing 7×10^5 vfp (+)/ml of virus, with a final concentration of 7×10^4 vfp (+)/ml, were added.

In the following days no mortality was noted in any tanks. A particular form of locomotory ataxy of the infected shrimps was seen, characterized by long periods of stasis on one side, followed by sudden movements with return to the normal position.

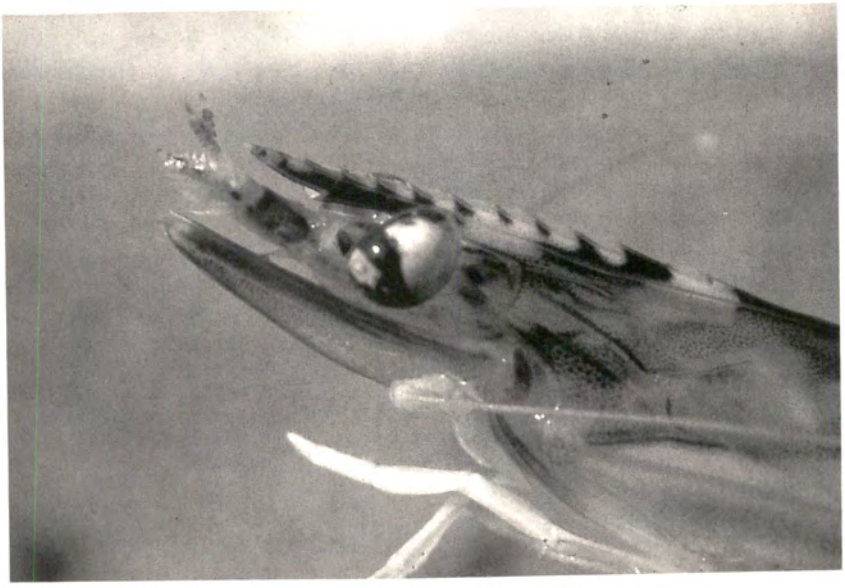

Photo 5. — Lesions by *Fusarium solani* : whitish corneal area.

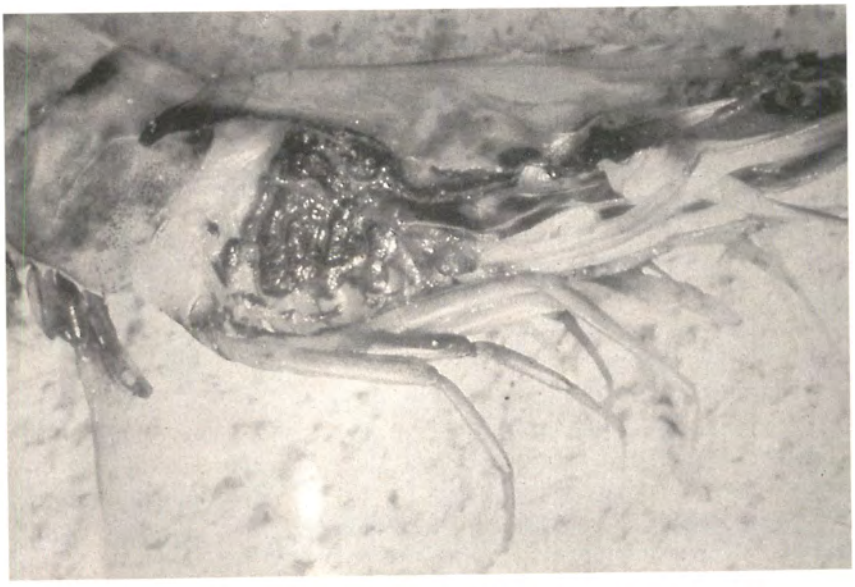

Photo 6. — Lesions by *Fusarium solani* : marked branchial blackening.

After 12 days, some of the infected individuals were fixed in 50 % glycerin and others in Davidson's for virus isolation and histological preparations, respectively.

Results

Investigations carried out by indirect immunofluorescence after growing in EPC and RTG2 cells never showed presence of virus, which evidently was reproduced and eliminated in a short time.

From the histological point of view we found differences in the structure of the hepatopancreas between infected and control animals. In the infected shrimps we noted a slight vacuolization of the tubuli cells.

Discussion

On the basis of infection tests on adult shrimps carried out with the same virus and the data reported in literature on crustacean virosis, we think that IPN virus has a limited pathogenicity. It is largely conditioned by environmental factors and can be of pathological significance in juvenile individuals, in which it can cause serious structural and functional degenerations of the hepatopancreas. It may be that the reduced pathogenicity of the IPN virus was caused by the high water temperature (25°C) during the trial.

TEST OF INFECTION BY *VIBRIO ANGUILLARUM*

We wanted to verify the pathogenicity of *Vibrio anguillarum* in shrimps; this facultative pathogen is the most frequently encountered bacterium in brackish and sea waters, and can be responsible for serious pathological conditions in sea water fish and shrimp culture.

Material and methods

A stock of *Vibrio anguillarum* isolated from shrimp and reactivated through intra-abdominal injection in adult shrimps was used to achieve infection. After 24 hours shrimps treated in this way were dead and pure *Vibrio anguillarum* were reisolated by cultures from hepatopancreas.

We used 2000 postlarvae PL25 acclimatized in tanks of the Fish Disease Laboratory : salinity 25 ‰, temperature 26°C, recycle with Eheim filters, feeding an experimental feed B 1000 μ.

Shrimps were divided into two groups :

1. the *control group* was placed for 30 sec. into a container with 9 l of water to which 1 l of Tryptone soya broth were added.

2. the *infected group* was placed for 30 sec. into a container with 9 l of water to which 1 l of Tryptone soya broth with a culture of *Vibrio anguillarum* containing 35×10^7 cfu (+)/ml, with a final concentration of 35×10^6 cfu (+)/ ml, were added.

In the following days we noted a serious mortality in the infected shrimps, which in the period of one week reached up to 80 % of the individuals.

The main signs of the condition were non-feeding, motory ataxy, lethargy and finally death, with sometimes reddening of the caudal part of the abdomen and telson.

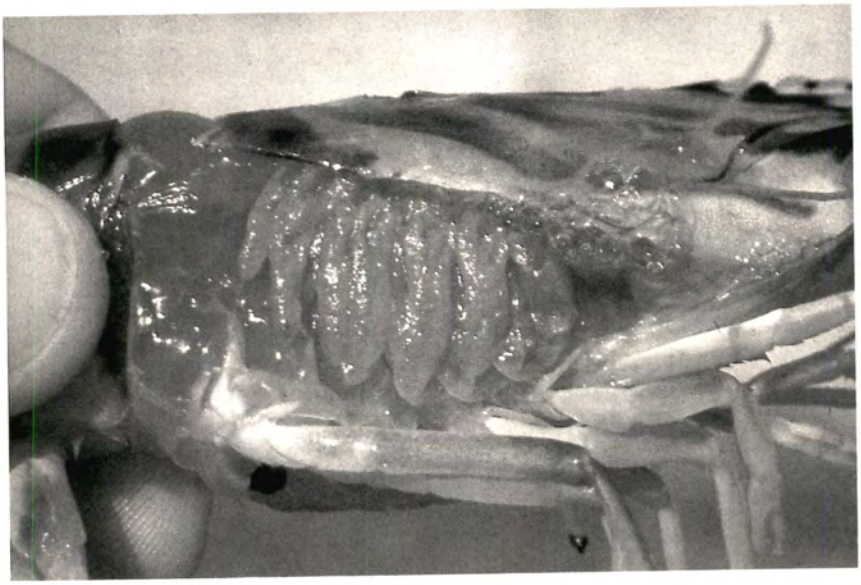

Photo 7. — Branchial blackening in individual with marked infection by *Zoothamnium.*

Photo 8. — White individuals, probably fed in an ascorbic acid deficient diet.

From the hepatopancreas of dead shrimps microbiological cultures were carried out on Tryptone soya agar, Marine agar and TCBS.

All cultural, microscopic and biochemical tests always confirmed the purity reisolation of *Vibrio anguillarum.*

The biochemical characteristics of the isolates are schematized in Table 1.

TSA 22-25°C	+
TSA 37°C	+/−
TSA NaCl 3,5 % 22-25°C	+
TSA NaCl 7 % 22-25°C	−/+
TCBS 22-25°C	+ (yellow)
Gram	−
Motility	+
O-F test	F
Catalase	+
Oxidase	+
Glucose (gas)	−
Nitrate reduction	+
Vibriostat 0/129	S
Galactosidase	+
Arginine dihydrolase	+
Lysine decarboxylase	−
Ornithine decarboxylase	−
Citrate	+/−
H_2S	−
Urease	−
Tryptophane desaminase	−
Indole	+/−
Voges-Proskauer	+/−
Gelatin proteolysis	+
Glucose (A)	+
Mannitol (A)	+
Inositol (A)	−
Sorbitol (A)	+/−
Rhamnose (A)	−
Saccharose (A)	+
Melibiose (A)	−
Amygdaline (A)	−/+
Arabinose (A)	−/+

Table 1. Biochemical characteristics of *Vibrio anguillarum* used for the test.

Discussion

The result of the infection test and the demonstration of the actual pathogenicity of *Vibrio anguillarum* in shrimps, show the particularly significant role that this bacterium can have in this kind of shrimp farming.

VACCINATION TEST AGAINST *VIBRIO ANGUILLARUM*

Considering the demonstration of the pathogenicity of *Vibrio anguillarum* and the difficulties which may arise from the need of therapeutic treatments with chemioantibiotics in pond rearing, we have thought it useful to try active immunization (vaccination) of postlarvae.

There are few bibliographic data on the immune system of shrimps which report on the type of immunity and period during which this immunity persists.

We based our trials on personal communications by foreign colleagues who applied the same method of immunization normally used on fish

and which in shrimps would give an immunity limited in time but sufficient to cover the period of 4 months required in our country for bringing shrimps to market size.

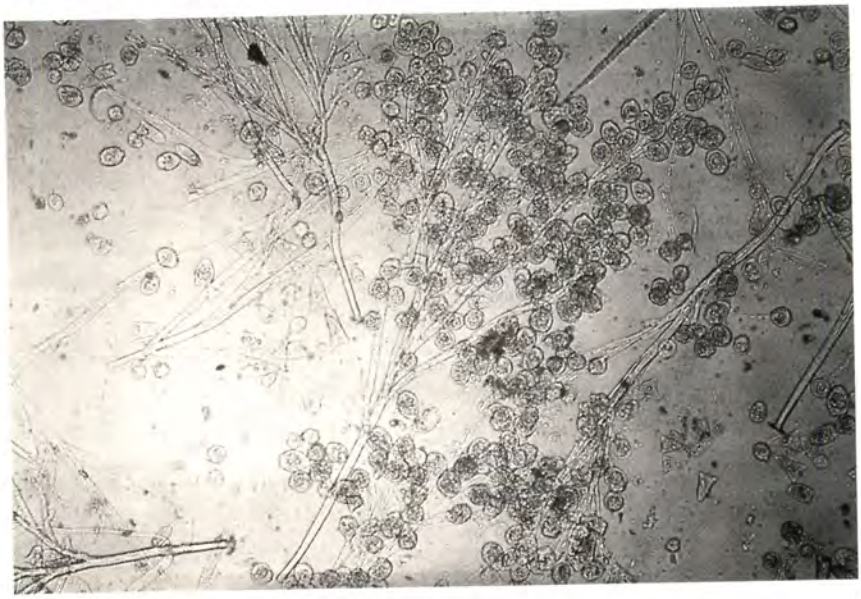

Photo 9. — Stalked colonies of *Zoothamnium* (scraping from exoskeleton).

Material and methods

We used 500 postlarvae PL30 acclimatized in tanks of the Fish Disease Laboratory : salinity 25 ‰, temperature 24°C, recycle with Eheim filters, feeding an experimental feed B 1000 ñ supplied by the Italian NRC, subsequently replaced by mussel meat.

Shrimps were divided into two groups :

1) the *control group* was dipped for 60 sec. in a container with 1.5 l of water.
2) the *vaccinated group* was dipped for 60 sec. in a vaccinal solution consisting of 1575 ml of water and 175 ml of a commercial vaccine against vibriosis; we followed the method used for trout vaccination.

After 30 days, time sufficient for the formation of antibodies in trout, we carried out infection test using the same stock of *Vibrio anguillarum* used for the preceding test.

Both vaccinated and control individuals were then placed for 30 min. in a container with 4.5 l of water to which 500 ml of infectant broth (Tryptone soya broth with *Vibrio anguillarum* culture) were added containing 66×10^7 cfu (+)/ml, with a final concentration of 66×10^6 cfu (+)/ml.

Mortality of control group began on the first day after infection and reached 30 % after 9 days. By cultures carried out on dying and dead shrimps it was always possible to reisolate *Vibrio anguillarum.*

Mortality of 14 % occured also in the vaccinated group, but *Vibrio anguillarum* was reisolated only sporadically.

Discussion

In this experiment the results are incomplete and need further verifications. Infection was only partially successful and we were not able to understand the 14 % mortality in the vaccinated group.

RECOMMENDATIONS AND SUGGESTIONS

The type of penaeid farming carried out in Italy has made an exhaustive study of the pathologies usually occuring in intensive culture impossible. Nevertheless, some pathological conditions were observed, the one caused by bacteria being the more serious.

Farmers are greatly interested in this culture which offers remarkable profits, limited biological risks and very short production cycles. Unfortunately, Italy, like many other countries interested in the culture of these crustaceans, cannot produce juveniles and all restocking material must be imported.

That being stated, strict sanitary inspections on restocking material are necessary at the borders. It is desirable as well that protected areas be arranged where shrimp cultures for the production of livestock for restocking are free from contagious diseases material should be transmissible disease transferred.

This meeting could be the starting point for planning the necessary measures to obtain protected areas, bearing also in mind what the Commission of European experts is doing in view of the elimination of the frontiers among the European Economic Community Countries in 1992.

Similar diagnostic techniques with standard antigens and sera should be adopted by the reference laboratories of the various countries. This could be a good opportunity to start an International Reference Centre on sea crustacean pathology.

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Penaeid pathology in Israel : problems and research

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Abstract — *A comparative search for the shrimp most suitable for mariculture in Israel has led to the import of exotic species. Unfortunately, these shrimp were carriers of at least three pathogenic viruses. Infectious Hypodermal and Hematopoietic Necrosis (IHHN) virus, Hepatopancreatic Parvo-like Virus (HPV) and Monodon-type Baculovirus (MBV) caused cross-infections among both imported and local penaeids. Since these episodes, general disinfection operations were carried out and quarantine measures were strictly enforced for any newcomers. However, penaeid shrimp culture is to date subject to persistent MBV infections. Aiming at developing more rapid and sensitive diagnostic methods, trials to initiate cell cultures from* ***Penaeus semisulcatus*** *were carried out. This in order to produce a suitable substrate for the* ***in vitro*** *replication of MBV and possibly other viruses and the production of a fast developing cytopathic effect. Explants derived from hemopoietic and ovary tissues and the lymphoid organ, which are regarded to possess high generative capacity, were used. Primary cultures of cells, presumably hemocytes or lymphocytes, were established and maintained for 3 weeks. Sub-culturing attempts, however, failed. Sporadic fusariosis was also diagnosed in* ***P. semisulcatus*** *cultures. Macroconidia and hyphae typical of* ***Fusarium solani*** *were observed incapsulated within a large number of hemocyte clots produced by the host.* ***F. solani*** *is not unknown in Israel where it occasionally affects vegetables and causes keratomycoses in humans. Whether any of these strains, that all fit the same taxonomic location, can be, or become under particular conditions, pathogenic to such a variety of distantly related hosts remains to be seen. The hazard shrimp farmers stand when handling infected shrimp is discussed.*

The climatic conditions in Israel's southern region, where most of the mariculture industry is located, are characterized by a sunny weather for most of the year and a very short and mild winter. The Gulf of Eilat is the northernmost part of the Red Sea and represents a very particular tropical biotope, with water temperatures between 23 and 26°C all year round and salinity of 40 ± 1 ‰.

The need to compare different species of shrimp in the continuous search for those most suitable for mariculture in these environmental conditions has led to the import of exotic species of penaeid shrimp. Although the potential danger of introducing infectious diseases into Israel and the concomitant hazard of spreading them in the local aquatic habitat were considered, the etiology of most viral diseases was unrecognized until

a few years ago, and no particular precaution had been taken against them. In 1980 *Penaeus stylirostris* was imported from Hawaii and *Penaeus monodon* from South Africa. In 1981 another shipment of *P. monodon* was imported from the Philippines.

Their growth rate was often erratic and in general they performed poorly. Weak and unable to preen properly, the shrimp were frequently infested with fouling epibionts such as algae (Fig. 1), both benthic (*Enteromorpha sp.*) and planktonic (in particular diatoms), peritrich protozoans

Figure 1. — Fouling epibiontic algae growing on a diseased *P. semisulcatus.*

(*Zoothamnium sp.*) and filamentous bacteria (*Leucothrix*, prob. *mucor*). Repeated treatments with KMnO4 occasionally alleviated the pressure on the shrimp by these epibionts. *Leucothrix* was found to be able to decompose urea. Its urolytic ability gives this bacterium a physiological and ecological advantage. In fact, in conjunction with the relatively high pH of the sea water, the requirement for urea can be replaced by ammonia. It can be assumed that *Leucothrix* requires ammonia as a nitrogen source for its respiration and growth. Since both substances are secreted by the shrimp, a logical explanation can be suggested for its rapid spreading as an epibiont pest whenever environmental conditions in the shrimp tanks deteriorate. *Leucothrix* growth was inhibited by 1 ppm of Flumequine. It was eventually realized that all these organisms were secondary invaders and the shrimp were actually infected with pathogenic viruses. *Penaeus monodon*-type baculovirus (MBV) was diagnosed in *P. monodon* and In-

fectious Hypodermal and Hematopoietic Necrosis (IHHN) virus was diagnosed in *P. stylirostris*. By then, however, IHHN virus had also infected both the MBV-infected *P. monodon* and *Penaeus semisulcatus*, the latter a local species, which were cultured in the same facilities. The entire stocks of MBV-IHHN infected shrimp were destroyed and the system was dried, cleaned, and re-stocked 6-8 months later with *P. semisulcatus*, which was successfully cultured for three consecutive generations (Colorni et *al.*, 1987).

The last shipment of exotic shrimp (*P. monodon*) arrived in Eilat from Kenya in January 1986. This time a quarantine unit, specially designed to prevent the possibility of releasing the shrimp or their associated pathogens into the environment, had been set up. It was located downstream in relation to all the other facilities, and outlet water that would normally reach the main sewer system and return to the sea was diverted to an earthen seepage well. The use of hand-nets and other maintenance tools was limited to this unit. A basin with disinfectant solution was placed at the entrance for immersing footwear, and strict instructions were given to the personnel in charge of handling the shrimp to wash with antiseptic soap before attending to other shrimps. All these precautions proved fully justified.

Single basophilic inclusion bodies, typical of Hepatopancreatic Parvo-like Virus (HPV), a virus pathogenic to many penaeids including *P. semisulcatus*, were detected in the epithelial cells of the hepatopancreatic tubules of the shrimp. Besides HPV, which causes atrophy and necrosis of the hepatopancreas, other pathological conditions were observed in these shrimp, such as the so called « Red Disease », characterized by massive inflammation and necrosis of the hepatopancreas.

At present, only *P. semisulcatus* is reared in our facilities. This species was caught as brood stock off Haifa Bay in the Mediterranean Sea. While no viral infections were ever detected in samples of shrimp freshly caught, once in Eilat (on the Red Sea), the shrimps often develop MBV. Its origin is unclear, and two theories have been formulated to explain its persistent appearance in our system. According to the first, the disease is caused by a local viral strain, present in a latent form in the wild shrimp and becoming symptomatic in the stressful culture conditions. According to the second theory, it is still the same virus introduced into the system by diseased exotic shrimp, that survived perhaps carried by other Crustaceans, such as barnacles, cirripeds, copepods, etc. which cohabit our shrimp tanks and ponds, or possibly due to its resistance in the environment.

Also, some peculiar inclusion bodies somewhat similar to, but not typical of, MBV, severe Hemocytic Enteritis, and abnormal hepatopancreas structures were occasionally observed in *P. semisulcatus*. Butylated hydroxytoluene (BHT), a dietary antioxidant widely used as a food preservative for its antioxidizing properties, has been found to be a potent inactivator of lipid-containing mammalian and bacterial viruses (Snipes et *al.*, 1975) and to protect chickens exposed to Newcastle Disease Virus (Brugh, 1977). Since MBV also contains a lipidic component, an experiment was set up with MBV-infected *P. semisulcatus* to evaluate the antiviral potential of BHT. Amounts of 0.5, 1.0, and 2.0 ppm of this substance were incorporated in the regular feed and the shrimp were observed for over

6 weeks during which periodical samples of hepatopancreas were taken for histological examination. BHT, however, apparently made the pellets so unpalatable that a high rate of cannibalism occurred in the experimental tanks, and no definite trend could be observed.

All of the viruses detected in Eilat are characterized by the formation of intranuclear inclusion bodies in the target cells. This finding is consistent and typical enough in location, morphology and staining characteristics to be considered a good diagnostic criterion. However, formation of inclusion bodies is not a constant characteristic for every virus. Furthermore, diagnostics of MBV through histology, malachite green staining, acridine orange fluorescence (Fig. 2), or simple smear, although useful when intranuclear bodies become detectable (at medium or high intensity infections), are inadequate when screening for early or carrier state infections (Diamant and Colorni, 1987).

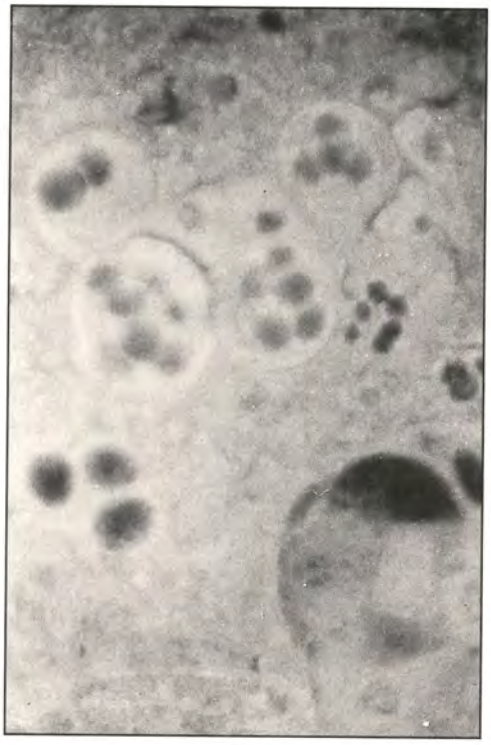

Figure 2. — MBV occlusion bodies as revealed by the acridine orange fluorescence method (courtesy of Dr. A. Diamant).

Development of sensitive detection methods for shrimp viral latent infections depends largely on the availability of purified viral particles. Crustacean *in vitro* cell culture which could support virus replication is still at a very early experimental stage. However, some preliminary trials to

initiate cell culture from our stock of *P. semisulcatus* in Eilat have yielded promising results (Rosenthal and Diamant, in press). Cultures were initiated from hematopoietic tissue/lymphoid organ and from ovarian tissue. Although these tissues do not support, at least *in vivo*, the growth of MBV as hepatopancreatic epithelium does, they typically possess high generative potential. Hepatopancreas in any case was found unsuitable for *in vitro* culture, due to its high content of lytic enzymes. The best results were obtained using cell culture medium M-199 supplemented with 15 % fetal bovine serum and 5 % heat inactivated shrimp hemolymph.

Streptomycin and kanamycin effectively prevented bacterial contamination. The cells migrated from the explants and formed fairly dense, though not confluent, monolayers (Fig. 3). However, no mitotic figures could be discerned. The cultured cells were maintained for 3 weeks, but attempts to sub-culture them were unsuccessful.

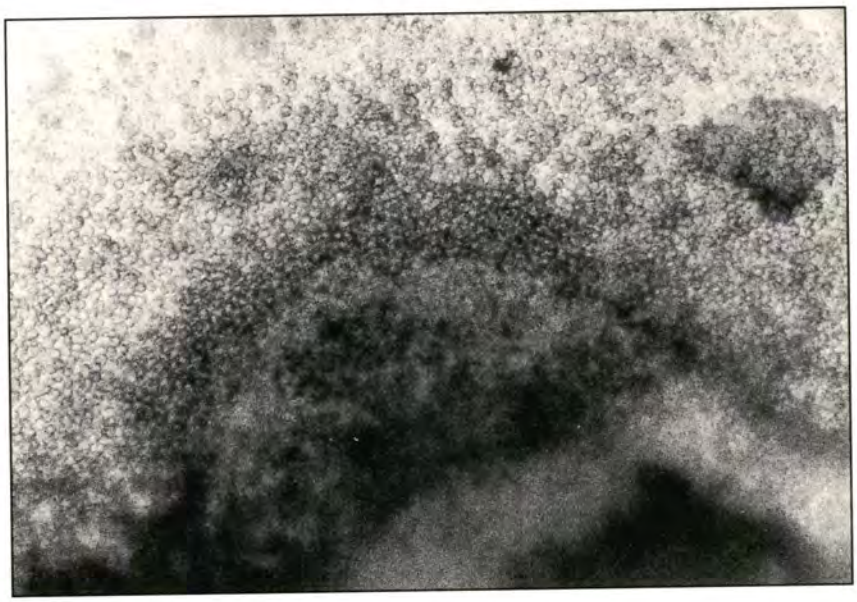

Figure 3. — Migrating cells from an explant of *P. semisulcatus* hematopoietic tissue/lymphoid organ (courtesy of Dr. A. Diamant).

The first case of shrimp fusariosis was diagnosed in Israel in 1988, in *P. semisulcatus* (Colorni, in press). An adult male from one of the rearing ponds displayed a large melanized lesion on one side affecting the cephalothorax and first abdominal segment.

The chitinous cuticula appeared degraded and the ulceration ran deep into the underlying tissues. Fungal hyphae and canoe-shaped macroconidia (Fig. 4) typical of *Fusarium solani* were observed, often encapsulated within a large number of hemocytic clots produced by the

inflammatory response of the host. Hyphae embedded in the underlying muscular fibers were surrounded by somewhat less or no inflamed tissue, suggesting their more recent growth and a gradual failure by the host to resist mycelial invasion. The report of this sporadic case in Israel extends the range of this mycosis to this geographical area. *F. solani* is not unknown in Israel where it has been associated with wilts and rots of dozens of plants. *F. solani* is also one of the most widespread causes of fungal eye infection in humans throughout the world. At least 3 cases of keratomycosis have been reported in Israel in recent years. Two cases were severe to the point that the infected eye had to be enucleated. Whether any of the strains above mentioned, which all fit the same taxonomic location, can become under particular conditions, pathogenic to such a variety of distantly related hosts remains to be seen. *F. solani* is a classic opportunist, and at least with regard to human pathology, is capable of producing disease only when carried into susceptible ocular tissues through an accidental injury to the eye or when the natural resistance to infection in the individual is abnormally low. In any case, since shrimps struggle and splash vigorously when they are harvested and superficial scratches and punctures by the prickly protuberances and appendages of these animals are a common occurrence, the hazard shrimp farmers face in handling infected shrimp should not be underestimated.

Figure 4. — Typical macroconidium of *F. solani* in *P. semisulcatus.*

Another fungus was detected in the eye stalk of a second shrimp from a different tank. The organ was reduced to a necrotic stump after experimental ablation had been performed to induce ovarian maturation and spawning. Histological sections stained with periodic acid-Schiff (PAS) clearly show presence of hyphae. However, the canoe-shaped macroconidia typical of *F. solani*, were not observed in this sample. Inoculation of pathological material onto Sabouraud, oatmeal and tellurite agars prepared with 25 % filtered sea water produced a slow growing salmon-colored mycelium similar to that of *F. solani*. Later though, basidia were produced and the fungus was thus identified as a basidiomycete. Fragments of mycelium grown on Sabouraud agar were inserted underneath the cuticula through a small cut between the first and second abdominal segments, but attempts of reinfecting the shrimp in this way all failed. Thus, the possibility of an external contamination during culture cannot be ruled out in this case, but it is noteworthy that, similarly to *Fusarium spp.*, the basidiomycetes also include many plant and insect pathogens.

Another frequent disease affecting the eyes of shrimp in Eilat appears as a « white-grayish patch ». Histological sections of the lesions show a

« shaft », of atrophic tissue down to the retinular layers (Fig. 5). Larger lesions show a thickening of the eye cuticle and tissue atrophy down to the medullar layers (Fig. 6).

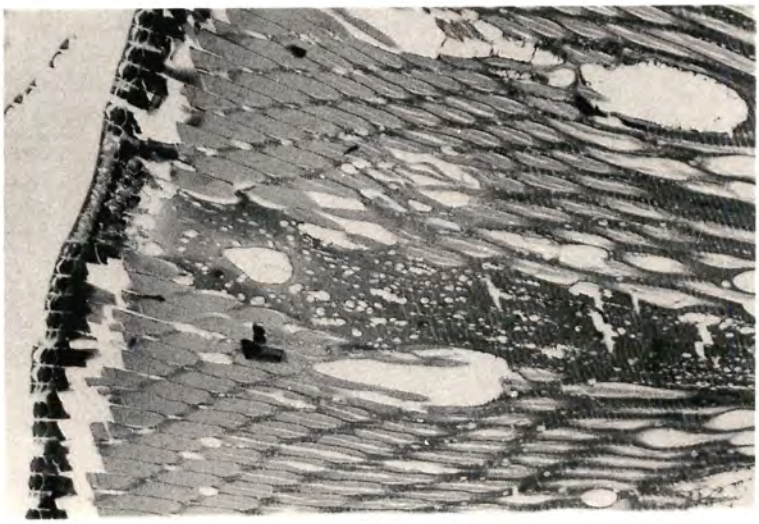

Figure 5. — « White-grayish patch » eye lesion, early stage.

Figure 6. — « White-grayish patch » eye lesion, advanced stage.

This condition is similar to that described by Laramore et *al.* (1977) in *Penaeus vannamei.* The etiology of these lesions is still unclear, but apparently these atrophic tissues present a suitable substrate to secondary infections by *Fusarium.*

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Diseases of cultured prawns in Australia

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Abstract — ***Plebejus Baculovirus*** *and other MBV-related viruses have been found in* ***Penaeus plebejus, P. monodon*** *and* ***P. merguiensis****. They appear highly pathogenic in certain situations currently undefined. We have found Hepatopancreatic Parvo-like Virus (<hpv) in wild* ***P. esculentus*** *and* ***P. merguiensis*** *but not yet in cultured prawns. The amphophilic intranuclear inclusion bodies we find in* **P. esculentus** do not appear to have a viral cause.

Black Gill disease, caused by a build-up of bacteria and ciliates on the gills, occured in ***Metapeneus macleayi*** *in ponds in New South Wales and was associated with high organic load and poor water exchange. Heavy infections with an apostome ciliate similar to* ***Synophrya hypertrophica*** *were found in the same area on* ***M. macleayi*** *and* ***P. plebejus.***

Fungal infections not yet identified to genus are found on and under the carapace of larval and adult prawns. Conditions of undetermined cause that we have seen include swollen uropods, soft shell, craped abdomen and red prawn. High mortalities occur in the early larval stages in hatcheries. These have been associated with bacterial necrosis and bacterial Haemolytic enteritis.

INTRODUCTION

Studies on the diseases of prawns in Australian aquaculture began in 1984 with the school *Penaeus macleayi*, which was grown at the first Australian prawn farms in Yamba, northern New South Wales. A commercial prawn hatchery was established in the same year in Darwin and protocols were set up to test prawns for disease when they were moved interstate. There are now about 30 prawn farms along the east coast of Australia, mostly in Northern Queensland. Post-larval prawns are supplied to the industry by at least 13 hatcheries.

We monitor the health of prawns on several farms and that of prawns moved interstate. Diseases we have found, together with some seen by other workers, were summarized in Paynter and Lester (1988). Below is modified and updated version of that review.

VIRUS INFECTIONS

A virus, *Plebejus baculovirus* (PBV), was found in the epithelium of the digestive gland of *Penaeus plejebus*, and a similar one in *P. monodon* and *P. merguiensis*. Mysis and early post-larvae were more heavily infected than juveniles and adults (Lester et *al.*, 1987); Doubrovsky et *al.*, in press). The viruses are similar to the *Monodon Baculovirus* (MBV) but differ in two ways. They frequently produce a single occlusion body rather than multiple occlusions, particularly in *P. plebejus* where over half the infected cells contain only a single inclusion. The capsid envelope of the virions has two electron dense bands regardless of the fixatives used (Doubrovsky et *al.*, in press; L. Owens, pers. com.) whereas this feature is rarely seen in MBV (Lightner et *al.*, 1983; Johnson and Lightner, 1988).

In our preliminary cross infection experiments we successfully transferred virus from *P. monodon* to other *P. monodon* (by feeding post-larvae infected material) but were unable to transfer virus from *P. plebejus* to *P. monodon*. It is possible we have two MBV-like viruses in Australia.

High mortalities in some Australian hatcheries have been attributed to these viruses, which appear to multiply rapidly in stressed individuals. Heavily infected post-larvae of *P. plebejus*, have cloudy digestive glands, spiral in the water column and swim sideways along the surface before dying. The rate of infection diminishes with age and the viruses do not appear to produce clinical disease in juvenile and adult prawns. Hatcheries which have experienced an outbreak drain all water, then chlorinate and dry all surfaces and equipment before restocking. In some cases this has removed the virus from the hatchery.

MBV-like viruses have been reported from hatcheries and farms on most parts of the east coast of Australia. They appear to be endemic rather than introduced : imports of live penaeids from overseas are prohibited.

We have not yet found MBV-like virus in *P. esculentus*, though this species is reported to be a host for MBV elsewhere (Johnson and Lightner, 1988). Inclusion bodies which are amphophilic and intranuclear have been found several times in the epithelium of the digestive gland. On three occasions they have been examined found. The inclusions, which we refer to as Shann Bodies after the name of the original supplier, appear to be the result of aberrant protein synthesis. Prawns showing the condition have normal growth and mortality rates (Lester et *al.*, 1987).

Hepatopancreatic Parvo-like Virus (HPV) has been found in wild caught *P. esculentus* from Moreton Bay, Queensland (Paynter et *al.*, 1987) and wild caught *P. merguiensis* from Mackay, Queensland (Roubal et *al.*, in press). It has not yet been detected in cultured prawns.

Bacterial infections

Bacteria are often assumed to be the main cause of high mortalities in Australian hatcheries. Larval mortalities have been associated with bacterial necrosis and bacterial hemolytic enteritis (I. Anderson, pers. com.). Frequently, filters are used to limit the number of bacteria entering

hatchery water. Some hatcheries routinely use broad spectrum antibiotics in larval rearing tanks and this has increased survival rates.

Surface filamentous bacteria of the *Leucothrix* type are commonly found on prawns from hatcheries and ponds, but they do not appear to influence their health.

Fungal infections

An *Atkinsiella*-like fungus formed discrete craters up to 2 mm across in the carapace of adult *P. esculentus* from a holding tank. Hyphae did not penetrate into the underlying tissue.

A *Lagenidium*-like fungus has been found on the eggs and in the body of larvae and post-larvae of *P. plebejus* (N. Preston, P. Ketterer, pers. com.). Hyphae invaded and damaged extensive areas of host tissue. High mortalities were observed.

Peritrich ciliates

Peritrich protozoans of the genera *Cothurnia, Epistylis, Vorticella* and *Zoothamnium* are common on Australian prawns and have been found from most farms. Recent reviews of the taxonomy of *Vorticella* and *Cothurnia* are given by Warren (1986) and Warren and Paynter (in press), respectively.

Prawns may carry so many peritrichs that they look furry, but they are not harmed unless the protozoans on the gills block respiration. Peritrich abundance can be a useful indicator of poor and pond water quality and they may also reflect the frequency at which prawns are moulting. They thus can give early warning of an imminent health problem.

Peritrichs are usually controlled by changing the water.

Apostome ciliates

An apostome ciliate similar to *Synophrya hypertrophica* was found in the gill tissue of juvenile *M. macleayi* and juvenile *P. plebejus* from ponds in northern New South Wales. Heavily infected gills turn black and much tissue is destroyed from melanin produced when the prawn reacts to the parasite. The capacity of the gills to absorb oxygen decreases and this may stress the prawns.

Apostome infections are difficult to control though high water exchange rates after the prawns moult may help to flush the infective stages from ponds.

Bryozoans and algae

Bryozoans, filamentous algae and *Zoothamnium* sp. were abundant on the carapace of juvenile *P. esculentus* from a southeast Queensland farm. Affected prawns kept in the laboratory did not moult properly and

died. Possibly the stress of cold weather stopped the prawns feeding, they became weak and failed to moult, and this allowed the epibionts to accumulate. Increased water exchange, the addition of EDTA to the pond (to stimulate moulting) and the onset of warmer weather removed the problem.

Black gills

Juvenile *Metapenaeus macleayi* developed black gills and died in the summer of 1987 in ponds in northern New South Wales. The gills were clogged with organic debris, and overgrown with peritrichs, bacteria and filamentous algae. The gills tips were melanised and dead, probably because of the anaerobic conditions generated by the detritus. It is likely the prawns died from hypoxia. The condition rapidly disappeared when the pond water was exchanged.

Blistered tail

This occurred in *P. monodon* in a pond in New South Wales as the temperature dropped in autumn. Large prawns (45 g) developed swollen uropods within which were a gelatinous matrix, blood cells and some bacteria. The edges of the tail had become melanised. The condition probably arises from tail damage during of shortly after moulting.

Cramped abdomen

Prawns with cramped abdominal muscles are regularly seen in ponds especially during summer. It appears to be related to environmental stress.

Soft shell

Juvenile *P. monodon* (4-5 g) at a southeast Queensland farm were soft shelled, anorexic, lay near the pond edge and eventually died. The prawns had been fed Taiwanese pellets and trash fish including orange roughy *Hoplostethus atlanticus*. The skin of orange roughy is known to contain a waxy ester which has a laxative effect on humans. It may have an adverse effect on prawns.

The symptoms disappeared when trash fish was removed from the diet and water exchange increased.

Red prawn

Juvenile *P. monodon* and *P. esculentus* have been found with red discolouration of the body, especially around the edges of the tail, along the dorsal abdomen and on the legs. No mortality in ponds has been associated with the condition. It is believed to relate to rancid fish or shell fish in the diet; the condition does not occur among prawns fed fresh or cooked feed.

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12

Recent advances in Australian prawns diseases and pathology

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Abstract — *Australian penaeid mariculture is undergoing a rapid expansion. Disease is always limiting in animal production and new syndromes occur in fledgling industries. This paper describes the latest findings in this area. RNA proliferation was seen in the ovaries of* ***Metapenaeus ensis*** *from the Torres Strait and was believed to have a viral aetiology. Idiopathic hypodermal, haematopoietic and myocardial necrosis has been associated with a number of conditions. Haematopoiectic changes include vacuolative necrosis, reactive hyperplasia with nuclear transformation tending towards anaplasia and rarely, small eosinophilic inclusion bodies. A coccidian was found in the hepatopancreatic cells of* ***Penaeus merguiensis*** *and elicited a marked cellular response with hyperplasia and nuclear transformation. Dinoflagellates have also been found attached to nauplii. Bungoo or rhinoceros disease has also been seen in northern hatcheries.* ***Vibrio damsela*** *was shown to be a primary pathogen of nauplii and caused a septicaemia. Vacuolation and shrinkage of the ganglionic neuropiles as well as muscular fragmentation and coagulative necrosis were common. Other bacteria caused similar changes but were unable to produce a septicaemia.*

Problems with ***Macrobrachium rosenbergii*** *in northern Australia include mid-cycle disease, microsporidiosis and bopyridiasis.* ***M. rosenbergii*** *from Malaysia have been infected with Hepatopancreatic Parvo virus.*

INTRODUCTION

Australian penaeid mariculture is undergoing a rapid expansion. Disease is always limiting in animal production and new syndromes occur in fledgling industries. Such has been the case in Australia. A recent review describes some of the diseases of penaeid prawns seen in north Queensland (Owens and Hall-Mendelin, 1989). This review updates that previous report and complements the material on other Australian penaeid diseases by R.G.J. Lester in this book. Only new or unreported entities will be included herein. Diseases reported in the previous review include Plebejus baculovirus, Hepatopancreatic Parvovirus, Chlamydia, Microsporidia, bacteria, Lagenidium, hypodermal necrosis, cramp tail and vitamin E deficiency.

MATERIALS AND METHODS

The greatest majority of prawns were submitted from aquaculture farms. Live prawns were slowed by cooling and then fixed in Davidson's fixative after the cephalothorax had been split longitudinally. Normal histology and staining was performed as in Culling et *al.* (1985).

A carton of commercially caught *Metapenaeus ensis* from Torres Strait were purchased from a trawler operator at Weipa in June 1982. The samples were already frozen and later fixed in 10 % buffered normal formalin. The first abdominal segment was cut transversely and prepared using standard histological techniques. Material for electronmicroscopy was deparaffinized, postfixed in osmium tetroxide and embedded in Spurrs resin (Culling et *al.*, 1985). Sections of approximately 70-90 nm were cut with the ultramicrotome and stained with uranyl acetate and lead citrate before viewing under the transmission electronmicroscope. Semi-thin epoxy resin sections (1-2 ñm) were stained with Toluidine blue and Fuchsin following the method of Burns and Bretschneider (1981).

For histopathology, protozoea were fixed in 10 % seawater formalin and postfixed in Davidson's fixative with eosin added. The larvae were then washed in tap water and 10 % agar was poured on top. After the agar cooled, it was fixed in formalin and processed using standard histological techniques. Three ñm sections were cut and stained with either haemotoxylin and eosin or gram Twort.

RESULTS

I Adults prawns

Wild caught female spawners of *P. merguiensis* were showing low tolerance to transportation. Moribund animals showed a spectrum of conditions of which two were most common. The first involved a haemocytic infiltration into the antennal gland tubules and sinuses. Nuclear transformation was common with some reactive hyperplasia and in one case, bright eosinophilic material was being deposited; a precursor to melanization. Within the lumen of the tubules were rounded, structureless objects attached by a stalk to the epithelial cells. These objects became swollen and then detached. Within the sphere, banana shaped secondary cyst formed like those of the genus *Dissodinium*, parasitic dinoflagellates of the Blastodinida (Drebes 1985). Parasitic dinoflagellates were common on wild nauplii stages of prawns and the current case may indicate the method transmission from spawner to larvae. Of the normal histological stains, only gram and silver methenamine were useful for staining the dinophytes. With gram, the primary cysts were neutral but the secondary cysts were unstained which allowed good contrast. The trophont stage stained heavily with silver much like a fungi.

The second condition that was common in moribund *P. merguiensis* was changes in the lymphoid organ. The wole organ was middly hypertrophied. Cellular changes were graded from haemocytic infiltration, nuclear

and cellular transformation with reactive hyperplasia and basophilic darkening of the cytoplasm with a tendency to anaplasia, and some of these areas organized into spheroids which were not located around a central vessel, and lastly vacuolative necrosis of the hyperplastic areas.

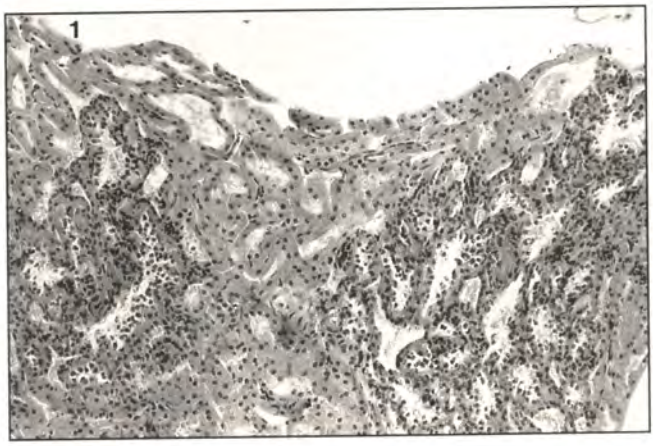

Figure 1. — Cellular infiltrate of antennal gland of *Penaeus merguiensis* associated with dinoflagellate infection. × 165.

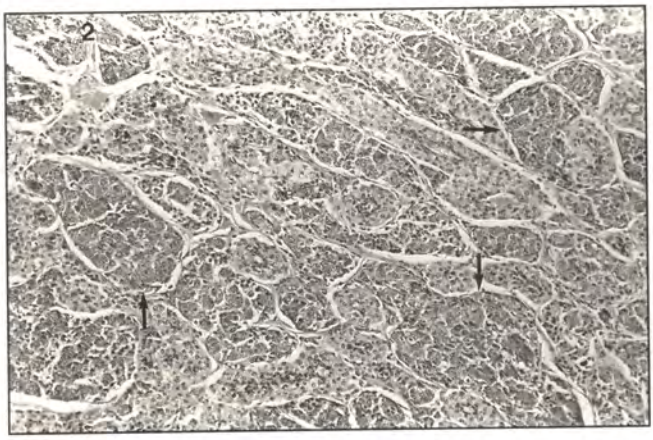

Figure 2. — Areas of cellular and nuclear transformation with tendency to spheroids (arrows) of lymphoid organ of *Penaeus merguiensis*. × 154.

Within the transformed areas, basophilic, cytoplasmic inclusions were common and infrequent eosinophilic, intranuclear Cowdrey type A inclusions were in areas with vacuolative necrosis. Both types of inclusions stained with phloxine and not with Macchiavello (inclusion body strains).

One of these cases had diffuse, multifocal necrotic areas within the maxilliped haematopoietic tissue.

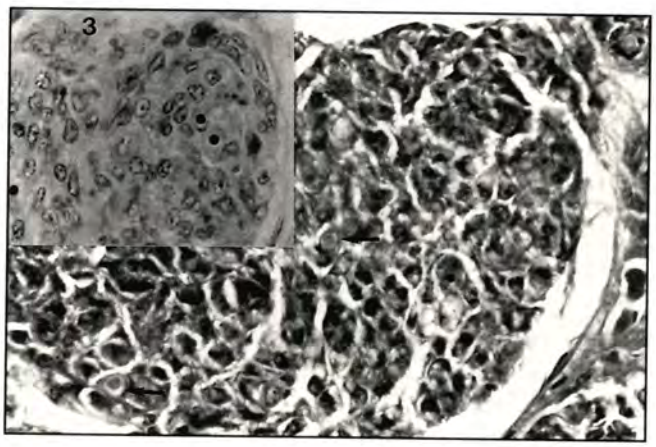

Figure 3. — Insert : Basophilic, cytoplasmic inclusion bodies in the lymphoid organ areas of cellular transformation. × 686. Main figure : Eosinophilic Cowdrey a's intranuclear inclusion body (arrows) in lymphoid organ spheroid transformation area. × 686.

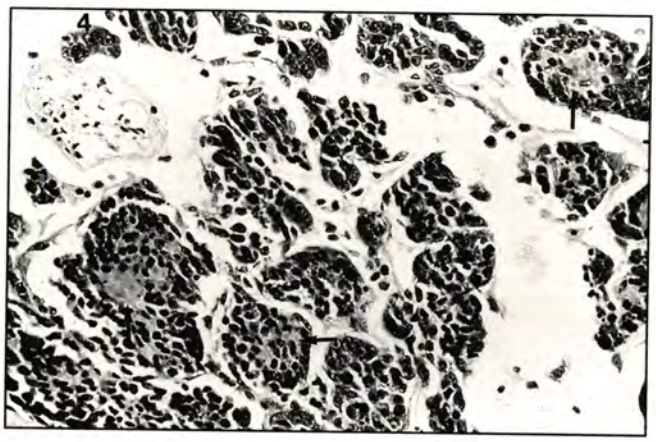

Figure 4. — Maxilliped haemopoietic tissue with diffuse focal eosinophilic necrotic areas (arrows) in *Penaeus merguiensis.* × 384.

Large eosinophilic, intranuclear and perinuclear inclusion material was in the ovary of 16 of 81 *Metapenaeus ensis.* The inclusion material first becomes visible as cells passed from the germinal zone, with a single nucleolus and chromatin. The inclusion material was intimately involved with the nucleolus. As the nucleoli split and became mutiple around the periphery of the nucleus (Tom et *al.*, 1987), the amount of inclusion material multiplied. No oocytes having inclusion material reached vitellogenic stage 2 (reducing PAS positive) of Tom et *al.* (1987), suggesting

halted development or dispersion of the inclusion material. Infected cells accumulated in tracts similar to germinal zones and often connected with germinal zones. In ultrathin sections, cells in these tracts were characterized by lack of cell membranes. In heavily infected prawns, the basophilic cytoplasm of immature oocytes also showed weak inclusion material stain. Some infected cells became hypertrophic before rupturing due to the inclusion material.

Other staining characteristics of the inclusion material include being positive for phloxine, pyronin and weakly positive for Ziehl Nielsen (Table 1). The material stained negative with Macchiavello, Feulgen, Methy-Green, Pas, Giemsa, Luxol fast blue and Herxheimer. The material was unreactive with Gram stains.

These results suggested the material to be RNA (Pyronin) and not DNA (Feulgen, Methyl-Green), lipid (Herxheimer, Luxol), bacteria (Gram), fungi (PAS), rickettsia (Macchiavello, Giemsa) or mycoplasms (Ziehl Nielsen). With the semi-thin sections cut from the resin blocks, the inclusion material stained with fuchsin and darkly with Toluidine blue.

Tab. 1. — Histochemical characterization of Ovarian Inclusion Material of *Metapenaeus ensis*

Stain	Result	Compound stained for
Paraffin Sections		
Haemotoxylin & eosin	eosinophilic	
Phloxine/Tartrazine	phloxophilic	inclusion bodies, fibrin
Macchiavello	-ve	inclusion bodies, Rickettsiae
Feulgen	-ve	DNA
Methyl Green/Pyronin	+ve	RNA DNA/RNA
Gram	nonreactive	bacteria
PAS	-ve	fungi,mucins
Giemsa	-ve	Rickettsiae, inclusion, bodies, bacteria
Ziehl Neelsen	weakly +ve	acid fast bacteria, mycoplasm
Luxol fast blue	-ve	myelin, phospholipids, nissl substance
Herxheimer	-ve	lipids
Trichrome (M.S.B.)	+ve (scarlet red)	inclusion material
Epoxy resin sections		
Toluidine Blue	+ve	
Fuschsin	fuchsinophilic	

Under the electron microscope, the inclusion material was electron dense and was again perinuclear and intranuclear. Due to the poor fixation procedure, resolution was severely compromised. However, some structures were visible including membranous labyrinths, a net-like proliferation of nuclear membranes associated with inclusion material very similar to

those alter nations caused by *Baculovirus penaei* (Couch, 1974) and *Plebejus baculovirus* (Lester et *al.*, 1988). We have called this syndrome RNA proliferation.

II Larval and Juvenile Prawns

Bright eosinophilic cytoplasmic material was found by Jan Paynter of the University of Queensland in the hepatopancreas of juvenile *Penaeus plebejus* that were grown in water heated by a power station. The material had little structure with very small basophilic speckles. Cells were greatly hypertrophied, the cellular nucleus was displaced to the margin and the hepathopancreas had more vacuolated B- cells than normal. Serial sections revealed early globular stages progressing to an open anastomosing primordium and lastly to many small (5 μm) sporozoites. It is believed to be a type of coccidia as the early stages were similar to poultry coccidia and the production of sporozoites also suggest a coccidia. The stages observed were very similar to the coccidia *Ixories psycchropotae* from a holothurian (Massin et *al.*, 1978).

Bungoo or rhinoceros disease was first discovered in mysis of *P. monodon* by Lindsey Trott and Matt Kenway of Australian Institute of Marine Science. The antennae droop or stand upright, wither and become necrotic and can eventually kill the larvae. In juvenile *P. esculentus* it was not fatal but antennae were no more than stubs. The causative agent is a protozoan of unknown affinities. It has been reported in the Philippines and Thailand were the names come from.

Experiments with bacteria of the genus *Vibrio* on protozoea of *P. monodon* by Paul Muir and David Sutton of James Cook University of North Queensland have shown *V. damsela* to be a primary pathogen and caused a septicaemia. The post common change was vacuolation of the ganglionic neuropiles and concurrent shrinkage of the neuropile with *V. damsela* and *V. harveyi.* Fragmentation and loss of the muscle blocks due to coagulative was common with all *Vibrio* spp. but not with *Pseudomonas nautica.* There was atrophy of the hepatopancreas which was most marked in the *V. damsela* and *V. harveyi* treatments. Cuticular necrosis was not seen. In terms of histological change and progressing from mild to severe, the bacterial treatments were ranked as follows : control, *P. nautica,* S.P. *Vibrio, V. harveyi* and *V. damsela.*

III Macrobrachium rosenbergii

Little culture of the giant freshwater prawn has been attempted in Australia. However, disease has been a large problem in all such attemps. The first such problem was with the bopyrid *Probopyrus buitendijki.* A hatchery in Darwin was supplying infected postlarvae to grow-out ponds from Atherton Tablelands to the Maroochy River, Queensland, a distance of 3000 km. The unsightly parasite had to be removed manually at the time of marketing and the deformed carapace caused some consumer concern. One facility had a severe recurring problem with microsporidiosis which finally forced the farm to close. The genus of the microsporidian was not determined but the *Thelohania* has been recovered from freshwater crayfish

that cohabit the same areas as *M. rosenbergii.* Mid cycle disease (MCD) has been reported in both north Queensland and Western Australia (Louise Evans, Curtin University of Technology, pers. com.). The hepatopancreatic epithelium showed progressive atrophy and increased vacuolation whilst muscle bundles also atrophied. *Vibrio alginolyticus* was isolated from the animals in north Queensland.

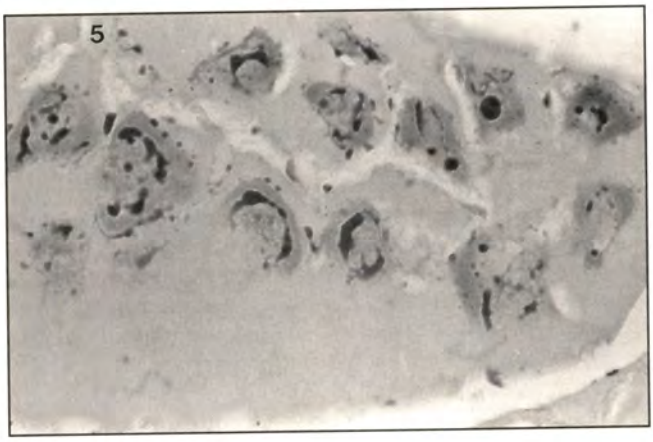

Figure 5. — Intranuclear and perinuclear RNA proliferation in the ovary of *Metapenaeus ensis.* × 274.

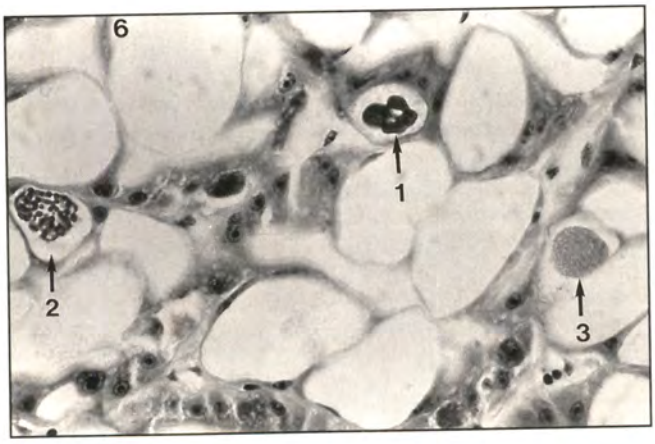

Figure 6. — Coccidian-like organism in the hepatopancreas of *Penaeus plebejus* showing three diffferent stages of development.
1. Primordium.
2. Anastomosing and condensing primordium.
3. Sporozoite formation. × 603.

Ian Anderson of the Queensland Department of Primary Industries Aquaculture Health Program identified large basophilic intranuclear inclusion bodies in hypertrophied hepatocytes of *M. rosenbergii* from farms

in Malaysia. Although electronmicroscopy was not performed, the inclusions were indistinguishable from Hepatopancreatic Parvovirus of penaeids.

DISCUSSION

I Bacteria

Enterobacter aerogenes has been isolated the most frequently from MCD. MCD is characterized by the large amount of coccobacilli found within the lumen of the hepatopancreas (Brock 1988). In the present case *V. alginolyticus* was isolated from freshwater prawns showing signs of MCD. *V. damsela* did cause a septicaemia in penaeid prawn protozoea, in line with studies of *V. alginolyticus, V. anguillarum* and *Aeromonas* sp. (Lightner and Lewis 1975). *V. damsela* has been implicated as a pathogen for damsel fish (Love et *al.*, 1981) and barramundi (Glazebrook and Campbell, 1987). Prawns affected by bacteria are typically off-feed. Some of the changes herein caused by the pathogenic bacteria were consistent with those changes caused by starvation (ie hepatopancreatic atrophy and muscle catabolism). The changes to the neuropile suggest the involvement of a neurotoxin.

II Syndromes

Haematopoietic tissue necrosis has been associated with both infectious hypodermal and haematopoietic necrosis virus (IHHN) (Lightner et *al.*, 1983) and Monodon baculovirus (Nash et *al.*, 1988). There was no mention of lymphoid changes associated with these viruses. However, spheroids within the lymphoid organ have been strongly associated with idiopathic generalized inflammation of *P. monodon* and *P. penicillatus* in the gills, antennal gland, heart, and subcuticular tissues (Lightner et *al.*, 1987). All these tissues are attacked by IHHN. IHHN is now believed to be a virus of Indo-West Pacific origin (Lightner, pers. com.) and therefore endemic prawns have a long history of exposure to the virus and noticeably produce Cowdrey type A inclusions. Furthermore body of IHHN (Lightner, 1985) was very similar to those found in the lymphoid organ of *P. merguiensis* in the study.

The RNA proliferation is not a normal part of ovarian maturation in *M. ensis* (Yano, 1985). Special histological stains have excluded all infectious agents except viruses as being the cause of the inclusion material. Also, changes seen under TEM resembled changes caused by virus (Couch, 1974, Lightner et *al.*, 1983). Of the seven well known viruses of penaeid prawns only one, IHHNV, attacks mesodermal and ectodermal tissues. All the others, many of the possible viruses (Lightner, 1985) and some environmentally caused inclusion bodies attack endoderm tissue, especially the hepatopancreas. RNA proliferation attacks mesodermal tissue.

Whilst RNA proliferation can be intranuclear, its preferred site appears to be adjacent to the nucleus in the cytoplasm but always

associated with nucleoli. Similarly, late in IHHNV infections, cytoplasmic replication occurs (Lightner, 1985). IHHNV is thought to probably be a picorna virus (RNA virus) (Lightner, 1985) and RNA proliferation, as the name suggest, is positive for RNA.

IHHNV primarily affects juvenile prawns with the numbers of inclusions becoming less as the epizootic progresses. Survivors are thought to be asymptomatic carriers for the rest of their lives. RNA proliferation seems to offer a possible mechanism for the carrier status and spread of the virus to operate besides cannibalism or necrophagia. As RNA proliferation is located in the ovary, spawning will release virions into the environment with eggs so the infection of a new generation of prawns seems assured. However, vertical transmission within the ovary seems unlikely as heavily infected oocytes do not seem to complete vitellogenesis. Possibly, single or low numbers of virions may be incorporated in normal eggs for later activation.

RNA proliferation and IHHNV are similar because (a) both attack mesodermal tissues (b) both produce eosinophilic inclusion bodies first in association with the nucleus and later in association with the cytoplasm (c) IHHNV is thought at present to be a picorna virus (RNA) and the proliferation inclusion material is RNA (d) EM changes were similar to those observed for IHHNV and (e) the inclusion material is located in a site which would be a logical follow on from early epizootic events.

In conclusion, both these above syndromes suggest a IHHN- like virus is present in Australia, but the local prawns have evolved in contact with the virus and may not display the characteristic epizootics and histology.

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13

A review of the known hosts, geographical range and current diagnostic procedures for the virus diseases of cultured penaeid shrimp

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Abstract — *At least six virus diseases are presently known in cultured penaeid shrimp. Each of these six penaeid virus diseases (BP = **Baculovirus penaei**; MBV = **P. monodon**-type baculovirus (including PBV of Australian penaeids); BMN = baculoviral midgut gland necrosis; HPV = hepatopancreatic parvo-like virus; IHHNV = infectious hypodermal and hematopoietic necrosis virus; and REO = reo-like virus of the hepatopancreas) may be comprised by a multitude of individual strains, some of which are highly pathogenic to some penaeids, while being of little importance to others.*

*BP is widespread in its distribution in cultured and wild penaeids in the Americas, and except for Hawaii, it has not been observed elsewhere. MBV-type baculoviruses have a diverse host range and wide distribution on the Indopacific coasts of Asia, Australia, and Africa and in Southern Europe. Reports of BMN have been confined to **P. japonicus** cultured in Southern Japan. HPV has a geographic range similar to that of MBV in Asia and Australia. IHHNV has a world-wide distribution in cultured penaeid shrimp, but its distribution in wild penaeids remains virtually unknown. The only occurrences of IHHNV (or a similar agent) in shrimp culture facilities using only wild broodstock have been in Southeast Asia. Little is known about REO, with the only reports of viruses of this type coming from cultured penaeids in France, Malaysia, and Hawaii. Three basic diagnostic procedures are used in screening penaeid shrimp stocks for virus infections :*

1) *direct samples for microscopic (wet-mount) examination or histopathology for signs of virus infection (e.g. polyhedral occlusion bodies);*
2) *enhancement of infection by severe crowding « stress » followed by microscopic examination or histopathology;*
3) *bioassay of a suspect shrimp population with a sensitive indicator species followed by sampling and histopathology; more rapid and sensitive advanced diagnostic procedures based on serological and gene probe technologies are being developed, but are not yet available to the industry.*

INTRODUCTION

Six virus diseases are presently recognized in the penaeid shrimp (Table 1). These six viruses are : BP = *Baculovirus penaei* (Couch, 1974); MBV = *P. monodon*-type baculovirus (Lightner and Redman 1981); BMN = baculoviral midgut gland necrosis (Sano et *al.*, 1981); IHHNV = infectious hypodermal and hematopoietic necrosis virus (Lightner et *al.*, 1983a); HPV = hepatopancreatic parvo-like virus (Lightner and Redman, 1985); and REO = reo-like virus (also known as RLV) of the hepatopancreas (Tsing and Bonami, 1987) (Fig. 1). Each virus may actually comprise a multitude of individual strains, some of which are

Tab. 1. — The known penaeid viruses

Virus	Virion Size	Approximate Nucleic Acid	Probable Classification
IHHNV	20 nm	ssRNA (?)	Picornavirus (?)
HPV	22 nm	ssDNA (?)	Parvovirus
REO	60 nm	dsRNA	Reo-like virus
BP	~ 75 × 300 nm	dsDNA	Baculovirus; occluded
MBV	~ 75 × 300 nm	dsDNA	Baculovirus; occluded
BMN	~ 75 × 300 nm	dsDNA	Baculovirus; non-occluded

IHHNV = Infectious hypodermal and hematopoietic necrosis virus
HPV = Hepatopancreatic parvo-like virus
REO = Reo-like virus
BP = *Baculovirus penaei*
MBV = *P. monodon*-type baculoviruses
BMN = Baculoviral mid-gut gland necrosis

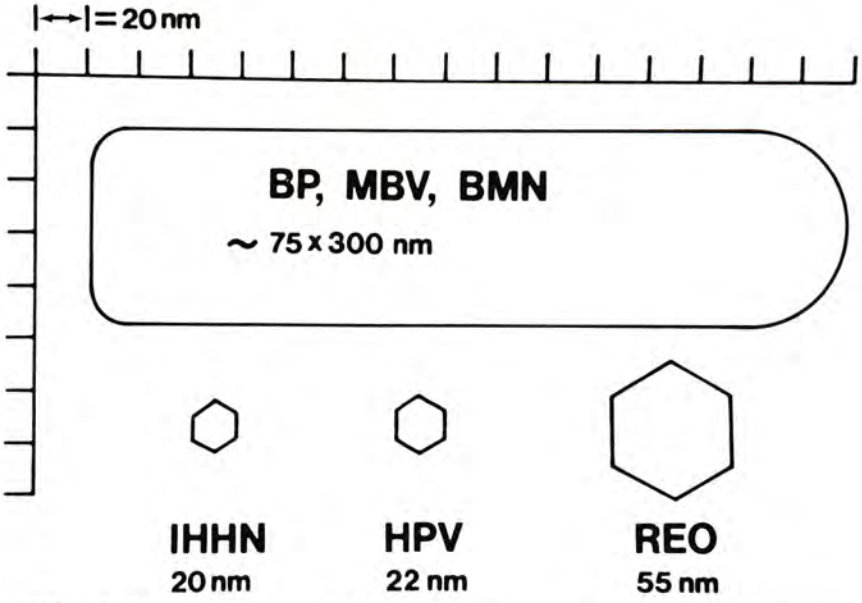

Figure 1. — A schematic representation of the six known viruses. All are shown in relative size.

highly pathogenic to some penaeids, while being of little consequence to other penaeids (Table 2). In this review *Plebejus Baculovirus* (PBV) of Australian *P. plebejus* (Lester et *al.*, 1987; Doubrovsky et *al.*, 1988) is considered to be an MBV-type baculovirus.

Tab. 2. — The penaeid viruses and their natural and experimentally infected hosts

Host Subgenus	VIRUS*					
And Species**	BP	MBV	BMN	IHHNV	HPV	REO
Litopenaeus						
P. vannamei	+ + +	+		+	+	
P. stylirostris	+ +			+ + +		
P. setiferus	+			+ (e)		
P. schmitti	+ +					
Penaeus						
P. monodon	+	+ +		+ +	+ +	+ +
P. esculentus		+			+ +	
P. semisulcatus		+		+	+ + +	
Fenneropenaeus						
P. merguiensis		+ +			+ + +	
P. indicus					+ +	
P. chinensis (= *orientalis*)					+ +	
P. penicillatus	+ +	+ +			+ +	
Marsupenaeus						
P. japonicus			+ + +	+ + (e)		+ + +
P. plebejus		+ +				
Farfantepenaeus						
P. aztecus	+ + +			+ (e)		
P. duorarum	+ + +			+ (e)		
P. brasiliensis	+ +					
P. paulensis	+ +					
P. subtilis	+ +					
Melicertus						
P. kerathurus		+				
P. marginatus	+ + +					
P. plebejus		+ +				

* Abbreviations
BP = *Baculovirus penaei*
MBV = *P. monodon*-type baculoviruses
BMN = Baculoviral mid-gut gland necrosis
IHHNV = Infectious hypodermal and hematopoietic necrosis virus
HPV = Hepatopancreatic parvo-like virus
REO = Reo-like virus
\+ = Infection observed, but without signs of disease
\+ + = Infection may result in moderate disease and mortality
\+ + + = Infection usually results in serious epizootic
e = Experimentally infected; natural infections not yet observed

** Classification according to Holthuis, 1980, FAO Species Catalog.

DIAGNOSTIC PROCEDURES

Three basic diagnostic procedures are currently in use to screen penaeid shrimp for virus infections :

1) Direct samples for microscopic (wet-mount) examination and/or histopathology or electron microscopy.
2) Enhancement of infection followed by sampling and histopathology and electron microscopy.
3) Bioassay of a suspect shrimp population with a sensitive indicator species combined with direct sampling and examination of the indicator shrimp for signs of infection using wet-mounts or histopathology.

Direct Diagnostic Procedures

Random or (preferably) nonrandom samples of shrimp, or shrimp feces, are selected in the direct sampling procedure from culture tanks, ponds, or cages and examined directly for signs of BP or MBV in wet-mounts, or they may be preserved in Davidson's AFA or in 10 % buffered formalin (Humason, 1967) for histological evaluation. The sensitivity of this procedure is limited, and it will only demonstrate shrimp with viral infections that are acute or subacute in a population with a high incidence rate. All six types of penaeid virus infections (IHHN, REO, BP, MBV, BMN, and HPV) may be diagnosed successfully with direct samples, but such samples have also produced false negative diagnoses on populations later shown by electron microscopy, enhancement, or bioassay diagnostic procedures to be positive for one of these virus diseases.

Enhancement Diagnostic Procedures

A quarantined population in the enhancement procedure is reared under relatively crowded and stressful conditions. Post-larvae are best used for this test, which normally requires 30 to 60 days. Random samples are taken at intervals throughout the test period, or moribund animals are nonrandomly sampled when they are observed. Samples may be prepared for direct wet-mount microscopic examination for occlusion bodies diagnostic of BP and MBV-caused diseases, or for histology for diagnosis of IHHN disease in *P. stylirostris*, *P. vannamei*, and *P. monodon*. Demonstration of unapparent infections due to HPV and REO may also be possible by the enhancement procedure. Enhancement is not a suitable procedure for demonstration of IHHNV in asymptomatic carriers (i.e. subadult or adult *P. stylirostris* IHHN epizootic survivors, or in species such as *P. vannamei* which are readily infected by the virus, but seldom show diagnosable infection after the early juvenile stages).

Bioassay Diagnostic Procedures

Carriers of IHHNV may be detected by bioassay with sensitive « indicator » shrimp. Indicator shrimp in this procedure (juvenile *P. stylirostris* of 0.05 to 4 g body weight) may be exposed to samples of suspect carrier shrimp by one or more of three methods.

1) Injection with a cell-free filtrate prepared from a homogenate of suspect carrier shrimp (the indicator shrimp will show signs of IHHN disease within 5 to 15 days if the suspect shrimp were infected with IHHNV).
2) Rearing in the same tank suspect carrier shrimp with indicator shrimp (the indicator shrimp will show signs of IHHN disease within 30 to 60 days).
3) Feeding minced carcasses of suspect carrier shrimp to indicator shrimp (the indicator shrimp will show signs of IHHN within 15 to 30 days).

In actual bioassay tests, the latter technique of exposure (i.e. feeding carcass fragments to the indicator shrimp) has become the method of choice.

Current Disease Specific Procedures

Actual diagnosis of infection by BP, MBV, HPV, IHHNV, and REO is dependent on microscopic or histologic demonstration of the particular cytopathology that is unique to each disease. Gross signs and behavior are usually not sufficiently specific in shrimp with infection by these penaeid viruses to be used reliably in diagnosing these diseases.

BP and MBV

Patent acute BP and MBV infections may be readily diagnosed by demonstration of their characteristic occlusion bodies in either wet-mounts of feces, hepatopancreas, or midgut, or in histological preparations of the latter two organs. BP occlusions are distinctive tetrahedral bodies (Couch, 1974, 1981) easily detected by bright field or phase microscopy in unstained wet-mounts of feces or tissue squashes while MBV occlusions are spherical and therefore difficult to distinguish from lipid droplets, secretory granules, etc. The use of a stain, like 0.05 % aqueous malachite green, in preparing wet mounts for MBV diagnosis aids in demonstration of the occlusion bodies (Lightner et *al.*, 1983c). Presumably, the protein making up the occlusion absorbs the stain more rapidly than does most other material in the feces or in host tissues, contrasting them relative to other materials present within a few minutes.

BP and MBV occlusion bodies in histological preparations appear as prominent eosinophilic (with H & E) usually multiple inclusion bodies within the hypertrophied nuclei of hepatopancreatic tubule or midgut epithelial cells. Often the affected nuclei have a peripherally displaced compressed nucleolus and marginated chromatin, giving affected nuclei a « signet ring » appearance even before occlusion bodies become well developed. Brown and Brenn histologic Gram stain (Luna, 1968), although not specific for baculovirus occlusion bodies, tends to stain occlusions

more intensely (either red or purple, depending upon section thickness, time of decolorization, etc...) than the surrounding tissue, aiding in demonstrating their presence in low-grade infections.

Transmission electron microscopy of BP and MBV infected cells show large numbers of rod-shaped baculovirus particles both free and occluded within the protein matrix of the occlusion body.

BMN

BMN affects the same target organs as does BP and MBV, but unlike BP and MBV it does not produce an occlusion body. Hence, its diagnosis is dependent upon history, clinical signs, and on wet-mount observations and histopathology of the hepatopancreas. Sano et *al.* (1984) in Japan have also reported development of a fluorescent antibody diagnostic technique for BMN. By wet-mount microscopy or histology the principal diagnostic feature of BMN is hypertrophied nuclei within infected hepatopancreatocytes (Sano et *al.*, 1981 and Momoyama, 1983). These enlarged nuclei have marginated chromatin, a laterally displaced or disassociated nucleolus, but lack occlusion bodies.

HPV

Diagnosis of HPV is dependent upon the histological demonstration of single prominent basophilic (with H & E), Feulgen positive (Luna, 1968) intranuclear inclusion bodies in the hypertrophied nuclei of infected hepatopancreatic tubule epithelial cells. Consequent lateral displacement and compression of the nucleolus and chromatin margination are also prominent features of such infected cell nuclei. Early in their development, HPV inclusions are small eosinophilic bodies centrally located within the nucleus and closely associated with the nucleolus.

TEM of HPV-infected hepatopancreatocytes shows the inclusion body to contain virus-like particles of 22 to 24 nm in diameter. HPV is presumed to be a parvovirus because of its size, its Feulgen reaction, and its host cell cytopathology, that are quite similar to the reported characteristics of the parvovirus group (Kurstak et *al.*, 1977; Longworth, 1978; and Paradiso et *al.*, 1982).

IHHNV

Diagnosis of infection by IHHNV is dependent upon histological demonstration of prominent eosinophilic (with H & E), Feulgen negative intranuclear inclusion bodies within chromatin marginated, hypertrophied nuclei of cells in tissues of ectodermal (epidermis, hypodermal epithelium of fore and hindgut, nerve cord, and nerve ganglia) and mesodermal origin (hematopoietic organs, antennal gland tubule epithelium, connective tissue, and striated muscle). Usually the midgut, midgut ceca, and the hepatopancreas (endoderm-derived tissues) are unaffected, except in severe cases where hepatopancreatic involvement has been observed (Lightner et *al.*, 1985). These inclusions match closely the characteristics

Tab. 3. — Natural and introduced geographic distribution of the penaeid viruses

Virus	Distribution in Wild Penaeids	Introduced Distribution
BP	Americas : Atlantic side; from SE U.S., Gulf of Mexico, Caribbean into Brazil Pacific side; from Peru and Ecuador through Central America into Mexico Hawaii	No major introductions
MBV	IndoPacific : P.R. China, Taiwan, Philippines, Malaysia, Singapore, Thailand, Sri Lanka, Indonesia Australia Africa : S. Africa Middle East : Israel, Kuwait Mediterranean : Italy	Pacific : Tahiti, Hawaii Americas : Mexico, Ecuador, Brazil, SE U.S.
BMN	Asia : Japan	
HPV	IndoPacific : P.R. China, Taiwan, Philippines, Malaysia, Singapore, Indonesia Australia Africa : Kenya Middle East : Israel, Kuwait	Americas : Brazil, Ecuador Pacific : Hawaii
IHHNV	Asia : Malaysia, Indonesia, Philippines, Singapore	Americas : SE U.S., Caribbean, Brazil, Venezuela, Ecuador, Peru, Central America Pacific : Hawaii, Guam, Tahiti Asia : Taiwan Middle East : Israel
REO	Asia : Japan, Malaysia	Pacific : Hawaii Europe : France

of the type A intranuclear inclusion body class described by Cowdry (1934). Basophilic chromatin strands are occasionally visible by light microscopy within IHHN intranuclear inclusion bodies. These chromatin strands are a prominent feature of IHHN intranuclear inclusion bodies by TEM.

IHHN intranuclear inclusion bodies are common early in acute infections, later decreasing in number, and are followed by necrosis and inflammation of target tissues. Affected cells may also have highly vacuolated cytoplasm with cytoplasmic bodies that range from eosinophilic to basophilic. Although the prominent intranuclear inclusions present in shrimp infected with IHHNV are evidence of nuclear involvement, assembly of the virus occurs in the cytoplasm of affected cells. The size and morphology of the virus, 17 to 26 nm in sections and 20 nm in

purified preparations, and its replication within the cytoplasm support the tentative classification of IHHNV with the picornaviruses.

REO

Diagnosis of REO at present is dependent upon gross signs and demonstration by electron microscopy of large cytoplasmic inclusions containing 50 to 70 nm diameter virus particles in the cytoplasm of F-cells and R-cells of the hepatopancreatic tubule epithelium. The non-enveloped, icosahedral virions of REO measure about 60 nm and 50 to 70 nm in diameter, respectively, in purified preparations and in tissue sections. In all species in which REO has been recognized, REO lesions in the hepatopancreas are usually eosinophilic, but not readily apparent. Hence, most infections are overlooked until found by electron microscopy (Tsing and Bonami, 1987; Lightner, 1988).

HOSTS AND GEOGRAPHIC DISTRIBUTION

The host geographic range of the known penaeid viruses has been updated several times recently (Couch, 1981; Johnson, 1983; Lightner, 1983; Lightner et *al.*, 1985; and Lightner, 1988). Surveys and investigations of mortality problems undertaken by the authors' laboratory and other research groups in various shrimp growing areas have provided new data on several of the virus diseases that affect cultured penaeid shrimp. This review of the penaeid viruses emphasizes the current diagnostic procedures for the penaeid viruses, their natural hosts, and their natural and introduced geographic distributions.

BP

BP is widely distributed in cultured and wild penaeids in the Americas, ranging from the Northern Gulf of Mexico south through the Caribbean and reaching at least as far as the State of Bahia in central Brazil. On the Pacific Coast, BP ranges from Peru to Mexico, and it has been observed in wild penaeid shrimp in Hawaii. BP has not yet been observed in wild, cultured or imported (from the Americas) penaeids outside of the Americas. Recent new information on the host and geographic distribution of BP has come from Brazil and Mexico. In South America (Ecuador and Brazil), BP was found to infect larvae and post-larvae of six penaeid species. In Ecuador BP was found to infect imported larval *P. monodon* in a hatchery in which BP was enzootic in its stocks of *P. vannamei* (Philippe Danigo, pers. com., December 19, 1984, SEMACUA, Ecuador). BP has been found in at least two hatcheries in Brazil in native *P. schmitti*, *P. paulensis*, and *P. subtilis*, and in introduced *Penaeus vannamei* and *P. penicillatus*. Five of these species (all but *P. vannamei*) represent new host species for the virus (Table 3). BP was found for the first time in Mexico in cultured larval and post larval *P. stylirostris* at a facility near Guaymas, Sonora on the West Coast of Mexico (Lightner et *al.*, 1988). Because the affected facility had no history

of stock importations, BP must be assumed to be enzootic in wild penaeids in the region.

MBV

MBV-type baculoviruses are similar to BP in their diverse host range and in their wide distribution on the IndoPacific coasts of Asia, Australia, and Africa, and in Southern Europe. However, unlike BP, MBV has been observed in the Americas in imported stocks and in an American penaeid exposed to the virus. Although MBV was first discovered in a quarantined population of *P. monodon* that had originated from Taiwan (Lightner and Redman, 1981; Lightner et *al.*, 1983c), it had not actually been demonstrated in Taiwan until it was found to be widely distributed in Taiwanese shrimp farms in a 1986 survey of the country (Lightner et *al.*, 1987). Studies in 1987 linked MBV to serious disease losses in many Taiwanese farms (S.N. Chen and G.H. Kou, unpublished communication, National Taiwan Univ., Taiwan).

Since the information on MBV was last summarized, MBV has been found in Texas, Ecuador, and Brazil in imported stocks of *P. monodon.* Of possible significance in Ecuador was the presence of MBV-like (spherical) occlusion bodies found along with a heavy BP infection of juvenile *P. vannamei* being cultured with MBV-infected *P. monodon.*

A similar agent, found first in *P. plebejus* and thus called *P. plebejus* baculovirus (PBV), was found in cultured penaeids in Australia (Lester et *al.*, 1987). A similar baculovirus was also found in Australian *P. monodon* and *P. merguiensis* (Doubrovsky et *al.*, 1988). Other than its presence in a new host species, the agent of PBV differs little from MBV in host cell cytopathology and in the morphology of the virus, and it may represent a strain of the MBV-type viruses rather than a separate distinct species.

BMN

BMN has been reported only in *P. japonicus* cultured in Japan, where it is considered a major problem in the larval and early postlarval stages of that species (Sano et *al.*, 1984, 1985; Sano and Fukuda, 1987). Despite numerous introductions of *P. japonicus* stocks (larvae, postlarvae, and broodstock) to Hawaii, France, Brazil, and other locations during the past two decades, BMN has not been detected in that species or in other penaeids cultured in the Americas.

HPV

HPV has a geographic range in Asia and Australia similar to that of MBV, and like MBV it has been introduced to the Americas with imported penaeids. More recently, HPV was found for the first time in dual infections with MBV. It was found in post larval and juvenile *P. monodon* sampled from farms in the Pingtung area of Southern Taiwan. This region in 1987 had experienced serious disease losses in its farms due, at least in part, to MBV. The severity of HPV infections in some of the shrimp

sampled suggests that HPV, while unrecognized, may have contributed to the 1987 epizootic.

Reports of HPV in captive-wild *P. esculentus* in Australia (Paynter et *al.*, 1985), in *P. monodon* imported to Israel from Kenya (Colorni et *al.*, 1987), and in captive-wild and hatchery reared *P. indicus* and *P. merguiensis* in Singapore (Chong and Loh, 1984) have expanded the known host and geographic distribution of this virus (Tables 2 and 3). In the Singapore study, of four shrimp farms surveyed, HPV incidence was highest (> 50 %) in the two farms that reared hatchery-derived post-larvae, and lower (< 15 %) in the two farms which cultured only feral shrimp collected by tidal entrapment (Chong and Loh, 1984). This suggests that HPV is transmitted either vertically from parent broodstock, or horizontally from shrimp to shrimp with efficiency only during the larval stages.

HPV has been observed in the Americas. In Brazil in 1987, HPV was found in stocks of *P. penicillatus* imported from Taiwan. At the same culture facility, HPV was found in light infections in juvenile *P. vannamei*, which had been exposed to infected *P. penicillatus* indirectly as a result of normal farming practises. The discovery of HPV in cultured shrimp in Brazil represents the first time this pathogen has been documented in the Americas and in an american penaeid (S. Bueno, R. Meyer, and D. Lightner, unpublished observations). More recently, HPV lesions have been found in *P. vannamei* cultured in Ecuador (Bell and Lightner, unpublished data). The numerous introductions of *P. monodon* from Southeast Asia into Ecuador may have been the source of HPV.

IHHNV

IHHNV has a world-wide distribution in cultured penaeid shrimp, but its distribution in wild penaeids remains virtually unknown. Infection by the virus causes serious disease in *P. stylirostris*, and acute catastrophic epizootics in intensively cultured juveniles of that species. In other penaeids, IHHNV has been reported to cause infection and disease (Brock et *al.*, 1983; Lightner et *al.*, 1985; and Lightner, 1988), but disease severity does not approach that observed in *P. stylirostris*.

The natural host(s) and natural geographic distribution of IHHNV is largely unknown. However, the occurrence in Southeast Asia (Singapore, Malaysia, Indonesia, and the Philippines) of IHHNV (or a similar agent) in shrimp culture facilities using only wild *P. monodon* broodstock suggests that this region is within the virus' natural geographic range, and that *P. monodon* may be among its natural host species.

Since 1985, no new hosts for IHHNV have been demonstrated. However, the geographic distribution of the virus in culture facilities has continued to expand. In Mexico in 1987, IHHN was found in an imported population of post-larval *P. vannamei* at a facility in Baja California (Lightner, unpublished data). Likewise, IHHN was found to be present in imported quarantined stocks of *P. vannamei* in a 1986 survey of Taiwanese shrimp culture facilities, but not in cultured stocks of other penaeid species, including *P. monodon*, at the farms surveyed (Lightner et *al.*, 1987).

REO

REO is the newest of the penaeid viruses. It was discovered in 1984 by Tsing and Bonami (1987) in juvenile *P. japonicus* in France using electron microscopy, and subsequently, in the same species in Hawaii using the same technique (Lightner et *al.*, 1985). In related work Tsing et *al.* (1985) suggested a possible link between infection by REO and « gut and nerve syndrome » (GNS), an idiopathic condition found in chronically ill populations of *P. japonicus* cultured in Hawaii (Lightner et *al.*, 1984). Most recently REO, or a closely related form, has been found associated with a serious disease syndrome in pond-cultured *P. monodon* in Southeast Asia (Nash and Nash, « in press »).

DISCUSSION AND CONCLUSION

The present diagnostic procedures for the penaeid virus diseases are largely dependent upon history, clinical signs, and histopathology. Electron microscopy is also of importance in some diagnostic applications. Techniques like enhancement and animal bioassays when coupled to histopathology add sensitivity to these diagnostic procedures.

These procedures, however, are very limited. Examination of relatively small samples is one important limitation; the length of time required to carry out routine histopathology and/or electron microscopy is another factor limiting their practical usefulness. Also, the cost of histopathology, electron microscopy, of maintaining enhancement and bioassay areas, and the limited availability of specific pathogen-free indicator shrimp for bioassays, all add to the list of reasons why better, more rapid, more sensitive diagnostic procedures are needed. Methods using tissue culture, serologic methods, and gene probe diagnostic techniques that have become common place in human and veterinary medicine, are being developed for penaeid shrimp. For example, the first documented success at producing primary cell cultures from shrimp was recently reported (Chen et *al.*, 1986). However, while development of these techniques for use with shrimp is underway, none are yet routinely available to the diagnostic labs of the industry. As the new diagnostic methods do become available, they should provide more rapid diagnoses than do current methods; they should be very sensitive and easily standardized among various labs that use them; and they should be inexpensive and simple to run.

The practise of transporting penaeid stocks between facilities and/or different geographic regions has resulted in the introduction of five of the six known penaeid shrimp viruses to regions where they may not have previously existed. Four of the six known types of penaeid viruses are apparently not native to the Americas, but of these four, three (IHHNV, MBV, and HPV) have been introduced with shrimp intended for aquaculture. Whether or not these introduced viruses have escaped the culture facilities to which they have been introduced and have become established in local wild penaeid stocks is not known.

Evaluation of non-native penaeids by the rapidly growing shrimp culture industry is an essential component to the future growth and

development of that industry. Introduction of pathogens like IHHNV to regions where it previously did not occur can have catastrophic consequences to the industry (Lightner et *al.*, 1983a, 1983b; Lightner, 1988). Prevention of such exotic pathogen introduction is dependent upon the use of quarantine, certification and inspection policies, and procedures that are supported by reliable diagnostic tests. Mechanisms have been proposed by a number of international groups to reduce the risks of importation of exotic pathogens and pests with transfers of aquatic species. One example (Sindermann, 1988) entitled, « Revised Code of Practice to Reduce the Risks of Adverse Effects Arising from Introduction of Non-indigenous Marine Species », was adopted in 1979 by the member countries of the International Council for the Exploration of the Sea. The practises outlined in this code, if practised, are effective for their intended purpose. Adoption and implementation of such a policy by the penaeid shrimp aquaculture industry of the Americas may be in the best interest of that emerging industry.

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CRUSTACEAN SESSION
DISCUSSION

Lightner — The first topic in the agenda is : « What specific pathogens of penaeid shrimp should be listed as being of major importance to the international culture industry and which one should have only regional importance or, in other words, which ones we might call certified pathogens and which ones are important internationally or regionally. The catastrophy that has happened in Taiwan last year, with monodon type baculovirus implicated as the cause, suggests that we would not like to transfer that virus into areas where it does not occur. P. *stylirostris* and *P. vannamei* in Mexico seem to be free from IHHN virus, a situation we would like to keep that way.

Weppe — What are the Pathogens of importance we should concentrate our efforts on ?

Lightner — IHHN is an extremelly important pathogen to Mexican shrimp both in supermitensive and intensive. The question is whether it is also important in more extensive systems like in Ecuador. It seems it is since nobody in that country produces monocultures of *P. stylirostris*, so there is circumstancial evidence that it is important even in low density cultures. The best survival of *P. stylirostris* in Ecuador runs only around 20 % of stocking, and if you then sample those shrimps that survive, they are positive for IHHN virus.

Weppe — What virus do you consider most dangerous ?

Lightner — It depends upon the species you are culturing. If you culture *P. vannamei* in Ecuador, there are times you would consider BP as the most dangerous.
If you culture *P. monodon* in Taiwan, you may say it is MBV.
If you culture *P. stylirostris* in Hawaii, then IHHN is the most dangerous virus. It depends on where you are.

Weppe — The viruses are more dangerous in intensive cultures. That is not the case of Ecuador.

Lightner — That is right. In Ecuador the problem is more in the hatchery.

Miahle — All these viruses belong to groups that are known to be highly pathogenic to other invertebrates such as insects. From Dr. Lightner's work it appears that the most important representatives of pathogenic viruses are present in shrimp. These viruses should be considered highly pathogenic.

Lightner — The baculoviruses seem to be pathogenic to at least warm-water species. As for the Parvo viruses, I am not so sure about. There are some indications that they are pathogenic, but there are also nearly as many indications that they are just there. Evidence is increasing that they are important pathogens, but time will show us how really important they are.

Weppe — In heavily MBV infected *P. monodon* from Sri Lanka, I found unsually small occlusion bodies. Do you think there are different strains of the same virus ? What is your explanation ?

Lightner — Because of the geographic distribution of the stocks that are carrying these viruses and the millions of years that have involved, there are likely

to be quite a few strains for example of MBV, that might differ considerably in their pathogenicity and specificity. What we lack are the means of showing that these are indeed different strains. We do not have gene probes or antibodies yet. How can we tell other than by morphological differences ? It is a difficult question right now.

Grizel — In what direction should then shrimp culture develop ? Prophylactic measures are important. We should propose rules to increase control and limit.

Michel — Too little too late. The viruses are already there. In Taiwan, for example, they are relying completely on imported broodstock, because they are unable to produce PLs for their own farms. What do you expect them to do ?

Grizel — What can be done then ? Just diagnosing the disease ?

Colorni — A possible strategy could be to change the species cultured, replacing it with another more resistant. Obviously, the not so remote danger of getting rid of one pathogen and introducing a new one should be taken into account.

Grizel — This may be a solution, but you may simply post pone the same problem. In mollusc culture in France for example we have replaced *Crassostrea anguillata* with *C. gigas*, then what ? We do not have another spare species.

Baudin-Laurencin — There are populations which can be considered virus-free, for example *P. monodon* here in Tahiti. If these stocks are truly disease-free, then they can be kept as some sort of « banks » from which you can obtain healthy stocks for a new start. Another problem, for example in Taiwan, is how long it takes to the virus to lose its infectivity in the environment ?

Lightner — I do not think anybody knows. The practise they sometimes use of lining the pond bottom between crops is apparently very effective in decreasing the frequency of virus appearance, and of course disinfecting hatchery tanks with chlorine and detergents is also helpful. Another point about Taiwan which I have overlooked but may be as important as how much virus was there, is also the lack of people who knew how to diagnose it. They had problems for years but until about 1984 virtually no one in that country was even looking for viral diseases in shrimp.

Miahle — That is an important point. If we want to have a better control over these diseases, the number of people able to diagnose them should increase. Most of the scientists working in diagnosis of shrimp diseases are probably present here : Weppe for Tahiti, Lightner for the US and a lot of other countries. Lesler and Owens for Australia, Colorni for Israel. This is similar to what is happening with bonamiosis in France. People dealing directly in production are not able to recognize their problems at an early stage and to take the suitable steps.

Elston — In Ecuador we are working with wild stocks of *P. vannamei* 60 % of which is infected with BP. If you take some precautions in the hatchery you can produce millions of PLs from this infected broodstock without any problems. In the last 3-4 years there has been no outbreack of the disease : it must be there, but it does not show This is what the farme is mostly interested in.

Breuil — If the viruses are not inside the eggs, simple disinfection of the eggs should prevent vertical propagation of the virus, as in fish eggs.

Lightner — A characteristic of shrimp eggs is that when they are fertilized the cortical reaction makes them sticky for a while. It may be then that some viruses get trapped in individual eggs. If you separate the eggs from the faeces you should at least reduce the level of infection. The most successful

hatcheries in Ecuador are using various techniques such as hatching the eggs in screen baskets with water running.

Weppe — What is it known about immune memory in penaeids ?

Lightner — A few people are working on immune response in crustaceans. Promissing work is being done. Antivibrio vaccines are on the market and some shrimp farmers believe they work. What is missing is some of the control studies and challenge models to prove that the vaccinated animals are indeed immune. There are many testimonies that these vaccines are good but there are not many published data to support them.

Colorni — The taxonomy of Vibrios is still a mess. Even if these vaccines work with a certain strain of Vibrio, they may not necessarily work with a strain by the same name from a different region.

Lightner — As for virus vaccines, I am not aware of anyone actively working on them. So far no one has succee in establishing a cell culture from shrimp. For some reason the cells are not growing. Often they get contaminated with bacteria or fungi. The primary sub-culture methods that people are using are from embryos, ovary tissue, lymphoid organ, haematopoietic tissue and heart with probably lymphoid cells attached.

Elston — Chen is using lymphoid organ. I think his success is due to the fact that his cultures are nearly neoplastic, the cells metastasize, but no mitotic figures are present and eventually the cells degenerate.

Lightner — The tumor that Jim Brock found in some shrimp in Hawaii, a haematopoietic sarcoma of the maxillopeda that looks like a lymphoma in vertebrates could probably be a good candidate for establishing a cell line in a short term.

Colorni — In the case of MBV, the target organ is the hepatopancreas, so even if you manage to culture cells from ovary or lymphoid tissue, you may still have a problem.

Lightner — Chen tried MBV on lymphoid cells and got uptake, but it could not pass the nuclear membrane. He got infection but no penetration into the nucleus.

Elston — I agree that neoplastic tissue is probably a good candidate. We tried that with molluscs, but if you look back at the history of the development of insect tissue culture, which is very recent, you will see that quite a lot of effort was in basic nutritional studies and many nutritional combinations were tried. I think that basically we are similarly dealing with a cell nutrition problem.

Weppe — In conclusion are viral diseases the most important diseases of shrimp ?

Lightner — They are important because we can not control them, treat them or even diagnose them properly. From other points of views, economical for example, bacterial diseases are equally important.

Elston — In Ecuador, people use a lot of antibiotics without really knowing the effects on the bacterial flora and without any planification.

Lightner — I know of hatcheries that have no history of use of antibiotics at all, just husbandry pratises. Other hatcheries can not produce PLs at all without antibiotics. Apparently these bacteria are very much opportunistic.

Michel — We use two antibiotics : Chloramphenicol and Furazolidone. Although we have some problems of bacterial resistance at least with Chloramphenicol

col, these substances work. We cannot explain why, but we know that if we do not use them, the larvae soon die. If you compare bacterial levels in treated and untreated tanks, there is no appreciable difference; it seems that the balance among different bacterial populations is critical.

Colorni — I once fed larvae of *M. rosenbergii* with *Artemia salina* heavily contaminated with Vibrios, but I was not able to influence much the larvae intestinal flora. Mortality rates were similar to those obtained with larvae fed with disinfected nauplii.

Owens — A thing to consider is that when you culture bacteria you obtain perhaps 10 % of what is really there. When you use antibiotics you change the relative composition of that 10 %. There are genera and species you do not even know there are.

Colorni — It is also possible that during passages on artificial media to isolate pure colonies, bacteria modify some characteristics, including perhaps, what makes pathogenic. This may explain the frequent lack of success in reproducing experimentally the disease.

II. PATHOLOGY

II.2. PATHOLOGY OF FINFISHES

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Pathology of marine warmwater finfish in Israel : problems and research

A. COLORNI

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Abstract — *A condition in* ***Sparus aurata*** *larvae termed « distended gut syndrome » (DGS) has become a major problem in the past three seasons. Unable to digest rotifers and brine shrimp nauplii, the larvae develop a swollen abdomen, exhibit a disoriented spinning motion and are swept passively with the current. Bacterial or viral infections were suspected. Chloramphenicol-supplemented larval food did not affect the prevalence of DGS. Virus-like particles 80 nm in diameter were observed in necrotic cytoplasm of midgut wall cells; however, attempts to grow this putative virus* ***in vitro*** *were unsuccessful. Environmental and nutritional factors were also considered, but the etiology of this disease is still unknown. Systemic granuloma, a metabolic disorder of* ***S. aurata*** *that causes severe nephropathy and urolithiasis reappeared again in our cultures after a six year interval during which not a single case was diagnosed. The disease was believed to be related to unbalanced, deficient or spoiled diet. In the light of the most recent episodes, however, it is clear that diets alone cannot be held responsible for the conditions. Erythrocytic congestion, lamellar anevrysm and clubbing, trombosis formation, epithelial hyperplasia and degenerative processes were often observed in the gills of nursery grown fish. While these symptoms appeared sometimes in conjunction with identifiable biological agents, (e.g. epitheliocystis clamydia-like organism,* ***Amyloodinium ocellatum, cryptocaryon irritans****), water supersaturation, stocking density and other environmental factors were often held responsible for their development.*

The rapid development of intensive mariculture worldwide over the past two decades has not been followed by an equally rapid development of veterinary medicine for aquatic animals. This problem is felt even more in tropical mariculture, where tradition does not date back as far as that of cold water species.

The first stocks of gilt-head sea bream *Sparus aurata* were brought to Eilat as fingerlings from Bardawil Lagoon on the Mediterranean coast of Sinai. Some of these fish succumbed to stress during the trip and some soon after their arrival at Eilat due to secondary bacterial infections (Colorni et *al.*, 1981). The great majority, however, survived, pointing at this robust lagoon fish as the right choice for a mariculture industry on the Red Sea. These wild stocks carried a wide array of parasites : over 14 species were identified at the time (Paperna, 1983). The endoparasites

were mainly trematode metacercariae that require a benthic mollusc to complete their development. Since such an intermediate host was absent from our system, these larval helminths gradually disappeared.

Today only ectoparasitic species, such as *Trichodina, Trichodinella* (Ciliata), *Colponema* (Flagellata), and the monogeneans *Gyrodactylus* and *Furnestinia echeneis* (Diplectanidae) proliferate on the fish and, with the exception of *Colponema* whose pathogenicity has not yet been established, still cause some problems. These infections though are sporadic, usually easily diagnosed, and their appearance is dealt with efficiently by formalin treatments. On the contrary, the chlamydia-like organism of epitheliocystis has been responsible for high rates of mortalities in fingerlings in the past years. Epitheliocystis infection occurs in gills of *S. aurata* either as a « benign » infection, with a few or several infected cells (« cysts ») and a limited epithelial tissue response (Fig. 1), or as a « proliferative » hyperinfection with severe hyperplasia of the gill epithelium (Fig. 2) (Paperna, 1977). So far no treatment has been found for this condition.

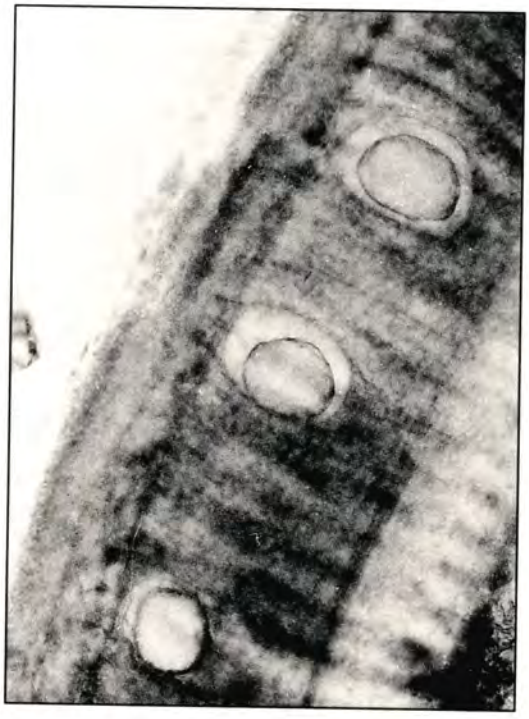

Figure 1. — Gill epitheliocystis, light infection.

Invasive bacteria are occasionally responsible for infections which occur especially after handling operations. The great majority of these bacteria belongs to the genus *Vibrio* and causes acute septicemia in the sea bream and in the other species of fish cultured in Eilat. However, when

healthy fish are challenged with these strains (*V. alginolyticus, V. parahaemolyticus, V. anguillarum* or *anguillarum*-like) by intraperitoneal injection, the fish do not develop clinical symptoms and the bacteria are not recovered from the fish blood. Difficulty in experimentally reproducing septicemic conditions indicates a lack of primary pathogenicity and the requirement of a physiological stress on the host before these strains are able to manifest their opportunism.

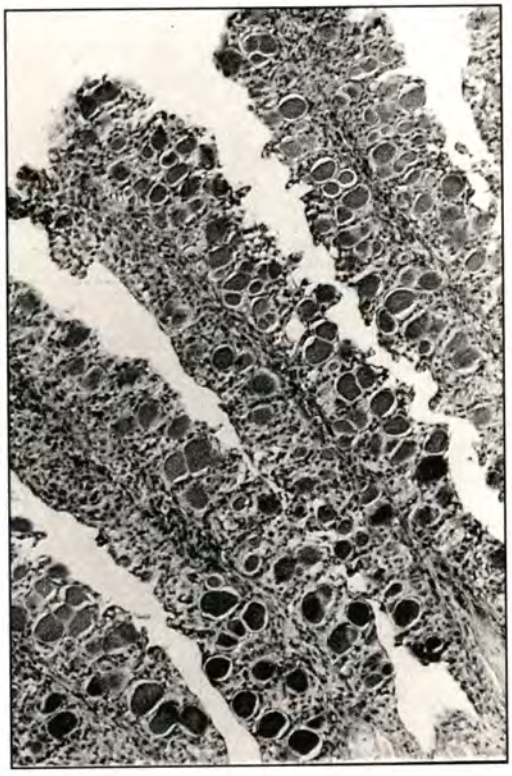

Figure 2. — Gill epitheliocystis, hyperinfection.

Another serious problem affecting practically all open ponds in Eilat is the gas bubble disease. During summer, the intense solar radiation, coupled with the high organic load of the pond water (uneaten food, fish feces, etc.), produces algal blooms and an incessant photosynthesis. Consequently the fishes breathe in a highly oxygenized water all day long. At sunset photosynthesis stops, water exchange continues and the algae start using, rather than producing, oxygen. A rapid external drop in oxygen pressure occurs, with which fishes are not able to cope. Consequently, the fish supersaturated blood fills up with emboli that reach and block capillaries. Downstream tissues are left ischemic, and the consequences become evident in post-mortem examination, in particular of the gills that present conspicuous erythrocytic congestion, telangiectasis, lamellar aneurysm and other degenerative processes. Branchiopathy then predisposes

the fish to infection of microorganisms that are normally kept at asymptomatic levels. Two commercial algicides, Diurex and Terbutrex, were tested but while a limited effect was observed on the algae at the recommended dosages, higher concentrations were severely detrimental to the fish that soon displayed signs of stress (accelerated breathing, darkened color, nervous swimming by the surface) and anorexia for several days after the treatment.

At present, the most troublesome fish diseases in our cultures are of two kinds : a) those caused by parasites with a complex life cycle that can nevertheless be completed in our system, and b) those whose etiology is still unknown and thus we do not know how to prevent.

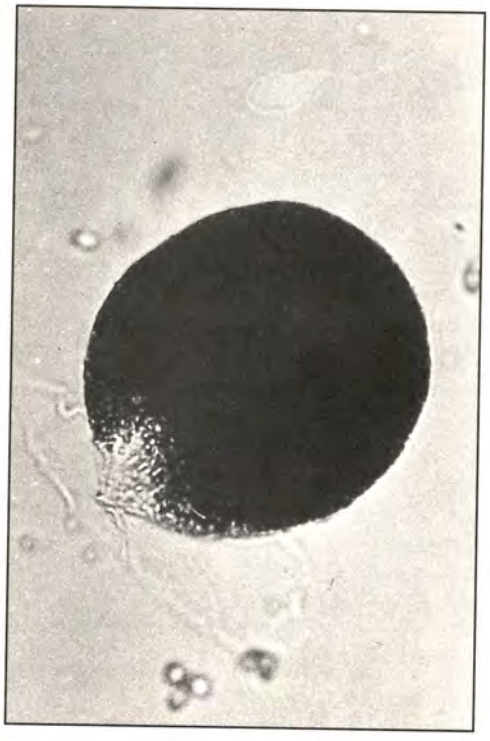

Figure 3. — Trophont of *Amyloodinium ocellatum*.

The dinoflagellate *Amyloodinium ocellatum* (Fig. 3) and the ciliate *Cryptocaryon irritans* (Fig. 4) are two classical examples of the first kind. Although phylogenetically very distant from one another, these two parasites have a similar life cycle, with a parasitic stage on the fish (trophonts), a reproductive stage off the fish (tomonts), and an infective free swimming stage (dinospores and tomites, respectively). No other intermediate host is necessary to close their life cycle, and in the warm water of Eilat, in a confined environment of a tank or of a pond, their proliferation can rapidly reach devastating proportions, undoing within days the work of months or even years. *A. ocellatum* is treated by continuous exposure of the fish to copper sulfate (Paperna, 1984). This

chemical, however, acts relatively slowly, is often toxic at therapeutic concentrations (0.5-1.0 mg/l), and is rapidly sedimented in sea water by magnesium carbonate. *C. irritans* can be treated by rapidly reducing water salinity to at least 10 mg/l for 3 hours four times at 3-day intervals. The efficacy of this treatment lies in the fact that the osmotic shock destroys the tomonts before they can complete their division process (Colorni, 1987). However, this method is practical only when euryhaline fish and limited volumes of water need to be treated. A certain degree of resistance to both parasites has been observed in fish that have survived previous infections, pointing at the possibility of vaccination as the future control method. *A. ocellatum* has been successfully grown *in vitro* on a cell line of catfish gill tissue (Noga, 1987), and large quantities of pure, sterile parasite can be made available as antigens. Parallel experiments are in progress in Eilat with *C. irritans.* As an example of diseases of still unknown etiology, systemic granuloma, a metabolic disorder of *S. aurata* believed to be related to unbalanced, deficient or spoiled diets, reappeared after a six-year interval during which not a single case had been diagnosed.

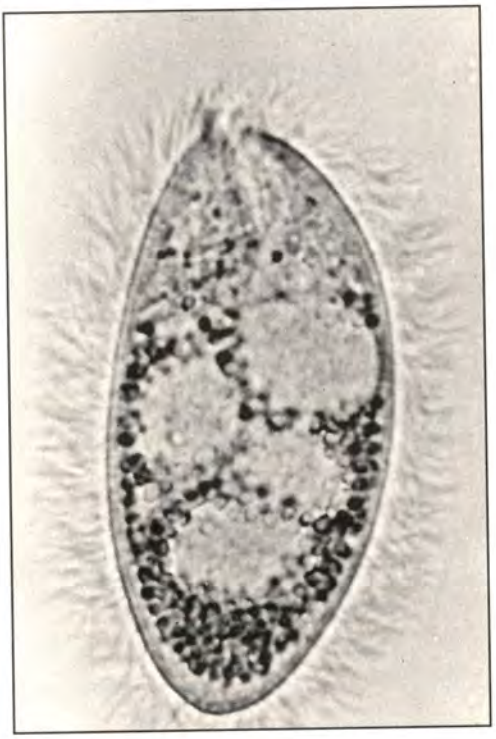

Figure 4. — Tomite of *Cryptocaryon irritans.*

Affected fishes develop severe degenerative changes and necrosis of the renal tubules with eventual collapse of the tubular system (Fig. 5) and formation of granulomata. The condition later spreads to the spleen (Fig. 6) and liver. Severe damage and deformations occur in the eyes (Fig. 7) (Paperna et *al.*, 1980). Presence of viruses, bacteria or fungi has not been detected so far in the affected organs. A similar condition

has been described in the same fish in Spain (Acuigrup, 1983). Quite remarkably, in Eilat this condition affected only *S. aurata* and *Acanthopagrus bifasciatus*, the latter a sparid from the Red Sea that may be considered as the tropical counterpart of the former. During a nutrition experiment carried out in July 1988, a batch of about 300 fishes of 5 to 10 g developed this condition within a short time. The fishes most severely affected, both in number and degree, were the ones that received a high percentage of squid meat in their diet. Degree of severity was arbitrarily determined according to the kidney condition and the characteristic presence in it of tyrosine crystals (Fig. 8) at the first stages of the disease.

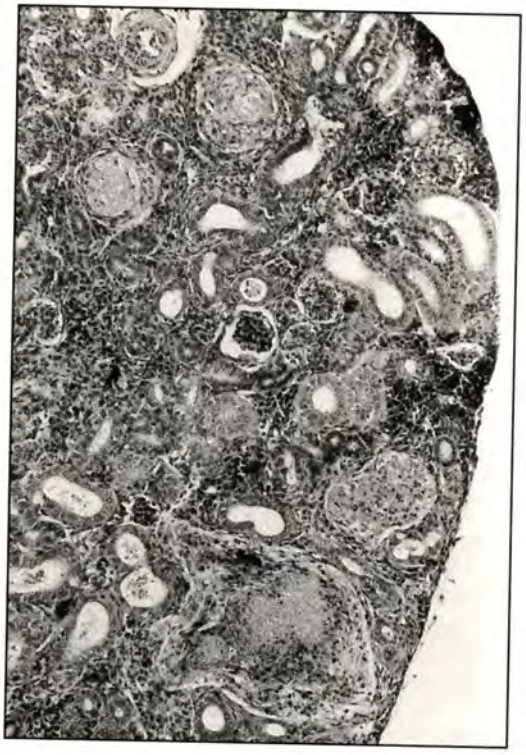

Figure 5. — Granulomatous kidney.

However, altogether a relatively low number of fishes was found to be unaffected in this batch, including the control fishes, whose feeds contained no squid. In the past, empirical change of feeds to fresh diet resulted in regression of the disease incidence which suggested a relation of this condition to dietary problems (Paperna et *al.*, 1980; Acuigrup, 1983). However such relation was never proved and the etiology of this disease is still unknown. More recently, a « granulomatous hypertyrosinemia » was induced in turbot (*Scophthalmus maximus*) fed for over 5 months with an ascorbic acid deficient diet (Messager et *al.*, 1986), while injections of ascorbic acid in the affected fish brought about normalization of tyrosinemia and regression of ocular lesions (Messager, 1986). Vita-

min C is a very labile substance, whose activity is conditioned by time, temperature, oxygen, pH, light, etc. In our feeds it is lipid-coated to prevent its dispersion into the aqueous medium before ingestion by the fish. Although the possibility exists that the vitamin C batch used was somehow faulty, it is puzzling that the same batch has been used for the preparation of other feeds, but no new cases have been diagnosed since this episode.

Figure 6. — Granulomatous spleen.

Another condition that affects the gilt-head sea bream was called Distended Gut Syndrome (DGS) and is associated with mass mortalities of the larval stage. To date, the disease has been shown to affect larvae 9 to 32 days old. The afflicted larvae develop a swollen abdomen, exhibit a disoriented, rolling or spinning motion, and are swept passively with the current. In most larvae, the feeding response seems to persist as if out of control, and the alimentary tract becomes engorged with large clumps of undigested or semi-digested rotifers or brine shrimp nauplii. The severely dilated mid and posterior gut contains vast populations of gram negative bacteria, mainly *Vibrio* spp. *Epithelial hyperplasia* is often associated with leukocyte infiltration into the lamina propria of the mid gut. Vacuolation of hepatocytic mitochondria and a marked reduction of zymogen granules in the pancreatic acinar cells are also commonly seen. Microvilli look disrupted and a paucity of pinocytotic vacuoles in the absorptive cells is

evident, indicating an impairment of the normal absorptive processes. Spherical, membrane-bound electron-dense inclusions, approximately 85 nm in diameter were observed in cells of the midgut epithelium. Tissue ultrafiltrates from homogenates of larvae with acute DGS symptoms were inoculated in nine different cell lines, but attempts to grow this putative virus *in vitro* failed. It is noteworthy, however, that the cell lines used in these challenges were all from non sparid fish and it is thus possible that a virus was present but unable to replicate (Diamant, in press).

Figure 7. — Typical appearance of the eye in *S. aurata* affected by systemic granuloma.

Prevalence of body deformities is high in hatchery spawned sea bass while is more moderate in gilt-head sea bream. Its correlation with environmental and nutritional factors, including ascorbic acid deficiency, is presently under study in Eilat. Fishes with opercular deformities, scoliosis, swimbladder defects and other abnormalities demonstrate growth retardation and become very susceptible to bacterial and parasitic infections. These fishes, nicknamed « sentinel fish », are usually the first to show signs of distress or to die when conditions in the system deteriorate. A normal swimbladder is paramount for proper shape and growth. A partial correlation could be established between spinal deformities in gilt-head sea bream fingerlings and non-differentiation of swimbladder epithelium, whose cells, rather than developing into a functional cuboid lining, become hypertrophic and gradually degenerate. Apparently,

swimbladder pressure is important for the normal development of the spine in early life stages. However, scoliosis and non- or partial inflation of the swimbladder can also occur independently in both fishes. The stimuli required to ensure proper development of this organ are presently being studied.

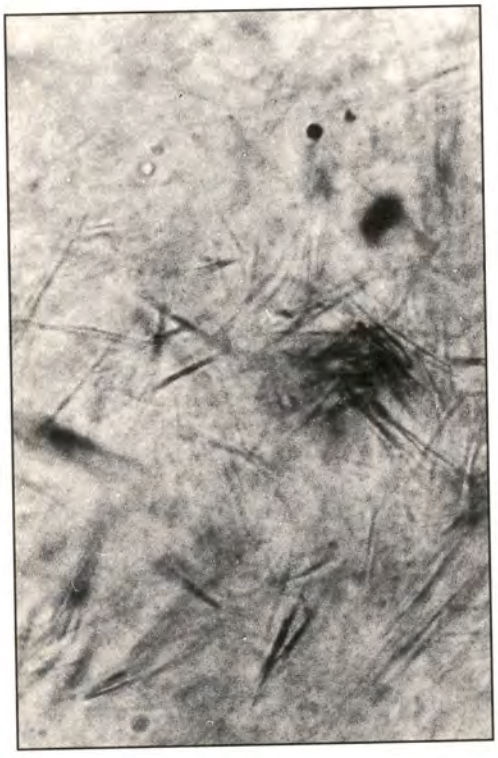

Figure 8. — Characteristic tyrosine crystals from a fresh smear of kidney tissue (early stages of systemic granuloma).

The choice of gilt-head sea bream and European sea bass as the main species to be cultured in Eilat was dictated by both the sturdiness of these two species and the price they can fetch in the European markets. However the natural habitat of these two fishes is the temperate water of the Mediterranean Sea. Higher temperatures have, for example, a strong inhibitory effect on the oogenesis of the sea bass (Y. Zohar, unpubl.), which may be an important limiting factor for the culture of this species in our region. Perhaps some of the deformities or diseases of nuclear etiology simply represent failures of the fish physiology to adapt to the high salinity and temperatures of the Red Sea.

Spontaneous tumors rarely occur in our cultures. A case of lamelo-blastic fibro-odontoma of the lips (Paperna et *al.*, 1977) and a branchial osteochondroma (Nash and Porter, 1985) were diagnosed in *S. aurata*, while a large tumor, probably arising from the thymus gland, was more recently diagnosed in *Dicentrarchus labrax*.

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Marine finfish pathology : specific problems and research in the French West Indies

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Abstract — *The finfish species reared in marine intensive conditions in the French Caribbean Islands are imported (European SeaBass, American Red Drum and* ***Oreochromis*** *Hybrids) or local (Carangids and Lutjanids) species. The European SeaBass (****Dicentrarchus labrax****) is susceptible, during its first year of life, to a contagious disease characterized by whirling and hyperexcitability, associated with severe lesions of the brain, viral particles being pointed out in the damaged nervous cells.The only way to contend with this disease is to undertake preventive actions (excellent sanitary management and reinforced diet with vitamins and immunostimulants). The red Drum (****Sciaenops ocellatus****), in its hatchery phase, is highly susceptible to parasitism (****Amyloodinium ocellatum*** *and* ***Microsporidia****-like organisms) that adapted treatments are able to control. In its nursery and grow-out phases, nutritional deficiencies can induce vertebral column spontaneous fractures, making necessary extemporaneous vitaminic addings to the diet. This technique allows to prevent as well the Scale Loss-Blindness-Melanism Syndrome of Lutjanids, characterized by melanism and blindness with retina degeneration. The Oreochromis Hybrids, the Carangid Palometa (****Trachinotus goodei****) and the Lutjanid Yellowtail Snapper (****Ocyurus chrysurus****) are susceptible to parasitism by the Monogenean Fluke* ***Neobenedenia melleni****, and, for the latter species, by the Ciliate Protozoa* ***Brooklynella hostilis*** *and* ***Cryptocaryon irritans****. Depending on the parasite, the fish species and the rearing condition (tank or cage), different preventive and curative treatments have been carried out. All these primary diseases create favourable conditions for bacterial contaminations, which were always found secondary.*

INTRODUCTION

The Finfish species reared in marine intensive conditions in the French Caribbean Islands are both :

— imported ones, such as European SeaBass (*Dicentrarchus labrax*), American Red Drum (*Sciaenops ocellatus*), and « Florida » Oreochromis Hybrid (*O. mossambicus* o X *O. hornorum* o)

— and local ones, such as Carangid Palometa (*Trachinotus goodei*) and Lutjanid Yellowtail Snapper (*Ocyurus chrysurus*).

The first three ones are reared with a purpose of production, the two others are in an experimental phase. Rearing technologies, which have been adapted from an initial pattern, are similar. Consequently, the diseases encountered are in keeping with these technologies as well as with the animals themselves.

The IFREMER Laboratory of Pathology has a very practical goal, which is to prevent the rearings from disease; this requires the three following steps :

— first, identifying the outstanding pathological risks for each rearing stage of each species;

— second, finding out or adapting specific curative and preventive treatments to each problem;

— and third, inserting the preventive techniques into the rearing technology itself, in a routine way.

At the present state of knowledge, the Outstanding Primary Diseases for each rearing stage of each species are given in Table 1. It is obvious that all these primary diseases create favourable conditions for bacterial contaminations, which were always found secondary.

Tab. 1. — Outstanding diseases in the F.W.I. rearings

Species / Diseases	European Seabass	Red Drum	Florida Hybrid	Carangids	Lutjanids
Whirlingh	H N G				
Parasitosis	B	H	G	B N G	N
Environmental <nutritional> Diseases		N			B G

B Breeders storing
H Hatchery stage
N Nursery stage
G Grow-out stage

THE WHIRLING DISEASE OF THE EUROPEAN SEABASS *(Dicentrarchus Labrax)*

A/ Epizootics since 1983

In the summer of 1983, appeared in the cages of European SeaBass a new disease which seemed to be linked to the temperature rising over thirty degrees centigrade (Picollier, pers. com.). It was called « Summer

Disease ». It was an apparently contagious disease which affected only young of the year animals and led them to death (morbidity = mortality).

During the summer of 1984 (June to October), the total mortality due to this disease reached eighty percent of the yearling livestock.

For the summer of 1985, preventive measures (Gallet de St Aurin, 1985) probably allowed to minimize the incidence of the disease, which was however active (ten percent mortality from June to October).

In the early 1986, a very similar syndrome affected the fingerlings in the nursery and the larvae in the hatchery, each cohort being affected younger than the previous one (the last one to be affected was only 30 days old); furthermore, the survivors of this « Whirling Disease » did not suffer « Summer Disease » in cages afterwards.

B/ Symptoms

The main symptoms of both « Summer Disease » and « Whirling Disease » are the following :

- progressive darkening, beginning on the caudal part of the fish
- nervous troubles, alternating with stages of remission : total loss of equilibrium; apparent blindness; hyperexcitability, in particular to noise or changes in the light intensity; and sudden and violent muscular contractions.

  In all cases, death occurs within one week after the beginning of the nervous symptoms.

Figure 1. — Longitudinal section of the brain of a 10g diseased fish, showing spongiosis (S) in the optic tectum granular layer (HES × 40).

C/ Lesions

Histological studies showed, in addition to muscular degeneration (Gallet de St Aurin et *al.*, in press), specific lesions in the brain of diseased fish :

- a Spongiosis, which in the cage fish is limited to the optic tectum granular layer (Figure 1); in the fingerlings and larvae, this

spongiosis can become so extensive that the whole nervous tissue (including the spinal cord and the retina, which is a part of the mesencephalon) is affected (Figure 2).

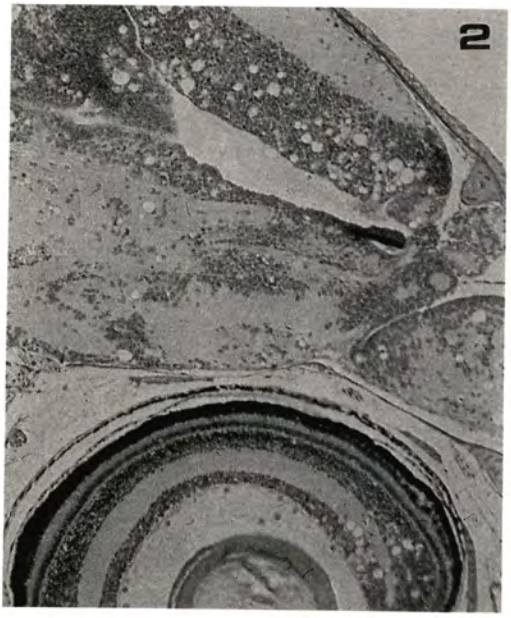

Figure 2. — Longitudinal section of the anterior part of the brain of a 45 days old diseased larvae (HES × 100).

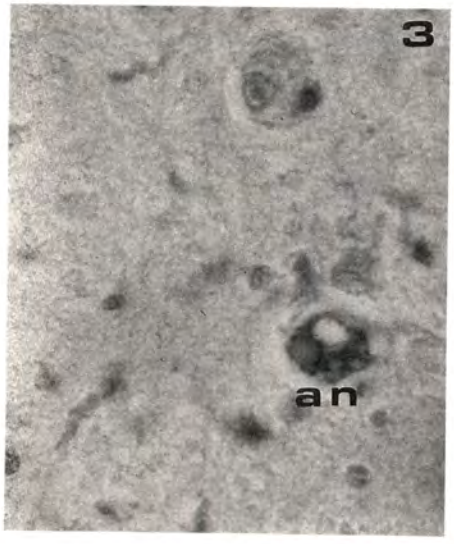

Figure 3. — Vacuolized and inclusion-bodies containing cytoplasm of an abnormal neuron (a n) surrounded by normal ones (HES × 1000).

— the presence of abnormal neurons in different parts of the brain (optic tectum, tegmentum, cerebellum, vagal lobes, medulla oblongata and spinal cord) : they are enlarged cells with cytoplasmic vacuolation and presence of basophilic round-shaped inclusion-bodies (Figure 3). The vacuoles are sometimes very extensive (Figure 4). The younger the affected fish is, the more extensive and destructive the lesions are.

Electron Microscopy studies were performed on the brain of seventy days old healthy and diseased fingerlings. Healthy fish showed normal neurons (Figure 5). On diseased fish, the studies showed a modified shape of the affected neuronal cells and their inside nucleus, and the presence of round-shaped osmiophilic inclusion-bodies (one micrometer average diameter) in the cytoplasm (Figure 6).

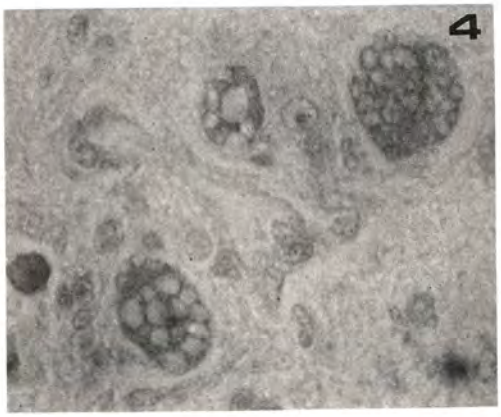

Figure 4. — Extensive vacuolation of enlarged affected neurons (HES × 1000).

Figure 5. — Normal neurons. G : × 5900.

At a higher magnification, the inclusion-bodies appear to be limited by a unit-membrane and to be filled with icosahedral particles (mean

diameter of the envelope : 35 ± 5 nm) (Figure 7). These particles sometimes have a very dense pseudo-cristalline array, and were identified as viral units. In some cases, the unit-membrane was interrupted, and the particles were spread into the neuronal cytoplasm (Figure 8).

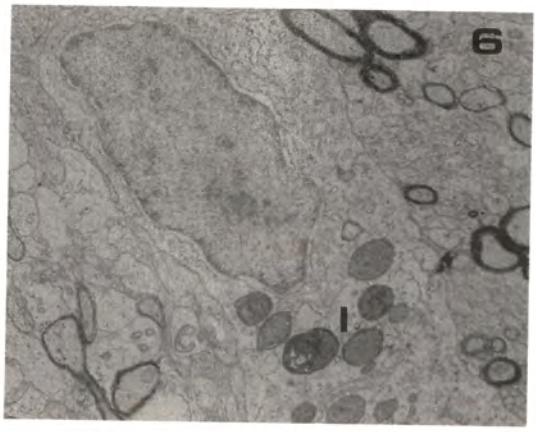

Figure 6. — Affected neurons : modified shape of the cell and the internal nucleus, and round-shaped osmiophilic inclusion-bodies (I) in the cytoplasm. G : × 5900.

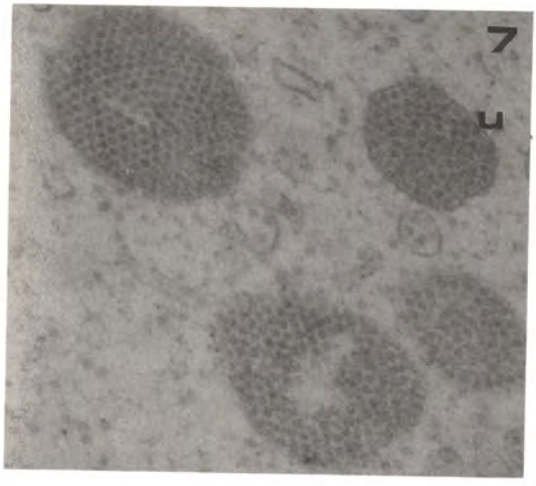

Figure 7. — Higher magnification of inclusion-bodies, showing the unit-membrane (U), and the inside icosahedral viral particles (external diameter : 35 nm). G : × 67000.

D/ Discussion

At that state of knowledge, some questions are to be discussed :

1) Are the viral particles the aetiological agents of the « Whirling Disease » ?

Among the results (Gallet de St Aurin et *al.*, in press), it was found that :

— the cell-culture trials always failed

— thus far, the disease has not been experimentally reproduced.

However, the presence of viral particles in the cytoplasm of strongly affected neurons of fish showing nervous troubles, and their absence in the brain of apparently healthy fish, brings a quasi-certainty about the role of these particles in the morbid process. Also the following facts :

— progressive contagion,

— higher and higher virulence (fish affected younger and younger; nervous tissue lesions more and more extensive), come in aid of an infectious aetiology.

The failure of experimental reproduction of the disease indicates that probably other factors are necessary for its clinical manifestation; some authors showed that concomitant factors were involved in the expression of viral diseases (Schwedler and Plumb, 1982; Wolf, 1984).

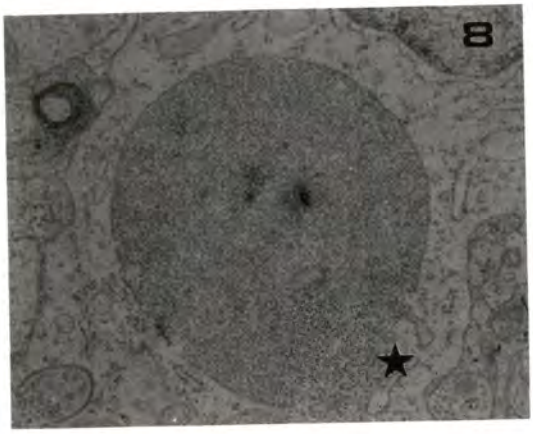

Figure 8. — Inclusion-body with interrupted unit-membrane and spreading of the virions into the neuronal cytoplasm (*). G : × 12500.

2) Are the « Whirling Disease » of larvae and fingerlings, and the « Summer Disease » of cage fish, the same syndrome ?

These diseases have strong similarities in their epidemiology and symptoms, and above all induce identical histological lesions in the brain of the affected individuals.

Another argument in favour of the hypothesis of the uniqueness of these diseases is the fact that the cohorts previously affected by « Whirling » were not susceptible to « Summer Disease » some weeks later; as if these fish had developed an immunity resistance against this disease.

An Electron Microscopy study could not be performed on neurons of « Summer Diseased » fish. Only this would have brought the proof of the uniqueness.

3) What do such lesions look like ?

In Slow Virus Encephalitis such as KURU or CREUTZFELDT-JAKOB diseases in human beings, or SCRAPIE in sheeps, the lesional pattern is alike the above described one (Gajdusek, 1967 ; Carrier et *al.*, 1973); though the aetiological agents of these diseases are prions, which are not complete virions.

Nevertheless, the SeaBass Disease could be useful as an experimental model for the study of these Encephalitis (Mikol, pers. com.).

4) In a practical point of view, what was possible to do to contend with this disease ?

- the shore-side buildings (hatchery and nursery) were closed for several months.
- for the cage-rearing stage :
- new areas were looked for (lower temperatures, longer distance to the shore-side)
- preventive actions were undertaken before and during the critical summer period (from early May to October), consisting in a daily survey, an excellent sanitary management and reinforced diet with tonic and stimulant chemicals, such as :

  Vitamin C : 100 mg/kg, 3 days a week

  Vitamin E : 40 mg/kg, 3 days a month

  Fish autolysate : 600 mg/kg, once a week

  Levamisole : 100 mg/kg, twice a month

As seen above, these actions, undertaken in 1985, probably allowed to greatly reduce the mortalities during the summer period.

PARASITICAL DISEASES

The second kind of Outstanding Primary Diseases, and the most frequent, is Parasitism. It affects all the species at one or several rearing stages (Table 1). The tropical intensive mariculture conditions are highly favourable to direct external parasitosis, caused by Protozoa or Monogenean Flukes (Table 2, Figure 9).

A/ Parasitosis by *Neobenedenia melleni*

Its very wide repartition area and the various fish species it attacks (Jahn and Kuhn, 1932; Nigrelli and Breder, 1934; Nigrelli, 1935; Loyau, 1985; Conroy, pers. com.) make this parasite a real danger for many tropical fish cultures. In Martinique, among the reared species, only the red Drums have never been found with the parasite on them; the most susceptible are the Florida Hybrids.

As it is easy to treat and to prevent by hyposalinity (freshwater dips), this parasitosis is really dangerous only in cages. The infestation is favoured by a high density of fish, a small mesh size or a low quality food such as long-stocked pellets (Soletchnik et *al*, 1988b). Also a previous

disease will favour its occurence (Raymond, 1988). Two peaks of high infestation were revealed : March to May and August to October (Loyau, 1985).

Neobenedenia melleni is a Plathelminthus from Monogenea class and Capsalidae family. The adult is egg-shaped and can reach 5 mm in length. The parasitical cycle lasts 3 to 6 days at 29°C (Loyau, 1985).

Tab. 2. — Parasitical risks in the F.W.I. rearing conditions

Rearing conditions	Effects on pathogens	Expected parasitosis
Water	Effective vector	
High and stable temperatures	Rapid proliferation	
Some are weakened	Permanent source	Direct (Monoxene)
Many animals	Easy contagion	Ecto parasitosis
Organic matter	Supports for free stages	Protozoa
Closed or semi-closed systems	Increased efficiency	Monogenean flukes

REARING CONDITION	CAGE	TANK		HATCHERY	
PARASITE	Neobenedenia mellini  adult 0,3-5 mm	Brooklynella hostilis  70 x 40 µ	Cryptocaryon irritans  50-450 x 30-50 µ	Amyloodinium ocellatum  30-70 x 150 µ	Microsporidia - like  3-6 granulations in cyst (200-500µ)
HOST(s)	FLORIDA HYBRID LUTJANIDS EUR. SEABASS CARANGIDS	LUTJANIDS EUR. SEABASS	LUTJANIDS EUR. SEABASS CARANGIDS	RED DRUM	

Figure 9. — Parasites affecting the rearings.

The first symptom is an abnormal behaviour of fishes. They rub against net (« flashing ») and jump out of the water. Anorexia and hyperproduction of skin mucus are also observed. Then appear haemorrhagic ulcers on the sides, fin rot (mainly on anal and caudal fins), thickening and opacification of the eye cornea. At this stage, it is easy to see many parasites on eyes, skin and fins. Ulcers are very quickly invaded by bacteria (*Vibrio, Aeromonas, Pseudomonas*) and mortality increases.

Prevention in cages is based on avoidance of favouring factors, by limiting the density of fish, providing a good quality food and sanitary management. These measures are able to keep the parasitical infestation at a low level, on the less susceptible species (Soletchnik et *al.*, 1988b). They are not sufficient for Florida Hybrids, for which an early treatment, before anorexia, is possible with Trichlorfon (Dylox) in the food (50 mg/kg of fish, four times at 3 days intervals), each month during the critical periods (Loyau, 1985).

B/ Parasitosis by Ciliate Protozoa

The most dangerous parasites encountered in tank rearings, other than in the Red Drum Hatchery, are the Ciliate Protozoa *Brooklynella hostilis*, and above all *Cryptocaryon irritans* (Loyau, 1985; Gallet de St Aurin et *al.*, 1986). This last parasite has a worldwide repartition area, affecting a large number of species of marine aquaria and cultures (Brown, 1951; de Graaf,1962; Nigrelli & Ruggieri,1966; Wilkie and Gordin, 1969; Sindermann,1977; Herwig,1978; Violetta, 1980; Hignette, 1981; Huff and Burns, 1981; Paperna, 1983; Loyau, 1985; Weppe, pers. comm.).

B. hostilis was previously reported from aquarian fish (Lom & Nigrelli, 1970).

In Martinique, Lutjanids and European SeaBass did suffer high infestations by *B. hostilis*. Also *C. irritans* affected Lutjanids, Carangids and European SeaBass in tanks. The infestations could occur all the year round, but they were more frequent on the summer time (Loyau,1985).

Both parasites are localized on gills, causing respiratory troubles; but *C. irritans* also invades skin, and lives embedded under the host's epithelium (Colorni, 1985), that makes it much more destructive and also harder to reach by chemicals.

A *B. hostilis* infestation can easily be treated by a 200 ppt Formalin 15 mn bath; complete eradication is obtained by repeating this treatment 3 times at 48h intervals. On the other hand, the only efficient treatment used against *C. irritans* was a 12 days continuous exposure to Tris buffered Copper Sulfate, at a rate of 0.15 mg of Copper ion Cu++ per litre. Obviously this is a very heavy constraint.

So a quarantine process has been developed, to be used whenever new fish are to be put in tanks. Based on reported studies on the biology and attempts to control *C. irritans* (Nigrelli and Ruggieri, 1966; Canella, 1972; Blasiola, 1978; Herwig, 1978; Cheung et *al.*, 1979; Hignette, 1981; Huff and Burns, 1981; Colorni, 1985) and local observations (Loyau, 1985), this 12 days quarantine has to be done in a special tank which had been previously disinfected and dried out, and which receives filtered water under 5 micrometers; at days 1, 5, 8, and 11, the fish are subjected to a 200 ppt Formalin 30 mn exposure, followed by a 5 mn freshwater dip and an antiseptic (50 g/m3 Furaltadone Chlohydrate, or 2 ppt Quaternary Ammonia) 30 mn bath. Transfer occurs at day 13 to the rearing tank receiving filtered water under 5 micrometers; at day 14, a last antiseptic bath allows to avoid bacterial infection.

C/ Parasitosis in red Drum Hatchery

The red Drum larvae were affected by the Dinoflagellate *Amyloodinium ocellatum* and by what we tentatively identified as a Microsporidia-like organism.

A. ocellatum has a very wide repartition area and affects a large number of fish species (Blasiola, 1978; Overstreet, 1978; Lawler, 1979; Cheung et *al.*, 1981; Baticados and Quinitio, 1984 ; Barbaro and Francescon, 1985). The red Drum is noticed as a susceptible species by American researchers (Johnson, 1987; Arnold, pers.comm.; Roberts, pers. com.).

In Martinique, the European SeaBass breeders had suffered an *A. ocellatum* infestation (Gallet de St Aurin, 1985).

The eggs of red Drum were imported from the U.S.A., and it was not determined whether the parasites were imported as well, or were local strains. The larvae were affected as early as day 18 post hatching.

The Microsporidia-like organisms were found on 20 days old larvae (S.I.M., 1988).

The clinical manifestations of both parasitosis are identical : invading gills, skin and fins, they induce respiratory troubles by themselves and by the thick layer of mucus secreted by the host in response to their irritating presence.

According to reported studies on the biology and control trials on these parasites (Overstreet and Whatley, 1975; Bulla and Cheng, 1976 and 1977; Lawler, 1977b; Overstreet, 1978; Johnson, 1984; Paperna, 1984a and 1984b; Colorni, pers. com.) and to local observations (Gallet de St Aurin, 1985; S.I.M., 1988), attempts to treat these parasitosis in red Drum hatchery were carried out, after conclusive in vitro tests. But, due to the weakness of so young larvae and the difficulty to handle them, these treatments induce mortalities by themselves, which is not satisfactory.

A strict sanitary prevention remains the best way to avoid troubles. In particular, to avoid entering of parasites, the water in which the eggs are shipped needs to be filtered under one micrometre before shipment. Also the rearing water and the live preys should be under control. To avoid proliferation, a limited density of larvae (under 5 individuals per litre) and a good water quality maintenance are necessary. To avoid spreading of any parasites which could have entered the hatchery, time partitioning between batches, space partitioning between tanks (each one having its own equipment, which is disinfected after each use), a daily sharp observation of the animals, and in case of disease in one tank, the early discard of this tank, are necessary measures.

ENVIRONMENTO-NUTRITIONAL DISEASES

The third group of Outstanding Primary Diseases is the environmental (including nutritional) one. These diseases affected in Martinique mainly the red Drum in nursery and the Lutjanids in grow-out cages (Table 1).

A/ The Scale Loss- Blindness-Melanism Syndrome

1) Description of the disease

This disease affected the Lutjanids, and at a lesser extent the European SeaBass; it appeared after a few months of cage rearing and feeding on pellets (Gallet de St Aurin et *al.*, 1986). The affected fish were found lonely at the water surface, dark coloured, very slow moving, and emaciated. Scales were lost in patches on the fish sides, the lower lip was ulcerated, and from the anus was often issueing green-whitish faeces (Raymond, 1988). Fish were dying mostly by secondary bacterial infection.

2) Lesions

Necropsy of the dying fish showed a few unspecific signs, and mainly the absence of mesenteric fat (Raymond, 1988).

Histological studies were performed on slightly affected fish :

- no lesions were found in the integument; only the melanocytes were expanded and numerous
- in some cases, a lysis of the intestinal mucosa was observed
- a retina degeneration was evidenced for all the observed diseased fish (Figure 10); more obvious in the central retina area, this degeneration affects all the layers internal to the pigmentary epithelium : these layers become thinner and thinner, and merge together, making the thickness of the retina about one third of the normal value; the photoreceptor cells, mainly the Rod-cells, are altered and become scarce (Raymond, 1988).

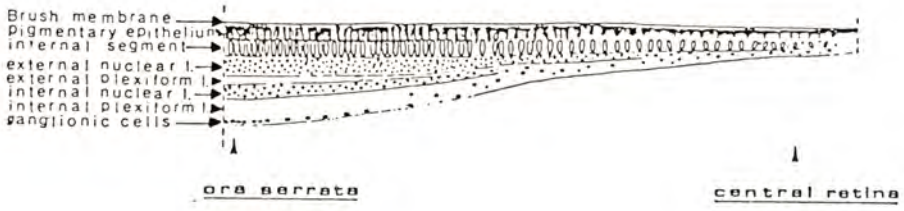

Figure 10. — Retina degeneration.

3) Pathogenic Scheme

All the experimental findings (Raymond, 1988) go to prove that the pathogenic scheme is the following :

The initial retina degeneration (which is the only specific lesion) induces blindness, and then melanism by humoral route. But also starvation, which itself explains a biliary retention which is responsible for the lysis of intestinal mucosa (observed in some cases), and emaciation and lack of mesenteric fat. Blindness explains as well the lower lip ulceration (which is the consequence of knocking against the net), and the poor

mobility. Parasites and Bacteria find very favourable conditions and induce secondarily the scale losses, and new ulcerative lesions.

4) Proposed Aetiology

According to the literature (Lawill et *al.*, 1977; Lanum, 1978; Penn, 1985), such a retina degeneration is probably due to a phototraumatism by high light intensity, the fish being reared in shallow cages in clear water.

This type of phototraumatism is greatly favoured by the high temperature, and the oxidizing of membrane lipids (Farnsworth & Dratz, 1976; Weigand and Giusto, 1983; Bazan et *al.*, 1984), which occurs for example when the given diet is Vitamin E and/or Vitamin C deficient, as both Vitamins act as membrane anti-oxidative (Ashley et *al.*, 1975; Farnsworth and Dratz, 1976; Amemiyat, 1981; Joel et *al.*, 1984; Organisciak, 1985; Cowey, 1986).

B/ Spontaneous Vertebral Column Fractures

1) Description of the Disease

The red Drum and the European SeaBass in their nursery stage were affected by a disease which induced low but constant mortality (one to two percent of the livestock per day). It appeared generally 15 to 20 days after weaning on pellets. The affected juveniles were seen motionless in an oblique posture, dark coloured in the caudal half part of the body, and sometimes showing a lateral curvature (Figure 11). When forced to swim away, they could only move the anterior fins, which suggests paralysis.

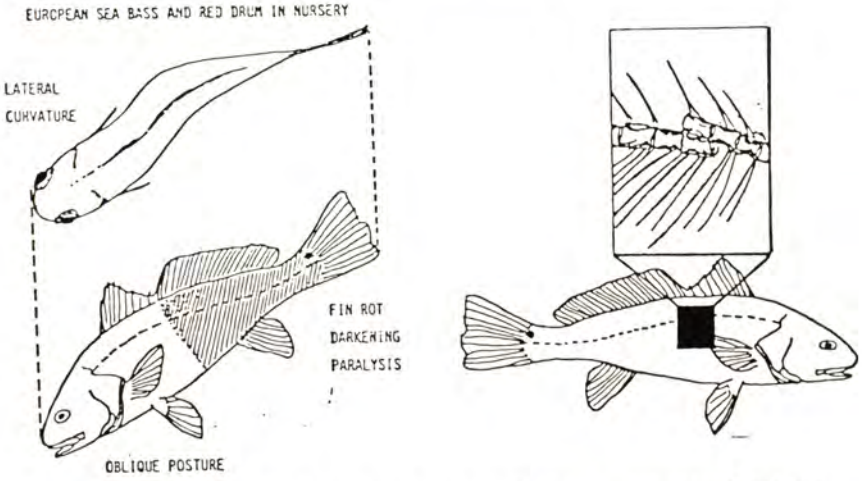

Figure 11. — Symptoms and lesions of « vertebral column spontaneous fractures ».

2) Lesions

Necropsy and radiography of the affected fish showed a fracture of the vertebral column, located between vertebrae six and twelve (Figure 11).

Radiography performed on apparently healthy fish from the same batch showed, in 12 percent of the cases, a scoliosis or a lordosis (unpublished data).

3) Proposed Aetiology

Scoliosis and lordosis in fish are assigned to various causes (Hoffman and Dunbar, 1961; Ashley et *al.*, 1975; Kloppel and Post, 1975; Overstreet, 1978; Halver, 1980; From et *al.*, 1985). Between them, a Vitamin C deficiency is known to induce a deficient synthesis of the connective tissue, and then squelettal deformities and frailty (Koenig, 1984). In our case, during the fast growing nursery period, a spontaneous fracture will occur in the maximal bending zone of the body of some fish, when swimming.

C/ Prevention of Scale Loss-Blindness-Melanism Syndrome and Spontaneous Vertebral Column Fractures

Both above mentioned diseases, so different in their clinical manifestations, have in common a nutritional aetiological factor, which is a Vitamin C deficiency. The use of commercial pellets available in the French West Indies, even without any storage, seems to be inadequate for supplying the fish vitaminic needs (Koenig, 1984); high temperature and moisture greatly reduce the conservation time of vitamins, specially Vitamin C (Halver, 1980; Messager, pers. com.); Vitamins C and E have synergical activities (Halver, 1980; Cowey, 1986; Cillard, 1987).

According to the literature and to the tests carried out in Martinique (Raymond, 1988; Gallet de St Aurin, unpublished data), the prevention of the above diseases can only be obtained by extemporaneously oil-coating the daily diet (on a seven days a week feeding basis) with the following amounts of Vitamins :

— during the nursery stage, beginning as early as the weaning time :
100 mg Vitamin C per Kg of fish
20 mg Vitamin E per Kg of fish

— during the grow-out stage :
30 mg Vitamin C per Kg of fish
2 mg Vitamin E per Kg of fish

CONCLUSION

All the above mentioned pathologies could be linked to a definite aetiology, which is often concealed by secondary pathogenic causes; thanks to an accurate diagnosis, they could be contended with, in a more or less successful way. On the other hand, in larval rearings, the results in terms of survival remain highly variable from one batch to another, suggesting the occurence of « pathological events » which, due to the size of larvae and to the rapidity of the course of such events, remain too often misunderstood. The researches have now to be focused on this field.

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A field report on vibrio disease of Seabass (*Dicentrarchus labrax*) in the South of France

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Abstract — ***Vibrio anguillarum*** *was isolated from diseased seabass reared in aquatic farms (hatcheries and grow-out farms) located along the French Mediterranean coast. A representative strain (V62) differs from the French* ***V. anguillarum*** *strain (V408) by its ability to grow on a 0 % salinity medium. The pathogenicity of the representative strain for sea bass was confirmed by injections and bath challenge. The V62 strain reacted with* ***V. anguillarum*** *antiserum (V408) and 84 % of the isolated strains from diseased sea bass agglutinated with anti V62 serum.*

INTRODUCTION

Six French aquatic farms (3 hatcheries and 3 grow-out farms) located along the Mediterranean coast have been found to be faced with bacterial disease of seabass since 1986.

* In hatcheries (water temperature 20°C), all the rearings are affected with bacterial problems following the weaning period (0.2 to 0.5 g). A good antibiotherapy (Oxytetracycline 70 mg/kg/day for 8 to 10 days) is able to stop the disease. Nevertheless, mortalities still reach 15 to 20 % (from 0.2 to 2 g). Diseased animals show haemorrhagic symptoms (red mouth disease) and ulcerative lesions of the fins.

* In grow-out farms, mortalities usually occur in Spring or Autumn, after handling or sudden thermic variations. Estimated mortality reaches about 10 % (on an average general scale) during the first year of rearing, but only occurs in few cases during the second one. Diseased fishes show marked ulcerative lesions near the ventral zone or along the body side (Ulcer disease). A good antibiotherapy (Oxytetracycline, Furazolidone or Oxolinic acid) is also needed to stop the disease.

MATERIEL AND METHODS

Bacterial investigations

Bacterial samples

One hundred and thirty three bacterial samples, isolated from the blood, liver and skin of live diseased animals were analysed by standardized kits (API 20E, API 20NE, ADH-LDC-ODC kits from the Pasteur Institute). A presumptive bacterial identification (*Vibrionaceae*, *Pseudomonaceae* and *Enterobacteriaceae*) was done by using Bergey's manual procedure. For the *Vibronaceae* the most accurate identification was made according to West and Colwell's scheme (1984) and to Reichenbach and Dworkin's (1981) for the Myxobacteria. Presumptive *Vibrio anguillarum* strains were then checked with the French vibrio 408 antiserum (Baudin-Laurencin 1981), and with the US V/408, V/775 antiserum.

Filamentous-like bacterias isolated from the skin did not show chararacteristics of mixobacterias when cultured on Anacker and Ordal medium (Anacker and Ordal 1959). These motile, oxydo- fermentative and O129 sensitive bacterias were further classified as Vibrio species.

Pathogenicity tests

Intraperitoneal inoculation

A saline (2.5 % NaCL) suspension containing 10^6 CFU/ml of the representative V62 strain was injected in the abdominal cavity (IP inoculation of 3.10^5 bacteria per fish) of 30 seabass weighing from 14 to 20 g. The water temperature was maintained at 20°C. A bacterial control is made on the kidney of moribond fishes.

Scarification, intradermal and bath challenge

Scarification : fishes weigh 15 g have been scared on the body side. The lesion is then plugged with a sterile gauze (control group) or with a gauze soaked with salt water containing 10^8 bacterias/ml. The water temperature is maintained at 20°C (group 1) or raised from 19 to 25°C (group 2), or from 15 to 21°C (group 3 and control group).

Intradermal (ID) inoculation : 0.1 ml of a saline (2.5 % NaCL) suspension containing 10^6 bacteria/ml is injected to six fishes weighing 250 g (ID inoculation of 105 bacterias per fish). The control group is inoculated with a sterile saline (2.5 % NaCL) solution. The water temperature is 20°C.

Bath challenge : 30 seabass weighing 1 g have been bathed for 1 hour in salt water containing 107 bacterias/ml. The water temperature is 20 °C.

Epidemiology

Serological characterization of the *V. anguillarum* strain previously isolated (V62) was done by using an anti V62 strain rabbit serum supplied

by the Laboratoire National de Pathologie des Animaux Aquatiques (LNPAA) de Brest (Baudin Laurencin pers. comm.). Seventy presemptive V. anquillarum strains showing the same biochimical character as the V/62 reported strain, have been tested by using the V/62.

Geographical repartition of the disease : various hatcheries and grow-out farms of seabass affected with vibriosis have been controled by using the anti V62 serum in 1987.

RESULTS

Bacterial investigations

Strains of *Vibrio anguillarum* (representative strain V62) were isolated from 45 % of the samples (fig. 1). The biochemical characters of the representatives strains (*V. anguillarum* V/62, *V. alginolyticus* and *V. parahemolyticus*) are listed in table 1. The V62 strain reacts with the V408 antiserum as well as with the V775 one.

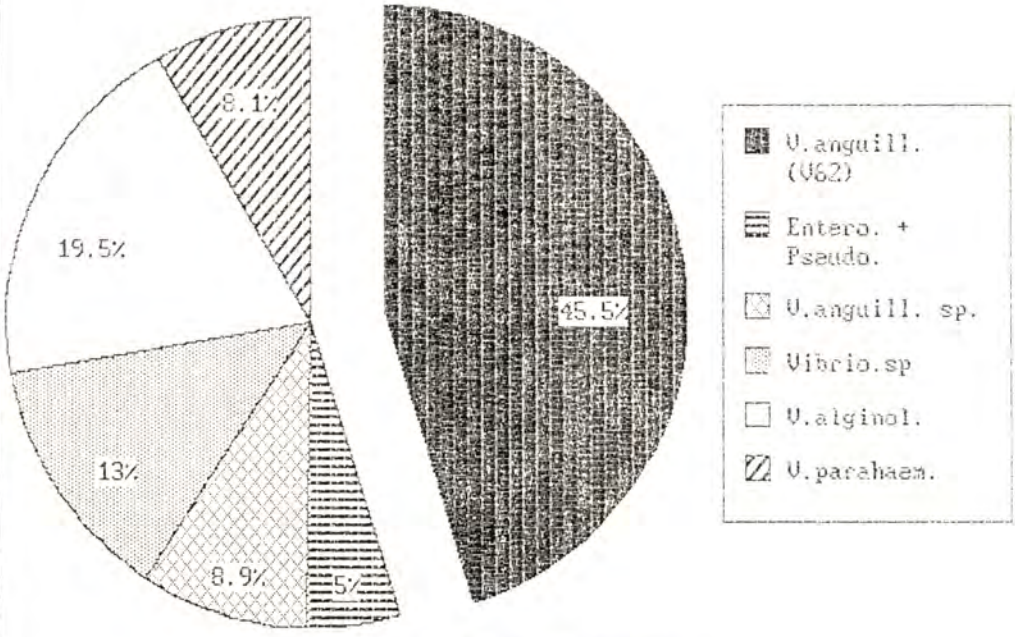

Figure 1. — Total bacteria distribution.

Bacterias identified as *V. fishery*, *V. vulnificus* or *V. proteolyticus* were classified in the *Vibrio* sp. group.

The percentage of the different bacterial species depends on which organs are sampled (fig. 2). *Vibrio anguillarum* is predominant in the blood and liver of infected animals and is present but not predominant in the skin, where *V. alginolyticus* is found in 36 % of the samples.

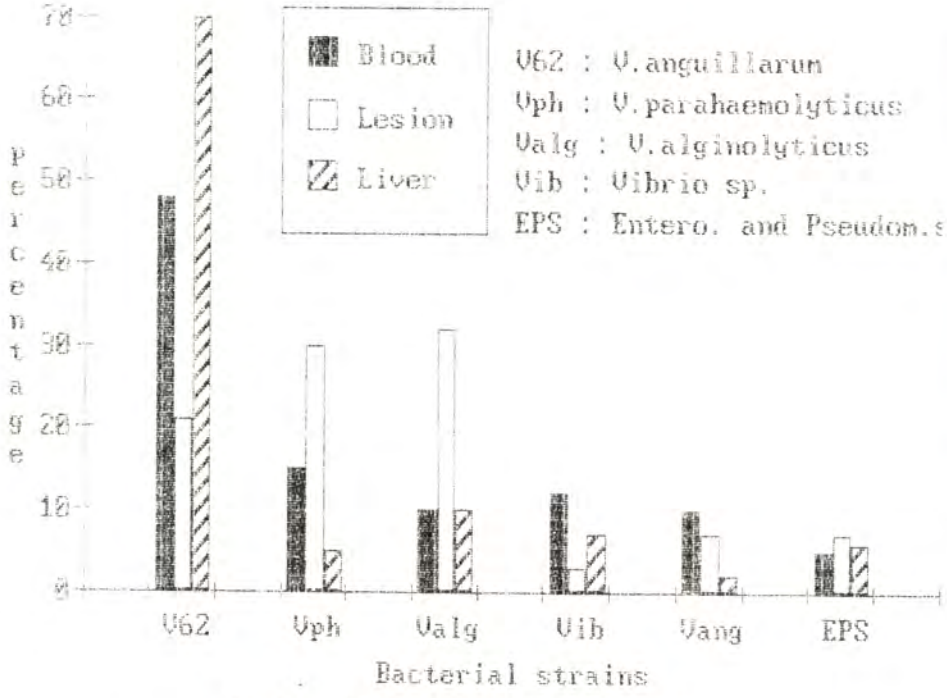

Figure 2. — Bacterial distribution in organ samples.

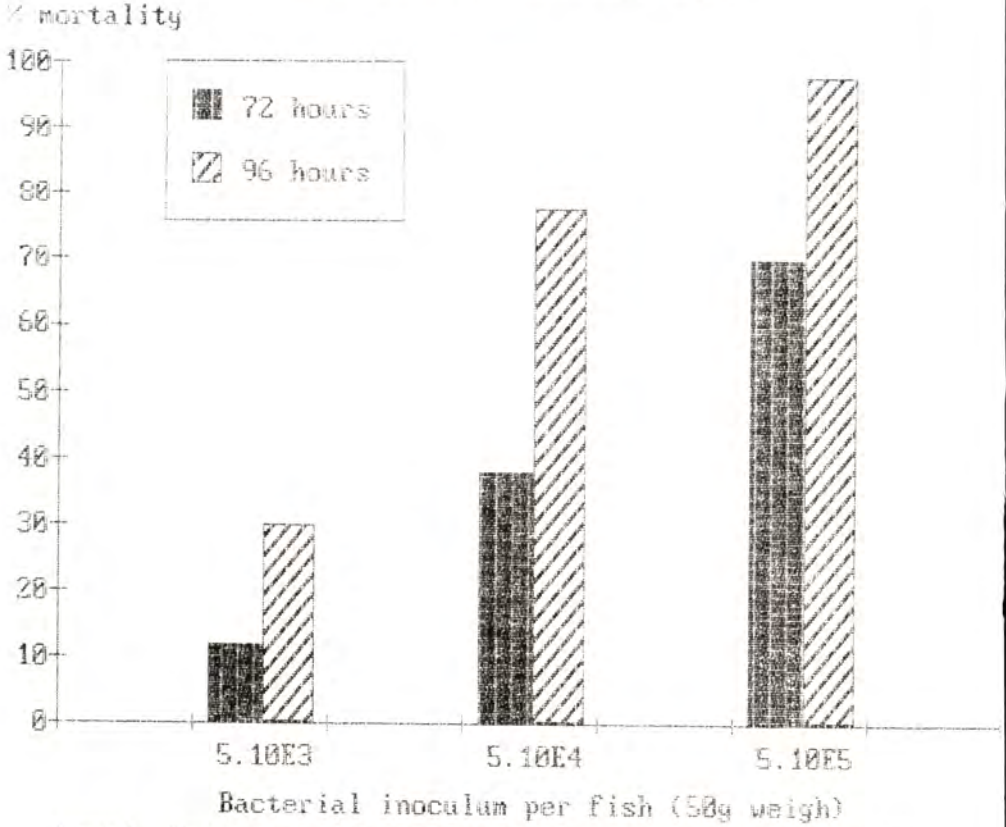

Figure 3. — Percent mortality induced at 72 and at 96 hours by an intraperitoneal inoculation of *V. anguillarum* (V62) to sea bass.

Pathogenicity tests

Intraperitoneal inoculation

Among three different strains tested (*V. anguillarum* V62, *V. parahaemolyticus*, and *V. alginolyticus*), only the V62 induced mortalities within a week with haemorrhagic lesions. *V. anguillarum* V62 is isolated from the blood and kidney of moribond fishes. All the animals which were inoculated with the other strains survived like the control group (IP inoculation with a sterile saline solution). The results of inoculation with the V62 strain including two doses are shown on fig. 3.

Tab. 1. — Biochemical characters of reference strains

V62	V. alg	V. paraph	
Cytochrome oxydase	+	+	+
Nitrate reduction	+	+	+
0/129 sensitivity : 10 µg	+	nd	nd
400 µg	+	+	+
Swarming	− +	−	
Luminescence	−	−	−
Arginine dihydrolase	+	−	−
Lysine decarboxylase	−	+	+
Ornithine decarboxylase	−	+	+
Growth at 37°C	+	+	+
Growth at 42°C	−	nd	nd
Growth at % NaCL : 0 %	+	−	−
2 %	+	+	+
6 %	+	+	+
8 %	+	+	+
10 %	−	+	−
Voges-Proskauer reaction	+	+	+
Gas from glucose fermentation	−	−	−
Fermentation to acid :			
L-arabinose	−	−	−
m-inositol	−	−	−
D-mannose	+	+	+
Sucrose	+	+	+
Amygdaline	−	−	−
Utilization as sole source of carbon			
Cellobiose	−	−	+
D-gluconate	+	+	+
Sucrose	+	+	+
D-xylose	−	−	−
Enzyme production :			
Alginase	−	−	−
Amylase	+	+	+
Gelatinase	+	+	+
Lipase	+	+	+

nd : not done
V : variable

V62 : *V. anguillarum*
V. alg : *V. alginolyticus*
V. paraph : *V. parahaemolyticus*

Scarification, intradermal and bath challenge

Results are shown on table 2.

An intradermal (ID) inoculation with 10^5 bacteria/fish as well as a 1 hour bath in salt water containing 10^7 bacterias/ml induces mortalities within 3 days. Fishes whose skin had been scarred and exposed to a bacterial suspension of 10^8 CFU/ml, reacted differently depending on the the water temperature of the test. At 20-21°C, fishes which had been stressed by rising the water temperature (group 3) are more sensitive than the others (group 3); moribond fishes do not show ulcerative lesions of the skin. Only fishes scared at 20°C and then placed at 25°C (group 2) have developed ulcerative lesions of the skin.

Tab. 2. — Scarification, intradermal and bath challenge with the reference strain V62

Bacterial concent.	Test group	Nb	Wgt (g)	Temp (°C)	Temp variat.	Mortality (%)	Skin lesion
10^8/ml	SC 1	10	15 g	20°		20 %	–
	SC 2	10	15 g	15°	+5°	70 %	–
	SC 3	40	15 g	20°	+5°	100 %	++
Control	SC	30	15 g	20°	+5°	0 %	–

SC = Scarification test

10^5/anl	ID	6	250 g	20°		50 %	–
control	ID	6	250 g	20°		0 %	–

ID = Intradermal inoculation

10^7/ml	1 h	30	1 g	20°		70 %	+
control	12 h	30	1 g	20°		0 %	–

Bath challenge

EPIDEMIOLOGY

Among 70 presumptive *V. anguillarum* V62 strains tested, 59 strains (84 %) are agglutinated by the specific antiserum. However, crossed reactions exist between the two antisera tested (V408 and V62), and suggest antigenic communities between these two strains. Other isolated strains (*Vibrio* sp., *V. alginolyticus*, *V. parahaemolyticus*, *Pseudomonas sp.* and *Enterobacteria* sp.) did not react the V62 antiserum.

Geographical distribution of vibriosis : rearings of sea bass affected with vibriosis are shown on fig. 4.

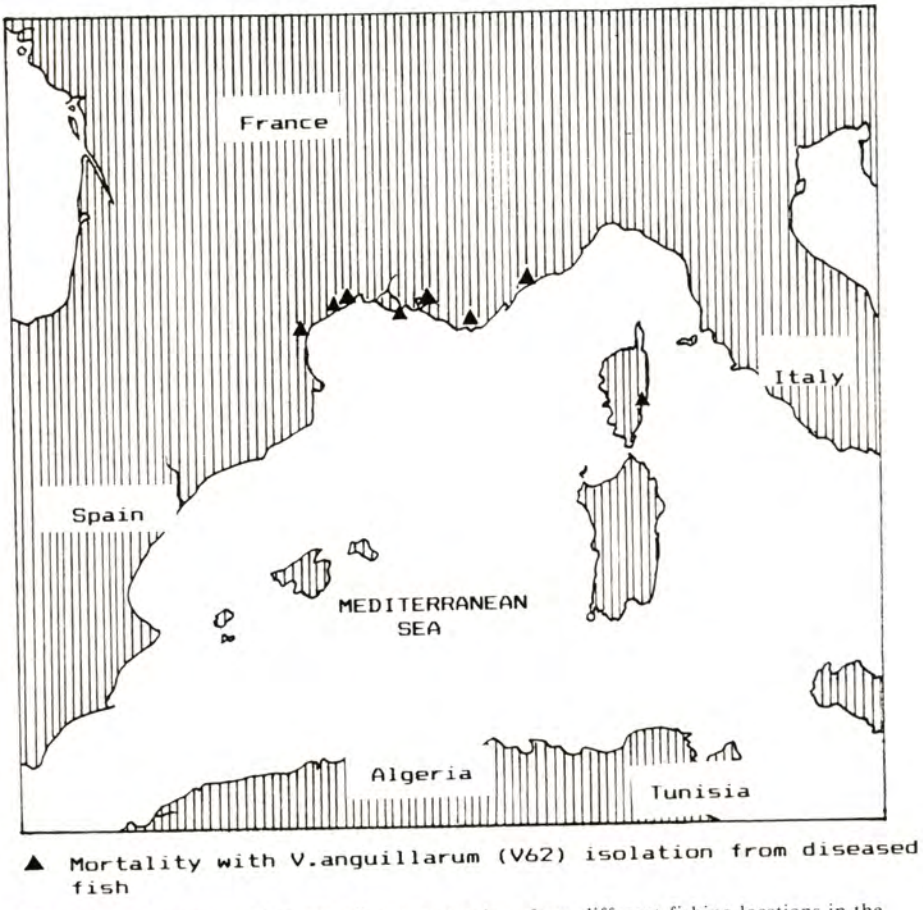

Figure 4. — Occurence of *V. anguillarum* on sea bass from different fishing locations in the South of France.

DISCUSSION

Many marine fish species may develop an ulcerative disease : salmon (Evelyn 1971, Morrisson et *al.*, 1981), Pacific halibut (Levine et *al.*, 1972), cod (Jensen & Larsen, 1982), sea bream (Colorni et *al.*, 1981), grey mullet (Burke et *al.*, 1981), turbot and eel (Colwell and Grimes, 1984). Previous bacterial investigations of diseased animals have involved several genera : *Vibrio* (Burke et *al.*, 1981; Colwell and Grimes, 1984; Colorni et *al.*, 1981; Phelepp and Martin, 1985; Phelepp et *al.*, 1985, Nounou ,1985), *Aeromonas* (Alvarez and Conroy, 1987, Llobrera et *al.*, 1987), *Pseudomonas* (Wakabayashi et *al.*, 1972; Nounou, 1985), *Mixobacteria* (Demoury, 1987). Our bacterial identification may be regarded as a presumptive one; neverthe-

less, *Vibrio anguillarum* has been isolated in different aquatic farms suffering from bacterial disease. The V62 strain differs from the representative French strain of *V. anguillarum* V408 (Baudin Laurencin, 1981) by a few biochemical characters (negative arabinose and amygdaline assimilation, growth on 0 % salinity medium). Arabinose negative *V. anguillarum* strains have already been isolated from grey mullet (Burke *et al.*, 1981), catfish (Lewis, 1985) and molluscs (Bolinches et al., 1986); and *V. anguillarum* strains able to grow on a 0 % salinity medium have been reported. Pathogenicity tests with the V62 strain have induced mortalities as with other *V. anguillarum* strains inoculated to salmon (Evelyn, 1971), or turbot and trout (Baudin Laurencin, 1986). Experimental challenge with *V. anguillarum* is also known to induce ulcerative lesions on grey mullet (Burke *et al.*, 1981) and on winter flounder (Levine *et al.*, 1972).

Vibriosis is presently the main pathological problem for the rearing of sea bass in France (fig. 4). This pathology may increase in the future as recent data show that trout (Baudin Laurencin, pers. comm and turbot (Ifremer, Palavas), are also sensitive to the V62 strain. Attemps are made to set up vaccination of the rearings.

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Two examples of nutritional pathology related to vitamin E and C deficiencies

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Abstract — *Symptoms recalling a nutritional pathology and more precisely a process of lipid peroxydation were observed during the last years in seabass* ***Dicentrarchus labrax*** *and other fishes cultured in tropical marine condition,* ***Ocyurus chrysurus*** *and* ***Lutjanus analis****. They included dark coloration, skin ulceration, lethargy, anorexy, emaciation. Histological examinations showed hepatic fatty degenerative lesions, pancreatitis, muscular degeneration and retinal atrophy. Additional vitamins E and C in the food suppressed tha pathological symptoms. Several experiments were carried out using sea bass which received a lightly oxidized food. The previously evoked clinical or histological signs were not achieved in spite of an increase of the level of hepatic, muscular or blood malondialdehyde and of the conjugated dienes of the perivisceral fat. In the same time, hepatic and muscular tocopherol decreased. These experiments also showed the feasibility and the interest of different analyses in the characterization of such a pathology : hemolysis test and activity of enzymes such as erythrocytic catalase or superoxyde dismutase and plasma glutathione peroxydase.*

Another nutritional disease is worth describing again, the Granulomatous Hypertyrosinaemia. It was essentially investigated in turbot ***Scophtalmus maximus*** *but other fish species such as sea bream* ***Chrysophris aurata*** *are often affected. The disease is both characterized by an increase of the plasma tyrosinaemia and the coming out of visceral and muscular granulomatous nodules. Microscopic needle shaped crystals of tyrosine may be found in lesions and in subcorneal deposits. The experimental ascorbic acid deficiency induces the pathology and on the contrary a sufficient providing of vitamin C rapidly leads to normal tyrosinaemia and reduced eye lesions. As in mammals, ascorbic acid seems to plan an anti-oxydant role in protecting the p-hydroxyphenyl-pyruvate dioxygenase, the enzyme acting in the main way of the tyrosine metabolism. The tyrosine crystals could directly induce an inflammatory specific response with development of granulomatous nodules.*

In practise, the knowledge of the risks and the distribution of a good quality food must prevent such diseases.

INTRODUCTION

It may be surprising to imagine that the present good knowledge of fish nutrition may let subsist any possible nutritional pathology. However, vitamin E and C deficiencies have been recently mentioned in 2 diseases described on marine fish raised in the West Indies : Summer Mortality of seabass *Dicentrarchus labrax* (Gallet de Saint Aurin, 1987) and Blindness-Melanism Syndrome (Raymond, 1968).

This review presents several experiments made in LPAA to check these aetiological hypothesis. Attempts to reproduce clinical signs described on these diseases are made by feeding fish vitamin C and E deficient diet, further containing oxidized lipids.

Granulomatous Hypertyrosinaemia is then briefly described as directly related to vitamin C deficiency.

A number of the collected data are here presented in order to discuss possible nutritional aetiologies and mechanisms of the diseases described in the West Indies.

I. PATHOLOGY OF CULTURED FISHES IN THE WEST INDIES

1.1. Observations

« Summer mortality of seabass »

The disease occurs on net-pen reared fish less than one year old when water temperature is over 29-30°C, from July to September. An unusual behaviour is mentioned : whirling swimming or, at rest, recognizable position : oblique, head down, sometimes belly up. The fact that fish are repetitively hitting the net can be related to blindness. Anorexia is noticeable.

Histopathological examinations were carried out on different organs :

Liver : lipid vacuolisation is particularly significant during summer season, simultaneously with degeneration phenomena (nucleolus swelling, chromatin margination). There is some ceroid deposits.

Kidney : vacuolisation of tubular epithelium, increasing throughout the hot season.

Stomach : degeneration of gastric glands.

Guts : desquamation of intestinal mucosa with atrophy of villosities and epithelial necrosis.

Pancreas : atrophy, cytoplasmic densification, pycnosis.

Spleen : congestion and hemosiderin deposits in macrophages and melanomacrophage centres.

Muscle : cloudy swelling, vacuolization, necrosis.
Eyes : retinal atrophy (2 cases).

The lower haematocrit, haemoglobin and erythrocyte number characterize an anaemic condition, while the decreasing levels of plasma total protein and cholesterol indicate a metabolic disorder. Cytolysis (high Na^+ and K^+ with hydrolysis of intracellular adenosine triphosphate increases with clinical signs. Abnormaly high amounts of malondialdehyde (MDA) are detected in liver of fish presenting clinical symptoms.

« Blindness-melanism Syndrome »

The disease was firstly described as a « Loss of Scales Syndrome » by Raymond (1987) on indigenous fishes of the West Indies, especially *Ocyurus chrysurus* and *Lutjanus analis.* Fish show a loss of appetite, melanism, and an important decrease of weight. Some of them can hardly catch the pellets which also suggest blindness. Ulcerative skin lesion are often seen on the head, latero-dorsal body part, and fins. Ocular lesions such as keratitis and aphaky are sometimes detected.

In the liver, the excessive accumulation of fat in cytoplasm is often accompanied by nuclear atrophy and sometimes pycnosis. More seldom, intrahepatocytic ceroid deposits can be observed. The kidney presents a hyalin droplet degeneration of tubular epithelial cells, particularly on *Ocyurus chrysurus.* In the guts, desquamation of enterocytes begins on the top of villosities and possibly extends to almost the whole intestinal epithelium with, in that case, a flattening of the mucosa. Sub-epithelial structures remain untouched. A muscle degeneration appears on white as well as on red muscle and under different forms : hyalinisation, vacuolization, centronucleation. The retinal atrophy seems to be specific to the disease, which justifies its name : Blindness-Melanism Syndrome. The lesion is gradually developing, first on retinal center and then towards *Ora serrata* : the most internal tissue layers are first touched (ganglionic cells, inner plexiform and nuclear layers) and then external to be disorganized and fragmented. No more nucleated structure can be seen at the last stage.

A high level of tyrosinaemia is observed on the most affected fish. Further, low levels of plasma glucose, proteins, and cholesterol illustrate a nutritional disorder.

No evidence of any septicaemia is made on sea bass, neither on indigenous fishes. Parasitism is somewhat inconstant. Clinical, histopathological and biochemical data tend to prove nutritional (particularly vitamin C and E deficiencies) and environmental (light and temperature) aetiologies. This hypothesis is enhanced by the fact that distribution of food enriched with vitamins did reduce the problems. Accordingly, it seemed quite appropriate to verify this hypothesis with experiments reproducing field conditions : partially oxidized food, containing pretty low levels of vitamin E and C, hot temperature and high light.

1.2. Attempts to experimental reproduction of the disease (Table 1).

Experiment A :

Five hundred 80 g seabass raised in gradually increased temperature (until 31°C) are vaccinated against *Vibrio anguillarum* and kept in a heated

open circulating water system. Three batches are made, each of them divided into 4 identical tanks (4 repetitions). The basal diet contains 12 % of voluntarily oxidized oil *in vitro*. Its lipid fraction is then characterized with the following indices median values : peroxyde value (mEq./Kg of lipids) : 15.5; n moles MDA/g : 55. This diet is given to the B group. C and CE groups are fed the B diet supplemented with vitamin C (minimum measured 1 700 mg/Kg treated instead of 96 in B) and CE group with vitamin E (m.m. 550 mg/Kg instead of 36 in B and C).

Tab. 1. — Experiments A, B, C. Experimental conditions and results

EXPERIMENTS	A (batch B)	B (Batch Ox)	C (Batches Ox)
FOOD CHARACTERISTICS *			
MDA (nmoles TMP**/g)	55		500
POV (mEq/Kg Lipid)	15.5		350
VITAMIN E (mg/Kg)			
- supplementation	40	40	0
- measured in food	36-56		5
VITAMIN C (mg/Kg)			
- supplementation	710	710	50
- measured in food	96-404		
BHT (mg/Kg)			
- supplementation	100	100	0
CHOLINE (g/Kg)			
- supplementation	3.1	3.1	0
FISH : Seabass			
Initial mean weight (g)	102	3	86
Final mean weight (g)	170	30-50	162
WATER TEMPERATURE (°C)	28-31	18	20
EXPERIMENT DURATION (weeks)	13	45	11
RESULTS			
Hepatic E vitamin (µg/g)	56.2 ± 15.6	46.5 ± 21.5	3.4 ± 0.9
Muscular E vitamin (µg/g)	3.63 ± 0.93	2.5 ± 21.5	0.78 ± 0.13
Muscular TMP (n.mole/g)		3.71 ± 1.44	8.12 ± 5.23

* Characteristics of basal diets, with oxidized oil added and vitamin E and C deficiency
** TMP : tetramethoxypropane.

After 13 weeks, fishes supplemented with vitamin C (batches C and CE) have got double liver concentrations of vitamin C (70 mg/Kg). In batch CE, liver and muscular values of vitamin E are 3 times higher (respectively in liver and muscle : 156 and 13 mg/Kg) than in B and C.

However, general health status of fish is not affected : biometric and plasma parameters (glycaemia, total protein, cholesterol) are identical at the end of the experiment. No gross or histological lesions are observed. The levels of agglutinating antibody (anti-*Vibrio anguillarum*) remain the same in the 3 batches. There is no difference in values of hepatic lipids

nor in fatty acid composition, and MDA levels in liver. But, levels of MDA are superior in B fishes muscle and plasma B fishes ($P < 0.05$).

Experiment B (G. STEPHAN, 1988)

500 seabass (mean body weight : 3 g) are randomly divided into two 200 litres tanks. The temperature (18°C) is maintained constant throughout the experiment in an opened sea-water system. One group (T) is fed the basal diet with fresh oil added. The other one (Ox group) is fed the basal diet but with slightly oxidized oil. As in experiment A, no alteration of w3 total fatty acids was recorded. The batch T is maintained under alleviated light strength whenever the batch Ox is under constant artificial light.

Throughout this experiment, no mortality is noticeable. After 10 months, fish weight ranged from 30 to 50 g without any difference between the 2 batches. Fishes in batch Ox are darker than in batch T. No gross lesions are detected. Liver MDA values are increased at the end of the experiment, but similarly in the 2 batches (230 - 330 n moles TMP/mg fresh tissue). Conjugated dienes of perivisceral fat are significantly more abundant on batch Ox. On the other hand, gluthation peroxydase activity in plasma is decreased but there is no difference on erythrocytic enzymes (catalase, SOD). Liver concentrations of vitamin E remain similar in the 2 batches, but muscular values are significantly lowered on fish Ox.

Experiment C

This experiment is presently conducted in LPAA. Two batches (Ox) of seabass (90 and 190 g) are fed an oxidized diet, synthetized anti-oxydant -vitamin E - choline- deficient, poorly supplemented with vitamin C. Two control batches are fed corresponding regular food. Water temperature is around 20°C. After 11 weeks, a first sample does not show any difference in growth rate, biometrical data, haematological values hemolysis test, plasma transaminases and creatine-kinase, plasma glucose and total protein.

On the contrary, hepatic and muscular concentrations of vitamin E are dramatically lowered on fish not supplemented with vitamin E while muscular MDA level is increased.

1.3. Discussion

Most of the pathological signs described on fishes raised in the West Indies could not be reproduced in the laboratory. It is likely that experimental conditions were not drastic enough to test protection mechanisms.

In experiment A, fish size is rather important when fishes have to face high temperatures. They have probably got sufficient vitamin C and E stores. Body weight and body stores have been invoked by Cowey et *al.* (1981) and Lowell et *al.* (1984) as two important factors in the success of experimental reproduction of vitamin deficiencies. For Mocia et *al.* (1984), metabolic requirements of vitamin E and C on fish are highly dependent on their relative growth and the amount of body storage of vitamins. For them, differences on this particular point and also on

experiment duration (24 weeks instead of 16) can explain why they observed symptoms of vitamin E deficiency on rainbow trout while Cowey et *al.* (1981, 1983) could not. Nevertheless, at the end of our experiment A, hepatic and muscular levels of vitamin E on non supplement fish are down to 56 µg/g and 3.6 µg/g, respectively (from initial values 85 µg and 9.5 µg/g). These values are close to those detected on Atlantic salmon (45 and 1.4 µg) by Poston et *al.* (1976) and on carp (27 and 2.8 µg) by Watanabe et *al.* (1970), associated with clinical signs. Similarly, vitamin C levels in liver go down to 33 µg on non supplemented fishes, which approximate the 30 µg/g threshold value of deficiency defined by Saroglia and Scarano (1984) on sea bass.

Furthermore, concentrations of MDA (reflecting accumulation of degradation products of oxidized lipids) increase in plasma, and particularly in muscle of fishes not supplemented with vitamin E. If we had carried out the experiment on a longer period of time, it is possible that more important metabolic disorders would have occured, even inducing some clinical symptoms on the non supplemented fishes.

In experiment B, no clinical but biochemical disorders are detected on fishes fed an oxidized diet. On this batch, the level of hepatic vitamin E does not change a lot perhaps because of a still remaining sufficient amount of vitamin E and/or of other anti-oxydant products in food but the level of muscular vitamin E is significantly lower. An explanation could be a lesser transportation of vitamin E towards surrounding tissues. In fish indeed as in mammals, alphatocopherol is transported by low-density lipoproteins (Hung et *al.*, 1982). In other respects, *in vitro* assays showed the interaction of oxidized products upon the structure and the synthesis of lipoproteins. In the Blindness-Melanism Syndrome observed in the West Indies fishes, the retinal histopathology and the preventive effect of a vitamin E supplementation show some likeness to the human abetalipoproteinaemia (Raymond, 1988). Ox fishes are also characterized by a decrease of the glutathione peroxydase (GPX) activity. It could be related to a lesser bio-avaibility of selenium if in sea-bas as in catfish (Gatlin et *al.*, 1984) GPX exists in plasma only as a selenium dependent form. This decreased activity of the enzyme can lead to a lesser protection to food hydro-peroxides for the intestinal epithelial cells and explain the higher values of conjugated dienes in perivisceral fat.

In experiment C, the higher oxidation of lipids, the vitamin E deficiency and the lack of synthetic anti-oxidant in the food more rapidly induce the fall of both hepatic and muscular vitamin E. The increase in MDA level seems to be a first indication of the accumulation of decayed products of oxidized lipids.

II. GRANULOMATOUS HYPERTYROSINAEMIA (GH)

2.1. Observations

Tixerant et *al.* (1984) were the first to link a so called Granulomatous Syndrome observed on farmed turbots (*Scophthalmus maximus*) to a disorder in tysosine metabolism. Clinical signs of the disease are essentialy : 1) White yellowish or orange nodules, mostly on kidney but also on

the other viscera and muscle. 2) Subcutaneous white deposits, around the articulations or under the cornea, possibly hiding the pupil. It is also possible to remark cutaneous melanism, loss of weight, hepato-splenomegaly, adbominal dropsy and presence of urinary calculus. At microscopic examination, the white deposits show bushes of needle-shaped crystal, 30 - 40 µm long, located around the melanomacrophage centres or around the nodules. These crystals cannot be seen on histological sections (after paraffin embedding). However, two basic types of granulomatous nodules (GN) can be identified : 1) Solid homogenous GN 50 to 200 µm in diameter, constituted of elongated epithelioid cells concentrically arranged within a thin basal lamina. 2) Cystic or necrotic GN, larger than the previous type with a peripheral lamellar cell arrangement and a necrotic centre empty or containing cellular debris. An infra-red spectrometry allows to first identify L-tyrosine as the primary component of vesical calculus. Plasma and kidney analysis by ion exchange chromatography gives evidence of abnormally elevated levels of tyrosine. By its clinical aspects, the disease seems to be very similar to the nutrionally induced Granulomatous Condition in farmed turbot (Richards et *al.*, 1984) although the writers do not report any crystal in tissues. The Systemic Granuloma of sea-bream *Chrysophris aurata* (Paperna et *al.*, 1980) also appears to be related, since the presence of tyrosine is detectable in kidney and in urinary calculi. In other respects, hypertyrosinsemia was detected in *Charax puntazzo* (Messager, unpublished data) and, as above related, in the West Indies farmed *Ocyrurus chrysurus* and *Lutjanus analis.* More recently, examination made on sea bream from Turkey suggested this kind of pathological disorder (LPAA, unpublished data), and a presumptive evidence of the disease appeared in halibut *Hypoglossus hypoglossus* (Egidius, pers. com.).

2.2. Experimental approach

This work was made in order to identify, pathological processes and to bring solutions for its control. Considering routine catabolism of tyrosine in mammals, and hypertyrosinaemia process in humans, Messager, (1986) makes the hypothesis that the catabolism disorder of tyrosine on turbot could be related to vitamin C deficiency. This reductor agent is necessarily involved in the second pathway of tyrosine catabolism, to protect the parahydroxy-phenyl-dioxygenase, enzyme which catalyses the oxydation of p.HPP acid in homogentisic acid. To check this hypothesis, twenty four 130 g turbots exhibiting clinical signs of GH (subcorneal deposits, melanism, loss of weight) receive intraperitoneal injection : 0.2 ml of a solution of ascorbic acid for 14 of them (10 mg ascorbic acid per fish), or the same amount of saline for control fish. Tyrosinaemia is estimated by the fluorimetric method on blood samples before any injection and 7, 15, 30 days later. Seven days after ascorbic acid injection, 11 out of the 14 treated fish have got a normal tyrosinaemia (less than 60 µñ moles/l) which they keep until the 30th day. The injection of saline in control fishes does not affect tyrosinaemia which remains around 2000 - 3000 µ moles/l. A remarkable regression of corneal lesions is also observed on treated fishes. After 30 days, hepatic levels in vitamin C are the following : 2.3 µg/g on treated fishes.

A second experiment is conducted by Messager et *al.* (1986). Its purpose is to experimentally reproduce natural disease in feeding fish on ascorbic acid deficient diet. Five hundred 200 days old turbots are divided into two size groups S and T (average body weight, 12 and 18 g). Fishes of each group are distributed into five 150 l tanks in an open sea-water system. Water temperature is 18°C. Fishes are fed on a food supplemented or not in vitamin C. At day 155, noticeable hypertyrosinaemia is detected on fishes fed on non supplemented food only : 67 % on group S and 27 % on group T. Some fishes also show subcorneal or renal deposits of tyrosine crystals and/or microscopic granuloma in kidneys : 67 % on group S and 6 % on group T. Compared to control fishes (1500 mg ascorbic acid/Kg dry food) the non-supplemented groups exhibit significantly lower growth. In other respects supplemented diets with 750 mg/Kg of ascorbic acid or ascorbyl palmitate give results similar to control. On the other hand, diet supplemented with ground fish and 600 mg/Kg ascorbic acid gives a poor growth and some cases of hypertyrosinaemia.

2.3. Discussion

Results confirm that a vitamin C deficiency in turbot can be the cause of hypertyrosinaemia and of tissual tyrosine deposits. According to the lysosomial theory of Goldsmith (1978), these crystals are then able to induce local inflammatory granulomatous response. Nodules, such as observed in GH were also produced in turbot intraperitoneally injected with talc or BCG suspensions (Balouet et *al.*, 1986). A nutritional aetiology (but not the role of ascorbic acid) was already suspected by Paperna et *al.* (1979) for the Systemic Granuloma of sea bream. As a matter of fact disease was experimentally carried out on sea bream (Paperna et *al.*, 1984) and on turbot (Richards et *al.*, 1984) fed on an altered food. It was suggested that prolonged storage could be accused.

It is well known that ascorbic acid is one of the most unstable dietary components. Hilton et *al.* (1977) showed the importance of its destruction during the preparation and the storage of a fish food. According to these authors, incorporated water is the main cause of the ascorbic acid degradation. Messager et *al.* (1986) show that almost the total amount of incorporated ascorbic acid quickly disappears in humid pellets incorporating or not ground fish.

However, several factors may be implicated in the process of the disease. In the above related experiment, the characteristic macroscopic nodules of the natural disease are not observed. On the other hand, smaller fish appear to be more sensitive. Fish age seems to be determining : Systemic Granuloma could not be reproduced in sea bream older than one year (Paperna et *al.*, 1984). Water temperature and particularly food consumption appear to be also important (Messager, 1986). Indeed, enzymatic inhibition level and further, the quantity of non catabolized tyrosine and tyrosine deposits, are dependent on the amount of ingested tyrosine or phenyl-alanine. As a matter of fact, throughout the course of the disease, while the nutritional status is impairing, and in spite of the setting up of the lesions, hypertyrosinaemia can regress and disappear. Sometimes weakest and thinnest turbots show remaining kidney lesions attesting a previous GH, but exhibit a subnormal blood tyrosine level.

CONCLUSION

The two diseases observed in the West Indies show a few similarities (hepatocyte and muscular degeneration, retinal atrophy), which can partly be attributed to vitamin E and C deficiencies. In the two cases, the pathology was limited on the field by supplementing the food with vitamin E and C. It does not necessarily mean that nutritional factors can utterly explain the described symptoms and lesions. The neurotrop virus reported by Gallet de Saint Aurin et *al.* (1987) may already take part in the « Summer Mortality of seabass ». There is no doubt that environmental aetiology plays a role in retinal atrophy (Raymond, 1988).

The carried out experiments did not reproduce the clinical signs of these diseases, but probably because the experimental methods did not gather the bad field conditions together. However, biochemical results show the begining of a pathological process susceptible to go forward to the symptoms described in the field pathology.

The role of ascorbic acid in the tyrosine catabolism explains why the vitamin C deficiency is directly related to the determinism of the GH. The observed hypertyrosinaemia in the West Indies cultured fish is certainly the mark of such a deficiency. Stress increases utilization of ascorbic acid (Wedemeyer, 1969) and the severe summer conditions and/or pathological agents can act in such a way.

A number of authors have discussed mechanisms of vitamin E and C interactions and their importance as chain breaking antioxidants in the in vivo autoxidation of polyunsaturated lipids of cellular membranes (Tappel, 1968; Lambelet et *al.*, 1985).

Alpha-tocopherol is pretty resistant in a food suitably protected from oxidative process. In the opposite, the unstability of vitamin C in the presence of various environmental factors (light, temperature, humidity, pH) explains still frequent deficiencies. They are attested by GH in some fish species but they are also undoubtedly related to many other pathological phenomena.

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Virus diseases in temperate and tropical farmed fish : state of current knowledge

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Abstract — *It is over 30 years since the development of fish tissue cultures led to the first confirmation of a viral infection in fish (infectious pancreatic necrosis). Since that time, tissue cultures and, more usually, monolayer cell cultures, have been developed from a large number of fish species and have enabled the isolation from a large number of fish species and have enabled the isolation and study of 30 or so viral infections of fish, predominantly those of freshwater, catadromous and anadromous species, and particularly temperate species in the northern hemisphere. However, evidence for numerous other viral infections in fish has also been obtained without successful virus isolation through the visualisation of virus particles by electron microscopy or through experimental transmission of the disease using ultra-filtrates of infected tissues.*

The virus infections for which there is most information are those causing serious diseases in freshwater aquaculture rather than in mariculture, no doubt because of the much higher economic importance of the former, historically, in countries with the necessary laboratory resources for fish virology work. Some of the most sophisticated research techniques in medical and veterinary biotechnology, such as gen sequencing and cloning, recombinant vaccines and monoclonal antibodies are now being employed in the study of some of these fish viruses, especially those of farmed salmonids.

In stark contrast there is a paucity of knowledge on virus infections in cultured marine fish : most of the known viral diseases of marine fish have been found in wild rather than farmed stocks. Information on virus infections in fish farmed under tropical conditions is even more limited due to the relative lack of investigations so far. However, activity in this area will increase as fish farming in the tropics intensifies, serious outbreaks occur and the socio-economic importance of the industry increases thereby justifying the cost of adopting the same techniques which have given success in the study of virus infections in temperate fin fish species.

Example from the range of investigation techniques available are presented and some current approaches to control of virus diseases in fin fish are discussed.

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The "Office International des Epizooties" its role in improving awareness and prevention of international transfers of diseases in aquaculture

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Abstract — *The Office International des Epizooties (O.I.E.), is an international veterinary organisation (established in 1924) comprising 110 member countries worldwide. Its main objective is to promote awareness of serious animal disease problems associated with trade in live animals and of means for control and prevention. It acts as a central source of information on the occurrence and progress of epizootics of listed diseases and the methods being applied for their control in individual countries. This information is disseminated through a monthly bulletin and a yearly report on the animal health situation worldwide. The Animal Health Code lays down recommended procedures for health surveillance of animals for domestic and international trade.*

The O.I.E. has dealt with fish disease matters for many years through its Fish Diseases Commission (F.D.C.), which produces an annual report on the main developments regarding current diseases, new pathogens, diagnostic methods and control methods worldwide. The F.D.C. has produced a separate section on fish diseases in the Animal Health Code and has recently had its responsibilities enlarged to encompase diseases of molluscs and crustacea for which it is currently extending the Code. This will produce a model on which a standard health certification system for international trade in live aquatic animals might be based. In the meantime, disease specialists in aquaculture are encouraged to fully utilise the disease information collection and dissemination service of the O.I.E. : the more it is used the more effective it will be. To further assist discussion of the problems, an O.I.E. international meeting on disease transfer associated with international trade in live animals for aquaculture is being planned for 1991 in Paris.

FISH DISEASES SESSION
SUMMARY OF DISCUSSION

The discussion period did not focus on specific areas, but covered a wide range of concerns. The general lack of information on tropical fish diseases and the small number of known laboratories and researchers in the field was noted. The following were important points from the discussion :

1. — Specific positive steps to advance tropical fish disease studies

   A. — Develop a network (list) of researchers and laboratories with an interest in tropical fish diseases.

   B. — List known tropical fish diseases of importance. Include host and geographic range, effect on host, mortality rate, diagnosis and therapeutic methods.

   C. — Compile information on mortality rates and economic losses due to tropical fish diseases.

   D. — The specific technical information (specific diseases and their effects) will be manageable if compiled on a regional rather than global basis.

2. — General ideas to advance fish disease prevention

   A. — List steps which can be taken to reduce risks of infectious diseases.

   B. — List groups of pathogens which appear to have wide range of host specificity (e.g. rhabdoviruses).

A general problem which was recognized is that most of the expertise and facilities for fish disease research and control are in temperate rather than tropical regions. Publication and distribution of the proceedings of this workshop and taking the steps listed above will help to draw attention to the needs of tropical aquaculture.

II. PATHOLOGY

II.3. PATHOLOGY OF MOLLUSCS

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Status and future of molluscan pathology in North America

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Abstract — *Serious diseases have plagued the North American oyster industry for at least 75 years. These include Malpeque Bay disease, M.S.X. disease, perkinsiosis, nocardiosis, oyster velar virus disease and others. Some of these diseases have proven intractable since they are caused by infectious agents which have not yet been transmitted in the laboratory or which cannot yet be cultivated outside of the host animal. The increased importance of aquaculture of these species and the advent of modern molecular technologies in biology have provided both the impetus and the means to now advance the science of molluscan pathology.*

The relative importance of each molluscan disease in North America and specific objectives to enhance the management of the diseases will be discussed. For example, the potential and actual application of antibody technologies and cytofluorometry to diagnose clinical stages of hemic neoplasia in ***Mytilus*** *will be described. The use of molecular probes to detect the presence of infectious agents or the presence of abnormal host gene transcripts is another method which can potentially make health management more efficient.*

The development of resistant strains of molluscs is an important aspect of health management which should receive more attention. Specific criteria of resistance must be identified and, if possible, correlated with ***in vitro*** *measurements of functional aspects of immunity or with the presence of gene transcripts which are indicative of resistance. A priority list of recommended future research will be presented.*

Serious diseases have plagued the North American oyster industry for at least 75 years. These include Malpeque Bay disease, MSX disease, perkinsiosis, nocardiosis, oyster velar virus disease and others. Some of these diseases have proven intractable because they are caused by infectious agents which have not yet been transmitted in the laboratory or which cannot yet be cultivated outside of the host animal. The increased importance of aquaculture of molluscan species and the advent of modern molecular technologies in biology have provided both the impetus and the means to now advance the science of molluscan pathology. In the following treatment, the classic diseases affecting wild harvested populations of molluscs in North America are discussed as well as those diseases

which have been recently recognized as the intensive culture of molluscs is increasingly practised and advanced.

MALPEQUE BAY DISEASE

This is a widely known but poorly understood disease that caused severe mortalities in American oysters (*Crassostrea virginica*) in Malpeque Bay in the Canadian maritime province of Prince Edward Island starting in 1915 and continuing through the 1930s. The geographical expansion of the disease, first observed a year after substantial plantings of seed oysters imported from the United States, is considered evidence for an infectious cause of the disease. Over 90 % of original stocks were reported to have succumbed to the disease. The oysters affected by the disease reportedly show visceral shrinkage, a translucent quality, reduced growth, and failure to spawn. The cause of Malpeque Bay disease has never been determined with certainty.

MSX DISEASE OF THE AMERICAN OYSTER
(Crassostrea virginica)

This disease is caused by *Haplosporidium nelsoni (Minchinia nelsoni)* referred to as multinucleate sphere unknown (MSX) (Haskin et *al.*, 1966). The plasmodial form of the parasite invades all but the epithelial tissues of the oyster but apparently requires another host species (as yet unknown) in order to complete its life cycle. Sporulation is rarely observed in the epithelial tissues (Farley, 1965, 1967). MSX disease was first recognized in Delaware Bay in 1957. It destroyed the Delaware Bay industry with mortalities of oysters reaching 90 % to 95 % by 1960 (Andrews and Wood, 1967; Ford and Haskin, 1982). Resistance to the disease has occurred in some stocks of oysters subjected to continuing infection over the years (Haskin and Ford, 1982; Ford and Haskin, 1987). The disease has occurred in other locations in Atlantic North America and beginning in about 1980, a recurrence of the disease was observed in both Chesapeake and Delaware Bays, associated with a drought. Salinity and temperature are important in determining the severity of MSX disease (Ford, 1985). In general, the disease is rarely acquired below about 10 ppt (parts per thousand); salinities of about 15 ppt are required for the parasite to appear in substantial numbers in host tissues and serious mortalities occur only above about 20 ppt.

There is some indication that the disease may be limited above a salinity of 30 ppt. Diagnosis is based on histological examination or on the observation of parasites in preparations of oyster blood.

PERKINSIOSIS OF THE AMERICAN OYSTER
(Crassostrea virginica)

This disease is caused by *Perkinsus marinus* (Apicomplexa) (*Dermocystidium marinum, Labyrinthomyxa marina*) that infects almost all tissues

of the oyster (Mackin et *al.*, 1950; Levine, 1978). Transmission occurs by direct contact in water or by the gastropod vector, *Boonea impressa*, (White et *al.*, 1987) but is limited by the parasite's inability to tolerate low salinities and low temperatures (Andrews, 1966). The disease occurs during high temperature months of the year and is more severe in highly concentrated populations of oysters. Mortalities can reach 100 % and have been reported to be 30-50 % in the first year and up to 95 % in the second year in oysters introduced to an infected area. The disease does not cause serious mortalities below salinities of 12 to 15 ppt but can persist in overwintering oysters in salinities below 5 ppt. The disease has had a resurgence in the last 5 years in the Chesapeake Bay. Diagnosis is facilitated by the use of the fluid thioglycollate method which allows spores to enlarge vegetatively in anaerobic conditions (Ray, 1966), and subsequently be manipulated to undergo zoosporulation (Perkins and Menzel, 1966).

SEASIDE HAPLOSPORIDIOSIS OF AMERICAN OYSTERS
(Crassostrea virginica)

This disease is caused by *Haplosporidium costale (Minchinia costalis)*. In 1959 it was originally described as seaside organism (with the acronym SSO) because of its occurrence in relatively high salinity waters on the seaside coast of Virginia and Maryland in contrast to *Haplosporidium nelsoni* (causing MSX disease) which is found in more inland waters such as Chesapeake Bay (Andrews, 1982). The disease caused three years of serious mortalities from 1959 to 1961 but has not been as severe and recurrent a problem as MSX (Andrews et *al.*, 1962; Andrews and Castagna, 1978). Annual mortality rates can reach 50 % in seaside bays of Virginia. The parasite infects all tissues of the oysters except the epithelium and causes substantial synchronous mortalities when sporulation occurs. Diagnosis is based on histological examination.

BONAMIASIS OF THE EUROPEAN FLAT OYSTER
(Ostrea edulis)

This disease, caused by *Bonamia ostreae*, infects blood cells of the oyster. Serious mortalities occur in newly infected populations. The disease is best known for its substantial impact on the European industry, particularly in France, where it was first identified in 1979. It is transmitted by water contact but close proximity to infected oysters is believed to be necessary. The disease occurred in flat oysters in California in the 1960s, but was then known as « microcell disease » (Katkansky et *al.*, 1969; Katkansky and Warner, 1974). The disease spread from a California hatchery to Brittany, France, where it initiated the well known epizootic (Pichot et *al.*, 1980). Bonamiasis was transplanted to Washington state from the California hatchery in the late 1970s and remains an important disease in the Pacific Northwest (Elston et *al.*, 1986). Some degree of resistance to the disease has occurred in North American stocks of oysters which have had long-term exposure (Elston et *al.*, 1987a; Holsinger, 1988).

Studies in Washington state showed a 20 %, 7 % and 4 % mortality rate, respectively, for 2-, 3- and 4-years- old infected oysters. The effects of the disease are mitigated in off-bottom culture. Bonamiasis can cause significant mortalities between 12° and 20°C but not at higher temperatures. A related *Bonamia* occurs in New Zealand. The possibility must be considered that *Bonamia* represents a cosmopolitan parasite of another host and that multiple infections have taken place in flat oysters around the world. Diagnosis is performed by an immunodiagnostic assay developed by the French or by histological analysis (Bucke and Feist, 1985).

VELAR VIRUS DISEASE OF PACIFIC OYSTERS
(Crassostrea gigas)

Oyster velar virus disease (OVVD) is known only as a hatchery disease, and is caused by an iridovirus (Elston and Wilkinson, 1985). It infects the epithelium of the velum, mouth, esophagus and, rarely, the mantle of the larvae. The disease has only been reported from Washington state. Since there has been extensive commerce of this oyster historically, it is likely that the disease is much more widespread than is presently known. Larvae of the Pacific oyster, *Crassostrea gigas*, are the only species and life stage known to be infected by the disease. Some similar viruses have been observed in adult Pacific and Portuguese oysters in France but their relationship to OVVD has not been determined. Oyster velar virus disease can cause nearly 100 % mortality in affected hatchery tanks. The disease typically appears in the March to May time period, but it has also been reported throughout the summer. Observations of mortalities in the spring, always in larvae greater than 150 µm in shell length and at least 10 days post-spawning, when grown in the 25 to 30°C temperature range are suggestive of OVVD. Virus infected cells on the velum of sick larvae detach and form the characteristic « blisters ». Deciliation of the velum also occurs but it should be noted that loss of cilia can result from other causes. Presumptive diagnosis can be made histologically by observation of the intracytoplasmic inclusion bodies.

DENMAN ISLAND DISEASE OF THE PACIFIC OYSTER
(Crassostrea gigas)

Denman Island Disease, also referred to as « microcell » disease is a little studied but apparently important disease of the Pacific oyster (Quayle, 1961, 1982). The term « microcell » has also been used to refer to bonamiasis (caused by *Bonamia ostreae*) of the European flat oyster. Since the diseases and their causative microorganisms are unrelated, the term microcell should be abandoned to avoid further confusion between these two diseases. The causative agent of Denman Island disease infects the glycogen storage cells of the oyster and is now known as *Mikrocytos mackini* (Farley et *al.*, in press). The disease was first reported from Pacific oysters, *Crassostrea gigas*, from Denman Island in British Columbia in 1960. Since then it has been noted at other sites in the Strait of Georgia

in British Columbia. Mortality rates of up to 53 % in a single season have been reported but the severity fluctuates from year to year. Infection and loss to the disease increased at lower tide levels when oyster mortality was monitored at the 4.0, 2.5 and 1.0 foot levels (Bower, in press). The disease is characterized by the appearance of round, yellow to green lesions or pustules (1 to 3 mm in diameter) occurring on the body surface. Similar lesions occur in several other oyster diseases.

PACIFIC OYSTER *(Crassostrea gigas)* NOCARDIOSIS

Nocardiosis is a disease of the Pacific oyster, *Crassostrea gigas*. The causative agent was recently isolated and identified as a member of the genus *Nocardia* (Friedman et *al.*, 1987). Previously the disease has been known as « fatal inflammatory bacteremia », « focal necrosis », and « multiple abscesses » (Glude, 1974; Elston et *al.*, 1987b). The disease causes typical small, round yellow lesions (which are, in fact, abscesses) on the body surfaces of the oysters, often observed on gaping individuals. « Multiple abscesses » described from Matsushima Bay, Japan, appears to be the same as nocardiosis (Imai et *al.*, 1965, 1968). On the west coast of North America, the disease has been reported from sites in California to British Columbia. The Pacific oyster, *Crassostrea gigas*, is the principal oyster affected by the disease, although a few specimens of the European flat oyster, *Ostrea edulis*, cultivated near areas of infected Pacific oysters have been reported to have a similar disease. The severity of the disease in individual oysters and the high prevalence in some populations suggest that it is an important mortality factor in some cases. In one study it was reported to occur in about 30 % of oysters sampled from Washington sites during September and October. The disease is a summer and fall phenomenon, typically observed from August through November.

HEMIC NEOPLASIA OF VARIOUS SPECIES OF BIVALVE MOLLUSCS

Hemic or hemocytic neoplasia (HCN) is also referred to as hemic proliferative disease, leukocytic neoplasia, sarcomatous neoplasia, sarcomatoid proliferative disorder, disseminated sarcoma and atypical hemocyte condition. Research on *Mya arenaria* (the soft shell clam) has suggested that the disease is caused by a retrovirus, but this is not yet confirmed in other species (Oprandy et *al.*, 1981). The disease is transplantable with whole cells and transmissible with cell free homogenate, in some species, at least (Elston et *al.* 1988a; Twomey and Mulcahy 1988). The disease affects many species throughout the world (Peters, 1988). The species of commercial importance which are affected in North America are *Crassostrea virginica, Mya arenaria, Mytilus edulis, Ostrea lurida* (Farley, 1969a, b; Frierman and Andrews, 1976; Elston et *al.*, 1988b). Dense farmed populations appear to be 100 % infected if individual animals are monitored over several months. Mortality rates due to the disease are

reported to approach 100 % over an annual period in some species, but these high mortality rates have not been associated with the disease in natural populations. Typically, the disease is reported to have highest prevalence and intensity during fall and winter months. The prevalence drops in the spring and summer period, possibly because heavily infected individuals die in the winter and the disease does not start another cycle of increasing infection until autumn (Mix 1983). Diagnosis can be made by microscopic examination of blood for the enlarged, transformed cells or by histological examination of tissues. Mitotic figures are common among the affected cells in advanced cases of the disease. Recent research has demonstrated a repeatable pattern in the formation of aneuploid DNA content of the transformed cells. In some cases affected individuals fail to produce mature reproductive follicles.

VIBRIOSIS OF LARVAL AND JUVENILE MOLLUSCS

Vibriosis is an opportunistic disease of the larval stage of many, if not all, bivalve molluscs and is also known to affect juvenile stages of the red abalone, *Haliotis rufescens*, in North American abalone operations (Elston, 1984). In a properly managed hatchery there should be only minimal loss due to the disease. However, the disease has been associated with some significant mortalities (Elston et *al.*, 1981). Three general sources of bacterial contamination and expansion exist in hatcheries : (1) from brood stock, (2) from algal cultures and (3) from incoming seawater or the seawater system. The disease probably results from a variety of species of *Vibrio*, the most notable of which is the recently described *Vibrio tubiashi* (Hada et *al.*, 1984). It is likely that strains which have not received species designations are important in the disease as well.

HINGE LIGAMENT DISEASE OF JUVENILE BIVALVE MOLLUSCS

This disease causes erosion or destruction of the ligament that binds the two valves of bivalve molluscs together (Dungan, 1987; Dungan and Elston, 1988). Myxobacteria or « gliding bacteria », the causative agents of the disease, are known to have the ability to decompose many highly organized and complex biological structures made of protein such as the hinge ligament of bivalve molluscs (Dungan et *al.*, in press). Infections with such bacteria are often found within the ligaments of juvenile clams, oysters or scallops which are dying in nursery areas. Once the ligament is destroyed, the mollusc is unable to open its valves for feeding and respiration. In addition, it is possible that the destruction of the ligament allows other bacteria to infect the tissues of the animals. Juvenile bivalves from the east and west coasts of North America have been examined and found to have the disease. It has been found in the following species : Pacific oyster (*Crassostrea gigas*), American oyster (*Crassostrea virginica*), European flat oyster (*Ostrea edulis*), hard clam (*Mercenaria mercenaria*), Japanese littleneck clam (*Tapes philippinarum*), Pacific razor clams (*Siliqua patula*) and bay scallops (*Argopecten irradians*). In many cases, aquaculturists have reported the complete loss of large groups of clams and oysters

from this disease. Usually the bivalves affected by the disease are from settlement size to 1 cm in shell height. The smaller animals are probably more susceptible. No typical seasonal cycle of the disease has been determined. It can occur year-around possibly because juvenile molluscs are usually grown in a controlled environment, often with heated water. Research on the disease has shown that the hinge ligaments are degraded at an increasing rate as the water temperature increases over the range from 5°C to 20°C and that the normally hard ligament, when infected with the gliding bacteria, can become jelly-like at water temperatures as low as 10°C. The disease may be controlled by disinfection of the outer surface of the bivalves. The most effective disinfectant has been sodium hypochlorite. While treatments may have to be adjusted to meet individual circumstances, a suggested starting point is 25 parts per million sodium hypochlorite dip for three minutes daily.

OTHER DISEASES

There are many other reported diseases of bivalve molluscs in North America which are of lesser significance or are not extensively studied. As the field of molluscan pathology develops, some of these diseases will likely receive more attention from the research community.

APPLICATION OF NEW TECHNOLOGIES

There is clearly an opportunity to apply new technologies in order to advance the science of molluscan pathology and health management in mollusc husbandry. More basic research is needed to understand physiological and pathological processes in these animals. The need for invertebrate tissue culture is as great as ever for the study of obligate intracellular parasites and viruses. Molecular biological tools offer an opportunity to understand certain basic mechanisms in disease progression and to identify infectious agents. For example, use of gene probes in the study of bivalve hemic neoplasia may help reveal the relationship of this disease to other cancers and the potential presence of gene transcripts originating from an integrated viral genome. Use of monoclonal antibodies has potential for both fundamental and applied benefits. These probes can be used to identify epitopic relationships between cell and tissue types and thus indicate ontogenetic and pathogenic relationships of tissues. Monoclonal antibodies have recently been used to develop a diagnostic kit for the detection of *Bonamia ostreae* in commercial stocks of oysters (Mialhe et *al.*, 1988a). Conceivably within the foreseeable future, it will be possible to transfer genes for specific traits into invertebrate animals. Research to achieve this objective should receive priority for the long term development of the invertebrate aquaculture industry. Already, on a technologically less complex plane, triploid oysters are being produced on a commercial scale and providing an improved product.

NEEDS IN MANAGEMENT OF HEALTH IN MOLLUSC HUSBANDRY

In addition to the need to utilize new technologies, there are several other important needs for the advancement of the applied side of this science. One important area is to develop accurate quantitative data on mortality rates and growth loss due to each disease. Surprisingly, little of this type of information is available. Without such information, the importance of some diseases is overstated while that of others may be understated. Perhaps even more importantly, such quantitative information should be available so that diseases can be prioritized by their economic impact and this information used to allocate research funds.

Another area is the formation of long term research programmes aimed toward the development of disease-resistant stocks of molluscs. Technologies for gene transfer, should they develop for invertebrates in the near future, may help shortcut this process. Nonetheless, we need to begin to identify desirable traits and combinations of traits in molluscs in order to understand how these affect the overall performance of the animals.

The science of molluscan pathology is on the threshold of remarkable new advances. The increasing numbers of investigators in the field and the use of new technologies are signs of the progress the field is beginning to experience. These advances will increase our understanding of fundamental biological processes in these animals, with potential application to pathological processes in higher animals, as well as increase our ability to manage the health of husbanded molluscs.

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Parasites and diseases of commercially important molluscs in New Zealand

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Abstract — *Mass mortalities among flat oysters (**Tiostrea lutaria**) in Foveaux Strait, southern New Zealand, between early 1986 and the present, are primarily associated with a haplosporidan, **Bonamia** sp.. Highest infection rates occur in the densest beds, with more than 80 % mortality in these areas, and movement of high density/high infection foci over c.10 km in 3 years. In some oysters large numbers of coccidian merozoites may also be a cause of mortality. Rickettsial-like organisms, microsporidans, bucephalid sporocysts and neoplasis also contribute to the poor health of these stocks.*

__Bonamia__ has also caused 40 % - 60 % mortalities in two or three stocks of __O. lutaria__ held on a mussel farm 800 km north east of Foveaux Strait. A third, slow-growing, local stock held among heavily infected oysters experienced no mortalities. Future studies on this stock, and options for management of the Foveaux Strait fishery are discussed.

Mass winter mortalities have been reported among native rock oysters, __Saccostrea glomerata__, until recently the basis of oyster farming. Investigations by Jones (pers. com.) found gaping in these oysters was associated with an idiopathic adductor muscle necrosis with pustular areas adjacent to brown rubber-like blisters on the shell. Similar blisters occur in the shell of the currently farmed __Crassostrea gigas__ and may be associated with ectosymbionts. Shell disease, of probable fungal aetiology, occurs in __Haliotis iris__, but other than intranuclear inclusions in haemocytes, disease is not known from this species.

Mortalities and disease have not been reported in the intensively-farmed green-lipped mussel (__Perna canaliculus__) or blue mussel (__Mytilus edulis aoteanus__), but digenean sporocysts are common in these species.

All these bivalve species may contain the copepod, __Pseudomyicola spinosus__, in the gut, but pathogenicity has not been demonstrated. Pea-crabs are also common as ectosymbionts, but in __Perna__ the gregarine __Nematopsis__ cycles through the mussel and crab. The general health of the stocks is discussed.

INTRODUCTION

There have been relatively few studies on the parasites and diseases of molluscs in New Zealand. Several mass mortalities, particularly of

bivalves, have been reported, but sparseness of population and the terrain have made it difficult to obtain adequately fixed material for diagnostic studies. Commercially dredged or farmed species are more readily obtainable and their diseases and parasites are considered here.

OYSTERS

Tiostrea lutaria

This species forms the basis of a dredge fishery at the south end of the South Island in Foveaux Strait. Normally 23 boats dredge < c. 138 x 106 oysters/year from autumn (1 March) to late winter (31 August). The fishery has experienced mass mortalities about every 20 years, but the cause of most of these events is unknown. However, mortalities in 1962-64 were attributed to *Bucephalus* sporocysts and cercariae heavily infecting the oysters (Howell, 1963). Two small fishes (*Acanthoclinus quadridactylus* and *Tripterygion* sp.) have experimentally been shown to act as second intermediate hosts, and *Scorpaena cardinalis* and *Kathetostoma giganteum* as definitive hosts (Howell, 1966). Infection of oysters occurs in summer and development occurs at the expense of the gonad, with subsequent parasitic castration and death (Howell, 1967).

Tab. 1. — Incidence (% infection) of *Bonamia* at 8 stations in Foveaux Strait. N = northern, S = southern, W = western, E = eastern

Station	Beds	Month/year 9/86	1/87	6/87	8/87	11/87	4/88	12/88
13	E	2	3	12	20	6	4	2
14	E	4	4	18	38	8	30	8
17	S	0	0	4	4	4	8	4
19	E	4	6	10	22	12	61	4
21	N	0	0	5	0	4	4	2
27	S	0	0	12	28	2	8	0
36	W	–	35	32	28	18	18	6
38	W	–	35	18	32	14	16	6

Mass mortalities in autumn 1986 were attributed to a haplosporidan, ultrastructurally (Dinamani et *al.*, 1987) and serotypically (Mialhe et *al.*, 1988) very similar to, but distinct from, *Bonamia ostreae*, a very pathogenic species in Ostrea edulis in Europe (Pichot et *al.*, 1979) and North America (Elston et *al.*, 1986). *T. lutaria* sampled in winter contain a « lighter » form under surface epithelia and in blood spaces, but by late winter this becomes larger and irregular (Fig. 1), with many mitoses, leading to dense forms in gonads and deeper Leydig tissue in summer (Dinamani et *al.*, 1987). Change in distribution may reflect movement of infected haemocytes and egress may occur in shed gonad products, through digestive diverticulae or through the gills (Dinamani et *al.*, 1987). Heavy infections and mortalities occur in autumn (April/May). Infection « hot spots » have moved c. 10 km down-current in 3 years, but both incidence and intensity of infection appear to be declining (Table 1).

Autumnal mortalities may also be attributable to merozoites of an apicomplexan (Fig. 2). The parasite initially radiates out of the ventral « blood sinus », between Leydig cells, in a stellate configuration. After phagocytosis by haemocytes the merozoites divide (Fig. 3) until the cell ruptures causing a responding haemocytosis, destruction of Leydig tissue and tissue loss (Fig. 4) and its replacement by amorphous eosinophilic and fibrous tissue. The parasite infects 80-100 % of oysters, is associated with tissue damage in 10-25 % of these, and numbers build up over summer, almost certainly causing mortalities in autumn. Ultrastructurally the merozoite has a typical apical complex, c. 84 sub-pellicular microtubules, and division is by *Semiopen pleuromitosis* (R. Entzeroth : pers. com.). With decline in *Bonamia* and increase in the apicomplexan, the latter currently appears to be the major pathogen in the oysters. *Bucephalus* occurred in < 4 % of oysters in 1986-88 samples, compared with 18-47 % in 1963-64 (Howell, 1967), and cannot be considered a major contributor to recent mortalities.

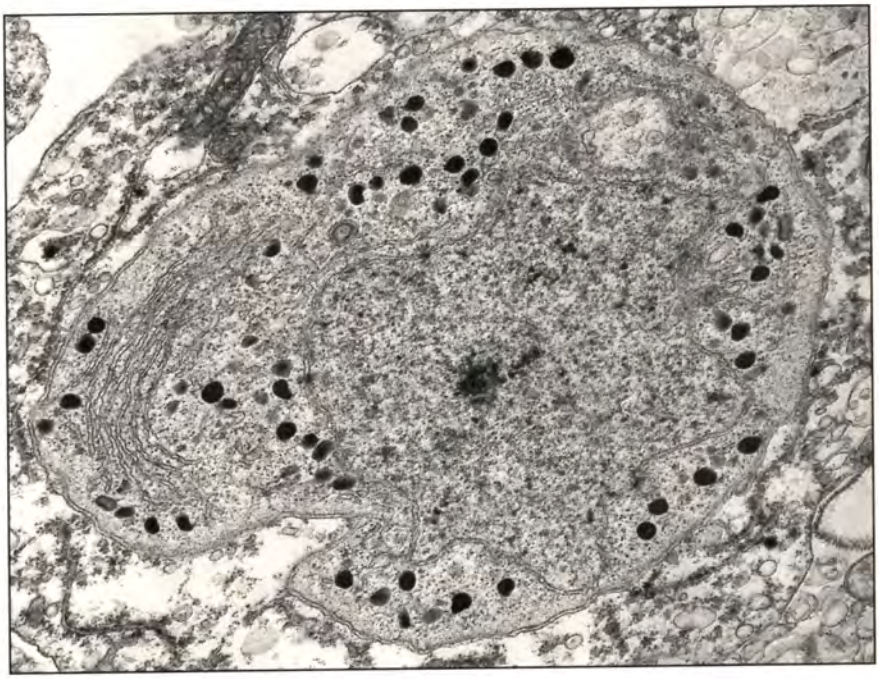

Figure 1. — Irregular plasmodial form of *Bonamia*, 5.5µm dia., observed in early spring (September).

Other parasites are unlikely to have contributed directly to mortalities. A microsporidan, *Microsporidium rapuae*, (Jones, 1981), occurred throughout the year in 10-40 % of stations sampled in 1986-88. Pre-spore and spore stages occurred in Leydig tissue around the stomach. Infiltrating haemocytes were occasionally observed phagocytosing spores from ruptured cysts, leaving a sheath of haemocytes and fibrous tissue around the

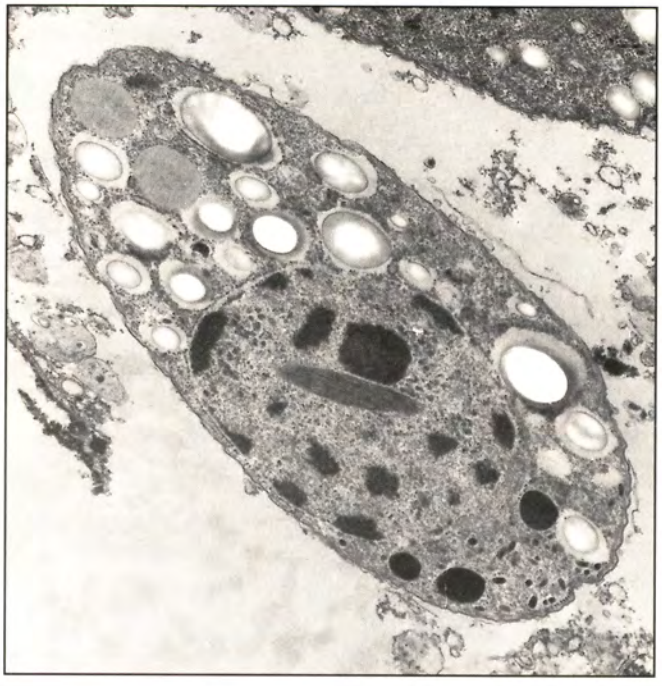

Figure 2. — Merozoite of the apicomplexan, 6.5μm long. Note intranuclear fibrils.

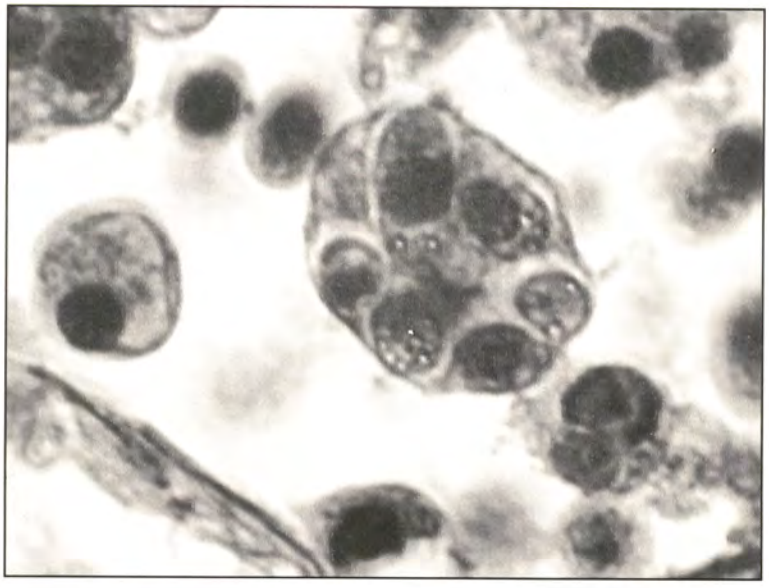

Figure 3. — Haemocyte replete with apicomplexans.

stomach. Oval or reniform bodies containing rickettsia-like organisms were observed intermittently among the epithelial cells of digestive diverticulae in spring and summer samples (November-February), but were absent from oysters in winter (June) samples. Rarely, thick-walled spores of an unidentified species have been observed among the digestive diverticulae (Fig. 5). The life-cycles of the apicomplexan, *M. rapuae* and the rickettsia-like organisms are unknown.

In general the Foveaux Strait stocks of oysters are in poor health, but bad weather over 1986-1988 probably contributed to general loss of condition. With the exception of an outbreak of bonamiasis in an isolated bay at Port Underwood, at the north of the South Island, in winter 1988, *Bonamia* is only known from Foveaux Strait. Oysters in other areas occasionally have low levels of the apicomplexan, *M. rapuae* or the rickettsia, but disease is not apparent.

Saccostrea glomerata

Mass mortalities (< 100 %) among rock oysters were noted in 1971 and named « winter mortalities » because of similarities to mortalities in Australia (Jones, 1975a), although mortalities occurred in spring (August-October). Examination for parasites failed to identify the cause of disease. However, it was noted the adductor muscle contained « yellow necrotic pustules » that extended onto the mantle, and the inner surface of the shell had « brown rubber-like warts and spots ». Histologically the adductor muscle showed a gradual reduction in muscle fibres and an extensive haemocytosis (Jones, 1975a). The cause and current status of the disease are unknown.

Rock oysters are parasitized by the copepod *Pseudomyicola spinosus* which causes gut epithelia to change from columnar to low-squamous type, but the overall effect on the host is unknown (Dinamani and Gordon, 1974).

Crassostrea gigas

Disease surveys of Pacific oysters have shown the presence of *P. spinosus*, turbellarians, chironomids, nematodes, mudworms and pea-crabs which, with the exception of *P. spinosus*, are non-pathogenic ectocommensals (Dinamani, 1986).

ABALONE *(Paua)*

Haliotis sp.

Intranuclear inclusions are rarely observed in the haemocytes of *H. iris*, and « blisters » are occasionally reported on the foot of *H. iris*. Shell disease under and around the adductor muscle, of probable fungal aetiology, occurs intermittently. Otherwise disease is unknown.

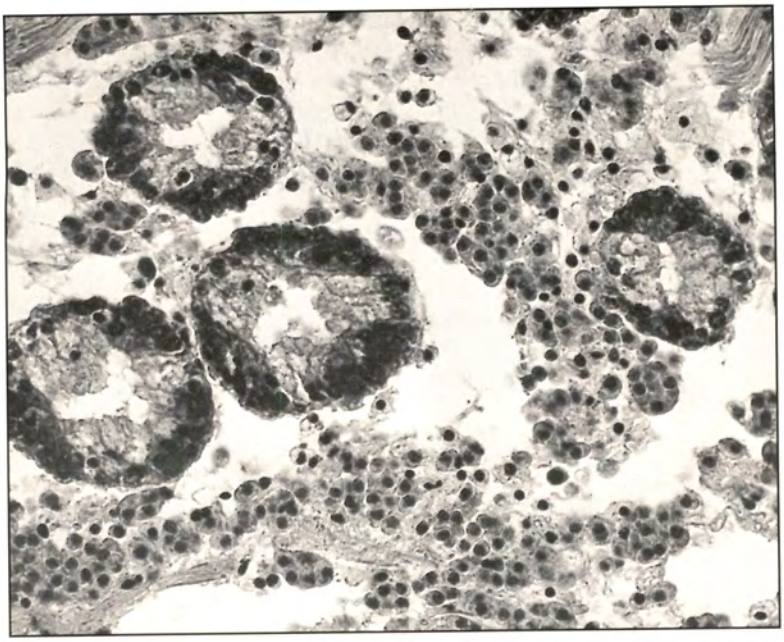

Figure 4. — Apicomplexans among digestive diverticulae. Note loss of ground substance.

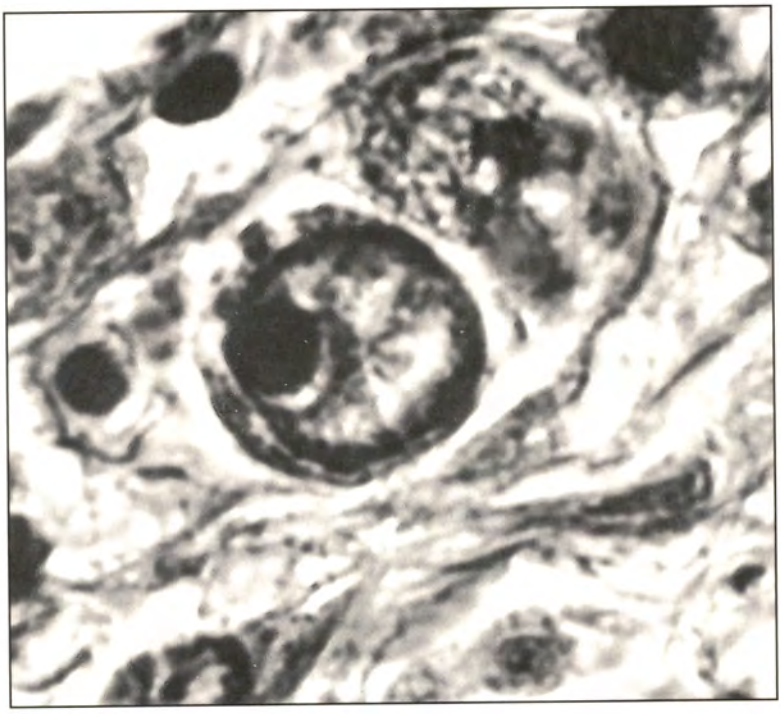

Figure 5. — Thick-walled spore (11.5µm dia.) with eccentric nucleus.

MUSSELS

Perna canaliculus

The green-lipped mussel forms the basis of a growing industry which produced 10500 tonnes of mussels in 1985. There have been no reports of disease-associated mortalities in aquaculture systems. Examination of wild and cultured *Perna* has revealed cercariae and sporocysts of *Tergestia agnostomi*, which uses mullet (*Aldrichetta forsteri*) as definitive host, a weakly parasitic copepod (*Lichomolgus*) on the gills, and *P. spinosus* infections (Jones, 1975a, 1978). A gregarine, *Nematopsis*, infects labial palps (Jones, 1975b) with transmission by its other host, the pea-crab (*Pinnotheres*) (Jones : pers. com.).

Mytilus edulis

Blue mussels are not favoured for aquaculture and are only known to harbour *P. spinosus*, *Tergestia* and pea-crabs (Jones, 1975a).

SCALLOPS

Pecten novaezelandiae

Scallops are reared for enhancement of the wild fishery. Although wild stock undergo large fluctuations in population, no diseases have yet been identified in this species.

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Diseases of cultured molluscs in Australia

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Abstract — *Three mollusc species are cultured commercially in Australian tropical and warm temperate waters : the Sydney rock oyster* ***Sacccostrea commercialis****, the pearl rock oyster is susceptible to two protozoans : Marteilia sydneyi, which is the cause QX Disease and infects oysters during the summer; and* ***Mikrocytos roughleyi*** *which is associated with winter mortality. It also harbours mudworm,* ***Polydora websteri*** *a parasite farmers try to avoid by growing oysters on racks. Transported pearl oysters* ***Pinctada maxima*** *die with heavy infections of* ***Vibrio harveyi****. Cultured giant clams* ***Tridacna gigas*** *are pestered by pyramidellid snails and some carry* ***Perkinsus*** *sp., a protozoan common in reef bivalves. Deaths in mollusc hatcheries have been associated with* ***Vibrio tubiashi****,* ***Vibrio*** *spp., and* ***Alteromonas*** *spp.*

INTRODUCTION

The value of cultured molluscs in Australia in 1987/88 was A$104 million p.a., equivalent to 91,300 million CPF or US$83 million. This included A$65 million CPF from the pearl oyster industry, A$30 million from the sale of Sydney rock oysters (*Saccostrea commercialis*) and A$8 million from Pacific oysters (*Crassostrea gigas*). Smaller but developing industries are based on the culture of mussels *Mytilis edulis* and mud oysters *Ostrea angasi* (Victoria), abalone *Haliotis ruber* (Tasmania), blacklip oysters *Crassostrea echinata*, milky oysters *C. amasa* and giant clams *Tridacna gigas* (Queensland).

Bivalve culture throughout the world has been plagued by epizootics, the most severe of which have been those caused by Protozoa. Australia is no exception. These and other diseases known from Australian molluscs are described below.

PROTOZOA QX DISEASE

Aetiology. The disease is caused by *Marteilia sydneyi* Perkins and Wolf, 1976, a protozan in the phylum Ascetospora.

Pathogenesis and epizootiology. The parasite multiplies within the digestive gland of *Saccostrea commercialis*, the Sydney rock oyster. First signs usually appear within a few days of a « fresh », i.e. a sudden drop in salinity (Lester, 1986a). Some oysters will die. Histological sections of survivors show foci of inflammation in the Leidig tissue around individual tubules. The parasite is presumably within these masses of haemocytes. Within 3 weeks parasites move to the cytoplasm of epithelial cells lining the tubule and begin to develop into sporangia.

In the summer oysters die about 6 weeks after infection. Some of those infected in early fall will survive through the winter. Oysters in the laboratory with light infections apparently shed all their parasites via the digestive tract and recovered (Roubal et *al.*, in press). Recovery from infection has not yet been observed in the field.

The parasite causes epizootics in estuarites in southern Queensland and northern New South Wales, down to the Mcleay River (Nell and Smith, 1988).

Clinical signs and lesions. When the digestive gland is cut open, its interior is pale yellow-brown rather than the deep green of healthy oysters. This is not a diagnostic test, however, as other conditions will also stop an oyster from feeding. In the winter, oysters with chronic infections are largely devoid of white gonad tissue; they appear watery and have an enlarged pale brown digestive gland. In the summer, the « fatness » or amount of gonad present is not a reliable guide as oysters close to spawning often become infected and may carry extremely high parasite loads beneath a full gonad.

Diagnosis. Confirmation of infection is based on the demonstration of sporonts. These are easily seen in wet smears of digestive gland examined under the high power of a microscope; the refractile granules show up particularly well. Sporangia and sporonts are also readily visible in the tuble epithelium of fixed tissues sectioned and stained with H. and E..

Treatment. No treatment is known.

Prevention. Oyster farmers in the endemic area try to avoid having oysters on leases in estuarine waters during the summer months, specifically from the beginning of January to the second week of April. Oysters in high salinity areas, though they grow less well, remain free of infection. The life cycle of *M. sydneyi* is not known. It probably requires an intermediate host (Roubal et *al.*, in press).

WINTER MORTALITY

Aetiology. This disease is believed to be caused by a minute protozoan *Mikrocytos roughleyi* Farley, Wolf and Elston, 1988, related to the oyster pathogens, *M. mackini* from North America, and *Bonamia* spp. from Europe and New Zealand. Their taxonomic position in the subkingdom Protozoa is unclear.

Pathogenesis and epizootiology. Parasites develop in the cytoplasm of haemocytes. The disease is reported only from S. commercialis from the George's River and adjacent estuaries of high salinity in New South Wales. It thrives in high salinity waters (30 to 35 °/oo), has an incubation period of 2.5 months and mortality does not occur in animals less than 3 years old.

Perkinsus olseni has been reported from four species of abalone (*Haliotis* spp.) from South Australia (Lester, Goggin and Sewell, 1988). The large trophozoites are typically extra-cellular and circulate in the haemolymph (Lester and Davis, 1981). In recovering abalone they are trapped and killed in tissue abscesses. The parasite is believed to be responsible for the die-back of greenlip abalone *Haliotis laevigata* off Stansbury, South Australia (Lester, 1986b).

Unknown *Perkinsus* species are wide-spread in bivalves on the Great Barrier Reef (Goggin and Lester, 1987) and have been linked to mortalities in giant clams. It seems more prevalent in the winter.

Treatment. In abalone, stress from high temperature seems to aid the spread of the parasite through the tissues (Lester and Davis, 1981). Removal of any source of stress on cultured animals may help to alleviate mortalities attributed to this parasite.

Prevention. Use filtered sea water. Avoid introducing infected molluscs.

COCCIDIA

An unidentified protistan resembling a coccidian was found in the ovary of blacklipped oysters *C. echinata* from Darwin harbour, Northern Territory (Wolf, 1977). The intracellular parasite was within the developing and mature ova of over half the female oysters examined. It was not detected in males.

SPHERICAL BODIES

Wolf (1978) reported spherical bodies 2 to 3 ñm in diameter in the digestive epithelium of moribund pearl oysters, *Pinctada maxima* from tropical Australia. Pass and Perkins (1985) concluded that they were probably normal constituents of the digestive cells.

METAZOA MUDWORM DISEASE

Aetiology. The disease is caused by four species of polychaete worms, of which *Polydora websteri* is thought to be the most damaging (Nell and Smith, 1988). It parasitizes *S. commercialis, C. gigas, Mytilus edulis* and *Ostrea angasi.*

Pathogenesis and epidemiology. Young worms creep under the mantle of the bivalve and develop a « U » shaped burrow from which they strectch

out to collect the detritus on which they feed. The mollusc tries to wall them off, together with the mud waste, by laying down shell over the burrow. In *S. commercialis* a large thin-walled « mud blister » eventually forms on the inside of the shell. Young oysters and heavily infected older oysters die. Mudworm has been blamed for the disappearance of the dredge oyster industry from Moreton Bay (Smith, 1982). Today, low levels of infection are a nuisance to those shucking oysters because blisters are easily broken and valuable time is lost washing mud off the meat.

Clinical signs. In oysters a dark blister 10 mm or more across is found on the inside of the shell. The mantle adjacent to the blister is often yellow and necrotic. Infected oysters are more likely to die from high temperature, low salinity, or storage out of water than uninfected oysters (Nell and Smith, 1988).

Diagnosis. The U shaped burrow is usually visible at least near the edge of the shell. The openings may be at the edge or, if the animal has grown since infection, may open to the outside some distance from the shell margin.

Treatment. Several crude methods have been used, e.g. oysters kept out of water in shade for 10 to 14 days; oysters dipped in dilute solution of phenol and detergent for 1 hr then left out of water for 1 day (Nell and Smith, 1988).

Prevention. To avoid mudworm disease, oysters are grown on racks so that they dry out each day. Under these conditions the larval worms are apparently not able to gain entrance.

TURBELLARIA

A large polyclad turbellarian, *Notoplana australis*, is found within the shells of weakened oysters, *S. commercialis*, on the New South Wales coast. Sometimes called the wafer or oyster leech, it feeds on the oyster but probably only enters the shell when the oyster is dying from some other cause (Prudhoe, 1982).

Small commensal turbellarians are occasionally found on the gills and in the gut of cultured bivalves but do no demonstrable harm (Lauckner, 1983; Goggin and Cannon, in press).

DIGENEA

Digenetic trematodes of the family Bucephalidae parasitize the gonads of oysters (Howell, 1966), scallops (Sanders and Lester, 1981) and tridacnid clams (Shelley et *al.*, 1988). The gonad is enlarged and eventually almost totally replaced by the branching sporocyste.

CESTODA

Larval tapeworms are common but do not cause problems. Encapsulated lecanicephalids (genera *Tylocephalum* and *Polypocephalus*) are abundant in the tissues of oysters from Queensland waters. Adults occur in rays.

PEA CRABS

The pinnotherid crab *Pinnotheres hickmani* is a frequent commensal in the mantle cavity of cultured mussels *Mytilis edulis* in Victoria. It reduces the meat yield of infected animals by up to 26 % (Pregenzer, 1981). A pinnotherid infection has been controlled with an insecticide in animals not for human consumption (Andrews et *al.*, 1968).

PYRAMIDELLIDAE

Pyramidellids are tiny gastropods that parasitize other molluscs. Large numbers of *Pyrgiscis* sp., 1 to 6 mm long, developed in the culture facility on Orpheus Island and killed juvenile giant clams (Cumming, 1988). During the day they hide outside the clam. At night they crawled to the lip of the shell and extended their probosces into the mantle to feed. Biological control using the crab *Thalamita sima* was suggested.

Pyramidellids reduce the growth rate of *C. virginica* and can transmit the protozoan *Perkinsus marinus* from oyster to oyster (White et *al.*, 1987).

BACTERIA VIBRIOSIS

Aetiology. *Vibrio tubiashi, V. anguillarum, V. siginolyticus* and *V. harveyi.*

Pathogenesis and epizootiology. Saprophytic vibrios and pseudomonads are an ongoing problem in mollusc hatcheries. Larval molluscs under adverse environmental conditions become covered in the bacteria. The gut and tissues are invaded and the larval molluscs die. The course of infection is rapid; mortality may be 100 % within 24hr.

Infections with *Vibrio tubiashi* and *Alteromonas spp.* were fatal to larval *C. gigas* in a Tasmanian hatchery. The pathogens were apparently taken in with food as fertilized eggs were bacteria-free. Fifteen to 100 % of larvae died (Garland, 1988).

Vibrio harveyi has been isolated from the haemolymph of dying adult pearl oysters. Up to 80 % of oysters frequently died after being transported from collection grounds to a lease site in Western Australia. The mortality was associated with cold water temperatures (19 %), crowding of oysters during transport, inadequate water circulation in carrier tanks, and infection with *Vibrio* sp. Experimentally, the *V. harveyi* isolated was shown to produce mortality in the oysters (Pass et *al.*, 1987).

Clinical signs and lesions. Larval molluscs show a decrease in motility and high sudden mortalities. They become pale and stop growing. Velum losses its cilia (Garland, 1988).

Adult pearl oysters had withdrawn mantles and brown-stained nacre between the withdrawn mantle and the edge of the shell. Foci of inflammation were evident in histological sections of the mantle and digestive gland. The epithelium of digestive tubules was atrophied.

Diagnosis. Direct microscopic examination of live affected larvae for swarming vibrios. Bacteriological culture.

Treatment. In hatchery, antibiotics (Chloramphenicol 10 ppm, erythromycin, neomycin); may be too late. For adult oysters, reduce stock density and improve water quality.

Prevention. Improve water quality.

VIRUS

Virus-like particles were found in the digestive gland of the pearl oyster (*Pinctada maxima*). They were in inclusions centrally located within hypertrophied nuclei in the epithelial cells of digestive tubules. Inclusions were basophilic or amphophilic with H. and E., and surrounded by a clear zone. They were not associated with any disease (Pass et *al.*, 1988).

PAPILLARY EPITHELIOMA

Papillary epitheliomas have been found in the mantle of *S. commercialis* from several locations in central New South Wales (Wolf, 1976). The tumours are ovoid or spherical, 1 to 16 mm in diameter, and have deep indentations like a cauliflower. They are composed of proliferating epithelium. They occur in older stunted oysters and are apparently unrelated to pollution.

MESENCHYMAL TUMOURS

Mesenchymal tumours have been reported from a pearl oyster *P. margaritifera* on the Great Barrier Reef. The tumours were firm, polyp-like growths attached by flexible stalks to the visceral mass near the gut loop and adductor muscle. Normal tissue elements with increased fibrosis and blood spaces composed the stroma and epithelium covered the surface (Dix, 1972).

Similar tumours were found in a Sydney rock oyster by Dinamani and Wolf (1973).

TRIBUTYL TIN

The tributyl tin (TBT) contained in many antifouling paints inhibits the growth of Sydney rock oysters at extremely low concentrations (5 ng TBTO/1; J.A. Nell, pers. com.). The sale of paint containing TBT is restricted in NSW and Tasmania.

HEAT KILL

Sydney rock oysters die from heat stress if a midday low tide coincides with a hot summer day. Trays are often covered in shadecloth or sprayed with water to reduce losses.

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Some aspects of the abnormal mortalities of the pearl oysters, *Pinctada margaritifera* L. in the Tuamotu Archipelago, (French Polynesia)

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Abstract — *The black-lip pearl oyster* ***Pinctada margaritifera*** *L. lives and reproduces with high performance in most of Tuamotu Lagoons. Technics to collect spats, to culture juveniles and to operate oysters were quickly found and applicated.*

Nevertheless, the sudden rise of this activity has been correlated with an anarchic development. For instance, large occupation of areas in the lagoons, cultivation of numerous oysters in confined areas, lack of knowledge on the basic culture technics in many farms.

In 1985, mass mortalities have appeared in some lagoons and 50 to 80 % of cultured stock died. Some investigations have been done but no explanation was found.

In 1988, the mortalities are still very high. An analysis of cooperative production results from 1979 to 1988 shows that many factors might explain in part these mortalities, like the graft operation and the environmental conditions.

INTRODUCTION

In 1985, mass mortalities have affected pearl oyster culture farms in the Tuamotu islands. Despite various studies on the etiology of the disease (Grizel, 1985) and on environmental disturbances (Chung Sao, 1986) during the high mortality period, no satisfactory explanation has been found. Rapidly, some measures to protect healthy lagoons were adopted like transfers prohibition, whatever the age and the origins of the pearl oysters.

Other researches done in Takapoto atoll, which was one of the most attacked islands, showed that the mortality was also high on the natural stocks of pearl oysters as well as different species of molluscs during the same period (Richard, 1987; Cheffort, 1988).

The biotic capacity of the lagoon of Takapoto has been consequently suspected to be responsible for high mortalities according to the rise of cultured pearl oyster biomass in some areas of the lagoons these last years (Intes, 1988).

Nevertheless, some indications have shown a spreading of the disease to other atolls with an epidemic pattern, independent of the cultured level in these lagoons. Also, since the first apparition of the disease, there are always high mortalities, but spaced in time and mainly on grafted oysters.

Zootechnical aspects have rarely been taken into account in the former studies. The following review is a preliminary study on the results of numerous farms with a comparison of different aspects on the mortalities of grafted oysters as the importance of graft technicians, of cultivation techniques and cultured areas (islands) also.

METHODS AND MATERIAL

Data from pearl oysters cooperatives have been analyzed from 1979 to 1988. These cooperatives are dispersed in all the Tuamotu islands. They are managed by the GIE Poe Rava Nui which organizes the harvest of pearls each year and computes all the data concerning the cooperatives.

The farms plotted in this study are not the same each year as well as their number.

Only data on grafted oysters have been analyzed. The mortality of the spats or the juveniles have not been checked because of insufficient data.

The cultivation time of grafted oysters is approximately two years after the operation. For each farm, the number of oysters varies from 200 to 3000 with a mean of 1000 oysters. The grafting seasons are generally similar every year, between May and August. However, over the past three years, due to the high number of shells to be operated on, the graft period takes place in the first half-year.

At the harvest, the number of live shells is counted but no data is available during the cultivation period. To distinguish between dead oysters and missing ones was not possible; however, each time the disappearance of oysters was explained (hurricane, stealing...), it was taken into account for the analysis.

Because graft technicians sometimes operate all together in the same islands or farms, comparisons between them are only on the 1986/1988 cycle for which we have separated data.

X2 test have been done on the percentages for the different analyses to test the homogeneity of the samples.

RESULTS

General survival rates

With the increase in cooperatives, the number of grafted oysters has been multiplied by 5 in 8 years (Fig. 1). So, the graft technician team has

been reinforced and the operation season extended. Until 1985, the mean survival rate was between 60 % and 70 % but in 1986, it dropped to only 35 %. As the pearl rates (the number of pearls over the number of live shells) are rarely more than 40 % with approximately 15 % of really marketable pearls, these low survival rates of grafted oysters represent a major problem for the future of this aquaculture industry.

Figure 1. — Evolution of the survival rates in the cooperatives since 1981.

Variations between islands

A few islands which have had pearl oyster activities since 1979 are plotted.

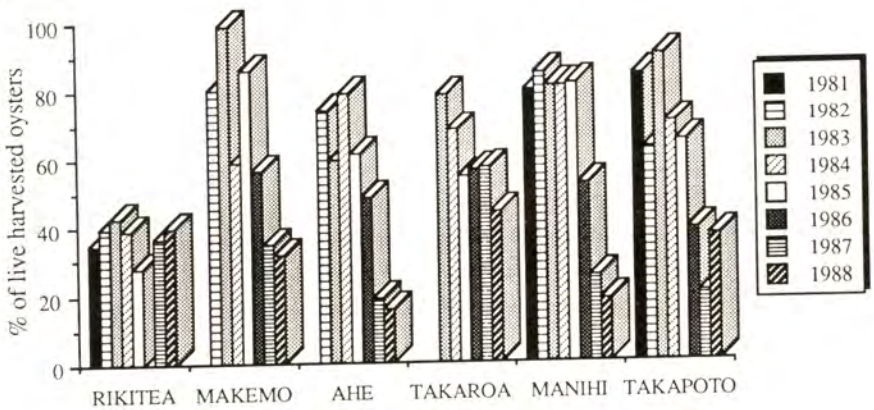

Figure 2. — Survival rates in different islands (1981/1988).

Except for Rikitea island, the other atolls show the same evolution (Fig. 2). The survival rates vary from 50 % to 90 % between 1981 and 1985. Since 1986 and mainly in 1987, in the atolls of Makemo, Ahe, Manihi and Takapoto, the survival rates became very low, often less than 40 %. Despite a similar general tendency, Takaroa seemed to suffer less mortality.

Concerning Rikitea island, the survival rates are a particular problem for this island since the beginning of the cultivation as they have never reached 45 %.

For 1988, the mortalities have greatly varied between exploited lagoons. However, except for Raraka and Katiu islands where the results were quite satisfying, all the other atolls had low survival rates, between 53.2 % in Hao to only 18 % in Manihi. (Fig. 3).

It is noted that the survival rates seem independent of the number of oysters grafted in each island.

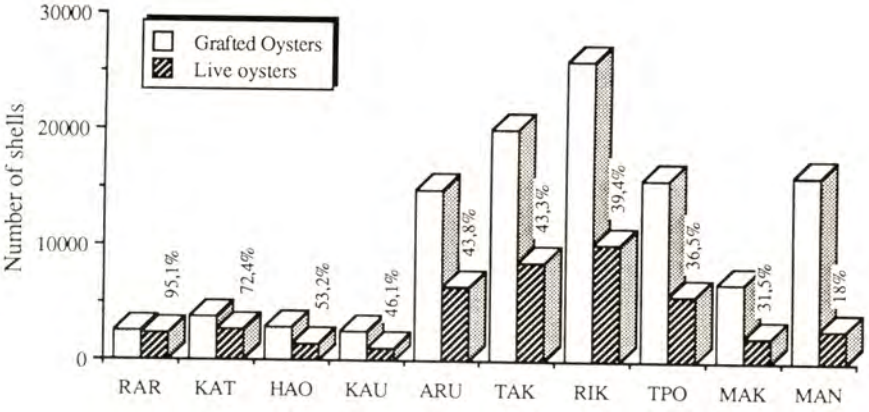

Figure 3. — Survival rates in the cooperatives from different islands in 1988.

Variations between graft technicians

Zootechnical stress during the five year culture period are numerous but among them, the graft operation seems preponderant. The harvest results of the 1986/1988 culture cycle have been plotted according to the graft technicians. (Fig. 4).

For 6 technicians, the survival rates greatly differ from 3 % to 48 %. However, 4 technicians have similar rates in a fork of about 10 %. This rate seems independent of the number of oysters operated.

As technicians often operate in a defined region, these results might represent the environmental variation as seen before, more than the influence of the graft technician.

The results of graft technicians in same islands at approximately the same period were compared. In this case also, the differences are often significant, particularly at Takapoto and Manihi where the mortalities were important (Table. 1).

The difference is still high with the comparison of technicians in the same farm. In one case, it was possible to plot the survival rates of 3 technicians. The results of the survival rates are statistically different ($P < \mathbf{0.001}$).

For one technician, it was possible to compare the survival rates in various islands, (Fig. 5). Even in that case, the variations are very important between islands and confirm that for a same technique and independently of the biomass of oysters, the mortalities may greatly differ.

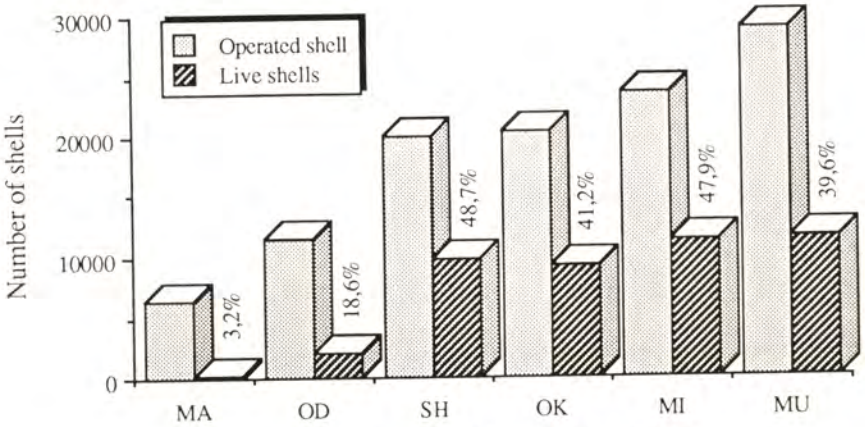

Figure 4. — Survival rates for different graft tecnicians.

Tab. 1. — Oyster survival rate results (%) for different technicians in various islands

	OD	MA	MI	SH	OK
TAKAPOTO	23,39	4,86	28,19	59,99	—
MANIHI	17,74	1,63	45,90	—	—
ARUTUA	—	—	—	43,69	44,27
TAKAROA	—	—	—	39,00	41,00

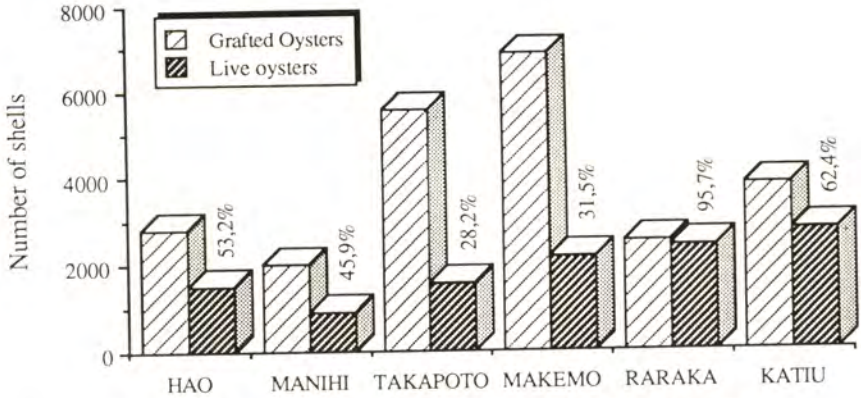

Figure 5. — Survival rates in different islands for the same technician.

The last factor used to appreciate the impact of the graft technicians and their techniques on the mortalities is the relationship between the pearl rate and the survival rate (Fig. 6). The coefficient of correlation obtained

by the Spearman nonparametric test for 6 technicians ($r = \mathbf{0.886}$; $P < \mathbf{0.02}$) shows a good correlation between these two rates, and lets us suppose that generally, the graft technique might affect the mortalities.

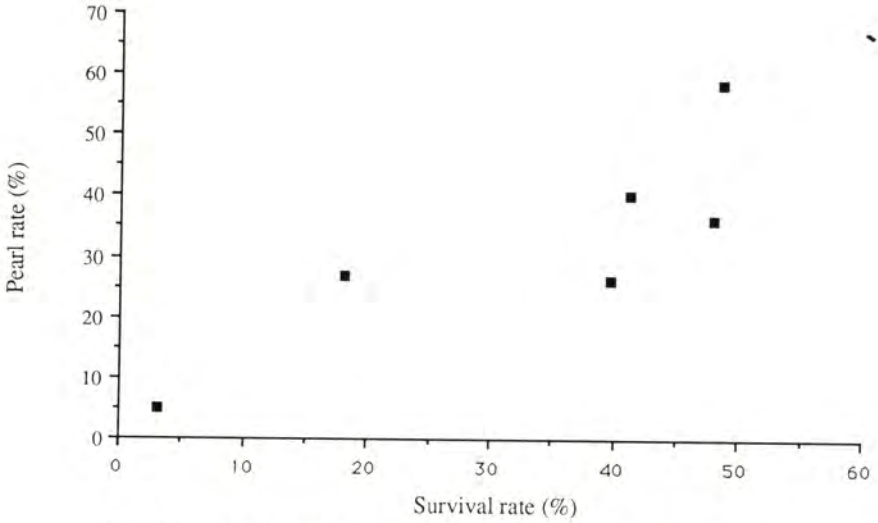

Figure 6. — Relationship between the survival rate and the pearl rate.

Variations between the farms

Although results of private farms are not well known, it is a real fact that their results (survival rates and pearl rates) are better than the cooperatives ones. The reasons which lead to this situation are numerous and will not be discussed here. Nevertheless, among the cooperatives, the results are often very variable, even with the same technician or in the same island.

Tab. 2. — Survival rates (%) in different farms

HAO	RIKITEA	TAKAROA	MAKEMO
51,00	45,2	45,1	27,25
51,00	58,3	47,25	57,55
48,45	45,7	47,45	38,2
59,00	15,4	48,25	27,9
47,9	35,45	34,4	33,65
73,00	51,9	24,85	17,5
42,6	13,9	45,00	—
61,3	—	46,9	—

Table 2 shows the comparison of survival rates in 4 islands with at least **6** farms and where the graft technicians have had average results. The farms have significant different results according to the X2 test for all the islands plotted except Takaroa. On this island with 9 farms, 7 have approximately the same average results, but with the low rates of the other

two, the test also confirms the heterogeneity of the samples. This might be the consequence of inadequate choice in the culture grounds (poor renewal of water or extra pollution). It might also be the consequence of bad cultivation techniques as some lagoons like Takaroa seem to have good hydrological conditions and a large pass. Here again, the results do not refer to the number of grafted oysters for each farm.

DISCUSSION

Concerning the pearl oysters culture, various types of diseases have been noted : (1) in Japan, Kotake et *al.* (1954) have suspected bacteria to be responsible for high mortalities in the rearing ground *of Pinctada fucata martensii* (Dunker); (2) in Australia, Dix (1972) has described tumors in *Pinctada margaritifera.* Wolf and Sprague (1977) have found an unidentified protist on the digestive gland of *Pinctada maxima* (Jameson) and Pass et *al.* (1988) have described virus-like particles in the same organ. Dybdahl and Pass (1985) and Pass et *al.* (1987) have also shown the importance of bacterial breakdown during transfers of oysters and the high mortalities which followed this stress in *Pinctada maxima*; (3) in the red sea, high mortalities have been observed on the rearing of *Pinctada margaritifera* in 1969 and 1973. Causes are still unknown but an unidentified protozoan parasite has been isolated, (Nasr,1982); (4) in India, mortalities were noted on *Pinctada fucata* after a long exposure to biofouling (Alagarswami and Chellam, 1976); (5) in French Polynesia, Grizel (1986) has shown the physiological disturbance of cultured *Pinctada margaritifera* and its relation with an abnormal lysosomal activity. He observed also mantle lesions in some samples. However, no direct relation with the mortalities has been clearly established.

There are numerous examples of failure in the culture of bivalve molluscs because of mass or long-lasting mortalities without any control or explanation (Van Banning, 1985; Sindermann, 1986).

Generally, epizootics, change in the environmental conditions, infectious pathogens or undetermined tumors and lesions are the causes of the diseases in the mollusc cultures (Balouet and Poder, 1985).

Many of the actual works on reared molluscan diseases highlight the importance of the environmental condition and the necessity to improve our knowledge about it. (Grizel, 1986; Maurer and Comps, 1986; Mori, 1986; Sindermann, 1986).

Also, prophylactic techniques or treatments are often difficult to find and employ and mean serious epidemiological studies (Grizel, 1986) or complex researches on the poorly known immune defenses of the molluscs. The most useful techniques are actually the management of cultured areas and the improvement of cultivation techniques in relation with the environment to avoid problems like overcrowding, limited biotic capacity, etc...(Heral et *al.*, 1983; Deslous-Paoli et *al.*, 1981 and 1982).

Evolution of the mortalities between 1981 and 1988

The rapid adjusting, in a few years, of simple techniques to cultivate the black lip pearl oysters in French Polynesia (see Coeroli et *al.*, 1983;

Coeroli and Mizuno, 1985; Cabral et *al.* 1985) has allowed the sudden rise of this aquaculture activity and particularly of small farms. However, it has been correlated with the growing of the oyster biomass, often in the same areas, and with a lack of knowledge on the basic techniques for the new farmers as the technical fishery service was too busy to control and advise all these new farms in this vast multiple island territory.

The high mortalities analyzed in this paper are obviously the result of a complex situation, where human factors cannot be neglected. However, the difference between the survival rates until 1984 and after is without any doubt the consequence of the growth on the cultivation of this species. Moreover, mainly because of the environmental features of its lagoons, the pearl oyster culture in the Tuamotu islands is typical and any extrapolation with diseases problems on pearl oysters in other countries, (as far as they are recognized), might be analyzed with great expectation. As we have had the opportunity to examine some data of the cooperatives, we have tried to understand the director indirect influences of various factors on the high mortalities of grafted oysters.

Distribution of the mortalities

The mortalities occur in many lagoons and cannot be correlated with disturbance of the environmental conditions, as this fact will mean synergical problems in more than ten lagoons with different hydrological and biological conditions, which is unrealistic. Nevertheless, in some overcrowding lagoons like Takapoto and Manihi, the deterioration of the environmental conditions were observed during the outbreak of the disease. Except Rikitea Island, where the mortalities were observed several years ago, there is however a similar temporal and symptomatic evolution of the disease in numerous islands which suppose a common problem for all of them.

As no infectious disease has been isolated despite sampling during the high mortality outbreaks, it seems unlikely that such a pathogen, if it really exists, was missed. If the hypothesis of an infectious disease cannot be completely excluded, particulary during the high mortality period, (1985/86), mainly because of the appearance of the mortalities in some lagoons after the transfers of oysters from attained lagoons (observations of the farmers), other causes can be advanced particularly concerning the grafted oysters.

However, as the differences on the survival rates are also important in various conditions for the same technician, the operation does not seem sufficient to explain the high mortalities in grafted oysters.

The influences of the cultivation techniques

Some of the mortalities are due to inadequate culture techniques. Numerous stressing factors, as regular washing, drilling of the shells, the graft operation and the cultivation duration (about 5 years), are important contributors to the mortalities. Moreover, overcrowding and irregular controls of shells, sometimes observed in cooperatives farms might induce unfavorable conditions and the weakness of the oysters, more susceptible to the culture stresses.

The importance of the pearl oysters origin

Since several years, most of the shells used for the operation came from the spat collection and are reared 2 to 3 years before the operation. In the lagoons where the survival rates were satisfying in 1988, the grafted oysters were issued from the natural stock (graft technician com.). It seems possible that selective pressures were less important on the collected oysters, leading to less resistant oysters during the various culture stresses. This fact will be in accordance with the regular bad results of all the graft technicians these last years, as they cannot obtain similar results as during the first year of the pearl oyster culture, when all the shells were issued from the natural stocks.

These results highlight the extreme complexity of the mortalities outbreaks over four years in the pearl farms of French Polynesia. The area diversity, the numerous biologic and hydrological characteristics, the particularity of the cultivation techniques all along the rearing time, the heavy stress of the grafting operation, the rise of the cultured biomass these last years and the overcrowding of some cultured areas are many factors that might have a great importance in the recent mortalities of grafted shells. The next step will focus on the observation of a few farms, with a particular attention on the zootechnical problems, the origin of the oysters and the chronology of these mortalities. These studies will be simultaneously done with researches on the biology of *Pinctada margaritifera* (reproduction, nutrition, genetics), the lagoon environment and particularly the change under the cultured ground and a new approach of the pathological and parasitic problems in cultured shells.

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Prophylactic strategies and zootechnic measures, recent advances

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Abstract — *The spread of major diseases of molluscs around the world and the increase of transfers need the development of adapted prophylactic strategies and the increase of research in epidemiology, immunology and genetics.*

The prophylactic strategies must be based on common laws with the establishment of a list of declarable pathogens, with basic sampling rules (sample size, frequency, definition of geographical areas) and with reglementation of the internal transfers. Moreover, specific adapted diagnosis should be developed to increase the sanitary control performance (e.g. ELISA test, DNA probe, cell culture).

Concerning the epidemiology, specific experimental plans should be realised, when abnormal mortalities occur in a growing area. In the case of an infectious disease the epidemiology studies should be concerned, the relationship beetween the culture techniques and the disease, the repartition of the disease, in terms of prevalence and infection ratios, experimental infection in the field and in the lab, the possible effects of the environmental components.

On the other hand, specific zootechnical research should be increased, particulary in genetics which is the main way to find applicable solutions to control the diseases. Selection of resistant strains, hybridization, gene transfer should permit the formation of new adapted strains. Finally, with the development of new models in mollusc pathology, like ***Bonamia*** *and Rickettsies and hemocytes, the study on defense mecanism, humoral and cellular response, enter in a new approach which should permit to understand better this mechanism and which should permit to select characters of resistance.*

INTRODUCTION

Over the past twenty years the development of hatcheries and the case of transportation facilities have increased the exchanges of alive animals between countries. Molluscs have followed the same way and the commercial exchanges concern the different age populations. In parallel, five recensed epizooties have occured in the world and some of them have spread in different countries. The best example concerns the parasite

Bonamia ostreae, native to the U.S.A., which was introduced to France with the importation of juveniles and extented to Spain, Netherlands, England and Ireland (Elston et *al.*, 1986; Mialhe et *al.*, 1988; Grizel, 1985).

In front of this situation, the laws appear completely inadapted, and the number of efficient applied actions to reduce the disease is very low. Also, it has become very important to develop new research in view of enhancing the zoosanitary control and to perform zootechnic prophylaxies.

ZOOSANITARY CONTROL

The zoosanitary control is based on laws which are very variable between countries. Moreover, none of them took into account the notion of pathogen. Now, with progress in the knowledges of pathogens, it is possible to establish a list of these. The interest of this list is considerable because it should permit to :

1. develop specific diagnosis methods, easier to use than histological techniques.
2. propose adapted sampling plans in definite geographical area in order to reinforce the quality of the control and to regularly obtain a map of the epidemic situation.
3. define correctly, disease by disease, the control rules (frequency, admissible prevalence levels) and the management rules for each disease (transfer, definition of free areas, contaminated areas and so on).

RECENT ADVANCES IN DIAGNOSIS METHODS

One of the characteristic in mollusc pathology is the absence of cell cultures used in Vertebrates for the diagnosis of some microorganisms. The common method remains the histological technique which is useful to observe different pathogens. But, on the other hand histology takes a lot of time, does not permit quantification and it is an expensive and non sensitive technique. These reasons have conducted Boulo et *al.* (1989) to perform a serological diagnosis based on the E.L.I.S.A. method to detect *B. ostreae*. This progress proceeds from the obtention of purified parasite (Mialhe et *al.*, 1985, 1988) which have opened important research ways in mollusc pathology. So, the main results concerning the diagnosis comparison between smears and E.L.I.S.A shows for an equal sensitivity a saving of time of around six hours and a cheaper cost (Boulo, 1989). In the case of low prevalence of *B. ostreae* and for an equal time control effort, the use of E.L.I.S.A. diagnosis kit gives a better response because the control can be easily made on a large sample.

Moreover, before choosing the E.L.I.S.A method, the I.I.F. technique with polyclonal and monoclonal antibodies has been tested. This method is more sensitive than the smears technique but compared to E.L.I.S.A it requires microscopic observation. In fact, I.I.F. stays very useful and efficient in the case of spat control for which the quantity of hemolymph ponctioned is not sufficient. Serological diagnosis, according to different

methods (sero agglutination, I.I.F., E.L.I.S.A.), used to depend on the host and on the parasite group (virus, bacterias, protozoan, etc...) and localization (internal or external). It could be also interesting in the near future to develop other new techniques, such as DNA probe and in every case to reinforce the research on the obtention of cell lines. The absence of this tool reduces considerably the potential of actions in mollusc pathology.

PROGRESS IN LAWS : HARMONIZATION

Different international organizations : Office International des Epizooties (O.I.E.), Conseil International pour l'Exploration de la Mer (C.I.E.M.) try to push the different responsibles and actors of the exchanges of animals to take into account the major risks presented by the diseases in aquaculture. The role of scientists in this action is very important to sensibilize the administration, inform and educate the farmers and perform diagnosis methods and sampling plans in each geographical area. Many countries have no laws and none of them has made a list of declarable and undesirable diseases. Nevertheless, some pathogens are now well known, like *Bonamia ostreae, Minchinia nelsoni, M. costalis, Marteilia refringens, Perkinsus marinus* and it could be possible to define, disease by disease, importation and transfer rules.

The putting together of ideas by the different organizations should be beneficial to an adapted aquaculture regulation. Compared to the zoosanitary regulation in force for vertebrates, the result is exactly the same. The only question is : does the country prefer to develop aquaculture or commercial exchanges ?

In the second case the philosophy becomes completely different and does not need a so strong regulation, but one must not forget the possible impact of a disease on wild stock.

ZOOTECHNIQUE MEASURES

Different zootechnic measures should be envisaged when an infectious disease appears. Some of them result in the acquisition of knowledge on parasites, mainly the cycle development, the infectious period and the relationships between the pathogen and the farming techniques (density, growth methods) and beetween the pathogen and the environment (t°, s ‰; O_2). Each disease is particular but general and adapted experimental plans can be used when abnormal mortalities occur in a geographical area.

Presently, the number of applicable zootechnic measures remains very low, due to the characteristics of the shellfish culture conducted in open sea and also to the farmers who do not apply unanimously the recommendations.

Also, in front of this different difficulty, it seems better to work on the host using the different techniques of genetics. Compared to the results obtained on insects and on plants, few works and results have been obtained on molluscs. The main data concern the formation of a strain

with increased resistance to *Minchinia nelsoni* (Haskin et Ford, 1979; Ford et *al.*, 1988). This selected strain came from broodstock picked up on oysters bed exposed naturally to the MSX disease. The successive breedings between oysters of this strain show an increase in survival ratio.

In spite of the results, these experiments remain critizable because it is not possible to analyse the genetic and other components, like veracity of the control, evolution of the infectivity of the parasite.

Still, this strategy can be used with some modifications in the concept.

The other interesting ways to exploit concern individual resistance, polyploïdization and gene transfer.

Individual resistance against a disease should be exploited using breeders which have survived inoculation by a parasite. Actually any results concerning molluscs have been reported, but after the reproduction of bonamiasis using purified pathogen (Bigot-Vuillemin, 1987) a monitored broodstock is in creation. It will be useful to examine the heritability of the resistant character.

Different techniques of polyploïdization have been applied successfully on different species, *Crassostrea virginica, C. gigas, Ostrea edulis* and *Ruditapes philippinarum* (Allen, 1986; Gendreau, 1988; Dufy, 1988).

As for fish, this experiment has been realized to increase the growth and the quality of the meat. However, in one case the polyploïdization of hybrids *Salmo trutta x Salmo salar* have given resistant individuals (Dorson et Chevassus, 1985).

It appears interesting to test hypothesis leading to an existing identical phenomenon with molluscs.

Finally, the research on gene transfer should be also very interesting. The advance of the concept and of the results on plants and insects requires attention, particularly the different models used to fight against some Virosis (Cuozzo et *al.*, 1988; Hemenway et *al.*, 1988).

An effort in this research should surely be beneficial and should permit to find applied solutions to resolve the disease problem.

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Infectious pathology in mollusc and shrimp hatcheries

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Abstract — *Development of aquaculture has been linked to zootechnical mastering of larval production in hatcheries.*

Now, expected progression will depend upon rearing new species or strains (hybrids, polyploïds, etc.) and reducing the risk of introducing pathogens (closed-circuit, biological filter, etc.). But, like in all animal or vegetal production, pathogens must always be taken on account as a potential risk. The importance and frequency of infectious diseases in hatcheries of molluscs and shrimps are poorly estimated in broodstocks and larval productions because of general lack of data on epidemiology and exact determination of the causes of mortalities. In some cases, viral, bacterial and fungal agents were involved in severe mortalities. It appears therefore necessary to develop methods suitable for qualitative and quantitative diagnosis of pathogens and for use in hatcheries (miniaturized systems for marine bacteria identification and quantification; enzymatic and fluorescent virus-immunoassays). The availability of these diagnostics as « kits » may be of major importance for zootechnicians in order to quickly detect and identify a pathogen. By this way, they would be able to elaborate prophylactic measures and regularly check sensitive points in hatcheries.

Regular and excessive use of antibiotics (antifungal or antibacterial) may thus also be reduced, permitting to limit release of these products in sea-water and, consequently, avoiding progressive selection of resistant-strains. At the same time, sea-water adapted formulations (microemulsions, etc.) of active products may lead also to decrease used-amounts and to improve efficiency of treatments.

Development of aquaculture has been linked to zootechnical control of larval production in hatcheries. Thus, for most economically important species, have become more independant of environment for regular supply of larvae. But this independance is relative, in so far as the hatcheries are more or less directly connected with their surroundings. This feature, which is essential concerning physico-chemical quality of sea water, appears primordial relating to pathogens due to the risk of epidemic outbreaks. Besides this direct sea-water pathogen introduction, broodstock, algae and *Artemia* may be a secondary contamination route and even an amplification step. Epidemiological investigations of mortalities occurring in hatcheries are still limited but sufficient to point out the involvement of infectious agents. Their actual impact is probably underestimated due

to the lack of adapted methods suitable for pathogen diagnostic by non-trained staff. It must also be emphasized that some « psychological » reticence expressed by stock-breeders and zootechnicians to take into account the pathological hypothesis during mortalities.

Most frequent and drastic pathogens involved in hatcheries are fungi, bacteria and viruses.

Among fungal agents, *Lagenidium* and *Fusarium* are worthy of notice for their pathogenicity in shrimp larvae but also juveniles and broodstocks. The spreading of spores by sea water and by aerosols and a supposed wide hosts range ensure fungi continuance.

Bacteria are ubiquitous microorganisms present in sea water, broodstocks, algae, *Artemia* (dessicated cysts and cultured nauplii) and finally in larvae tanks. *Vibrio* and *Aeromonas* (Vibrionaceae) are the most frequently determined species associated with mortalities. Unlike human and veterinary medicine, where some true pathogenic species are identified and alone to be considered, the bacteriological data are more confused in aquaculture. This fact is due to the absence of adapted tools for standardized biochemical identification and for rapid and automatized enumeration of bacterial flora in normal and abnormal breeding cycles. Moreover, when bacteriological investigations are performed in hatcheries, they generally concern sea water samples and thus may be unreliable, as some *Vibrio*-like bacteria need to be associated with a biological support like the cells of intestine. This binding capacity must be related to oligomeric protein production, by some *Vibrio* species, these proteins including toxic and binding subunits.

Among mollusc mortalities in hatcheries, only the oyster velar disease of *Crassostrea gigas* has a known viral etiology. This supposed iridovirosis was discovered through obvious macroscopical infection symptoms and easy light microscopic detection of viroplasms and even viral particles (340 nm). The potential risk of this disease for worldwide oyster farming must be seriously considered, keeping in mind, first the total disappearance of the Portuguese oyster *C. angulata* from atlantic coasts subsequently due to an Iridovirus epidemic, and secondly the transovarian transmission demonstration for similar viruses of insects. It may be assumed that more systematic and more sophisticated analysis (electron microscopy) performed during severe mortalities will lead to detection of other virus types yet identified in different molluscan species adult stages.

The role of viruses in crustacean hatchery mortalities is better studied. Baculoviruses, easily detected by light microscopy after inclusion bodies formation, are responsible for large epidemics, sometimes on a nation-wide basis in productive countries. Picornaviruses and Parvoviruses are also involved in mortalities. Transmission modalities are poorly understood because of the lack of experimental pathology methods and qualitative and quantitative diagnosis techniques. Nevertheless, transovarian transmission is supposed for Baculoviruses and alternate hosts, such as *Artemia* which are associated with several virus types, are possible.

A common opinion stated by hatchery zootechnicians concerns the dependance on stress activation of viral infection triggering. This feature, more assumed than experimentally demonstrated, is however essential to consider because it suggests that infected hatchery produced larvae, when

taken out of hatcheries, become healthy carriers or an infectious source in breeding areas.

Confronted to epidemics, hatchery conceptions and zootechnical processes have quickly evoluted trying to develop prophylaxis : individualization of units and tanks to limit infection spreading; systematic antibiotic treatments to prevent bacterial proliferation. More recently, progresses aiming at increasing the environment-independance have been related to biological filters and broodstock management.

In order to insure more reliable aquaculture productions, it is now advisable that zootechnicians and pathologists devote together their energies to the improvement of larval productions in hatcheries which are the initial steps of the breeding system.

Fungal diseases due to *Lagenidium* are relatively well-controlled using Treflan preventive treatment, and a recently elaborated microemulsified formulation is effective against *Fusarium conidia* propagation. A macroscopic and individual examination of breeders is useful to avoid hatchery introduction of highly infected and contaminating animals.

The prioritary researches in the bacteriology field aim at developing adapted tools for routine bacteriological hatchery surveys. Classical methods, using Petri dishes with solid media, appear indeed expensive, time consuming (preparation, inoculation) and reading is much too delayed (one to a few days) considering to bacterial proliferation time. Thus, a bacteriological unit in hatcheries is in practise non-operating. Miniaturized systems ready for use are in progress for quick total and *Vibrio*-like bacteria quantification according to the most probable number method. Such systems must permit a daily control of larvae tanks and of different crucial points (filters, algae, etc.). Any change in bacterial populations could be precocely detected, allowing a prompt intervention. In such a perspective of available « bacteriometer », it would be possible to conceive some changes in antibiotherapy. Applying antibiotics only when and where necessary would be of particular interest in reducing antibiotic use. This concept becomes urgent facing the aquaculture development and political will for antibiotic and chemical regulation.

Such antibiotic decrease may also be facilitated by the elaboration of special formulations with the following items :

- microemulsified formulations permitting easy and quick solubility in sea water whatever the spontaneous solubility of antibiotics. These kinds of formulations are well adapted to simultaneous disinfection of tanks and contaminated animals (external and also inside the digestive tract of molluscan and crustacean larvae). Moreover these formulations allow precise adjustment of selected concentrations avoiding any excess of non-soluble and residue-forming antibiotics.
- availability of a panel of antibiotics with different modes of action in order to reduce constant and similar selection pressures for resistance. It is important to keep in memory the extraordinary adaptation capacity showed by bacteria to resist antibiotics (detoxifying enzymes, transposon-mediated amplification, intra and interspecific plasmid-mediated transfer).

— suitability of bactericidal antibiotic formulations, alone or in association. Such formulations, carried out when an abnormal bacterial outbreak is detected (bacteriometer), are adapted to a true elimination of bacteria. Indeed, bacteriostatic treatments promote growth of resistant bacteria, leading thus to a progressive selection of more and more resistant bacteria populations.

Progresses in aquaculture bacteriology will have to take inspiration from human medicine. Microsystems for exact antibiotic sensitivity determination would be easier for zootechnicians, compared to classical disc antibiogramme method. Elaboration of miniaturized systems, specially conceived for biochemical identification of marine bacteria, would also be useful tools for standardized characterisation of pathogenic strains. Worldwide exchange of epidemiological information would lead to identification of opportunistic and perhaps true pathogenic bacteria. Specific and sensitive diagnostic methods could then be elaborated.

Concerning virological problems, the only way of investigate is prophylaxis. Close relationships will be necessary between zootechnicians and pathologists, first to develop experimental pathology methods (isolation and purification of viruses, laboratory reproduction of diseases, qualitative and quantitative diagnosis methods). Then, it will be possible to develop hybrid production and individual selection programmes, refering specially to insect or plant virology. Indeed, taking into account the molecular basis of virus specificity (cell receptors) and pathogenicity, some highly-resistant or refractory strains can be selected in short time.

More long-term studies concern gene transfer methodology by which viral DNA-sequences integration in host chromosomes may lead to resistant transformation of the host (parasite-derived host resistance concept).

Awaiting resistant-strain selection and in order to avoid large hatchery epidemics and international virus spreading, adapted-diagnostic methods must quickly be elaborated (immunodiagnostics using monoclonal antibodies, DNA probe methods).

MOLLUSC PATHOLOGY
DISCUSSION

Hill — How do we speed up selection for resistance ? Also what are MAB's being developed for ? Another priority is development of molluscan cell lines. Is anyone considering transgenic oysters or genetic engineering for resistance ? First can we discuss black pearl oysters and their problems. Have you done any pathology ?

Weppe — We have sampled 800 animals for pathology. (Histology, bacteriology and virology).

Hill — What was the mortality rate ?

Cabral — About 50 %.

Hill — Over what time ? It could be slow mortality over a long time.

Weppe — It is very difficult to say. We have sampled with Grizel all the stages of diseased animals before they died; we have not found any histological modification but always the large lysosomes with some autolysis in the digestive gland. But we do not know if we sampled normal and healthy animals to make the difference.

Hill — Did you sample black pearl oysters in other country without mortality problems ?

Weppe — I sampled wild specimens in Fiji, where no mortality was reported. Histological aspect was exactly the same.

Mialhe — What do you think of the viral infection of *Pinctada maxima* ?

Grizel — The disease in Australian oysters is noticeable. We have observed large lysosomes, autosomes, and autolysis. Mussels under stress show the same abnormalities in the digestive gland. We have examined oysters after the graft and have found mal-formation of the shell. After 1986 we have not observed shell abnormalities. *Ruditapes philippinarum* have a brown stain and this can be reproduced by injecting bacterial isolates.

Mialhe — It is, however, impossible to see bacteria in shells with brown ring; they may be destroyed by fixation.

Lester — A brown nacre stain has been reported in oysters in Western Australia.

Elston — There is the possibility of a water-borne toxin. Mucus is a response to irritation. Perhaps that happened in 1985. I agree with Grizel you should concentrate on the grafting.

Weppe — Yes, the grafting is certainly responsible of an increasing of the mortality. But a large part of the mortality occurs before grafting.

Hill — What about genetic factors ?

Cabral — Madame Blanc from Montpelier did a genetic survey for polymorphism. No possibility of larvae crossing with different lagoons. There is a possibility it is inbreeding but she showed it is not the case.

Michel — She found a strong difference between the Marquesas and Tuamotu. May be the larval viability is low thus maintaining diversity in any lagoon.

Hill — Has spat collection altered and is this a selective process in itself ?

Michel — Yes. But in lagoons where there is no spat collection there are few wild larvae.

Hill — May be you are selecting for weakness. What is the economic cost ?

Cabral — 3 000 of 10 000 people on Tuamotu are involved, with up to $25 m just for exportation. In grafted oysters there is 40-60 % mortality with only 40 % pearl-formation rate and only 10 % of those good quality.

Hill — What sort of increase in production could be achieved if this mortality problem was overcome ?

Cabral — It could be more than doubled.

Hine — Have you considered zinc toxicity ?

Cabral — Yes we have considered it, but data do not suggest it is the cause of mortality.

Hill — Can we discuss selection for resistance ?

Elston — There is resistance to Malpeque Bay disease after 40-50 years. We should look at speeding up the development of resistance. If you have to wait a year to determine resistance it takes a long time to breed resistant strains. We should determine immunocompetence.

Hill — You could determine phagocytosis index, bactericidal capacity, and chemoluminescence.

Mialhe — Chemoluminescence is different in molluscs. There are few hemocytes. In *Crassostrea gigas* it can vary greatly. We still have to determine immunological factors. First selection cases involve protozoans, it is more difficult with viruses. *Bonamia*/oyster relationships are complex; what for example is the function of blood enzymes ? May be we should consider humoral factors such as interferon.

Hill — We need virus models.

Mialhe — We, in the United States, Europe and New Zealand, have a common problem and should work on this.

Hill — Ralph (Elston) what have we learned about Malpeque Bay disease immunology ?

Elston — It has not been properly studied. We should look at resistance by selecting after challenge.

Lester — Could different species of oyster be crossed ?

Grizel — We have tried it without success, we must now try cytogenetics.

Mialhe — Between genera it is not easy.

Lester — As the parasites are so specific it might be quicker than using selection.

Grizel — It has taken seven generations to develop resistance against protozoans.

Elston — Natural processes are too long.

Mialhe — We have found *Ostrea angasi* is more susceptible than *O. edulis* to infections, so crossing may not be an advantage. In short term we need resistance.

Elston — If you can change management, in the short time, this is what must be done. We should look at linkage or (genetic) traits.

Hine — Susan Ford at Rutgers has noticed MSX-resistant *Crassostrea virginica* are slow-growing, and our, apparently, *Bonamia* resistant oysters are also slow-growing. These could be undesirable linkages.

Mialhe — We must remain optimistic.

Grizel — We should try cytogenetic techniques, polyploidy triploidy, introducing the sperm head of one species into the egg of another.

Hill — Does anyone know if there are genetic studies in molluscs ? There are in fish. One group is trying to alter a gene that controls haemoglobin and the ability of trout to tolerate low oxygen levels.

Mialhe — There are many such techniques with insects, such as mosquitoes. Insect geneticists are trying to develop mosquitoes that are bad vectors for *Plasmodium.* There are similar developments in chickens and plants. It will be harder with protozoans and rickettsias. There are also many studies on the genetics of *Drosophila.*

Hill — There is obviously a gap in research.

Grizel — We are working to find molecules to fight pathogens. But it will take 2 years, 3 years...

Hill — Can we move to cell culture. Is anyone claiming and showing progress ?

Mialhe — Lee Ellis at VIMS has taken embryonic cells from *Crassostrea* and tried to insert ras genes, oncongens, into them. We have to know what genes are involved in neoplastic transformation.

Hill — How can we encourage more work on this ?

Elston — How would you (Hill) justify it ?

Hill — We have to fund « fire-fighting » at the moment. We need the problem before we can act.

Mialhe — It will be easier in shrimps. It has been easy to establish insect cell lines.

Elston — You need a whole variety of molluscan lines; we may not need advanced technology, we need to understand basics like nutrition better.

Hill — Can we move to diagnostic kits ? These techniques can be done by technicians and are rapid.

Grizel — We must focus on the most important pathogens like *Bonamia.* Haplosporidans in the United States and Europe are also important. *Marteilia* is also important. *Minchinia* is being worked on but we need better purification. The same problem exists with iridoviruses. DNA probes may be better than ELISA techniques.

Hill — What will be the next kit from France ?

Grizel — May be *Marteilia.*

Mialhe — We need to determine the pathogen's role of rickettsias first. There are marketing problems. The kits must be available at the right time. MAB's from neoplastic mussels are not a commercial prospect.

Hill — Kits are important as they standardize certification procedures. It gives greater uniformity.

CHAPTER III

NUTRITION OF CRUSTACEANS

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Anatomy and physiology of digestive tract of Crustaceans Decapods reared in aquaculture.

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Abstract. — *The digestive tract of crustacea is complex. It is composed of a short oesophagum, a stomach with two bags, and internal wall with appendags specialized in grinding of feeds. These hard mastication pieces represent the gastric mill. Setae, filters included in the stomach structure allow the separation between food particles and liquid compounds. Those one are coming over through filters and more on to the digestive gland tubules or mid gut gland. In this organ, several types of cells play specialized functions : absorption, enzymes secretion, stocking function. Some cells of a new type, with undefined function, may be neurosecretions function, have been described recently. The end of tubules of digestive gland exhibits cells with embryonic characteristics. Digestive enzymes of crustacea are diversified : proteases, among which trypsin is the major one, carboxypeptidase, aminopeptidases and a protease of low molecular weight, 11 000, recently thoroughly examined. Enzymes related to lipid metabolism are lipases and esterases. Amylases, maltases, chitinases are well represented.*

Enzyme secretions vary according to factors of external environment. Digestive enzymatic activities are under control of hormones which are about to be checked more in detail.

INTRODUCTION

The digestive tract of some crustaceans decapods has been described with great detail for a very long time; some excellent works have been realized during the last century.

These works are, in their large majority, essentially anatomical, and they have not integrated the fact that they are of a physiological or a zootechnical interest.

With the actual development of the aquaculture of crustaceans, a better knowledge is needed concerning the anatomy and the functions of

the different parts of the digestive tract of the decapod crustaceans, especially those who are candidates to be reared in the future.

The following review is an attempt to clarify the common structures of the digestive tract in the Decapods, and to point out their functions, at the light of some recent researches and new discoveries.

The digestive tract is responsible, for many animals, and crustaceans in particular, for the nutritional function. This function, includes ingestion, transit of nutriments, mechanical digestion, chemical and biochemical hydrolysis, cellular absorption and transfert of excreta, occurs in the digestion tract. This one has several aspects typical of crustaceans. The lumen of digestion tract must be, as in other groups of animals, considered as being located **outside** the animal itself. This concept takes an original connotation for the whole group of crustaceans. This digestive tract has more or less entirely, characteristics of the **tegument** of Arthropoda. The luternal wall of crustacean digestion tract is in fact impregnated, in its fore and hind parts, with complexes of chitin and proteins which are removed at each moult with the exoskeleton.

Another original aspect of crustacean digestive tract relies on the fact that most reserves of the animal are accumulated here. They are utilized at each intermoulting cycle to build up for example, new tissues after ecdysis.

In upper crustaceans groups and particularly in decapods, there exists at the level of stomach walls, a set of hard chewing parts, which are rejected and rebuilt at each moult. Their position and their motility on one part, muscles and nerves which contribute to movements on the other hand, have original characteristics. In fact it is in the stomach that most chewing action on feed particules occurs, and their movements depend on special complex mechanisms.

Even digestive enzymes have their originality, for example special endocrine mechanisms which regulate their synthesis and proteases of low molecules weight. In spite of the diversity in organization schemas of each studied groups, there exists some common structures, at cellular level, which should be considered as characteristics of Crustacea, noticeably, within absorption stocking mechanisms.

Within upper Crustacea, predators and scavengers, sophisticated mechanisms of dilaceration and using calcified parts of stomach, change ingested feeds into very fine food particles. These particles will be selected at filter level which are in several parts of stomach.

Embryogenesis and **organogenesis of digestive** tract studies on organogenesis of the digestive tract of Crustacea are pretty scarce.

During embryogenesis, forgut or stomadeum, and hindgut or protodeum, are differenciated very early during embryo development as in the majority of groups of Crustacea.

Differentiation of digestive **tract during larval** development. After hatching, the digestive tract, very simple at start, becomes complex. Nauplii larvae have, after hatching a non functional digestive tract with a mouth but the **anus** will open after 2 or 3 moults.

This digestive tract is straight and little by little acquires its own movements and enzymatic activities. Its fore part widen into a stomach

sack which differentiates in two side pockets. It is progressively transformed until resembles the tract of the adult. This is true for hard parts which belong to gastric mill and the hepatopancreas.

ANATOMY OF THE DIGESTIVE TRACT IN HIGHER CRUSTACEANS

General structure

An adult decapod crustacean digestive tube is divided into 3 main parts : fore, mid and hindguts. Generally, the foregut is composed of the oesophagus, and a large part of the stomach where the masticating parts are present. The midgut has no chitin, but contains the many tubuled hepatopancreas which secretes digestive enzymes. The midgut is nearly straight and chitin is present. It is enlarged posteriorly into the rectum and terminates at the anus.

Mouth

The mouth is associated with specialized prehensible appendages, maxillula, maxilla, mandibles and maxillipeds. The anterior part of the mouth is reinforced by a hard labrum. There is a localized rare of glandular tissue present on either side of the mouth, as described in *P. aztecus*, made of large epithelial cells and a clear cytoplasm.

Oesophagus

In decapods, it is usually short, straight, positioned vertically and joining the mouth to the stomach. In cross section, an anterior roll can be seen as an extention of the labrum as well as two lateral rolls. The lumen is in an X shape.

It can be noted in the brachyurans (e.g. the crab *Callinectes sapidus*) that the oesophagus penetrates the antero ventral wall of the cardiac stomach. The anterior part tends to be lateral. The pyloric stomach is suddenly directed downwards, at the cardio pyloric juvenile crabs have also a well developed gastric mill, very similar to the adult. In lobsters factor (1981) clearly illustrated that the first larval stage of *Homarus americanus* does not have median, lateral or accessoring teeth in the stomach walls, although they are rigid setae, folds and septae.

For *Crangon sept.* Reynault (1972) showed that the foregut became more complex coinciding with larval growth with a lamellated stomach, which will form the gastric mill between the pylonic and cardiac zones. As with many Caridians, older stages have a reduced or absent gastric mill.

On the other hand, the Brachyurans, Anoriaurans and the Macrourans, the development of the gastric mill occurs during larval metamorphosis.

For *Palaemon serratus*, at the zoea 2 stage, a pair of symetrical tubules parallel to the tract appears and grows towards the rear. At zoea stage 3,

another smaller pair of symetrical tubules develop laterally and forward. At zoea 4, a new pair of tubules develop posteriorly. At stage zoea 5, the number increases to above 10 tubules. This will continue to increase during the whole grow stages of the larvae juvenile.

The thin integument and tissues of the larvae allows for observation of particle movement in the tract. They come and go between the stomach and the lumen, by the use of constricting waves. If the form tubule branches are in the form of an X, with the stomach in the centre, the particles pass from one tubule to the opposite one. If however, the number of tubules is high, observations become very difficult, even impossible. The system is made even more complex by the qualitative action and quantitative of digestive enzymes function. In the Macrourans *(lobster Homarus americanus)* the oesophagus is more ventral, and the cardium tends to be shorter than for crabs. For the rock lobster *Panulirus argus* the oesophagus is practically vertical and penetrates the cardium more posteriorly than in crabs or crayfish.

Histological studies show that the lumen is covered by cylindrical basal epithelium cells, with clear cytoplasm, with average height 40-60 M. This epithelium is itself recovered by a thin hyaline article composed primarily of chitin. Three types of muscle fibres are present in the connective tissue, enveloping the epithelium. The most internal are dialation, the middle are circular and the outer layers longitudinal. A few rare glandulor elements, comparable to tegumentary glands, are found in the oesophagus.

Stomach

The most important and detailed study of decapod stomachs dates back to over a century ago. Mocquard (1883) described more than 6O species, his observations and illustrations are the basis of all works on this topic, although the stomach of many species have never been examined neither detailed.

Penaeid stomachs are the most elongated among decapod stomachs. The oesophagus is associated with the heart region, where it forms a right angle. The stomach pocket has a narrow floor. The cardiac pocket is well developed anterior to the most forward ossicles. Half the anteroventral pocket is thick and calcifield folded towards the front, the intero-lateral cardiac disk which has on its dorsal side a simple and longitudinal of teeth. The pyloric section of the stomach is bent towards the back following a specific angle.

The most distinguishable characteristic in the stomachs of penaeid prawns is the reduction in the number of identifiable ossicles to 14 for *Penaeus* versus 33 in the Reptantia. They are less calcifield as well (Fig. 1).

— *Walls*

The stomach is covered, on all its inner surface, by chitin protein coat which is of exodermal origin. This coating does not exist in the endodermally derived intestine. As for the vertebrals, the passage from the oesophagus to the stomach is the cardia and pylore from the stomach to

the intestine. The anterior part of the stomach has thin walls, it is flexible a chamber where ingested food is masticated. The posterior region of the cardiac stomach and the pyloric stomach are reinforced and supported by a number of articulated calcorious pieces, disks and ossicles. There are thick due to a coat of chitin.

The mucosa is similar to that of the oesophagus. The walls are complex with various size lamellae, depending on its location. Threads and needles in the interior of the stomach also vary in size depending on their location. Dorsally, there is a saddle shaped gland, the cells resembling blood cells. This gland is considered as an haematopoetic organ.

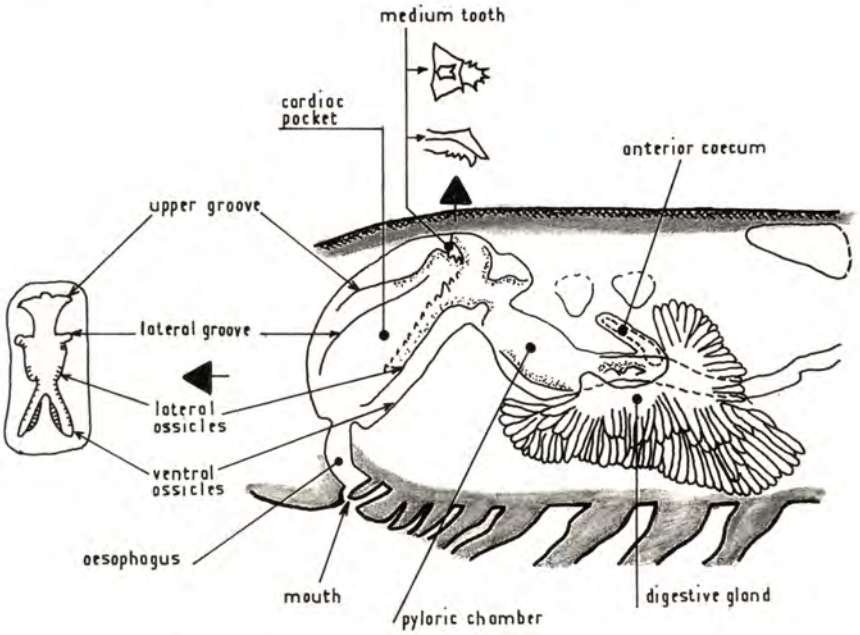

Fig. 1. — Drawing of the stomach of Penaeid crustacean.

— *Mastication parts of the stomach*

Geoffroy (1709) was the first to mention the presence of « dents stomacales » in Crustaces podophtalmaires (Crustacean with stalked eyes). Among the general characteristics of the digestive tract, it has been stated earlier that the anterior and posterior parts are coated with chitin protein material and associated with the exoskeleton, thus they participate in the periodical moults as well. The internal surface of the anterior part of the digestive tract is thus armed with protrusions capable of grinding the food. These projection are sometimes calcified and become skeletal articulated pieces of varying forms. Each piece is moved by individual muscles located outside the wall, and controlled by a group of characteristic nerves. These pieces and folds have group specific forms. Their internal surface are not smooth, and these invagination constitute a complex valvular system, particularly marked in the posterior region of decapod stomachs.

After Geoffroy, these masticating parts were studied by famous anatomists, like Reaumur (1712), Cuvier (1805), Milne-Edwards (1834) and Heckel (1836). The system was well described over the post century. It would seem that the stomach armature can be compared to a three branched claw through which the food must pass to reach the pyloric region (Milne Edwards 1834). On the other hand, Huxley (1857) compares the masticating parts to a mill, and the pyloric region to a filtre. Here, only fine particulate matter can pass and be digested. Thus decapod crustaceans can be considered filterfeeders.

These masticating parts have roles which are quantitatively and qualitatively different, depending on their respective positions. The first fifteen pieces located in the cardiac pocket, is often called the gastric mill. The anterior parts, stronger and more calcified, are ossicles called teeth. The second set of 18 pieces at most smaller and less calcified, participate in the filtering function of the pyloric region.

Many observations have led to recognize that the effectiveness of a stomach is in part due to its complexity. More over, the complexity of the mandibules varies inversely to that of the stomach.

The masticating parts are mobile, and with their respective movements, grind food into a soup of fine particles.

The general shape of the stomach and the position of its masticating parts varies from species to species. It is thus impossible in this context to give detailed descriptions of different species types, remembering also that many decapod species have been little studied in this field. Additionally the number of ossicles varies in decapods. The Brachyuran having much more.

— *Function*

It was thought that, for decapod Crustaceans, digestive liquids from the secretory gland flowed forward along ventral channels, and that digestion took place in the anterior pocket. The secretions, along with the food particles would then be carried towards the rear. They were to be filtered in the ventral section of the stomach, the finest particle and liquid products passing into the hepatopancreas tubules where absorption took place.

It is now believed that the system works differently. Food enters the stomach and moves towards the rear along the dorsal wall of the anterior pocket, then they pass through the gastric mill where they are ground before entering the pyloric pocket. The food is continually worked by lateral outgrowths (teeth and ossicles) and return anteriorly to pass through the system again. The liquid phase flows antero-posteriorly along ventro-lateral sides leading to the principal channels of the pyloric pocket excludes any particles larger than 1$\tilde{n}$m of the secretory gland. A filtre is located in the ventral region. These non digestible particles are rejected into the hindgut.

It is possible that chemical digestion begins in the stomach, helping to reduce the size of the particles. Transit time of food products lasts a few second, not exceeding a minute. Food may rapidly pass through the anterior diverticulum located dorsally to the pyloric pocket. Here, enzyme activors may be secreted in the stomach pH modified (Fig. 2).

Decapod crustacean stomachs have varying degrees of complexity. Ossicles and the gastric mill are often absent in Caridians, but are most complex for Brachyurans. There seems to be though compensatory factors : less the stomach contains ossicles more the mandibular appendages are complex.

These alimentary rejects not digested by internal filters become faeces.

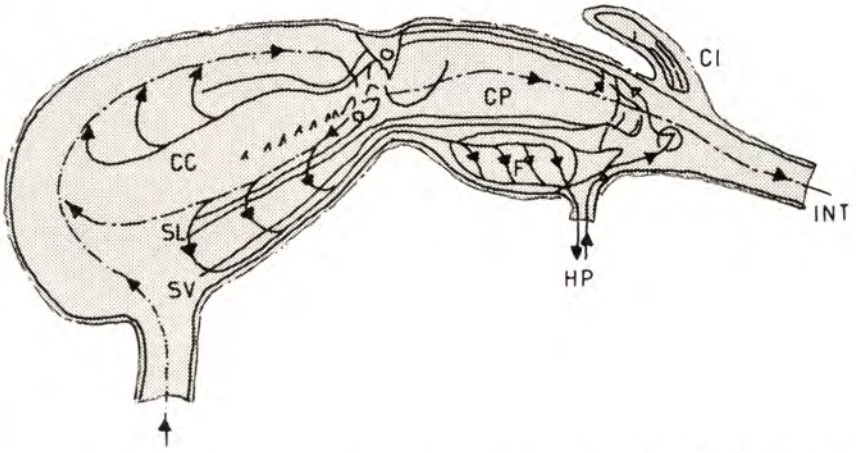

Fig. 2. — Diagram on circulation of digestive fluids in the stomach of Penaeid Crustacean.

Hepatopancreas or midgut gland

The hepatopancreas is considered a major organ in decapods, used in many metabolic functions : synthesis and secretion of digestive enzymes, absorption of digested dietary products, production of mineral reserves and organic substances, lipid and carbohydrates metabolism, role in distribution of stored reserves during the intermoult cycle, catabolism of organic compounds. In most decapods, it forms a pair of glands, occupying on either side of the stomach a large volume inside the cephalothorax. It represents 2-6 % of the total body weight. Each section is composed of 2-3 well distinguished lobes, associated with connective tissue. A network of longitudinal and circular muscles has been described for several species.

Generally, each half of the hepatopancreas opens into the digestive tract via principal ducts. Each duct divides into secondary and then tertiary tributaries until they end in small tubules that constitute the glandular mass.

In Penaeids, the collecting chamber is teardrop shaped and progessively elongates during growth (Nakamura and Yonekura, 1980).

Liquid and digested material exchange between the gland and the midgut occurs either anteriorly, lateraly or ventrolaterally.

The colours of the hepatopancreas is variable — brown, red, green, yellow, blue or tan — and depends mainly on the stored reserves. i-carotene, zeaxanthine and astaxanthine are the pigments present.

Around each hepatopancreatic duct are fine circular and longitudinal fibres, which are responsible for the movement of liquid and cells throughout the organ. Each duct must empty into the stomach the secretions produced by the gland and then fill up with digested material which will be absorbed. These muscular fibres allow for peristaltic and longitudinal movements of each duct, which have lumens of small dimensions.

The detailed innervation of this organ is little known. The crayfish *Cambarus virilis*, haemolymph irrigation occurs through first defined vessels (such as the hepatic artery) then less defined ones, the haemolymph flows between the ducts in the fibroelastic tissue in relation with the basal membrane.

The hepatic artery, after leaving the anteroventral part of the heart, divides into two and takes the haemolymph towards the hepatopancreas, thus being in close relation to the general haemolymph circulation.

Midgut coecae

In addition to the two hepatopancreatic masses, several decapod crustaceans possess coecae of variable length depending on the species. The crab *Cancer* has three elongated tubular coecae, two anterior symetrical ones, the other posterior. The lobster *Homarus* only has one short anterior coecum.

Intestine

The intestine extends all along the abdomen, from the posterior end of the stomach and terminates generally at the anus. The lumen made of secretory cells produces a mucuous substance which coats the non digested products from the stomach, then aiding their movement within the tract. The peritrophic membrane which envelops the excreta is found in nearly all crustaceans. Its synthesis and production cause a loss in the nutritional energetic costs of crustaceans.

Hindgut : rectum and anus

The hingut of decapods is located in the posterior half of the 6th abdominal segment and includes the rectum and anus. There is usually a gland present in this segment, oval shaped, a continuation of the midgut lumen. It is located dorsally and has a folded epithelium. The distal portion of this gland is in continuation with the hindgut.

A particular structure has been described in Penaeids two parallel channels form the hindgut lumen, and join with the rectum, where they come together to form a single channel.

The proximal third of the hindgut lumen is much larger than the last third, and the mucosa forms the internal folds. The rectum has a small

diametre and opens externally at the anus, between the telson and the uropods.

In Penaeids, the hindgut originates at the rectal gland which is dorsally located, and comprises the rectum and anal canal (Fig. 3).

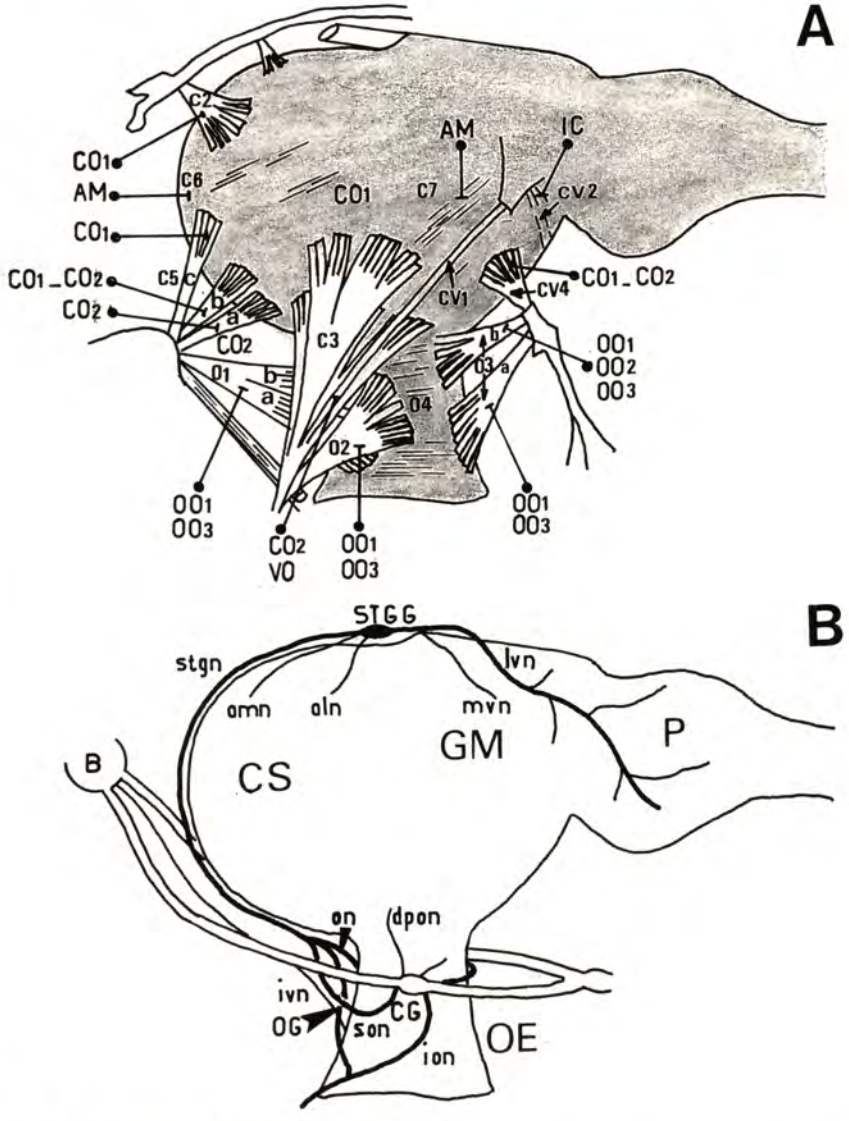

Fig. 3. — Neuromuscular organization of the foregut
A) Lateral view of the foregut with oesophageal and cardiac sac muscles. For each muscle, the motoneurones are indicated.
B) Lateral view of the stomatogastric nervous system. B, brain; CG, commissural ganglion; CS, cardiac sac; GM, gastric mill; OE, oesophagus; OG, oesophageal ganglion; P, pylorus; STGG, stomatogastric ganglion; amn, anterior medical nerve; DPON, dorsal posterior oesophageal nerve; ION, inferior oesophageal nerve; IVN, inferior ventricular nerve; LVN, lateral ventricular nerve; MVN, medical ventricular nerve; ON, oesophageal nerve; SON, superior oesophageal nerve ; STGN, stomatogastric nerve.

MICROSCOPICAL ANATOMY

Oesophagus

The walls are made of an epithelium covered with a 140 µm thick cuticle, itself subdivided in a homogenous exocuticle and an internal horizontally indented, and separated by a strongly PAS positive zone.

For *Scylla serratus* (Barker and Gibson, 1978), this cuticle is covered with fine canals (3 µm diametre), leading to gladular structures of 70-130 µ diametre, their secretions flowing towards the oesophageal lumen.

Their number decreases the stomach, where there are none.

The oesophageal musculative is made of bands of acidophilic circular fibres, 200 µm thick, and longitudinal fibres.

Stomach

The walls histologically resemble those of the oesophagus but the tegumentary glands do not exist and the muscle elements are more massive and differentiated Oka (1967) gave a good description of *Penaeus orientalis* stomach.

Hepatopancreas

The functions of this species variable shapped gland are diverse : absorption, enzyme secretions, storage, etc. It is evident that different types of epithelial cells are present to assure these functions.

The presence of characterized by microvilli cells indicate an absorption function which has been well described in several decapod groups. Secretory glands are also present, characterized by vesicles which rupture in the digestive tract. These two types of cells coexist in the midgut of lower crustaceans. For higher crustaceans, the different types of cells are differentiated at the apex of the hepatopancreas tubules, and their specialization increases towards the base of the tubules.

Classical cell type nomenclature is as follows :

- E cells : embryonic characteristics, apical
- R cells : described by Hirsch and Jacobs (1928, 1930) or Restrellen, which have a storage function;
- F cells or Fibrenzellen, have a fibrous appearence;
- B cells or Blasenzellen, have a secretory function, and contain one or more important secretory vacuoles.

These are confirmed and more detailed by Gibson and Barker (1979).

The E cells of the crab *S. serrata* are 25 µm tall and 5 µm wide. The proximal nucleus has a 4-5 µm diametre. Their apical surface shows no brush border. They are very often in the mitosis phase.

The R cells, of dimension 60 µm X 10 µm, are more abundant, and contain multiple vacuoles, filled with osmiophilic lipids. Their brush border is 1-2 µm tall. Small calcareous deposits and glycogen spheres are

also present. Ultrastructurally, these spheres are less electron dense than other deposits.

The immature R cells located near the apex of the hepatopancreas tubules are clear and of similar size to E cells, with numerous mitochondria apically and a well organized Golgi body basically. Their small nuclei contain less chromatin, and the endoplasmic reticulum (ER) is limited to several zones only. The microvilli are dense and well arranged. The mature cells have lipid droplets and glycogen particles. They are characterized by vacuoles which will progressively form a supranuclear inclusion containing metals such as copper, zinc and sulphur as well as other less abundant ones.

F cells are basophilic, and slightly larger (50-90 µm) than R cells. These cells are found in the central region of the tubules, between the R and B cells. The cells have a fine brush border, and a PAS positive vacuole near the nucleus.

B cells may reach a height of 30 µm and certain a large oval vacuole, with a maximum with of 50 µm. A group of numerous small vacuoles located near the apical surface, just below the brush border, may coalesce with the larger vacuole. Sometimes, entire B cells are found in tubule lumen.

More recently, a new type of cell - M cell - have been described in the hepatopancreas of *P. semisulcatus* (Al-Mohanna and Nott, 1985). They neighbour the base of the tubules, and have a storage function. They are rich in proteins, and are large vacuoles. Their spherical shape and their position differentiates them from the other epithelial cells. Their exitence, appearence and reserves vary throughout an internal cycle.

The association between the various cells have lead to several different interpretations. The most classical being R cells derived from E cells on the one hand, and B cells from E and F cells, on the other. Enzymes may be produced by several types of cells, at least F and B cells.

Crustaceans have different types of digestive secretions, a part from intracellular digestion. In most cases, the secretion is holocrine, that is the entire contents are rejected into the lumen. But merocrine secretions, or continual enzyme production by specialized cells, and apocrine secretions occuring near the apex have also been described.

Midgut and digestive coecae

The cells of the portion of the digestive tract located between the foregut and the rectum, that is between the posterior end of the cephalothorax and the junction of the 5th and 6th abdominal somites, and these of the digestive coecae have been described for *Homarus* and *Cancer* by Mykles (1979). The epithelium is made of simple layer of cubic cells covered with a thick cuticle. The nucleus is basal, and associated with 1-2 nucleoli. Additionally the lateral membranes of adjacent cells are highly interdigitate, which enhances the epithelial cohesion. That cohesion is reinforced by three types of specialized intracellular junctions, the most remarkable being the *macula adherens*, located level with the lateral membranes (separated by 9-10 nm) and have very electron dense material.

The cells possess microvilli and the basal cytoplasm contains numerous mitochondria, and a smooth ER. Elongated vacuoles, called pleomorphic vacuoles, most probably are at the origin of the peritrophic membranes [sone think that this material secretes peritrophic membranes].

At the base, nerve cells are present, possibly neurosecretory in function, haemocytes and other cells with possible endocrine functions.

Water and salts can pass through the epithelium, at rates depending on the stages of the intermoult cycle (Mykles and Thearn, 1978).

Hindgut

The epithelial cells have numerous mitochondria in their apical cytoplasm. It is probable that the epithelium is associated with water and ion transport. Water absorption in this part of the digestive tract helps compact and transfer excretia. This zone plays an important role in osmoregulation of different species, complementing the exchanges occuring in the gills.

DIGESTION PHYSIOLOGY

Mechanical digestion

Crustaceans often choose their prey or food with respect to dissolved substances emitted from these into the water, and detected by chaemoreceptory organs located around the mouth and anterior appendages. Larval stages or planctonic species choose adequate particles with the help of filtres and basket systems located on their anterior appendages.

Chemical digestion

Several digestive enzymes were studied last century (Hoppe-seyler, 1877). Few species however have to be studied in detail using modern techniques. Several proteases have been studied in detail, for Penaeid species, and the crayfish *Astacus* for example.

The carboxypeptidases A and B, and trypsic activity have been studied by gates and Travis (1969, 1973) and by Galgani et *al.* (1984). For Penaeids, some aminopeptidases and dipeptidases have been identified. Decapods also have a light (11000 molar mass) protease that is very active (Pfleidener et *al.*, 1967; Swilling et *al.*, 1985, Galgani et *al.*, 1985).

In decapods only, a slight collagenolytic activity is detected, in the crab *Uca*, it was studied by Eisen et *al.*, (1973).

Trypsine represents by far the principal proteolytic activity. It is composed of 6 isoenzymes, with a molar mass of 25000 daltons (Galgani et *al.*, 1985). The trypsine sequence has been revealed by Zwilling and Tomaseck (1970) and Titani et *al.* (1983). It is about 50 % homologous with beef trypsine.

Chymotrypsine is present in small amounts, sometimes absent (Eisen et *al.*, 1973), aminotransferases have been shown to exist in the crab *Carcinius maena* (Chaplin et *al.*, 1967).

Carbohydrates and polysaccharide digesting enzymes exist in all those crustaceans already studied : amylases, saccharases, maltases, laminarinases, chitinases, even cellulases in certain species.

Amylase (endo-ί-1, 4- glucanase) is also present. For *Palaemon serratus*, this enzymes molar mass is 50000 (Van Wormhoudt, 1980), and composed of 2-3 isoenzymes, depending on geographic location. This polymorphism can even be greater, *P. elegans* has 7 *ί amylase* isoforms. Three glucuronidases (23500, 275000, and 370000) exist in *P. serratus* (Treller and Ceccaldi, 1976).

Cellulose digestion by crustaceans enzymes is not clearly demonstrated except in wood borers like *Limnoria* (Ray and Julian, 1952).

Laminarinases or i-1,3-glucanases digest the storage polysaccharides or laminarines present in algae, fungi and marine protists. This family has been little studied in crustaceans.

Chitinases and chitobiases are present in all these crustaceans studied. For predators, this allows the digestion of pray exoskeletons or their own after ecdysis. A chitobiase of molar mass 110-125000 was studied by Brun and Wojtowicz (1976). These enzymes related to chitin digestion have been studied by Jeuniaux (1963) and Muzzaelli (1977).

Multiple functions oxydases (MFO) and nitroreductases were found in the hepatopancreas of the lobster by Elmamlouk and Gesser (1976).

At last, deoxyribonucleases, ribonucleases of molar mass 33,000; 25,000 respectively and phosphatases have been studied in several species.

But lipases have been little studied. Numerous esterases (ca.20) exist in each species however their specificity needs to be better understood. Lipid digestion and absorption is greatly aided by the presence of emulsifying agents with properties close to those of bile (Holwerda and Vonk, 1973).

The optimal pH for the activation of enzymes varies, but is, in many cases, higher than for vertebrates, and especially for mammals. The values range from 5.5 to 9.

Reserves

Absorption occurs in the cells, cylindrical and elongated, with a brush border. They contain droplets associated with carotenoid pigments, glycogen, calciumphosphate crystals (especially during the period immediately preceeding exuviation). Vacuoles containing irregular copper corpsules are detected with an electronic microscope. Zinc and magnesium are also present (Al. Mohanna and Nott, 1985).

Their content,(the content of these metals) varies along the intermoult stages, reaching their maximum concentration at stages C4-D0. The storage of material and its reüse immediately after exuviation to reconstitute new tissues is a major key in crustacean physiological equilibrium.

BACTERIAL FLORA OF THE DIGESTIVE TRACT

Their importance is very variable from one group to another. Generally, there is no true symbiosis as it occurs in termites for example.

Digestive tract bacteria can be a source of food or vitamins, or perhaps produce themselves digestive enzymes. But, as the food has a very short transit time in the digestive tract (Repper, 1978) such a direct enzymatic role would be rather limited.

WAYS OF USE OF NUTRIENTS

When a crustacean nutritional balance is established, the erse physiological functions undertaken by the animals must be accounted for a part of the ingested food assimilated and used :

- for mechanical energy, e.g. locomotion;
- for reproduction, especially in the biosynthesis ovarian tissue, or of testes;
- for proper growth, e.g. energy used for moulting hepatopancreas reserves, synthesis of muscular tissue, production of exuviae;
- for the synthesis of the peritrophic membrane;
- for osmotic regulation (e.g. chemical energy used for the maintenance of internal balance) and for excretion.

The other part is not assimilated, and is rejected excreta, or sometimes regurgitated after sorting in the gastric mill.

CONTROL OF THE DIGESTIVE FUNCTION

Variation

The mechanisms for digestion adaptation to variations in environmental factors and food characteristics constitutes an important chapter in the ecophysiology and ecobiochemistry of crustaceans (Ceccaldi, 1982, 1986).

Digestive enzymatic activities vary with the time of day and night, following internal circadian rhythms, with two peaks per day, depending on the phases of the intermoult cycle, but also on the phases of vitellogenesis. Larval development stages affect, both qualitatively and quantitatively, digestive enzymes, where amylases are first present in high concentration, then slowly giving way to proteases towards the middle of the larval to life. This is synchronous with changes in their diet.

In adults, food untake induces an increase in digestive enzyme secretion. The affinity of amylase and trypsine for their substrate is based on temperature, this being measured *in vitro.* Maximum affinity corresponds to optimum temperature of the species. Light photoperiod and quality also has an effect on enzyme activity, and also regulates the

circadian clocks. Van Wormhoudt and Malcoste (1976) showed that green wavelength acts as a stimulant whereas red wavelength acts as an inhibitor.

Amylase and protease activity is modulated by the composition of the diet (Lucien Brun et *al.*, 1984). This activity increases with respective increases in glucides or proteins in the diets, either natural or artificial, given to the animals. Above an optimum percentage, around 5-10 % for glucides and 40-50 % for proteins, their activity begins to decrease. Histologically, R- cells do not change shape during the 1-2 h. post-feeding. Then the smooth ER rapidly develops, and the Golgi system becomes very active and produce small multivesicular bodies. After 12 hours, the R- cells loose their hemidesmesomes, the ER becomes smaller, and the cells return to their original shape.

Control

Hepatopancreatic secretions are controlled by hormones. There is a double mechanism in the eye-stalk. A stimulant, of molar mass 500 daltons, containing 20-OH ecdysone is present. An inhibitor must also exist, acting on the Y-organ to inhibit production of ecdysteroids; its activity varies with the seasons and with the intermoult cycle. For *Palaemon serratus*, gastrine cholecystokinine have been found in the eye stalk, near the X, organ (medulla terminalis), the X organ (medulla externa) and the sinus gland. Similar peptides are present in the walls of the stomach (Favrel and Van Wormhoudt, 1986). For *Penaeus japonicus* quantitative and qualitative variations in these peptides have been observed after feeding.

These peptides have a role in the decapod stomach nervous system for example in the lobster, they act on the muscles controlling the rythm of the gastric mill teeth.

CONCLUSION

Zoological knowledge of crustaceans has lead to much research. However, more scientific studies have to be initiated in this field as well as an increase in crustacean physiological and biochemical knowledge (Ceccaldi, 1982). The discoveries and results of these studies will have an important impact in the field of marine biotechnics.

At last, these studies will lead to improvements in aquacultural development, exploitation management, and better understanding of the distribution.

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27

Protein requirements of Penaeid shrimp

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Abstract. — *Proteins are indispensable nutrients for growth and maintenance of live of all animals. The optimum protein levels in diets for shrimps are different among the various species. Squid meal is an effective protein source for many penaeids. The effects of dietary protein, lipid, and carbohydrate levels on the growth and survival of larvae of* ***Penaeus japonicus*** *were examined by feeding trials using purified diet with carrageenan as a binder. As a result, the effects of protein levels on growth and survival of* ***P. japonicus*** *larvae varied with dietary carbohydrate levels but not dietary lipid levels.*

Proteins having an essential amino acid profile similar to that of body ar like to have a high nutritive for shrimp. Therefore, the amino acid composition of body protein of shrimp was analyzed, and then diets using various protein sources to simulate the amino acid profile of the body protein of shrimp were chosen. As a result of feeding trials, the shrimp showed good growth and high survival when the amino acid profile of the diet simulated that of the body protein of shrimp.

The superiority of crab protein as a protein source in diets has been demonstrated in the feeding trial of some crustaceans. However, we have found that crab protein as a sole protein source is not good enough to sustain larval growth and survival of ***P.japonicus*** *probably due to both its physical property and unsatisfactory amino acid profile, but it can be used together with other protein sources in diets of larval* ***P. japonicus.***

ESSENTIAL AMINO ACID OF SHRIMP

Proteins are indispensable nutrients for growth and maintenance of life of all animals. As the shrimp and prawn have difficulties of efficiently utilizing free amino acid in diets (Deshimaru and Kuroki, 1974; 1975a,b; Deshimaru, 1982), the incorporation of radioactive acetate into individual amino acid of shrimps was investigated in order to determine requirements of essential amino acid. The shrimp and prawn were shown to require 10 amino acids, arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine and tryptophan (Cowey and Forster, 1971;

Shewbart *et al*, 1972; Miyajima and Broderick, 1977; Coloso and Cruz, 1980; Kanazawa and Teshima, 1981; Pascual and Kanazawa, 1986).

PROTEIN REQUIREMENTS OF PENAEID SHRIMP

Several groups of workers have reported the optimum protein levels in diets for Penaeids : *Penaeus japonicus* (52-57 % : Deshimaru and Yone, 1978), *Penaeus indicus* (43 % : Colvin, 1976), *Penaeus monodon* (46 % : Lee, 1971; 40 % : Aquacop, 1977; 40 % : Khannapa, 1977; 35 % : Bages and Sloane, 1981), *Penaeus aztecus* 23-31 % (Shewbart et *al*, 1973); 40 % : Venkataramiah et *al*., 1975), *Penaeus setiferus* 28-32 % (Andrews et *al*., 1972). *Penaeus californiensis* 31 % (Colvin and Brand, 1977), *Penaeus vannamei* (30 % : Colvin and Brand, 1977; 36 % : Smith et *al*., 1985), *Penaeus stylirostris* (35 % : Colvin and Brand, 1977), *Penaeus merguiensis* (50 % : Aquacop, 1978; 34-42 % : Sedgwick, 1979), *Metapenaeus monoceros* 55 % : Kanazawa et *al*., 1981), Metapenaeus macleayi (27 % : Maguire and Hume, 1982), and for freswater shrimp : *Macrobrachium resenbergii* (25 % : Clifford and Brick, 1978; 30-40 % : Ashmore et *al*., 1985).

The optimum protein levels in diets for Penaeid shrimp are different among the various species. I assume that the diversity of optimum protein levels for crustaceans is likely to come from a variety of factors, namely, the discrepancy in food habits, age of specimens, water temperature, protein sources used, and energy level of the diet.

EFFECT OF PROTEIN LIPID, AND CARBOHYDRATE LEVELS ON GROWTH OF PRAWN LARVAE

The effects of dietary protein, lipid, and carbohydrate levels on growth and survival of the prawn larvae were examined by the feeding trials using purified diets with carrageenan as a binder (Teshima and Kanazawa, 1984). In experiment 1, the larvae were fed 12 diets containing various levels of protein (Casein; 25, 35, 45 and 55 %) and lipid (6.5, 11.5 and 16.5 %) at a fixed carbohydrate level of 15 %. In experiment 2, the prawn larvae were given 9 diets containing various levels of protein (35, 45 and 55 %) and carbohydrate (5, 15 and 25 %) at a fixed lipid level of 6.5 %.

The effect of protein levels on growth and survival of the prawn varied with the dietary carbohydrate levels but not with the dietary lipid levels. The elevation of lipid levels from 6.5 % to 16.5 % did not improve growth and survival when the diets contained sufficient levels (15 % or more) of carbohydrate. Contrarily, the elevation of dietary carbohydrate levels from 5 to 25 % improved the survival of the prawn larvae when the diets contained low levels (35-45 %) of protein. On the basis of these data, the optimum protein levels for the prawn larvae were estimated to be around 45 %, 45-55 % and 55 % or more when the diet contained 25 %, 15 % and 5 % levels of carbohydrate, respectively (Fig. 1.).

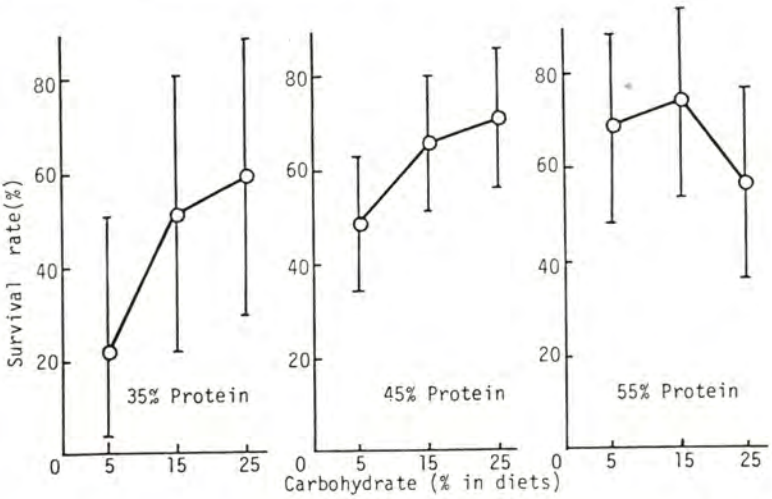


Fig. 1. — Effect of dietary Carbohydrate levels on the survival rates (means ê confidence limits at P=0.95) of *P. Japonicus* larvae.

PROTEIN HAVING ESSENTIAL AMINO ACID PROFILE SIMILAR TO THAT OF SHRIMP BODY

Generally, proteins having an essential amino acid (EAA) profile similar to that of whole body are like to have a high nutritive value for prawn and shrimp (Kanazawa, 1985; Kanazawa, 1986; Kanazawa and Teshima, 1988; Kanazawa, 1988). Therefore, the amino acid composition of whole body proteins of larval prawn *P. japonicus* was analyzed, and then four test diets containing various protein sources to simulate the amino acid profile of larval body protein were formulated using a computer. Four microparticulate diets were formulated with a soybean meal, chicken egg yolk, krill meal, white fish meal, skim milk, squid meal, brown fish meal, yeast powder, gluten meal, short-necked clam and fresh bonito milt (Tables 1 and 2). Feeding experiments were conducted using zoeal larvae of P. *japonicus* obtained from wild egg-bearing females. The zoea larvae were divided into lots of 100 individuals in 1-litre beakers containing sea water (specific gravity, 1.026) filtered a column of cotton and maintained at 29.0 + 1.0°C. Test diets were given to the prawn larvae at a feeding concentration of 0.08 mg/larva twice a day (Teshima and Kanazawa, 1983). The rearing water was renewed every day after the evaluation of growth and survial rates of larvae.

All test diets were based on carrageenan micro-bound diet with particle sizes of <53 μm for zoea 1 - —oea 2, 53-125 μm for zoea 3 — mysis 2 and 125-250 μm for mysis 3 — postlarva 1. Test diets were prepared according to the following process : mixing of powdered ingredients and carrageenan at 85°C, cooling in a refrigerator, freeze-drying, crumbling into particles, and sieving for the desired particle size. Dietary value was evaluated by the survival rate (%) and growth index of larvae,

Tab. 1. — Composition of test diet.

Ingredient (g/100 g)	Diet −1	Diet −2	Diet −3	Diet −4
Soybean meal		5.5	19.3	13.8
Chicken egg yolk powder	20.0	17.4	17.4	17.4
Krill meal	15.0	8.4	8.4	8.4
White fish meal			12.5	12.5
Skim lilk	15.0	3.1		
Squid meal	10.0	11.0	9.2	5.5
Browwn fish meal		19.1		11.4
Yeast powder	10.0	9.4	2.3	2.3
Gluten meal		1.4		
Short-necked clam extract	4.0	4.0	4.0	4.0
Pollack liver oil	7.0	7.0	7.0	7.0
Soybean lecithin	3.0	3.0	3.0	3.0
Cholesterol	0.5	0.5	0.5	0.5
Vitamin mixture	6.0	6.0	6.0	6.0
Mineral mixture	4.0	4.0	4.0	4.0
Cellulose	5.5	0.2	6.4	4.2
TOTAL	100.0	100.0	100.0	100.0
Carrageenan	5.0	5.0	5.0	5.0
Fresh bonito milt	25.0	25.0	25.0	25.0

Tab. 2. — Essential amino acid composition of relative ratio to menthionine.

Amino Acid	P. japonicus	Diet −1	Diet −2	Diet −3	Diet −4
MET	1.00	1.00	1.00	1.00	1.00
THR	1.16	1.22	.41	1.36	1.40
VAL	1.44	1.52	1.66	1.64	1.68
ILE	1.44	1.35	1.48	1.53	1.53
LEU	2.46	2.17	2.51	2.48	2.51
PHE	1.28	1.39	1.56	1.49	1.52
HIS	0.69	0.70	0.89	0.81	0.88
LYS	2.62	2.40	2.76	2.63	2.75
TRP	1.27	0.83	1.14	1.02	1.09
ARG	3.53	2.96	3.23	3.41	3.37

Tab. 3. — Effects of test diets on survival rate and growth index of prawn larvae.

Diet no	Feeding period (day)	Survival rate (%)	Growth index
1 a/b	10	91	7.0
	10	89	7.0
2 a/b	10	94	7.0
	10	90	7.0
3 a/b	10	73	6.8
	10	69	6.9
4 a/b	10	91	7.0
	10	95	7.0

1. Growth index : $zoea_1$, 1; $zoea_2$, 2; $zoea_3$, 3;
: $mysis_1$, 4; $mysis_2$, 5; $post\text{-}larva_1$, 7.

determined daily on 10 samples and on all surviving larvae at the end of the feeding trials when one of the experiment groups reached the stage postlarva 1. Statistical analysis of survival and growth data was performed by analysis of variance. The results of feeding trial are shown in Table 3. The larval prawn fed diets 1, 2 and 4 showed good growth and high survival rates when the amino acid profile of the diet simulated that of the body protein of larval prawn. However, growth and survival of diet 3 containing 19.3 % soybean meal were inferior to diets 1, 2 and 4 (Kanazawa et *al.*, in preparation).

NUTRITIVE VALUE OF METHIONINE ENRICHED SOYBEAN PLASTEIN

Nutritive value of methionine enriched soybean plastein for Tilapia, *Oreochromis niloticus* fry was reported (Teshima and Kanazawa, in press). Feeding trials P. *japonicus* juvenile were conducted by Teshima et *al.* (in preparation) to examine the effects of supplemental methionine as crystalline amino acid or enriched soybean plastein on weight gain, feed conversion efficiency, and protein efficiency ratio. Growth was not enhanced by supplementing diet with methionine enriched soybean plastein gave significant improvements. These results indicate that methionine in soybean plastein is more effectively utilized by the prawn than crystalline methionine. This study proves the possibility of using methionine-enriched plastein to improve the nutritive value of methionine-deficient vegetable protein sources.

NUTRITIONAL EVALUATION OF CRAB PROTEIN FOR PRAWN

The superioritu of crab protein as a protein source in semipurified diets has been demonstrated in the feeding trial of juvenile lobsters, *Homarus americanus* (Boghen et *al.*, 1982) and juveniles of other crustaceans species such as P. *monodon*, P. *vannamei*, P. *stylirostris* and M. *rosenbergii* also grew and survived well with feeding on crab protein based diets (Castell et *al.*, in preparation). However, Koshio et *al.* (in press) have found that crab protein as a sole source is not good enough to sustain larval growth and survival of P. *Japonicus* probably due to both its physical property and unsatisfactory amino acid profile, but it can be used together with other protein sources such as casein in microparticulate diets of larval P. *japonicus* (Table 4 and Fig. 2). While, crab protein was recommended as the sole protein diet formulations for juvenile P. *japonicus*.

EFFECTS AND FREE AMINO ACIDS ON GROWTH OF PRAWN

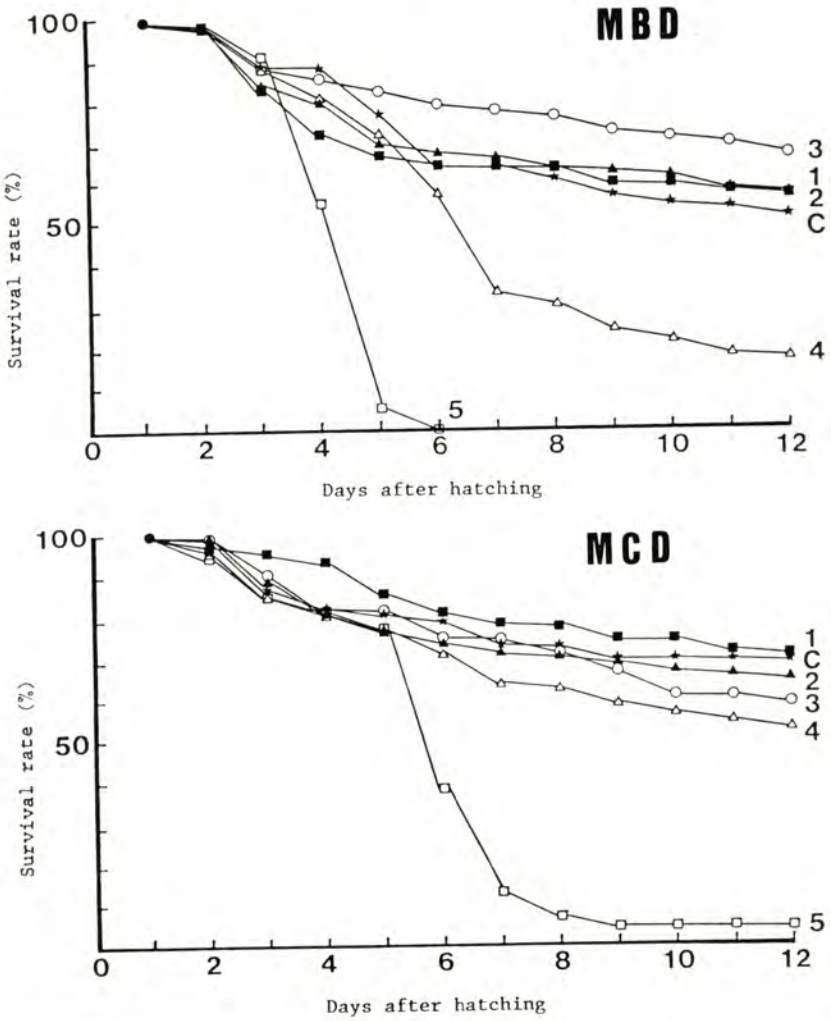
Deshimaru and Kuroki (1974, 1975a, b) and Deshimaru (1982) prepared a diet with a crystalline amino acid mixture instead of protein,

Tab. 4. — Formulation and proximate analysis of test diets.

Ingredient (g/100g)	Diet (MBD)[1]				
	1	2	3	4	5
Crab protein		15.0	35.0	55.0	6
Casein	51.0	39.2	23.4	7.6	
Amino acid mix.[2]	3.0	3.0	3.0	3.0	3
Alpha starch	5.0	5.0	5.0	5.0	5
Dextrin	4.7	4.7	4.7	4.7	4
Feed oil[3]	4.0	4.0	4.0	4.0	4
Corn oil	2.0	2.0	2.0	2.0	2
N^3-HUFA	1.0	1.0	1.0	1.0	1
Cholesterol	0.5	0.5	0.5	0.5	0
Soybean lecithin	3.0	3.0	3.0	3.0	3
Mineral mix.	5.0	5.0	5.0	5.0	5
Vitamin mix.	5.0	5.0	5.0	5.0	5
Glucosamine-HCl	1.0	1.0	1.0	1.0	1
Sodium citrate	0.5	0.5	0.5	0.5	0
Sodium succinate	0.5	0.5	0.5	0.5	0
Alpha cellulose	13.8	10.6	6.4	2.2	
TOTAL	100.0	100.0	100.0	100.0	1
Carrageenan	5.0	5.0	5.0	5.0	5
Crude protein (%)					
MBD	49.9	46.7	47.1	46.3	4
NCD	51.2	47.6	48.2	48.0	4
Crude lipid (%)					
MBD	11.2	7.5	11.2	10.2	8
MCD	2.1	2.8	4.7	4.4	3
Gross energy (Kcal/g)					
MBD	4.29	3.75	4.13	3.99	3
MCD	3.50	3.36	3.57	3.54	3

1 Five MBD and control diet were Cholesterol-lecithin (MCD)
2 L-Phenylalanine 0.24, L-Arginine-HCl 0.54, L-Cystine 0.3, L-Tryptophan 0.2, L-Histidine-HCl, H_2O 0.12, DL-Alanine 0.9, L-Aspartic acid-Na 0.42, L-Lysine-HCl 0.24, L-Valine 0.3, Glycine 0.18.
3 Riken Vitamin Ltd., CO., Japan.

and found that such a diet was unsuitable for sustaining growth and survival of juvenile prawn. The supplemental amino for larval prawn were examined using artificial diets containing carrageenan as a binder (Teshima et *al.*, 1986). The supplementation of a casein diet with crystalline L-arginine HCl improved its nutritive value better than only casein. In the feeding trial, about half the casein in the casein-based diets was replaced with a mixture of crystalline amino acid, either coated or uncoated with a nylon-protein membrane, and balanced to approximate the amino acid protein to that of prawn larval whole body protein. Diet containing crystalline amino acids gave survival rates and growth equal to or higher than the control group receiving live food. These results indicate that prawn larvae are probably able to utilize crystalline amino acid mixture in contrast to juvenile prawns which lack this ability.



Fi. 2. — Survival of larval *P. japonicus* fed MBD and MCD. The diet numbers shown are as follow : diet (CPC casein = 0.51), Diet 2 (15 : 39), Diet 3 (35 : 23), Diet 4 (55 : 8), Diet 5 (65 : 0). 'C' indicates a protein mixture diet (egg yolk powder 15.5, Casein 10.0, Albumin 7.0, Squid meal 15.0, Yeast powder 10.0, Krill powder 15.0, Scallop extract powder 4.0, Amino acid mix, 5.0).

THE DIETARY EFFECTS ON OVARIAN MATURATION OF PRAWN

The technique of eyestalk ablation or removal has been widely used in the operation of seedling production of shrimp due to its effect of induced maturation of gonad and spawning. However, the operation often causes the stress to spawners and therefore high mortality. Mussels, oysters, clams and squids are employed for the broodstock foods and it is suggested that those contain the substances which induce the ovary

maturation. The effects of food materials on ovary maturation were investigated in this study.

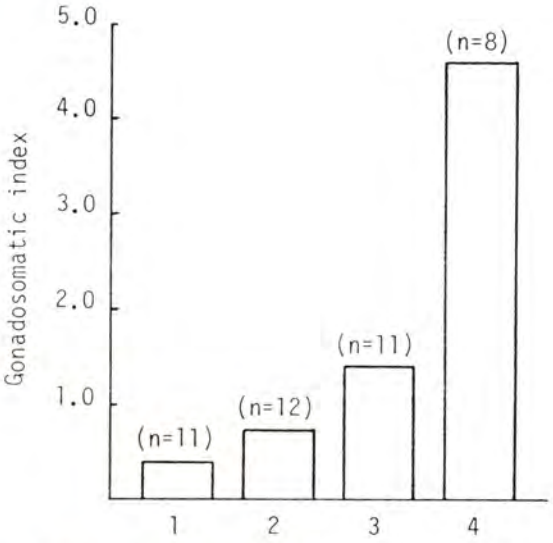


Fig. 3. — Variation of gonadosomatic index by destalking and feeding.
1. Initial (Before treatment)
2. Non-destalking and clam-fed
3. Destalking and unfed
4. Destalking and clam-fed.

Both eyestalks of shrimps (about 20 g) were removed by trying with a surgical thread after the acclimation to the experimental condition. Destalking group was divided into unfed and clam-fed groups, and control group was fed clam. After 10 day rearing period, the tissues were dissected out and gonadosomatic index (GSI) was measured (Fig. 3).

GSI of control groups was 0.69 whereas that of destalking groups was higher (1.42 in unfed and 4.54 in clam-fed groups, respectively). Furthermore, the effect of food after destalking was very important since GSI in clam-fed group was much greater than that in unfed one. It seems that the substances in clam meat, which induce the ovary maturation, are lipid fraction and low molecular nitrogen compounds.

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Lipid requirements of shrimp

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Abstract. — *A review of lipid requirements of shrimp and other crustacean species, when applicable, is presented. Qualitative requirements of crustaceans for cholesterol, fatty acids and phospholipids have been documented, but quantitative requirements generally remain underfined. Potential interrelationships between different classes of lipids that may influence requirements as well as differences due to age and species need to be investigated. A more accurate definition of lipid requirements can be achieved by supporting traditional evaluations based upon weight gain and survival with biochemical or histological evidence.*

INTRODUCTION

No absolute dietary lipid requirement for shrimp exists. Rather, provision of sufficient lipid is based upon the satisfaction of requirements for specific nutrients such as fatty acids, sterols and carotenoids and for energy. Requirements for these lipid classes may vary according to diet composition and the species under consideration. Research devoted to defining the best quantity and quality of dietary lipid has been principally confined to juvenile and subadult forms of shrimp. Most studies have used individuals having initial body weights below 10 g and generally within the range of 0.1 to 1.0 g. This review of the lipid requirements of shrimp also provides information from research results involving other crustaceans species whenever there is apparent aplicability.

REQUIREMENTS

Dietary Lipid

Past nutritional studies with crustaceans indicate that the best survival and growth responses are achieved when the dietary level of one or a mixture of oils is between 5 and 8 % (Table 1). In most of these studies, however, the level observed to be best is ultimately influenced by the quality and quantity of dietary protein, the amount, type and availability

Tab. 1. — Summary of observations concerning EFA nutrition of shrimp.

Investigation	Species	OBSERVATIONS		
		Preferred dietary PUFA 18 : 3n-3 (*) vs 18 : 2n-6 (+)	Limited conversion of 18 : 3n-3 to C20, C22 HUFA (+)	Greater nutritive value of 20 : 5n-3, or 22 : 6n-3 vs 18 : 3n3 (+)
Bottino *et al.* (1980)	P. setiferus P. azrecus P. duorarum	– –	+	– –
Guary *et al* (1976)	P. japonicus	*	– –	+
Kanazawa *et al.* (1977a)	P. japonicus	– –	– –	+
Kanazawa *et al.* (1977b)	P. japonicus	*	– –	
Kanazawa *et al.* (1978)	P. japonicus	– –	– –	+
Kanazawa *et al.* (1979c)	P. japonicus	*	– –	– –
Kanazawa *et al.* (1979d)	P. japonicus	– –	– –	+
Read (1981)	P. indicus	– –	+	– –
Colvin (1976)	P. indicus	– –	+	–

of other energy sources and the source of oil. Nevertheless, high dietary levels of oils are usually associated with significant growth retardation. As levels increase, the amount in the hepatopancreas has been found to increase correspondingly. Exceedingly high levels may not be effectively processed. In addition, food intake is generally thought to be a function of energy supplement and excess dietary lipid may therefore inhibit appetite (Church and Pond, >1982). A significant reduction in the growth rate of *Palaemon serratus* was observed when the level of corn or cod liver oil was increased from 7.5 % to 15 % (Forster and Beard, 1973). Deshimaru et *al.* (1979) found that when a dietary mixture of pollack liver oil and soybean oil was increased beyond 6 %, the growth rate of *Penaeus japonicus* was reduced. Castell and Covey (1976) used 1 %, 5 %, 10 % and 15 % levels of cod liver oil as a lipid source for adult *Homarus americanus* and found the 5 % dietary level to be most beneficial. Growth was not significantly improved when the cod liver oil was increased to 10 % and 15 % levels. Kanazawa et *al.* (1977a) observed a reduction in body weight gain of P. *japonicus* when a dietary level of 16 % was used. Davis and Robinson (1986) used menhaden fish oil as a lipid source (0 to 15 % in 3 % increments) for crayfish *Procambarus acutus acutus* and found reduced growth of crayfish fed diets containing 9 % or more lipid.

Growth of shrimp is superior when diets contain lipids (oils) of marine rather than vegetable origin (Guary et *al.* 1976; Kanazawa et *al.*, 1977a). Powdered pollack residual oil and short necked clam lipid were better dietary lipid sources than soybean oil for *Penaeus japonicus* (Kanazawa et *al.*, 1977a). A marine and vegetable oil mixture has generally achieved the best results (Read, 1981; Deshimaru et *al.*, 1979). Deshimaru et *al.* (1979) found that 6 % of a mixture of pollack liver oil and soybean oil provided in a ratio between 3 : 1 and 1 : 1 was associated with high growth and feed efficiency of *P. japonicus*.

Fatty Acids

Fatty acids may be divided into four different groups as follows :

(1) fatty acids that can by synthesized *de novo* from acetate. This group includes all even carbon number, straight chain, saturated fatty acids up to 20 or 22 carbons. The most abundant is 16 : 0 (palmitic acid). Shrimp, like other animals, apparently possess a delta- 9-desaturase enzyme system which can convert these saturated fatty acids to monoenoic (monounsaturated) forms,

(2) unusual fatty acid groups which include :

  (a) odd carbon number fatty acids such as 13 : 0, 15 : 0, 17 : 0 and 19 : 0.

  (b) non-methylene interrupted fatty acids which have two or more double bonds that are separated by more than three carbons;

  (c) cyclopropanoic and cyclopropenoic fatty acids,

(3) essential fatty acids (EFA) composed of two families of polyunsaturated fatty acids (PUFA). The linoleic (n-6) family has the first double bond at the sixth carbon from the methyl end of the molecule and has the greatest EFA value for homothermic animals. The linolenic (n-3) family has its first double bond between the third and fourth carbon from the methyl end. Neither of these two families of fatty acids are synthesized *de novo* by crustaceans ((Kayama et *al.* 1980) and the linolenic family has been observed to have the greatest EFA value for marine animals (Castell and Boghen, 1979),

(4) essential fatty acids (EFA) composed of the linoleic and linolenic, n-3 and n-6, families of highly unsaturated fatty acids (HUFA).

Castell (1983) and Chanmugam et *al.* (1983) indicated that the body tissue of marine crustaceans generally tends to contain proportionately higher levels of fatty acids of the linoleic family of HUFA and PUFA than that of freshwater crustaceans. The freswater species tend to have higher levels of acids of the linoleic family.

Kanazawa and Teshima (1977) injected juvenile *Penaeus japonicus* with acetate -14C and found activity almost exclusively associated with the saturated (16 : 0, 18 : 0) and monounsaturated fatty acids (16 : 1, 18 : 1 n-9, 20 : 1n-9). Kanazawa et *al.* (1979a) also showed that *P. japonicus* has the ability to incorporate palmitic acid (16 : 0) into saturated and monosaturated fatty acids, but little is transformed into PUFA or HUFA,

Tab. 2. — Summary of investigations that evaluated response of crustaceans to quantity and quality of dietary lipid.

Investigations	Species	Lipid Sources	Lipid Level (%)	Protein Source and Amount	Observations
Castell & Covey (1976)	Homarus Americanus	cod liver oil	1, 5, 10, 15	50 % casein	best weight increase with 5 % CLO
Davis & Robinson (1985)	Procambarus acutus acutus	menhaden fish oil 12, 15	0, 3, 6, 9, 12, 15 + 4.5 % gelatin	28.1 % casein 0-6 %; growth reduction at 9 % or more lipid	no difference among
Deshimaru *et al.* (1979)	Penaeus japonicus	pollack liver oil (PLO) + soybean oil (SO) 1 : 11 : 1)	3, 6, 9, 12 egg albumin	60 % casein and at 6 % PLO : SO (3 : 1 or	best weight increase
Kanazawa *et al.* (1977a)	P. japonicus	soybean oil, PRO SNCO	8, 12, 16	50 % casein	best growth at 8 % SNCO; growth inhibition at 16 %

PRO : pollack residual oil
SNCO : short necked clam oil.

linoleic acid (18 : 2n6), linolenic acid (18 : 3n3), eicosapentaenoic acid (20 : 5n3) or docosahexaenoic acid (22 : 6n3). These early studies indicated that PUFA and HUFA might be essential for the growth of *P. japonicus*.

In a series of experiments (Kanazawa et *al.* 1977b, 1979b, 1979c), dietary additions of 1 % levels of either 18 : 2n6 and 18 : 3n3 to diets containing either oleic acid, pollack *(Theragra chaleogramma)* residual liver oil, soybean oil, or short-necked clam *(Tapes phillipinarium)* lipids improved weight gain of *P. japonicus*. Shewbart and Mies (1973) introduced levels of linolenic acid ranging from 0.5 % to 5 % into a commercially produced shrimp feed and observed the growth response of *P.aztecus* juveniles. A level between 1 % and 2 % appeared to achieved the best response. Read (1981) found that the addition of 18 : 2n-6 and 18 : 3n-3 fatty acids to diets for *P. indicus* improved growth and survival.

The nutritive value of linolenic acid was found to be greater than that of linoleic acid for the prawn, *P. japonicus* (Kanazawa et *al.* 1977b). Guary et *al.* (1976) also found that better growth of *P. japonicus* was achieved with diets containing high levels of 18 : 3n3 than with those containing comparative levels of 18 : 2n6. Fenucci et *al.* (1981) indicated that the ratio between 1.18 and 1.00 for n3 fatty acids to 1 of 18 : 2n6 should give the best growth response for the growth of *P. stylirostris*. Martin (1980) fed *Palaemon serratus* diets containing different ratios of 18 : 2n-6 and 18 : 3n-3 by varying the relative proportions of soybean oil and linseed oil of the dietary lipid mix. The best growth was achieved with a 18 : 2n-6/18 : 3n-3 ratio of 2.2. Kanazawa et *al.* (1978, 1979d) have observed that n-3 HUFA (20 : 5n-3 and 22 : 6n-3) possess higher activity as essential fatty acids than that of n-6 (18 : 2n-6) and n-3 (18 : 3n-3) PUFA families.

The 20C and 22C HUFA generally demonstrate higher nutritive value than 18C PUFA (Table 2). These results suggest that the often observed growth promoting response to the addition of dietary oils such as shrimp head oil (3 % to *Macrobrachium rosenbergii*, Sandifer and Joseph, 1976), sardine or clam oil (4 % to *Penaeus japonicus*, Guary et *al.*, 1976) and cod liver oil (4 % to *Palaemon serratus*, Martin, 1980) is due to >- 20C n-3 HUFA. Sandifer and Joseph (1976) suggested that the functions of n3 fatty acids was for the biosynthesis of longer chain polyunsaturated fatty acids for tissue incorporation, whereas, the n6 fatty acids were utilized as energy sources. Kanazawa et *al.* (1977a) showed that the low nutritive value of soybean oil for prawns seems to be due to the small amount of HUFA. On the other hand, pollack residual oil containing large amounts of n3 HUFA improved prawn growth rate.

Shrimp appear to have little or no capacity to biosynthesize n-3 PUFA to n-3 HUFA. Kanazawa et *al.* (1979e) found that *Penaeus japonicus* had some ability to convert [1-14C] linolenic acid (18 : 3n-3) to 20 : 5n-3 and 22 : 6n-3. Kanazawa et *al.* (1978) concluded that growth of *P. japonicus* receiving either 18 : 2n6 or 18 : 3n3 dietary fatty acids was inferior to that achieved with 20 : 5n3 and 22 : 6n3. Although this species of prawn can elongate and desaturate 18 : 3n3 or 18 : 2n6 fatty acids, the requirements of the 20 and 22 C HUFA could not be optimally satisfied by dietary provisions of 18C PUFA.

Read (1981) indicated that juvenile *P. indicus* had a limited capacity to chain elongate and desaturate linoleic acid and linolenic acid to C20

and C22 HUFA. He also demonstrated that survival and weight gain associated with a dietary mixture of 18 : 2n6 and 18 : 3n3, 0.5 % + 0.5 %, respectively, was worse than 18 : 2n6 alone. Superior survival, feed conversion and weight gains were achieved when a combination of dietary 18 : 2n6 and 3 % of oils rich in HUFA were provided. Colvin (1976) fed *P. indicus* diets containing sunflower, linseed, soybean or groundnut oil at a 5 % level. No significant differences in growth were observed after a 35 days feeding trial. A comparison between the fatty acid profile of the experimental shrimp and that of wild caught individuals suggested a limited capacity for bioconversion of 18C n-6 or n-3 fatty acids to > – 20C n-6 or n-3 fatty acids.

Kanazawa et *al.* (1979f) investigated the nutritive value of shortnecked clam oil (*Tapes* oil) versus pollack liver oil for the growth of *P. japonicus*. They found that *P. japonicus* fed a diet supplemented with 1 % Tapes lecithin grew best. The lecithin was the best phospholipid source and contained proportionately greater amounts of 18 : 2n6, 18 : 3n3, 20 : 5n3 and 22 : 6n3.

Bottino et *al.* (1980) stated that shrimp, *P. setiferus*, brown shrimp, P. aztecus and pink shrimp, *P. duorarum* have little ability for the conversion of C18 unsaturated fatty acids into C20 and C22 polyunsaturated fatty acids. Kanazawa et *al.* (1977a) evaluated pollack residual oil, soybean oil and short necked clam oil as lipid sources for *P. japonicus*. They found that the greatest growth was obtained when prawn received 8 % short necked clam oil containing 1.1 % of 20 : 5n3 and 1.1 % of 22 : 6n3.

A quantitative requirement for any described essential fatty acid has yet to be established for any species of shrimp. Qualitative requirements for linolenic (18 : 3n3), linoleic (18 : 2n-6), eicosapentaenoic acid (20 : 5n-3) and docosahexaenoic (22 : 6n-3) acids have been documented. A specific requirement of the 18 C EFA when satisfactory levels of 20 : 5n-3 or 22 : 6n-3 are available has not been determined. Nevertheless, 20 : 5n-3 and 22 : 6n-3 are generally preferred to 18 : 3n-3. An exogenous source of the HUFA is generally necessary because biosynthetic conversion from 18 : 3n-3 is either absent or limited. Significant increases in weight (gain) growth have been achieved with relatively small additions of PUFA and HUFA. Levels as low as 0.075 % of the diet may be sufficient to satisfy the requirements for particular essential fatty acids. Experimentation leading to the precise determination of fatty acid requirements should be directed toward the provision of pure fatty acids in a triglyceride or methyl ester form, rather than through the provision of oils rich in fatty acids of particular interest. The percent composition of other dietary fatty acids should be maintained constant. Qualitative and quantitative analysis of the fatty acids in the experimental diet should be conducted. Tissue analysis of shrimp should always complement feeding studies and should be conducted prior to the initiation of an experiment, after a preconditioning period that precedes the feeding of the experimental diets and at the termination of the experiment. Relating tissue and dietary concentration of suspected EFA should assist in identifying biosynthetic pathways and other fatty acids that may serve as possible indicators of an EFA deficiency.

Dietary polyunsaturated fatty acids also appear to be necessary for successful ovarian maturation (Middleditch et *al.*, 1979, 1980). During induced ovarian maturation, through bilateral eyestalk ablation, the neutral lipids of the ovary of *P. setiferus* contained higher proportions of monoenes and 22 : 6n-3 and lower proportions of 20 : 4n-6 and 20 : 5n-3 than those of non-destalked prawns (Teshima et *al.*, 1988). Nevertheless, no true relationship between dietary PUFA and HUFA and the achievement of ovarian maturity has been established.

Sterols

Most animals are capable of synthesizing sterol from acetate, but shrimp, like all other crustaceans, are incapable of *de novo* sterol synthesis from acetate (Teshima and Kanazawa, 1971). Several estimates of the cholesterol requirement of shrimp and other decapod crustaceans have been reported (Table 3). Kanazawa et *al.* (1971a) showed that the prawn *P. japonicus* fed with a sterol-free diet had poor growth and survival rates, but grew well on a diet containing 0.5 % cholesterol. Other workers also demonstrated the necessity of dietary cholesterol for good growth of *P. japonicus* (Deshimaru and Kuroki, 1974), juvenile lobster *Homarus americanus* (Castell et *al.*, 1974; D'Abramo et *al.*, 1984) and crayfish (*Pacifastacus leniusculus)* (D'Abramo et *al.*, 1985a).

An optimum dietary level of 0.5 % cholesterol has been found for *P. japonicus* (Kanazawa et *al.*, 1971a) and *H. americanus* juvenile (Castell et *al.*, 1975). Other researchers have obtained the best growth of *P. japonicus* with a diet containing 0.2 % (Shudo et *al.*, 1971) and 2 % (Deshimaru and Kuroki, 1974) cholesterol. D'Abramo et *al.* (1984) demonstrated that a level of dietary cholesterol as low as 0.12 % was satisfactory for normal growth of lobster and indicated that a cholesterol level ranging from 0.19 to 0.59 % did not improve growth rate. The recent work of Kean et *al.* (1985) also suggested that the dietary cholesterol requirement for lobster, *H. americanus*, is probably between 0.25-0.5 %. D'Abramo et *al.* (1985) found that a dietary sterol level of 0.4 % is associated with the best growth of juvenile crayfish, *Pacifastacus leniusculus*. Briggs et *al.* (1988) added 0.5 % and 1.0 % cholesterol to a semi-purified diet containing 0.12 % cholesterol and did not observe any effects on growth or survival of juvenile prawn, *Macrobrachium rosenbergii.* Teshima and Kanazawa (1983) have demonstrated that the absorption rate of dietary cholesterol is improved by adding lipids such as palmitic acid, tripalmitin or chicken-egg lecithin. The wide range of results concerning sterol requirements may primarily reside in differences in ingredient composition, particularly other lipid components of the test diets. The duration of experiments may also be a principal factor influencing the interpretation of results.

Teshima and Kanazawa (1973) have demonstrated that crustaceans can utilize dietary C28 and C29 sterols, such as i- sitosterol, ergosterol and stigmasterol, by bioconversion (dealkylation) of these sterols to cholesterol. The prawn has been shown to be capable of effectively absorbing both dietary cholesterol and phytosterols such as ergosterol and i-sitosterol (Teshima et *al.*, 1974). However, *Penaeus japonicus* fed on diets containing either ergosterol, stigmasterol, or i-sitosterol have lower growth and

Tab. 3. — Summary of investigations that evaluated sterol nutrition of shrimp and other crustaceans.

Investigations	Species	Sterol (s)	Dietary level (s) (%)	Observations
Castell *et al.* (1975)	Homarus americanus	cholesterol	0.5, 1, 2	best growth achieved with 0.5 % level
D'Abramo *et al.* (1984)	H. sp.	cholesterol & phytosterol mix	0.12, 0.19, 0.39, 0.59	0.12 % dietary cholesterol satisfactory for normal growth. At least a portion and possibility all of the sterol requirement is specific for cholesterol.
D'Abramo *et al.* (1985)	Pascifastacus leniusculus	cholesterol & phytosterol mix	0.22, 0.47	at least 0.4 % total dietary sterol mixsterols required for survival ptimum dietary level 0.5 % to 1 % Phytosterol mixture as effective as cholesterol in satisfying a portion of the sterol requirement.
Kanazawa *et al.* (1971a)	Penaeus japonicus	cholesterol, ergosterol, cholesterol, other sterols	0.05, 0.1, 0.5, 1, 5	best growth achieved with dietary level of 0.5 % cholesterol,cholesterol, other sterols other sterols were inferior to cholesterol
Kean *et al.*	H. americanus	cholesterol	0, 0.25, 0.5, 1.0	best growth at 0.5 % level
Teshima *et al.* (1983)	P. japonicus	cholesterol	0, 1, 5	best growth and survival at 1 % level.
Teshima and Kanazawa (1986)	P. japonicus	cholesterol β-sitosterol 24-methyl- cholesta-5, 22-dienol	0.5	cholesterol at highest r most effect feed conver and protein.
Teshima *et al.* P. japonicus (1989)	cholesterol β-sitosterol		0.5, 1.O	No effective cholesterol best growth at 1.0 % lev.

survival than those fed on diets containing cholesterol (Kanazawa et *al.*, 1971b). Teshima et *al.* (1989) found sitosterol to be ineffective in replacing cholesterol at a level of 0.5 % in diets for juvenile prawns *P. japonicus*. Partial substitution of cholesterol with sitosterol was associated with reduced weight gain, feed conversion efficiency and protein efficiency ratio. The rate of larval development of this species generally decreased as the amount of sitosterol proportionately increased as a substitute for dietary cholesterol. Neither a mixture (9 : 1 W/W) of i-sitosterol and cholesterol, nor 24-methylcholesta-5, 22-dienol served as a completely effective substitute cholesterol (Teshima and Kanazawa 1986). Teshima et *al.*, (1983) indicated that when either ergosterol or 24- methylenecholesterol was used as sterol source for larval *P. japonicus*, each had a similar dietary value to that of cholesterol.

D'Abramo et *al.* (1984) observed poor growth and survival and large amounts of phytosterols in the body tissue of lobster when fed diets containing phytosterols with and without cholesterols. Phytosterols as exclusive dietary sources of sterols yielded poor growth and survival. The sterol requirement is not entirely satisfied by exclusive provision of a mixture of i-sitosterol or desmosterol. Most likely because of inefficient bioconversion. At least a portion or possibly all of the sterol requirement of juvenile lobsters is apparently specific for cholesterol. D'Abramo et *al.* (1985) found that a mixture of phytosterols could spare the cholesterol requirement and still maintain good growth of crayfish *Pacifastacus leniusculus*. Because of the nature of the diet, the possibility of a total satisfaction of the sterol requirement by dietary phytosterols could not be determined. The ability to effectively use dietary phytosterols as an exclusive or partial substitution for cholesterol appears to be possibly dependent upon substitution diet of the shrimp species. The recent investigations of Teshima and Kanazawa (1986, 1987) suggest that the inferior nutritional value of i-sitosterol for the prawn *Penaeus japonicus* may be attributed to greater turnover rates in the midgut gland and less residence time in the muscle.

Phosphoglycerides

There is evidence indicating that phospholipids are required by some crustaceans. Kanazawa et *al.* (1979f) found that lecithin (Phosphatidylcholine) and cephalin (phosphatidylethanolamine) fractions derived from the lipid of the short-necked clam, *Tapes phillippinarum*, significnatly improved the growth of *P. japonicus* when incorporated into artificial test diets at 1 %. Teshima et *al.* (1986) found that weight gain and feed conversion efficiency of *P. japonicus*, increased significantly when 3 % soybean lecithin was added to a diet. Similar beneficial effects of dietary phospholipid for *P. monodon* were observed by Pascual (1984). Conklin et *al.* (1980) found that inclusion of soy lecithin in a purified diet for culture of juvenile lobsters, *H. americanus*, was critical for survival. D'Abramo et *al.* (1981) later identified the active component of the soy lecithin to be phosphatidylcholine and demonstrated that phosphatidylcholine containing PUFA yielded the best survival. Lack of soy lecithin or the presence of alternative phosphoglyceride sources was associated

with reduced serum cholesterol levels and the rate of transport of this nutrient (D'Abramo et *al.*, 1982, 1985b). The reduction in serum cholesterol levels was later attributed to a decrease of lipoproteins that are believed to serve as vehicles for cholesterol transport and to have phosphatidylcholine as their principal lipid component. Teshima et *al.* (1986) found a lower retention of dietary lipids, especially cholesterol, in body tissue of *P. japonicus* when fed a diet lacking soy lecithin. Prawns receiving a diet with soybean lecithin had a higher concentration of phospholipids in their tissue than those fed a diet with no lecithin supplement. These data indicate that in some instances, phospholipid synthesis may be insufficient based upon observed increased in tissue levels corresponding to dietary increases. Insufficient dietary phospholipid also reduces the effective utilization of dietary lipids such as triglycerides and cholesterol.

Kean et *al.* (1985) demonstrated that juvenile *H. americanus* do not require dietary soy lecithin for survival when purified protein derived from rock crab, *Cancer irroratus*, replaced casein as the principal protein source in a purified diet. They also could not detect any significant growth enhancement when the lobsters were fed crab protein based diets supplemented with either soy lecithin or crab phospholipids at levels of 3.0 and 6.0 % of the diet. Supplementation of lecithin to a diet fed to *Macrobrachium rosenbergii* did not increase survival nor promote growth (Hilton et *al*, 1984; Briggs et *al.*, 1988).

A dietary source of phospholipids is an important factor in growth and survival of larvae of *P. japonicus* (Kanazawa, 1983; Kanazawa et *al.*, 1985). A 3.0 % addition of soy lecithin to a larval diet was essential for growth and survival from the nauplius to the postlarval stage and was most effective when other dietary lipid sources such as 18 : 1n-9 and HUFA, or pollack liver oil were provided. The phospholipid requirement of larvae of *P. japonicus* was estimated to be between 0.5 and 1.0 % of the diet. The most effective phospholipids were phosphatidylcholine and phosphatidylinositol, specifically those containing HUFA and PUFA in their ¿ and i positions.

There is no evidence of the production of bile acids by crustaceans, suggesting that the metabolic processes of emulsification, digestion and transport of lipids in crustaceans are unique. Lipid transport in shrimp is accomplished primarily by high density lipoproteins (HDL) (Teshima and Kanazawa, 1980a). Teshima and Kanazawa (1980b) found that high density and very high density lipoproteins in the serum of the prawn, *P. japonicus* contain substantial amounts (65-85 %) of polar lipids. Polyenoic fatty acids, principally docosahexaenoic acid, make up almost 50 % of the fatty acids of the lipids of the serum lipoproteins of this shrimp species. Efficient transport of lipids may reside in the provision of dietary phospholipids containing polyenoic fatty acids.

The need for dietary phospholipids appears to be related to the ingredient composition of the remainder of the diet. Lack of exogenous phospholipids has not been associated with mortality of any species of shrimp. Some growth enhancing effects associated with dietary phosphatidylcholine and phosphatidylinositol may be related to an efficient provision of choline and inositol which are at deficient levels in the diet.

Carotenoids

Pigmentation of shrimp is attributed to isoprenoid lipid compounds called carotenoids. Normal pigmentation of crustaceans can only be achieved through an exogenous source of these compounds. Carotenoids have been associated with enhanced growth, reproductive rates and fecundity in crustaceans. Astaxanthin is the carotenoid pigment most often associated with shell pigmentation. The degree of pigmentation appears to be related to the quantity and quality of dietary carotenoids (D'Abramo *et al.* 1983) and to other dietary nutrients. Otazu-Abrill et *al.* (1982) found that dietary methionine and isoleucine were associated with enhanced levels of pigmentation in *Palaemon serratus*.. The suggested nutritive role of carotenoids needs to be empirically documented.

CONCLUSION

A thorough knowledge of the lipid requirements of shrimp is lacking. Future investigations need to be directed toward a greater understanding of quantitative requirements. Determinations should not be merely limited to measurements of weight gain and survival but rather should be complemented with well documented, diet dependent, biochemical and histological differences. Results must also be submitted to the appropriate statistical analysis to determine if observed differences are truly significant. Precise quantification can only be achieved through such approaches.

Lipid requirements of shrimp may be dependent upon other lipid or non-lipid constituents of the diet or age and such interactions need to be considered. Larval stages of species of crustaceans most likely require higher levels of dietary lipid relative to juveniles and adults. These forms consume food containing high levels of lipid, particularly if the species is carnivorous. Finally, care should be exercised in planning the duration of an experiment. If insufficient time is allowed for a response to occur, the erroneous conclusions can accordingly result.

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Vitamin requirements of juvenile Penaeid shrimp

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Abstract. *— The results of supplementing crustacean feeds with vitamins are examined specifically from the standpoint of shrimp culture. Micro-nutrients selected for discussion include : water-soluble vitamins of the B-complex, choline and inositol, vitamin C and the fat-soluble group of vitamins; A, D, E and K. Ways in which utilization of vitamins and ultimately dietary demand are altered by physiological state, conditions of culture, as well as factors which impact on feed levels, are explored.*

INTRODUCTION

Considering the commercial importance of penaeid shrimp culture there are surprisingly few specifics on the value of supplementing formulated feeds with vitamins. One possible reason for this lack is the relative newness of the field. While there was some scattered early work on general aspects of crustacean nutrition (see Beerstecher 1952; Fisher 1960), nutritional research focusing on shrimp and other large benthic crustaceans didn't start until the early 1970's with the advent of appropriate test diets (Kanazawa et *al.* 1970; Meyers and Zein-Eldin 1972; Castell and Budson 1974). A second, and probably more significant, reason for this lack of research is that it was unwarranted. In spite of early concerns that development of effective shrimp feeds would require a sophisticated understanding of crustacean vitamin requirements (New 1976), a variety of formulated feeds are available for commercial culture of juvenile shrimp (New 1987; Lim and Persyn 1989). Typically, these feeds use vitamin premixes derived directly from formulations used in vertebrate feeds and which are of unknown efficacy for shrimp. However, this uncertainty has not hindered the progress of commercial culture. Over the last few years, intensive pond culture of penaeid shrimp, which is reliant on addition of formulated feeds, has become major industry in several parts of the world. Specific problems which conceivably might have been related to vitamin deficiencies have not been noted and there was no sense of urgency about

detailed studies on requirements. As a consequence, overall knowledge regarding vitamin needs of shrimp and related crustaceans remains limited.

While reliance on vertebrate nutritional patterns has worked reasonably well, some caution is appropriate in that significant differences in metabolic capability do exist between these two groups of animals. With this in mind, the present review on vitamin requirements of juvenile shrimp has two goals. The first of these goals is to set the sparse details of shrimp vitamin requirements within the general vertebrate model and to add appropriate insights from studies with other arthropods, primarily insects. The second goal is to attempt to prioritize research thrusts needed to sustain future growth of the penaeid culture industry.

DEFINITION OF A VITAMIN

While once it may have been fairly easy to define a vitamin, this is no longer true. Originally these nutrients were identified by their ability to alleviate specific deficiency symptoms when fed to various mammals and birds. Although absolutely vital for various aspects of growth, health or reproduction, only minute amounts were required in the diet. This was explained by the idea that the role of these essential nutrients was limited to serving as co-factors for enzymatic reactions. A total of 15 compounds are typically considered vitamins. Most of these factors : thiamin, riboflavin, niacin, vitamin B6, pantothenate, folate, vitamin B12, biotin, choline, myo-inositol (inositol) and vitamin C (ascorbic acid) are water-soluble. The remaining four vitamins; A, D, E and K, are fat-soluble.

While they can still probably be classed as either water-soluble or fat-soluble, little else regarding the traditional view of vitamins remains uncomplicated as more and more is gleaned about their biochemical action. Originally, essentiality was thought to be a consequence of an early evolutionary loss leading to a total absence of synthetic ability in multicellular animals. It has been found, however, that the situation is a great deal more complex. Some vitamins, such as niacin, can be synthesized by a number of animals, although typically in insufficient quantities to meet physiological demand without some dietary input. For most terrestrial vertebrates ascorbic acid (vitamin C) is not a dietary requirement at all in that synthesis is more than sufficient to meet typical physiological demand. The role of these factors is also being expanded as some vitamins have been found to act in ways other than as co-factors. Vitamins such as choline and inositol have been shown to have an important function as structural elements of membranes, while vitamin D acts primarily as a hormone in regulating calcium levels in birds and mammals.

As these new aspects of vitamin nutrition have been elucidated it has become harder and harder to define this collection of compounds so as to exclude other nutrients. For example, in the case of shrimp cholesterol might easily be considered a vitamin in the traditional sense. An inability to synthesize the basic ring structure of this fat-soluble compound is characteristic of all crustaceans, insects, and other arthropods which have been tested. Because of this metabolic lesion, a dietary requirement for cholesterol characterizes the entire phylum Arthropoda (see Dadd 1983).

While it is not known to act as a co-factor, cholesterol is an important constituent of biological membranes in an analogous fashion to choline and inositol. This sterol also is needed in arthropods as a precursor of ecdysone, the molting hormone. In this respect the role of cholesterol is very similar to the relationship between cholecalciferol (vitamin D) and the various active metabolites of this molecule which actually regulate mineral levels in terrestrial vertebrates. While one might quibble that the dietary level of cholesterol needed by crustaceans is not minute enough to include it with the vitamins, this distinction weakens considerably if one considers the levels of ascorbic acid or the amount of choline containing phospholipid presently being used in some crustacean diets. True, there isn't a well defined deficiency syndrome for cholesterol but this is unfortunately true for almost all vitamins when it comes to crustaceans.

NUTRITIONAL UNIQUENESS OF SHRIMP

In the above context, it can be seen that the collection of dietary compounds commonly discussed as vitamins in relationship to crustacean nutrition are delineated in large part by tradition based on studies with vertebrates. Thus, it becomes important to consider how useful is this model in understanding nutrient needs of animals in the invertebrate phyla Arthropoda. One important question would be; are there other nutrients, like cholesterol, which are unique to the nutritional needs of shrimp and other crustaceans.

In answering the above question it is particularly useful to look at nutritional studies with other arthropods. While what is known about the nutrition of shrimp and related crustaceans may be somewhat limited, a host of insect species have been studied. In all the studies with various arthropods there is no hint of an additional unique nutrient requirement characteristic of the phylum. This is not to suggest that the potential for improving shrimp growth by focusing on crustacean metabolic needs should be ignored. For example while it is likely that crustaceans are able to synthesize glucosamine, the primary organic component of the exoskeleton, addition of shell waste, chitin or glucosamine to shrimp feeds generally has proven to be beneficial for growth. This boost in growth rate most likely reflects the advantages of sparing metabolic effort for the biosynthesis of glucosamine (see Conklin, 1983).

One point that is seen in examining work with insects is the possibility that individual species or somewhat larger taxonomic groupings can have unique nutrient requirements. For example Dadd (1983) pointed out that, in addition to the 10 amino acids which are typically essential for all animals, a number of other amino acids are either essential or growth promoting in the diet of many insect species. Dadd (1983) notes the crayfish *Astacus leptodactylus*, like some species of mosquito, is unable to synthesize the amino acid asparagine. Provasoli and D'Agostino (1969) found the brine shrimp *Artemia salina* required the amino acid putrescine. Other fairly unique requirements of at least some strains of *Artemia* are nucleic acids, a requirement shared with some species of flies (see Hernandorena 1983). Other possible nutrients which may be of interest in

this regard are; p-amino benzoic acid, ὶ-lipoic acid, glutathione, spermidine, and carnitine.

At the moment, there is no indication that any of the above possible requirements are required by penaeid shrimp. Fairly purified diets already exist for crustaceans (Kanazawa et *al.* 1970; Conklin et *al.* 1980; Kean et *al.* 1985) which support juvenile growth and survival. The required amount of any such unidentified nutrients would have to be quite low in order to be masked by unidentified contaminants. In that no requirement is seen with purified diets it is also unlikely that such a requirement would impact animals reared on practical diets containing a variety of complex feedstuffs. As will be discussed, however, shrimp and other marine decapods may be unique in their need for dietary phospholipids.

Tab. 1. — Recommended individual vitamin levels (mg/kg dry diet) for chick, trout, and shrimp feeds plus a summary of known dietary requirements for the shrimp (SDR).

Vitamin	Chick[a]	Trout[b]	Shrimp[c]	SDR
Thiamin	15	10	120	R[d]
Riboflavin	15	20	40	—
Pyridoxine	6	10	120	R
Pantothenic	20	40	100	—
Niacin	50	150	150	—
Folic acid	6	5	5	—
Biotin	6	1	1	—
Vit B—12	<0.1	0.1	< 0.1	—
Choline	1,000	3,000	600	?
Inositol	R	400	2,000	R
Vit C	R	100	10,000	R
Vit E	50	30	200	—
Vit A (IU)	4,500	2,500	5,000	—
Vit D (IU)	400	2,400	1,000	—
Vit K	1.5	10	40	—

[a] Standard purified reference diet for chicks (p. 48, NRC 1984)
[b] Recommended feed levels for coldwater fishes (p. 41, NRC 1981)
[c] Mix ≠4 developed for marine shrimp research (Kanazawa, see p. 156, New 1987)
[d] R = required; R = not required; ? = data contradictory; — = requirement not determined; see text for discussion.

While shrimp may share with insects a common requirement for cholesterol, in some other nutritional respects insects may not be a good model for these marine crustaceans. Dadd (1983) has pointed out that insects lack the heavily mineralized exoskeletons characteristic of crustaceans. Recent research also indicates that insects may be uniquely different from other invertebrates as well as vertebrates in that they do not use vitamin B12 dependent metabolic pathways (Halarnkar et *al.* 1987). Thus, in this case, the vertebrate model may be closer to that of crustaceans than the insect model. For many nutrient requirements, however, there may be little difference among animal phyla. This is particularly true of most of the so called B-complex vitamins : thiamin, riboflavin, niacin, vitamin B6, pantothenate, folate, and biotin. This is undoubtedly due to the fact that most central metabolic pathways are common to all multicellular orga-

nisms and as a consequence, there is a similar interaction between vitamin-dependent co-enzyme systems and cellular functions. This commonality of qualitative B-complex vitamin requirements of vertebrates and invertebrates was suggested quite early (Beerstecher, 1950) and has seemed to hold up. Certainly the similarities outweigh differences when comparing the nutritional needs of insects, the arthropod group which has been studied in the most detail, to those of vertebrates (Dadd 1983).

While useful in anticipating qualitative requirements of many micronutrients, the vertebrate model is limited when predicting quantitative needs. In general much higher levels of some vitamins are added in crustacean feeds compared to those levels recommended for other animals (see Table 1). How much of this difference in premix vitamin levels is related to metabolic demand in these two groups is unknown. There are a host of factors which are known to impact on metabolic demand in vertebrates, and probably in crustaceans as well, although the magnitude has yet to be defined. Even for normal metabolic needs within a species there is variation among individual animals in response to nutrient levels. In addition to normal biological variation, physiological state most certainly will modify demand. In many animals nutrient needs increase dramatically during periods of rapid growth. In shrimp and other crustaceans this will involve not only the building of tissue mass but uniquely the periodic process of molting. Reproduction in crustaceans, as in other animals which deposit extensive nutrient reserves into eggs for later use during embryonic development, also undoubtedly increases demand. Other changes in metabolic demand can be induced by changes in the diet of the animal. For example, increasing the lipid level in the diet increases the demand for vitamins that have an antioxidant function. The culturist can also impact the demand for vitamins by stressing the animals. Some of this stress may be purposeful such as increasing density while at other times this may be due to an inadvertent deterioration of optimal culture conditions. Infectious diseases and parasites have been shown to increase vitamin needs in many animals probably as part of a physiological response attempting to overcome these agents. Because of the variety of interaction impacting on metabolic demand, differences between vertebrates and crustaceans can not be evaluated as yet, however, studies with other animals suggests overall changes in physiological demand would be relatively minor.

A more likely explanation of the relatively greater need for vitamins in shrimp feeds would lie in the area of delivery. While nutritive levels added to the test feeds of terrestrial animals tend to be fairly close to actual metabolic demand this is not the case for crustaceans. In all animal culture systems there will be some delivery losses in attempting to provide nutrients which are both stable and yet readily available biologically. While actual, as opposed to formulated, levels of vitamins in feeds may vary depending on manufacturing processes and storage conditions such losses can be controlled to some extent through the use of proper temperature and humidity. However, even with freezing there is some loss of very labile nutrients such as ascorbic acid (Slinger et *al.*, 1979; Soliman et *al.*, 1987). Depending on the method of manufacture, temperature and moisture regimes necessary to develop stable pellets are such that large amounts of labile vitamins can be lost even though processing time is

relatively short. As these losses can be predicted based on experience, supplemental levels of nutrients high enough to compensate are added to the feed. In addition, a so-called extra margin of safety is usually included which increases the levels of vitamins two or three times above what is likely to be lost during processing. This final increase allows stated minimums on bag labels to be met with confidence even though analysis is not carried out on each lot.

Unique to aquaculture are the large losses from formulated diets resulting from leaching of water-soluble compounds (Goldblatt et *al.*, 1979; Slinger et *al.*, 1979). In that fish share the aquatic environment with crustaceans they face many of the same challenges in acquiring nutrients. Also in contrast to birds and mammals, fish lack any nutritional complexity that might be associated with maintaining a stable internal body temperature. Thus although there is a variety of information available to the crustacean nutritionist from studies with other animals, that derived from studies with fish is of particular value. Information on feed parameters such as leaching of water-soluble vitamins which relate directly to the aquatic environment can be quite useful to the shrimp nutritionist.

Tab. 2. — Vitamin levels (mg/kg dry weight) of selected organisms applicable as penaeid shrimp feeds.

Vitamin	Clams			Brine shrimp	
	[a]	[b]	[c]	adults[d]	cysts[e]
Thiamin	3	1	—	27	8
Riboflavin	12	14	12	17	25
Pyridoxine	4	—	—	8	—
Pantothenic	27	—	20	68	77
Niacin	65	49	98	130	116
Folic acid	2	3	—	1	—
Biotin	—	—	—	1	—
Vit B-12	0.5	0.6	3	3	—
Choline	2,900	—	—	6,100	—
Inositol	—	—	—	1,200	—
Vit C	550	350	—	49	—
Vit E	5	—	—	—	—
Vit A (IU)	3,700	7,500	17,000	14,000	37
Vit D (IU)	44	—	—	—	—
Vit K	—	—	—	—	—

[a] presented in Sidwell et al. 1975 (p. 3 + 9; clams, miscellaneous species) values converted assuming 80 % H_2O.

[b] presented in Sidwell 1981 (p. 379; Clam, Venus, Littleneck, Japanese) valves converted assuming 80 % H_2O.

[c] presented in Exler 1987 (p. 172; clam, mixed species, raw) values converted to dry weightdpresented in Simpson et al. 1983 (p. 191; Gallagher and Brown 1975 and M. Gallagher, personal communication 1982).

[e] from Stults 1974 (p. 65) values converted to dry weight.

Losses due to leaching are of course proportional to the time it takes an organism to devour its food. Shrimp and other crustaceans, which are slow benthic feeders, require large amounts of water-soluble vitamins to be added to the feed in order to offset these heavy delivery losses. The magnitude of these losses can be seen by comparing the recommended feed

levels (Table 1) with levels representative of natural prey items. While direct quantification of the diet of shrimp in the wild has yet to be done, vitamin analyses for some live food organisms appropriate for shrimp rearing can be found in the literature (Table 2). Two important food items are clams and brine shrimp. Chopped pieces of fresh bivalve mollusks such as the short-necked clam *Venerupis philippinarum* have been found to provide an excellent source of nutrition for *Penaeus japonicus* juveniles (Deshimaru and Shigeno 1972) and some information on vitamin content is available. Brine shrimp, the small filter-feeding crustacean *Artemia salina* is used as food in rearing a wide variety of crustaceans as well as fish (Leger et *al.* 1986). Brine shrimp nauplii are used extensively in the rearing of larval shrimp. Unfortunately, specific information on nauplii is not available but the data from analysis of cysts is probably indicative of vitamin content at hatching.

In the following discussion it must be remembered that the amount of a vitamin added to experimental feeds must be sufficient to cover all the various avenues of loss in addition to meeting the metabolic needs of shrimp. These complexities which are seldom measured in conjunction with differences in culture techniques between laboratories probably explain the apparent wide range of requirement found in the case of some vitamins. This variability where several individual studies exist should serve as a note of caution in the cases where only a single study exists for a particular vitamin.

B-complex vitamins

All eight of the B-complex water-soluble vitamins are known to be required by fish although a requirement for each has not been established in every cultured species (NRC 1981, 1983). For the shrimp *Penaeus japonicus* vitamin requirements as indicated by studies with purified test diets have been suggested only in the case of thiamin, 60 mg/kg of diet and pyridoxine, 120 mg/kg (Deshimaru and Kuroki, 1979). Requirements were estimated on the basis of differences in growth and a decline in whole animal levels of the vitamin. Deficiencies of thiamin and pyridoxine in fish are often characterized by nervous disorders, however, no behavioral changes in shrimp were reported. Confirming studies by other investigators or studies with other species have yet to be carried out.

Over a 12 week period, the growth rate of juvenile shrimp reared on the thiamin-deficient diet was only slightly reduced and survival was equal to those groups reared on the supplemented control diet. Total body content of thiamin in shrimp reared on the deficient diet was 0.4 mg/kg. Shrimp provided with 60 mg thiamin/kg diet had a total body content of 1.0 mg thiamin/kg; tissues became saturated at 1.4 mg thiamin/kg as feed levels were increased above 120 mg/kg diet.

A lack of pyridoxine led to a decrease in both survival and growth. Tissue levels of shrimp grown on the deficient diet after 12 weeks was only 0.08 mg pyridoxine/kg of whole animal. Survival and growth were best at a pyridoxine-HCl level of 60 mg/kg feed. At this feed level the vitamin content of the shrimp increased to 0.43 mg/kg shrimp. Doubling the feed levels saturated the total body level at 0.54 mg/kg but growth was not improved.

Requirements for the other B-complex vitamins have not been established. While it would be interesting to have this information in order to compare vitamin requirements among various groups of animals, its value in formulating practical shrimp feeds is limited. Even if the level of supplementation for some of these vitamins is marginal, deficiencies are unlikely because of other sources available to the animal.

Bacterial synthesis of vitamins in the gastrointestinal tract of some animals can result in substantial vitamin contributions. The level of bacterial synthesis in crustaceans has not been examined with regard to vitamin production. In general, compared with most mammals and birds, the gastrointestinal tracts of shrimp and other decapods of commercial interest are relatively simple and microbial synthesis may not provide appreciable quantities of vitamins. On the other hand, whatever amounts produced is likely to be efficiently utilized in that delivery losses are eliminated. For aquatic organisms, microbiological growth on pelleted feed and other surfaces also can contribute nutrients even in relatively clean intensive systems. In pond growout systems extensive input of vitamins may be obtained through natural productivity. In the case of catfish no requirement for vitamin C could be established in pond fed fish until the density reached 4,000 kg/hectare (Lovell and Lim, 1978).

Even if the source of vitamins is limited to the feed, knowing the exact requirement for each of the B- complex vitamins would be of little advantage to the shrimp culturist. The role of these vitamins is primarily limited to that of co-factor and, although requirements can vary somewhat, the absolute amounts are still minute. In that the cost of these vitamins is relatively minor considering the overall cost of the feed it is economically feasible to continue the present practice of providing generous supplemental levels. Experience with fish suggests that this approach will work for shrimp feeds as well and there is no real concern with defining requirements any closer than what we have now. At least in the case of the B-complex vitamins the lack of further research would not appear to have any appreciable effect on future developments of the shrimp culture industry.

A potential problem with the above approach may be the tendency to provide excessive amounts -- « just to be on the safe side ». Although conventional wisdom would suggest toxicity is a concern only with fat-soluble vitamins it should be noted there are a number of cases in shrimp and prawns where decreased growth rates are noted as dietary levels of water-soluble vitamins are increased beyond an optimum. Kitabayashi et *al.*, 1971 reported an excess of vitamin C inhibited growth of *P. japonicus*. Doubling the recommended amount (120 mg/kg) of pyridoxine decreased growth performance of *P. japonicus* (Deshimaru and Kuroki, 1979). Heinen (1984) reported deletion of riboflavin from a vitamin mixture led to increased growth rates for juvenile freshwater prawns *Macrobrachium rosenbergii*.

Choline and Inositol

As most animals are able to synthesize either one or both of these nutrients, the possibility that shrimp require both choline and inositol is intriguing. This possibility, however, should be viewed with some caution

as information on these two vitamins is both limited and, in some cases, contradictory. The question of need is further confused by a possible dietary requirement for phospholipids containing choline or inositol rather than the vitamins by themselves.

Compared to other vitamins, the amount of choline found in biological tissue is relatively substantial. While there is no known co-factor function (Zeisel 1981), this trimethylated compound can serve as source of labile methyl groups in a number of enzymatic reactions and it serves as a precursor of acetylcholine, the neurotransmitter. As an element of phospholipids, lecithin and sphingomyelin, it is an important structural component of biological membranes. In crustaceans, phospholipids also serve as the primary lipid moiety of the hemolymph lipoprotein transport system (Lee and Puppione 1978; Teshima and Kanazawa 1980a).

Kanazawa and coworkers (1976) reported the provision of choline chloride at 600 mg/kg clearly improved growth and survival of *Penaeus japonicus* juveniles. However, later studies by Deshimaru and Kuroki (1979) found no need for choline even though they were working with similar sized animals and somewhat similar diets. These different results suggest shrimp may be able to synthesize choline under certain, as yet, undefined conditions. Many vertebrates which can synthesize choline require an additional dietary source during periods of rapid growth and when fed diets high in lipids. Some crustaceans are able to synthesize at least limited amounts of choline most likely via methylation of dietary ethanolamine as is the case in vertebrates (Bilinski 1962; Shieh 1969; D'Abramo and Baum 1981). While the synthetic potential of shrimp has yet to be examined it remains a possible explanation for the variable results to date. Ultimately, understanding the choline requirement in penaeid shrimp will require an examination of the impact of dietary lipids as well as other dietary factors like methionine which may be involved in its synthesis.

Myo-inositol is similar to choline in that it is an important constituent of cell membranes and may have some lipotropic activity. In this case, both groups of Japanese investigators (Deshimaru and Kuroki 1976; Kanazawa et *al.* 1976). agreed on the need for substantial amounts (above 400 mg/kg) of this cyclic sugar in the diet of the Japanese shrimp. Inclusion of inositol at 2,000 mg/kg of diet maximized both growth and survival of *P. japonicus.* One unique uncertainty with regard to inositol and practical feeds is its availability. Plant material, particularly seeds, contain high levels of inositol, however, it tends to be complexed as phytic acid, an indigestible form for non-ruminant farm animals. On the other hand, Vanderzant (1963) found phytic acid is readily utilized as a choline source by boll weevil larvae. Insects have also been used to examine the value of inositol as a carbohydrate energy source. Even though closely related to glucose this sugar is not utilized to any great extent by various insect species (see Dadd 1985). It would be of value to know how closely shrimp resemble insects with respect to the digestion and utilization of this nutrient.

While there is doubt as to the dietary requirement for choline and the requirement for inositol has yet to be confirmed, it is clear that additions of phospholipids containing choline (phosphatidylcholines) as well as inositol (phosphatidylinositols) to shrimp diets are beneficial.

Improved growth was first noted by Kanazawa and coworkers (1979) when they added a small amount (1 %) of the lecithin fraction obtained from the short-necked clam. Soy lecithin (commercial sources contain a mixture of phospholipids; predominantly phosphatidylcholines, phosphatidylethanolamines and phosphotidylinositols) has been found to be a useful source of these needed phospholipids for both larval and juvenile shrimp (Teshima et *al.* 1982, 1986a) as well as juvenile lobsters (Conklin et *al.* 1980; D'Abramo et *al.* 1981; Conklin et *al.* 1983). It is assumed that the beneficial effect of dietary phospholipids for penaeid shrimp and juvenile lobster reflects some intrinsically rate limited step in the synthesis of phospholipid number of studies have now been carried out detailing the role of these phospholipids in penaeid shrimp (see Teshima and Kanazawa 1980b; Kanazawa et *al.* 1985; Teshima et *al.* 1986a,b,c and d for details) and in homarid lobsters (see D'Abramo et *al.* 1982 and 1985). Basically, in these two marine crustaceans, dietary phospholipids have been shown to enhance the serum level of both phospholipid and cholesterol. While there is a slight improvement in uptake, work with radioisotopically labelled cholesterol suggests that the primary role of phospholipids is associated with serum transport of cholesterol from the midgut gland to other body tissues. The most effective phospholipids seem to be phosphatidylcholines and phosphatidylinositols containing polyunsaturated fatty acids. Lack of these particular phospholipids is thought to result in a cholesterol deficiency at the tissue level even though it may be present in the diet.

While research has highlighted the effect of certain phospholipids in enhancing cholesterol transport in shrimp and lobsters there are a number of aspects to their dietary role which remain unclear. To date, the dietary need for phospholipids appears unique to these two crustaceans, other vertebrates and insects being able to synthesize needed phospholipids. Also it should be noted that dietary lecithin has little or no effect on the growth, survival or cholesterol levels of freshwater prawn *Macrobrachium* (Hilton et *al.* 1984; Briggs et *al.* 1988). Recently it has been found that the phospholipid requirement varies depending on the protein component of the juvenile lobsters diet (Kean et *al.* 1985). When using casein as the primary protein component, absence of dietary phospholipids leads to greatly reduced serum cholesterol titer and to death of early juveniles at the time of molting. However, lack of lecithin has no effect on juvenile survival when purified crab protein is used as the primary amino acid source in place of casein. Addition of lecithin to the diets regardless of protein source still enhances cholesterol levels (Baum et *al.* submitted for pub.) indicating dietary lecithin in the lobster has several roles.

Vitamin C

Generally ascorbic acid synthesis has been thought to be characteristic only of higher vertebrates (amphibians through mammals). This traditional viewpoint developed as a result of enzymatic surveys (see Chatterjee et *al.* 1975) which extended results from dietary trials with man as well as laboratory and domestic animals. More recent work, however, challenges this paradigm and suggests a number of fish and invertebrates may also be capable of synthesis.

Ikeda and coworkers were the first to suggest fish could synthesize ascorbic acid following radioisotopic labelling experiments with common carp, *Cyprinus carpio* (Ikeda and Sato 1964) and later supported these results with enzymatic analysis. The enzyme gulonolactone oxidase (GLO) is the terminal enzymatic step in ascorbate synthesis and thus indicative of synthetic ability (Burns et *al.* 1956; Sato et *al.* 1976; Sato and Undenfriend 1978). Not only was GLO activity demonstrated in the carp but several other species as well including the goldfish *Carassius auratus* (Yamamoto et *al.* 1978). GLO activity in both carp and goldfish has been independently confirmed by other investigators (Thomas et *al.* 1985; Soliman et *al.* 1985). GLO activity among invertebrates has been noted by Wallace and coworkers (1985) in the horseshoe crab and synthesis of ascorbic acid has been indicated in *Homarus* using C-14 labelled glucose (Desjardins et *al.* 1985). Lightner and colleagues (1979) suggested limited synthesis was possible in penaeid shrimp. While it is premature to speculate on distribution of ascorbic acid synthesis, since relatively few finfish and shellfish species have been examined, this potential can no longer automatically be dismissed. Importantly, it should be noted that large scale phylogenetic patterns may not exist in that Soliman et *al.* (1985) found the ability to synthesize ascorbic acid could vary among species within a genus.

Although originally presence of GLO was thought to indicate vitamin C would not be required at all by a species, this premise should also be viewed with caution. Hanssen et *al.* (1979) demonstrated that even though the willow ptarmigan has high levels of GLO activity significant quantities of vitamin C are required in the diet of this bird to prevent deficiency symptoms. Although Sato and coworkers (1978) found the rate of synthesis in carp was sufficient to meet physiological demand under their conditions of culture this is not true for others (Kitamura et *al.* 1965; Dabrowski et *al.* 1988). The fact that *Tilapia aurea* requires vitamin C in its diet (Stickney et *al.* 1984) as does *Oreochromis mossambius* (Soliman et *al.* 1986b) suggests the reported GLO activity noted by Soliman and coworkers (1985) in these species also does not fulfill normal physiological requirements. Lightner et *al.* (1979) in discussing the potential for synthesis in *P. californiensis* and *P. stylirostris* suggested while physiological demands of adults could be met it was inadequate for rapidly growing juveniles.

Vitamin C is particularly known for its involvement in collagen formation. While collagen isn't the predominate structural element in shrimp it is in vertebrates, many of the vitamin C deficiency symptoms in shrimp may also be related to insufficient synthesis of this protein. Evidence for impaired collagen formation resulting from a lack of dietary vitamin C was seen in *P. californiensis*, *P. aztecus* and *P. stylirostris* juveniles by Lightner and colleagues (1979). Lack of collagen synthesis caused affected juveniles to suffer « black death », melanized lesions distributed throughout the collagenous tissue underlying the exoskeleton. Deshimaru and Kuroki (1976) reported symptoms of decolorization and development of an abnormal grayish-white color on the carapace margin, lower abdomen and tips of walking legs. A somewhat similar syndrome of black lesions along with deaths at the time of molt were reported for *Macrobrachium rosenbergii* juveniles (Heinen 1984).

Outside of the important role ascorbic acid plays in collagen formation, most of the mechanisms by which ascorbic acid acts in biological systems are poorly known (for a recent review see Englard and Seifter 1986). It is known that ascorbic acid is a co-factor in the synthesis of carnitine which is needed for effectively utilizing lipid stores for energy. Lack of carnitine may be involved in the reduced growth resulting from anorexia as a consequence of general lethargy. Recent work with fish has suggested that ascorbic acid is important in resistance to stress of various types including infection and in reproduction (see Halver et *al.* 1975; Hilton 1984; Lovell 1984; Sandnes 1984; Sandnes et *al.* 1984).

There is limited information on whole animal and tissue levels of ascorbic acid in shrimp (Guary et *al.* 1976; Magarelli and Colvin 1978). In examining the data of Guary et *al* (1976) it appears that hepatopancreatic levels below approximately 5.0 mg ascorbate/100 g wet tissue may be indicative of a deficiency as judged by reduced growth in *P. japonicus* juveniles. How much this might vary among different species is unknown. While this amount appears reasonable when compared to levels found in wild-caught crustaceans (blue crabs *Callinectes sapidus* and grass shrimp *Palaemonetes pugio*) as reported by Coglianese and Neff (1981) much higher levels have also been noted (Sinha and Mooswi 1978).

Information on tissue levels of ascorbic acid is further complicated by the fact that most assays did not include evaluation of possible contributions by ascorbate-2-sulfate (A2S). While this form is not efficacious for vertebrates requiring vitamin C, some fish species are able to utilize it. It is also used as a storage form of ascorbic acid in brine shrimp cysts (Mead and Finamore 1969; Golub and Finamore 1972). In salmonids, A2S was as effective as ascorbic acid itself in preventing the symptoms of vitamin C deficiency (Halver et *al.* 1975; Tucker and Halver 1984a and b, 1986). However, while salmonids can readily cleave the sulfate from ascorbic acid enzymatically (Benitez and Halver 1982), this may not be true of all fish (Tsujimura et *al.* 1981).

Suggested dietary levels for ascorbic acid in shrimp feeds are astounding compared to other animals. While the optimal amount of vitamin C in the diet of fish will of course depend on species, size, growth rate and culture conditions, generally a level of around 50 mg ascorbic acid/kg of diet is considered adequate (see Milliken 1982). Feed levels several orders of magnitude greater 10,000-20,000 mg/kg diet have been suggested for penaeid shrimp *P. japonicus* (Guary et *al.* 1976). Other results for this species are difficult to interpret. Deshimaru and Kuroki (1976) found, although best growth occurred with no added ascorbate, levels at least 3,000 mg/kg diet were needed to prevent mortalities. Lightner and coworkers (1979) found levels of 1,000-2,000 mg/kg diet were sufficient for *P. californiensis* and *P. stylirostris* but felt these species could be synthesizing part of their requirements.

The chemical nature of ascorbic acid undoubtedly contributes to the need for the very high levels of vitamin C in shrimp rations. Ascorbic acid is highly soluble in water and easily oxidized when in solution to dehydroascorbic acid. In biological systems such oxidation is easily reversed enzymatically with a glutathione dependent reductase (Hughes 1964; Yamamoto et *al.* 1977). This reductase allows for alleviation of

deficiency symptoms through the provision of either the oxidized or reduced from ascorbic acid. If dehydroascorbic acid is not reduced, however, further degradation results in the irreversible formation of diketogulonic acid and loss of vitamin activity. Such degradation is common under conditions routinely associated with the manufacture and storage of formulated feeds. In that ascorbic acid is both chemically labile and highly water-soluble provision of adequate dietary levels of vitamin C for cultured shrimp and other crustaceans has been of continuing concern. Loss of vitamin C activity in feeds due to processing, storage, and leaching can be a significant problem (Guary et *al.* 1976; Goldblatt et *al.* 1979; Hilton et *al.* 1977; Sandnes and Utne 1982; Slinger et *al.* 1979; Soliman et *al.* 1987). A number of approaches have been tried to reduce ascorbate loss (Murai et *al.* 1978; Brandt et *al.* 1985; Soliman et *al.* 1986a; Albrektsen et *al.* 1988), however, the general but not very satisfactory answer to this concern has been to supplement formulations used for intensive culture of fish and crustaceans with large amounts of ascorbic acid hoping a small percentage will reach the animals.

Vitamin C levels in the diet can be maintained much easier if one of the stable derivatives of ascorbic acid are used. While the potential advantages of ascorbate-2- sulfate have been pointed out by Halver and coworkers (Halver et *al.* 1975; Tucker and Halver 1984a and b, 1986) this form is not readily available commercially. Phosphates of ascorbate, however, seems to have a lot of potential (Shigueno and Itoh 1988; VTI 1988). Shigueno and Itoh (1988) found normal growth of *P. japonicus* juveniles could be achieved with as little as 215 mg/kg diet of Mg-L-ascorbyl-2-phosphate.

Fat-soluble vitamins

Predicting the probability of shrimp needing the fat-soluble vitamins; A, D, E and K is the most difficult task of all. Although the water flea *Moina macrocopa* was shown to require some fat-soluble vitamins when reared on an artificial media (Conklin and Provasoli 1977), little is known about the possible need for these vitamins in other crustaceans. Only a few insect species studied have required fat-soluble vitamins, however, the vast majority of insect studies have focused only on early juvenile growth and requirements for fat-soluble vitamins may have been missed (Dadd 1983). The vertebrate model may be of little value with regard to this group in that often these fat-soluble vitamins are involved in highly evolved regulatory systems for which there is no analog in crustaceans (Conklin 1983). Several authors have speculated that i-carotene or derived carotenoid may be an important dietary component of aquatic animals (Gilchrist and Lee 1972; Tacon 1981). Addition of β- carotene to the diet of the water flea *Moina macrocopa* enhanced production slightly (Conklin and Provasoli 1977) and Kanazawa (1985) has stated it is beneficial in larval diets for penaeid shrimp, on the other hand, no benefit has been noted with juvenile lobsters (D'Abramo et *al.* 1983). In that subtle behavior differences in feeding or sexual interactions expressed as reduced growth or reproduction would be difficult to distinguish from metabolic differences in response to a dietary lack of carotenoid interpretation of experiments involving these pigments is difficult. Firm evidence that carotenoids have

a vitamin function other than as a vitamin A precursor is lacking, however, carotenoids are clearly needed to maintain normal market appearance of shrimp and other aquatic animals (see Goodwin 1986).

It is likely that vitamin A is involved in the visual cycle of crustaceans. As most of vitamin A in arthropods is concentrated in the eyes, however, physiological demand to meet this need is likely be inconsequential (Conklin 1983). Marine fish oils are a good source of vitamin A and practical shrimp rations which incorporate these oils as a lipid source should meet this demand. The use of shrimp and other meals to enhance final pigmentation of the shrimp will provide another source of vitamin A.

Several authors have asserted that vitamin D is important in the diet of crustaceans (larval shrimp, *P. japonicus*; Kanazawa 1983, 1985 and juvenile lobsters, *H. americanus*; Stewart and Castell, 1979), however, details are not yet available. In mammals and birds, vitamin D metabolites regulate calcium levels through hormonal regulation of bone stores in order to maintain homeostatic tissue levels in the rest of the body. In that shrimp (Deshimaru et *al.* 1978) and other crustaceans (Hayes et *al.* 1962) apparently have ready access to significant amounts of dissolved calcium through the gills, the mammalian-avian model in the case of vitamin D would seem to provide little insight into the possible role of this vitamin in shrimp or other commercial decapods (Conklin 1983). Even in species of fish in which a dietary need has been shown (channel catfish, Lovell and Li, 1978; trout, Barnett et *al.* 1979) feed levels are quite low. In that marine fish oils and other marine feedstuffs used in practical crustacean feeds will have appreciable levels of vitamin D, additional supplementation is most likely to be unnecessary or at least superfluous.

Presently, there is no evidence that vitamin E has a specific vitamin function in shrimp, however, because of the importance of polyunsaturated fatty acids in the diets of crustaceans, it is assumed that vitamin E will be important as a metabolic antioxidant. With fish the requirement for vitamin E has been noted to increase when diets contain higher levels of polyunsaturated fatty acids (Watanabe et *al.* 1981 a and b; Cowey et *al.* 1983; Boggio et *al.* 1985). However, it is not always clear in these experiments if the diets were appropriately protected from oxidation during processing and storage. Fish oil rapidly undergo autoxidation if not protected with a synthetic antioxidant (Fritsche and Johnston 1988) and rancid oil may lead to a loss of vitamin E (Hung et *al.* 1981). In that natural levels of vitamin E in fish oils may not afford enough antioxidant protection, synthetic antioxidants such as ethoxyquin (Hung et *al.* 1981) or t-butylhydroquinone (Fritsche and Johnston 1988) should be added to these lipid sources upon receipt. Information as to the need for vitamin E in shrimp diets containing practical feedstuffs which have been appropriately protected with synthetic antioxidants would be of value particularly as changes are made in formulations with regard to ascorbic acid, another antioxidant source in feeds.

Finally, there is no evidence to date that the final fat-soluble factor, vitamin K is needed in the diet of crustaceans nor in any insect (see Conklin 1983). One amino acid absent from crustaceans is å-carboxyglutamic acid. Interestingly, the formation of this amino acid in vertebrates is uniquely vitamin K dependent. While most likely this vitamin is not

required by shrimp and other crustaceans, it apparently does no harm and is often included in the vitamin premixes.

CONCLUSION

Shrimp nutrition has clearly benefited from the information gained with other organisms particularly vertebrates. Effective diets have been formulated for commercial growout of juvenile shrimp even though specific requirements for vitamins are not known in any detail. While further research is indicated for several vitamin requirements studies detailing B-complex vitamins are not a high priority. Even for shrimp in high density culture systems which have limited access to other sources of these factors there is no indication levels should be increased above present amounts.

Further research is indicated in the case of choline and inositol. While it can be concluded that the use of soy lecithin which contains these two nutrients in shrimp diets enhances cholesterol utilization and growth rates, it is not clear that this is the only effect of this added phospholipids. Because of the uncertainties surrounding these two possible vitamins it is not possible to confidently replace all or part these phospholipids with an appropriate combination of other nutrients. This lack of flexibility in diet formulation remains a hinderance to future ration development.

Use of more stable forms of ascorbic acid will undoubtedly bring recommended feed levels more in line with other animal feeds as well as ensuring more reliability. Such changes may also inadvertently reduce antioxidant protection during diet manufacture and storage. Studies aimed at determining appropriate levels of synthetic antioxidants are important in insuring sensitive lipids as well as key vitamins are not degraded prior to consumption. There is increasing evidence that a number of vitamins such as C and E have a vital antioxidant role in protecting lipids in the tissue of aquatic animals (Cowey et *al.* 1985; Cowey 1986; Soliman et *al.* 1986; Sato et *al.* 1987). As interest of shrimp nutritionists moves on form juveniles to sexually mature adults and then larvae these antioxidant factors are likely to become even more important.

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Mineral requirements of Penaeids

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Abstract. — *Marine shrimps absorb minerals from their aquatic environment aside from the minerals that come from the food they eat. Thus, the dietary requirement of shrimps for certain minerals will depend on the amounts and availability of these minerals in the aquatic environment. Dietary sources for growth may be necessary due to losses during moltings.*

Most of the dietary studies for mineral requirements have been done under laboratory conditions with purified or semi-purified diets and hardly any information is available under practical culture conditions. Most published data for mineral requirements are for juvenile ***Penaeus japonicus.*** *There are few data for* ***P. monodon, P. californiensis, P. merguiensis, P. aztecus.***

Calcium and phosphorus are the minerals that have been studied the most. These two have been found to be related to problems of soft-shelling in P. monodon. Apparently calcium and phosphorus requirements are within the range of 1 to 2 %. The ratio of calcium to phosphorus in the diet is also an important factor in the efficient utilization of both minerals. It seems that a 1 :1 ratio provides for good growth. Phosphorus deficiency results in reduced growth while lack of magnesium brings about decreased growth, poor survival and reduced feed efficiency in ***P. japonicus.*** *Iron toxicity has also been observed in* ***P. japonicus.***

It might not be necessary to include some minerals in the diet of penaeids.

INTRODUCTION

Food is of utmost importance in the culture of any organism and this includes penaeids. However, in order to properly feed the organism and be able to plan efficient, low cost diets it is necessary to know the nutrient requirement of the species. This includes protein, lipid, carbohydrates, vitamin and minerals. This review is limited to the report of existing literature on the dietary mineral requirements of penaeid/shrimps, including the methodologies, composition of basal diets, mineral deficiencies and toxicities.

PHYSIOLOGICAL SIGNIFICANCE OF MINERALS

Minerals are essential components of bones, teeth, and exoskeleton. These are necessary for maintenance of osmotic pressure, acid-base balance, thus the regulation of pH of blood, haemolymph, urine, and other body fluids. They are also components of soft tissues, enzymes, some vitamins, hormones and respiratory pigments and are essential for muscle contraction and transmission of nerve impulses.

The cuticle of most crustaceans contain minerals primarily CaCO3 with small amounts of magnesium, phosphorus and sulfur. About 99 % of the total inorganic composition of the exoskeleton widely varies among species, location on the body and stage of the molt cycle (Conklin, 1981).

Around twenty two minerals, both macro and micro, have been found essential to animals, fish and shrimp. However, unlike finfishes, there are relatively fewer information on the mineral needs of shrimps. Most of the information available for mineral requirements have been done under laboratory conditions using purified or semi-purified diets and scanty information is available on elemental requirements under practical culture conditions. (Tacon, 1987). The inorganic component of feedstuffs or ash is composed of minerals of which seven are needed in greater quantities compared to the other 15 which are required in trace amounts hence the distinction between macro, and trace minerals. Calcium, phosphorus, potassium, sodium, chloride, magnesium and sulfur are considered macro elements while iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium and arsenium are trace elements. Some minerals identified as essential to shrimps, are : calcium, phosphorus, magnesium, potassium, iron, zinc, copper, iodine and selenium. (Tacon, 1987).

Although minerals are very important, the study of mineral requirements of shrimps has been quite neglected. Perhaps this is due to the fact that shrimps live in an aquatic environment and can meet part of its requirement from the water they live in and the food they eat. Marine shrimps live in an environment that is hypertonic and continually drink small amounts of water, thus their mineral requirements like calcium are partly met (Tacon 1987). On the contrary, freshwater shrimps drink little or no water. Phosphorus, is not abundant in seawater and has to be taken from the food ingested. Therefore, dietary requirement of shrimps for some minerals will depend on the amounts present in the aquatic environment (Cowey and Sargent, 1979).

Penaeids may need dietary sources of minerals for growth because of repeated moltings wherein minerals are lost (Kanazawa, 1985). The availability of minerals to shrimp is dependent on the dietary source and form of the mineral that is ingested, amount stored in the body, interaction of other elements present in the gastrointestinal tract and body tissues and mineral interactions with other dietary ingredients or metabolites (Tacon, 1987).

Soluble monobasic, inorganic salts or bioavailable organic salts must be provided in the diet of stomachless shrimps. Phosphorus and calcium availability and absorption is dependent on the presence of an acid-secreting stomach. (Tacon, 1987).

Of the penaeids, the mineral requirement of *P. japonicus* is relatively the most studied. Hence data for mineral requirements for *P. japonicus* has often been extended to other species such as *P. monodon.* In order to define the nutrients required formulation of a basal diet is necessary.

DIETARY REQUIREMENT STUDIES

As a starting point a diet either similar to the diet of the shrimp in the wild or simulated from those fed to other species close to it such as insects and other finfishes is formulated. Kanazawa and co-workers (1970) formulated diets patterned after the diets fed to silk worm, chinook salmon, brine shrimp and components of short-necked clam. The salt mixture that was used is in Table 1. The composition of the purified diet is in Table 2.

Tab. 1a. — Trace minerals.

$AlCl_3.6H_2O$	0.024
$ZnSO_4.7H_2O$	0.476
$MnSO_4.4\text{-}6H_2O$	0.107
CuCl	0.015
KI	0.023
$CoCl_2.6H_20$	0.140
Cellulose powder	0.215

Likewise, Deshimaru and Kuroki (1974) tested 14 diets containing various levels of protein, fat, carbohydrates and minerals using semi-purified ingredients. From the results of the study they were able to formulate a basal diet for *P. japonicus* composed of 54 % casein, 6 % egg albumin, 3 % soybean oil, 3 % cod liver oil, 6 % glycogen a mineral mixture of 19.5 %, and 8.5 % of other ingredients. The mineral mixture is presented in Table 1. Their control diet is shown in Table 3 wherein 12.6 % of the mineral mix in Table 1 was used.

Tab. 1. — Percentage composition of mineral mix.

Salt	% *	% **
K_2HPO_4	30	10
NaH_2PO_4		21.5
Ca $(H_2PO_4)2.H_2O$		26.5
$CaCO_3$	16.8	10.5
Ca-lactate		16.5
KCl	9.4	2.8
$MgSO_4.7H_2O$	14.8	10.0
Ferric sulfate		1.4
$Ca_3(PO_4)_2$		27.4
$MnSO_4.7H_2O$		0.2
Fe-citrate		1.2
Trace metals		1.0***
Mineral mix/100 g diet	7.7 %	19.5 %

* Percentage composition of salt mixture used by Kanazawa and co-workers (1970)

** Percentage composition of the salt mixture used by Deshimaru and Kuroki (1974)

*** Trace minerals used are : $AlCl_3.6H_2O$, 0.024; $ZnSO_4.7H_20$, 0.476; $MnSO_4.4\text{-}6H_20$, 0.107; CuCl, 0.015; KI, 0.023; $CoCl_2.6H_20$, 0.140 and cellulose powder 0.215 g.

Further work on effects of Ca, P, K, Mg and Fe and mixed trace metals was reported by Deshimaru and Yone (1978). The diet reported in 1974 was used as basal diet and the mineral mixture was modified to contain :

	%
Ca	2
P	1 :1 Ca/P ratio
K	1
Mg	0.3
Fe	0.02
Trace metals	0.08

The above was added at 21 % of the basal diet.

The amounts of each major elements in percent of mineral mix in the diet are Ca 2.4 %, P 2.1 %, K, 1.2 %, Mg 0.2 %, Fe 0.02. The elements were provided as a single salt. The presence of 7.2 mg Ca and 249 mg P from casein and albumin were disregarded in the computations.

The mineral mixture defined by Kanazawa et *al.* (1970) was used by Sick et *al.* (1972) in a preliminary study with *Penaeus aztecus* and *P. setiferus*. The basal diet consisted of the following :

Casein	50.2
Methionine	1.0
Glycine	0.1
Na glutamate	0.2
Citric acid	0.3
Succinic acid	0.3
Mineral mix	5.0
Fat	8.0
CHO	20.5
Collagen	4.0
Vit. mix	2.5
Cellulose	7.9

Although the diet with added mineral mix produced shrimps with higher biomass, the increase was not very significant and the authors believe this was due to lack of cholesterol in the basal diet.

Sedgwick (1980) studied the mineral requirements of *P. merguiensis* using the mineral mixture defined by Deshimaru and Kuroki (1974) and compared the growth of shrimps to fresh mussel (*Mytilus edulis*). Freeze dried mussel, 79 % was the sole source of protein in the diet with 0, 7 and 14 % of the mineral mixture. The diet with 7 to 14 % mineral mix with 3.5 % vitamin mix gave the best growth. There was extensive mortality in shrimps fed the diets with minerals alone. In a preliminary study at the Aquaculture Department, SEAFDEC, poor growth was obtained with juvenile *P. monodon* when only minerals were added to a practical diet. Tables 4 and 5 give the composition of some premixes (New, 1976).

Calcium and phosphorus are the most commonly studied minerals. Although calcium is present in large amounts in seawater and is available to *P. japonicus* as shown by the study of Deshimaru and Yone (1978) it is still necessary to study the relationship of calcium to phosphorus. Results of studies by Deshimaru et *al.* (1978) and Deshimaru and Yone (1978) showed that adding calcium did not increase growth of *P. japonicus* compared to those fed the diet without calcium. Furthermore, they suggest 2 % phosphorus in the diet. The fact that calcium can be obtained from

Tab. 2. — Composition of diet for *P. japonicus* (Kanazawa et *al.* 1970).

Substances	%
Glucose	5.6
Sucrose	10.0
Starch	4.0
Chitin	4.0
Glucosamine	1.5
Cellulose powder	4.0
Purified soy-bean protein	50.0
Methionine	1.0
Tryptophan	0.2
Amino acid mixture	0.2
Glutamic acid	0.1
RNA	—
Citric acid	0.3
Succinic acid	0.3
Fatty acid mixture	—
Oil (Soy-bean oil, refined)	8.0
Salt mixture	7.7
Vitamin mixture	2.6
Cholesterol	0.5
Total	100.0

Tab. 3. — Composition of the control diet (Deshimaru and Kuroki, 1974).

Squid meal	43.2
Shrimp meal	13.8
Gluten	2.7
Active sludge	4.6
Yeast *1	18.4
Salt mixture *2	12.6
Vitamin mixture	2.7
Starch	1.0
Cholesterol	1.0
Total	100.0

*1 Saccharomyces sp.

*2 $AlCl_3.6H_20$, 0.024; $ZnS0_4.7H_20$, 0.476; $MnSO_4.4\text{-}6H_20$, 0.107; CuCl, 0.015; Kl, 0.023; $CoC_{12}.6H_20$, 0.140 and cellulose powder 0.215 g.

*3 Thiamine-HCl, 5; Riboflavin, 16.4; Pyridoxine-HCl, 5; Nicotinic acid, 65.8; Ca-pantothenate, 24.7; Inositol, 328.8; Biotin, 0.5; Folic acid, 1.2; *p*-Aminobenzoic acid, 32.9 Choline Chloride, 657.7; Ascorbic acid, 822; — Tocopherol, 32.9; Menadione, 3.3; B-Carotene, 3.3; Calciferol, 0.5; Cyanocobalamin, 0.03 and cellulose powder 700 mg.

*4 This mixture was well-mixed with water and extruded from a mincing machine into a noodle-like shape (2 mm in diameter, 2 cm in length), then dried to less than 10 % moisture content.

the aquatic environment and is abundant in fish meal while phosphorus is lacking in the water makes phosphorus all the more necessary to provide for in the diet. On the other hand, Kanazawa et *al.* (1984) reported the need for 1 % calcium and 1 % phosphorus in the diet for *P. japonicus*. According to Shewbart et *al.* 1973), calcium, potassium and sodium chloride may not be needed by *P. aztecus* but phosphorus may be essential.

Due to the apparent discrepancy in the calcium requirements, which could be due to the type of calcium salts used-primary, secondary, or tertiary; Kanazawa (1985) suggests a reevaluation of the calcium requirements of *P. japonicus.*

Tab. 4. — Composition of some shrimp diet mineral premixes (g/100 g dry diet)⁺.

Composition	Premix number *			
	1	2	3	4
K_2HPO_4	2.310	0.500	1.500	0.600
$CaHPO_4.2H_2O$	—	—	1.370	0.548
$Ca_3(PO_4)_2$	2.110	0.750	—	—
KCl	0.724	—	0.470	0.188
$MgSO_4$	1.140	—	0.740	0.296
$CaCO_3$	1.293	—	0.840	0.336
$(C_3H_5O_3)_2Ca.5H_2O$	—	3.750	—	—
$FeCl_3$	—	—	0.070	0.028
$FeSO_4 7H_2O$	0.108	—	—	—
$MnSO_4 7H_2O$	0.015	—	0.010	0.004
Total premix inclusion in diet (%)	7.700	5.000	5.000	2.000

*1 Kanazawa et *al.* (1970); 2 Deshimaru and Shigueno (1972); 3 Sick et *al.* (1972); 4 Andrews and Sick (1972).
⁺ After New (1976).

Tab. 5. — Mineral contribution to shrimp diets by premixes (News, 1976).

	Premix number*				
	1	2	3	4	5
g/100 g dry diet					
Calcium	1.336	0.679	0.655	0.264	1.06
Phosphorus	0.832	0.239	0.514	0.206	1.06
Potassium	1.417	0.244	0.919	0.368	0.90
Magnesium	0.230	—	0.150	0.060	0.30
Socium	—	—	—	—	
Choloride	0.344	—	0.270	0.107	
Sulphate	0.951	—	0.594	0.237	
mg/kg dry diet					
Iron	216.6	—	240.9	96.4	0
Copper	—	—	—	—	0
Manganese	30.5	—	19.8	7.9	0
Zinc	—	—	—	—	
Cobalt	—	—	—	—	
Iodine	—	—	—	—	
Calcium-phosphorus ratio	1.61	2.84	1.28	1.28	

*1 Kanazawa et *al.* (1970); 2 Deshimaru and Shigueno (1972); 3 Sick et *al.* (1972); 4 Andrews and Sick (1972); (1974b); 5 Kanazawa et *al.* (1984).

Additional dietary calcium might be necessary to maintain a 1 :1 calcium : phosphorus ratio. Kitabayashi et *al.* (1971) reported an optimum ratio of Ca :P of 1 :1 for *P. japonicus.* Bautista (personal communication) showed that a 1 :1 ratio was necessary to prevent soft-shell disease in *P.*

monodon while for juvenile *P. californiensis*, Huner and Colvin (1977) reported that a 2.1 :1 Ca :P ratio was optimum for growth.

Potassium at 1 % has been shown to be necessary in purified diets (Deshimaru et *al*, 1978). On the other hand Kanazawa et *al.* (1984) reported the need for 0.9 % potassium, and 0.3 % magnesium, in dry diets for *P. japonicus.*

Tab. 6. — Dietary mineral requirement of shrimp (Tacon, 1987).

Element/Species	Dietary requirement	References
Calcium		
P. japonicus	1-2 %	Kanazawa et *al.* (1984)
	1.24 %	Kitabayashi et *al.* (1971)
	1.00 %	Kanazawa (1985)
	< 0.50 %	Deshimaru et *al.* (1978)
Phosphorus		
P. japonicus	1.04 %	Kitabayashi et *al.* (1971)
P. japonicus	2.00 %	Deshimaru and Yone (1978)
	1.00 %	
	1.2 %	Kanazawa et *al.* (1984)
Ca : P. ratio		
P. monodon	1:1	Bautista (Pers. comm.)
P. californiensis	2.42:1	Huner and Colvin (1977)
P. japonicus	1.2:1	Deshimaru and Shigueno (1972)
P. japonicus	1:1	Kanazawa et *al.* (1984)
P. merguiensis and *P. aztecus*	1.3:1	Sick et *al.* (1972)
	(0.66 %	and
	0.51 %)	
	ND[1]/	Shewbart et *al.* (1973)
Magnesium		
P. japonicus	0.30 %	Kanazawa (1984)
	ND[1]/	Deshimaru and Yone (1978)
P. merguiensis	.3 %	Aquacop (1978)
Iron		
P. japonicus	ND[1]/	Kanazawa et *al.* (1984)
	ND[1]/	Deshimaru et *al.* (1978)
Copper		
P. japonicus	ND[1]/	Kanazawa et *al.* (1984)
Potassium		
P. japonicus	0.9 %	Kanazawa et *al.* (1984)
	1.0 %	Deshimaru et *al.* (1978)
P. aztecus	ND[1]/	Shewbart et *al.* (1973)
Sodium chloride		
P. aztecus	ND[1]/	Shewbart et *al.* (1973)
Trace metals		
P. japonicus	0.2 % of the diet	Deshimaru et *al.* (1978)

[1]/ No dietary requirement demonstrated.

Most of the work done has been on juveniles and there is indeed a lack of information on mineral needs of larval and broodstock penaeids.

TRACE ELEMENTS

Except for the studies of Deshimaru and Kuroki (1974), Deshimaru and Yone (1978) and Kanazawa et *al.* (1970 and 1984) there is little information regarding trace element requirements. These are needed only in minute quantities, therefore, it is very difficult to study and demonstrate a mineral deficiency for trace elements such as copper, iron, and manganese. An amount of 0.2 % trace metals has been used in the diet by Deshimaru and Yone, (1978).

MINERAL DEFICIENCIES

Although minerals may be present in adequate quantities in feedstuffs for shrimp diets mineral deficiencies can occur under intensive culture conditions. The lack of certain specific minerals may be due to the presence of certain compounds that bind the elemental form of the mineral that is used in the feed, and antagonistic or synergistic reactions in the gastro-intestinal tract are factors that sometimes cause dietary mineral imbalances or deficiencies (Tacon, 1987). Kanazawa et *al.* (1984) reported reduced growth in *P. japonicus* when there is a deficiency in phosphorus and magnesium. Survival and feed efficiency are also decreased in magnesium deficiency.

Tab. 7. — Recommended mineral nutrient levels for carnivorous shrimp species. (Tacon, 1987).

Nutrient Level	Shrimp size class					
	Larval	PL1-25	PL25-1g	Juvenile	Grower	Broodstock
Major minerals, %						
Calcium, % max	3	3	2.5	2.5	2	2.5
Available phosphorus, % min	1.8	1.6	1.4	1.2	1.2	1.4
Potassium, % min	1.1	1.0	0.9	0.8	0.7	0.9
Magnesium, % min	0.18	0.15	0.13	0.10	0.08	0.13
Added dietary supplements						
Trace mineral, mg/kg	min					
Iron	100	90	80	70	60	100
Zinc	120	110	100	90	80	120
Manganese	60	55	50	45	40	60
Copper	12	11	10	9	8	12
Cobalt	1.2	1.1	1.0	0.9	0.8	1.2
Iodine	6	5.5	5	4.5	4	6
Chromium	1.0	0.9	0.8	0.7	0.6	1.0
Selenium	0.25	0.23	0.21	0.19	0.17	0.25

MINERAL TOXICITY

Studies done under laboratory conditions showed levels of iron above .006 to .012 % and manganese .01 to 0.1 % (Kanazawa et *al*, 1984)

retarded growth. Shown in Tables 6 and 7 is a summary of mineral requirements of shrimp (Tacon, 1987).

RECOMMENDATIONS

More work on mineral requirements of various penaeids species should be done using references diets and standardized methodologies such that results are comparable.

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The role of astaxanthin in shrimp pigmentation

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Abstract. — *Carotenoids are exogenously derived isoprenoid compounds which are responsible for pigmentation in crustaceans. While implants and bacteria are able to synthesize these carotenoids de novo, shrimps like other animals entirely depend on their dietary supply. The major carotenoid found in most crustacean tissues and responsible, e.g. for the typical colour of penaeids is astaxanthin (3,3'-dihydroxy-i, i-carotene-4,4'-dione). With increasing industrialization in shrimp farming there is a growing demand for synthetic, nature-identical carotenoids, not only for pigmentation, but also for the maintenance of growth and fertility.*

Following some general considerations on the importance of colour, pigmentation and carotenoid pigments some preliminary results from recent investigations on the improvement of pigmentation and the so-called blue shrimp syndrome will be presented.

INTRODUCTION

The cultivation of prawns and shrimps in brackishwater coastal ponds has been practised in different parts of the world for decades. Traditionally, however, extensive culture methods have been employed with minimal inputs of seedstock, fertilizer and feed where farmers raised incidental crops of wild shrimps in tidal fish ponds. In such extensive cultures, where a large amount of land is involved to the number of animals reared, shrimps feed on natural food in the pond and traditionally reached marketable size after a period of 6-12 months. As a consequence, yields per unit of land were typically low.

During the past ten years, however, there have been major technological advances leading to significant improvement of culture methods and shrimp production. Since then shrimp industry experienced an incredible boom. Shrimp farms have been established in over 40 countries increasing their contribution to the world shrimp production from 2.1 % in 1981 to more than 22 % in 1988. The bulk of farmed penaeid species consisting mainly of 3 species (Fig. 1), namely the giant tiger shrimp

(*Penaeus monodon*) contributing 33 %, the chinese white shrimp (*P. chinensis*) 22 % and the western white shrimp (*P. vannamei*) 18 % to the world production of penaeid shrimps. Though some first tremors have been experienced by the shrimp industry during 1988, at least partly caused by a significant increase in competition, diseases and quality requirements, shrimp farming is still expanding at such a pace that no one can keep up with the numbers.

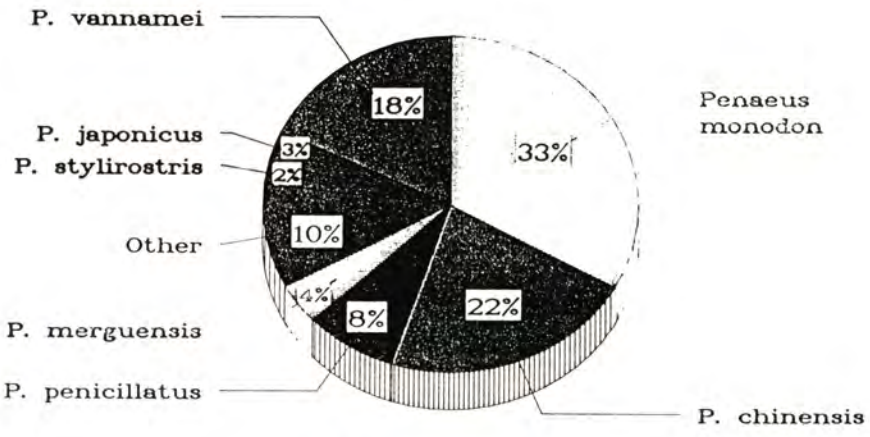

Fig. 1. — Production by species.

However, future outcomes in terms of profitability will largely depend on the productivity and the quality of the shrimp produced. Since the shrimps in close proximity, as e.g. in intensive culture systems (present trend in shrimp farming is still toward intensive and even super-intensive culture methods) compete more aggressively for the same resources in the given space and since the high density in the ponds practically eliminates the growth of natural food this outcome, as a consequence, will largely depend on the quality of feed used during the whole farming cycle involving maturation hatchery nursery and grow-out phases.

SHRIMP QUALITY CRITERIA

According to most Southeast Asian shrimp processors and importers, colour besides others (Table 1) is one of the most important, but widely neglected quality criteria for penaeid shrimps. In fact, today poor general pigmentation as well as a sort of blue discolouration also known as the so-called « blue shrimp syndrome » is one of the most alarming problems plaguing the shrimp industry in that region.

IMPORTANCE OF COLOURS AND PIGMENTATION

Colours are hardly just waste or luxury products of a profligate nature, but rather significant in supporting a multiplicity of biological functions. Pigments, and their respective colours often trigger various physiological processes and widely differing complex behaviour patterns which are essential to e.g. inter- and intraspecific communication, species recognition, courtship, reproduction and brooding as well as to the survival of the individual acting for example as lure, warming, camouflage and protection. One might, however, object that — in captivity — the mechanisms of protection and defense are no longer necessary. Sex attraction by colours in the mating season is also superfluous because of artificial insemination. Nevertheless, colours (pigments) as noted also influence many physiological processes and last but not least have a significant impact on man's choice of food.

Visual appearance, especially colour is one of the most important characteristics of foods in determining their selection prior to actual consumption.

As man in earliest times, the consumer, often unconciously relates the appearance of a natural colouration with e.g. the ripeness, freshness, taste and healthiness or simply with the quality of a product. Conversely, if colouring or pigmentation of a product is inappropriate or off-putting the consumer usually considers the product to be improperly processed, spoiled or of low quality.

Tab. 1. — Major Shrimp quality Criteria.

Size
Shell
Colour
General Appearance
Uniformity
Texture
Flavour/Odour

Tab. 2. — The Major types of Pigments found in Crustaceans.

Hemoglobins
Hemocyanins
Flavins
Cytochromes
Carotenoids
Melanins
Ommochromes
Pterins
Rhodopsin

THE ROLE OF ASTAXANTHIN IN SHRIMP PIGMENTATION

In crustaceans the basic nature of body colouration relies on specific pigments present in the subepidermal chromatophores and/or in the principal layer of the animals exoskeleton. Among the different types of

pigments found in crustaceans (Table 2), the carotenoids are by far the most significant ones in determining their pigmentation (Goodwin, 1954; Schiedt, 1987).

Carotenoids are exogenously derived isoprenoid compounds representing not only one of the most wide-spread groups of natural pigments, but probably also have the most varied structures and functions (see Latscha, 1989, for references). Regarding the structures, formally, all carotenoids can be derived from the acyclic C40H56 polyene lycopene by reactions involving e.g. hydrogenation, dehydrogenation, cyclization, chain elongation and oxygen insertion. Their wide range of colours from almost colourless to yellow and dark red is due to the varying chromophore in different groups of carotenoids and consisting in different groups of a varying conjugated polyene system in the molecule.

Carotenoids are of almost universal distribution in living matter occuring from the most primitive bacteria (*Archebacteria*) and procaryotic blue-green algae (Schizophycaea) to the highly developed flowering plants (Angrospermae) and from the protozoans right up to the mammals. It is estimated that, in nature, more than 100 million tonnes are produced annually, which represents an output of over 3 tonnes per second ! Since the earliest scientific studies on these lipophilic pigments about 600 naturally occuring carotenoids have been isolated and identified in the plant and animal Kingdom (Pfander, 1987).

Despite their wide distribution, however, the *de novo* synthesis of carotenoids is confined to certain microorganisms, fungi, algae and the higher plants. Animals, in contrast, unable to synthesize these pigments *de novo* fully depend on a dietary supply. In animals even a species-specific absorption of various carotenoids must be taken into account. In crustaceans, however, this is less pronounced since they apparently absorb e.g. i-carotene and astaxanthin as well as some other carotenoids. Once absorbed, the ingested carotenoids are either deposited as such or converted into species-specific compounds.

In crustaceans including the commercially most important penaeids, the most prevalent carotenoid found in the integuments is astaxanthin, 3,3'-dihydroxy-i,i-carotene-4,4'-dione (Table 3, Schiedt, 1987) representing about 65-98 % of the total carotenoids present and consisting of 3 sterioisomers (3S,3'S; 3S,3'R; 3R,3'R) as shown in Table 4.

As depicted in Table 3 three different forms of this particular pigment are recognized in crustaceans, namely diesters, monoesters and the free form. The esters generally representing the bulk of astaxanthin in the integumental tissues. Pigmentations in crustaceans or shrimps respectively is further complicated by the wide occurence of this pigment in carotenoid-protein complexes commonly termed carotenoproteins or chromoproteins which due to usually marked bathochromic shift in the light absorption maximum caused by characteristics of the pigment-protein bonding exhibit colours (e.g. green to purple) which largely differ from the colour of the pigment itself (Chersman et *al.*, 1967). It is obvious from these, that pigmentation in general, but particularly that of crustaceans is rather complex and largely influenced by a multiplicity of pigment-, feed-, animal-, and disease related as well as environmental factors of which the most important ones are summarized in Table 5, and giving rise to

Tab. 3. — Carotenoid Content and composition in Various Wild Species of Penaeidae.

Carotenoids	P. vannamei	P. monodon	P. japonicus	Metapenaeus monoceros
Content mcg/g tissue	56	52	38	44
Composition	%	%	%	%
β, β-Carotene	0.5	0.2	0.0	3.0
Yellow xanthophylls*	30.0	0.4	19,0	23.0
7,8-Didehydroastaxanthin	5.0	1.0	2.0	2.0
Astaxanthin total	65.0	98.0	79.0	72.0
Diesters	50.8	27.5	46.8	30.5
Monoesters	44.6	58.2	36.7	52.8
Free form	4.6	14.3	16.5	16.7

* Ester of fatty acids, no lutein, zeaxanthin, presumably three hydroxy groups.

Tab. 4. — Composition of Optical Isomers of Astaxanthin in Various Wild Species of Penaeidae.

Optical isomers	P. vannamei	P. monodon	P. japonicus	Metapenaeus monoceros
(3R, 3'R)	23*	19*	15*	20*
(3R, 3'S; meso)	44	45	40	42
(3S, 3'S)	32	36	45	38

* % of total astaxanthin.

significant variations e.g. between wild-caught and farmed animals (Table 6).

However, pigmentation of shrimps may be influenced by several factors, the achievement of an optimal and consistent pigmentation nevertheless is primarily a question of the amount and type of available carotenoids in the feed. In industrialized shrimp farming, the animals are deprived of their natural feed sources. If, therefore, the respective carotenoids normally present in the diet, or possibly a substitute, are not included in the feed, the carotenoid content will decrease and the integuments will fade as depicted in Fig. 2 (Latscha et *al.*, unpublished). Though the ultimate cause of mentioned « blue shrimp syndrome » is not fully understood at present, it is yet widely attributed to a most probable dietary lack of carotenoids like astaxanthin or its precursors. In fact recent investigations of blue shrimps have consistently revealed a significantly lowered total carotenoid content (Table 7) due mainly to a deficiency of astaxanthin in these animals. The inclusion of 50 ppm astaxanthin (CAROPHYLL Pink (R)) into the commercial diets in field trials performed in consequence resulted in the accumulation of exoskeleton pigments and the successful conversion of abnormally blue pigmented individuals of *P. monodon* into normal pigmented ones.

Tab. 5. — Factors influencing Pigmentation.

Pigment-related factors	type amount form stability
Feed-related factors	manufacturing composition intake/FCR biovailability administration period
Animal-related factors	species age/stage sex/maturation genetics tissue molling
Environmental factors	culture method soil condition water quality light intensity
Disease-related factors	eg. vibriosis
Consumer-related factors	

Tab. 6. — Astaxanthin Content of Wild and farmed Penaeus monodon.

P. monodon	Total Astaxanthin content (mg/kg)	
origin	mean	range
Wild catch	54.1	40.16-61.92
Farmed	18.7	9.96-20.90

Tab. 7. — Mean Carotenoid Content and Distribution in « Blue » and « normal » individuals of Penaeus monodon.

P. monodon	Carotenoids	% Distribution in	
individuals	mg/kg	Shell	Flesh
Normal	78.37	84.46	15.54
Blue	7.63	85.15	14.85

Mean body weight : normal : 37.64 g, blue = 40.64 g.

The provision of an accurate carotenoid source in the field, therefore, is important in yielding a natural pigmentation acceptable to the consumer as well as to improve the animals general performance.

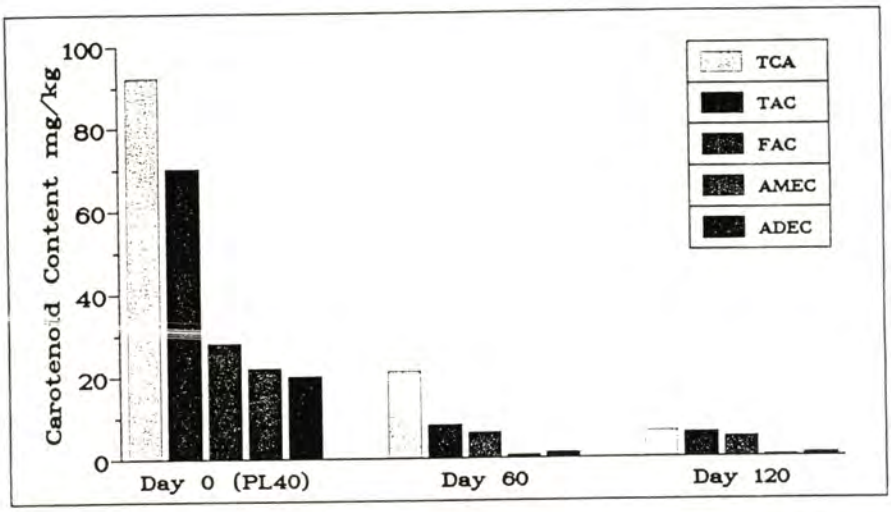

Fig. 2. — Decrease in the carotenoid content of *Penaeus monodon* fed a carotenoid deficient diet (Blue Shrimp Induction).

Acknowledgement. *In conclusion, I would like to thank all those colleagues who have contributed to the results presented in this paper.*

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Growth factors in Penaeid shrimp feeding

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Abstract. — *Some nutrients known as non essential in vertebrates could be essential in invertebrates and could be related to some « unknown growth factors » which were evidenced or suspected, mainly in mollusc or crustacean flesh or meal, by several authors working on different species of crustaceans. Since 1982 a set of experiments was carried out in our laboratory in order to isolate one of these factors found in squid meal. In all these trials, meal (prepared from fresh lyophilised squid) or extracts were tested in isoproteic and isolipidic casein based semi-purified diets. The first experiments showed that a growth enhancement amounting to 30 to 50 % per month in* ***Penaeus japonicus*** *factors (**sensu stricto**) by stimulating cell hypertrophy and not hyperplasy. The postprandial level of glucose and aminoacids in haemolymph was found to be increased by the « squid factor ». This effect is not explainable by a better digestion of feed, since no clear effect was found on digestive enzymes activities; furthermore no effect was found on the absorption of glucose or aminoacids neither* ***in vitro*** *nor* ***in vivo****. Therefore the action of squid factor seems to be related to metabolic phenomena and remains to be elucidated. The practical interest of this factor and that of feedstuffs containing possibly similar factors is discussed.*

INTRODUCTION

It is well known that the first authors who studied the nutrition of penaeid shrimp using mixed diets, i.e. Drs Deshimaru and Kanazawa took pattern by the knowledge in fish (Chinook salmon), insect (silk worm),and artemia nutrition. This very clever step allowed fast success in many fields. The good efficiency of shrimp semi-purified diets now used in many laboratories demonstrates that the essential nutrients taken into account in feed formulation fit well those actually required by shrimp. However, fewer things are known concerning so called « unknown growth factors » that can be found for example in fish meal, fish solubles or distillery by

products; their activity was partly explained by inorganic elements supply, balance of nutrients and positive influence on feed intake (Alenier and Combs, 1981). In shrimp feed the existence of such growth promoting factors remains an open question : natural food and mixed diets made from non purified feedstuffs seem to be more efficient than semi purified diets. This higher feeding value may be related to higher palatibility, better balance or availability of nutrients, influence on gut microflora, etc but, like in upper vertebrates unknown growth factors could also be implied.

The existence of such factors was suspected by FOSTER and Beard (1973) in mussel fed to *Palaemon serratus*; Fernandez and Puchal (1979) working in *Penaeus keraturus* made a similar hypothesis. In *P. japonicus* Cuzon *et al.*(1981) supposed the existence of an unknown growth factor in spirulina and lactic yeast; however, like previous authors, they did not conduct any experiments to demonstrate that the growth promoting effect observed was not attributable to any known nutrient or to balance between some nutrients. A beautiful demonstration of the role on organic compound in the growth of a crustacean was made in *Artemia salina*, of the Utah strain which cannot synthesize purines (Hernandorena, 1987). However there is no evidence of such a requirement in upper crustaceans, though some authors use nucleic acids as possible growth promoters in *P. japonicus* semi purified diets.

THE SQUID FACTOR

— The high feeding value of squid meal

Japanese researchers were first to demonstrate the high feeding value of squid meal or squid by products in *P. japonicus* (Kitabayashi *et al.* 1971; Deshimaru and Shigeno, 1972); this result was corroborated by Faranda et al. (1983). Later on similar reports were done in other species of penaeids such as *P. aztecus* (Fenucci and Zein-Eldin, 1976), *P. setiferus* (Fenucci et al., 1980) or *P. monodon* (LIM *et al.*, 1979).

Comparing the efficiency of different mixed diets tested in our laboratory, the superiority of feeds containing squid meal was evidenced (HEW, 1983). As a first hypothesis the amino acid balance and especially the lysine/arginine ratio was supposed to be responsible for the high nutritional value of this protein source. But, the effect of lysine/arginine ratio was found to be lightly pronounced (Hew and Cuzon, 1982, HEW, 1983).

— Evidence of a « growth factor » or of a « squid factor » (Cruz and Guillaume, 1983)

In the preliminary experiment whole squid was ground and divided into 3 fractions using a process deriving from Bligh and Dyer (1959) lipid extraction method slightly modified by us. This procedure more or less similar to that of Deshimaru's one (1982) led to lipids, proteins and hydroalcoolosoluble extract (Hae).These fractions were added two by two

or all together in isoproteic isolipidic diets well fortified in minerals and vitamins and other known nutrients. The omission of one out of the three components had variable effects on the growth of shrimp :

- the omission of squid lipid, replaced by cod liver oil, had no effect,
- the omission of HAE reduced food intake without changing growth, improving therefore food efficiency,
- the omission of squid protein replaced by fish protein had a very pronounced growth depressing effect without influencing food intake.

Tab. 1. — Amino acid composition of squid meal protein, squid protein fraction and COMNAR fish meal protein, the possibility first limiting factor of fish protein is underlined.

Amino acids	Squid meal	Squid protein	Fish meal
Taurine	3.17	0.96	—
A. Aspartique	6.07	8.13	8,18
Threonine	3.48	4.65	4.49
Serine	3.17	3.80	4.28
A. Glutamique	9.30	11.45	13.63
Proline	5.33	4.69	3.91
Glycine	4.01	4.49	4.70
Alanine	3.54	3.96	5.02
Valine	2.96	4.60	4.60
Isoleucine	3.21	5.14	3.95
Leucine	5.28	7.28	7.39
Tyrosine	1.80	2.59	2.57
Phenylalanine	2.64	3.69	3.56
Lysine	4.70	6.21	7.05
Histidine	1.20	1.61	1.82
Arginine	4.68	5.99	5.77
Orinithine	0.37	—	—
TOTAL	64.91	79.24	80.92
Protein (NX6.25)	75.30	89.11	88.93

This trial was replicated and brought evidence that the high nutritional value of squid meal was due neither to lipids (phospholipids cholesterol or highly unsaturated fatty acids) nor to food attractants. Since the fraction responsible for growth enhancement was the protein fraction, attempts were made to explain its effects through its content in essential amino acids.Analyses of amino acids were performed on squid and squid fractions and chemical indexes according to Mitchell were calculated. But taking into account the most deficient amino acid in the basal diet, i.e. isoleucine, supposing it was actually limiting, comparing it to its level in the squid supplemented diet (Table 1), only a small part of the growth difference was explained. In our condition, in opposition to those of Deshimaru (1982), the amino acid balance was not the cause of the high feeding value of squid protein. Therefore the hypothesis of an unknown factor was formulated.

— Study of the squid factor in penaeids

— *General material and methods*

All studies on the squid factor were carried out in laboratory conditions. According to experiments three kinds of basal diets were used : one regime was based on casein gelatin, cod liver and purified micronutrients and called « semi purified diet »; the other regimes, called « classical basal diets » (one for *P. japonicus* and one for tropical species) contained various usual feedstuffs. All substitutions were made in isoproteic and isolipidic diets.

Squid meal or extracts were prepared from freshly caught squid (*Loligo* sp.) from Brittany (West of France) with one exception where the experiment was carried out with frozen *Nototodarus sloani* from New Zealand.

All shrimp juveniles arised from IFREMER hatcheries and weighed less than 1 g to 4-5 g. All experiments lasted 4 weeks. The environmental conditions, number of shrimp and experimental procedures were described in our preceeding publication.

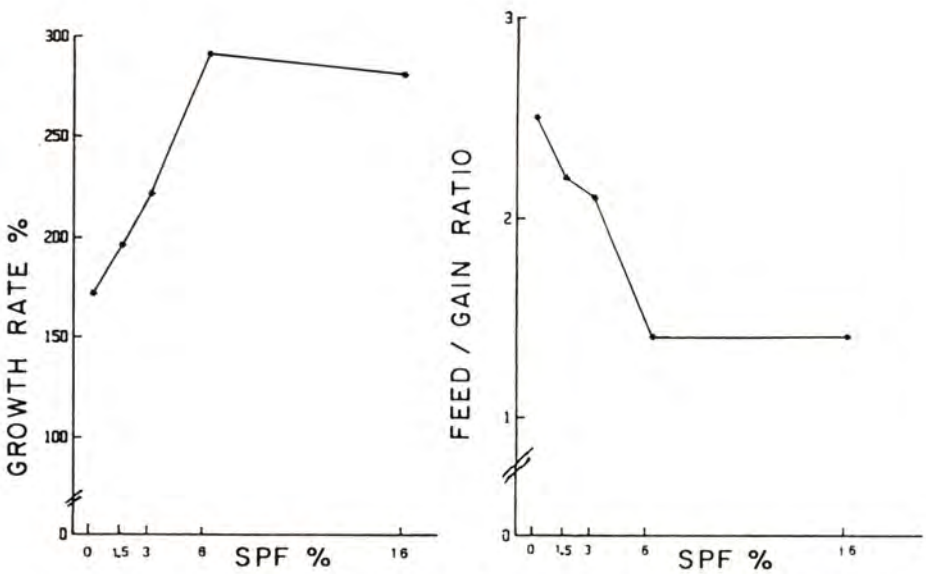

Figure 1. — Growth rate and feed conversion of *P. japonicus* in relation to the level of squid protein fraction.

— *Dose response curves in five species of penaeids* (Cruz-Ricque *et al.*, 1987b; Cruz-Suarez *et al.*, 1987)

The effect of graded levels of squid protein fractions (SPF) was tested first for *P. japonicus*, then for *P. monodon*, *P. stylirostris*, *P. vannamei* and *P. indicus*. In the first four species a very pronounced and very significant growth promoting effect of SPF appeared a plateau became visible around 6 % of SPF (Fig. 1 and 2). But the susceptibility of these species was very

different and *P.indicus* did not respond significantly to SPF supplementation; the most marked effects were obtained for *P. stylirostris* where the growth improvement was significant ($p < 0.05$) even with a supplementation of 1.5 % SPF i.e. with a very negligeable change of amino acid balance. Both series of experiments corroborate the very marked growth enhancing ability of squid protein irrespective of the composition of basal diet and even with a very low supply of essential amino acids.

— *Way of action*

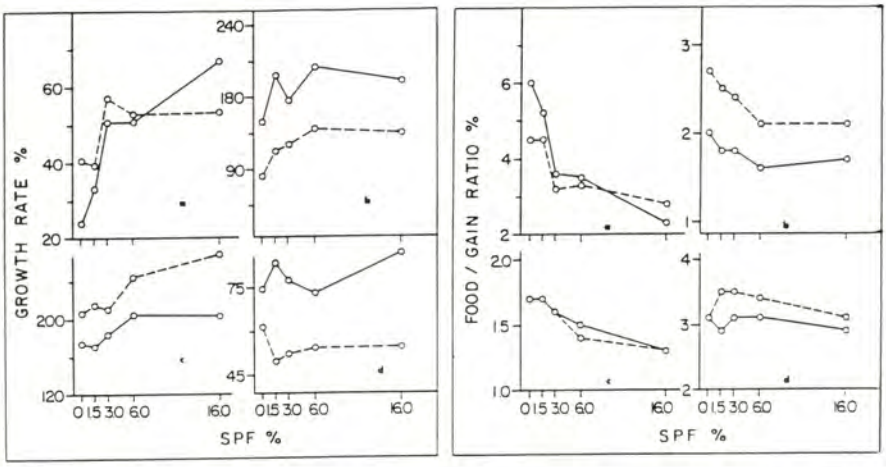

Figure 2. — Growth rate and feed conversion of four species of penaeid shrimp fed on diets containing different levels of squid protein fraction at two stocking densities.
a) *P. stylirostris*
b) *P. vannamei*
c) *P. monodon*
d) *P. indicus*
high stocking density
low stocking density

Growth factor *sensu stricto* or moulting factor - Tissue growth factors such as epidermal growth factor or nerve growth factor stimulate growth by cell division without inducing cell hypertrophy. Cell hypertrophy and hyperplasy were estimated in *P. japonicus* fed on diets containing or not SPF, using DNA and RNA content of whole shrimp as indicators of cell number and size respectively. The results clearly indicate that, DNA content being not increased, no hyperplasy was induced, while cell size was markedly increased (Cruz *et al.*, 1987 — Fig. 3).Therefore squid factor is not a growth factor *sensu stricto* (Cruz-Suarez *et al.*, 1987c).

Another way of action could be the stimulation of moulting by squid factor. This hypothesis was tested by Cruz *et al.* (1987a) in *P. vannamei.* In this trials shrimps were reared in individual tanks and weighed after each carefully recorded moulting. The experiment was conducted until all animals had moulted at least 3 times. No effect of diet (basal diet or 6 % SPF) was noticed on the duration of the intermoult periods, but the weight increment at moult was clearly improved by SPF supplementation

($p < 0.05$) (Fig. 4). Therefore squid factor does not act as ecdysones or other possible moult stimulating factor such as those tested by KANAZAWA *et al.* (1972) (without beneficial effect on weight gain).

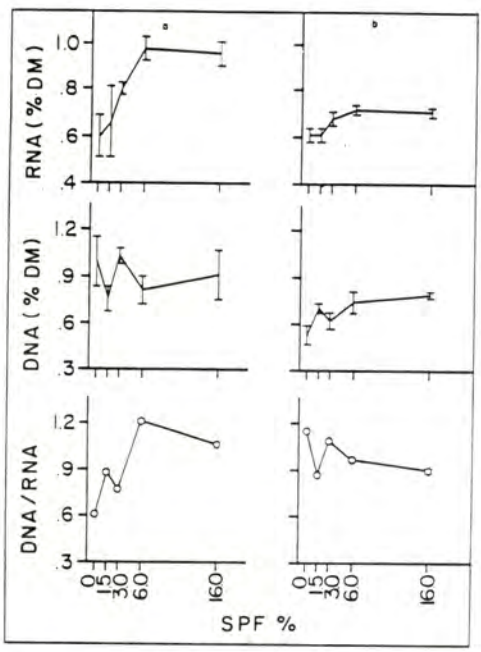

Figure 3. — Effect of dietary supplementation with various levels of squid protein fraction on RNA and DNA content of tissues in *P. japonicus*.

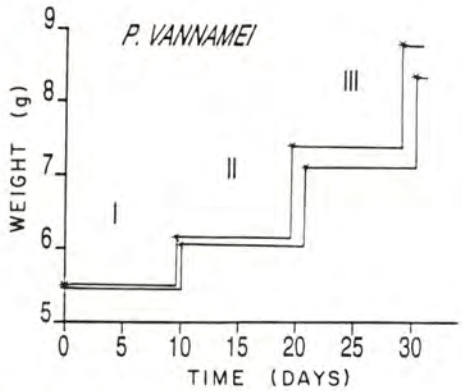

Figure 4. — Effect of the supplementation of diet with squid protein fraction on moulting frequency and weight increase at moult.

Feed attractant or related effect — In marine biology feeding behavior is often controlled or reinforced by feed activators or related compounds, at least in non predators. In the case of crustaceans, feed attractants or stimulants were shown to be free amino acids or bases

(Heinen, 1980) and may be indirect by efficient growth promotion (Deshimaru and Yone, 1978). As indicated previously, a feed activator effect was observed in our preliminary experiment when hydroalcoolosoluble extract was added to basal diet but this fraction did not enhance growth, a result in agreement with the statement of Deshimaru and Shigeno (1972) who noticed that high rate of feed-intake does not necessarily bring about rapid growth. Furthermore in all experiments where squid protein or squid extracts were tested, the increase of growth was always obtained without increase of food intake. Squid factor does not increase appetite.

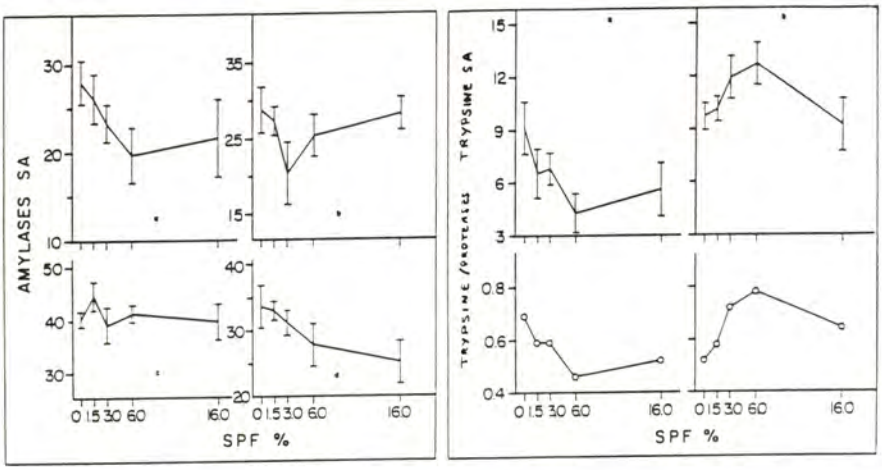

Figure 5. — Amylase, trypsine and trypsine/total protease ratio in shrimp fed diets containing different levels of squid protein fraction.
For amylase a) *P. stylirostris* b) *P. vannamei*
c) *P. monodon* d) *P. indicus*
For trypsine and trypsine/protease ration
a) *P. stylirostris* b) *P. indicus*

Improvement of digestion or absorption - In all trials feed efficiency was improved together with growth rate and, at first sight, it could be explained by an improvement of digestion or absorption of nutrients (Figure 1). No amylasic or proteasic activity was noticed on SPF or squid extracts; no effect either of SPF supplementation was observed on digestibility of food (unpublished). But these results do not allow to discard any effect on the kinetic of digestion or absorption. The activity of digestive enzymes (amylase, total proteases, trypsine) was studied in the digestive tract of shrimp belonging to four species during the day following food intake (Cruz-Ricque, 1987). Results were not quite similar in each species but, by and large, the specific activity was lower in groups fed on diets supplemented with SPF (Fig.5). The purification of *P. japonicus* trypsine was also performed and its activity was related to the actual enzyme protein; but, again, no clear relationship between growth stimulation and enzyme activity was found. However a surprising difference in *in vitro* activation of digestive enzyme activities was remarket (Fig. 6), this activation being more pronounced when shrimp had been fed on diets supplemented with squid extracts. This phenomenon has not been explained.

The time course appearence of glucose and free amino acids in haemolymph was measured in *P. japonicus* as well as in *P. vannamei*; the postprandial increase of both glucose and amino acids was more pronounced with SPF ($p < 0.05$) (Cruz-Ricque *et al.*, 1988 - Fig. 7). In a further study performed on *P. vannamei* a more pronounced increase of amino acids ($p < 0.05$) was observed when shrimp had been fed on SPF supplemented diet for 4 weeks, but was already noticeable after 4 days. It concerned both essential and non essential amino acids and was not related to the transport system (if similar to those of vertebrates) indicating that the absorption mechanism itself does not seem to be responsible for the beneficial effect of SPF (Revol and Guillaume, 1989).

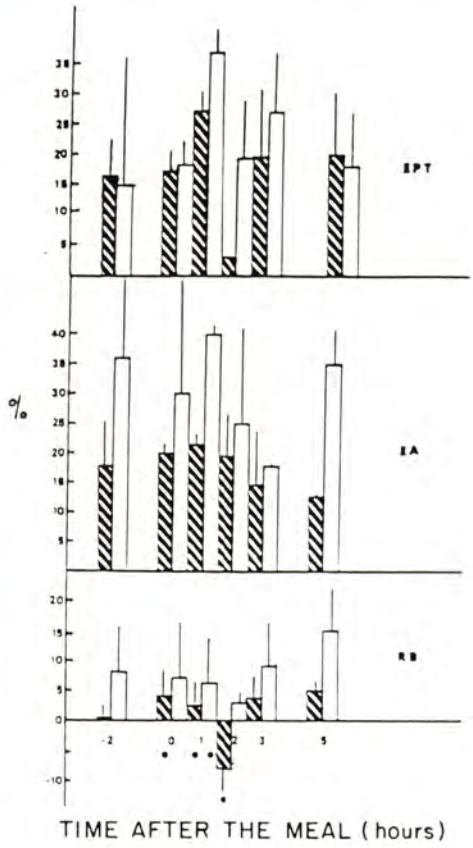

Figure 6. — Activation of amylasic and trypsic activities during incubation (in % of initial activity) in relation to time after the meal.

Otherwise attemps to estimate *in vitro* absorption of labelled amino acids either on gut or on isolated hepatopancreas cells failed to show any effect of SPF (unpublished data).

In conclusion the way of action of squid factor remains unexplained. This factor is very different from « unknown growth factors » that stimulate growth rate of chicks by 3 to 6 % only and do not improve feed

efficiency. It is different from nucleic acids. The hypothesis of its identification to a growth factor *sensu stricto*, or to a moulting hormone may be rejected. Several hypotheses remain to be tested such as a possible influence on gut microflora or an effect of hormones which may influence the organism after ingestion of animal meal such as fish meal (Pelissero *et al.*, 1988; Higgs *et al.*, 1982). Squid factor improves the nutrition of cells not their division, however the way of this improvement itself remains unknown.

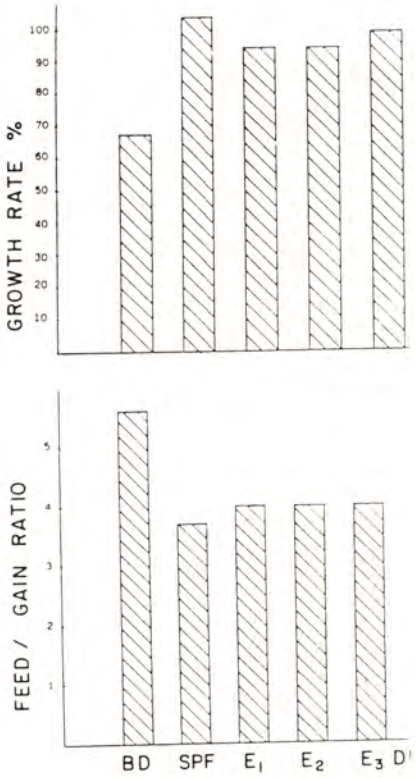

Figure 7. — Growth rate and feed conversion of *P. japonicus* juveniles fed on diets supplemented with various extracts of squid containing « squid factor » (more and more concentrated fractions).
BD = basal diet; for explanation of SPF, E1, E2 and E3 see table 1.

CONCENTRATION AND PURIFICATION

Because of a patent request no detail can be given on the procedure used for purification. However fractions with increasing activities were obtained using a first procedure (Table 2 — Fig. 8). Because of unsufficient reliability of the process a second technique was developed which is still being tested.

Untill now the most concentrated fraction obtained was efficient at 100-200 ppm but it was still a mixture of several compounds.

Tab. 2. — Concentration of squid factor : incorporation level of the various fractions obtained and estimation of the concentration factor.

	Incorporation level	Concentration factor
Whole squid (DM)	10	0.6
Squide protein (=SPF)	6	1
E1	1	6
E2	0.17	35
E3	0.02-0.01	300-600

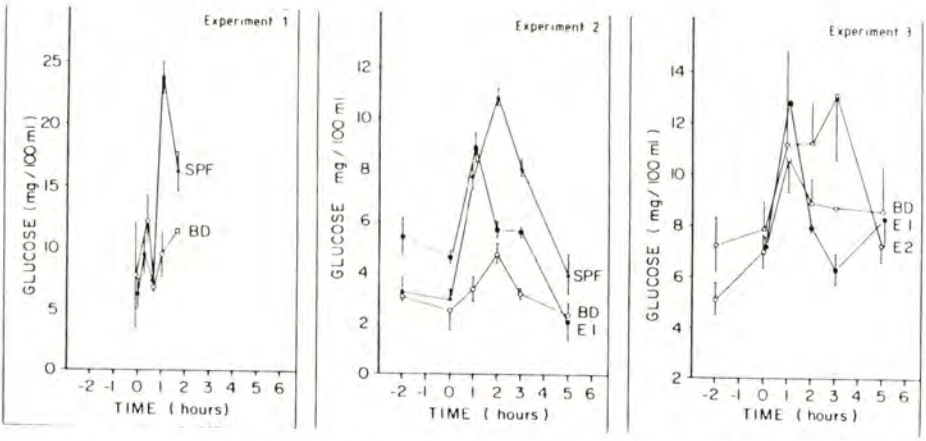

Figure 8. — Postprandial increase of haemolymph glucose level in *P. japonicus* fed on diets containing different squid fraction (same remarks as in figure 7 for explanation of symbols.

CONCLUSION

Neither the way of action of squid factor nor its chemical nature are known. But its efficiency was found in several dozen experiments or trials conducted on four species of penaeids. From a practical point of view the presence of « squid factor » in feedstuffs should be explored to gather information of their possible interest in shrimp diets. The identity of squid factor with the « unknown growth factors » suspected in mussel by Fernandez and Puchal (1979) and Foster and Beard (1973), or in lactic yeast and spirulina by Cuzon *et al.* (1981) should be verified. The very high feeding value of crab protein demonstrated by Boghen and Castell (1982) unexplained to our knowledge, could be related to a more or less similar factor.

The efficiency of squid factor in usual diets under practical rearing conditions was *a priori* not evident. An experiment of Cruz-Ricque et *al.*, (1987a) demonstrated that the effect of squid factor was at least as pronounced in a pond (with some natural productivity) rather in tanks (as sole food) when SPF supplemented versus SPF free diets were compared. But this conclusion is perhaps not valid in any rearing condition and new experiments remain to be performed before growth enhancing factors could be taken into account in shrimp diet formulation.

But from a scientific point of view the purification of this factor should be achieved and its way of action should be elucidated for a better understanding of shrimp nutrition.

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Reference diet for Crustaceans : principles of experimentation

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Abstract. — *As a result of discussions among crustacean nutrition researchers at the annual meetings of the World Aquaculture Society (WAS) in 1984, feeding trials were conducted with over 22 different crustacean species to compare the potential use of two diets as Standard Reference Diets (SRD) in crustacean research. The two diets (BML 81 S, a casein/albumin based formula developed at the University of California, Bodega Marine Laboratory, and HFX CRD 84, a crab protein concentrate based diet formulated at the Canadian Department of Fisheries and Oceans Halifax Laboratory) were about equal in performance for most species tested. Though neither diet was an ideal SRD, the HFX CRD 84 formulation based upon a purified protein of marine origin was accepted as the interim SRD for use in aquatic crustacean studies.*

As a further aid in making comparison among species and laboratories, it was proposed that standard sets of experimental diets be produced and distributed to various laboratories around the world. The first such set of 12 diets was produced in 1987 and contained varying levels of protein, lipid and carbohydrate to evaluate optimum protein/energy ratio. Feeding studies have been completed with these diets being fed to ***Homarus americanus*** *at the DFO laboratory in Halifax, and* ***Penaeus orientalis*** *at the Yellow Sea Fisheries Research Institute Laboratory at Qingdao in China. Studies are in progress with* ***Astacus astacus*** at the University of Stockholm in Sweden. These diets have also been supplied to researchers working with several other species of crustaceans.

Although many aspects of experimental design are controlled by unique biological and behavioural characteristics of each crustacean species, it is recommended that careful consideration be given to proper standards for all aspects of research on nutrient requirements of aquatic crustaceans including water systems design, water quality control, environmental conditions, pre-experimental culturing conditions, statistical methods of data analyses and reporting results.

As a further aid in improving the methodology and experimental design used in crustacean nutrition research, it is recommended that researchers in this field form an International Crustacean Nutrition Working Group to review existing information on nutrient requirements and research techniques and in developing improvements.

INTRODUCTION

There have been difficulties in comparing results of nutrition research conducted with various crustacean species and at different laboratories. These difficulties have been associated with the lack of standardization in experimental diets, standard technologies, culture conditions, animal stocks, culture history, age or size at initiation of the experiment, and other aspects of experimental design used (New, 1976a, b). In 1976, the World Mariculture Society (now World Aquaculture Society, WAS) established a Nutrition Task Force to review nutrition research techniques with aquatic species and to make recommendations for scientists conducting this type of study. The reports of both this Nutrition Task Force (Conklin and Beck, 1979) and of the EIFAC, IUNS and ICES Working Group on standardization of methodology in Fish Nutrition Research (EIFAC, 1980) emphasized the importance of standardization in nutrition studies of aquatic species.

At an informal discussion on crustacean nutrition during the 1984 World Mariculture Society (WMS) meeting in Vancouver it was concluded that the establisment of a Standard Reference Diet (SRD) for crustaceans in general or species specific SRDs would be a very important first step in standardizing the nutrition research conducted by scientists of the WMS. It was decided to evaluate two diets that had been developed for lobster (*Homarus* sp.) as potential SRDs for a wide variety of other crustaceans. Sufficient quantities of HFX CRD 84 (a crab protein concentrate based diet developed at the Dept. of Fisheries and Oceans Halifax Laboratory) and BML 81 S (a casein/albumin diet formulated at the University of California Bodega Marine Laboratory) were prepared at the DFO Halifax laboratory and distributed to researchers around the world (Castell et *al.*, 1989a). Though both diets were designed for studies with lobsters they each supported reasonably good growth and survival of a wide range of crustacean species (Bordner, 1989; Castell et *al.*, 1989a; Morrissy, 1989, Reed and D'Abramo, 1989). At the Crustacean Nutrition Workshop held during the WAS 1986 annual meeting in Guyaquil, Ecuador it was decided that while either diet might serve as adequate SRD the HFX CRD 84 would be accepted as the first internationally recognized SRD for crustacean nutrition research.

The objective of this report is to discuss the further improvement in standardization in crustacean nutrition technology, including consideration of (a) an improved SRC for crustaceans in general, (b) development of species specific SRDs, (c) the use of standard sets of experimental diets to investigate specific nutrient requirements of several different species, (d) provision of analyses of nutrient content of SRDs as well as ingredients used in the manufacture of SRDs, (e) standards in experimental animals for each important species, (g) standard methods of evaluating nutrient value of diets, (h) standards in reporting results and (i) statistical interpretation of results. Many of the researchers involved in aquatic crustacean nutritional requirements have limited training in nutritional sciences.

It has frequently been necessary to develop diets for animals used in disease, genetics, toxicology or other aspects of crustacean culture with species where little or no nutritional requirement information is available.

Since nutrition research of aquatic species is relatively recent, reference to standards developed for nutrition with small animals on land will be used as models for suggestions put forth in this report. Where applicable the terminology will conform to the recommendations ofthe American Nutrition committee on Nomenclature (1987 a,b). In spite of more than a century of nutrition research on rat and nutrition, it was only 12 years ago that the American Institute of Nutrition Committee on Standards for Nutritional Studies (AINCSNS, 1977) proposed a Standard Reference Diet (AIN-76) for these rodents. When fed for prolonged periods, this purified Reference Diet resulted in some nutritional deficiency signs and modifications to the SRE (AIN-76) were suggested in a later report of the AIN Committee on Standards for Nutritional Studies (1980).

Tab. 1. Formulations proposed for evaluating the relative nutritional values of crab protein concentrate from Halifax and squid protein concentrate from France.

Ingredient	HFX CRD 84 (40)	(30)	(20)	(40)	(30)	SPC 89 (20)
Crab protein concentrate	40.0	30.0	20.0			
Squid protein concentrate				40.0	30.0	20.0
Wheat gluten	5.0	5.0	5.0	5.0	5.0	5.0
+ Corn starch	15.0	20.0	25.0	15.0	20.0	25.0
Dextrin	5.0	5.5	6.0	5.0	5.5	6.0
Alpha-Cellulose	17.8	19.3	20.8	17.8	19.3	20.8
Cod Liver Oil	6.0	8.0		6.0	8.0	10.0
Corn Oil	3.0	4.0		3.0	4.0	5.0
Cholesterol	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix (Modified Bernhart-Tomarelli)	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix CRD	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin E (DL Alphatocopherol)	0.2	0.2	0.2	0.2	0.2	0.2
Choline Chloride (70 %)	1.0	1.0	1.0	1.0	1.0	1.0

Finally, this report will suggest establishment of an international committee for the compilation of standard research methodologies and terminology for aquaculture researchers starting with nutrition methodologies. This working group would start by collecting suggestions from documents such as the ICES Aquaculture Glossary (Rosenthal 1986) American Institute of Nutrition Experimental Animal Nutrition committee (1987) and the Institute for Laboratory Animal Resources, National Academy of Sciences Guidelines for Satisfactory Nutritional Practises in Experiments Using Small Animals (Cf. AINCSNS 1977) as well as the several reports on standardization in aquaculture nutrition Conklin and Beck, 1979; EIFAC, 1980; Metailler, 1987.

STANDARD REFERENCE DIETS

Both tentative SRDs for Crustacean research fit the AIN definition for a Purified Died (AINCSNS 1977); « composed primarily of refined ingredients, i.e. refined protein, carbohydrates and fat, with added mineral

and vitamin mixtures ». Information on amino acid, fatty acid, mineral, vitamin and proximate composition of both formulations is available (Castell et *al.*, 1989a, Reed and D'Abramo, 1989).

Though either diet appeared acceptable as a SRD for a wide range of crustacean species, and the HFX CRD 84 formulation has been recommended as an interim SRD. There are difficulties with both diets. The casein/albumin based diet (BML 81) requires the addition of 6-7 % soy lecithin to prevent molt death when fed to juvenile lobsters, *Homarus* sp. (Bowser and Rosemark, 1981; D'Abramo et *al.*, 1981). If the lecithin is deleted and replaced with purified fatty acid studies, most lobsters fed these modified diets would father acids for Fatty acid esters, as would be necessary for essential diet of « molt death » syndrome. The soy lecithin is not required in diets using the crab protein concentrate (CPC) as the principal source of protein (Kean et *al.*, 1985), however, the CPC, used in HFX CRD 84, contains relatively high mineral content which makes modification of this formulation for the study of mineral requirements impractical.

One important aspect of a SRD is that it or the ingredients used in its production should be readily available and consistent in nutrient composition. Though the crab, *Cancer irroratus*, that is used for producing the CPC in HFX CRD 84, is abundantly available as a by-catch of the lobster fishery in eastern Canada, only relatively small quantities of the CPC are available from Novo Scotia Crustacean Feeds, a small company that utilizes the feed production and laboratory facilities of the Dept. of Fisheries and Oceans Laboratory, Halifax for producing feeds and feed ingredients. The limited supply and high price of HFX CRD 84 will limit its world wide application as a SRD for crustacean research. This formulation will continue to be made available to those wishing to use it as a SRD, but it will continue to have limited application until a larger facility is developed for the production of CPC, or a substitute source of purified protein that produces good growth and survival of aquatic crustaceans, is developed. It is possible that a purified squid protein, such as incorporated into purified diets for penaeids by the researchers of France (Cruz-Ricque *et al.*, 1987; Cruz- Suarez *et al.* 1987), would be of equal or better nutritional value compared with the CPC from Halifax.

In the feeding studies comparing HFX CRD 84 and BML 81 S, two different formulations were being tested. There were differences in the protein, carbohydrate, lipid, vitamin and mineral supplements in these formulations. If the squid protein concentrate from France is to be truly compared with crab protein concentrate from DFO, Halifax, all other dietary ingredients should be constant. It would be interesting to compare these protein sources at several different levels of supplementation to evaluate the relative nutritional value in test diets for different crustacean species. The six diets proposed in Table 1 are approximately isocaloric and could be used to compare the two proteins in feeding studies with several species. For *Homarus americanus* optimal growth was obtained with 30 % CPC rather than the 40 % used in HFX CRD 84 (Castell et *al.*, unpublished results). It is possible that a lower level of protein would improve the SRD for use with other crustacean species. Following a co-operative international multi-species study, the best of the squid or crab based formulations

could be accepted as the new SRD. Identification of differences in species responses to either the protein/energy or the crab versus squid protein formulations would be the first step in identifying species specific SRDs.

UNREFINED SRD

HFX CRD 84 and BML 81 S are purified diets and have value as SRDs for relatively small scale laboratory studies with crustaceans, but they are much too expensive to use as SRDs in pilot scale or commercial culture feeding trials. The AINCSNS (1977) also proposed the terms « Unrefined or Non-purified Diet » for formulations composed predominantly of unrefined plant and animal materials. They proposed a diet designated NIH-07 as a Standard open formula diet which allowed for a less expensive reference diet that was reproducible among different laboratories and over time. Again it is probable that eventually it will be beneficial to develop an unrefined SRD for each important crustacean species being studied, but it would be convenient to start with one reproducible, high nutritional value, relatively expensive Unrefined Crustacean Diet as a standard for evaluating other practical or commercial crustacean feeds.

Table 2. Formulation of the Dept. of Fisheries and Oceans, Halifax unrefined reference diet.

HFX-EXD-85	
Ingredient	%
Freeze-dried crab meal	50.0
Wheat gluten	5.0
Wheat middlings	20.0
Alpha-cellulose	11.8
Sodium alginate (Kelgin HV)	2.0
Herring Oil	4.0
Corn Oil	2.0
Vitamin Mix (Same as for HFX CRD 84)	2.0
Carophyll red	0.1
Bernhart-Tomarelli modified salt mix	2.0
Alpha-tocopherol	0.1

Based upon the success of the HFX CRD 84 purified SRD in lobster studies, an unrefined formulation using freeze-dried crab meal has been tested at the Halifax, DFO Laboratory. When fed to juvenile lobsters, this formulation (HFX EXD 85, Table 2) produced greater growth that significantly exceeded that of animals fed either HFX CRD 84 or BML 81 S (Figure 1). While both of the purified SRDs resulted in white lobsters, the HFX EXD 85 fed animals were naturally coloured. This diet was about one tenth the price of HFX CRD 84 and produced as fed feed conversion values as low as 1.25 (weight feed/live weight gain). A vaccine development company, Aqua-Health has routinely used this formulation to grow juvenile lobsters from 4th stage up to one year of age for vaccine testing experiments. These animals have experienced good growth and high

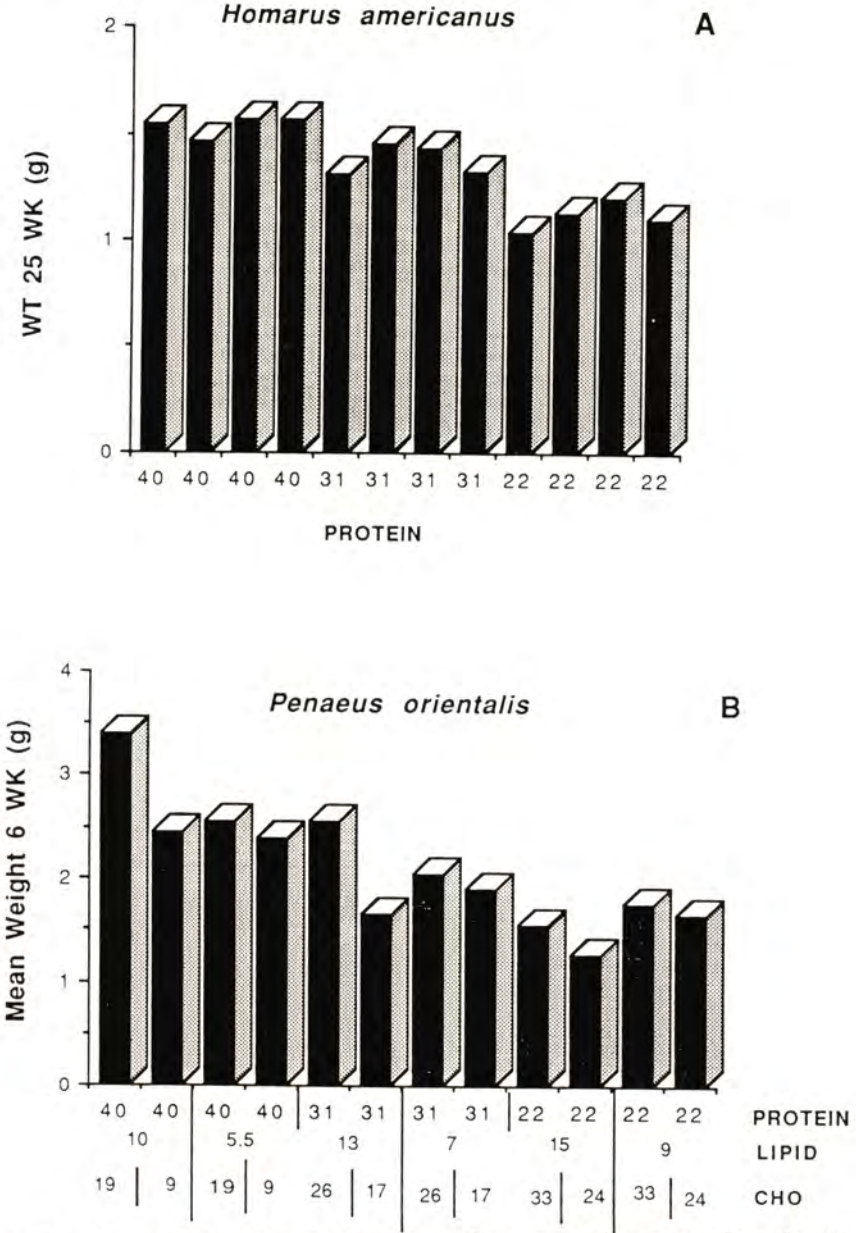

Fig. 1. — Mean final weights of (A) lobsters, *Homarus americanus* after 25 weeks and (B) prawn, *Penaeus orientalis* after 6 weeks feeding with diets varying in protein, lipid and carbohydrate.

survival even when exclusively fed HFX EXD 85 in excess of one or more years. Nova Scotian Crustacean Feeds can also make limited quantities of this feed available for testing as a « practical » or unrefined reference diet for other crustacean studies.

STANDARD EXPERIMENTAL DIETS

Many factors involved in diet production may affect the nutritional value and animal responses to sets of diets designed to evaluate the nutrient requirements of various aquatic crustaceans. Some of these factors can lead to inconsistencies in interpretation of results obtained at different laboratories with regard to the requirements of specific nutrients. A few examples of factors affecting results are :

- interference by other dietary ingredients. Diets containing lecithin or a mixture of phospholipids would not provide any evidence for a requirement for supplemental choline chloride or inositol. Absence of antioxidants or supplements of some mineral salts can lead to autoxidation of highly unsaturated fatty acids and a decreased growth and survival with a diet that should produce enhanced growth and survival if the essential fatty acids were properly protected from oxidation.
- the physical characteristics of the feed pellets are affected by the type of binders, the processing temperature and equipment, particle size of the feed ingredients, moisture content and by the over all formulation of the diet. In the study of aquatic crustacean nutrition the physical characteristics of the feed pellets are especially critical. Most crustaceans find their feed by chemoreception. It may take several minutes to several hours in the aquatic environment before the animal begins feeding on the feed pellet. The initial chewing is done externally by a combination of mandibular appendages. Water soluble nutrients may be leached into the environment and become unavailable to the test animal. This loss will be affected by the physical characteristics of the diet. The plasticity and adhesiveness of the diet will affect the amount of feed which crumbles and is lost during the external chewing of the pellets.
- even commercially available purified feed ingredients may vary in physical properties and nutrient composition. Thus diets prepared in accordance with the same formulation in different laboratories, with identical feed processing equipment may still vary slightly in final nutrient content and nutritional value.

Because of these and other factors which affect results of nutritional experiments with crustaceans it was suggested that not only should we consider use of a Standard Reference Diet, but we should consider standard sets of experimental diets produced at one facility to minimize interferences in interpreting results.

NEWSLETTER QUESTIONNAIRE

In order to maintain a communication among those interested in the development of a SRD for crustaceans and other forms of collaboration, a Crustacean Nutrition Newsletter was established. In 1985 a questionnaire sought input on the suggestion of taking diet standardization one step

further to standard sets of experimental diets. There were 24 scientists who expressed an interest in participating in testing of standardized experimental diets designed to compare specific nutrient requirements of various crustacean species. When asked to list the various aspects of nutrition in order of importance, the number one and two priorities were essential fatty acid (EFA) requirements and optimum protein/energy ratio. Since the pure fatty acid esters required for EPA studies are extremely expensive, it was decided that the first International Co-operative Standard Crustacean Nutrition Study should focus on protein/energy ratios.

Table 3. Diet formulations for international co-operative crustacean nutrition study designed to evaluate the effect of varying protein/energy ratio.

The following values, were used for calculating digestible energy values :

Nutrient	Digestibility	Kcal/g	Ingredient	Purity
Protein	96 %	5	Crab protein conc.	90 % protein
Lipid	90 %	9	Corn starch	92 % CHO
Carbohydrate	65 %	4	Corn oil	100 % lipid
			Cod liver oil	100 % lipid
			Cholesterol	100 % lipid

Basal diet ingredients	%
Wheat gluten	5
Dextrin	5
Mineral mix (as in HFX CRD)	4
Vitamin mix (as in HFX CRD)	2
Vitamin E acetate	0.2
Choline chloride (70 %)	1.0
Cholesterol	1.0

INGREDIENT	1	2	3	4	5	6	7	8	9	10	11	12
CPC	40	40	40	40	30	30	30	30	20	20	20	20
Corn starch	15	5	15	5	23	13	23	13	31	21	31	21
Lipid mix[1]	9	9	4,5	4,5	12	12	6	6	15	15	8	8
Alpha-cellulose	17,8	27,8	22,3	32,3	16,8	26,8	22,8	32,8	15,8	25,8	22,8	32,8
TOTAL							81,8					
Estimated :												
Protein	40	40	40	40	31	31	31	31	22	22	22	22
Carbohydrate	18,5	9,2	18,5	9,2	25,8	16,6	33,1	23,9	33,1	23,9	33,1	23,9
Lipid	10	10	5,5	5,5	13	13	7	7	16	16	9	9
Kcal/100 g	321	297	284	260	321	300	273	251	321	297	265	241
Protein/energymg/Kcal	125	134	141	153	97	103	114	123	69	74	83	91

[1] Lipid mixture is a 2 : 1 mixture of cod liver oil.

PROTEIN/ENERGY RATIO

Sufficient quantities of 12 diets with three levels of protein (40, 31 and 22 %) and two levels of lipid and carbohydrate at each protein level were produced in the DFO, Halifax laboratory to permit up to 12 different species to be studied with the identical set of diets (Table 3). These diets

have been distributed to scientists in the United States of America, Australia, Sweden and China. There are still several 500 g, nitrogen flushed, vacuum sealed, plastic coated packages of each of these diets stored at 40°C that would be available to any other researchers wishing to compare responses of their species with results for *Homarus americanus*, *Penaeus orientalis*, *Astacus astacus* and other crustacean species fed these test diets. Though the results of these studies will be the subject of future publications, it is interesting to note that considerable differences exist in the apparent protein/energy optimum of *Homarus americanus* (Castell, DFO Halifax, Nova Scotia, Canada; unpublished results) and *Penaeus orientalis* (Xu Xueliang, the Yellow Sea Fisheries Research Institute, Qingdao, China; unpublished results). The prawn was able to derive a growth advantage of the highest energy diet at the higher protein level (Figure 2A) while this high lipid level provided no growth enhancement of the lobster compared with the lowest energy diet at the highest protein supplementation (Figure 2B). Thus although this high energy diet (which

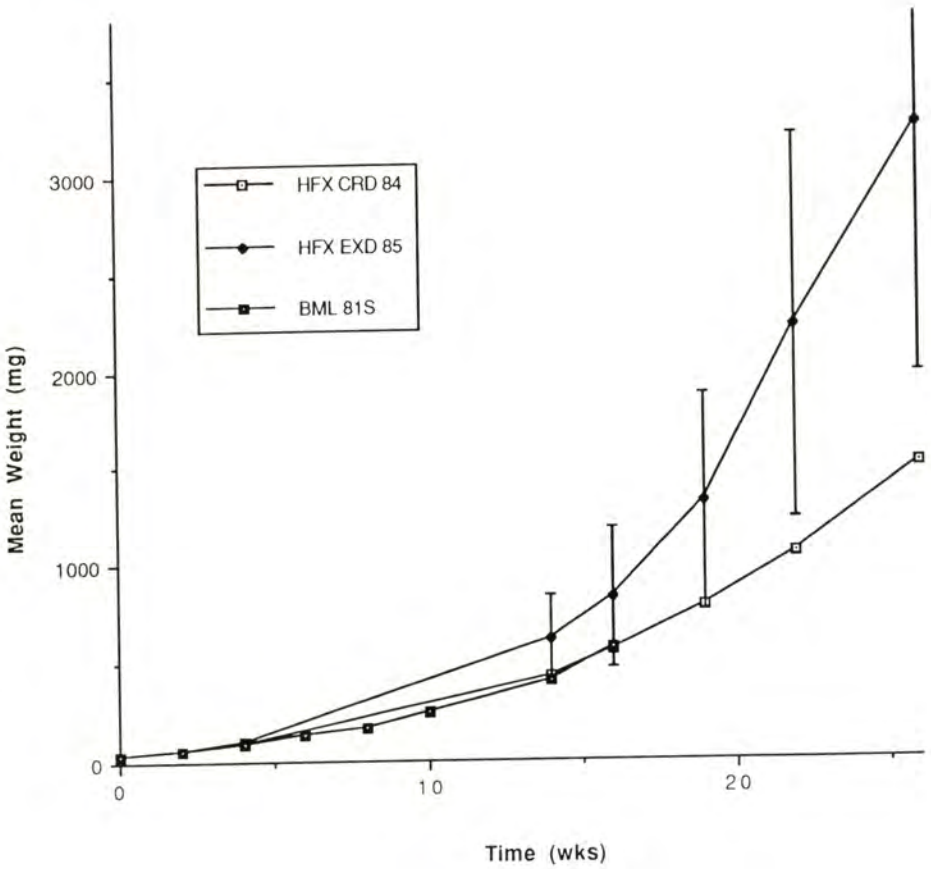

Fig. 2. — Growth curves of lobsters fed either of the purified Standard Reference Diets (BML 81 S or HFX CRD 84) for 26 weeks after reaching 4th stage, compared with growth of lobsters from the same family fed the unrefined Reference Diet (HFX EXD 85).

is HFX CRD 84) was optimal for *Penaeus orientalis* but it contained excess lipid which the *Homarus americanus* was unable to utilize effectively and which was accumulated in the digestive gland.

Table 4. Preparation for dietary lipid study 1988.

Basal diet ingredients	% of dry diet
Crab protein concentrate	40
Gelatin	10
Com Starch	15
Dextrin	5
Cellufil	17,3
Mineral mix (as in HFX CRD)	4
Vitamin Mix (« » « »)	2
Cholesterol	0,5
Total	93,8

Note : Gelatin, corn starch, dextrin and cellufil must be solvent extracted (boiling ethanol) prior to making basal mixture to remove traces of lipids.

Ingredients to be added during final diet preparation.

Ingredient	%
Vitamin E acetate	0,2
Choline chloride (70 %)	1,0
Expt. Lipid Mixture	5,0
Total	6,2

Percent of triglycerides to be added to 12 experimental diets for juvenile lobster and prawn.

Diet	1	2	3	4	5	6	7	8	9	10	11	12
16 :0	2,5	2	2	2,35	2,35	2,15	2	2	2	2	2	2
18 : 1	2,5	2	2	2,35	2,25	2,15	2	2	2	2	2	2
18 : 2n-6	0	1	0	0	0	0	0,5	0,25	0,1	0	0	0
18 : 3n-3	0	0	1	0,3	0,5	0,7	0,5	0,75	0,9	0	0	0
20 : 4n-6	0	0	0	0	0	0	0	0	0	1	0	0,5
22 : 6n-3	0	0	0	0	0	0	0	0	0	0	1	0,5

ESSENTIAL FATTY ACIDS (EFA)

As part of a Canadian International Development Research Center funded project a similar set of 12 diets varying only in EFA content are being tested with *Homarus americanus* at the DFO, Halifax Laboratory and with *Penaeus orientalis* at the YSFRI, Qingdao, China (Table 4). This set of diets was designed to compare the relative EFA values of 18 :2n-6, 18 :3n-3, 20 :4n-6 and 22 :6n-3 as well as various mixtures of the 18 and longer carbon chain n-6 and n-3 triglycerides. On the assumption that the n-3 fatty acids will have greater EFA value, this experimental diet set also

contains five different levels of supplemental 18 : 3n-3 as the only polyunsaturated fatty acid triglyceride.

It is possible that cost of dietary ingredients will be a limiting factor in this set of standardized experimental diets. Just the pure lipids used in these 12 diets for use with prawn (*P. orientalis*) and lobsters (*H. americanus*) cost over $4000.00 CDN. If there are researchers who wish to test any or all of the diet formulations listed in Table 4 and are willing to pay the cost of ingredients, we would be willing to produce sufficient quantities to meet their needs.

STANDARD EXPERIMENTAL CONDITIONS

In studies of nutrient requirements the researcher may either attempt to provide environmental conditions that stimulate maximum growth or that approximate the conditions under which the species being investigated would be cultured for commercial production. In either case, those conditions will be very specific for the species in question. To facilitate comparisons among different laboratories which study the same crustacean species, it would be helpful to identify the temperature, salinity, photoperiod, photo intensity, tank colour and shape, feeding ration, feeding frequency, water exchange rates and other environmental factors which provide optimum growth and survival rates. Though it is unlikely that identical conditions could be established at each laboratory, it would be useful to list recommended standard environmental conditions which approximate the optimal conditions for each species being investigated.

STANDARD REFERENCE ANIMALS

Unlike the situation in nutrition research with rats, most crustacean nutrition studies are conducted with wild captured animals or the offspring of wild broodstock. Much of the variance in responses to differences in diets may be explained by the diversity in the genetic make up. Though often unavoidable, this genetic diversity may result in differences in response to diets from one experiment to the next in the same laboratory as well as differences among laboratories working with the same species. As with studies on rats, chickens and other land based animals, it would be useful to establish standard strains of experimental crustaceans.

STOCK OF GENETIC IDENTITY

Because standard reference strains of most aquatic crustaceans do not exist and could not be established in the near future, it is important to identify as accurately as possible the origin of the stocks that are used in current nutrition studies. Information on source of stock should be provided in any publication of research results. Whenever possible, the

same source for experimental animals should be used in each subsequent study or source and reasons for changes among experiments should be given.

PRE-EXPERIMENTAL REARING CONDITIONS

The diet and culture conditions of animals prior to the initiation of a feeding study will affect their responses in a nutrition trial. It may be useful to establish a set of recommended culture conditions for each species of crustacean that becomes the subject of nutrition and culture research in several laboratories. Clearly these pre-experimental rearing conditions are an important element in the experimental design and cannot be ignored.

SIZE AND AGE

There are differences in the growth and patterns of metamorphosis among the various crustacean species. It is most probable that physiological and nutritional requirements vary with developmental stages of aquatic crustaceans. It is therefore very important to identify the age, size and developmental stage of the animals used in each experimental study. Whenever possible, animals at the same stage should be used for comparisons among experiments at the same laboratory or among laboratories studying the same species.

EVALUATING GROWTH RESPONSE

In addition to the complications associated with metamorphosis and different developmental stages, crustaceans experience a very different weight gain growth curve than most other animals involved in nutrition research. Because of the restrictions of an exoskeleton, crustaceans must moult before a major increase in body size. Application of classical exponential growth equations to crustacean growth patterns must neglect some of the vital growth information that could be used interpreting growth responses to nutritional differences among diets. It would be more informative if a growth model were developed for crustacean work that allowed comparisons of variations in weight gain per molt at each developmental stage, length of inter/molt periods, as well as changes in weight gain per molt and intermolt period with time in response to dietary differences.

NON-DIETARY SOURCES OF NUTRITION

In addition to losses of dietary nutrients through leaching and crumbling, crustacean nutrition researchers must be concerned with non-dietary sources of nutrient that can interfere with experimental results.

Algae, mold, fungus or bacteria may grow on the surfaces of experimental holding containers and provide an uncontrolled source of nutrition to the experimental animals. To minimize this, uneaten food and faeces should be daily removed from the animals environment. We transfer our lobsters to clean holding trays every two weeks at weighing time and physically clean and disinfect the used trays.

There may be suspended or dissolved nutrients in the water supplied to the experimental animals. D'Abramo *et al.* (1988) provided an excellent description of the system that they have developed for conducting nutrition research with *Macrobrachium rosenbergii.* They have taken great pains to insure that each animal receives the same quality and volume of replacement water. Water is passed through filters and UV sterilization treatment to minimize water borne nutrients. In our studies with *Homarus americanus*, Halifax Harbor water first passes through large pressurized sand gravel filters before being heated to 20°C in heat exchangers. It then passes through a second sand gravel filter before treatment with a 5M pore size cartridge filter. This water then is treated with activated charcoal and UV irradiation. In each laboratory involved in crustacean nutrition it will be necessary to take steps to minimize the non-dietary sources of nutrition.

INDIVIDUAL HOLDING vs POOLED POPULATIONS

Some species of crustaceans are so cannibalistic that nutrition studies are only possible if animals are reared in individual containers. This disadvantage has provided some advantages in analyses of results. It is possible to record weight gains, molt frequencies, intermolt periods and other nutritional response data on an individual animal basis because of the experimental constraints that the animal's behaviour has placed upon us in the study of *Homarus americanus* for example. Such individual animal data is more sensitive to differences among treatment and gives a more accurate picture of within treatment variances. This raises the question of whether, even with noncannibalistic crustaceans, holding animals individually might improve the statistical interpretation of results. This subject should be carefully evaluated by biostatisticians and animal behaviour experts for each species being studied.

FUTURE OF INTERNATIONAL COOPERATION

Many of the criticisms regarding experimental design and the lack of standardization, which New (1976a, b) reported with regard to shrimp and crustacean nutrition research over 13 years ago, are applicable to many current research reports. If there is to be significant progress made in understanding dietary requirements of commercially important cultured crustacean species, it would be advisable to encourage greater international cooperation. The three day « Nutrition of Crustaceans » workshop organized by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) February 24-28, 1989 in Tahiti is an important step in developing improved international cooperation. The continued improve-

ment of standardization of experimental designs and cooperation might be further aided by the formation of an **International Crustacean Nutrition Working Group**. This group should undertake the following tasks.

1. The development of an improved, readily available, reproducible, open formula, purified affordable Standard Reference Diet for aquatic crustacean research,
2. Development of species specific SRDs that are designed to match known nutrient requirements of important cultured crustaceans,
3. Publishing a summary report on all known nutrient requirements of important crustacean species,
4. Maintaining a catalogue of purified and unrefined crustacean diet formulations, given as complete bibliographical information and nutrient content information as possible for each formulation,
5. Establishing a manual of recommended techniques for the study of nutrition of aquatic crustaceans.

THE ROLE OF THE WORLD AQUACULTURE SOCIETY

Since its establisment of the Nutrition Task Force in 1976, the World Aquaculture Society has been actively encouraging improvements in standardization of experimental design applied in aquaculture research. Each year since 1983, the WAS has conducted a 2 hours nutrition workshop as part of its annual meeting. The Crustacean Nutrition Newsletter is published by the WAS to assist in information exchange regarding the use of SRDs in crustacean nutrition research. Several of the manuscripts on the BML 81 S vs HFX CRD 84 will be published in the Journal of the World Aquaculture Society. It is therefore proposed that the International Crustacean Nutrition Working Group be formed as a subgroup of the WAS and submit an annual report at the Annual Meeting of WAS (the next meeting being June 10-16, 1990 in Halifax, N.S., Canada).

CONCLUSIONS AND RECOMMENDATIONS

1. A STANDARD REFERENCE DIET is desirable for comparisons among experiments, species and laboratories involved in crustacean nutrition research. HFX CRD 84 is currently accepted as a Standard Reference Diet for crustacean nutrition research by a number of prominent researchers.
   - The crab protein concentrate made form the rock crab (*Cancer irroratus*) is in too limited commercial supply, is high in mineral content (about 10 % ash) and consists of a mixture of several different types of protein. An alternative purified protein for use in crustacean SRD is being sought.
   - Though HFX CRD 84 provided reasonable growth and survival of several freshwater and marine crustaceans it is probably

not an optimal formulation for any of these species : not even the lobster for which it was first formulated.

2. A STANDARD UNREFINED (OR PRACTICAL) REFERENCE DIET would be less expensive to produce than a purified reference diet and would be useful in comparing values of commercial, closed formula diets.
3. Complete information on formulations, diet and ingredient nutrient composition, digestibilities, vitamin and mineral premixes and other nutritional criteria must be available for any proposed SRDs.
4. Comparisons of results among different laboratories and species could be facilitated by the production of standard sets of experimental diets designed to test for specific nutrient requirements.
5. Following the lead of the American Institute of Nutrition, the World Aquaculture Society should establish an International Crustacean Nutrition Working Group to (A) conduct collaborative research programmes to establish general as well as species-specific SRDs, (B) to catalogue experimental diet formulations, (C) to recomment standards in experimental design and analytical procedures for application in aquatic crustacean nutrition research and (D) to continually update the known requirements and recommended minimums for nutrients in various cultured crustacean feeds.

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A brief review of nutritional studies for *Penaeus monodon*

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Abstract. — *In order to develop effective formulated for* ***Penaeus monodon****, sufficient information is needed on its nutritional requirements. Unfortunately, research on this area has been very limited. This paper outlines the results of such research. Since data on the nutritional requirements of* ***P. japonicus*** *are relatively well established, some of these data are also reviewed in this paper and are used as a basis for assessing the nutritional requirements of* ***P. monodon****. Protein, lipid, carbohydrate, mineral and vitamin requirements are evaluated from the parameters of growth survival and feed efficiency. Studies on the effects of enzymes and the development of microparticulate diet for larvae are also summarized.*

INTRODUCTION

Due to the high market value and great demand for the grass prawn, *Penaeus monodon*, in the world market, it has become one of the most promising prawns in the southwest Pacific region. It is ideal for culture because of some of its advantageous features such as rapid growth, tolerance to high temperatures and a wide range of salinities, large size and simple pond construction requirements. It is quite appealing to consumers because it has an attractive red color when cooked. Because of the early success of its artificial propagation and the availability of support industries such as manufacturers of feed and aquaculture facilities, the country's *P. monodon* culture industry has become well established and Taïwan is now regarded as one of the major producers of cultured prawn in the whole world (Liao et *al.* 1969, Liao and Chao 1983, Liao and Chiang, 1986, Liao, 1988a).

In the past, when *P. monodon* was commonly cultured under extensive culture systems, not much attention had to be paid to nutritional studies, since the extensive system relied mainly on natural food. However, with the shift from extensive to semi-intensive and intensive culture systems, nutritionally balanced artificial diets became crucial. It became increasingly necessary to determine the nutritional requirements of this

prawn to be able to formulate an effective and economically efficient diet for it.

At present, the nutritional requirements of *P. japonicus* are well know and reported. Several reviews on this subject already been published, notably those by New (1976), Kanazawa (1985a, 1985b) and Liao (1988b). The paper by New (1976) covered prawns in general, while the one by Liao (1988b) focused particularly on larval rearing of *P. japonicus*. Unfortunately, for *P. monodon*, research on this field has hitherto been rare. Nevertheless, based on the nutritional requirements of *P. japonicus*, the requirements for protein, lipid, carbohydrate, minerals and vitamins of *P. monodon* will be discussed and evaluated from the parameters of growth, survival and feed efficiency. Since information is very limited, the discussion will include works referring to different stages of growth of the above species.

PROTEIN REQUIREMENT

Gross protein

Protein is an essential component in a diet. Under an intensive culture system, feeding becomes more costly because it requires the use of artificial feed with several components, of which protein is the most expensive. This expensive protein fraction should therefore be utilized at an optimum level for growth, rather than for maintenance of the prawn. In this regard, the feed cost may be reduced by considering the optimum level of protein and the protein-sparing effect of nonprotein nutrients such as carbohydrates and lipid.

The optimum protein level usually varies depending on the source of nitrogen, the composition and ratio of essential amino acids, the cultured species and its stage of growth, the culture environment, the culture method, the energy value of the diet and many other factors. It has been ascertained that there is a correlation between feed efficiency and the amount of crude protein in the diets. Also, it has been known that diets containing suitable amounts of protein are capable of showing favorable feed efficiency. Kitabayashi et *al.* (1971d) reported that the optimum protein level for *P. japonicus* is estimated to be 53.5 %, while Shigeno et *al.* (1972) indicated that diets containing crude protein higher than 60 % have better feed efficiency for the same species. Similarly, Deshimaru and Kuroki (1974a) and Deshimaru and Yone (1978c) found that the suitable protein content is between 48 and 57 % and that a 52 % protein diet produces the maximum weight gain. On the other hand, in *Metapenaeus monoceros*, a diet containing 55 % protein gives the best growth (Kanazawa et *al.*, 1981). *P. brasiliensis* requires 54 % protein, while *P. penicillatus* requires 22 % protein for best growth (Liao et *al.*, 1986, 1988a).

As for *P. monodon*, Lee (1971) reported that better growth was obtained with 45 % protein in the diet. However, Lin et *al.* (1981) contended that protein in the diet of *P. monodon* should be about 35 %. Bages and Sloane (1981) revealed that the range of 35-45 % protein level in the diet produces the maximum weight gain in 40 days old postlarvae. Alava and Lim (1983) recorded an improvement in the growth, feed

conversion ratio (FCR), protein efficiency ratio (PER) and survival rate of *P. monodon* juveniles fed with a 40 % protein diet. More recently, Liu and Cheng (1988) proved that a 45 % protein diet showed significant effect on growth Table 1 shows the desirable level of protein in some penaeid prawns.

Table 1. Summary of data available on optimum dietary protein levels for penaeid prawns.

Species	main diet ingredient	Optimum protein level (%)	Author
P. japonicus	Casein	64	Deshimaru and Kuroki, 1974
	Squid meal	60	Deshimaru and Shigeno, 1974
P. monodon	Casein	52-57	Deshimaru and Yone, 1978
	egg albumin	46	Lee, 1977
		40	Khannapa, 1977
		35	Bages and Sloane, 1981
	White fish meal	35	Lin et *al.*, 1982
		45	Liu and Cheng, 1988

The efficiency with which protein is assimilated by prawns is most likely affected by the relative proportion of lipids and carbohydrates in the formula as well as the amino acid composition of the protein source. Bautista (1986) found a significant change in growth of *P. monodon* juveniles despite a reduction in protein content of the diet from 50 % to 40 % at an energy level of 330 Kcal/100g (Fig.1). He suggested that protein may be separed by carbohydrate or lipid as long as the calorie requirement was met, thus permitting more efficient utilization of protein. If the diet

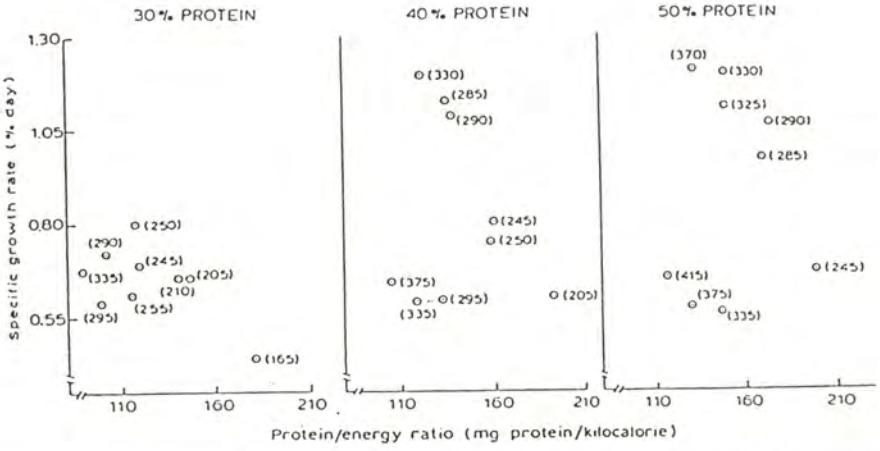

Fig. 1. — Specific growth rate of *Penaeus monodon* juveniles fed with diets with varying protein/energy ratios. Numbers in parentheses are energy levels (kcal/100 g) (Bautista 1986).

does not contain sufficient energy, protein may be used for supplying energy rather than for promoting growth. Therefore, one way of making the feed more economically efficient is by using the calorie sources as energy source and sparing the protein for growth.

Weight, feed efficiency and protein utilization of *P. monodon* juveniles increased with a constant dietary protein level and a increasing dietary energy level of up to 412.6 Kcal/100g (Hajra et *al.* 1988). In addition, a low calorie diet results in a high feed conversion ratio and hence, in low efficiency, implying that prawns consume more food to overcome energy insufficiency. From this, it appears that control of food consumption through dietary energy density is possible and is economical.

As to the source of nitrogen, Kanazawa (1985b) suggested that some extent, substituting soybean meal for the more expensive marine animal meals at a rate of 20-30 % is a feasible way to reduce prawn feed cost. Yang et *al.* (1988b) further indicated that the substitution rate could be increased to 40 % without significantly decreasing the growth rate. The effectiveness of soybean meal used as protein source for the feed of *P. monodon* was confirmed by Yang et *al* (1988a), and also by Wu and Yang (1989).

Table 2. Essential amino acid requirements of *Penaeus japonicus* and *P. monodon* (Kanazawa, 1985b).

Amino acid	*Penaeus japonicus*	*Penaeus monodon*
Alanine	–	–
Aspartic acid	–	–
Cystine	–	–
Glutamic acid	–	–
Glycine	–	–
Proline	–	–
Serine	–	–
Tyrosine	–	+
Arginine	+	+
Histidine	+	+
Leucine	+	+
Isoleucine	+	+
Lysine	+	+
Methionine	+	+
Phenylalanine	+	+
Threonine	+	+
Tryptophan	+	+
Valine	+	+
Author	Kanazawa and Teshima	Coloso and Cruz

+ : Essential; – : Non essential

AMINO ACIDS

Shigeno et *al.* (1972) reported that the diets which gave the best results were those that most closely approximated the amino acid composition of the prawn. Kitabayashi et *al* (1971c) contended that both arginine and methionine, at a concentration of 0.83 % and 0.52 %, respectively, have growth-promoting effects on *P. japonicus.* Kanazawa and Teshima (1981)

proved that the essential amino acids (EAA) of *P. japonicus* are as follows : valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, threonine and tryptophan. They indicated that the essential amino acids of *P. monodon* are approximately the same of those of *P. japonicus*. Deshimaru et *al.* (1985) also observed that the content and/or balance of the EAA is one of the major factors relating to the nutritional quality of the diet of *P. monodon* (Table 2).

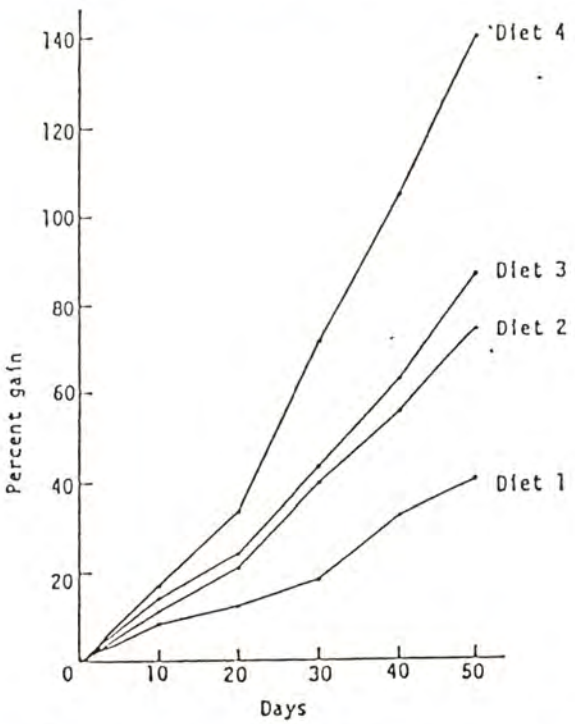

Fig. 2. — Effects of dietary linoleic and linolenic acids on the growth of *Penaeus japonicus*. Diet 1 : 5 % oleic acid; Diet 2 : 4 % oleic acid and 1 % linoleic acid; Diet 3 : 4 % oleic acid and 1 % linolenic acid; Diet 4 : 5 % pollack residual oil (Kanazawa et *al.*, 1977).

LIPID REQUIREMENTS

Essential Fatty Acids (EFA)

Nutritional studies on lipids have shown that several crustaceans require essential fatty acids (EFA) for their normal growth and that nutritive values of lipids for crustaceans are highly influenced by the dietary content of EFA. Teshima et al. (1976), Kanazawa and Teshima (1977) and Kanazawa et *al.* (1977b. 1979b) suggested that the linoleic (C18 : 2w6), linoleic (C18 : 3w3) and w3-long chain highly unsaturated fatty acid (HUFA) may be essential for the prawn, *P. japonicus* and that the nutritive value of linolenic acid was higher compared with linoleic acid (Fig. 2). Moreover, Kanazawa et *al.* (1978, 1979d) indicated that docosa-

hexaenoic (C22 : 6w3) and eicosapentaenoic (C20 : 5w3) are more effective as EFA's rather than linoleic or linolenic acids. This is probably because *P. japonicus* is able to convert 18 : 3w3 to 20 : 5w3 and 22 : 6w3. The optimum content for each w3 is about 1 % (Kanazawa et *al.* 1979a) (Fig. 3, 4, 5, 6 and 7).

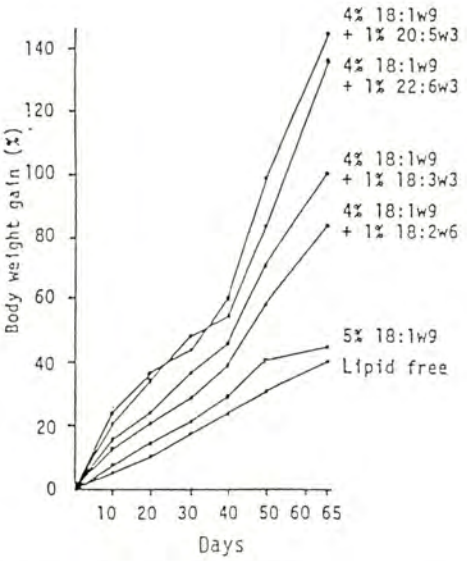

Fig. 3. — Growth of *Penaeus japonicus* fed with diets containing fatty acids (Kanazawa, 1985b).

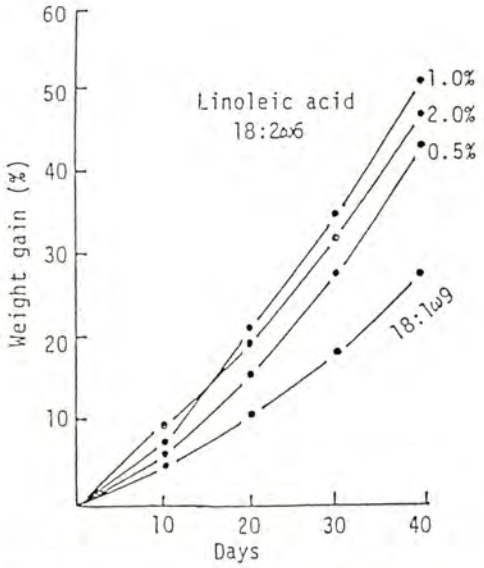

Fig. 4. — Effects of dietary linoleic acid on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

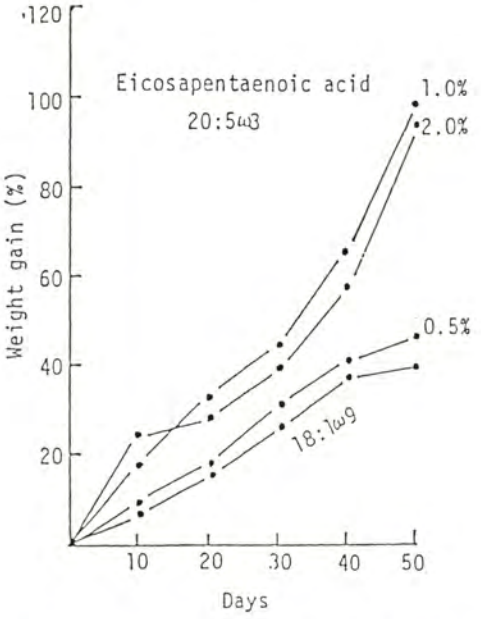

Fig. 5. — Effects of dietary eicosapentaenoic acid on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

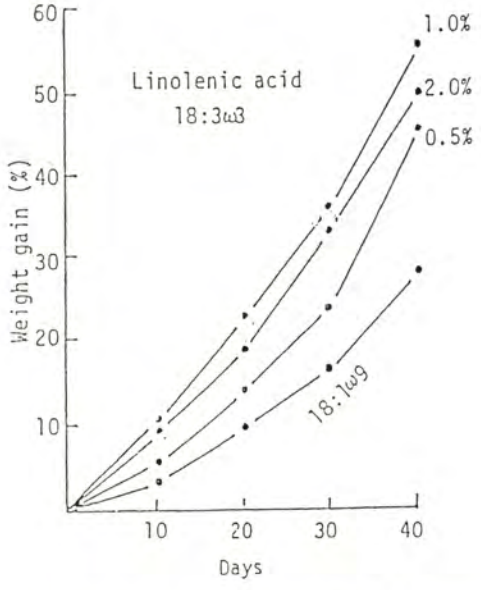

Fig. 6. — Effect of dietary linolenic acid on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

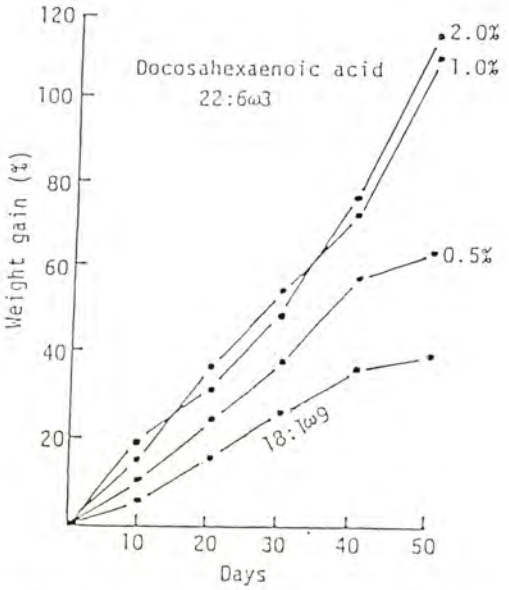

Fig. 7. — Effects of dietary docosahexaenoic acid on growth of *Penaeus japonicus* (Kanazawa, 1985b).

Regarding the effects of different dietary lipids on the growth of *P. japonicus*, Kanazawa et *al.* (1977a, 1979c) observed that the addition of either pollack residual oil or short-necked clam lipid (as compared with soybean oil) increased the percentage of 20 : 5w3 and 22 : 6w3 in lipids of the whole body of *P. japonicus*. Consequently, the fatty acid composition in lipids of *P. japonicus* is remarkably affected by dietary lipids. Deshimaru et *al.* (1979) mixed pollack liver oil and soybean oil in ratio ranging from 3 : 1 to 1 : 1 at 6 % lipid level. Use of these as dietary lipid source for *P. japonicus* resulted in good growth performance and feed efficiency for this species. Due to lack of HUFA, soybean oil may not be as good as squid visceral oil, as a source of lipid diet for *P. monodon* (Wang and Yeh 1987, Wang 1988, Wang and Yeh 1988).

Fatty acid such as C18 : 2w6, C18 3w3, C20 5w3 and C22 : 6w3 are considered as the EFA's for *P. monodon* (Kanazawa et *al.* 1979d), Wu (1983) reported that the C22 : 6, C22 : 1, C20 : 5, C18 : 3, C18 : 2, C18 : 0, C16 : 1, C16 : 0 and C14 : 0 acids were the main fatty acids of the lipid of *P. monodon*. The lipid composition of neutral and polar lipids is shown in Tables 3 and 4.

Bautista (1986) indicated that the inclusion of 15 % lipid in the diet was detrimental to the growth of *P. monodon*. This means that when *P. monodon* is fed with lipids higher that the amount they can normally tolerate, lipids may accumulate rapidly in their body and may cause an imbalance in the protein/energy ratio. This finding is consistent with that of Deshimaru and Kuroki (1974a). The latter reported that diets containing pollack liver oil of up to 6 % would improve the growth rate of *P. japonicus*, while at the 12 % level, it will inhibit growth. Wu (1986) also pointed out

that 6 % lipid level contained in the diet would bring about the best growth in *P. monodon.*

Table 3. Lipid composition of neutral lipids in *Penaeus monodon* (Wu, 1983)

Lipids	Weight (%)					
	Muscle		Exoskeleton		Viscera	
	Male	Female	Male	Female	Male	Female
MG	tr	tr	8.6	8.1	tr	tr
ST	49.7	47.1	49.0	46.1	14.8	16.4
FA	10.7	6.1	tr	tr	3.7	2.6
DG	23.5	31.2	25.7	20.5	9.1	6.6
TG	16.1	15.6	14.7	22.0	69.5	72.0
SE	tr	tr	1.6	3.3	2.2	2.1

Table 4. Lipid composition of complex lipido in *Penaeus monodon* (Wu, 1983)

Lipids	Weight (%)					
	Muscle		Exoskeleton		Viscera	
	Male	Female	Male	Female	Male	Female
PE	19.6	22.0	22.5	22.9	26.2	16.8
PD	5.8	5.5	3.3	3.5	2.6	3.7
PC	56.8	51.8	63.9	61.2	61.0	68.9
Ps	11.8	13.1	6.4	5.9	4.8	4.8
Unknown	6.0	7.5	3.9	6.5	5.4	5.8

Sterol requirements

It was found that when prawn was fed with sterol-free diets, the sterol content of the body tissues decreased after feeding. However, in the case of the prawn fed with sterol-added diets, the sterol contents before and after feeding were the same. Furthermore, when the prawn was fed with sterol-added diets, it was found that 96-99 % of the sterol content was composed of cholesterol (Kanazawa et *al.* 1971b).

Table 5. Effect of dietary cholesterol levels on the growth and survival of *Penaeus japonicus* (Kanazawa et *al.*, 1971a).

Cholesterol added (g/100 g of diet)	Experimental period (days)	Nº of prawnat start	Rate of survival (%)	Rate of growth (%)
0.05	30	24	82	45
0.1	30	24	88	57
0.5	30	24	88	84
1.0	30	24	92	84

Teshima and Kanazawa (1971) also reported that cholesterol was the major sterol (73-100 %) in crustaceans. The growth and survival of *P.*

japonicus supplied with cholesterol-added diets are better compared with those fed with cholesterol-free diets (Kanazawa et *al.* 1971a). Table 5 shows the effect of cholesterol levels on growth of *P. japonicus*. Crustaceans are incapable of synthesizing cholesterol and require dietary sources of cholesterol or other sterols for normal growth and survival. Kanazawa et *al.* (1971a and b) showed that *P. japonicus* fed with diets containing ergosterol (C28), stigmasterol (C29) and i-sitosterol had survival rates similar to those of *P. japonicus* fed with cholesterol in the diet. Where growth rate was concerned, these sterols were inferior to cholesterol (Table 6). Teshima and Kanazawa (1987) confirmed that *P. japonicus* can convert i-sitosterol to cholesterol, possibly via the 24-methylene-cholesterol.

Table 6. Effect of various sterols on the growth and survival of *Penaeus japonicus* (Kanazawa et *al.*, 1971a).

Sterol added 0.5 g/100 g of diet	Rate of survival (%)			Rate of growth (%)		
	Experiment			Experiment		
	1	2	3	1	2	3
Cholesterol	95	86	88	72	98	56
Ergosterol	94	87	92	51	79	48
Stigmasterol	96	83	88	62	67	56
β-sitosterol	89	83	92	56	29	50

Number and average weight of surviving prawn were determined at the end of 30 or 40 day feeding trials.

Several authors (Kanazawa et *al.* 1971a, Shudo et *al.* 1971, Deshimaru and Kuroki 1974b, Teshima et *al.* 1974) estimate the optimum desirable content of cholesterol, as an effective growth-promoting factor, to be in following percentages : 0.5 %, 0.1 %, 0.05 %-1.0 % and 2.1 %. For *P.monodon*, more than 94 % of the sterol content was found to be cholesterol and the optimum level of cholesterol content was around 0.5 % (Wu 1983, 1986) (Table 7).

Table 7. Sterol contents of *Penaeus monodon* (WU, 1983).

Lipids	Weight (%)					
	Muscle		Exoskeleton		Viscera	
	Male	Female	Male	Female	Male	Female
Free ST	0.62	0.38	0.42	0.30	2.92	2.03
Total ST	0.63	0.38	0.45	0.33	2.78	2.35

Phospholipid Requirements

In one study (Teshima et *al.* 1986a), one group of *P. japonicus* was fed with 3 % soybean lecithin diet (phospholipid-supplemented diet or

PL-supplemented diet) and another group was fed with no supplemental phospholipid diet (PL-deficient diet) for 30 days. It was found that the PL-deficient significantly reduced the weight gain and feed efficiency (Fig.8). Further, the prawn contained a lower concentration of phospholipids, such as phosphatidylcholine (PC) and phosphatidylinositol (PI) in the whole body than the prawn receiving PL-supplemented diet. It was concluded that the nutritional role of dietray Pl was possibly related to the transport of dietary lipids, such as cholesterol and triglyceride, in the body. On the other hand, the retarded growth of *P. japonicus* receiving PL-deficient diet can be attributed to the insufficient mobilization of dietary lipids (Teshima et *al.*, 1986a, b, c and d, 1987).

The optimum level of PL for *P. japonicus* is about 1.0 % (Kanazawa et *al.* 1985), while for *P. penicillatus*, the value was estimated to be 1.25 % (Jenn and Chen 1988). Deshimaru et *al.* (1985) suggested that both polar-lipids and sterols may be essential ingredients in the diet of *P. monodon* and of *P. japonicus*.

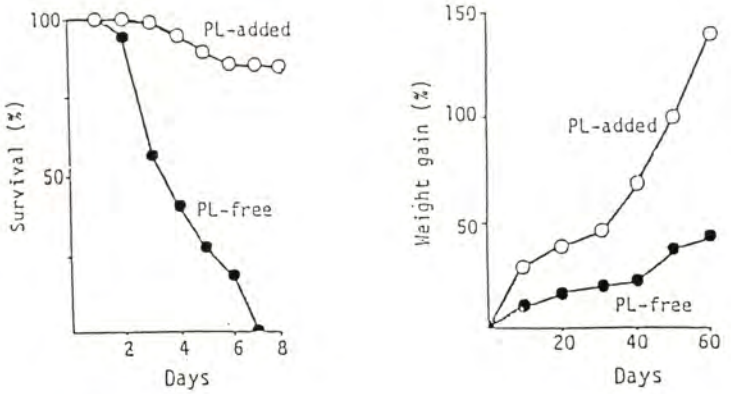

Fig. 8 A. — Survival rate of larval *Penaeus japonicus* fed on PL-added and PL-free diets (Kanazawa, 1985b).

B. — Weight gain of juvenile (1g) *Penaeus japonicus* fed on PL-added and PL-free diets.

Carbohydrate Requirement

As mentioned above, energy production from protein oxidation is both nutritionally and economically wasteful. Therefore, it is necessary to supply protein for growth by optimizing the level of nonprotein energy sources. The type and level of carbohydrate in the diet have been proven to affect the growth and survival of *P. japonicus* (Deshimaru and Yone 1978b).

According to Deshimaru and Yone (1978b), the desirable sources of dietary carbohydrate for *P. japonicus* are sucrose and glycogen, while less suitable sources are starch, dextrin and particularly glucose. The growth, weight gain, feed efficiency and mortality of *P. japonicus* fed with various

carbohydrate sources are shown in fig. 9 and 10. Abdel-Rhaman et *al.* (1979) observed that the dietary glucose was absorbed rapidly in comparison with disaccharides and polysaccharides, but Kanazawa (1985b)

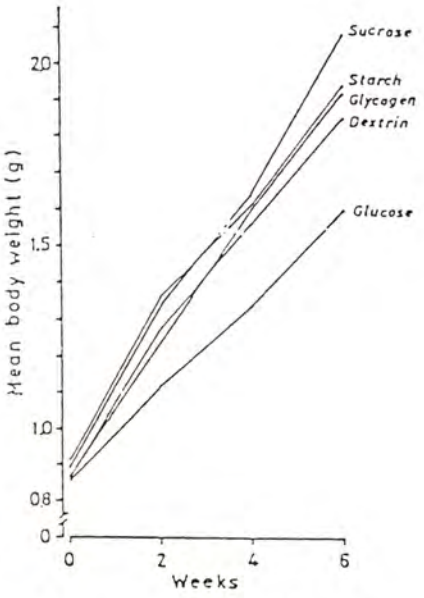

Fig. 9. — Growth curves of *Penaeus japonicus* fed with the test diets (Deshimaru and Yone, 1978).

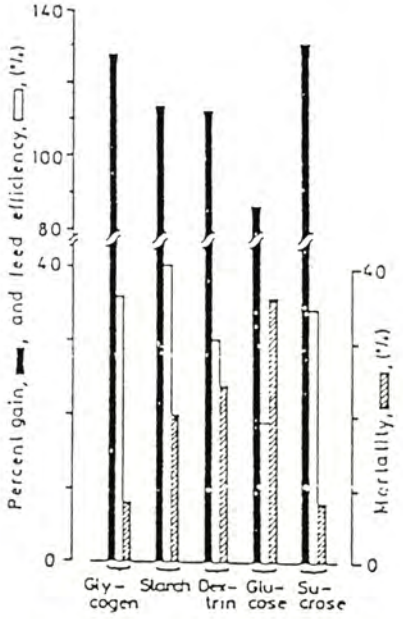

Fig. 10. — Percent gain, feed efficiency and mortality of *Penaeus japonicus* fed on the test diets (Deshimaru and Yone, 1978).

pointed out that the absorbed glucose was probably not utilized efficiency for the growth of *P. japonicus* (Fig. 11). He also demonstrated that diets containing 19.5 % maltose brought about the best growth performance for this species. Similar results were obtained by Deshiamru and Kuroki (1974a) for the same species.

Glucosamine can also be used as carbohydrate source at a content level of 0.53 % (Kitabayashi et *al.*, 1971a). Its growth-promoting effect has been reconfirmed by Kanazawa (1985b), as shown in Fig. 12.

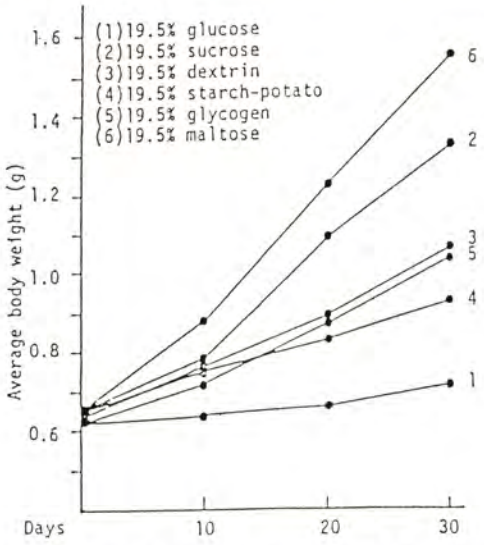

Fig. 11. — Effect of dietary carbohydrates on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

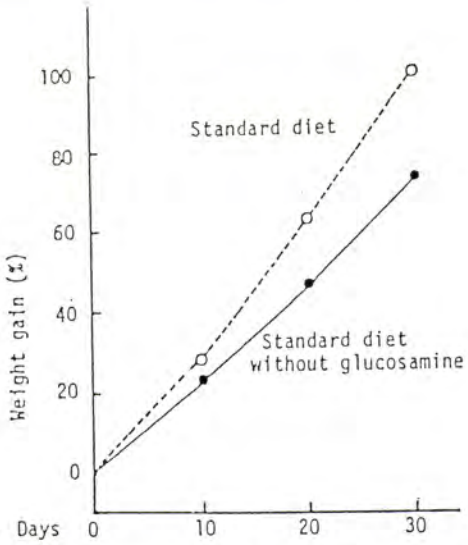

Fig. 12. — Effect of dietary glucosamine on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

For *P. monodon*, Pascual et *al.* (1983) found that the highest survival rate (56 %) was obtained in juveniles fed with a diet containing 10 % sucrose (Fig. 13). However, the growth was generally poor on all the trials using carbohydrate-containing diets (Table 8). In addition, histopathological changes were observed at all levels of carbohydrate content. Pascual et *al.* (1983) suspected that sucrose is the « best » source of carbohydrate for *P. monodon.*

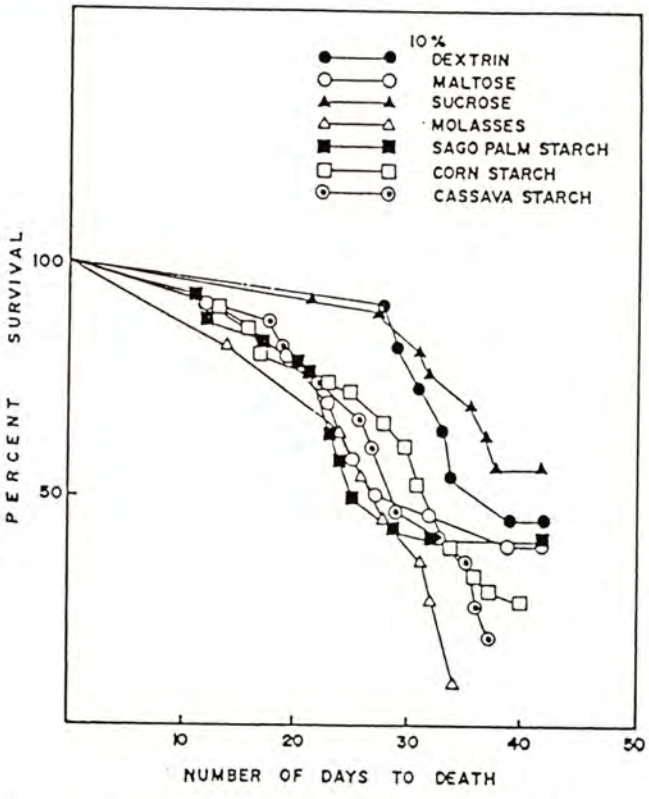

Fig. 13. — Survival vs. time before death curves of *Penaeus monodon* juveniles fed with various carbohydrates at 10 % level in the diet (Pascual et *al.*, 1983).

Table 8. Mean survival rate and weight gain of juvenile *Penaeus monodon* fed with diets containing various carbohydrate levels, after 6 weeks of rearing (Pascual et *al.*, 1983).

Carbohydrate Level (%)	Survival rate (%)		Average weight gain (%)	
	10	40	10	40
Dextrine	(22) 36	(23) 23	24	34
Maltose	(23) 35	(23) 0	5	—
Sucrose	(23) 56	(22) 38	7	28
Molasses	(22) 0	(21) 0	—	—
Sago palm starch	(22) 42	(22) 0	7	—
Cornstarch	(23) 27	(22) 32	14	26
Cassava strarch	(23) 0	(21) 0	—	—

Supporting this view, Alava and Pascual (1987) observed that *P. monodon* fed with a diet containing 20 % trehalose brought about the highest gain and survival, followed by sucrose ; glucose ranked last. Carbohydrate, at its optimum value, could be used as precursor for the various metabolic intermediates necessary for growth, i.e., dispensable amino acids. However, if the amount is too high, energy utilization tends to be less efficient, resulting in poor digestibility. An optimum dietary sugar level for *P. monodon* is therefore considered to be 20 % (Bautista 1986; Alava and Pascual, 1987).

MINERAL REQUIREMENTS

Little is known about the mineral requirements of prawn, due to the limited studies in this field. Kitabayashi et **al.** (1971a) considered that 1.04 % of Phosphorus (P) and 1.24 % of calcium (Ca) are indispensable in the diet for *P. japonicus.* Kanazawa et **al.** (1984) observed that the suitable levels of Ca, P, Potassium (K) and Magnesium (Mg) in diets for *P. japonicus* juveniles were 1.0 %, 0.9 % and 0.3 % respectively. Deshimaru and Yone (1978a) suggested that 2 % of P, 1 % of K and 2 % of trace metals should be supplemented into a purified diet for favorable growth of *P. japonicus,* while Ca, Mg and Iron are indispensable. The data available for the mineral requirement of *P. japonicus* are shown in Table 9.

Table 9. Mineral requirements of juvenile *Penaeus japonicus* (Kanazawa, 1985b).

Minerals	Requirements (%)	
	Kanazawa	Deshimaru and Yone
Ca	1.0	Dispensable
P	1.0	2.0
K	0.9	1.0
Mg	0.3	Dispensable
Mn	Dispensable	—
Fe	Dispensable	Dispensable
Cu	0.06	—
Trace metals	—	0.2

VITAMIN REQUIREMENTS

The growth of *P. japonicus* was remarkably accelerated by adding ascorbic acid into a diet containing a fixed amount of glucose (Kitabayashi et *al.*, 1971b). Kanazawa et *al.*(1976) estimated the adequate levels of choline chloride and inositol in the diet to be 60 mg and 200 mg, respectively, for each gram of the diet. In addition, the desirable level of dietary ascorbic acid, inositol, thiamine (B1) and pyridoxine (B6) were approximately evaluated at 300 mg, 400 mg, 6-12 mg and 12 mg, respectively (Deshimaru and Kuroki 1976, 1979).

The vitamin deficient diet could cause high mortality in *P. japonicus* larvae and may inhibit the larval development; most likely, the prawn larvae will fail to reach the postlarvae stage. Kanazawa (1985a) studied the effect of vitamin on the growth and survival of *P. japonicus.* The results of his experiments are shown in Fig. 14, 15, 16, 17 and 18. Desirable vitamin requirements for both larvae and juveniles of *P. japonicus* are shown in Table 10.

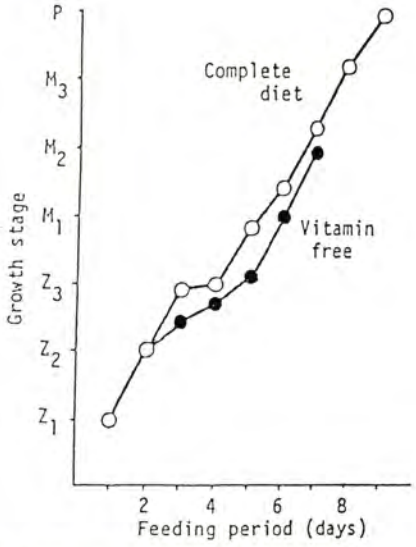

Fig. 14. — Effect of vitamin on the growth of *Penaeus japonicus* (Kanazawa, 1985b).

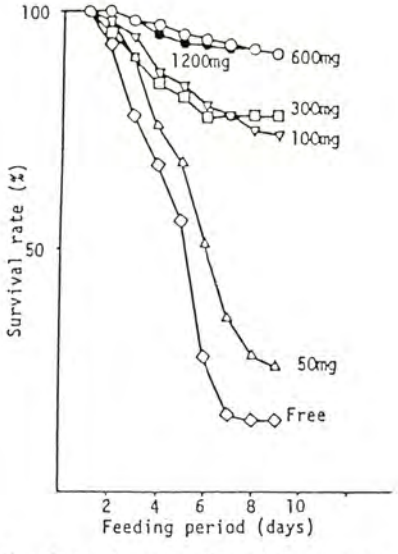

Fig. 15. — Effect of choline-Cl levels on survival of *Penaeus japonicus* (Kanazawa, 1985b).

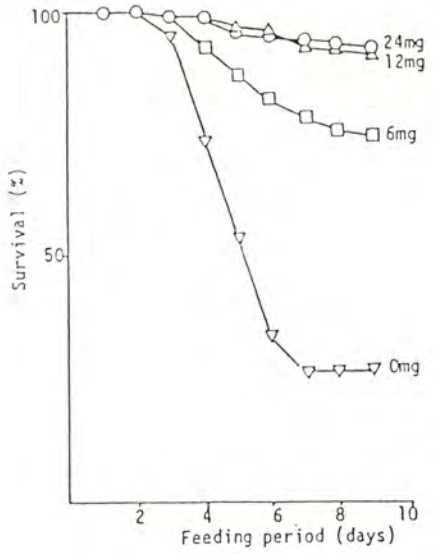

Fig. 16. — Effect of pyridoxine-HCl levels on survival of *Penaeus japonicus* larval (Kanazawa, 1985b).

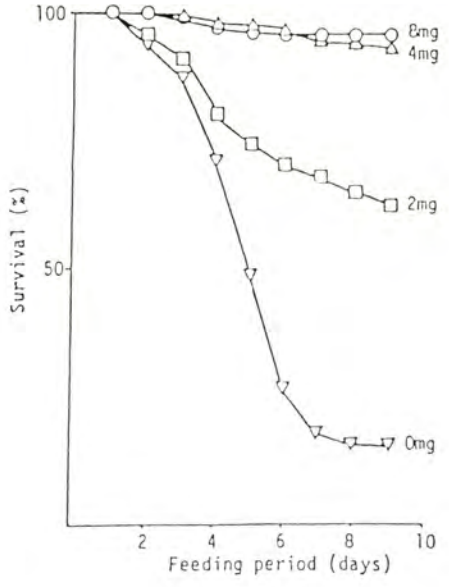

Fig. 17. — Effect of thiamine-HCl levels on survival of *Penaeus japonicus* larval (Kanazawa, 1985b).

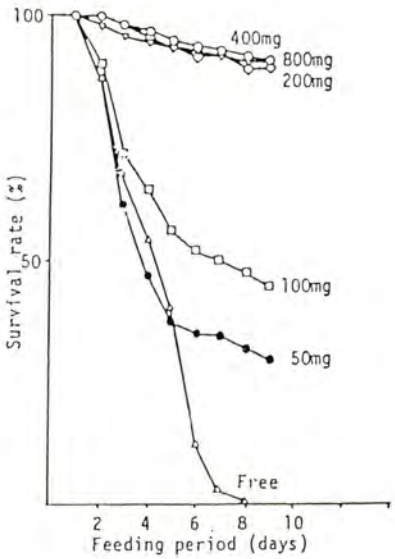

Fig. 18. — Effect of inositol levels on survival of *Penaeus japonicus* (Kanazawa, 1985b).

Table 10. Vitamine requirement of juvenile *Penaeus japonicus* (Kanazawa, 1985b).

Minerals	Requirements (%)	
	Kanazawa	Deshimaru and Yone
Ascorbic acid	1000-2000	300
Choline	600	Dispensable
Inositol	200	400
Thiamine	—	6-12
Pyridoxine	—	12

EFFECT OF ENZYMES ON DIGESTION

Enzymes originating from animals food sources (exogenous enzymes) have been shown to affect prawn digestion positively. Maugle et *al.* (1982) indicated that protease and amylase act to control the utilization of dietary food *P. japonicus.*. Furthermore, Maugle et *al* (1983) reported that bovine trypsin dietary supplements may have activated the endogenous protease zymogen of *P. japonicus*, resulting in increased growth and a complete cycle of protease activities. They also found that the hepatopancreas was the primary site of enzyme secretion (Fig. 19). In contrast, Lin and Chen (1988) contended that the growth of postlarvae of *P. monodon* was not correlated with midgut gland amylase or protease activities, although the inclusion of exogenous enzymes *(P. monodon* hepatopancreas acetone powder) in the diets significantly promoted growth. Hence, further studies

concerning the effect of exogenous enzymes on the endogenous enzymes should be encouraged.

Vogt et *al.* (1985) suggested that R-cells of the hepatopancreas could be used to monitor the nutritional value of prawn diets. The effect of feed was visible on the cellular or organ level after only a few days of feeding, whereas the effect on the individuals (organism level) occured after 10 days. Therefore, it is suggested that histology may be applied to nutrition studies to provide supplementary information to statistical and biochemical parameters (Vogt et *al.*, 1986).

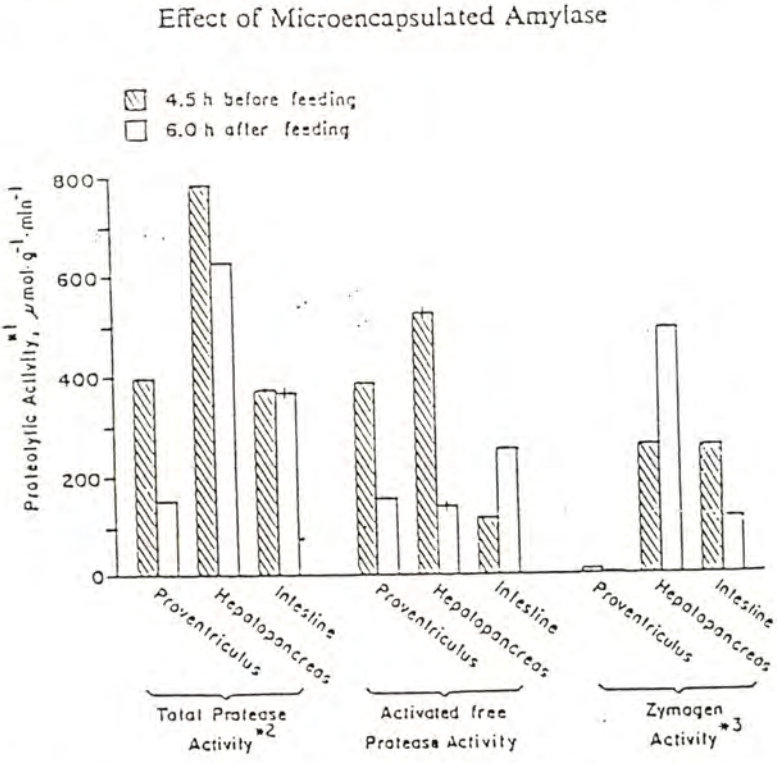

Fig. 19. — Activities of protease and protease-zymogen in the proventriculus, hepatopancreas, and intestine of the shrimp 4.5h before and 6.0h after feeding with a control diet which did not contain digestive enzyme microcapsule supplement (Maugle, 1983).
* 1. As μmol tyrosine liberated per g wet tissue per minute
* 2. Activated free protease activity + protease-zymogen activity
* 3. Total protease activity - activated free protease activity.

In the hepatopancreas, except for the protease and amylase, a specific trypsin-like enzyme was visible in the cell near the sides of the intestine (Liu 1986, Liu and Shyong 1987). Through histological methods, it was also found that there were small blind tubules in the hepatopancreas of *P. monodon.* In addition, protease in the hepatopancreas was categorized as a carboxypeptidase A-like, B-trypsin-like and C-trypsin-like enzymes (Liu and Shyong 1987).

DEVELOPMENT OF LARVAL MICROPARTICULATE DIET

Research on compounded diet for *P. monodon* larvae has remained scarce. Forster (1973) reported that *P. monodon* larvae fed with a compounded diet showed good survival but slow growth, as compared with those fed with fresh food. Khannapa (1977) found that 30 % protein in diet is suitable for *P. monodon* larvae. Bages and Sloane (1981) further demonstrated that feeding *P. monodon* postlarvae exclusively with the compounded diet retarded growth, with the survival rate between 39 and 74 %.

Results of research on compounded diet have not been very encouraging, as can be gleaned from the above studies. Natural food has still proven to be more effective than artificial food. Nevertheless, because the use of natural food entails many inconveniences, research on the development of artificial diet must be emphasized. Fortunately, researchers are still persevering towards this direction and some novel products have recently emerged.

Microparticulate diet (MD) is a newly developed product found effective for prawn larvae. Several types of microparticulate diets, such as artificial plankton B.P. (Liao et *al.*, 1988b), nylon-protein microencapsulated diet (Nylon MED), zein microcoated diet (Zein MCD) and carrageenan micro-bound diet and others have been used successfully in rearing prawn larvae. In one study which used microparticulate diet, the survival rate obtained was 94 % (Kanazawa et *al.* 1982). The dietary value of these microparticulate diets is almost comparable with that of live food, such as *Chaetoceros* sp. and *Artemia salina* (Kanazawa et *al.* 1982, 1985; Teshima et *al.* 1982; Teshima and Kanazawa 1983).

Microparticulate diet for *P. monodon* larvae was also tried and the survival rate of experimental MD and control (*Skeletonema, Artemia*) groups were 40 % and 20 %, respectively (Kuo 1986). In addition, a simple but satisfactory microencapsulation method for laboratory nutrition studies with postlarvae of *P. monodon* was introduced by Chen and Tsai (1986), who revealed that the w3 polyunsaturated fatty acid requirement of *P. monodon* is no less than 0.5-1 %, as reported for *P. japonicus*.

Likewise, the microencapsulated feed could be used to replace part or all of the conventional live food diet. The survival of the *P.monodon* larvae fed with MED may be superior to that larvae fed with live food. In addition, the application of MED reduces labor, raising its economic value (Jones et *al.*, 1987). A similar result was obtained by using cross-linked protein-walled microcapsules to feed mysis and postlarvae of *P. monodon*; 80-100 % growth and survival rates were obtained (Clark et *al.*, 1987). Liao et *al.* (1989b) found that the particle size of artificial diet was not a limiting factor in the growth and survival of *P. monodon* larvae. Moreover, Liao et *al.* (1989a) proved the feasibility of using several kinds of microbound diet (MBD) in rearing *P. monodon* larvae (Zoea to postlarvae), since the survival rates in all MBD groups were higher than 50 %.

CONCLUSION

To date, although some progress has been achieved in this respect, knowledge on the nutritional requirements of *P. monodon* is still too insufficient to provide reference for effective diet levels. More extensive and detailed research in nutrition must be carried out, covering such topics as feeding behavior and preferences of prawn, optimization of the nutritional requirements, nutritional variation during manufacturing of MED, mechanism of digestive enzymes and acceptability of feed given.

While *P. monodon* culture has now become well established and has come to occupy an important place in the entire prawn production industry, new problems continue to emerge and challenge its stability. Since nutrition is a highly crucial factor, with feed accounting for about half of total production cost, the industry will need all the research and development support it can get in this area.

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The nutritional requirements of the Japanese shrimp *Penaeus japonicus*

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Abstract. — ***Penaeus japonicus*** *is probably the best known species among penaeids or even among shrimps. In juveniles, the protein requirement was best studied and Japanese shrimp appears as the most demanding penaeid species. Some discrepancies remain among estimations of the protein requirement. They may be related to several factors such as energy level of the diet, digestibility or biological value of protein or other nutritional factors. The essential amino acids are well known, however from a quantitative point of view the requirements in each of these amino acids remain to be determined. The fatty acid utilization and mainly the role of essential fatty acids was well studied :* ***Penaeus japonicus,*** *like most of the sea fish species, has a low ability to elongate and desaturate C18 highly unsaturated fatty acids. The essentiality of both cholesterol and phospholipids was well demonstrated and represents one of the most original features of penaeid nutrition. The feeding value of several carbohydrates was studied and phenomena such as the « diabetic » behavior of the shrimp, its low amylase activity, the beneficial effects of glucosamine, were pointed out. However much information on the actual capability of kuruma shrimp to use carbohydrates from practical sources are still lacking. Furthermore almost nothing is known on energy requirements or recommendations. Vitamin nutrition of* ***P. japonicus*** *was rather poorly studied, mainly from a quantitative point of view. Mineral nutrition retained little attention but several original traits were elucidated such as the detrimental effect of dietary iron, the rather high phosphorus requirement and the ability to use phosphorus from various sources which is very different from that of upper vertebrates. In summary much information on the requirements of* ***P. japonicus*** *juveniles is now available but often remains limited from a quantitative point of view. On the other hand, the nutritional requirements of larvae are much less known and breeder's ones are almost unknown.*

INTRODUCTION

Several reviews or bibliography were recently devoted to penaeid shrimp nutrition (New, 1976, 1980; Kanazawa, 1982; Guillaume, 1987). Among the different species of penaeids interesting aquaculturists, *Penaeus japonicus* was mostly studied. Since the first efficient mixed diets were used by Kanazawa et *al.* (1970), most nutritional requirements of

juveniles studied, at least for macro ingredients. The nutrition of larvae is much more difficult to approach, but recent success in larval rearing with microbound diets or microcapsules have also information in this field. This review gives an overview of the known requirements of *P. japonicus* with emphasis on their quantitative estimations without giving details on mechanisms involved.

THE PROTEIN REQUIREMENT

Gross protein requirement

— Gross protein requirements of juveniles

Like most aquatic animals penaeid shrimps require a high protein level in their diet. In *P. japonicus* juveniles the optimal level was studied by Deshimaru and Shigeno (1972), Balacz *et al.* (1973), Deshimaru and Kuroki (1974a), and Deshimaru and Yone (1978b). From their results (Table 1) it is obvious requirement is at least 52 % of the diet when measured in small shrimp fed on casein-albumin diets, both for maximal growth and best feed efficiency. In these conditions levels of protein exceeding 60 % lead a clear depressing effect on growth. Nevertheless in a precious review, Deshimaru and Shigeno (1972), referring to a set of experiments, insisted on the improvement of feed efficiency obtained when crude protein content exceeded 60 %. Therefore the optimal level seems to be more elevated for best food to gain ratio than for growth.

As mentioned by Kanazawa (1982) this requirement is the strongest among all other penaeid species, a phenomenon that was often explained by nutritional habits. In fact this requirement should be related to specific growth rate and therefore to age or size as demonstrated in *P. stylirostris* by Colvin and Brand (1977) and in *Crangon crangon* by Regnault and Luquet (1974).

Table 1. Dietary protein requirement of *P. japonicus.*

Initial Weight	Source of protein	Optimal levelq	Author
—	Various	>60	Deshimaru and Shigeno 1972
—	Shrimp meal	>40	Balasz et *al.* 1973
0.6 g	Casein albumin	46	Deshimaru and Kuroki 1974a
0.8 g	Casein	52	Deshimaru and Yone 1978b
(Zoe)	Casein 5 +Carbohydrates 15	55 45-55	Teshima and Kanazawa 1984
(Zoe - mysis 2)	Casein 25 Hen egg protein	45 >50	Besbes 1987

— Gross protein requirements of larvae

For larvae (Table 1) two experiments carried out in this field have shown a lower requirement during the early stages, *ie in zoea* which is

herbivorous. More precisely the work of Teshima and Kanazawa (1984) demonstrated the sparing effect of carbohydrates against protein. Results of Besbes (1987) indicate that a microbound diets containing 44 % protein even sustains faster growth than a diet containing 50 % of protein from casein and hen egg until mysis 3 stage.

Possible dietary factors of variation - The role of essential and semi essential amino acids

The protein requirement depends on many nutritional factors such as lipids, carbohydrate content or energy level. But, with the exceptions mentioned above, the sparing effect of these nutrients has been little studied. The protein requirement is also very dependent on essential amino acids (EAA) content and balance. The well known qualitative requirement in EAA, related elsewhere, will not be commented on but the work of Deshimaru and Shigeno (1972), and Deshimaru (1982) on the balance of EAA deserves some attention since it led to the conclusion that short necked clam protein is the best protein fitting the requirements of *P. japonicus* or, in other words, the protein with best EAA profile. Calculating the sum of sulfured and aromatic amino acids, supposing they were semi essential as they are in vertebrates these data are proposed in table 2 in a form suitable for linear programming.

Table 2. Profile of essential and semi essential amino acids in the protein of short necked clam *(Ruditapes philippinarum)* after Deshimaru, 1981.

	Profile of EAA and SEAA in clam protein		Suggested level for P. japonicus
	% of protein	% of EAA + SEAA	% of diet
Methionine	3.02	5.98	1.57
Methionine + Cystine	3.28	6.49	1.70
Threonine	5.82	11.52	3.03
Valine	3.87	7.66	2.01
Isoleucine	7.11	14.06	3.70
Phenylalanine	3.78	7.36	1.96
Phenylalanine + Tyrosine	8.30	16.42	4.32
Lysine	7.50	14.84	3.90
Histidine	2.25	4.45	1.17
Arginine	7.98	15.79	4.15
Tryptophane	0.90	1.78	0.47

However it must be noticed that these recommendations do not correspond to individual amino acid requirements as used in the formulation of upper vertebrate diets by linear programming; the only supplementation in EAA were done in *P. japonicus* by Kitabayashi *et al.* (1971) (arginine and methionine) and by HEW and Cuzon (1982) (lysine and arginine). But these experiments did not bring estimations of the requirements in individual EAA or of SEAA; the very slow growth of kuruma shrimp when fed on regimes where protein was replaced by a mixture of amino acids (Deshimaru and Kuroki, 1975) is perhaps the main cause of this deficiency.

THE ENERGY REQUIREMENT, CARBOHYDRATES AND LIPIDS

Energy needs

Little data is available on energy metabolism in crustaceans (Capuzzo, 1982) except at the biochemical level where the energy yielding pathways were studied, mainly by Zandee (1966) in *Astacus* sp. But such studies are lacking in *P. japonicus*. Even the optimal protein/energy ratio level remains to be determined in this species. Theorically the energy value, either digestible or metabolizable, is difficult to calculate due to the lack of balance studies. However, the digestible energy level of efficient semi purified shrimp diets apparently ranges from 14 to 17 MJ DE kg-1. Nevertheless the energy value of classical diets is more difficult to calculate since the digestibility coefficients of nutrients remain unknown in most feedstuffs.

Carbohydrate supply

Carbohydrates are very efficient energy sources in most animals for standard metabolism, muscular energy expenditure, and other processes needing ATP. But, large terrestrial carbohydrates such as starch are not very digestible in marine animals because of lower amylase activities. On the other hand very available sugars such as glucose are too quickly absorbed and may induce hyperglycaemia. Such a phenomenon was demonstrated in *P. japonicus* by Abdel Rahman *et al.* (1979) who observed that 10 % glucose inhibited growth while polysaccharides (ά starch, dextrin and glycogen) and mainly disaccharides (maltose and sucrose) sustained good performances. These results demonstrate the existence of « diabetic » phenomena in shrimp similar to those known in fish. They also suggest the importance of further studies on the digestibility of carbohydrates in vegetable feedstuffs and on their metabolic effects in shrimp.

Chitin being a polymer of glucosamine the addition of this aminated sugar was tested by Kitabayashi *et al.* (1971) who observed a beneficial effect of the supplementation. The authors even determined the optimal dietary level of glucosamine (0.53 %) and noticed that chitin had a growth depressing effect. Unfortunately studies of Deshimaru and Kuroki (1974b) failed to corroborate the essentiality, or semi-essentiality, of glucosamine in *P. japonicus*, perhaps because of differences in diet composition.

Chitinase and cellulase activities were found in the digestive tract of some crustacea; however practical implications of such activities, if occuring in *P. japonicus*, remain to be studied.

In conclusion carbohydrates sustaining good growth in semi- purified diets for *P. japonicus* are known, but fewer quantitative informations are available. In larvae (as mentioned in the previous paragraph) diets may contain as much as 35 % carbohydrates; in juveniles the optimal level seems to be close to 15-20 %, but acccording to Maugle *et al.* (1983), this level could be brought to almost 30 % without marked growth depressing effects with the addition of amylase; such an addition, needing microen-

capsulation would lead to a sparing of protein amounting to 30 % of the supply.

Lipidic nutrition

— *Gross lipid optimal level*

The need for total lipid is devoid of scientific meaning, especially when energy level carbohydrate and protein contents are not stated precisely. Nevertheless several experiments have shown that growth performances tend to decrease when the dietary lipid content exceeds 10 %. Provided essential nutrients are in sufficient amount, very good performances are registered with 6 to 8 % of total lipid.

— *Requirements for essential fatty acids*

The qualitative needs of crustaceans for EFA were reviewed by several authors including Castell (1981) and Galois (1987); since the very demonstravive experiments of Kanazawa et *al.* (1979b,c), and Jones *et al.*, (1979). *P. japonicus* is known to be unable of synthezing 18 :2 n-6, 18 :3 n-3, 20 :5 n-3 (EPA) and 22 :6 n-3 (DHA), which can be considered as « essential » (broad meaning); the relative efficiency of several fatty acids in promoting growth or decreasing mortality was also tested both in juveniles (Kanazawa *et al.*, 1979a) and larvae (Jones *et al.*, 1979). From these data it is well demonstrated that long chain highly unsaturated fatty acids (Hufa) give best results and are the main requirement in essential fatty acids (EFA); linolenic acid which is bioconverted in.EPA and DHA at a too slow rate is less efficient; if supplied without EPA or DHA, even at a high percentage, it remains markedly insufficient for maximal growth promotion.

The role of linoleic acid and other fatty acids of the n-6 series is less clear : it is less efficient than linolenic and n-3 Hufa and in normal condition 20 : 4 n-6 is at a rather low percentage in shrimp lipids. But Deshimaru et *al.* (1979) demonstrated that a mixture of vegetable and marine oil was more efficient in growth promotion than pure marine oils. Therefore the n-6 family may be required, though at a much lower level than n-3 for the synthesis of some prostaglandines deriving from 20 : 3 n-6 or 20 : 4 n-6. Unfortunately almost nothing is known in this field. Furthermore little data is available on the possible antagonistic effect of excess of n-6 Hufa on elongation desaturation of n-3 Hufa.

— *Requirement for phospholipids*

The requirement for phospholipids, in opposition to that of EFA, is very specific of crustaceans. It was noticed by Kanazawa *et al.* (1979d) when studying the high nutritive value of *Tapes* lipids. Its nature, its importance and its quantitative values were further studied by Teshima and Kanazawa (1980), Teshima *et al.* (1986), etc.. The higher efficiency of phospholipids containing choline or inositol (phosphatidylcholine and phosphatidyl-inositol) rich in HUFA was demonstrated as well as their role in the transportation of cholesterol and other lipids (Teshima and Kanazawa, 1980; Teshima *et al.*, 1986b).

From a formal point of view phospholipids should be classified among the semi essential dietary components, at least in juveniles, since their biosynthesis from fatty acids, glycerol choline and inositol is possible, though very slow. But in larvae the essentiality of phospholipids is much more pronounced since Kanazawa *et al.* (1985) observed a complete and early mortality in larvae fed on microbound diets well fortified in linoleic, linolenic and n-3 HUFA, as well as in cholesterol but devoid of phospholipids. The addition of soybean phosphatidylcholine had a very marked action on both survival and growth.

The values given in table 3 show that the recommendations appear higher in larvae than in juveniles.

Table 3. Suggestions for dietary levels of essential lipids for *P. japonicus* juveniles and larvae.

Juveniles	n-3 + n-6 Essential fatty acids	0.5-1	Kanazawa et *al.* 1979a
	Phospholipids	1	Kanazawa et *al.* 1979d
	Cholesterol	0.2	Shudo et *al.* 1971
		0.5	Kanazawa et *al.* 1971
		2.1	Deshimaru and Kuroki 1974b
Larvae	(n-3- Hufa acids (EPA + DHA)	1	Kanazawa et *al.* 1985
	Phospholipids	3.5-6	Kanazawa et *al.* 1985
	Cholesterol	1	Teshima et *al.* 1983

The essentiality of cholesterol, apparently specific of all arthropods, is well known both in juveniles (Teshima and Kanazawa, 1971; Teshima, 1982) and larvae (Teshima *et al.*, 1983). Nevertheless this precursor of sex or moulting hormones and vitamin D, which has also a structural function in membranes can be obtained by bioconversion of many sterols from plant origin, mainly from ergosterol, stigmasterol and i sitosterol, both in juveniles and larvae (Teshima, 1982; Teshima *et al.*, 1983).

The values indicated in table 3 correspond to best estimations of the requirements of juveniles and larvae.

— *Interrelationship between essential lipids*

The efficiency of lecithin in juveniles depends, among other factors, on their content in EFA. Therefore the requirement for phospholipids is qualitatively bound to that of HUFA. More striking is the transportation of cholesterol depending on phospholipids present in the haemolymph, there is an inverse relationship between the « needs » in both nutrients;

this interrelationship was demonstrated in *P. vannamei* juveniles by Clarck and Lawrence (1988). A similar very clear interaction was revealed by Kanazawa *et al.* (1985) in their study on EFA and phospholipids of larvae : if the diet contained 6 % soybean phosphatidylcholine (PC) growth and survival were almost identical with 0.5 and 1 % n-3 Hufa, while with 3.5 % of PC 0.5 % of n-3 Hufa already appeared insufficient.

VITAMIN NUTRITION

No complete study on qualitative requirements for vitamin was published for *P. japonicus* as far as WE. know. But a distinction must first be made between the A and B groups : in the former group vitamins A and D do not appear strictly essential since they can be derived from carotenoids and cholesterol respectively. The capacity of bioconversion of several carotenoids into vitamin A appears broad in upper crustacea and many authors use i caroten instead of vitamin A with very good results. The ability of shrimp to derive vitamin D from sterols probably exists though little is known on the role of this vitamin in crustacea and, *a fortiori*, on the quantitative needs of penaeids (Fisher, 1960). Vitamin E was the first vitamin which was demonstrated to be essential in crustacea; it plays a very important role in reproduction, its deficiency inducing male sterility in *P. indicus* as shown in our laboratory (Cahu and Fachfach, 1989), but no data are available for *P. japonicus*. As far as we known vitamin K is perhaps not required by crustaceans, where it even could lead a detrimental effect (Fisher, 1960).

Table 4. Vitamin requirements of juvenile *Penaeus japonicus*.

Vitamin	Requirement (mg %)			
	Kanazawa et al. 1976	Guary et al. 1976	Deshimaru and Kuroki 1979	Civera 1989
Ascorbic acid	—	1000-2000	300	—
Choline	60	—	Dispensable	—
Inositol	200	—	400	>400
Thiamine	—	—	6-12	—
Pyrodoxine	—	—	12	—

In the B group

A. the essentiality of riboflavin, pantothenic acid, nicotinic acid, biotin, folic acid and vitamin B12 remains to be demonstrated,

B. it is likely since these vitamins were found to be required in most insects studied

C. paraaminobenzoic acid essentiality is doubtful if we refer to insects (House, 1974). The requirements for other water soluble vitamins are shown in table 4.

Ascorbic acid plays a very important role in practise because of its lack in most dry feedstuffs and it is very unstable during feed processing and storage. Because of these characteristics recommendations probably

correspond to strong overestimations of the actual requirements (or to safety margins).

MINERAL NUTRITION

Most fields of mineral nutrition have still been less studied than vitamin nutrition in sea shrimp since seawater is the supplier of most required inorganic elements except phosphorus.

For this reason, the requirement for phosphorus, as well as for calcium, retained the attention of several authors, but their results show a great discrepancy (Table 5). The causes of such a discrepancy may be numerous but we have to mention the very clear results of Cheng (1986), and Civera and Guillaume (1989) indicating that the availability of P was very variable among different salts. One reason of such a variability, i.e. the absence of acidic pH in the « stomach », appears as previously underestimated. If very available sources of P are chosen, the actual requirement of *P. japonicus* for this element is probably below 1 % of the diet. On the other hand the optimal Ca/P ratio seems to be very difficult to determine since very good growth can be obtained without dietary Ca, this element being easily extracted from seawater (Deshimaru and Yone, 1978a).

Table 5. Calcium and phosphorus requirement of *Penaeus japonicus* and suggested Ca/P ratio.

Requirements			Sources		Authors
Ca	P	Ca/P	Ca	P	
1.24	1.04	1:1	$CaHPO_4.2H_2O$ $CaCO_3$	$CaHPO_4.2H_2O$	Kitabayashi et al 1971
0	2	—	$CaCO_3$	$NaH_2PO_4.2H_2O$	Deshimaru and Yone, 1978a
—	—	2:1	$CaCO_3$	$CaHPO_4$	Cuzon 1982
1-2	1-2	1:1	Several	Na_2HPO_4 NaH_2PO_4	Kanazawa et al 1984
0	1.5	—	Several	Na_2HPO_4	Cheng and Guillaume 1984
—	0.56	—	$CaCO_3$	Na_2HPO_4 phytate.	Civera and Guillaume

The usefulness of other minerals in dietary supplies is poorly known; iron can have a negative effect (KANAZAWA *et al.*, 1984) while other micro elements seem to be supplied in sufficient amount by seawater. In semi purified diets improvements of performances were obtained with the supplementation in potassium and magnesium; the optimal levels being of 0.9 and 0.3 for these elements respectively according to Kanazawa (1984). However, most trace elements as well are extracted directly from seawater and do not need to be added to the usual diet.

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MISCELLANEOUS COMPONENTS

Very little is known on the role of fiber for *P. japonicus.* This component has received limited attention in other shrimp also, but beneficial effects of fiber were registered both for *Macrobrachium rosenbergii* and *P. aztecus.*

Concerning carotenoids that are added either pure or through concentrated sources in practical diets, little information is available (Otazu Abrill and Ceccaldi, 1984) despite of considerable economic importance of these compounds ubiquously found in crustaceans.

The existence of possible « growth factors » will be discussed elsewhere, but it may be recalled that nucleic acids are sometimes added to semi purified diets as growth promoters.

CONCLUSION

During the past two decades an impressive amount of knowledge was gathered in the field of *P.japonicus* nutrition, most of the work being done by the Japanese technicians. Of course many facets have not yet been studied, but the essentiality of nutrients known to be so in vertebrates was verified (except for some vitamins and minerals) and for the most important nutrients requirements have been estimated, while nothing was known 20 years ago. Some very original nutritional features were discovered such as phospholipid requirement and role in different steps of lipid metabolism. The very high value of the protein requirement in *P. japonicus* was also underlined and has led to the conclusion that this species was the most demanding penaeid. More recent exploration of larval needs have also been very successfull.

Unfortunately almost nothing is known in breeder nutrition : as far as we know it is still impossible to obtain good spawning in *P. japonicus* without using natural food. On the other hand for accurate least cost formulation of juvenile foods most estimations of the requirements should be submitted to further studies. New (1976) stated that « perhaps the most urgent task is the determination of quantitative amino acids requirements ». We think this statement remains valid; but more information is also needed in the field of energy requirements which correspond to the most expensive part of diets.

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AQUACOP. IFREMER. Actes de Colloque **9** pp. 393-394.

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Development of diets for *Penaeus aztecus, P. setiferus, P. vannamei* and *P. stylirostris* juveniles and postlarvae

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Abstract. — *Laboratory Studies of penaeid feeding and nutrition were performed at the Galveston Laboratory of NMFS between 1963 and 1985. Earliest studies used field-collected* ***Penaeus aztecus*** *postlarval and juvenile stages of both wild and laboratory-spawned* ***P. aztecus*** *and* ***P. setiferus*** *and laboratory-spawned* ***P. vannamei*** *and* ***P. stylirostris*** *were later included.*

A standard cold-extruded laboratory diet containing sun-dried shrimp meal, defatted rice bran, menhaden meal, ¿-soy protein, soybean lecithin, vitamins, menhaden fish solubles (as attractant) in an alginate binder was formulated (with Dr. Samuel Meyers, LSU) and tested in various shapes and forms with both native species. This standard diet later modified by the addition of squid meal.

Penaeid species apparently differed in their growth responses to squid meal. ***P. setiferus*** *juveniles required somewhat lower percentages than* ***P. aztecus.*** *Cheaper vegetal proteins (¿-soy, treated soy meal, cottonseed meals) were increased at the expense of marine animal sources), varying the ratio of animal : vegetal protein.* ***P. aztecus*** *required an increase in total protein as vegetal protein increased. Responses to altered A : V protein ratios differed between species and among size groups within species, smaller sizes within species usually requiring a higher proportion of marine animal protein. Neither short-term protein and carbohydrate assimilation nor attractivity of various feeds to larger* ***P. stylirostris*** *correlated with growth over a longer time period. Fatty acid analysis, though suggestive of need for polyunsaturates, did not correlate well with growth.*

Of various live and natural organisms tested as replacements for ***Artemia,*** *rotifers showed the most promise for postlarval* ***P. setiferus.*** *Other studies of this penaeid species and* ***P. vannamei*** *suggested that reducing total* ***Artemia,*** *but supplementing with high quality animal-protein feeds (A :V = 4.5 :1) for a limited period (13 days) could be followed successfully with prepared feed with lower animal protein (A : V = 1.5 : 1). Such a feeding regime, seemingly more efffective at temperatures of 30° both reduces dependence on live feed and limits total feed cost.*

The apparent changes in protein requirement with size and species may be correlated with the biology of the species cultured. More detailed knowledge of the biology of these organisms and their responses in nature will inevitably result in better nutritional understanding.

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Microparticulate feeds for Penaeid larvae

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Abstract. — *The mass production of penaeid larvae still depends on live foods such as diatoms, **Chlorella** and **Artemia**. However, it requires much facilities, maintenance expenses, and labor to produce a desired amount of live foods safely and constantly. Also, the nutritive value of planktonic organisms is occasionally variable and this makes the use of live food for mass culture restrictive. Therefore, it is necessary to develop microparticulate diets as a substitute for live foods to further increase the productivity of seed for shrimp culture.*

Several types of microparticulate diets, micro-encapsulated diet, microbounded diet, and micro-coated diet, were prepared and their dietary values for the larvae of shrimp were evaluated. Micro-bound diet containing kappa-carrageenan as a binder supported the high growth and survival rates of shrimp larvae from Zoea 1 to post-larva 1.

Protein resources having a high nutritional value such as krill meal, squid meal, fish meal, scallop meal, short-necked clam extract, chicken egg, casein, soybean meal, and yeast are used for microparticulate diets of larval shrimp.

*Recently, the mass seed production of **Penaeus japonicus** was carried out by using microparticulate diets. As a result, 11,911,000 postlarvae (survival rate of 96 %) were produced in a 400 ton tank.*

MICROPARTICULATE DIETS

The mass production of penaeid larvae still depends on live foods such as diatoms, *Chlorella* and *Artemia*.

However, it requires much facilities, maintenance expenses, and labor to produce a desired amount of live foods safely and constantly. Also, the nutritive value of planktonic organisms is occasionally variable and this makes the use of live food for mass culture restrictive. Therefore, it is necessary to develop microparticulate diets as a substitute for live foods to further increase the productivity of healthy seed for shrimp culture.

The various types of microparticulate diets reported are categorized into three groups, micro-encapsulated diet, micro-bound diet and micro-coated (Kanazawa and Teshima, 1983; Kanazawa, 1985a; Kanazawa, 1986a; Kanazawa and Teshima, 1988). Micro-encapsulating a solution, colloid or suspension of diet ingredients with a membrane. Micro-coated diets are prepared by coating micro-bound diet with some materials such as zein or cholesterol-lecithin. The details of the procedures for preparation of microparticulate diets are described elsewhere (Teshima et *al.*, 1982; Kanazawa and Teshima, 1983; Kanazawa, 1985b).

Table 1. Composition of Carrageenan micro-bound diet.

INGREDIENT	(g/100g)
Skim milk	52.0
Chicken egg yolk (dry)	10.0
Egg albumin	20.0
Amino acid mixture	5.0
Pollack liver oil	5.5
Soybean lecithin	1.5
Mineral mixture	1.0
Vitamin mixture	5.0
TOTAL	100.0
Kappa-Carrageenan	5.0

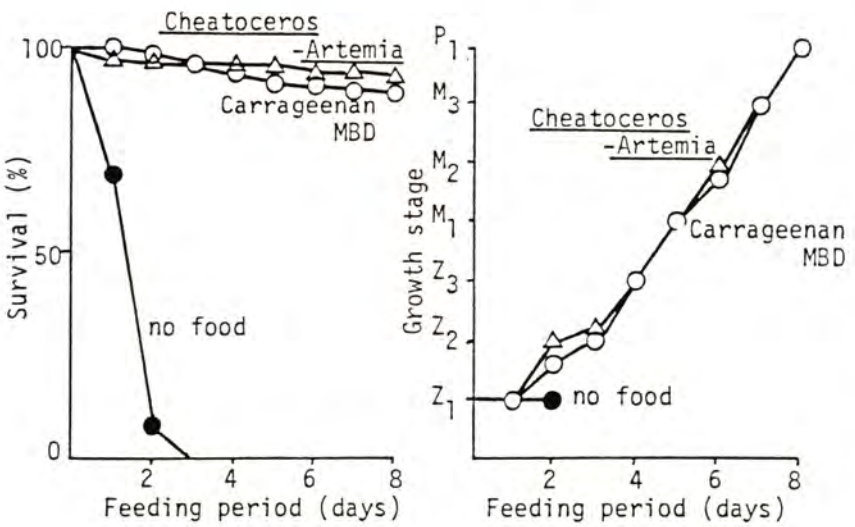

Fig. 1. — Survival and growth of larval prawn fed on micro-bound diet.

The nutritional components of the microparticulate diets for shrimp larvae should be determined on the basis of the requirements of the larval shrimp for protein, amino acid, lipid, carbohydrate, vitamin and minerals. However, as the requirements of the larval shrimp are still undefined, protein resources having a high nutritional value such as krill meal, squid

meal, scallop meal, short-necked clam, chicken-egg, skim milk, casein and fish meal are used (Kanazawa, 1985c). Micro-bound diet containing Kappacarrageenan as a binder supported the high growth and survival rates of prawn, *Penaeus Japonicus*, larvae from $zoea_1$ to $postlarva_1$ (Table 1 and Fig. 1) (Kanazawa, 1985b).

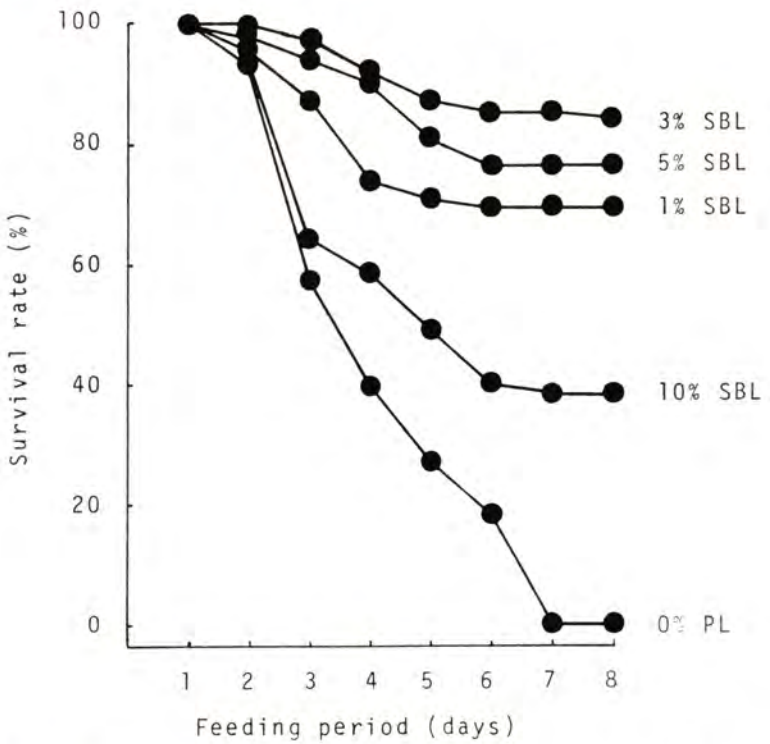

Fig. 2. — Survival rates (%) of the prawn larvae receiving varying levels of soybean lecithin (SBL). O % PL indicates a phospholipid-deficient diet.

PHOSPHOLIPID REQUIREMENTS

Some phospholipids in diets have also been demonstrated to be indispensable for su staining growth and survival of crustaceans such as the prawn (Kanazawa, 1983a, B; Kanazawa, 1984; Teshima, 1985; Teshima and Kanazawa, 1988) and the American lobster (Conklin et *al.*, 1980; D'Abramo et *al.*, 1981).

Feeding trials using the prawn, *P. Japonicus*, larvae were conducted to examine the effects of several dietary phospholipids on the growth, survival, and body lipid composition (Teshima et *al.*, 1986b). The deficiency in dietary phospholipids caused a total mortality within 6-7 days. When the prawn larvae were fed carrageenan micro-bound diets with varying levels of supplemental soybean lecithin, the highest survival rates were obtained on diets with 3 % soybean lecithin (Fig. 2). Soybean

phosphatidylcholine and soybean phosphatidylinositol showed a higher nutritive value than bonito egg phosphatidylcholine and soybean phosphatidylethanolamine at a 3 % supplemental level (Fig. 3). The deficiency in dietary phospholipids resulted in a slight decrease in the concentrations of steryl esters, free sterols, phosphatidylcholine, and phosphatidylinositol in the bodies. The concentrations of phospholipids such as phosphatidylcholine seemed slightly higher in the prawn larvae receiving supplemental soybean phosphatidylcholine than other supplemental phospholipids such as soybean phosphatidylinositol, soybean phosphatidylethanolamine and bonito-egg phosphatidylcholine.

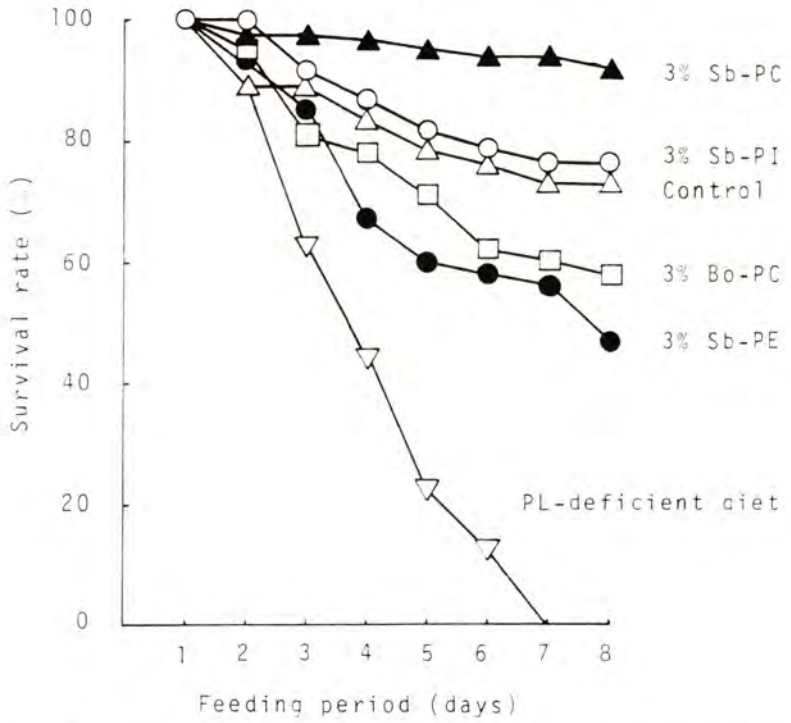

Fig. 3. — Survival rates (%) of the prawn larvae receiving several phospholipid sources Control : live feed (*Chactocero* + *Artemia*).

However, the body lipid compositions, on the whole, were not variable notably with the kinds of supplemental phospholipids examined. Also, the sum of 20 : 5W3 and 22 : 6W3 proportions of body phospholipids was slightly higher in the prawn larvae receiving soybean phosphatidylcholine rather than in those receiving other supplemental phospholipids.

The effects of dietary phospholipids on weight gain, retention of dietary lipids and body composition of the prawn juvenile were examined (Teshima et *al.*, 1986a).

The prawn *P. Japonicus* was reared with diets containing 3 % soybean lecithin (diet A) and no supplemental phospholipids (diet D) for 30 days. The deficiency of phospholipid in diets significantly reduced the weight

gain and feed conversion efficiency. The prawns receiving diet D without supplemental phospholipid contained a lower concentration of phospholipids such as phosphatidylcholine and phosphatidylinositol in the whole body than the ones receiving diet A with supplemental phospholipid. The retention (%) of dietary lipids, especially cholesterol, in the body was also significantly lower in the prawns receiving diet D than in those receiving diet A. These data indicate that the juvenile prawn requires dietary sources of phospholipid for good growth; suggesting that the dietaryphospholipid may be necessary for the effective utilization of lipids such as triglycerides and cholesterol in the diets by the prawn. However, it was not clear why dietary phospholipids exerted a growth-enhancing effect in the prawn *P. japonicus*. To confirm this, the postprandial variation in radioactive lipid classes was investigated after feeding of tripalmitin ^{-14}C and cholesterol ^{-14}C with or without dietary phospholipids.

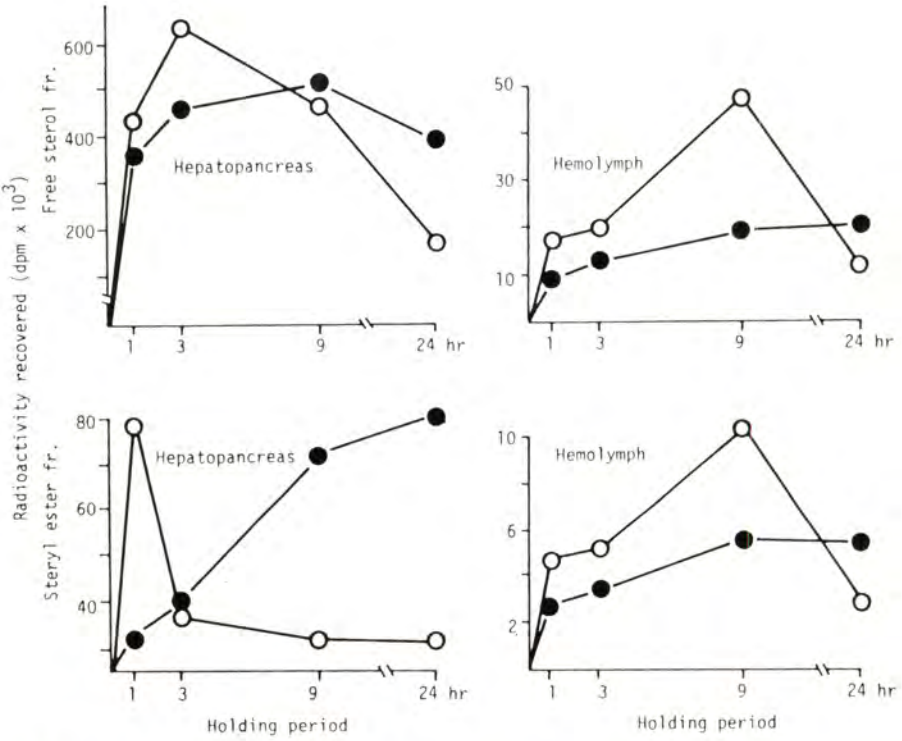

Fig. 4. — Postprandial incorporation of radioactivity into free sterol and esterified sterol fractions of the hepatopancreas and hemolymph in *P. japonicus* prawns after feeding of cholesterol-4-14 C in (0) phospholipid-added diets and (0) phospholipid-deficient diets.

The effects of supplemental phospholipids on the assimilation and transport of tripalmitin ^{-14}C in relation to the nutritional role of phospholipids in diets of the prawn *P. Japonicus* were examined (Teshima et *al.*, 1986c). The prawns were fed on the diets containing tripalmitin ^{-14}C with 3 % soybean lecithin (diet A) and without supplemental phospholipid (diet D) and then the incorporation of radioactivity into the organs and

tissues was examined after 1, 3 and 6 h of feeding. When the prawns received tripalmitin ^{-14}C, the inclusion of phospholipids (3 % soybean lecithin) in the diets resulted in an increase in radioactive phospholipids, especially phosphatidylcholine, in both the hepatopancreas and hemolymph. The supplementation of phospholipids to diets also brought on an increase in radioactive triglycerides in the hepatopancreas but not in the hemolymph.

Cholesterol ^{-14}C was orally administrated to the prawn to clarify the effects of dietary phospholipids on the mobilization of sterol in diets to various organs and tissues (Teshima et *al.*, 1986d). The prawns were fed on the test diets with 3 % soybean lecithin and without supplemental soybean lecithin and then dissected 1, 3, 9 and 24 h after feeding (Fig. 4). When fed the phospholipid added diet, the radioactivities of hepatopancreatic free and esterified sterols increased quickly and reached maximum levels 3 and 1 hours after feeding, respectively and then decreased, while those of free and esterified sterols in the hemolymph increased with the lapse of time and reached maximum level 9 hours after feeding. Thus, when fed the phospholipid-deficient diet, dietary cholesterol remained as a free sterol for a long time in the hepatopancreas and entered into the hemolymph slowly, and also the formation of cholesterol esters in the hepatopancreas proceeded at a slow rate. These results suggest that dietary phospholipids such as soybean lecithin contributes to the smooth mobilization of dietary cholesterol in the body especially from the hepatopancreas to the hemolymph. Growth of the prawns receiving the phospholipid-deficient diet was conceived to be retarded owing to the insufficient transport of dietary cholesterol rather than triglycerides in the body.

VITAMIN REQUIREMENTS

Kanazawa (1986b) have examined the requirements of larval *P. japonicus* for various vitamins by using microparticulate diets with carrageenan as a binder. As a result, the prawn larvae were found to require i-Carotene, thiamine, riboflavin, pyridoxine, nicotinic acid, folic acid, biotin, cyanocobalamin, choline, inositol, ascorbic acid, vitamin D and vitamin E. The shortage of one of these vitamins resulted in the cessation or retardation of metamorphosis and in high mortality during larval development.

The quantitative requirements of larval prawn for several vitamins have been done. The requirements of vitamin are as Table 2 : Thiamine-HCl. 4mg %; riboflavin, 8 mg %; pyridoxine-HCl, 12 mg %; nicotinic acid, 40 mg %; biotin, 0,2 mg %; choline chloride, 600 mg %; inositol, 200 mg %; Na-ascorbic acid, 1 000 mg %; vitamin E (tocopherol), 20 mg %. The requirements for some vitamins were apparently higher for *P. japonicus* larvae than for juvenile. It is conceivable, however, that some vitamins may have leached into the water before eating. This means that the vitamin requirements of larval prawn mentioned above should be regarded as « practical demand for rearing of the larvae ».

Table 2. — Vitamin requirements of larval prawn. *P. japonicus.*

VITAMIN	mg/100g of DRY DIET
Thiamine-HCl	4
Riboflavin	8
Pyridoxine-HCl	12
Nicotinic acid	40
Biotin	0.2
Choline chloride	600
Inositol	200
Na-Ascorbate	1 000
Tocopherol	20

MASS SEED PRODUCTION OF PRAWN WITH MICRO-BOUND DIET

We have attempted to rear larval prawn, *P. japonicus* with microparticulated diets. As a result, seed production of prawn was successfully achieved by partial or even complete substitution of microparticulate diets for live food.

Larval stage used	$Zoea_1$ stage
Number of larvae	410 000
Experimental period	16 days
Tank	15 ton
Water temperature	26.8 ± 1.5 °C
Feeding level	$Zoea_1$-$Zoea_3$: 0.16 mg/larva/day $Mysis_1$-$Mysis_3$: 0,20 mg/larva/day $Postlarva_1$-$Postlarva_3$: 0.24 mg/larva/day > $Postlarva_4$: 0.3 mg/larva/day
Feeding frequency	< $Postlarva_4$: 4 times/day > $Postlarva_5$: 5 times/day

Table 3. — Rearing and feeding methods of larval prawn.

Experiment I

In experiment I, 410,000 $zoea_1$ larvae were kept in a 15 ton tank. The feeding experiments were carried out under the conditions listed in table 3 (Kanazawa, 1985b). Kappa-Carrageenan micro-bound diet was used in this seed production. Control group of larvae was fed on diatom *Chaetoceros gracilis* until $mysis_2$, on *Artemia salina* until $postlarva_5$, and then on commercial diet until $postlarva_8$. From $zoea_1$ stage, the larval prawn reached $postlarva_8$ using only kappa-carrageenan micro-bound diet. As a result, 307, 500 postlarvae (survival rate of 75 %) were produced (Fig. 5 and 6). In the control group of live food, diatoms did not grow owing to the rain and the larvae almost died at $mysis_1$ stage.

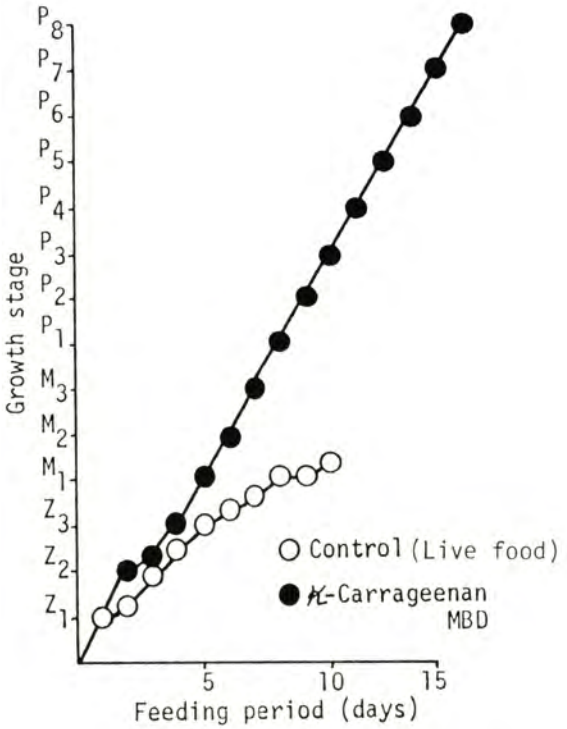

Fig. 5. — Growth stage of larval prawn fed on micro-bound diet.

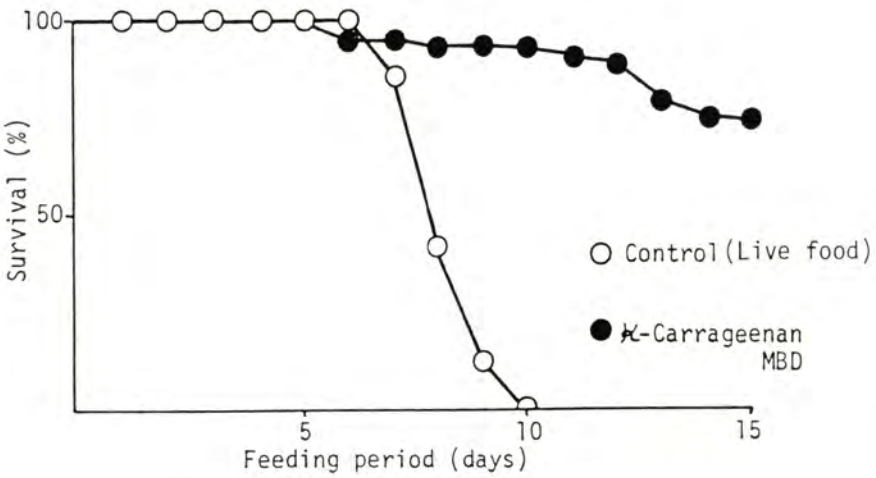

Fig. 6. — Survival rate of larval prawn fed on micro-bound diet.

Experiment II

12,400,000 larvae of *P. japonicus* were reared from zoeal stage to postlarval0 stage with a mixture of microparticulate diet and live food in 400 ton tank. As a result, 11, 911,000 postlarvae (survival rate of 96 %) were produced.

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Selected ingredients for shrimp feed.

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Abstract. — *The selection of ingredients is a high priority for formulating and processing shrimp feeds. One of these selected ingredients is fishmeal which represents 30-40 percent of the whole formulae. It is reviewed some specifications on fishmeal quality in order to determine which one is the best among white and brown fishmeal.*

Another ingredient of major importance is soyabean meal which can repleace part of fishmeal only. It seems important to select a good quality soyabean meal.

Yeasts are a potential source for protein supply and benefits for shrimp growth are substantial. But again the response of the animal is in relation with the quality of the product (lactic yeast versus brewers yeast for example).

As a last example of selected ingredients is shrimp meal which has long been representing a major component in shrimp diet with levels of inclusion as high as 30 percent. The real benefit of shrimp meal in a diet is discussed.

A short review is given on other ingredients like, squid meal, mussel meal and leaf protein concentrate, casein and gelatine.

INTRODUCTION

There are many ways to start up with a species for which a good nutrition is required.

So far the knowledge on ingredients is of prime importance for the formulation of shrimp diets, as far as multi-ingredients formulae are concerned. In case of purified diets some protein sources like casein, albumin, gelatine, have been proved successful (Kanazawa, 1972; Deshimaru, 1975) and more recently crab protein (Boghen et *al.*,1982; Castell, 1987) which is about to be used as a reference protein source.

Artificial diets are composed of several ingredients including squid meal, fish meal, yeast,.(Deshimaru, 1974; Shigueno, 1975, Aquacop, 1976).

It is the intermediate step before formulating commercial feeds in which fish meal represents the major component (Table 1).

MATERIALS AND METHODS

Most of experiments are conducted in 250 litres tanks as previously described (Aquacop, 1976, 1978, 1986), and standard conditions are obtained for the seawater, temparature, salinity, oxygen, pH, N-NH3, N-NH4, N-NO2.

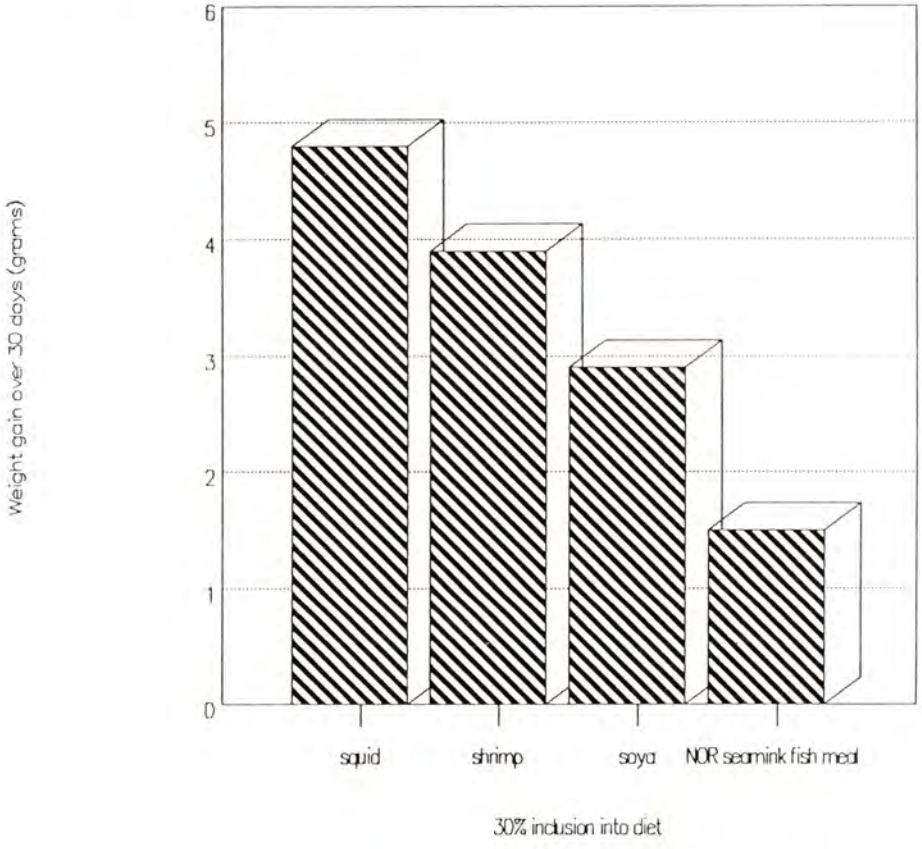


Fig. 1. — Comparison of the effectiveness of protein sources on growth of *P. stylirostris.*

RESULTS

Each ingredient is tested for its efficiency when included at a relatively high level in the feed, at least 30 %. And each protein source ingredient is compared to another in order to get a classification of these protein sources ingredients.

This method is not a precise one and it needs to collect other data to state clearly on each ingredient considered as a protein source (Fig. 1).

Nevertheless, indications given by such a procedure are proved to be useful for the formulation of artificial feeds.

All these studies on classification of raw ingredients for the diet of shrimp in order to select the most productive ones for growing animals are conducted with an average protein level generally well above the determined optimum level, in order to be sure no limiting effect results on a given amino acid of the diet (Table 2).

	%
CPSP 80	15
Wheat gluten	20
Wheat flour	15
Blood meal	3
CaHPO4	3.5
Oyster shell	0.5
Cellulose	2.5
Fish oil	1.75
Lecithin	1.75
Potato starch	3
Vit. mix.	4

Table 1. Basal diets for ingredients study on *P. stylirostris.*

Table 2. Proximate analysis and amino acid composition of experimental feeds according to the selected protein source in g/100 g.

	Norsea mink meal	Soy Concentrate	Squid meal	B/B Shrimp meal	CPSP 80	Caseine
ARG	3	4	3	2	3	3
HIST	1	2	1	1	1	2
LYS	3	3	3	2	3	3

DISCUSSION

Establishment of protein requirements within a system must be on the quality of the protein source (Colvin and Brand, 1984); when studying for protein requirement of PL's of *P. Californiensis*, they concluded at an optimum around 30 % when feed conversion ratio was improved regularly from 30 up to 40 % of the diet.

One can wonder if higher protein level have promoted better growth and consequently better feed conversion ratio ?

In the case of *P. Vannamei* raised experimentally at COP or in commercial ponds, it has been shown an evolution of growth rate according to concentration of protein in the feed. In early seventies, the marine ration 25 was used (Ralston Purina), in semi intensive conditions, commercial rations provided to shrimp raised in ponds with natural productivity.

Growth performances were satisfying with around 3-4 g, average weight increase per month. Then, early 1980's, a commercial feed (President shrimp feed) 40 % higher in protein provided better growth rates in

experimental tanks, 3-4 g per month without natural productivity. At last, more recently, 1984 onwards, another commercial feed, a Japanese one (Nippaï shrimp feed) was successfully experienced in order to reach what could be called maximum growth, i.e. 6 g average weight increase per month. Such an evolution underlines a real discrepancy between optimum of protein in a shrimp feed, for economical reasons and optimum of protein for maximum growth of penaeid shrimp. Such a statement is more accurately demonstrated thanks to the following experiment (Cam, Cuzon and Aquacop, 1989).

An appreciation of ad libitum feeding level allows to calculate several levels of restricted feeding and correlate this to growth performances of *P. Vannamei* over a month.

A relationship is clearly shown between the amount of ingested feed and growth performances. One distribution versus two distributions of extruded feed help to understand the growth improvement of 1 or 2 grammes weight increase over a month when two meals are provided instead of one Meanwhile, feed conversion ratio is approximately constant at 2.2 and slighlty improves when two meals are given, FCR = 2.1.

Another study on *P. japonicus* (Deshimaru, 1972) gave similar results = relationship between ingested level of feed and growth rate in one case, improvement of FCR with increase of protein in the diet in another case.

« The overall capacity of the gastro intestinal tract to digest protein is very high since levels of protein up to 60-70 % of the diet are apparently digested as well as are lower levels. It seems to be difficult to exceed the digestive capacity of an animal with a diet composed of readily digested components » (Nesheim, Scott and Young, 1982).

Aside the research for optimum combination of ingredients for maximum growth of penaeid shrimps, one of the other aspect of applied nutrition of the shrimp is the effectiveness of a given raw ingredient on growth and survival of shrimp.

It is the case with two major components of shrimp feed, soya bean meal and fish meal.

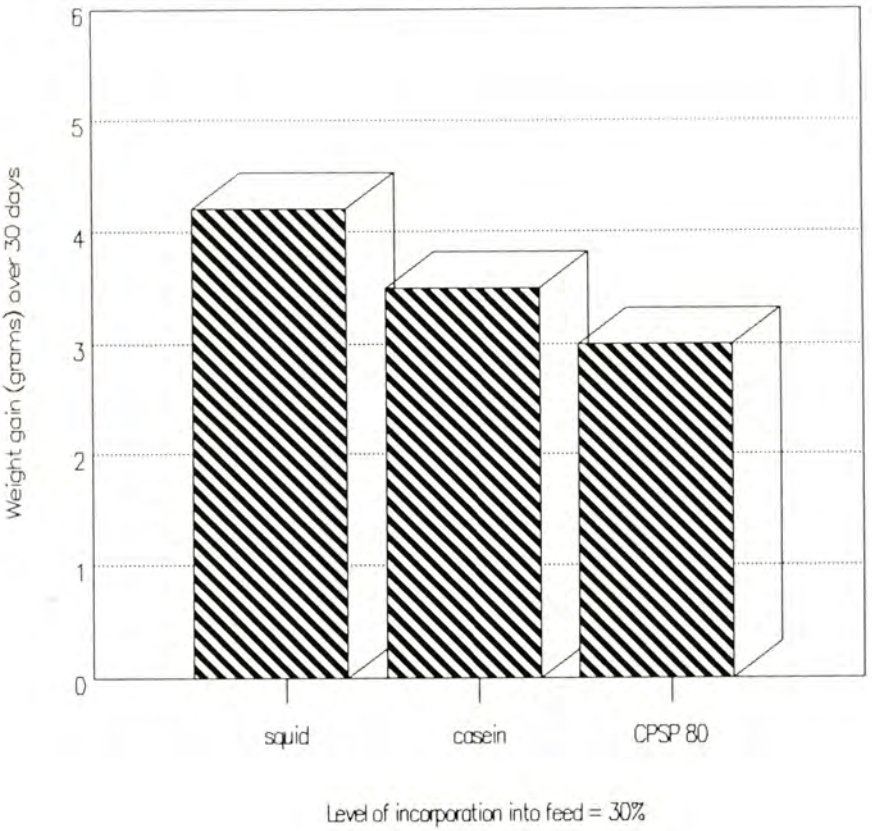
One of the techniques involved is what is called dose-response test for one ingredient in case of US soya bean meal, a range of 5 to 20 % is calculated at the expense of a combination of protein sources (FPC, shrimp meal, yeast, blood meal).

There is isoproteic value and isoenergenetic value of the 4 formulae. A one month growth test trial shows a regular decrease of growth performances when soya bean mean replaces the combination of other protein sources when no stunted growth was expected (Aquacop, 1983) unpublished results.

There is a possible indication of antinutritional effect of large amounts of soya bean meal in *P. vannamei* feeds for juveniles though some experiments on post-larvae seem to indicate a possible high level of soya-bean meal into the feed (A.D. Lawrence, 1988).

A second ingredient of importance is fish meal which has long been representing a major ingredient of fish and shrimp diets.

In Tahiti, a selection of Norway fish meal was done in 1983 and in order to check its influence at relatively high level in the feed of *P.*



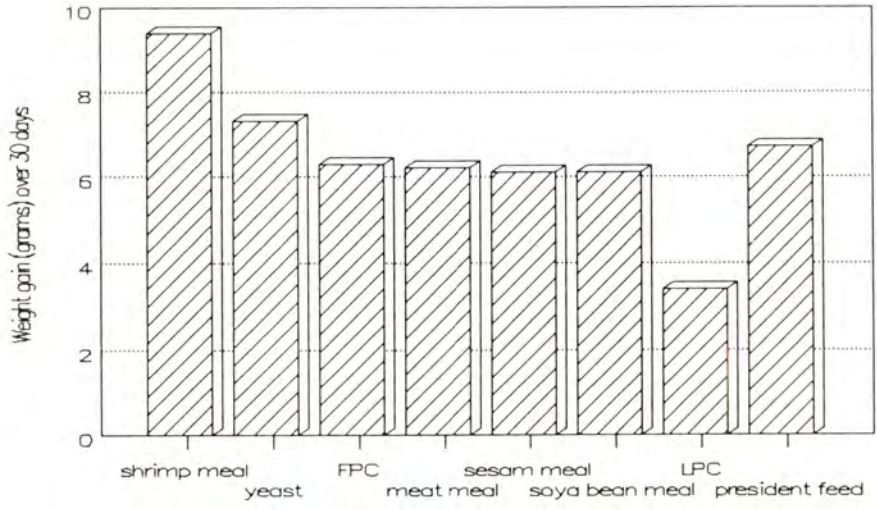


Fig. 2. — Comparison of protein sources for *P. monodon.*

vannamei, according to a similar protocole as the one used for soya bean meal test. The replacement of a mixture of other ingredients by Norway fish meal in *P. vannamei* feed do not affect growth rate of shrimp up to 20 % inclusion. Beyond that level, a slight negative effect is observed and it is explained by the fact that quality of Norway fish meal does not compensate the mixture quality of other ingredients, including fish protein concentrate. Formulations of shrimp feeds are exhibiting large amounts of fish meal. Japanese manufacturers are selecting white fish meal with low histamin content for *P. japonicus*.

Ecuadorian manufacturers are largely relying on local supply of regular fish meal for producing feeds for *P. vannamei* raised at low density in large ponds.

Taiwanese manufacturers import fish meal from Japan and especially from Indonesia, Perou, Chili in order to keep up their formulations for *P. monodon* at around 40 % crude protein. They emphasized a lot on criteria for selection of quality fish meals and they produced in 1985 a table given for classification of fish meal of different origins. This classification enables us to separate brown fish meal from white fish meals. Such data should be more widely distributed for helping manufacturers to select more precisely their stocks of fish meals for producing their shrimp feeds.

It is the case in Tahiti and New Caledonia where 7 differents spots can be considered in the South Pacific Area to select a supplier of fish meal.

More over other protein sources are essential for the production of shrimp feeds, there are shrimp meal, yeast, squid meal, mussel meal. Various qualities of shrimp coming from Louisiana, Alaska, Ecuador, Groënland principally where tested at same level inclusion in a shrimp diet with same growth performances.

The raw material seems highly dispensable in a shrimp feed and that was shown at 30 % level inclusion in a *P. monodon* diet in comparison with several other protein sources at same protein content, shrimp meal allowed shrimp to grow faster, yeast were coming right after. Yeast is another ingredient which has good potential regarding to the growth of shrimp; some commercial feeds are probably including up to 20 % of the whole formulae. Again its quality is strictly related to growth performances of penaeid shrimp (Fig. 2).

At last, molluscs meals are of prime importance, mussel meal, and especially squid meal (Cruz et *al.*, 1986; Cruz et *al.*, 1987) which seems to act as a growth promotant on several shrimp species. It is worth talking of a squid effect which could be a more general mollusc effect related to shrimp growth.

CONCLUSION

The good knowledge on major ingredients and adequate supply of quality raw materials help dramatically to formulate and produce shrimp feeds with a good potential for growth. One of the best example is given

with Taiwanese Practical feeds for *P. Monodon* (Chuang, 1988) which includes 30-40 % fish meal, 7-15 shrimp meal, 5-15 % soya bean meal, fish solubles and squid liver, cereals and premixes. In order to summarize all this information, a table of selected ingredients for shrimp feeds is established (Table 3) and will be revised annually according to specifications given by a Nutrition Working Group.

Table 3. Selected ingredients for shrimp feeds.

1. Fish meals. Marine protein	
NSM	10-25 %
WFM	
Tuna Fish meal	
Chilean Fish meal	< 40 %
Peron Fish meal	< 20 %
Alaska Fish meal	
NZ Talley Fish meal	15 %
Fish protein concentrate	8-20 %
2. Shrimps meals	
Blum and Berjeron	30 %
Alaska	30 %
Ecuador	30 %
3. Squid meal	
Japan	5-40 %
India	5-40 %
4. Yeasts unicellular proteins	
Lactic yeast	20 %
Torula yeast	< 15 %
Brewers yeast	10-15 %
Bakery yeast	20 %
5. Meat/bone meal : Conventional protein sources Blood meals	
MBW 48 % cp	< 15 %
Blood meal	
- drum dried	3-4 %
- atomized	10 %
Chicken of falls meal	10 %
6. Soya bean meal	
Regular SBW 48 % cp	20 %
Soy protein concentrate	10 %
7. Leaf Protein Concentrate	< 6 %
8. Wheat Gluten	5-20 %

All this information should be more and more available and accurate in order to allow the use of a linear programme with more and more insistance; that would be particularly helpful for feed manufacturers.

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Summary of recommendations and discussions from the round table discussion

J.D. CASTELL

1. ABSTRACTS

1.1. Recommend that criteria for estimating effectiveness should include :

1.1.1 stimulation of a initial change in behaviour
1.1.2. movement of shrimp or prawn toward the feed pellet
1.1.3. handling of the pellet
1.1.4. consumption of pellets
1.1.5. continued consumption of pellets over time
1.1.6. growth ennancement.

1.2. Two distinct types of attractants were recognized

1.2.1. Extracts from natural feed ingredients, like fish solubles.
1.2.2. Distinct formulated mixtures of known molecules such as amino acids, fatty acids, aldehydes, ketones etc...

1.3. A further distinction was suggested between (1.3) molecules which are dispersed in the water to attract shrimp to feeds and 1.3.2. flavour compounds which induce the shrimp to actually consume the pellet.

1.4. A list of papers on clams attractants for crustaceans will be prepared by the working group.

1.5. A table of chemically defined attractants that have been effective for crustaceans will be prepared.

1.6. Commercially available attractants for crustaceans include 1.6.1 Finnstin produced by the finnish sugar company, and 1.6.2. a squid liver powder used in Japan and Malaysia and 1.6.3. various artificial bait compounds. A list of all known such products will be prepared.

1.7. The synthetic mixture of betaïne, glycine, taurine, alanine, choline etc... in the proportions suggested by him will be accepted as an initial attractant for purified crustacean diets. It is expected that species specific standard attractants mixtures will eventually be identified as it is suspected that there will be differences in attractant to the various species.

1.8. The ICNRWG should clearly identify the importance of attractants to stimulate the development of commercially available effective attractants.

2. TECHNOLOGIES AND INTERACTIONS

1. Introduction : The main concern seemed to be with heat labile nutrients, especially the losses of vitamin during feed processing and storage.

2. Regarding vitamin C the group recommended.

2.2.1. A list of commercially available forms of vitamin C.
2.2.2. All informations on bioavailability of the various form of vitamin C to crustaceans should be listed.
2.2.3. Though the process of coating a vitamin/lipid mixture onto feed pellets after processing might reduce processing losses, we do not know enough about this process and potential for oxidative losses associated with this process to make any strong recommendations at this time regarding processing technology.
2.2.4. The cold extrusion process may also told some promise for reduced processing bases of nutriments.
2.2.5. There is a need for more research on the interaction between feed processing technology and nutrient retention and availability to crustaceans.

3. MATURATION DIETS

3.1. Factors that are assumed to be especially important in maturation diets :

3.1.1. Availability and form of vitamin C
3.1.2. The fat soluble vitamins A and E
3.1.3. The content and relative proportion of n-3 and n-4 PUFA and HUFA
3.1.4. Carotenoid pigments and their potential physiological role
3.1.5. Both quantity and quality of protein
3.1.6. Sterols and Phospholipids
3.1.7. Requirements for all other nutrients.

3.2. Criteria for evaluation of maturation diet quality

3.2.1. Gonado somatic index
3.2.2. Egg production numbers and frequency of spawning
3.2.3. Successful hatching or hatchability %
3.2.4. Survival to specific larval stages such as Zoea I or Post Larvae
3.2.5. It is very probable that distinct criteria will be species specific.

3.3. Indirect or non nutritional factors which may be important :

3.3.1. Heat of processing and storage losses of nutrients
3.3.2. Textures, moisture content, water stability attractants

3.4. Consideration must be given to the time for initiation of feeding and duration of feeding maturation diets.

3.5. Maturation diets may be live or natural feeds, unrefined formulated diets or a combination of the two. Some success has been reported with all these combinations of maturation diet.

3.6. This is an important area for future crustacean nutrition, especially considering the current critical shortage of shrimp seed stocks for commercial culture.

3.7. We must make a list of the informations on species formulation and nutrient composition of the maturation diets that have been successfully used to date.

3.8. Initial focus should be on commercially important species such as P. *Vannamei*, P. *stylirostris*, P. *monodon* and P. *japonicus*

4. INTERNATIONAL WORKING GROUP ON RECOMMENDATIONS FOR CRUSTACEAN NUTRITION

4.1. It was unanimously agreed by the work-shop participants, that there was a need for the formalization of a working group on crustacean with specific objectives for future cooperation and collaboration among crustacean nutritionists.

4.2. That group was formally incorporated by vote from the work shop participants and Dr. Gerard Cuzon was elected by unanimous vote of the newly formed working group.

4.3. It was agreed that our initial objective was to make lists of species specific nutrient requirement our chairman assigned the following responsibilities.

4.3.1. Protein requirements	Dr. A. Kanazawa
4.3.2. Lipids	Dr. L. D'Abramo
4.3.3. Minerals	Dr. F. Pascual
4.3.4. Growth factors	Dr. J. Guillaume
4.3.5. Carotenoid proteins	Dr. T. Latscha
4.3.6. Vitamins	Dr. D. Conklin
4.3.7. Maturation diets	Dr. H.J. Ceccaldi
4.3.8. Binders	Dr. Z. Zein-Eldin
4.3.9. List of commercial feed producers	Dr. M. Girin
4.3.10 Attractants	Dr. J. Castell

4.4. To accomplish this initial objective it will be necessary to assemble a complete bibliography by assigned topic and crustacean species. This will be the task of each assigned committee member with the assistance of all other working group members.

4.5. The report of the group should focus on practical application in crustacean culture. A second objective should be to itentify quality criteria for the various ingredients used in manufacture of crustacean feeds.

4.6. We should established a standardized format for the tables using AIN recommendations with regard to nomenclature and units of measure. We will use standard accepted journal abbreviations for bibliographies rather than spelling all names completely as required by the WAS journal.

4.7. Plans of actions, time tables on deadlines and other assigments will be developed by the closing of the workshop on Tuesday February 28th.

4.8. Grandfather of crustacean nutrition : From his first publication on the use of a purified experimental diet for *Penaeus japonicus* in 1970, Dr. A. Kanazawa has been one of the most outstanding contributers to the field of crustacean nutrition. In spite of the fact that all of the other reviews at the workshop in Tahiti were quoting from Dr. A. Kanazawa's work, he still managed to provide two papers with all new research finding from his laboratory in Kagoshima. In recognition for his exceptional contributions to crustacean nutrition, the International working group on Crustacean Nutrition bestowed upon him the honorary title of GRANDFATHER of Crustacean Nutrition.

CHAPTER IV

FINFISH

IV.1

Review of knowledge on aquaculture for the principal species of tropical fish

IV.2

Constitution of broodstock,
Reproduction in captivity

IV.3

Larviculture, production of juveniles

IV.4

Nursing and grow-out

IV. FINFISH

IV.1. REVIEW OF KNOWLEDGE ON AQUACULTURE FOR THE PRINCIPAL SPECIES OF TROPICAL FISH

ADVANCES IN TROPICAL AQUACULTURE Tahiti, Feb.20 - March 4, 1989
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Status of knowledge on farming of Seabass (*Lates calcarifer*) in South East Asia

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Abstract — *A review is made on the various techniques of farming seabass (**Lates calcarifer**) in South-East Asia, namely, Thailand, Malaysia, Philippines, Indonesia and Singapore. The management and husbandry aspects are discussed, and the potential of farming this fish under highly intensive conditions is mentioned.*

INTRODUCTION

Lates calcarifer, commonly called the giant sea perch, seabass or barramundi, is an important coastal, estuarine and freshwater fish in the Indo-pacific region. It supports extensive commercial and recreational fisheries in Australia and Papua New Guinea, and is farmed in Thailand, Malaysia, Indonesia, Singapore, Hong Kong, Taiwan, and more recently, in Australia, in both brackishwater and freshwater ponds, as well as in cages in coastal water (Kungvankij et *al.*, 1984; Grey, 1987 ,). The fish has a delicately-flavoured flesh, is popular in the region, and has a high market price whenever it is available. It has a fast growth rate, grows to a large size, and can be bred in captivity, thus making it very suitable for aquaculture.

World fisheries production of seabass in 1983 was reported by F.A.O. (1985) to be 14895 tonnes, of which 11456 tonnes (77 %) was contributed by South-East Asia, with Indonesia producing 11010 tonnes (from both inland waters and marine) and Malaysia 446 tonnes. In the same year, farmed seabass production in South-East Asia was 2416 tonnes, the producing countries being Indonesia (1105 or 46 %), Thailand (1084 or 45 %), and Malaysia (227 or 9 %) (SEAFDEC, 1985). Singapore's production of farmed seabass was 100 tonnes in 1983, rising to derived from by-catch from brackishwater culture of milkfish. This is also the case for the Philippines, but production statistics are not available. Farmed seabass

are usually marketed at around 500-800 g, while wild-caught ones usually weigh 7 kg or more.

Thailand is well known for its seabass culture as it was here that culture techniques were first developed -in the 1970's (Wongsomnuk and Manevonk, 1973). Culture of this fish has been fairly widespread since then, but, in the past, has been conducted mostly in connection with other types of culture and on a very small scale (Department of Fisheries, 1984). Presently, Thailand produces more than 100 million seabass fry annually (Anon, 1985), and the culture is on a larger scale and done on its own. In 1983, there were 1000 seabass farms in Thailand with a total farm area of approx. 9 ha (Sirikul et *al.*, 1988).

FARMING PRACTISES

Seabass may be farmed in ponds or in netcages, with the latter being more predominant. In the former, seabass is farmed either in brackishwater or freshwater ponds, while, in the latter, it may either be in fixed or floating netcages in coastal waters. Farming of seabass may also be carried out in brackishwater ponds like shrimp ponds, but this is not common, and has been reported only by Department of Fisheries (1984) for Thailand. Even then, this practise is not widespread in Thailand, and is used only by some of the shrimp pond operators there.

Site selection

a) Salinity

Being a euryhaline species, seabass can be farmed either in freshwater, brackishwater, or seawater. In parts of Thailand, seabass is reported to be farmed in freshwater ponds, while in Tahiti, seabass is successfully cultured in seawaters of 35 ppt (Fuchs, pers. comm., 1989). However, the fish is more commonly farmed in salinities ranging from 10-30 ppt. Hence most of the farms are located either along the coast or in coastal waters.

b) Temperature

Seabass grows best in warm waters of 26-32°C, and growth is slowed down under cooler water conditions. Mortalities arise when temperatures drop below 20°C over prolonged periods. Optimum temperature range is reported to be 26-32°C (Kungvanki et al., 1984). Minimum water temperature for seabass was found to be 15°C, beyond which the fish died within minutes (Wu, pers. comm., 1989).

c) Water quality

Other water quality parameters suitable for the rearing of seabass, as given by Kungvankij et *al.* (1984), are pH 7.5-8.5; dissolved oxygen 4-9 ppm; ammonia (NH3-N) < 1 ppm; H2S < 0.3 ppm; and turbidity < 10 ppm. The level of dissolved oxygen is usually high, around 7-8 ppm, in floating netcages due to the continuous exchange of water in the netcages from tidal influx. Other parameters can also be met

with good tidal flow. Wu,(pers. comm., 1989) found that seabass can tolerate dissolved oxygen levels of 1 ppm for short durations of less than 30 minutes.

d) Water exchange

Sites should be located in areas with good water exchange, i.e. having wide tidal fluctuations of 2-3 m and/or strong currents ranging between 1-2 knots (50-100 cm/sec). If floating netcages are used, the nets must be at least 2 m off the bottom so that currents can sweep away the siltation and detrital wastes that accumulate on the seabed. In fixed netcages, the nets should be at least 1 m off bottom. However these are often resting on the bottom resulting in heavy siltation of both net and substrate. Ponds located along the coast or mangrove areas will have placed where water can enter with the flooding tides. A good site is one where tidal exchange can be effected 20 times monthly.

In Singapore, sites for floating netcages are located in a narrow waterway, called Strait of Johor, to take advantage of the fast tidal movements (1-1.5 knots) yet relatively deep (8-10 m- and sheltered (wave heights not exceeding 1 m) conditions, the latter condition to minimize strain on the wooden frames and anchor ropes of the floating netcage. Floating netcages are therefore best placed to take advantage of the features in the topography and hydrography of various sites, as opposed to more permanent establishments like ponds.

e) Soil and other characteristics

This criteria does not apply directly to the sitting of netcages, since coastal seabeds usually consist of marine clay. However netcages can be located in areas with sandy bottoms. For sitting of earthern ponds, the soil at the proposed pond site should be loamy, with sufficient clay content to ensure good water holding capacity. Brackishwater ponds are usually located at sites similar to those of shrimp ponds, i.e., in coastal mangroves and close to the sea, and the problem of acid soil, frequently encountered with mangrove soils, may also arise.

Farm sites should be away from possible sources of pollution, e.g. sewage and/or industrial discharges. The incidence of biofouling organisms should also be assessed, as a high rate of biofouling would require considerable net cleaning effort.

Design of culture structure

a) Ponds

Earthern ponds are used, and these are usually rectangular in shape, ranging in area from 800 m^2 to 2 hectares. Pond depth is about 1-2 m. According to Department of Fisheries (1984), ponds in Thailand are of 2 categories : the small ponds of 800-1600 m^2, and the large ponds of 0.3 ha and more. The pond may be built by excavation or by having the dikes above ground. Each pond is provided with an inlet and outlet gate to facilitate water exchange. Pond bottom is flat and slopes towards the outlet gate.

b) Fixed/floating netcages

Generally, the fixed/floating netcage farm comprises several units of netcages suspended from a floating raft and anchored to the seabed, as in the case of floating netcages, or several units of netcages tied to wooden or bamboo poles implanted into the seabed, as in the case of fixed netcages. In Singapore and Malaysia, all netcage farms are floating ones, with size (in terms of area occupied by the netcage) ranging from 40-1600 m2. Fixed netcage farms are seen in Thailand's Songkhla Lake, although this method is said to be not as popular as pond or floating netcage culture because of the difficulty of finding suitable production sites (Sirikul et *al.*, 1988).

The raft frame may be constructed of wood, bamboo or galvanised iron. Although bamboo is frequently cheaper in those countries with a ready source of the material, it can last only for a short period -about a year in Malaysia (Hussin, 1987), and wooden or galvanised iron frames, which are longer lasting, are used. In the Singapore experience, wooden frames, with adequate maintenance, can last for at least 5 years. Galvanised iron pipe rafts could be protected by a coat of anti-corrosive paint to extend their life-span. All the rafts described are kept afloat by polystyrofoam blocks, plastic or metal drums.

Netcages are frequently made of polyethylene material. Square netcages are used in most cases. The size may range from 3 m x 3 m, as commonly used in Singapore and Malaysian farms, to 5 m x 5 m and 10 m x 10 m, as used in some Singapore and most Thai farms. Netcages for floating netcage culture usually have depths ranging from 2-3 m, with 1.5-2.5 m immersed in water. Those that are fixed are deeper, being usually 4-5 m deep, so that the top 1 m stands above the water surface at high tides. Nets are of various mesh sizes, depending on the initial size of the stocked fish.

Anchors are usually placed at the 4 corners and mid-positions of the raft, with length of each anchor rope usually 4 times the depth of water at the site during high tide. The anchors consist of concrete blocks or cast iron ship anchors.

Stocking

a) Shrimp ponds

Seabass may sometimes be cultured in shrimp ponds. The seabass juveniles are stocked after the shrimps have been harvested. Shrimp ponds range in size from 0.3-20 ha. Stocking size is about 1-2 cm body length, and stocking density about 1 per m2. There is no record of unit production levels for such culture.

b) Brackishwater ponds

For culture in brackishwater ponds, the culture may either be monoculture or polyculture, the latter being done in combination with a forage fish, like tilapia (*Oreochromis mossambicus* or *O. niloticus*), whose juveniles serve as food for the seabass.

Stocking size and density depend on the category of pond used. For small ponds (0.08-0.16 ha), seabass juveniles of 10-15 cm (about 20 g) are stocked at 0.5-1 per m^2 (0.4-0.75 per m^3), while larger juveniles of 20-30 cm (about 100 g) are stocked at 0.25-0.5 per m^2 (0.2-0.4 per m^3) for the larger ponds (> 0.3 ha). For polyculture, seabass juveniles are stocked at 10-15 cm size at 0.3-0.5 per m^2 (0.2-0.4 per m^3) and the ponds would have to be stocked with tilapia brooders at 0.2 per m^2 (0.1-0.2 per m^3) at sex ratio of 1 :1, about 2 months prior to the stocking of the seabass juveniles to produce the tilapia juveniles (Department of Fisheries, 1984).

An experiment by Rayong Brackishwater Fisheries Station, Thailand, in 1983 showed that ponds initially stocked at 2 per m^2 (1.3 per m^3) with 20 g frys could reach a unit production of 1.3 kg/m^3/yr (Sakaras, pers. comm., 1986) compared to 0.3 normally reached in small ponds practising monoculture (Department of Fisheries, 1984).

c) Fixed/floating netcages

In Thailand, stocking density in cages is initially between 40-50 per m^3 for the first 2-3 months, and thereafter reduced to 10-20 per m^3 (Kungvankij et *al.*, 1984). In Singapore, similar stocking protocol is followed : initially at 40-50 per m^3 from 20-100 g, then to 33 per m^3 from 100-700 g. Some farmers practise a second thinning to 27 per m^3 at 300 g.

An even higher initial stocking was tested by Sakaras (1987) in Thailand. Using experimental netcages of 1.3 per m^3, he demonstrated that initial stocking can be significantly increased to 77 and even up to 231 per m^3 with high survivals. His studies showed that unit productions of 71 and 196 kg/m^3/yr could be achieved at 77 and 231 per m^3 stocking respectively, as compared to 20-24 and 27 kg/m^3/yr unit production levels achieved by commercial farmers in Thailand and Singapore respectively. The study also showed that stocking should be done with larger-sized juveniles (16 cm or 60 g) as this gave higher final mean weight than those of smaller-sized juveniles (12 cm or 22 g).

Feeding

a) Shrimp ponds

The seabass frys are not given any supplementary feed and they rely solely on the natural food (mixture of young shrimp and fish) in the pond.

b) Brackishwater ponds

The fish cultured under monoculture are fed according to the same protocol in use for those cultured in netcages, and the feeding regime will be mentioned hereunder (c). Those raised under polyculture with tilapia feed on the tilapia frys produced by the tilapia brooders stocked in the same pond as the seabass. In some cases tilapia is raised in separate ponds and the frys are collected as food for the seabass (Department of Fisheries, 1984).

c) Fixed/floating netcages

The fish cultured under monoculture conditions rely mainly on

supplementary feed provided by the operator, this being in the form of chopped trash fish. In Thailand, feeding rate of 8-10 % of body weight is applied for fish less than 100 g, 5 % for fish > 100-600 g, and 4 % for fish > 600-1,000 g (IBID). In Singapore, feeding regime may be practised as following : 10 % of body weight for fish of 20-< 100 g, 8 % for fish of 100-< 300 g, 3-5 % for fish of 300-< 500 g, and 3 % for fish of 500-700 g. In all instances, the feed is usually given twice daily, in the morning and afternoon, till satiation each time.

The use of artificial feeds, like semi-moist feeds and dry pellets, is still mainly restricted to the experimental level, although some farmers have been reported to have used semi-moist feeds by incorporating a dry mash of fish meal, rice bran, etc..., to minced trash fish.

Diseases

Commonly encountered diseases are described by Ruangpan (1987), and Chong and Chao (1984). Three major diseases encountered are : Cryptocaryoniase caused by the ciliate *Cryptocaryon irritans*, Vibriosis caused by the bacteria *Vibrio* spp., like *V. parahaemolyticus* and *V. alginolyticus*, and Lymphocystis caused by the virus *Lymphocystis*.

Economic returns

The Cost Benefit Analyses for various farming methods in Thailand and Singapore have been investigated. Sale price (ex-farm) of seabass in Thailand is about US$ 2-3/kg. In Singapore, the fish fetches a premium price when sold live or freshly killed, with a sale price of US$ 6/kg. According to Kungvankij et al. (1984), a pond culture system in Thailand producing 14 tonnes/annum requires a working capital of US$ 22700, and would cost US$ 2.40 to produce a kilogramme of fish, while a floating netcage system producing 8 tonnes/annum requires a capital of US$ 12500, and cost of production would be US$ 2.30/kg. For a smaller floating netcage farm able to produce 0.35 tonnes/annum, Tookwinas and Charearnrid (1988) estimated working capital to be US$ 360 and cost of production to be US$ 1.50/kg. Pollock and Quinn (1984) calculated that a fixed netcage system producing 12 tonnes/annum requires US$ 9500 for working capital, and cost of production would be US$ 1.10/kg. In Singapore, a floating netcage farm able to produce 30 tonnes/annum would require a working capital of US$ 149700 and cost of production would be US$ 4.30/kg. Through large-scale farming, e.g. using floating raceway or semi-submersible netcage systems recently developed in the Nordic countries, cost of production, under Singapore conditions, could be reduced to US$ 3.10/kg. Such systems would be able to produce, theoretically, 200 tonnes/annum. However substantial investment of US$ 1.15 mil. as working capital is required.

CONCLUSION

Seabass farming has come a long way since the early 70's when its culture was confined to Thailand and Indonesia. Since then the species

has been identified as a potential aquaculture species, and farming activities have spread over many of the South-East Asian countries, like Malaysia, Singapore, and Philippines.

However several limitations to its widespread culture remain.

1) The species is not commonly farmed in the region. Some countries, like the Philippines, have just introduced the farming of this fish, and the farmers need to develop a hands-on experience of the farming methodology. In others, like Indonesia, the farmers need to acquire the hatchery technique of producing the frys as the critical shortage of seed has been identified as a constraint to seabass culture in that country (Ismail and Danakusumah, 1987).

2) The species is not tolerant to prolonged cold water conditions below 20°C. This seriously limits its culture in countries with cold seasons to the warmer seasons only, thereby decreasing the economic viability of farming this species. Seabass cultured in prolonged cold water conditions also grow more slowly as they become off-feed.

3) The high cost of trash fish that is used as feed for the seabass cultured is another constraint. Cost of trash fish is the single most expensive item of the cost of production, comprising about 40 %. Any increase in the cost of the trash fish would significantly affect the cost of production.

4) The farming is presently confined to a small-scale level, each farm producing no more than 50 tonnes annually. To match higher market demand, it is possible to scale-up the production with the use of larger netcages, like those used in European countries. However, for such a farming, the use of dry feed for feeding the fish is absolutely essential. This is however not presently commercially available.

Future research and development work on seabass should therefore be directed towards resolving some of these limitations.

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Review of knowledge on Grouper aquaculture in South East Asia

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Abstract — *The grouper is a popular marine food fish of high market value in Southeast Asia. Groupers have been farmed in netcages in coastal water for a longtime. The species which have been reared in tropical countries are estuarine grouper, **Epinephelus malabaricus**, black spotted grouper, **E. salmoides** and brown spotted grouper, **E. tauvina**. Some countries, like Indonesia and Singapore have attempted to rear leopard grouper, **Plectropomus leopardus**. In southern part of China and Japan the red spotted grouper, **Epinephelus akaara**, have been cultured in floating netcage.*

*Induced breeding trials for **E. tauvina** have been conducted since 1977 in Singapore. The induced spawning by hormone injection have been reported successful in Singapore, Kuwait and Thailand. However, the nursing of fry up to fingerlings are still under experiment. Therefore, uncertain supply of fingerlings from the wild has been the main problem for fish culture through out the region.*

INTRODUCTION

The grouper is a popular marine good fish in many parts of the world such as in Kuwait, Indonesia, Malaysia, Thailand, the Philippines, Hong Kong, Taiwan, Republic of China, Japan and Mexico. Their habitat are coral reefs and stony environment. Majority of groupers belong to the genus *Epinephelus* (Brais, 1987).

The grouper is characterized by an oblong body usually with spots and blotches and having a very large mouth (Brais, 1987). A highly carnivorous organism, it feeds on fish, crustaceans and cephalopods.

Some species of grouper such as estuarine grouper, *E. malabaricus*, black spotted grouper, *E. salmoides*, brown spotted grouper *E. tauvina*, red grouper *E. morio* and red spotted grouper *E. tauvina*, red grouper *E morio* and red spotted grouper *E. akaara* have been found to be suitable for intensive cage culture in coastal water (Chen et *al.*, 1977, Kohno et *al.*, 1988, Brais, 1977 and Tookwinas et *al.*, 1988). The culture of grouper

can be conducted both in cages and pond. However, cage culture is more popular than pond culture in many countries. The major constraints to its large-scale development are, however, the shortage and uncertain supply of fingerlings from the wild.

Artificial breeding has been done in many countries, such as in Singapore, Thailand, Kuwait and Japan. The hatchery techniques are still under experiment. However, the research efforts have therefore been directed at larval rearing techniques aimed at achieving self-sufficiency and independent supply of fingerlings.

CULTURE SPECIES AND CULTURE METHODS

Culture species

Grouper have been cultured in Southeast Asia for more than 10 years. *Epinephelus tauvina* was the first recorded species for culturing in Kuwait, Singapore and Thailand, while, *E. salmoides* have been cultured in Penang, Malaysia (Chua, 1978). At present, many species of grouper have been cultured in some Asian countries (Table 1). However, only *E. tauvina*, *E. salmodies* and *E. malabaricus* have been cultured in commercial scale in Southeast Asia and middle east. *E. akaara* have been cultured in Japan and China (Tseng and Foon, 1983).

Tab. 1. — Species of groupers cultured in some countries in Asia

SPECIES	COMMON NAMES	COUNTRIES	REFERENCES
1. - *Epinephelus*			
E. malabaricus	Black spotted grouper	Thailand Philippines	Tookwinas et al., 1988
E. salmoides	Estuarine grouper	Malaysia Thailand	Chua and Teng (1978)
E. tauvina	Brown spotted grouper	Singapore Kuwait	Chen et al., 1977
E. akaara	Red spotted grouper	Japan Hong Kong China	Tseng and Poon (1981)
E. amblycephalus	White-spotted green grouper	Hong Kong Philippines	Tseng and Poon (1983) Kohno et al., 1988
E. bleekeri	Yellow-spotted grouper	Philippines Hong Kong, Thailand	Kohno et al., 1988 Personel information
2. - *Plectropomus leopardus*	Leopard grouper	Indonesia, Singapore	
3. - *Cromileptes altivelis*	Hump-backed Grouper	Thailand	Pakdi et al., 1985

Culture methods

Cage culture has been practised in many countries such as Thailand, Malaysia, Singapore, Philippines, Indonesia and Hong Kong, while the

pond culture has been reported to be practised in Philippines (Kohno et *al.*, 1989). Cage culture has some advantages (Tookwinas and Charearnrid, 1988) which include following :

- cage culture are usually set in sites with better aquatic environmental condition. Therefore, cages can be stocked with more fish than ponds,
- the cost of cage preparation is much more cheaper than the cost of pond construction,
- cage culture would not need water changing and elaborate preparation, which makes the cage culture operation less costly than pond culture.

SEED PRODUCTION TECHNIQUES

Broodstock development

Grouper broodstock would be ready to spawn at an age of approximately 3 years (3.0-4.0 kg in body weight) (Ruangpanit et *al.*, 1988). Grouper is a protogynous hermaphrodite i.e. it matures as a female but transforms into a male when it grows bigger and older (Chen et *al.*, 1977). Therefore, male grouper can be obtained by accelerating the process of sex-reversal of 3 years old female through oral application of methyltesterone at the dosage of 1 mg/kg for a period of about 2 months (Rungpanit et *al.*, 1988 and Ratanachot and Pakdee, 1986).

Tab. 2. — Summary of hormonal treatments for induced spawning of grouper

SPECIES	FIRST DOSES	FINAL DOSES	INTER-VAL PERIOD (HRS)	REFERENCES
E. tauvina	500 IU HCG + 3 mg P.G. (female)	1000 IU GCG + 3mg P.G.	24	Kungvankij et *al.*, 1986
	500-1000 IU HCG + 50-150 mg P.G.*	—	12-24	Chen et *al.*, 1977
E. malabaricus	400-500 IU HCG + 2 mg P.G. (female)	800-1000 IU HCG + 4 mg P.G.	24	Chulavitayanuko et *al.*, 1985
		500 IU HCG + 2mg P.G. (male)	—	Chulavitayanuko et *al.*, 1985
E. salmoides	1 IU HCG (female)	—	—	Huang et *al.*, (1986)

* 1. The number of injections and timing of the final injection depended on the response of the fish to initial treatment.
2. Pituitary gland extracts were usually given with HCG in the final injection.

Induction of spawning

Induced spawning by environmental manipulation is now mostly employed. The technique includes controlling the feeding at 1-2 percent of

total body weight; the feeding is done once a day in the afternoon. The sea water is daily changed approx. 30-50 percent (Ruangpanit et *al.*, 1988). In the past, induced spawning by hormonal injection and artificial fertilization were conducted (Table 2).

The parent fish can spawn naturally in captivity for several days continuously during both lunar phases, full and new moon, a month, and every month from November to April of the following year. The hatching rate obtained at the National Institute of Coastal Aquaculture (NICA) was 57.16 % in 1988 (Rungpanit et *al.*, 1988).

Embryonic development and larval rearing

The hatching mechanism, embryonic development and larval rearing have not been studied in detail. The techniques for larval rearing of seabass have been applied, however, larval rearing techniques are under experiment. Only one crop of fry survived in the NICA hatchery in 1988. The average survival rate of 50-day old grouper fry was only 2.2 % (Rungpanit et *al.*, 1988).

FLOATING CAGE CULTURE OF GROUPER

Site selection

Floating cage should be set up in clam water, e.g. in a bay, behind an island or at a river mouth. This is to avoid damage caused by strong waves and current. The criteria for selecting a suitable site for cage culture of grouper (Tookwinas et *al.*, 1988 and Kohno er *al.*, 1988) are the following :

- salinity. This should be of range, 20-32 ppt. However, a site salinity of more than 10 ppt can be suitable for culture in the Philippines (Kohno et *al.*, 1988),
- tide and water depth. Water depth should be more than 2-3 metres. This is due to the usual size of culture cage which is 5 m x 2 m and 2 m deep. The tidal fluctuation should allow the water depth to be at least 2 meters at the low water of spring tide,
- current and waves. Area should be protected from strong winds, waves and current. An ideal area would be a protected bay, sheltered cove or inland sea,
- water quality. The site should be relatively free from domestic, industrial and agricultural wastes and other environmental hazards,
- water circulation. The site should have enough water circulation to improve on poor water quality that could occur at some period in the culture due to the decomposition of waste material which often accumulate beneath the net cage.

The water quality parameters which are considered of minimum range for cage culture are shown in Table 3.

Tab. 3. — The suitable water quality for cage culture of grouper (Tookwinas et *al.*, 1988)

PARAMETERS	RANGES
pH	7.5-8.3
Dissolved Oxygen	4.0-8.0 mg/l
Water salinity	20-32 ppt
Water temperature	26-32°C
Ammonia - nitrogen	less than 0.02 mg/l
Hydrogen sulfide	none
Current	normal

Material for cage preparation

Galvanized iron (GI) or wooden parts are used for the cage frame in Thailand, Singapore and Malaysia. The cage is kept afloat by styrofoam drum, plastic carbuoy or bamboo. In Philippines, wooden parts are used for the frame. Styrofoam drum, plastic carbuoy or bamboo are also used for supporting the cage frame (Kohno et *al.*, 1988).

Cage is usually 5 × 5 × 2 m in Thailand (Tookwinas et *al.*, 1988). However 3 × 3 × 3 m are also used in the Philippines (Kohno et *al.*, 1988).

Stocking density

At present, grouper fry are collected from the wild for culturing. The fry at size of 7.5-10 cm are usually collected by fish trap from coastal water near magrove aeras. The fry should be stocked in nursery cage. Stocking must be done separately for each size group. This is to avoid the cannibalistic behaviour of the species.

The stock density up to the marketable size varies from 10 to 100 fish per m^3 (Tookwinas et *al.*, 1988; Sakaras and Kumpang, 1988 and Tanomkiat et *al.*, 1987). However, the actual stocking density varies. This is due to insufficient supply of the fry.

Feeding

Grouper is a carnivorous and voracious fish taking live fish and crustaceans as food (Kohno et *al.*, 1988). However, it is not difficult to train the grouper to feed on trash fish. For first two months of culture, feeding rate is 10 percent of body weight. After that, it can be reduced to about 5 percent of body weight (Kungvankit et *al.*, 1986).

Supply of trashfish is always insufficient and expensive in some seasons and areas. The artificial diets can be recommended for feeding. It is easy to train the grouper to feed on artificial diet (Tanomkiat et *al.*, 1987). The growth rate is similar to the fish fed on trashfish (Table 4).

Tab. 4. — Growth of grouper at different stocking densities in cages (Sakaras and Kumpang, 1988 and Tanomkiat et *al.*, 1987)

CULTURE PERIOD (Days)	STOCKING DENSITY	
	$58/m^3$ *a	$100/m^3$ *b
0	83.7	26.9
30	158.7	45.6
60	186.5	65.9
90	243.9	98.7
120	283.7	137.0
150	296.8	217.1
180	355.8	312.4
210	433.9	387.6
250	/	586.6

* a : fed with trashfish
* b : fed with artificial diets.

Marketable size and rearing period

Marketable size fish varies from 0.5 to 1.3 kg. In the Philippines, marketable size fish of high demand ranges from 0.5 to 1.0 kg. In Thailand, 1.3 kg fish are usually exported live by air to Hong Kong.

Fish cultured in net-cage can reach 586.6 g in 8 months of culture period (Table 4).

POLYCULTURE OF GROUPER AND OTHER FISH

Polyculture of grouper and tilapia have been reported in Philippines (Manzano, 1985 and 1986). A ratio of 1 grouper to 20 tilapia proved to be the most effective in earthern pond. Grouper yield is greater since they fed on tilapia fingerlings.

The basic construction of the polyculture pond is similar to milkfish or prawn ponds. A suitable site with salinity higher than 10 ppt is preferred. However, feeding techniques, water changing management, growth rate and food conversion ratio (FCR) should be studied in more detail.

MARKETING

Grouper is more expensive than most other fish species in Thailand. The local demand is rather limited. At present, production from cage culture in Thailand is exported live by air to Hong Kong. The demand is year round. Therefore, the income from grouper could be more than from other species (Tookwinas, 1988). In Singapore, the production from cage culture is only sold live in the local market (Teo and Wah, 1988).

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Biological and economic factors in the selection of cultured fish species and the development of a bio-economic model

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Abstract — *The selection of candidate species for mariculture is determined by a number of biological factors, e.g. foraging efficiency, growth efficiency, mortality, grow-out period and susceptibility to environmental fluctuations, disease and crowding, as well as economic factors e.g. the supply and price of fry, grow-out cost and price of adult. A computer model incorporating these major factors is being developed, to determine and compare the potential of different culture species. Results of environmental tolerance experiments on 16 marine fish species revealed that some species, e.g.* ***Mylio macrocephalus****, are euryhaline and may be cultured in areas with fluctuating salinities. Other species, e.g.,* ***Epinephelus tauvina*** *and* ***Lates calcarifer*** *are hypoxia tolerant and can be cultured in waters where oxygen depletions may occur. These two species are, however, particularly sensitive to cold and may have over-wintering problems. The application of environmental tolerance data in selecting appropriate candidate species to suit different hydrographic conditions at different culture sites is demonstrated.*

INTRODUCTION

Selection of appropriate species is one of the most important factors in determining the success of fish farming. The desirable economic and biological attributes of a cultured species are given in Table 1. Economically, the price of fry and grow-out costs should be relatively low while adults should be well received by the market and fetch a high price. The supply of fry should be stable and non-limiting. Biologically, cultured species should be able to withstand prevailing environmental fluctuations, and have high growth and foraging efficiencies, low mortality, a short grow-out period, and low susceptibilities to crowding and pathological infections. The biology of the cultured species, especially with regard to

nutritional requirements, disease diagnosis, prevention and treatment should be well known, although such data are generally not available for most farmed tropical species. This paper elaborates upon the rationale for species selection in Hong Kong, which is largely based on the fish environmental tolerances and comparisons of profit return, using a bio-economic model.

Tab. 1. — Desirable biological and economic attributes of cultured species

BIOLOGICAL ATTRIBUTES	
Foraging efficiency	High
Growth efficiency	High
Tolerance to environmental changes	High
Susceptibility to disease and crowding	Low
Grow-out mortality	Low
ECONOMIC ATTRIBUTES	
Supply of fry	Unlimited and steady
Price of fry	Low
Grow-out period	Short
Grow-out cost	Low
Price of adult	High

SPECIES SELECTION BASED ON ENVIRONMENTAL TOLERANCES

The first pre-requisite for a successful cultured species is the ability to withstand and grow under environmental conditions prevailing at the culture sites. This is particularly important in regions with marked seasonality and/or in estuaries, where cultured fish are often subjected to wide temporal and spatial environmental fluctuations. The paucity of data on environmental requirements and tolerances of marine fish, particularly warm water species, however, often leads to selection based on experience rather than scientific data.

In Hong Kong, all marine fish farming activities are carried out in 28 designated fish culture zones, amongst and within which, spatial and temporal variations in hydrography are large (Fig. 1). Fish culture zones in the western approaches, for example, may be subject to large annual fluctuations in salinity (30 ppt in winter and 7 ppt in summer when discharges from the Pearl River is maximal). Annual water temperature ranges from 12°C to 32°C in all fish culture zones, and fluctuations are particularly marked in waters less than 3 m deep (depths above the thermocline). Dissolved oxygen levels at the culture zones generally vary from 3 to 8 mg $O_2 l^{-1}$, and in zones where waters are eutrophic or organically enriched, oxygen depletions (< 1 mg $O_2 l^{-1}$) caused by algal blooms and red tides may occasionally occur, leading to fish kills.

In view of the above problems, a series of environmental tolerance experiments have been carried out for 16 commonly cultured species (Woo & Wu, 1982; 1984; Wu & Woo, 1982, 1984; Wu, 1988), in order to provide a scientific basis for selecting species best suited to the particular environmental conditions at different sites.

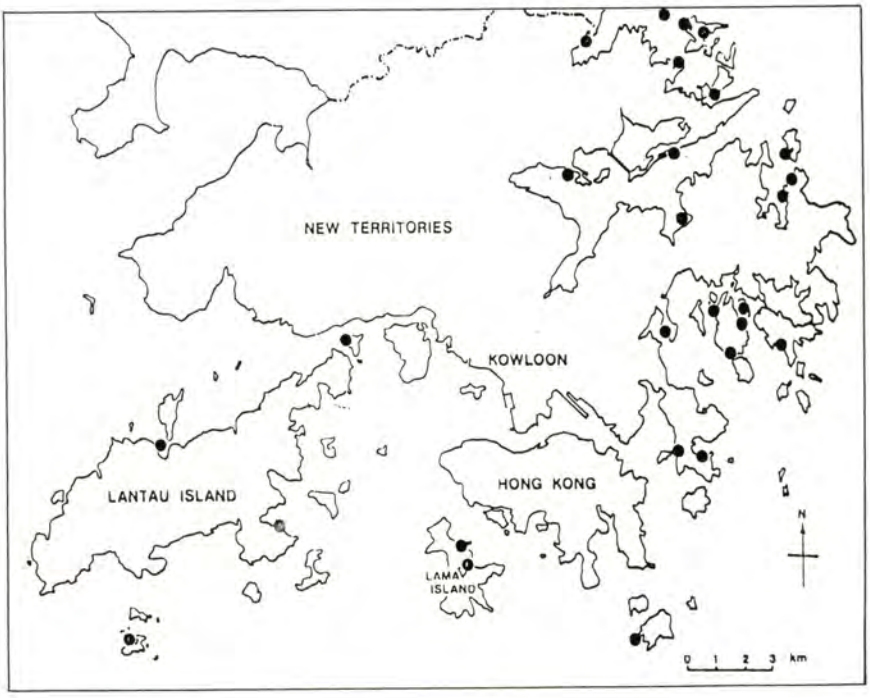


Figure 1. — A map showing the distribution of the 28 fish culture zones in Hong Kong and general hydrographic conditions.

The mortality, behavioural changes and physiological responses of nine species of marine fish under various levels of hypoxia were compared (Table 2). Large variations in tolerances between different species were found, and some species exhibited remarkable tolerances to hypoxic stress. For example, *Epinephelus tauvina* and *Lates calcarifer* survived without symptoms for 7 hours when subjected to 1 mg $O_2\,l^{-1}$, and only 10 % mortality was found for the former species at 0.5 mg $O_2\,l^{-1}$. In contrast, *Chrysophrys major* and *Rhabdosarga sarba* exhibited abnormal behaviour, followed by death, within minutes when subjected to the same level of hypoxic stress. The above laboratory findings are further supported by fish kill statistics in Hong Kong : *C. major* and *R. sarba* always suffering heavy losses in fish kills caused by hypoxia, while other species are normally affected to a lesser extent. Conversely, *L. calcarifer* and *E. tauvina* which have never been implicated in any fish kills due to oxygen depletions, showed a high tolerance to hypoxia in the laboratory (Wu, 1989).

Tab. 2. — Time (in min.) for 50 % of various experimental fish to show mortality (LT_{50}) and abnormal behaviours (BC_{50}). For mortality/behavioural changes ocurring in less than 50 % of the population throughout the experimental period, actual mortalities are shown in brackets (After Wu, 1989)

SPECIES	0.5 mg O_2 l^{-1}		1.0 mg O_2 l^{-1}	
	BC_{50}	LT_{50}	BC_{50}	LT_{50}
Chrysophrys major	TQC	TQC	20	60
Rhabdosarga sarba	TQC	TQC	130	225
Siganus oramin	TQC	7	10	177
Lutjanus ruselli	TQC	14	76	(30 %)
Epinephelus akaara	70	92	110	160
Mylio macrocephalus	30	100	NBC	NM
Epinephelus awoara	150	200	190	270
Lates calcarifer	373	393	NBC	NM
Epinephelus tauvina	(10 %)	(10 %)	NBC	NM

* TQC = Too quick to count NBC = No behavioral changes
NM = No mortality

Detailed biochemical studies on *Ephinephelus akaara* and *Mylio macrocephalus*, respectively exposed to hypoxic conditions of 4.0 to 2.5 mg O_2/l revealed no increases in serum and tissue lactate, and only slight changes in other tissue metabolites and electrolytes indicating that they obtain enough oxygen to prevent anaerobiosis under such regimes (Woo and Wu, 1984).

The overall results therefore suggest that hypoxia tolerant species such as *Lates calcarifer* and *M. macrocephalus* may be cultured in eutrophic waters where oxygen depletions are more likely to occur, while sensitive species such as *C. major* and *R. sarba*, should not be cultured in such environments.

Wu and Woo (1982) showed that ten out of thirteen tested species are euryhaline, and survived without abnormal behaviour and tissue hydration for more than two weeks in salinities > 10 ppt. Further experiments carried out upon *E. akaara* and *M. macrocephalus* showed only transient disturbance of various electrolytes and metabolites at salinities > 12 ppt, suggesting that physiological disturbance is unlikely to occur at salinities above this (Woo & Wu, 1982). It therefore appears that salinities above this regime would not normally limit culture most of these species.

Tolerances to cold (12°C) were compared for 8 species. Experimental results showed that *S. oramin*, *L. calcarifer* and *E. tauvina* are relatively sensitive to cold, while *E. akaara*, *E. awoara* and *M. macrocephalus* are more tolerant (Table 3).

The laboratory findings were clearly supported by fish kill statistics in Hong Kong : a high mortality of the former three species was found during cold spells when water temperatures fall below 15°C for a prolonged period (> 7 days). Contrarily, *Mylio macrocephalus*, *Epinephelus*

Tab. 3. — Time (in min.) for 50 % of experimental animals to exhibit abnormal behaviors (BC_{50}) at 12°C (n = 20). (After Wu, 1989)

Species	BC_{50} (%)	Mortality (%)
Siganus oramin	TQC	20
Lates calcarifer	1	—
Epinephelus tauvina	20	—
Rhabdosarga sarba	50	—
Chrysophyrs major	89	—
Epinephelus akaara	NBC	—
Epinephelus awoara	NBC	—
Mylio macrocephalus	NBC	—

* NBC = No behavioral changes, TQC = Too quick to count.

akaara and *Epinephelus awoara* which showed no abnormal behaviours at 12°C in the laboratory, have never been reported upon in fish kills caused by cold spells. The results therefore indicate over-wintering problems for *L. calcarifer* and *E. tauvina* in Hong Kong. Shortening of the grow-out period by, for example, importing larger fingerlings might minimize the risk of fish kills for these two species in a severe winter.

The upper lethal temperatures of *Chrysophrys major* and *Mylio macrocephalus* were found to be 32°C and 36°C respectively (Woo and Fung, 1980). The low tolerance of *C. major* to high water temperatures suggests that this species is less suitable for culture in shallow waters, i.e. < 3 m, the shallowest thermocline in the coastal waters of Hong Kong, where water may easily be heated up by solar radiation in the summer, to a temperature beyond the lethal limit for this species.

BIO-ECONOMIC MODEL

The ultimate success of fish farming is determined by the economic return which is, in turn, dependent upon a number of biological and economic factors, namely, food conversion ratio, grow-out period, age-specific mortality, price of fry and adult, grow-out cost (labour and feed), etc.... Each of these parameters varies with different species. For example, the price of *C. major* fry is $1 each and 80 % can be expected to grow to marketable adults in 12 months when the market price will be HK $40/kg. The price of fry for *E. tauvina* is much higher (HK $14 each), the grow-out period is longer (18 months) and only 62 % can be expected to grow to marketable adults, but their price is HK $94/kg. The price for *E. akaara* is HK $16 each, the grow-out period is the longest (24 months) and only 58 % can be expected to grow to market adults, but their price is very high (HK $ 135/Kg). The grow-out costs for these three species is also different because of differences in their daily feed ratio, grow-out period and age-specific mortality and growth (Table 4; Fig. 2).

Tab. 4. — Some biological and economic parameters of six common cultured species in Hong Kong

Species	Price of fry ($.no^{-1})	Price of 450g adult ($.kg^{-1})	Grow out period (M)	Survival rate (%)	Daily feed (% body wt)
Chrysophrys major	1	40	12	80	6
Epinephelus akaara	14	135	24	58	4
Epinephelus tauvina	12	94	18	62	5
Rhabdosarga sarba	3	44	18	?	?
Mylio macrocephalus	2	75	24	?	?
Lates calcarifer	3	58	12	?	?

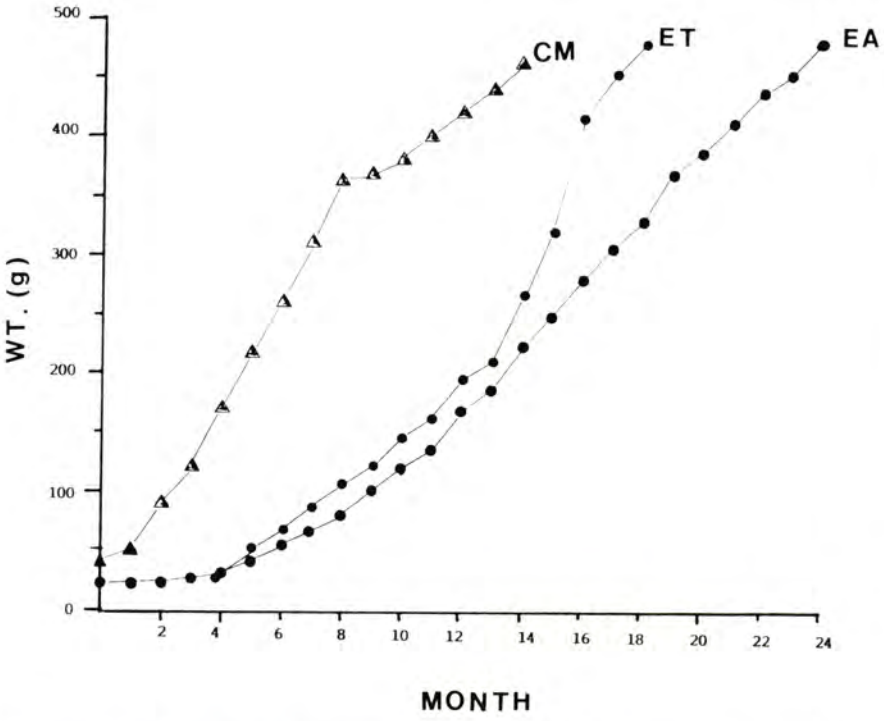
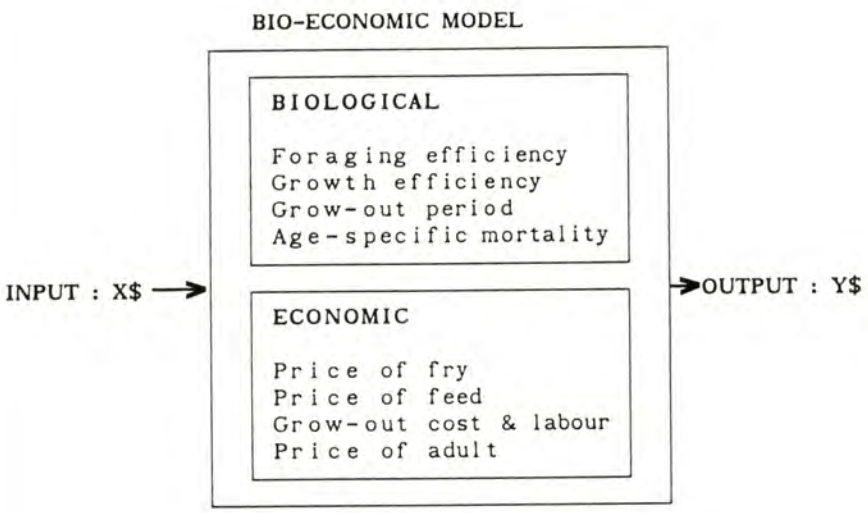


Figure 2. — Age specific growth curves for *Chryophrys major* (CM), *Epinephelus tauvina* (ET) and *Epinephelus akaara* (EA).

A computer model has been developed, in which the above biological and economic factors have been taken into consideration, for computing and comparing the profit return (profit per $ investment per unit time) of different cultured species under varying conditions (Fig. 3). Such an analysis provides a comparison of potential economic return for different species under different conditions, e.g. change in the price of feed, fry or

adult, reduced grow-out mortality, etc... The most profitable candidate species under a particular suite of environmental and economic conditions can therefore be identified. By comparing the profit return of different species under varying conditions, the major limiting factor for culturing each species can also be identified and further research developed to solve the problem.



$$\text{PROFIT RETURN (\%/yr)} = \frac{12\ (Y-X)/X}{\text{GROW-OUT PERIOD (M)}} \times 100$$

Figure 3. — Bio-economic model for determing profit return of different cultured species.

At present, analyses have been carried out for *C. major*, *E. tauvina* and *E. akaara*. For the values of biological and economic parameters given in Table 2 and Figure 3, and for the present feed cost of HK $ 2 Kg, the calculated profit returns for the three species are : - 18.6 %, 33.0 % and 37.1 % per annum respectively. Assuming that the cost of feed (trash fish) increases from $ 2/kg to $ 3/kg, the profit return per annum of the three species will change to : -16.2 %, 12.2 % and 25.1 % respectively, giving a negative profit return for culturing *C. major*. Assuming that the price of *E. akaara* fry increases from $14 to $16 each, the profit return per annum for *E. akaara* will decrease to 29.4 %. In such case, culturing *E. akaara* would then be less attractive than *E. tauvina* because of the lower profit return. Further analyses are being carried out and extended to other species.

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Review and current status of the aquaculture potential for the Mahimahi, *Coryphaena hippurus*

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Abstract — *The Mahimahi, **Coryphaena hippurus**, has excellent potential for aquaculture due to its fast growth (2 kg at 6 months, 9 kg at 1 year), good food conversion efficiency (30 %, wet weight), high fecundity with natural captive spawns (200 000 eggs/female/2 days year round for a 1 year old female), and high price (US$9.00/kg wholesale in Hawaii).*

Using current technology (pelleted feed conversion of 1 :1; feed cost US$1.10/kg; stocking 1.5 fingerlings per m^2) 3 crops a year would yield 85 000kg/ha/yr with a potential profit of US$500 000/ha/yr. The status of two commercial ventures is discussed.

The hatchery methods presented here are adequate for pilot-scale production. Hatchery success depends on meeting relatively fastidious dietary and environmental requirements. Optimum turbulence is higher than expected, and food preference is not a valid indicator of optimal diet. Optimum diet is rotifers, d2-d8; copepods, d6-d21; brine shrimp, d10-d25; newly hatched mahimahi larvae, d18-d40; wean to non-living food, d25-d35.

From first feeding through day 40, larvae gain 20 % body weight per day with a food conversion efficiency of 0.3 at ambient Hawaii temperatures (23 to 26°C).

INTRODUCTION

Mahimahi is an excellent candidate for aquaculture and stock enhancement programmes because of its high price ($5-$12/kg to the fisherman), high demand, foreign trade deficit (90 % comes from Taiwan), decline in catch per unit effort, fast growth rate (4-5 pounds at six months), and good (30 %) food conversion efficiency. The growth rate and food conversion efficiency of mahimahi far exceeds that of the yellowtail jack, *Seriola lalandei dorsalis*, (0.4-0.8 pounds at six months, 12 % f.c.e.) when cultured under identical conditions (Kraul, 1985). Similar jacks, *S. quinqueradiata*, are the most profitably cultured marine fish in the world (Mat-

susato, 1984). Stock enhancement of marine fishes is still in developmental stages, but mahimahi is among the most likely species to succeed (Polovina, 1986). Hawaii's nutrient-poor waters (Hirota et *al.*, 1980) and strong offshore currents probably limit larval survival and recruitment of pelagically spawning fishes. Stock enhancement might have significant impact in this recruitment limited fishery. Mahimahi have a two months hatchery phase to 10 cm FL, and reach minimum market size (2.3 kg) four months after release (Uchiyama et *al.*, 1986). Projected natural mortality over this period is less than 50 %, based on Pauley's (1979) empirical relationships, so chances of successful enhancement are good. If salmon grew as fast as mahimahi (i.e. returned after 4 months instead of 12) but still had the same monthly mortality rate, salmon releasers might get 22 % tag return instead of 1 %. The major limit to beginning enhancement and grow-out studies is the lack of a dependable supply of fingerling mahimahi.

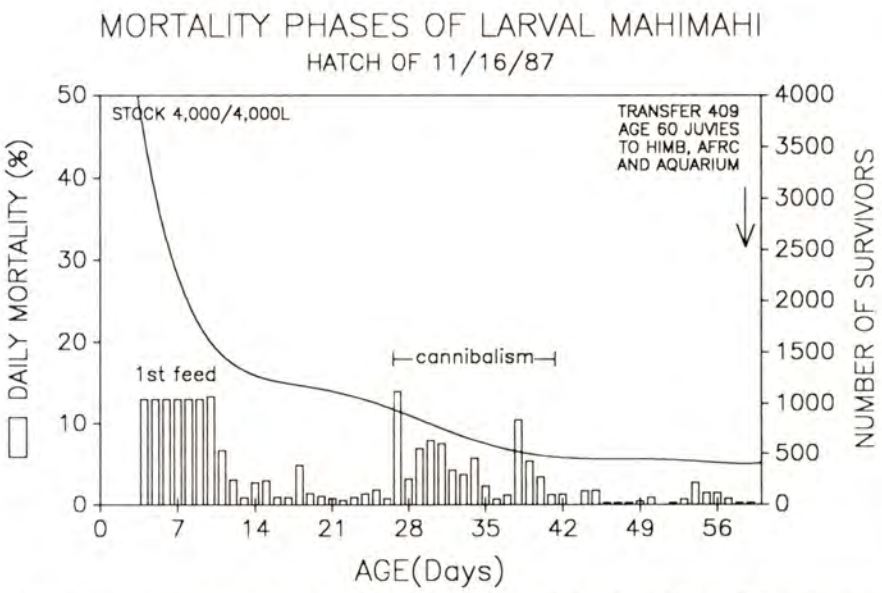


Figure 1. — Typical mortality phases of larval mahimahi, hatch of 11/16/87. Solid curve tracks number of survivors. Vertical bars show daily percent mortality.

BACKGROUND

Since the observation of the mahimahi's frequent natural spawnings in captivity (Soichi, 1978), and the development of techniques for rearing the larvae (Hassler and Rainville, 1975), several projects have reared a small number of fish from eggs through sexual maturity, and discovered good and bad features of this fish. Hagood et *al.* (1981) observed a 33 % wet weight food conversion (equivalent to 0.6 :1 dry food :wet fish) efficiency for juvenile fish. Szyper et *al.* (1984) found that juveniles grew well on a commercially available diet. Schekter (1982) successfully reared 165 F2 juveniles, but experienced inconsistent larval production.

In previous years, research at the Waikiki Aquarium found : survival of yolksac larvae was improved with heavy turbulence; first feeding larvae survived better using rotifers as a first food (despite a preference for copepods); second and third week larvae survived better using copepods instead of rotifers or brine shrimp (Kraul et *al.*, 1989); and postlarval mortality was closely associated with microbial and nutritional changes during weaning from live plankton.

We currently obtain about 20 % hatchery survival to day 30, and 10 % survival through day 6 (Figure 1). By 60 days, juveniles are capable of consuming chopped squid or pellets, and probably large enough for ocean release trials. Hatchery yields of 10 % (one 60-days juvenile per 10 litres) are adequate for testing pilot scale tagging and growout economics, and represent state-of-the-art technology (the « industry » standard is 1 %). Higher yields are being tested for stock enhancement and profitable aquaculture. Our tests at higher stocking densities are compromised by limited amounts of our reference diet, i.e. copepods and yolk-sac mahimahi. Unfortunately, successful weaning of juveniles onto nonliving foods presently requires access to these live plankters from days 6 to 30.

CURRENT HATCHERY PROBLEMS AND RESEARCH

To improve hatchery production, we are attempting to find alternate foods, and learning to diagnose and control diseases. Our successful diet demonstrates that we can achieve nutritional adequacy with live foods, but we need an effective pelletized food in order to reduce hatchery costs and make production more dependable. We also need more studies of bacterial pathogenicity.

In our tests to date, mass cultured brine shrimp has not supported good larval survival, possibly due to a lack of appropriate HUFAs (highly unsaturated fatty acids). The hatchery yield limit imposed by copepod culture (days 6-25) and yolksac larva production (days 18-30) might be bypassed by using *Artemia* spp. (brine shrimp) nauplii enriched with nutritional supplements. This method is useful for other fishes (Gatesoupe, 1982), and is being tested on mahimahi at the Aquarium and other facilities.

Non living foods are often rejected by mahimahi less than 30 days posthatch, and those we have tested appear to be nutritionally deficient. We obtain good survival by avoiding weaning from live foods until day 30. Use of yolksac mahimahi as a food for postlarval mahimahi from day 18 to 30 has been very successful, but depends on a large and consistent supplies of mahimahi eggs. We have not tested this method sufficiently to determine its cost in mass culture. With our successful live diet as a reference, we are defining the nutritional requirements of young mahimahi. Professor Harry Ako, University of Hawaii Agriculture Biochemistry Department, is measuring fatty acid, amino acid, and phospholipid profiles of our successful live diet. These profiles are then used to synthesize pellets. These foods are then tested for ingestion response (the limiting factor in all foods tested so far) and survival. Preliminary results are

encouraging. As shown in Figure 2, and Table 1, some pelleted foods promote survival and growth of mahimahi juveniles. Both Noraqua and Southern Sea Farms are presently using fish-meal-based pellets with some success.

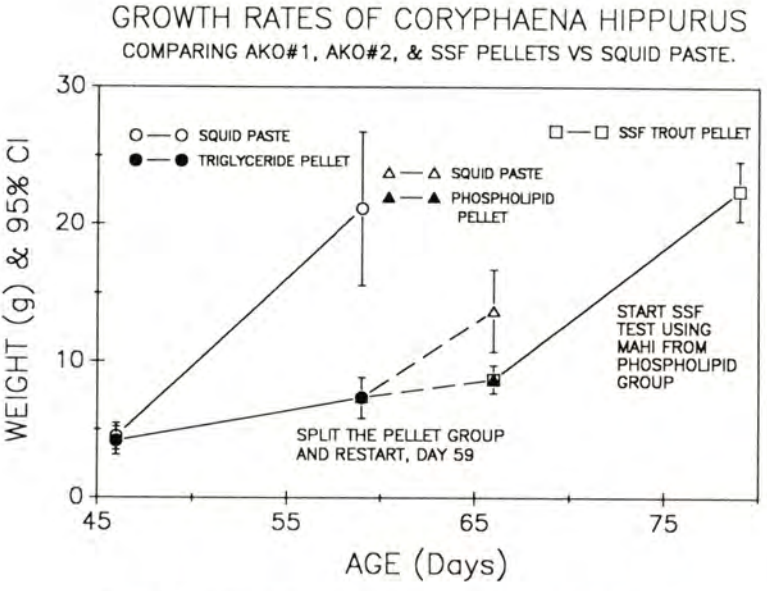


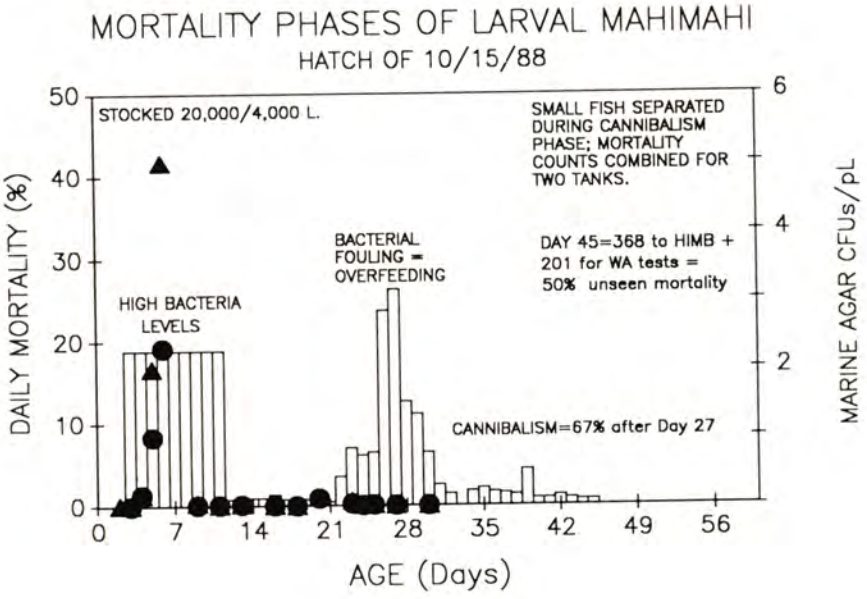
Figure 2. — Growth rates of mahimahi, using pelleted feeds.

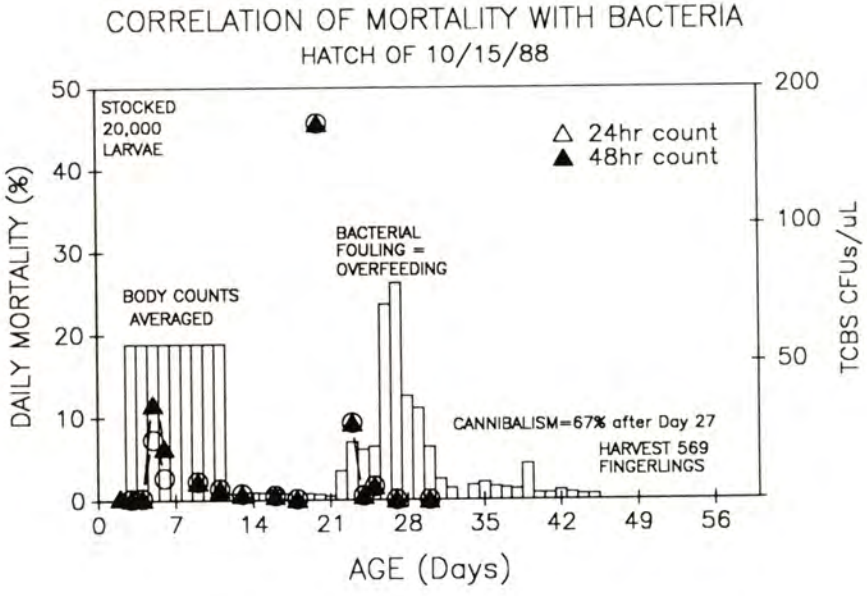
Tab. 1. — Growth rates of *Coryphaena hippurus*, comparing 3 trial pellets versus squid paste. See Figure 2 : preliminary conditions were not equal for all tests

	SQUID	AKO≠1	AKO≠2	SSF TROUT
Percent survival	86	76	64	90
Daily % Wt. gain	12.6	4.2	0	7.6
FCE	.21 w/w	.11 w/d	−.02	0.62 w/d
FCR	4.8 w/w	9.3 d/w	−58	1.6 d/w

Bacteria significantly affect larval survival. Our most successful rearing method specifies Prefuran treatment of all live foods. All of the live foods we use successfully now were unsuccessful for five years. We attribute our recent successes to prophylaxis and antibiotic treatment. We have isolated one bacteria type (*Pasteurella* sp.) associated with mortalities, discovered its antibiotic sensitivity (it is Prefuran resistant), and partly tested therapeutic responses of antibiotics. Vibrio species occasionally correlate with larval mortality, and it is quite likely that other bacteria are also pathogenic. Our goal is to prevent disease outbreaks with good

hygiene, and to be able to respond to disease organisms with specific antibiotics when necessary. Larval mortality does not always correlate with bacterial density (Figure 3a, b, c). However, some mortalities do correlate, and non-nutritional disease is the only theory that explains the variability in our mortality during the past 6 years (Figure 4).





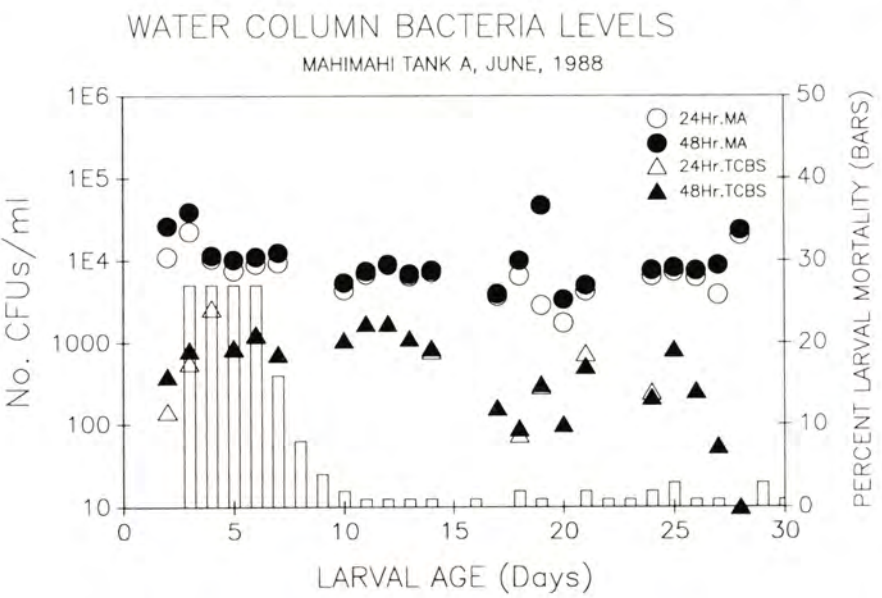


Figure 3. — 3a. Mortality phases of larval mahimahi.
3b. Correlation of larval mortality with suspended bacteria.
3c. Water column bacteria level.

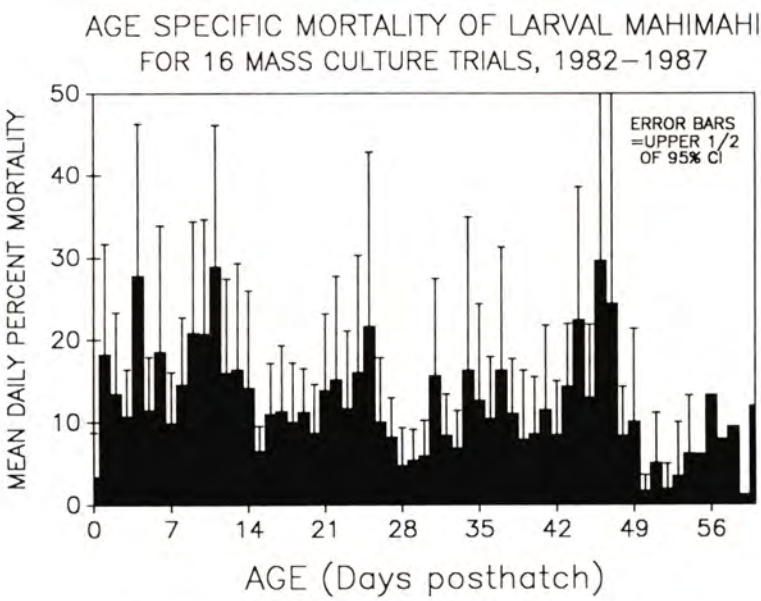


Figure 4. — Variability in larval mortality with age.

HATCHERY METHODS

The following material is condensed from the CRC Handbook of Mariculture, Volume II : Finfish (in review).

Water requirements

We use up to 250 lpm of sea water (SW), which is 1 % of the volume of our tanks each minute. Most of this goes to our broodstock tank, where adult biomass can be 100kg/10m^3 (100 fish, 1 kg each per 20 m^2 × 0.5 m deep). Mahimahi are very sensitive to dissolved oxygen : Levels above 6 ppm should be maintained; mahi are stressed below 5 ppm. Salinities of 15 to 35 ppt are acceptable, and preliminary results show that levels below 5 ppt may also be acceptable. Temperatures of 13 to 28°C have not killed adult mahimahi, but growth slows below 25-27°C.

BROODSTOCK HUSBANDRY

Adult mahimahi can be purchased from fishermen, who use barbless hooks and a large live well. Fish survive capture best if they are less than 45 cm FL. Transport methods for adult mahimahi have been mastered by Southern Sea Farms, Inc. of Western Australia.

Postlarvae can be dip-netted from flotsam on windward shores of Oahu, or from floating debris and sargassum. The most dependable way to get broodstock is to raise them from eggs. Homegrown breeders have no parasites, and are adapted to tanks. Important factors in maintaining healthy, productive broodstock are water quality, food quality, tank design, and tank cleaning.

Mahimahi can turn in a very tight circle, and a few large adults have been kept alive a few months in a rectangular tank in Okinawa. However, broodstock survive much longer in circular tanks. The best tanks have a central barrier to force the fish to swim around the outside, rather than cut straight across and smash into a perpendicular wall. We still lose a few 1-3 lb. fish, but have kept broodstock healthy and spawning through 15 months (9.5 months of daily spawning).

An all-fiberglass 6.6 m doughnut style tank needs only 5-10 minutes cleaning per day. In our outdoor system, we transfer adults to the inner tank every 2 weeks, by raising the water level and baiting or crowding them through a mid level door. The outer tank is then drained and cleaned with sodium hypochlorite. Cleaning schedule varies with temperature, sunlight, amount of feed, and nutrient load. Uncleaned tanks endanger broodstock survival, especially when eggs and sperm are in the water (i.e. every day), and especially if incoming water stops flowing, or contains nutrients.

Female mahimahi spawn at the Aquarium every other day throughout the year. First spawnings yield 15000-30000 eggs per female. Female growth rate slows at maturation because they discharge 5 % of their body

weight as eggs every spawning. By the time males are 15 kg. (15 months or less), captive females are 4.5 kg, and produce at least 200 000 eggs every 2 days.

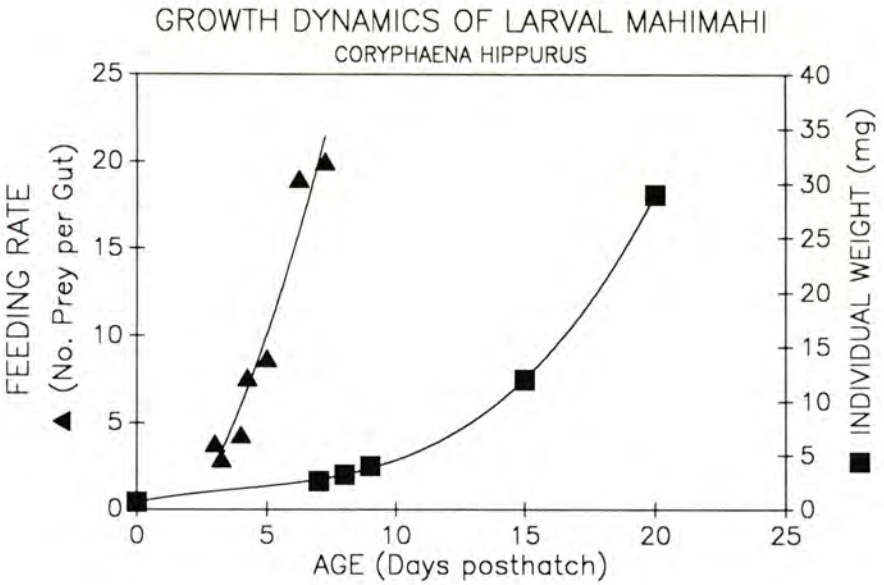


Figure 5. — Growth Dynamics of Larval Mahimahi, *Coryphaena hippurus*. Triangles track feeding rate. Squares track larval weight gain.

LARVAL REARING

Planning and Growth

The growth rate of mahimahi is phenomenal, decelerating very gradually compared to other fishes. From first feeding (0.67 mg blotted fresh weight) to 6 months (1.7 kg), average daily weight gain is 8.6 % : i.e. $(1.7 \times 10^3 \text{ g}/0.6 \times 10^{-3} \text{ g})$ exp 1/180 days. However, during their first three weeks, weight gain exceeds 25 % per day, and live food production has to keep pace. A 25 days old PL can weigh 60-300 mg, depending on food, temperature, and health. Mahimahi can exceed 1 gram at 30 days, 40 grams at 60 days, 300 grams at 90 days, and 2.4 kg at 6 months. Food conversion efficiency (FCE) is about 30 %, so larvae need to eat at least 83 % of their body weight (0.25/0.3 = 0.83) to gain 25 % of their weight daily. We offer 100 % daily. Satiation feeding is okay, as long as phytoplankton density in the rearing tank is greater than 5000 cells/ml. Note in Figure 5 that food consumption increases astronomically as larvae grow. Growth rate is affected by tank size, water quality, temperature and feeding.

Mahimahi go through several different hatchery phases. Larval behaviour changes as mahimahi grow from the yolksac to feeding to

postlarval (fry, or PL) to juvenile (fingerling) stages. Water quality control and optimal diet change as behaviour changes. In this text, larval phases are divided according to diet type. Hatch usually occurs in the night or early morning. The daytime period following hatch is called « day 0 », i.e. less than 1 full day after hatch. Best survival is obtained by feeding rotifers on days 2-8, copepods day 6-21, brine shrimp day 10-25, mahi hatchlings day 18-40, and nonliving foods after day 30. Food consumption during days 15-20 is around eight grams of copepods and 50 g of brine shrimp nauplii (blotted fresh weight) per 1000 fish. This food can be provided with 80 litres of copepod culture and 25g of brine shrimp cysts. For 22 days old mahimahi at 30 mg each, growing 18 % per day with a 30 % FCE, you'll need to provide 30000 hatchlings per 1000 PLs per day. By day 30, you'll need 110000 hatchlings per day, and by day 45 (maximum live food consumption) you'll need 1.4 million healthy hatchlings each day, as a sole food for 1000 PLs. The development of a successful artificial diet will greatly reduce these requirements. Until such a development, we recommend weaning to chopped or blended squid (with vitamins added, or with supplemental fish, chicken, or beef liver), beginning about day 30.

Egg collection and incubation

Eggs, (1.3-1.6 mm diameter) are incubated two days (one night) in a submerged, heavily aerated, screened vessel, with 10 % seawater exchange per minute. Prior to hatch (day -1), we net up to 400000 eggs into a 200 l. static incubator, aerate heavily, and provide 4-8 Lpm of new seawater.

Feeding the Larvae

The First Week Posthatch : Two (26-27°C) to three (24-25°C) days after hatching, mahimahi larvae develop pigmented eyes and functional mouth parts, and can be netted into rearing tanks for first feeding. Best rearing tanks are cone bottom, gel coated fiberglass, with central aeration (no diffusers). The central standpipe should have 60-100 ñm nytex screen to prevent plankton loss during flushing. Add 2 % algae (at 0.5-1 million cells/ml) on day 2, and 1 % daily through day 25, if needed, to maintain 5000 to 20000 algal cells/ml. Add 1-2 rotifers per millilitre (R/ml) after stocking the larvae. Mahimahi will find 1R/100 ml. Plan on 500 R/larva, though they usually eat 100-200/day at first. Ten litres of rotifers culture, at 100 rotifers/ml (0.18 g wet weight per litre) stocked on day 2, should reproduce within the larval rearing tank and provide a continuous supply of food for 1,000 larvae through the first week.

Day 6-22, The Copepod Phase : This phase can be a time of heavy losses if larvae are not well fed prior to metamorphosis. Around 12-14 days, pelvic fins emerge, and the straight gut twists and adds several organs. Postlarvae (PLs) spend more time near the tank bottom, and have to be avoided or returned during vacuuming.

Day 18-45 Postlarvae : PLs should be fed mahimahi hatchlings as soon as they will eat them. Ours start at day 18 (about 15 mm TL at 24-25°C). Day 2 larvae weight about 0.7 mg wet; day 0 « yolk sac larvae » weight 0.8-0.9 mg each. Note the example feeding rates from the « Plan-

ning and Growth » paragraph. By day 30, or sooner, PLs should be weaned to squid paste, or a pelleted feed. Mahimahi PLs will eat flake foods and freeze dried commercial foods by day 18, but we have not yet found a food of this type that promotes good growth and survival for young PLs.

Cleaning and Disease Treatment

Sea water (SW) exchange should be gradually increased from 10 % on day 3, to 30 % per day (0.36 volumes) days 7-10, 60 % (0.9 volumes) days 11-13, 90 % (2.3 volumes) days 14-18, 98 % (4 volumes) days 18-21, and 99.9 % (7 volumes, i.e. 20 lpm in a 4000 litre tank) after day 21 (based on a stocking density of 1-2 larvae per litre). Vacuum the bottom of the rearing tank (a swimming pool vacuum attachment works well) into a screened box to see if larvae or food are on the bottom. Food and larvae can be rinsed and returned, especially after day 14, when larvae become more valuable and more tolerant of handling. Scrub the bottom and sides daily with a scrub pad on a stick (eg. « Doodlebug »).

Usually, the first signs of impending disease are reduced growth and a noticeable number (10 %) of nonfeeding larvae. Our diagnostic procedures are still in the development phase, and new pathogenic bacteria species arise fairly often. Therefore, in order to react effectively against larval pathogens, we currently recommend routine identification of bacteria species and antibiotic sensitivities prior to mortality (i.e. every day, especially days 15 through 30). We have not found any single antibiotic that is effective against all pathogenic bacteria. Disease can sometimes be avoided without drugs by keeping larvae well fed and uncrowded, by frequently vacuuming bottom debris and flushing out dissolved organics and particles that act as bacterial media, and by meticulously avoiding introduction of pathogens. Our major sources of catastrophic diseases are live plankton cultures, and poorly stored non living foods.

SUMMARY

Disease, and reliance on live plankton cultures are the factors that limit our hatchery production yield. Successful transition to nutritious non living foods signals the onset of the juvenile grow-out (or ocean release) period. The aquaculture potential of mahimahi is still being tested, but the prognosis is good enough to encourage at least 2 commercial ventures to continue development. Statements from these companies follow.

STATEMENTS FROM COMMERCIAL VENTURES

NORAQUA

A Norwegian company doing mahimahi research in Florida and the Bahamas for the past 4 years.

The following status is quoted from Randy Hagood

— Satisfactory control of spawning and larval rearing.
— Grow-out to 10kg has been done, most likely production size less than 5 kg.
— FCR now down below three with fish meal based feeds.
— Have tested net-pen grow-out in Bahamas with encouraging results (if you overlook the sharks and 13 degrees water temperatures).
— Have decided to develop a pilot scale commercial farm with on-shore grow-out tanks.
— Most likely site-Hawaii, and hope to build farm this year.

SOUTHERN SEA FARMS LTD (SSF)

An Australian company based in Perth, SSF started research and development in late 1985.

The January 1989 issue of Fish Farming International, pages 12-13 have a good description of SSF's status. SSF is a public corporation and their 1988 Annual Report has very nice pictures and a comprehensive report of activities. Steve also reports :

SSF now has 3 pilot stage grow-out trial sites along the west coast, spanning 20-30°C. They are testing sea cages and fiberglass tanks so far, and all 3 sites are doing well. The Albany site (South) is a salmon cage farm : mahimahi are resistant to a local isopod that kills salmon, and mahimahi are stronger than salmon when accidentally dropped on the rocks; mortality was 5 % the 1st week, with no further mortality to the present 2 months.

Quoting from page 1 of the SSF 1988 Annual Report :

« SSF is on the verge of commercially farming.mahimahi. »

« Significant achievements during the year are summarized as follows :

Upgrading of the hatchery facility at Yanchep and expansion of its production capacity to enable pilot study grow-out trials to commence;

The vital discovery of a link in the complex live food chain of Mahimahi larvae;

Development of new live food enrichment techniques, and their subsequent modification to suit Mahimahi larval culture;

Development of specific inert pelletized food and the technology to wean the domestic strain of Mahimahi juveniles onto hard pellets at an early age;

The continuing co-operation and joint research with Curtin University of Technology and the Queen Elizabeth II Medical Centre into the morphology and histology of early larvae;

The commencement of a worldwide search for farming sites to efficiently service major international markets.«

PROFIT POTENTIAL WORKSHEETS

Although we can now produce enough juvenile mahimahi to test tagging and aquaculture, actual grow-out trials are not documented. The following cost spreadsheets are presented as drafts, using what we know about mahimahi to date. The author is not an accountant or economist. The purpose of this presentation is to promote workshop discussion of realistic costs for aquaculture.

ANNUAL COSTS FOR A PILOT SCALE MAHIMAHI FARM

Cost Item	Annual cost Current Technology Stock 250 fry *pers 200 m² tank*		Annual cost Predicted Technology Stock 1000 fry *per 200 m² tank*
Feed	11250	FCR = 1 :1	45000 US$1/TON
Fry	5625		22500 in-house
Misc.	10000		10000
Electr.$0.11/kwh	40000	2000 gpm SW = 40kw	40000
part. labor			
Staff (3)	54000		54000
Maintenance	2000		2000
Marketing			
TOTAL VARIABLE	122875		173500
Management (1)	30000		30000
Land	750	$1500/acre/yr or	750
Depreciation	37250	(10yrs on capital	37250
Interest			
Fees			
Insurance			
Accounting			
TOTAL FIXED	68000		68000
TOTAL COSTS	190875		241500
Prod. value	90000		315000
Number of fish out	4500	1500 × 3 crops/yr	18000
Production (lbs)	22500		90000
Mkt. value ($/lb)	4		4
Net profit	−100875		73500

CAPITAL COSTS, 1/2 acre		
Buildings		
Office/lab	35000	12ft × 36ft trailer + office supplies
Hatchery	60000	fiberglass roof, open sides, slab
Equipment		
Freezer	20000	Oahu Sales Inc, 8' × 12' 3HP, 4.4kw
Pumps	40000	4 × 15HP(500gpm) = 33kw, + 5kw aeration
Generator	25000	40kw, used (80kw = $25000)
Truck	10000	utility pickup
Hoist	5000	Fish transfer/harvest (global catalog)
Grounds		
Grading	50000	Pre-slab leveling, etc.
Plumbing	25000	(12" × 400') + (4" × 1000') + (2" × 400')
Electric	15000	trenching, installation, hardware
Concrete	40000	($1.60/sq.ft.) 420cu.yds $85/yd
Install concrete	40000	hyperlon = $25000;FRP = $480000
Shade/shelter	7500	($0.33/sq.ft.)
TOTAL	372500	

HATCHERY PRODUCTION COST

For taggable or stockable juvenile mahimahi. Draft of August 31, 1988. This does not include the cost of research. Several cost options are presented, but the most likely cost for large scale commercial hatchery production is option « D ».

CURRENT STATUS AT WAIKIKI AQUARIUM

1.	PRODUCTION = 400 fish/4,000L tank/mo	
2.	LABOUR = 1.6 PEOPLE × ($1500 + 30 % benefits)	$3120
3.	UTILITIES = 10 % OF No.2 =	312
4.	SUPPLIES (Mostly broodstock food)	268
5.	COST PER 400 FISH =	$3700
	= $9.25 EACH	

Production is limited by tanks, space, and labour.
Both staff must be skilled.
Fry Food = [20g × 1/0.3FCE × 1LB/454g × $0.76/lb] = $0.11/fish.

CURRENT POTENTIAL (Current technology, bigger facility)

1.	PRODUCTION = Same as « D », below = 60000	Juv./mo
2.	LABOR = (as per « D », below) + $1300	$7150
3.	FOOD = [60000 × $0.11] + $1300 BrdStk	7900
4.	UTILITIES AND SUPPLIES	2450
5.	DEPRECIATION ON $250000/20 YRS	2500
6.	COST PER 60,000 FISH (20g)	$20000
	= $0.33 EACH	

Production is based on staggered batches (130 000 l tank per week yielding 15 000 juveniles at the end of 2 months). Larval technician cares for 8 tanks per day. Broodstock technician cares for 10 tanks per day, with 1 male + 11 females per tank. Labour for larval rearing = 1 hour cleaning and monitoring per tank. Labour for broodstock = 10 min feeding + 15 min cleaning + 20 min egg collection and hatch treatment = 45 min/tank. To feed 60 000 PLs during the hatchling a food phase (days 20-45, 4-tank avg. wt./PL = 0.5 g) requires 0.5 g × 20 % growth/day × 1/0.3 FCE × 1000/0.9 g per 1000 hatchlings = 370 hatchlings per average PL = 22 million hatchlings per 60 000 PLs = 110 females × 400 000 eggs per spawn. Females weight about 4 Kg, males about 8 Kg. Each tank is fed 3 Kg of squid at $0.67/lb = $133/tank/mo.

Some costs might be saved if eggs were collected opportunistically from a grow-out farm.

CURRENT ENHANCEMENT POTENTIAL

Unweaned PLs, age 25 days, using current technology, genetically tagged, released into food-rich, non-predatory environment*.

1. PRODUCTION =
   15000 fish/30000 l tank/mo × 8 tanks per person/day
   = 120000 fish/mo.
2. LABOR = Same as « D », below = $5850
3. FOOD per 120000 PLs : 660
4. UTILITIES and SUPPLIES 1090
5. DEPRECIATION on $200000/20 years 2000
6. COST PER 120,000 FISH 0.1g $9600
   = $0.08 EACH

Food cost = $5.50/1000 PLs = (0.16gBS cysts/0.1gPL)
Hatchlings not needed as food. *FR/NP environment may not exist; no tests to date.

POTENTIAL COST, USING COMMERCIAL FOOD (If it were available)

1. PRODUCTION = 15000 FISH/30,000 L tank × 8 = 120000 per 2 months, = 60000 Juveniles/month.
2. LABOR = [$2500 + 1000 + 1000] × 1.3 $5850/Mo
3. FOOD = [60000 × $0.10] + $150 BrSt 6150/Mo
4. UTILITIES and SUPPLIES 1000/Mo
5. DEPRECIATION on $200000/20 YRS 2000/Mo
6. COST PER 60000 FISH, 20 g $15000
   $0.25 EACH (Taggable juvenile)
7. COST PER 1g FISH, Age 30 days = $7450/120000 =
   $0.06 EACH (Stockable PL)

Production limit based on :

1 hour cleaning & monitoring per 30,000 L tank = 8 tanks per day per person (for 0-30 days larvae/PLs); maintaining 1 broodstock tank (1M + 2-3 F) + 1 brood grow-out tank; 1 full day food preparation, culture work, broodstock, administration = 2 people per day. Staff = 1 manager + 2 technicians (minimum) to cover 7 days workweek. PL food cost based on price of squid ($0.76/lb). Commercially available hatchery feeds now cost $36-58/lb, and they do not work for mahimahi.

POTENTIAL BENEFIT/RETURN

1. 6mo old mahi = 5lb × $3.00/lb = $15 each fish
2. 1yr old mahi = 20lb × 3.50/lb = $70 each fish
3. 4mo old mahi = 1lb × 2.50/lb = $2.50 each fish

Acknowledgements. *This research was supported by grants from the State of Hawaii Department of Land and Natural Resources Aquaculture Development Program contract numbers 21087, 18882, 15856, and 17149, and National Oceanic and Atmospheric Administration grants NA81-AA-A-00070 and NA A5 AA-D-SG082.*

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Selection of finfish species as candidates for aquaculture in French Polynesia

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Abstract — *A programme of selection of finfish was conducted by IFREMER from 1983 to 1987 to identify the most suitable species for aquaculture in French Polynesia. A first bibliographical selection, based on the economical interest of species and the existence of previous researches in Tahiti or abroad allowed to retain 4 local families (Carangidae, Coryphaenidae, Serranidae, Siganidae) and 2 imported ones from South-East Asia (Centropomidae, Cichildae).*

In a second step, species were selected according to the following criteria : growth potentiality, adaptation of fry to dry pelleted food, reproduction and spawning capacity, larval rearing and fry production, pathology and resistance to stress, economic interest on local market.

A classification in 3 groups is consequently proposed.

*— Species with a very high potential for aquaculture, allowing development projects on a short term basis : the Seabass (**Lates Calcarifer, Centropomidae**), originating from S.E. Asia.*

*— Species, potentially interesting but presenting some drawbacks, as difficulties to perform larval rearing (grouper **Epinephelus microdon, Serranidae** and dolfin fish : **Coryphaenae sp., Coryphaenidae**) or presenting pathological problem (red Tilapia strain **Oreochromis sp.** during grow-out in sea water cage).*

*— Species considered as difficult to rear and not retained like **Siganus argenteus, siganidae** (unsuccessfull larval rearing and poor economical value) or **Caranx ignobilis, Carangidae** (no acceptability of compounded diets).*

The result of the tests are synthetized with more details for Seabass considered as the most promising species for the area.

The future development of finfish in Polynesia is emphasized and a project of Sea bass netcage culture in Polynesian lagoon is presented.

INTRODUCTION

Situated in the center of the Pacific Ocean, between 8 and 27° of latitude south, French Polynesia became an Oceanic area of economical interest after the extension to 200 miles of his economic exclusive zone.

The exploitation of his natural fish resources, in a territory of 3.3 million of km 2 reached a total of 8000 tonnes in 1987 including 4000 tonnes exploited by industrial Japanese and Corean vessals, 2000 tonnes of pelagic fish (Tuna, Bonitos) caught nearly Polynesian Islands and 1700 tonnes of lagoonal fish traditionnaly captured by traps and nets in the Tuamotu Archipelagos (Service de la Mer et de l'Aquaculture, 1987).The recent tourism development in the last ten years increased the demand of fresh good quality products that traditionnal fishing methods are not totally able to supply due to the seasonnal fluctuations of the catching and the risk of ciguatera contamination encountered in many atolls.

The project of developping finfish aquaculture, using floating netcages, largely extensed in many tropical Asean countries, as Singapore, Hong-Kongand Thaïland (Chua, 1986), was recently investigated by the Centre Oceanologique du Pacifique (IFREMER) in Tahiti, considering the favorable characteristics of many lagoons and Islands of French Polynesia as :

- continuous reneval of water by the action of waves over the reef,
- good protection from ocean swells,
- easy accessibility of water and constant temperature around 27 to 28°C,
- abundance of sites.

This new aquaculture project could offer good quality products, strictly calibrated and available all over the year. It could also help to create new economical activity, fitting the capacity and the need of the Islands populations, in participating to their retention and even their return in the Islands.

The programme, initiated by IFREMER in 1982, to select the most suitable finfish species for Aquaculture thank to the financial support of EEC had three mean objectives :

1. introduction of new fields of activity in the oversea French Polynesia
2. development of scientific cooperation with other tropical countries allowing the improvement in establishing rearing techniques as maturation, nutrition in the future
3. the transfer of knowledge to foreign countries.

It was divided in 4 steps, each corresponding to a 3 or 4 years research programmes.

From 1983 to 1987, the programme of selection allowed to retain a limited number of species considering several economical and biological factors. Both local and imported species were studied and 4 local families (Carangidae, Coryphaenidae, Serranidae and Siganidae) and 2 imported ones from South East Asia (Centropomidae and Ciclinae) were chosen in a first estimation among the hundred identified by Nash (1988) as potentially interesting and largely present in South East Asia (Rabanal, 1988). The results of the test are emphazised for each of the six families and a final classification in 3 groups is proposed, considering their availability to be reared and the difficulties encountered to control the whole cycle of their production in French Polynesia.

In a second step, more specific researches were conducted to develop rearing techniques on the most potential species : *Lates calcarifer.* Details of the experiments realized during the nursing (3 to 6th month) and the grow out phases (6th to 12 th month) of this species, are emphazised and detailed. Research effort was focused on the adaptation of fry and juveniles to dry pelleted diet, transferring the existing rearing techniques developed with success by IFREMER on other species as European sea bass : *Dicentrarchus labra* and Sea bream : *Sparus auratus* to the local conditions of Tahiti, caracterized by the lack of fresh fish meat supply and the cost of labour.

The demonstration of the technical and economical feasibility of sea bass net cage culture at a pilot scale level is also presented as a possible future development of finfish aquaculture in Polynesian lagoons.

MATERIAL AND METHODS

Facilities

The facilities built to conduct the finfish programme include an experimental hatchery equiped with larval rearing and weaning tanks 0,5 to 1.5 m^3 capacity and a production unit of live preys : rotifers, *Brachionus plicatilis* and artemia, *Artemia salina.* A nursery unit situated out-door with 12 circular tanks protected by a shadow net allowe to wean and grow the fry up to 50 g mean weight, feeding then with dry pellet on automatic feeders. The grow-out facilities included a series of experimental floating netcages 8 to 24 m^3 each, equiped with automatic feeders and timer and connected to the shore by a floating pontoon. A 40 mm protecting net provided an efficient protection against predators. The broodstocks were maintained in 5 circular fiberglass tanks, 15 m^3 capacity, to conduct breeding and reproduction experiments. Three laboratories of pathology, nutrition and water quality control of IFREMER were associated in these researches.

Rearing conditions

- *Origin of animals.* Fry and adults of local species were captured in the wild around Tahiti (Rabbitfish, Jack Fish, Dolphin fish) or in the Tuamotu archipelogos (Grouper). The imported ones (Seabass, red Tilapia) were air-shipped from Singapore at different stages (eggs, larvae, fry or juveniles). Flight duration, superior to 20 hours did not induce any particular mortaiity when strict packing and density were respected : 1 to 1500 eggs or larvae per litre, 300 fry per litre.
- *Stocking density.* In order to standardize the rearing conditions low density was recommended : 30 larvae per litre during larval rearing. 1 to 8 fry per litre during weaning and nursing phase, 5 to 10 kg per m^3 at the end of the grow-out phase. Higher density was occasionally tested for Seabass grow-out in cage where final density reached 30 to 35 kg per m^3.

- *Water quality.* Ocean sea water was pumped in the lagoon at a mean deep of 20 metres. Frequent monitoring revealed a constant water quality and stability of temperature (26 to 28°C) and salinity (35 ppt).
- *Food and feeding regime.* 3 kinds of diets were tested during nursing and grow-out phases :
  1. fresh bonitos-meal
  2. semi-moist pelleted diet including a mixture of 50 % of bonitos meal and 50 % of Norway fish meal
  3. dry diet pelleted in Tahiti and including 56 % of protein and 16 % of lipid in the starter diet or 52 % P and 12 % L in the grower one (Table 1).

Tab. 1. — Proximate analysis (as fed) of compounded diets pelletized in Tahiti for experimental nursing (Starter diet) and grow-out tests (grower diet)

	STARTER DIET %	GROWER DIET %
Protein	58,1	51,8
Lipid	14,4	12,1
Ash	9,5	9,0
Moisture	6,5	7,8

The amount of diet was ajusted to the feeding behaviour of each species and to the size of the fry. This amount was generally decreasing from 10 to 2.5 % of the body weight per day at the end of the grow-out period.

Design of experiments

1. Selection of species

Each species was selected according to following biological and economical criteria :

(1) growth speed and time to reach the marketable size;

(2) adaptation to dry pelleted food;

(3) capacity of reproduction and spawning in captivity;

(4) larval rearing and control of fry production;

(5) resistance to disease and stress;

(6) economical criteria as price of the product and impact on the local market were also studied.

After selection, a classification in 3 groups was proposed considering the ability of each species to be reared in captivity and their potential in aquaculture :

group 1. very high potential

group 2. interesting potential but difficulties encountered at different stages of the rearing cycle

group 3. low potential.

2. Status of Seabass research in Tahiti

During the second phase of the programme, research effort was concentrated on Seabass nursing and grow-out.

During the nursing phase (3rd to 6th month), 2 topics were studied.

a — Effect of grading frequency on survival and growth of seabass fry :

In 3 preliminary experiments, the whole population was graded, every 15 days during 3 months and dispatched in different circular tanks. Grading system was similar to the grader described by Vasselin (1984). In the last experiment, ungraded and graded batches of fry of the same population were maintained at a constant density of 500 fry/m^3. Fry were fed with dry pellets (starter diet) automatically distributed 8 hours per day and the quantity were adjusted after each grading in order to maintain the conversion ratio inferior to 1.5 : 1.

b — Effect of feed distribution and feed formulation :

In one experiment, young fry (0.7 g mean weight) and 70 days old were fed on starter diet using 2 feeding distributions : automatic feeding during 8 hours at a constant level of 10 % of the body weight per day or self-feeding, allowing a distribution by the fry themselves. Survival, growth and feed efficiency were monitored after 20 days on 4 replicates.

In 2 other experiments, 2 feed formulations, starter or grower diets were compared on 2 sizes of seabass fry (1 and 6 grams respectively) during 2 months. The effect of protein level between 52 and 56 % tested on survival and growth was analyzed on 4 replicates.

During the grow-out phase (6th to 12 th month), one experiment was carried-out in cage (8 m^3), testing 3 initial densities, 15, 30 and 60 fry per m^3. It lasted 6 months with a month lag between the two replicates, due to the limited number of fry of the same weight (30 g around). Dry grower diet was distributed every 30 minutes during a 12 hours period and the quantity varied from 3 to 1.5 % of the body weight per day.

Two other trials were conducted in larger cages (24 m^3), at a higher density of 70 fry per m^3 in order to confirm the results of the first experiment slave; feed formulation and distribution were similar to the previous test.

Data analysis

All the data collected were analysed for the following : survival rate (%) with a daily control of the mortality; mean fish weight by frequent sampling of significant number of fry; weight gain and daily weight increase; food conversion ratio in as fed and daily feeding rate.

One way analysis of variance was used to test the differences between treatments in the seabass nursing and grow-out experiments.

RESULTS

Selection of candidate species for aquaculture

The results of the test of selections are synthetized in table 2 and a classification of the species in 3 groups is consequently proposed.

Tab. 2. — Synthesis of selection of the most suitable species for aquaculture in French Polynesia

	SPECIES	SEABASS	RED TILAPIA	GROUPER	DOLPHIN FISH	RABBIT FISH	JACKFISH
Growth	A > 500 g B > 300 < 500	A	B	B	No Trial	B	B
Adaptation Dry Pellet	A = easy B = progressive C = impossible	A	A	B	No Trial	A	C
Maturation Spawning	A = natural B = induced C = no obtained	B	No Trial	A	A	A	C
Larval rearing Fry Production	A = easy B = feasible C = unsuccessfull	B	No Trial	C	C	C	No Trial
Pathology	A = resistant B = sensitive	A	B (salinity)	A	B	A	B
Price/Kg	A = high B = medium C = low	A	B?	B	B	C	C
Group 1. : High Potential Group 2. : Drawback Group 3. : No retain.		group 1	group 2	group 2	group 2	group 3	group 3

Seabass : *Lates calcarifer*, imported from Singapore and classified in group 1, has the highest potential with a fast growth speed (mean weight of 500 grams obtained in one year from the eggs), feeding fry with artificial compounded diet. Maturation and spawning have been obtained in captivity and larval rearing has been demonstrated to be feasible. His resistance to stress and disease was satisfying and it has been sold at a high price (up to 7 US $/kg on the local market.).

Group 2 included 3 species : grouper (*Epinephelus microdon)* and dolphin fish (*Coryphaena sp)* are two very potential local species for aquaculture but difficulties have been encountered to control larval rearing and fry production (Table 2). Red tilapia hybrid : *Oreochromis sp.* also imported from Singapore, has an interesting grow-out potential in sea water, but has a poor resistance to sea water when grown in net cage at

a density superior to 10 kg per m^3. Regular mortalities with necrosis were observed during the whole cycle of rearing, reducing the survival at 30 % after 6 months of growing.

Rabbitfish : *Siganus argenteus* and Jackfish : *Caranx ignobilis* were considered as difficult to rear and were classified in group 3. The first one had an unsuccessful larval rearing and a poor economical value, the second refused any kind of compounded diet although his good behaviour in net cages (Table 2).

Details of the results of selection are indicated for each criterion in following tables :

Criterion 1 : Growth speed (Table 3)

Growth speed of seabass fry, fed on local compounded diet was fast (weight gain : 100 grammes per month) compared to red Tilapia (51g) and rabbit fish (37g). Jack fish was potentially interesting (weight gain : 61 g per month) when fry were fed on a semi-moist pelleted diet confirming previous work obtained by Aquacop (1979). Grow-out potential of grouper and dolphin fish were not investigated due to the lack of wild fry available but preliminary results obtained with larger groupers gave a very first indication of his growth potential (weight gain 34 g per month).

Tab. 3. — Comparative growth speed of selected species fed on dry grower diet or semi-moist pelleted diet

CRITERION 1. GROWTH SPEED

SPECIES	SEABASS	RED TILAPIA	JACK FISH	GROUPER	RABBIT FISH
ORIGIN	SINGAPORE	SINGAPORE	TAHITI	TAHITI	TAHITI
Initial mean weight (g)	30	3	20-30	300	6
Rearing Conditions	Cage	Cage	Tank	Tank	Tank
Food	Dry Pellet	Dry Pellet	Semi-Moist	Semi-Moist	Dry Pellet
Duration (Months)	6	6	12	7	6
Survival	97	36	80	100	72
Final mean weight (g)	640	310	760	530	230
Weight gain (g. month)	102	51	61	34	37
Conversion Ratio	1,6 : 1	3,2 : 1	11,1 : 1	5,5 : 1	2,7 : 1

Criterion 2 : Adaptation to dry pellet

All the species, except in the case of Jack fish, easily accepted dry pelleted diet after a short adaptation. The trials conducted to wean wild fingerlings of Caranx on dry or re-hydratable pellet using chemical or natural attractive substances were unsuccessful. After 4 months, survival rate was inferior to 40-50 % and the daily weight gain was around 0.1 to 0.2 grammes compared to 1 g per day when fed with semi-moist pellet.

Criterion 3 : Maturation and Spawning

Except in the case of Jack fish, all the species naturally matured and spawned in captivity using or not hormone injections. Characteristics of spawning and fecondity rate are presented in table 4. The size and morphological aspect of dolphin fish brooders, with 300 grammes mean weight spawning females suggested that this species was the pompano Dolphin Fish or *Coryphaena equiselis* (Palko, 1982). The Tilapia hybrid breeding was not investigated, because bibliographical studies (Fitzgerald, 1979; Lester, 1983), suggested that no special difficulty was encountered when maturation was conducted in freshwater.

Tab. 4. — Maturation spawning and larval rearing results during selection of candidate species for aquaculture

CRITERIA 3 - 4
MATURATION / LARVAL REARING

SPECIES	SEABASS	GROUPER	DOLPHIN FISH	RABBITFISH
Origin of Brooders	Singapore	Tuamotu Archipelagos	Tahiti	Tahiti
Period of reproduction	October to May (To confirm)	December to May	December to February (Trial COP)	December to February
Spawning behaviour	Induced spawning LHRH (a) Hormone	Natural	Natural	Natural
Characteristic (Kg)				
Male	2,5 to 5	1,65 ± 0,26	0,30 ± 0,1	0,25 ± 0,03
Female	2 to 6	1,51 ± 0,24	0,32 ± 0,1	0,30 ± 0,05
Fecondity EGGS per Kg Female	780000	645000	225000	1500000
Fecondation rate	Up to 98 %	94	91	91
EGG Diameter (mm)	0,79 ± 0,02	0,857 ± 0,014	1,31 ± 0,02	0,650 ± 0,02
Origin of Larvae	Singapore or Tahiti	Tahiti	Tahiti	Tahiti
Initial Density Number/Litre	20 to 30	20 to 40	10 to 20	20 to 40
Survival	Up to 80 % (D.20)	0 % Day 24	0 % days 15	0 % Day 19
Peak of Mortality	10 to 15	3 to 5 10 to 25	5 to 15	3 to 5
Size Larvae (mm) Day 0	2,82	2,2	4,54	2,6
FOOD SEQUENCY	Rotifer D3 to 15 Nauplii D8 to 25 Artemia 2 Days old Artemia D15 to 25	D3 to 25 D12 to 25	D3 to 8 D5 to 15	D3 to 19

Criterion 4 : Larval rearing and fry production

The results of larval rearing experiments, conducted on 4 of the 6 species, suggested that only seabass larvae were successfully reared up to methamorphosis (Table 4). Survival rate reached 50 to 80 % on day 20 with eggs and larvae imported from Singapore.

Rabbitfish, Grouper and Dolphin fish larvae were particularly difficult to rear and no successful survival rate was obtained after 20 to 25 days although, some methamorphozed grouper larvae were observed.

Weaning larvae after the first month of rearing on a dry pelleted diet, including a high percentage of protein was feasible with seabass and several batches were produced. The over-lapping from live prey to artificial pelleted diet allowed to obtain weaned fry with a survival rate superior to 50 % and a final mean weight of 1 to 2 grammes after the third month. Frequent grading were provided, every 8 to 10 days period to prevent strong cannibalism appearing as soon as lived preys were replaced by artificial dry pellet.

Fresh water red Tilapia hybrid larvae (mean weight, 0,7 grammes), imported from Singapore were gradually adapted to seawater in 10 days. Results of survival (94 %), growth speed (final mean weight = 2,6 grammes) and feed efficiency (conversion ratio inferior to 2 : 1), after one month, revealed a satisfying acclimatation of these larvae to seawater conditions of Polynesia, when reared in tanks.

Wild fry of Rabbitfish were easily observed in dense school, 2 or 3 months after the spawning season but mass collection was limited by the difficulties encountered to predict time of schooling and strong fluctuations from year to year.

Criterion 5 : Pathology, resistance to stress

The parasite : *Cryptocaryon irritans*, commonly observed in tropical waters was a major pathogen agent in Tahiti. All the species, excepted red Tilapia, were very sensitive when maintained in tanks, specially Dolphin and JackFish (Table 5). Nematode and cestode parasites were also encountered in large number in wild grouper brooders originated from Tuamotu archipelagos and all the tentatives to eradicate revealed unsuccessful. Handling Jackfish and Dolphin fish was difficult and necroses often followed by death were observed when manipulated. Other handling problem was noticed with Rabbitfish which presents a venomous barb on his dorsal fin.

All the species were adapted to the salinity of Polynesian water except the red Tilapia strain imported from Singapore which presents necrosis when reared at high density in netcages.

Criterion 6 : Price on local market

Seabass had the highest price (up to 7 US $/Kg) on local market (wholesale) and is a successful seafood product due to its delicious taste and presentation. Dolphin Fish and Grouper, commonly caught around Tahiti and Tuamotu Archipelagos are sold at a mean price of 5 to 6 US $/Kg. The price of Tilapia (6 US $/Kg) has to be confirmed on a larger

quantity as common Tilapia *(O. Mossambica)* is not consummed in Tahiti. Jackfish and Rabbitfish have the lowest price of 3 to 4 US $/Kg.

Tab. 5. — Parasitism and resistance to handling of the selected finfish species

CRITERION 5
PATHOLOGY — RESISTANCE TO STRESS

	SEABASS	RED TILAPIA	JACK FISH	GROUPER	RABBITFISH	DOLPHIN FISH
PARASITISM						
EXT : *CRYPTOCARYON IRRITANS*	+ +	0	+ + +	+ +	+	+ + +
INT : NEMATODE	0	0	0	+ + +	0	0
CESTONE	0	0	0	+ + +	0	0
HANDLING	0	0	+ + +	0	+ + (Venomous Barb)	+ + +
SALINITY 35 %	0	+ +	0	0	0	0

+ + + Very high sensibility
+ + Average sensibility
+ Low sensibility
0 No sensibility

Status of seabass research in Tahiti. Nursing phase (3rd to 6th Month)

Effect of grading frequency

The results of the three preliminary experiments conducted in tanks, with a grading frequency of the whole population, every 15 days, are indicated in Table 6. Survival rate exceeded 85 % and the mean weight of fry was superior to 25 grammes after 3 months at a final density of 5 to 8 Kg per m3. The weight dispersion indicated by the final coefficient of variation fluctuated from 32 to 64 % depending on the trials. Food efficiency was satisfying and the conversion ratio was inferior to 1,5 : 1 for a daily feeding rate decreasing from 10 to 4 % of the body weight per day.

In the last experiment (Table 6), if we consider the lost of 10 % of the animals by accident in the second ungraded tank on day 60, survival results were not strongly affected when fry were not graded in tanks as soon as the feeding regime was satisfying. Mortality related to cannibalism appeared after the 2nd month in both tanks affecting 20 to 30 % of the population, against 7 % in the control. Growth and weight dispersion were depending on the treatments with a better final mean weight (45 against 27 g) but a higher dispersion when fry were not graded. Frequent grading of fry allowed to maintain a better homogeneity of the population as indicated by the weigh frequency polygon of each batch (Figure 1). Food intake was not affected by the treatment and the final conversion ratio did not exceed 1 to 1,1 : 1.

In comparison, the nursing of seabass in net cages, as early as the 3rd month (Table 6) induces strong mortality with necrosis and cannibalistic behaviour in our rearing conditions. Survival did not exceed 30 to 50 % when fry were ungraded and in the two trials, final mean weight reached 60 to 70 g correlated to low survival. Nevertheless, food efficiency was satisfying and the conversion ratio was not surperior to 1,5 : 1.

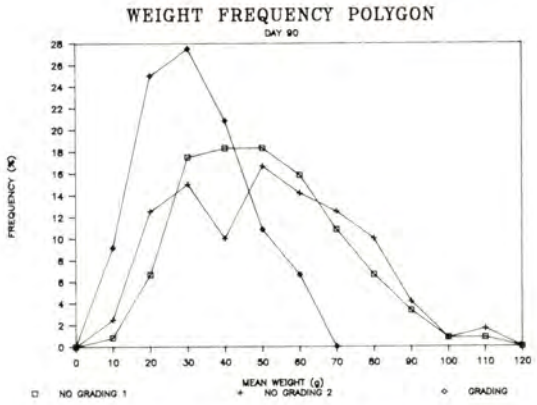

Figure 1. — Weight frequency polygon of Sea bass fry after 3 months of experiments. Two batches were not graded (1 - 2)and one (3) was graded every 15 days.

Effect of feeding regime and feed formulation

Survival was not affected when young fry were fed with automatic feeders or with self-feeders and no cannibalism was noticed (Table 7 - Trial 1). After 20 days, the final mean weights were not significantly different (3,4 and 3,7 g respectively). The advantage of using a self-feeder appeared on the feeding efficiency with significant better conversion ratio : 0,97 : 1 against 1,4 : 1 when fry were fed with automatic feeder. This preliminary experiment suggests that seabass fry are able to adapt their consumption as early as the 2nd month of rearing (Figure 2), when self-feeder is used. At the end of the experiment daily feeding rate was exactly adjusted to 7 % of the body weight for the fry fed with self-feeders.

The comparison of two formulations, starter or grower diet (Table 1), on survival and growth of seabass fry during nursing are indicated in Table 7 (Trial 2 and 3). For 2 weight ranges (1 and 6 grammes, initial weight), in the 2 trials, survival was very high and mortality was limited to 2 or 3 animals, during the 2 months of experiment. Significant growth differences appeared on youngest fry when fed with the higher protein level (56 % compared to the grower ($F = 7,74$ $DF = 6$ and $P < 0,05$) and conversion ratio reached 1.1 : 1 and 1.3 : 1 respectively. In the case of larger fry (initial mean weight 6 g), results of growth speed and food efficiency were similar (Table 7), suggesting that seabass fry could be fed with grower diet as soon as their mean weight reaches 5 to 6 grammes.

Tab. 6. — Result of the effect of grading frequency on survival, growth and feed efficiency of seabass fry. Experiments are conducted in circular tank, 1,5 m^3 of capacity or in cage, 8 m capacity

TREATMENT	REARING STRUCTURE	DURATION (Days)	INITIAL Number	SURVIVAL (%)	INITIAL WEIGHT (g)	DAILY WEIGHT INCREASE (% Day)	FINAL WEIGHT (g)	FINAL COEF. of variat.	CONVERSION Ratio (asfed)	REARING VOLUME (m^3)	DENSITY Kg/m^3
Grading	Tank	95	5.855	Variat. 86	1,1	3,3	25,6	39	0,9 : 1	21	6,1
Every	Tank	80	4.627	95	181	3,2	23,8	64	1.4 : 1	13,5	8,1
15 Days	Tank	70	1.496	86	0,3	6,5	27,6	32	1,2 : 1	7,5	4,7
No Grading	Tank	90	450	83	0,8	4,4	44,9	44	1,01 : 1	1,5	11,2
No Grading	Tank	90	450	63 *	0,8	4,5	46,0	50	1,01 : 1	1,5	9,4
Grading	Tank	90	1.113	93	0,8	3,9	27,4	47	1,05 : 1	4,5	6,3
No Grading	Cage	90	3.600	30	1,2	4,5	70,1	27	1,2 : 1	8	9,5
No Grading	Cage	90	3.600	56	2,3	3,7	64,0	28	1,1 : 1	8	16,1

* lost of 10 % of the population on day 60 by occident.

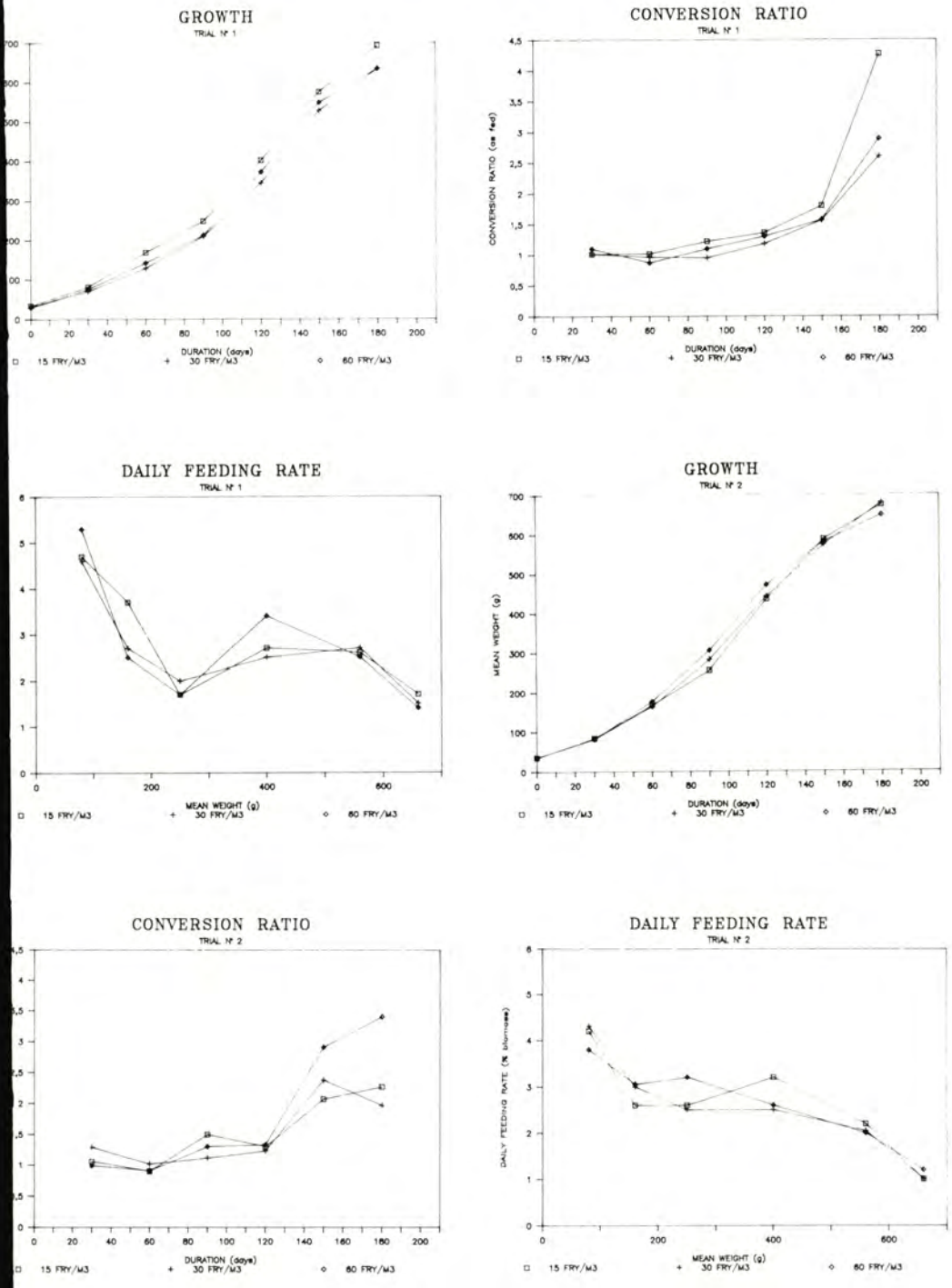

Figure 2. — Growth, conversion ratio and daily feeding rate of sea bass fry, reared in 8 m^3 netcages at initial densities of 15, 30 and 60 fry per m^3, during 6 months and fed on a 52 % protein and 12 % lipid compounded diet (Trial 1 and 2).

Tab. 7. — Results of the effects of the feeding regime and feed formulation on survival, growth and feed efficiency of seabass fry. Experiments are conducted in cylindro-conical tanks (150 liters capacity) in trial 1 and in rectangular tanks (800 liters capacity) in trials 2 and 3

TRIAL	TREATMENT	DURATION (Days)	INITIAL Number	SURVIVAL (%)	INITIAL Weight (g)	FINAL Weight (g)	DAILY WEIGHT Increase (Day)	CONVERSION Ratio	DENSITY Kg/m^3
1	Automatic	20	4 x 60	99,9	0,8	3,4	7,2	1,4 : 1	1,4
	Self-feeder	20	4 x 60	99,1	0,8	3,7	7,6	1,0 : 1	1,5
2	Starter	50	4 x 60	99,6	1,0	12,5 ***	5,05	1,01 : 1	3,7
	Grower	50	4 x 60	99,2	1,0	11,1	4,8	1,3 : 1	3,3
3	Starter	44	4 x 60	100	6,7	33	3,6	1,0 : 1	9,8
	Grower	44	4 x 60	100	6,7	31	3,5	1,0 : 1	9,3

(*** F = 7,74 DF = 6 P = 0,05).

Tab. 8. — Effect of the density on survival, growth and feed efficiency of seabass fry in 8 m^3 net cage fed on a 50 % protein and 12 % lipid compounded diet.
TRIAL 1 : Starting Oct. 24. 1985
TRIAL 2 : Starting Nov. 24. 1985

INITIAL STOCKING DENSITY (Fry/m^3)	DURATION (Months)	INITIAL Number	% Survival	INITIAL MEAN WEIGHT	FINAL MEAN WEIGHT	DAILY WEIGHT (% days)	DAILY FEEDING (% biomass)	CONVERSION RATIO (asfed)	FINAL DENSITY (Kg/m^3)
				TRIAL 1					
15	6	120	89	30,6 ± 1,2	692 ± 22	1,7	2,6	1,7 : 1	9,3
30	6	240	97	30,6 ± 1,2	634 ± 14	1,7	2,6	1,5 : 1	18,4
60	6	480	96	30,6 ± 1,2	633 ± 10	1,7	2,6	1,7 : 1	36,4
				TRIAL 2					
15	6	120	97	32,4 ± 1,2	674 ± 17	1,6	2,8	1,6 : 1	9,9
30	6	240	97	32,4 ± 1,2	677 ± 16	1,6	2,7	1,5 : 1	17,6
60	6	480	98	32,4 ± 1,2	648 ± 10	1,6	2,7	1,7 : 1	38,0

Tab. 9. — Grow out of seabass fry in 24 m^3 net cages, fed on a 50 % protein and 12 % lipid compounded diet.
Trial 1 : Fry originated from Singapore, arrival July 1987
Trial 2 : Fry originated from Singapore, arrival August 1987.

INITIAL STOCKING DENSITY DENSITY (FRY/m^3)	DURATION (months)	INITIAL Number	SURVIVAL	INITIAL MEAN WEIGHT (g)	FINAL MEAN WEIGHT (g)	DAILY WEIGHT INCREAS (% days)	DAILY FEEDING RATE (% biomass)	CONVERSION RATIO (Asfed)	FINAL STOCKING DENSITY (Kg/m^3)
				TRIAL 1					
70	6	1700	94	28,9 ± 2,0	321,3 ± 22	1,3	2,1	1,9 : 1	21,5
70	6	1700	62	33,2 ± 3,6	315,8 ± 15,3	1,3	2,4	2,9 : 1	13,9
					TRIAL 2				
68	5	1642	96,8	75,1 ± 6,1	403,2 ± 20,7	1,1	2,1	1,9 : 1	26,7
68	5	1642	96,8	77,5 ± 6,0	406,5 ± 22,1	1,1	2,0	1,9 : 1	27,0

Grow-out phase (6th to 12th months)

General results of effect of the density in net cage on survival and growth of seabass fry are indicated in Table 8. Survival rates, in all cages, exceeded 85 % after 6 months and were not correlated with the initial stocking density in a range of 15 to 60 fry per m^3. Mortality was related to accidental loss during net shifting. The final mean weight were very similar and varied in a range between 630 and 690 g corresponding to a daily weight increase of 1,6 to 1,7 % per day (Figure 3). No significant growth difference appeared as shown by results of analysis of variance ($F = 2,217$ $DF = 2,3$ NS $P < 0,266$), although the final density ranged 9,3 to 38 kg per m3. Food efficiency was satisfying and conversion ratio were not exceeding 1,7 : 1, feeding fry from 5 to 1,5 % of the body weight per day during the experiment (Figure 3).

The results of the 2 grow-out trials of seabass conducted in larger cages (24 m^3 capacity) and at high density (70 fry per m^3) are indicated in Table 9.

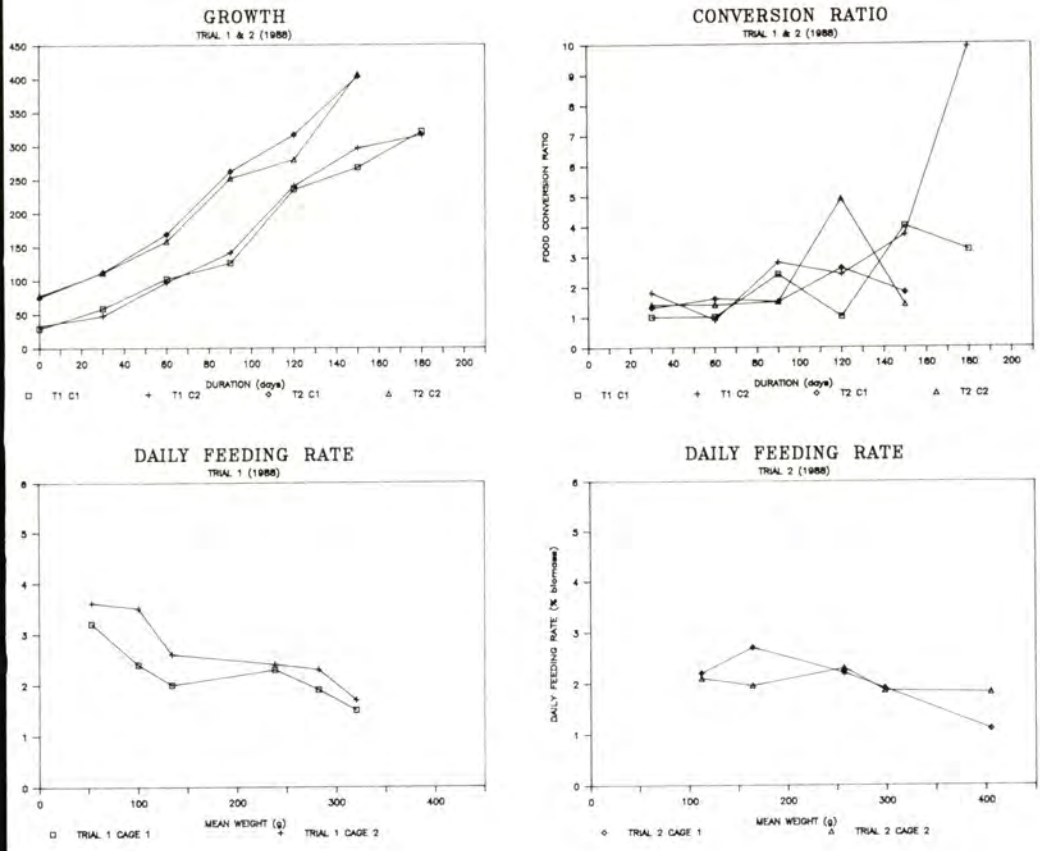

Figure 3. — Growth, conversion ratio and daily feeding rate of sea bass fry, reared in 24 m^3 netcage at an initial density of 70 fry per m^3, during 6 months and fed on a 52 % protein and 12 % lipid compounded diet (Trial 1 and 2).

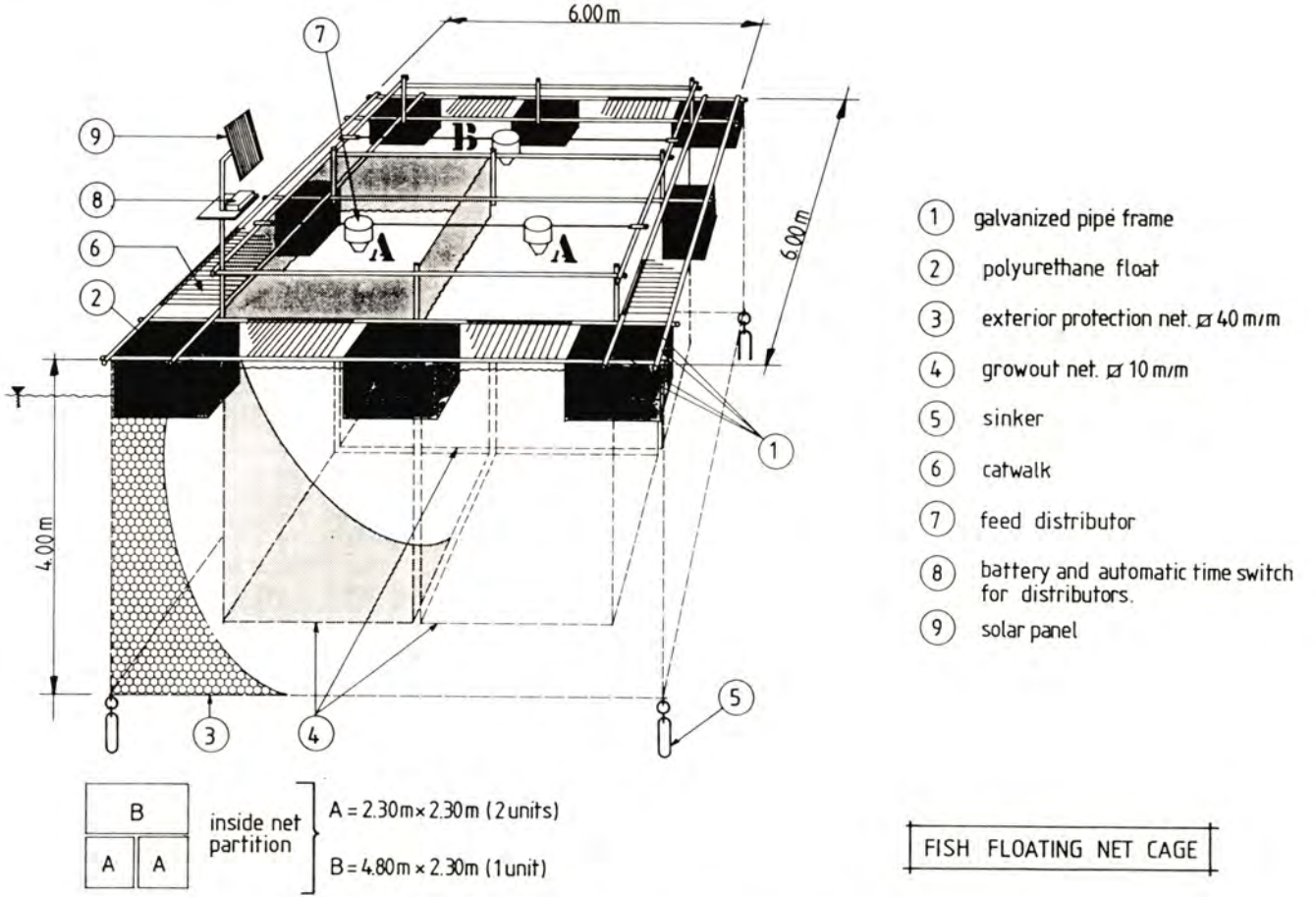

Figure 4. — Detail of the pilot-scale structure and equipment (capacity 75 m^3) for sea bass grow-out.

In both cases, survival was excellent, except in the second cage of trial 1 were 25 % of fry were eliminated after 3 months due to their low growth related to malformations. Final mean weight varied in a range of 316 ot 403 g and the daily weight increase was not exceeding 1,1 to 1,3 % (Figure 4).

The final density was 27 kg per m3 for the best trial and the conversion ratio varied from 1,9 : 1 to 2,9 : 1. Growth speed differences compared to previous experiment conducted in 8 m^3 cages suggest that fry quality and size of the cages are two parameters which need to be ıvestigated in the futur.

DISCUSSION

Among the criteria retained to select suitable finfish species for aquaculture, a number of biological factors have to be considered as growth efficiency, mortality, grow-out, disease and crowding (Wu, 1989). Economical factors as the supply and the price of the fry, the price of adult, the cost of production have a major importance in the insular situation of French Polynesia which is confronted to the problem of high freight cost, low availability of lands and high price for food and energy.

The results of the tests of selection, conducted by IFREMER from 1983 to 1987 in Tahiti have clearly revealed that only a limited number of local finfish species had a high potential for aquaculture in French Polynesia. One of the major difficulties were related to the lack of fry supply from the wild and the problems encountered to control the seed production in captivity.

Rabbitfish, *Siganus argenteus*, is the only local species which presents the characteristic to form dense schools of fry in the vicinity of reef flats after the spawning season. Thece formations seem to be related to lunar cycle (Duray, 1988) but this supply strongly fluctuate from year to year with frequent break down of the recrutment, restricting the possibility of developing a large aquaculture project based on a supply of wild fry. For the other local species, research efforts have been carried-out for a better understanding of the cycle of reproduction as for grouper *E. microdon* (Debas et *al.*, 1989) but larval rearing is not yet controlled and only a limited number of metamorphosis larvae have been obtained in captivity.

The importation of two species, originated from South-East Asia, Seabass, *Lates calcarifer*, and red Tilapia hybrid, *Oreochromis sp*, allowed to extend the programme of selection to species of economical interest where feasible seed production techniques have already been developed : Seabass in Thailand (Srikul, 1987); red Tilapia in Taiwan and Singapore (Pullin, 1984; Cheong, 1987).

After screening several biological and economical criteria on the six local and imported species, four have been retained as potentially interesting for aquaculture in French Polynesia.

The tests of selection revealed that seabass, *Lates calcarifer*, performed well and was easily adaptable to the environmental conditions of French Polynesia. The advantage of this species was related to his ability

to actively eat on a dry pelleted diet as early as the 2nd month of rearing. Marketable size (4 to 500 g mean weight) was obtained in about 12 months from the eggs, raising fry in net cages at high density which confirm preliminary tests conducted in Tahiti (Fuchs, 1986) and in Australia (Mackinnon, 1986). First breeding and larval rearing experiments have already been achieved and encouraging results have been obtained testing traditional Seabass larval rearing (Maneewong, 1986) or adapting techniques developed by IFREMER on other species as European seabass (Chatain, 1986; Ronzani Cerqueira, 1986). Interest of this species for the local market has been demonstrated and a regular supply is actually requested at a high price.

In comparison, the three other selected species, red Tilapia, Grouper and Dolphin Fish, although their high potential for aquaculture, present several drawbacks indicating that more researches have to be resumed up to solve a number of critical points.

Grouper, *Epinephelus sp* is a species of economical interest in Asia and many laboratories are developing important research on breeding and larval rearing to overcome the problem of seed production like in Singapore (Chen, 1979), Hong Kong (Tseng, 1983), Malaysia (Chua et *al.*, 1980) and Koweit (Akatsu et *al.*, 1983). Local grouper, *Epinephelus microdon* appears to be a good candidate for aquaculture in French Polynesia due to its economical interest and its ability to be raised in captivity. Research efforts have been focused on the identification of the cycle of reproduction and the description of the sex reversal phenomeneous of this hermaphrodite species (Debas et *al.*, 1989). Maturation and spawning have been achieved in captivity without encountering difficulties in opposition to other grouper as *E. tauvina* where the obtention of ripe male is a major problem. Larval rearing results suggest that *E. microdon* larval are difficult to raise. This fact actually limits his propagation in aquaculture. More investigations with possible cooperations with foreign laboratories are needed to increase the knowledges on this interesting species before reaching the larval rearing results obtained with *E. tauvina* in Koweit (Saif et *al.*, 1986). Grow-out potential has also to be investigated to confirm or not the preliminary results revealing the lower growth speed of *E. microdon* compared to other grouper species like *E. tauvina.*

Dolphin Fish, *Coryphaena sp*, is certainly a very exciting species, regarding its growth potential (2 to 6 kg in one year) compared to the other finfish species. Recent advances in Hawaii demonstrated that breeding and fry production were under control at an experimental scale when precises and detailed rearing conditions were provided (Kraul, 1989). Survival rate reached 10 % on day 40 when fry were fed on fresh natural food. Preliminary investigations have been conducted in Tahiti after the successful catch of some Dolphin fish juveniles, identified as Pompano *C. equiselis.* Their behaviour has been observed during 3 months and some maturations and spawning obtained but larval rearing results revealed unsuccesssful despite numerous feeding regime trials. This species has been retained as potentially interesting but an important research effort is necessary in order to develop adapted rearing technics. Its growth potential in floating net cage at high density has also to be verified, considering the agressive behaviour of males, observed as soon as sexual maturity appears.

Red Tilapia hybrid, *Oreochromis sp*, largely reared in Taiwan and recently adapted to sea water in intensive conditions in Singapore (Cheong, 1987) has a good potential regarding its growth speed and the easiness to produce large number of fry. The difficulties encountered in Tahiti were related to regular mortalities observed during grow-out in sea water net cages at high densities. The hypothesis of a low resistance of this strain to the rearing conditions and particularly to the high salinity was emphasized, although L. Cheong (1986) obtained good survival and growth in sea water ponds at a final density superior to 30 Kg per m3. More investigations have to be done to select a suitable strain adapted to our conditions, for example the Florida red Tilapia strain considered by Watanabe (1989) as a good candidate due to its resistance to intensive rearing conditions in sea water both in tanks or cages. Its economical interest has also to be demonstrated because the strong variation of the red color of the selected strain could break the value although its interest has been demonstrated.

The tests of selection revealed that local Rabbit fish and Caranx species, apparently interesting, were difficult to rear in captivity. The preliminary results obtained by Pillai (1962) on Rabbit fish suggested that this family had a great potential in aquaculture with an abondance of wild fry and the possibilities to feed fry with low protein diets or algae. The tests of selection, conducted in Tahiti on *Siganus argenteus*, previously studied by Tobias (1976) and Burgan et *al.*, (1979) revealed three major problems : larval rearing is not successful, compared to the encouraging results obtained by Duray (1988) on *Siganus guttatus*, the handling is delicate and his economical value is low. Among carangidae, many species have a high potential in aquaculture as Yellow tail, *Seriola quiqueradiata* in Japon, *Trachinotus carolinus and T. falcatus* in French West Indies (Soletchnik, 1988). The case of local *Caranx ignobilis* is different and this species has not been retained in the selection tests althought preliminary encouraging results have been obtained by Aquacop (1975), feeding fry with semi-moist pelleted diet. Maturation and larval rearing have never been observed in captivity and fry are not available in the wild in large number. Feeding fry with dry pellet is difficult and nutritional studies would be necessary to develop an artificial pelleted diet adapted to the requirement of the species.

CONCLUSION

Seabass : *Lates Calcarifer*, imported from Singapore appears to be the most potential finfish species in aquaculture for the next few years in French Polynesia and a project of net cage culture at a pilot-scale level has been recently drafted. The mean objective of this 3 years programme is to demonstrate the technical feasibility of its grow-out and to verify the economical interest of its aquaculture in French Polynesia. Annual productions of 20 tonnes in 5 units is expected with the participation of territorial organisms in charge of the development and the training of futur farmers.

Each pilot built in galvanized pipe frame and equiped with protected net and automatic feeders (Figure 4) is calculated to produce 2200 Kg of marketable size of Seabass (450 to 500 grams, mean weight) per cycle of 6 months, in 75 m^3 net cage, at a final density of 30 Kg per m^3. A quantity of 6000 fry are requested for each cycle of production in a pilot. Financial estimation gives a cost of investment of around 10,000 US $/unit and a cost of production of 6 US Dollars per Kg corresponding ot a net gain of 1 to 1,5 US$/Kg. In this calculation, the cost of pellet and fry corresponds to 50 and 58 % of the operating cost. Priorities of research have consequently been identified as follows :

- larval rearing and fry production cost could be easily reduced, transferring and adapting the technologies of microparticulate diet developed with success on other species as European seabass, *Dicentrarchus labrax* in France (Person Le Ruyet, 1989). Partial substitution of live preys by artificial diet would be of major interest and preliminary experiments are encouraging.
- the transfert of seabass fry in net cage as early as the 2nd month of rearing would drastically reduce the cost of production but cage design and rearing conditions have to be improved as it has been recently done in Singapore (Lim et *al*, 1986).
- nutritional requirements of seabass, initiated by Chou (1985) and AQUACOP et *al.*, (1989) have also to be completed to propose a performing formulation adapted to the needs with lower cost ingredients instead of expensive fish meals.

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Snook (*Centropomidae*) and Grouper (*Serranidae*) mariculture in the Gulf of Mexico and Caribbean Basin

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Abstract — ***Centropomidae*** *and* ***Serranidae****, sometimes collectively misnamed « Sea basses », are suitable for aquaculture in pens, ponds, and raceways around the tropical and subtropical latitudes of the world. Western Atlantic Ocean species were recently considered for farming. South Florida, Mexico, Central and South America and the Antilles areas of the Caribbean Basin have extensive areas suitable for farming groupers and snook. Principal species of interest are the common snook (****Centropomus undecimalis****), the fat snook (****C. parallelus****), the Nassau grouper (****Epinephelus striatus****), Black seabass (****Centropristis striatus****), Gag (****Mycteroperca microlepis****), and jewfish (****E. itajara****). Technology for experimental culture currently exists, but pilot nor production scale information is lacking. Adult snook and grouper are collected from wild stocks, and induced to spawn with hormones. Fry are reared in tank culture or ponds. Small numbers of fingerlings were reared in tanks. One-hundred thousand common snook phase I fingerlings were reared in 3 earthen ponds of 0.5 and 1.0 ha, respectively. Controlled maturation and spawning methods are currently being developed for both snook and grouper species. Captive broodstocks of black seabass and gag were induced to spawn in tanks using photoperiod and temperature as stimuli. Fry viability ranged from 0 % in gag to 80 % for seabass. Feed conversion for snook weighing 16-725 g ranged from 0.7-1.1 Growth, in small scale grow-out experiments, is fair (450 g in 1 year). Existing aquaculture technology for grouper and snook is summarized and compared.*

ADVANCES IN TROPICAL AQUACULTURE. Tahiti, Feb. 20 - March 4, 1989.
AQUACOP. IFREMER. Actes de Colloque **9** *p. 486.*

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Status of red Drum culture in the U.S.A.

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Abstract — *An overview of the methods of producing red drum are discussed. Although red drum culture is new compared to the culture of other species there is much interest in the production both in earthen ponds and in recirculating intensive systems. Various strategies for both methods will be discussed. This includes stocking rates, optimum growth, temperature, salinity and growth rates.*

ADVANCES IN TROPICAL AQUACULTURE. Tahiti, Feb. 20 - March 4, 1989.
AQUACOP. IFREMER. Actes de Colloque **9** pp. 487-498.

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Aquaculture of red Tilapia (*Oreochromis sp.*) in marine environments : state of the art

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Abstract — *The Caribbean Marine Research Centre is conducting research on the development of methods for intensive culture of euryhaline red tilapias in marine environments for application to Caribbean islands and similar regions with limited freshwater resources.*

The effects of salinity on growth and reproductive performance of Florida red tilapa have been determined under controlled laboratory conditions. Salinity tolerance has been studied in relation to early salinity exposure and to ontogenetic development. The results provide a basis for the development of methods for seawater adaptation that minimize reliance on freshwater during the hatchery phase of production and that improve survival and growth in seawater.

A commercial-scale tilapia hatchery is in operation on Lee Stocking Island (Bahamas), supporting research on seed production and grow-out technology under saline conditions. Research has emphasized seed production methods that conserve freshwater through culture intensification, including use of high brood fish stocking densities and artificial egg incubation, and utilization of recirculated brackishwater. The feasibility for large-scale fry production using brackishwater (12 ppt) has been demonstrated.

Grow-out of Florida red Tilapia fingerlings in seawater (37-41 ppt) has been studied in 23-m^3 above ground pools and in 1 m^3 floating cages at densities of 25/m^3 and 300/m^3, respectively, using prepared diets containing 30-32 % protein. In pools, fish fed at satiation rates were reared from 1.3 to 467 g mean weight over 170 days with a survival of 89.7 % and a food conversion ratio of 1.6. In floating cages, fish fed at similar rates were reared from 9.1 to 150 g mean weight in 84 days, with a survival of 98.2 % and a feed conversion ratio of 2.0. The faisability of reducing cost by using diets containing protein levels as low as 20 % for grow-out has been demonstrated.

The results support an excellent potential for utilization of saline-tolerant red tilapias for intensive culture in tropical marine environments.

INTRODUCTION

The Caribbean Marine Research Centre (CMRC) is a private, non-profit research organization based in Riviera Beach, Florida, with principal research facilities on Lee Stocking Island, Exuma Cays, Bahamas. Since July 1984, CMRC has undertaken a programme of research aimed at developing methods for intensive saltwater culture of euryhaline tilapias for application to Caribbean Islands and similar regions where freshwater resources are often limiting (Watanabe *et al.*, in press-b).

Red hybrid tilapia are gaining popularity among culturists due to their resemblance to premium marine species such as sea bream (*Chrysophrys major*) and red snapper (*Lutjanus campechanus*) (Fitzgerald 1979; Liao and Chen 1983; Fassler 1984; Stickney 1986) and excellent growth and feed conversion rates in freshwater (Liao and Chen 1983). An excellent potential for market development exists for fresh product in Japan (Fassler 1984) and the U.S. West Coast (Trosclair 1988). In Caribbean Islands such as the Bahamas (J. Thompson), Curacao (H. Martina and R. Hoseth) and Martinique (J.-P. Marion), a domestic market for red tilapias has been created by the inability of marine catches to satisfy demand for fish (personal communications). Commercial tilapia farming in the Caribbean is successfully established in Jamaica, where red hydrid strains are preferred by consumers (Chakallal and Noriega-Curtis in press). Export of red tilapia fillets to Florida has recently begun. Production for 1987 is estimated at 2,600 mt from 720 ha of fresh and brackishwater (0-10 ppt) ponds. As water resources in Jamaica are scarce, water supply is considered as an important constraint to further expansion of aquaculture (Chakallal and Noriega-Curtis in press).

While the genetic heritages of the existing varieties of red tilapias are not well documented, their derivation is generally attributed to cross-breeding of mutant reddish-orange *Oreochromis mossambicus* (a normally black species) with other species including *O. aureus, O. niloticus* and *O. hornorum* (Fitzgerald 1979; Behrends *et al.*, 1982; Galman and Avtalion, 1983). The suitability of red hybrid tilapias for brackish - and seawater culture is suggested by the salinity tolerance exhibited by these parental species, which are known to be moderately (*O. niloticus* and *O. aureus*) to highly (*O.mossambicus* and *O. hornorum*) euryhaline (Philipart and Ruwet 1982; Stickney 1986).

The feasibility of rearing red hybrid tilapia in brackish and seawater was first studied by Liao and Chang (1983) who reported good growth of Taiwanese red tilapia (*O. mossambicus* and *O. niloticus*) at salinities of 17 ppt and 37 ppt, although fish appeared susceptible to handling stress. Seawater-rearing studies of Taiwanese red tilapia in Kuwait (Hopkins *et al.*, 1985) showed that survival at 38-41 ppt was impaired at water temperatures below 24°C.

A red tilapia strain originating in Florida, USA was selected by CMRC for seawater culture trials in the Bahamas due to the high salinity tolerance of both its parental species (*O.hornorum and O. mossambicus*). Following a preliminary study which showed higher growth and feed conversion of juvenile, monosex males in brackish and seawater than in

freshwater (Watanabe *et al*,. 1988a), detailed studies on culture methodology were initiated. Experimental work at CMRC has sought to obtain basic information on the biology of the Florida red tilapia with respect to salinity tolerance as well as to assess production performance in seawater.

Origin of the « Florida red » tilapia strain

According to commercial culturists responsible for their development (C. Harris and R. De Wandel personal communication), red tilapia hybrids originating in the United States were first developed in the late 1970's by a commercial fish breeder (M. Sipe) in Florida. Inbredding of a population of *O. mossambicus* produced a mutant with reddish-yellow pigmentation, which was selectively bred to enhance the red and yellow coloration, resulting in a market decline in growth and body shape. In order to restore these qualities, mutant male *O. mossambicus* was crossbred with female *O. hornorum* (a black colored species) to produce a first generation (F1) red hybrid. F1 red hybrids were sold to producers for grow-out. In addition, black *O. hornorum* females and red *O. mossambicus* males were sold as broodstock to farmers, who could produce hybrids for grow-out, but could not generate additional pureline broodstock. In 1981, other commercial culturists began a selective breeding program with the F1 hybrids and, by the end of 1983, developed a relatively true breeding strain of red tilapia. Fish descended from F1 progeny became known to aquaculturists as the « Florida red » strain.

THE EFFECTS OF SALINITY ON GROWTH OF FLORIDA RED TILAPIA

Growth of juvenile, monosex males at different salinities

Little information is available on the influence of salinity on growth in tilapias. For most species, optimal ranges of salinities for growth have been inferred from data on natural distributions and/or fragmentary experimental evidence.

The effects of salinity on growth of juvenile, monosex-male Florida red tilapia were studied under controlled photoperiod (12 L : 12 D) and temperature (28°C). A high euryhaline capacity of the Florida red tilapia strain was evidenced by faster growth rates in brackish and seawater than in freshwater, although results appeared to be modified by stocking density (Watanabe *et al.* 1988.a). At a high density (20 fish/200 l tank), growth in freshwater was comparable to growth at 10 ppt and above. Growth under 36 ppt at a low density (10 fish/tank) was lower than that at a high density. At an intermediate density (15 fish/tank), however, there was a clear trend toward increased growth with salinity due to increased feed consumption and declining feed conversion ratios with salinity (Watanabe *et al.* 1988a). These results support previous reports of faster growth in brackish and seawater than in freshwater in certain tilapias including *O. mossambicus* (Canagaratnam, 1966; Jurss et al. 1984) and Taiwanese red tilapia hybrids (*O. mossambicus* and *O. niloticus*) (Liao and Chang 1983).

The influence of behavior on growth at different salinities

The apparent density-dependent differences in growth response to salinity observed in these studies suggested that behavioral factors influenced these results. Further investigations revealed that agonistic encounters among fish as well as percentages of fish with damaged fins (due to agonistic encounters) declined with salinity, suggesting that growth response to salinity was influenced by inhibitory effects of territorial aggression, which was mitigated by increasing salinity (Watanabe *et al.*, 1988b). This suggested that agression impairs growth by lowering feed consumption (appetite) and increasing conversion ratios. Hence, as agression was mitigated by increasing salinity, growth was improved. That behavioral interaction may exert inhibitory effects on growth which vary with salinity. It was previously suggested for Taiwanese red tilapia (Liao and Chang 1983).

COMMERCIAL-SCALE HATCHERY FOR SALTWATER TILAPIA CULTURE

The general procedures developed by CMRC for culture of Florida red tilapia in seawater are relatively simple : Spawning occurs naturally in brood tanks maintained under low salinity (3-6 ppt groundwater). Periodically, free-swimming fry are collected from the pools while unhatched eggs and yolksac fry are removed from mouthbrooding females and incubated artificially. Yolksac-absorbed fry are fed a diet containing an androgenic hormone (17 alpha-ethynyltestosterone) for 28 days to transform genotypic females to phenotypic males (Guerrero 1975). Monosex culture prevents unwanted reproduction at an early age which results in overcrowding and stunting during grow-out and minimizes the possibility of reproduction outside of the hatchery and the likelihood of unwanted introductions. After sex-reversal, fry (approximately 0.8-0.9 g in weight) are acclimated to seawater over a period of 1 week, then transferred to nursery tanks where they are grown to large fingerling sizes (approximately 5-10 g) prior to stocking in tanks or sea cages for grow-out.

An experimental tilapia hatchery supporting research on seed production and grow-out technology under saline conditions, has been in operation since April 1987 on Lee Stocking Island, Bahamas. The design and operation of this commercial-scale facility, consisting of six 34 m2 broodfish tanks, sixteen 560 l rearing tanks for sex reversal of fry, and eight 4.9 m3 tanks for seawater acclimation of sex -reversed fry, is described in details by Ernst (In press). The hatchery incorporates a system for recirculation of water through biofilters, a critical design feature in the Bahamas where limited groundwater resources must be conserved. Multiple recirculation systems permit simultaneous testing of separate salinity regimes for broodstock holding and sex-reversal so that optimal salinities for maintaining broodstock and rates of acclimation of fry to seawater may be determined experimentally. Spawning, incubation of eggs and sex-reversal of fry may be conducted at any salinity up to that of full seawater (36-37 ppt).

DEVELOPMENT OF METHODS FOR ADAPTATION TO SEAWATER

While the suitability of the Florida red tilapia strain for seawater grow-out has been demonstrated by high growth rates and feed conversion efficiencies, the hatchery phase of production remains restricted to water of lower salinities. The requirements for low-salinity water for maintaining broodstock and for early fry rearing restricts the siting of future hatcheries to areas where low-salinity water is available, ultimately affecting the ability of farmers to obtain fingerlings. Methods for seawater adaptation have been developed that minimize reliance on low-salinity water during the hatchery phase of production and that maximize survival and growth following transfer to seawater.

Selection of optimal life stage for seawater transfer

Low-salinity water requirements during the hatchery phase of production may be reduced by acclimating stocks to seawater at early stages of development (Watanabe *et al.*, 1985a-b). However, as salinity tolerance in tilapias varies ontogenetically, survival and growth in seawater may be affected by the life stage at which acclimation to seawater is initiated (Watanabe *et al,*. 1985b).

Salinity tolerance was determined in Florida red tilapia, spawned under 5 ppt, at 10, 25, 40, 55 and 70 days post-hatching, using the 96 hours median lethal salinity (MLS- 96) index. A trend toward increased tolerance with age was observed, with tolerance improving markedly from 40 days post-hatching (Watanabe *et al,*. unpublished date). To assess the influence on culture performance of age at which transfer to seawater (37 ppt) is initiated, survival of progeny acclimated to seawater beginning at 10, 25 and 39 days-hatching was compared. Survival to 48 days post-hatching improved as transfer was delayed, from 20.0 % in progeny beginning acclimation at 10 days post-hatching, to 55.9 % in progeny beginning acclimation at 39 days post-hatching. The results demonstrate that premature transfer to seawater can impair survival and that, selection of proper transfer time, based on knowledge of ontogenetic variation in salinity tolerance, can improve survival.

Production of seedstock in brackishwater

Another approach to reducing low-salinity water requirements during the hatchery phase of production is to maintain and spawn broodstock at elevated salinities. This approach is generally limited by the fact that, in tilapias, normal reproduction is inhibited by increasing salinity (Ridha *et al,*. 1985; Watanabe and Kuo, 1985).

The reproductive performance of yearling Florida red tilapia broodstock was studied in laboratory aquaria at salinities of 1 (freshwater), 9, 18, 27 and 36 ppt under controlled photoperiod (14 L : 10 D) and temperature (28 °C) (Watanabe *et al.*, in press-a). Spawning was observed at all salinities, although an inhibitory effect of salinity on reproductive perfor-

mance was manifested by a trend toward lower fertilization, hatching, and survival of prejuveniles with increasing salinity. Fry production per unit female weight declined at salinities above 18 ppt. The results suggest that Florida red tilapia broodstock may be maintained under salinities as high as 18 ppt without impairing fry production, further suggesting that hatchery production in brackishwater would be practical in areas where freshwater resources are limiting.

Influence of spawning salinity on survival and growth in brackish and seawater

To assess the influence of spawning salinity on survival and growth in brackish or seawater, growth of juveniles, spawned at salinities of 4 and 18 ppt, were compared at rearing salinities of 18 ppt and 36 ppt, in 200 l aquaria under controlled photoperiod (12 L : 12 D) and temperature (28° C). Under both rearing salinities, growth was significantly higher for progeny spawned at 18 ppt than those spawned at 4 ppt, suggesting that progeny spawned under elevated salinities are better adapted for growth in brackish and seawater (Watanabe *et al.*, 1989).

In another experiment, growth of juvenile progeny spawned and sex reversed at salinities of 2 ppt and 1.8 ppt was compared in 24 m3 outdoor pools at 36 ppt. When water temperatures exceeded 27° C, growth and survival were not significantly different between these groups. However, when temperatures abruptly fell below 25° C, growth and survival remained significantly higher among progeny spawned at 18 ppt (Watanabe *et al.*, 1989). This suggests that brackishwater-spawned progeny possesses a higher resistance to cold-stress in seawater than freshwater-spawned progeny. As Florida red tilapia have been overwintered in seawater tanks (37 ppt) in the Bahamas under water temperatures as low as 16° C without adverse effects (Watanabe et al., unpublished data), low-temperature tolerance may be related to the rate of temperature decline, rather than to a critical lower limit.

Large scale seed production in brackishwater

At the CMRC tilapia hatchery, broodstock are maintained in 34 m^3 broodtanks at a ratio of 180 females to 60 males. Based on the results of laboratory studies in which successful reproduction in brackishwater and improved seawater survival and growth of brackishwater-spawned progeny were observed, brood tank salinities were increased in 1988 to 12 ppt by mixing groundwater with seawater. Rates of seed (eggs, yolksac fry and free-swimming fry) production in broodtanks were monitored under the egg removal method of broodstock management, in which eggs as well as yolksac fry are removed every 16 days for artificial incubation. Data for an 84 days period during March-May 1988 showed that each broodtank produced a mean of 188,000 eggs and yolksac fry and 79,808 free swimmming fry (3188 seed/day or 93.8 seed/m^3/day) (Watanabe *et al.*, unpublished data), a higher production rate than reported for intensive seed production of *O. niloticus* (73 seed/m^3/day) (Hughes and Behrends, 1983). Average survival of eggs and yolksac fry through artificial incuba-

tion was 66.5 %, while survival through the 28 days sex-reversal period was 73.9 % for artificially incubated fry and 49.7 % for naturally incubated fry, yielding 1572 sex-reversed fry/day, or 46.2/m^2/day. The feasibility for large-scale production of Florida red tilapia fry in brackishwater (12 ppt) was demonstrated.

Tab. 1. — Summarized data on seawater rearing experience with monosex-male Florida red Tilapia hybrids in 23.2 m^3 aboveground pools and in 1.0 m^3 floating cages[a] (Data are based on 4 replicate culture units)

	Culture system	
	Floating cages	Aboveground pools
Stocking data		
Initial wt. (g)	9.1	1.3
Density (number/m^3)	300	25
Initial biomass (kg/m^3)	2.73	0.03
Growth data		
Culture duration (days)	84	170
Feeding rate (% body wt./day)[a]	9.9 declining to 3.3	>30 declining to 1.5
Daily weight gain (g/day)[b]	1.68	2.74
Specific growth rate (%/day)[c]	3.34	3.46
Harvesting data		
Survival (%)	98.2	89.7
Final wt. (g)	150	467
Final biomass (kg/m^3)	35.2	10.5
Feed conversion ratio (dry wt./wet wt.)	2.0	1.6
Culture conditions		
Salinity (ppt)	34 - 41	36 - 39
Temperature (°C)	26 - 33	22 - 30
Dissolved oxygen (ppm)	3.0 - 5.2	4.3 - 6.7

[a] Fish were fed commercially prepared diets containing 32 % (floating cages) or 30 % (aboveground pools) protein.
[b] (final wt - initial wt.)/number of days.
[c] 100 (Ln final wt - Ln initial wt.)/number of days.

REARING EXPERIMENTS IN MARINE CAGES

An experiment was conducted to assess the feasibility of rearing Florida red tilapia in floating cages at a marine site on Great Exuma, Bahamas. Survival and growth of monosex males (9.1 g mean weight) were studied in cages (1 m^3, 12.7 mm mesh) stocked at density of 300/m^3. Fish were fed three times daily a commercially prepared diet containing 32 % protein at a satiation rate. Over 84 days, feeding rate declined with increasing fish size from 10.5 % to 3.3 % body weight/day. After 84 days, mean fish weight was 150 g and average daily weight gain was 1.82 g (J. Clark *et al*,. unpublished data) (Table 1), a growth rate comparable to that observed in seawater pools (Ernst *et al.*, in press) when data on growth over a similar size interval were compared. Feed conversion ratio for the 84 days period was 2.04.

Temperatures remained above 28° C during the first 62 days but showed relatively abrupt declines during the final 22 days, with minimum temperatures falling to 26° C during this period. Salinity fluctuated over a relatively wide range (34-41 ppt) and was affected by tides and rainfall. Ambient dissolved oxygen levels ranged from 4.0 to 5.8 ppm, while in-cage levels were below ambient and decreased over time as cage biomass increased, falling to a low of 3.0 ppm and indicating that cage carrying capacities were approached. While no information is available in critical dissolved oxygen levels for tilapias reared in seawater cages, a dissolved oxygen of 3.0 ppm is considered a minimum below which adverse effects appear during freswater cage culture (Coche 1982). When the experiment was terminated after 84 days, survival was 98.2 % and final biomass was 35.2 kg/m^3. Evidence of disease (i.e., clouded eyes, external hemorrhagic areas, and reduced feeding) observed during the final 14 days of the study were likely related to stress induced by the combined effects of declining in cage dissolved oxygen and declining temperature.

Although the feasibility for culture of Florida red tilapia in floating marine cages was demonstrated by high growth and feed conversion rates, further studies are required to assess long-term growth under conditions in which dissolved oxygen is not limiting. In cage dissolved oxygen can likely be improved by utilization of lower stocking densities and increasing cage mesh size with fish growth, to improve circulation.

REARING EXPERIMENTS IN SEAWATER POOLS

Survival and growth of monosex male Florida red tilapia (1.3 g mean weight) was studied in 23 m^3 above ground pools stocked at a density of 25 fish/m^3 (Ernst *et al.*, in press) (Table 1). Pools were provided with aeration and with flow-through seawater (37 ppt) at 2.5 - 5.0 exchanges per day. Temperatures ranged from 22.0 to 29.5°C during the experiment, while dissolved oxygen averaged 5.6 ppm.

Fish were fed daily a commercially prepared diet containing 30 % protein at a satiation rate, defined as the percentage of body weight consumed in three 30 minutes feeding periods. Over 170 days, feeding rate declined with increasing fish size from >30 % to 1.5 % body weight/day. After 170 days, mean fish weight was 467 g at a survival rate of 89.7 %. Average daily weight gain was 2.74 g, a growth rate superior to those reported for Taiwanese red tilapia reared brackish and seawater (0.43-1.21 g/day) (Liao and Chang, 1983; Neriwether *et al*,. 1984; Hopkins *et al.* 1985) and comparable to that achieved with Taiwanese red tilapia under intensive freshwater culture, where fish are grown from 1 to 500 g in 150 to 180 days (2.77 - 3.33 g/day) (Liao and Chen, 1983). Feed conversion ratios for the 170 days period was 1.6. A high growth capacity and excellent feed conversion ratios in seawater from fingerling through market stages were demonstrated.

GROWTH, FEED AND PROTEIN UTILIZATION ON DIETS WITH DIFFERENT PROTEIN LEVELS IN SEAWATER POOLS

Growth, feed and protein utilization of monosex male Florida red tilapia fingerlings (10.6 g mean wt.) fed isocaloric diets with different protein levels (20 %, 25 % and 30 % protein) were studied through adult, marketable sizes in seawater pools (10 m^3) stocked at a density of 25 fish/m^3 (A. Clark *et al.*, unpublished data). Pools were provided with aeration and with flow-through seawater (37 ppt) at 7 exchanges per day. Average maximum/minimum temperatures were 32 ± 1°C / 28 ± 1°C during the experiment, while dissolved oxygen averaged 5.1 ppm.

Growth rates were high for all diets, with mean weights ranging from 440 to 464 g after 120 days, and survival ranging from 97.0 - 97.5 %. Feed consumption decreased with increasing body weight, with average consumption declining from 11.3 to 2.57 % body weight/day during the experiment. While there were no significant differences among diets in mean daily weight gain (3.60 - 3.75 g/day) or feed conversion ratios (2.01 -2.20), protein efficiency ratio was significantly higher in the 20 % protein diet (2.41) than at higher protein levels (1.74 - 2.04). The results demonstrate that Florida red tilapia can be reared in seawater from fingerling through marketable sizes more economically levels and suggest that reasonable growth rates may be maintained at protein levels lower than 20 %.

FUTURE RESEARCH REQUIREMENTS

Available evidence suggests that in tilapias, resistance to cold-stress is lowered under high salinities due to the interactive effects of temperature and salinity on osmoregulation (Allanson *et al,*. 1971; Tilney and Hocutt, 1987). Whereas *O. aureus, O. niloticus* and Taiwanese red tilapia hybrids (*O. mossambicus* x *O. niloticus*) exhibited heavy mortalities in seawater cages (30 ppt) under seasonally declining temperatures, considerably fewer mortalities were observed among fish reared at salinities of 8 ppt (Ting *et al,*. 1984). Furthermore, red tilapia growth rates were higher at 16 ppt than at 8 ppt or 30 ppt under these conditions. Increased incidence of disease under seasonally declining temperatures was previously observed in Florida red tilapia reared in seawater pools (Ernst *et al,*. in press).

Studies are required to assess the effects of salinity on low-temperature tolerance in Florida red tilapia, to identify causative agents of overwintering diseases, and to develop methods for prevention or treatment. Introgressive breeding may be a potentially important technique for developing cold-tolerant strains (Behrends and Smitherman, 1984).

Studies are also needed to determine the combined effects of dissolved oxygen, salinity, temperature and photoperiod on survival and growth of Florida red tilapia in order to define environmental conditions suitable for saltwater culture of this strain and to develop methods for maximizing survival and growth by environmental control.

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Selection of finfish species for aquaculture development in Martinique (F.W.I.)

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Abstract — *Since 1981, in Martinique, a programme of selection of fishes suitable for aquaculture has been conducted. The criteria were adaptability to specific tropical environments and to rearing conditions. Zootechnical and socio-economical constraints have led to the choice of three species, endemic and exotic : the palometa (**Trachinotus goodei**), the red drum (**Sciaenops ocellata**) and the red florida hybrid (**Oreochromis** sp.).*

Palometa has shown good growth performances : 400 g in 7 months from 17 g mean weight fingerlings. It accepts a commercial artificial diet and is quite resistant to pathologies. Control of maturation and spawning either natural or induced by hormonal injection appeared rather difficult. For two years trials held over, 88 females were injected, giving only 43000 viable eggs. That is the reason why larval rearing could rarely been tried. Other techniques to control the reproduction should be investigated.

Red drum is an exotic species from the gulf of Mexico. Thanks to the numerous works from the USA, encouraging results have been obtained in a short time on larval rearing and grow-out. Eggs were imported from Texas and Florida. Best survival rate after two months rearing reached 17 percent (fry from 2 to 5 g). In grow-out, the expected result is 300 g within 6 months from hatching.

*Red tilapia was introduced in Martinique in 1986. The strain reared is the red florida hybrid : (**Oreochromis mossambicus** X **O. urolepis hornorum**) X (**O. aureus**). Broostock, larvae and fingerlings are reared in brackish water (19 ppt). Grow-out is conducted in tanks (recirculated freshwater and seawater) and in net cages in the sea.*

*Prospects of a prompt development of the aquaculture of palometa are poor. Reliable rearing technology has to be set up for this species. On the contrary, tilapia hybrid culture is on the development either in sea water cages or as an alternating crop for giant freshwater prawn (**Macrobrachium rosenbergii**) farms. In the same time red fish is on the experimental phase with three sites of grow-out with a local commercial diet. These two latter species appear well suited for aquaculture development.*

INTRODUCTION

Martinique, French West Indies, appears as a specially suitable place to develop aquaculture. Its situation, among the Caribbean islands, its privileged relation with France, and its geographical characteristics offer many advantages to develop such an activity. The eastern coast provides a lot of sites well protected by coral reefs. A clean and warm oceanic stream supplies throughout the year good quality water with relatively constant temperature and salinity. The limited tourist and industrial development protects this island against pollution. The population is traditionnally an important consumer of seafood. But local fisheries only supply 50 % of the demand. Thus, a large quantity of seafood is imported... Martinique profits by the help provided by the French Government and especially the presence of IFREMER research centre.

In 1981, a programme began for the selection of the most suitable finfish species for aquaculture in tropical areas. This programme started with native species selected for their availability in the wild and their high value on the local market. These species were : *Ocyurus chrysurus* (yellowtail snapper), *Lutjanus analis* (mutton snapper), *Lutjanus apodus* (school master), *Lutjanus synagris* (lane snapper), *Lutjanus griseus* (gray snapper), *Trachinotus goodei* (palometa) and *Trachinotus falcatus* (permit). Wild-caught juveniles were placed in net floating cages and fed a commercial compounded food. Survival and growth were monitored to determine their rearing abilities. Experiments on maturation and spawning were attempted to know about their reproduction.

After a first selection, the programme extended to exotic species as red drum (*Sciaenops ocellata*) and the red florida hybrid (*Oreochromis* sp.). Lot of works were achieved in spawning and rearing these species and the feasibility of culturing red drum and red tilapia has already been proved successfull in the USA. In Martinique, the first experiments were focused on intensive larval rearing. Presently, a programme is underway on broodstock management and reproduction for both species. In the same time, several pilot-scale farms have been initiated to determine economic feasability to rear red tilapia as fish food for human consumption.

SELECTION PHASE

The main criteria of selection can be summarized in three parts : grow-out, maturation and spawning, and fingerlings production.

Grow-out phase

For both native and exotic species, the grow-out trials were always conducted in net floating cages moored in open water (René et Haffner, 1982). These facilities are less expensive for construction and power and do not require coastal land for which demand and price are high. An exception is made for red tilapia raised also in fresh water as an alternating crop for the giant freshwater prawn (*Macrobrachium rosenbergii*). In that

case tanks or ponds are used. The diets were commercial pellets previously formulated for the european seabass (*Dicentrarchus labrax*) (50 % protein) and for the red tilapia (35 % protein).

The species were preselected for their adaptability to cage rearing and to formulated pelleted food (table 1). This table summarizes the effort on these species and overall characteristics with emphasis on growth performances.

As a result of these trials, *Lutjanus synagris* and *L. apodus* averaging less than 250 g in two years were eliminated. *Ocyurus chrysurus* growth is somewhat higher but not very fast. However it has been kept for further experiments due to the abundance of juveniles in the wild and its high market price.

Lutjanus analis, L. griseus and *Trachinotus falcatus* present a fast growth but are rather rare in the wild. So their selection will be determined by the next criteria.

Tab. 1. — Synthetic results on grow-out phase of preselected species

SPECIES	Origin	Growth experiments period	Number of fish (g/ month)	Growth from fingerlings	Pathology
Trachinotus goodei	Wild	10/81-11/84 05/85-8/86 06/86-12/86	60 50 120	300/6	Parasitism
Trachinotus falcatus	Wild	10/81-11/84	40		
Ocyurus chrysurus	Wild	10/81-01/85 10/85-9/86 06/86-12/86	20000 500 500	300/18	Nutritional disease
Lutjanus *analis* *griseus* *apodus* *synagris*	Wild	10/81-4/84	1000	350/12 370/24 250/24 140/21	Nutritional disease
Sciaenops ocellata	Hatchery	07/87-1/88 08/88-1/89	4000 6000	500/6	No
Oreochromis	Hatchery	Since 1987		350/6	Parasitism in full sea water

Maturation and spawning

The selection under these criteria was determined by the availability of broodstock. If collection efforts for broodstock were not successful, age of first maturity was determinant. Experiments were conducted on maturation to know about the spawning season, the response to environmental stimuli and/or to hormonal induction. Table 2 summarizes the

Tab. 2. — Main sexual characteristics and reproduction methods

SPECIES	Origine broodstock	Age first maturity	Spawning season	Broodstock studied	Spawning	Main problems
Trachinotus falcatus	Wild-caught juveniles	4 years (ADAM data)	—	0	No	—
Trachinotus goodei	raised in cages	1-1,5 year (IFREMER data)	Throughout the year (IFREMER data)	100	Hormonal injection	Viability of eggs
Ocyurus chrysurus	Wild-caught Juveniles and adults	2 years (IFREMER data)	March October (IFREMER data)	200	Natural & hormone	Low fecundity
Lutjanus analis	None	5-6 years (Claro, 1981)	March-August (Claro, 1981)	0	No	—
Lutjanus griseus	None	1 year (Claro, 1983)	July-october (Campos, 1975)	0	No	—
Sciaenops ocellata	Juveniles from hatchery Texas raised in cages	2-5 years (Chamberlain, 1986) artificial	Fall Natural or 1989	o 20 o excepted	No	
Oreochromis sp.	Juveniles from hatchery (Jamaïca) raised in tanks in Martinique	4 months (ADAM data)	Throughout the year	100 o 500 o	Natural (brackish water)	Low fecundity

different characteristics of sexual life and the trials carried out on the selected species. On three of these, no attempts were conducted because of the lack of broodstock. One because of the little number of fish studied (*L. griseus*), the other because of the late age of first sexual maturity (*L. analis, T. falcatus*). So, these species have not been selected for the moment.

Trachinotus goodei presents early age of first maturity and responds to hormonal induction. But very few eggs of good quality have been obtained. Trials are underway to improve egg viability. *O. chrysurus* was spawned using either injection of HCG or without induction (natural spawn).

Spawns of red drum are expected in 1989 by manipulating temperature and photoperiod cycles. Broodstock has been raised in ADAM and IFREMER facilities. Substancial progress has been quickly achieved in spawning tilapia.

Larval rearing-Production of fingerlings

Larval rearing trials have been conducted on four species; *T. coodei, O. chrysurus* and *Oreochromis* sp. from local spawns, *S. ocellata* from imported eggs (Texas and Florida). Larval culture was carried out in intensive way in hatchery. A flow-through system supplied clear sea water. The alimentation scheme included live food (rotifers and artemia) and commercial weaning pellets. The criteria of feasibility were success of first feeding, survival at metamorphosis, fast weaning and overall survival. The level of research and the problems encountered were different for each species (table 3.).

Tab. 3. — Synthetic results on larval rearing with selected species

SPECIES	Number of trials	Origin of eggs	Number of trials giving fingerlings	Critical stage	Problem
Trachinotus goodei	3	Local spawn	0	First feeding	Quality of eggs
Ocyurus chrysurus	27	Local spawn	1	First feeding	Feeding quality
Sciaenops ocellata	11	USA (texas) Florida	7	Metamorphosis	Cannibalism & pathologies
Tilapia	Routine	Local spawn	All	No	—

The feasibility of rearing red drum and red tilapia larvae has already been proven successful, even if further progress and improvement have to be developed. On the contrary, it seems rather difficult to get yellowtail snapper fingerlings with such a method. That is the reason why, in relation with its slow growth, this species has been eliminated. It is too early to say something about *T. goodei* according to the few larval rearings attempted on this species.

SELECTED SPECIES

Presently, as a result of the selection, works in Martinique are focused on three species : the palometa (*Trachinotus goodei*), the red drum (*Scianops ocellata*) and the red florida hybrid (*Oreochromis sp.*).

THE PALOMETA : *Trachinotus goodei*

Results

The palometa fed commercial pellets (50 % proteins) in cages reached 300 g in 6 months from 15 g juveniles. Another trial demonstrated that trash fish as food provides a similar growth (260 g in 5 months). Overall mortality from juveniles to marketable sized fish (300-400 g) fed pellets never exceeded 17 % and 10 % fed trash fish. The conversion rate, when fed trash fish, is about 2.3 :1.

Tab. 4. — Control of reproduction trials on *Trachinotus goodei*

EXPERIMENTS	Broodstock Feeding	Hormone injected	Dose per Kg	Number of trials	Number of spawns	Viability rates (%)	Improvment
1	Fresh food	–	–	7 months	1	70	Natural management
2	Trash fish	HCG	2 x 50 to 600 UI	10	6	0	–
3	Trash fish + fresh food + pellet	HCG	2 x 500 UI	10	3	0	Food
	IDEM	LHRH(a)	10 mg	10	3	0	Hormone
4	Pellets	HCG	2 x 500 UI	10	4	0	–
	Fresh food	HCG	2 x 500 UI	10	3	0	Food
5	Fresh food	HCG	2 x 500 UI	38	14	16-62 % (8 spawns)	No stress

The monitoring of fishes by regular biopsies suggests that the palometa can be found at final ripening stage throughout the year with two peaks of higher sexual activity (August and February). Age of first maturity occured at 1 to 1.5 years when fishes reach a weight of 300 g for males and 350 g for females.

One spawning only occured in a seven months experiment under natural environmental conditions in tank. Egg viability was 70 %. But this species was spawned many times using injection of hormones. For two years, 88 females were induced by hormonal injection (table 4), 33 spawnings occured and only 7 with viable eggs. Viability rates range from 16 to 62 %.

Discussion

The growth of palometa fed commercial pellets (Bachellier and Thouard, 1983) and trash fish (Soletchnik, 1988) is somewhat higher than the growth obtained in Venezuela by Gaspar (1977), and Gomez and Cervigon (1984) with fresh fish as food. If the feed conversion rate looks satisfying (2.3 :1), it has not been possible to determine the conversion rate with pellets because of the important loss of food in the cages. Sinking pellets seem inadequate for these fishes of pelagic behaviour. Part of pellets fall through the bottom net before being eaten. A very slow automatic feeder or the use of buoyant pellets should alleviate this difficulty. A technological effort has to be achieved in that way. During the grow-out phase, the palometa have suffered pathological events, due to parasitical infestation by the monogenean fluke *Neobenedenia melleni.* Efficient treatments exist to get rid of this parasite (fresh water dip or Trichlorfon) (Loyau, 1985). Five years of experiments in rearing *T. goodei* have shown that this parasitism was not restrictive (Gallet et *al.*, 1986).

It seems really difficult to spawn palometa naturally without hormonal treatment (1 spawn in 7 months). This has also been observed by MOE et *al.*, (1968) working on pompano (*Trachinotus carolinus*). Although progress were achieved in spawning this fish by hormonal treatment, spawning frequency remains moderate and most spawns are unviable. Ovules are frequently retained reaching an overripening stage. Additionally, ovarian regression was observed as for *T. carolinus* (Hoff et *al.*, 1978). Whatever the improvement in food and whatever the hormone used (Soletchnik, 1988) egg viability remains nil. According to Hoff et *al.*, (1978) excessive handling stress can bring about regression or bad quality spawnings. In experiment 5 (table 4) the main objective was to reduce handling stress and the results were little better. Another trial is underway with older animals. They might produce more and better spawns as noticed by Moe *et al.*, (1968) on *T. carolinus.* Recent works suggest that spawning might be related to moon cycles.

Unfortunately, because of bad quality eggs, very few larval rearing attempts have been made on this species.

THE RED DRUM : *Sciaenops ocellata*

Results

Imported from Texas (University of Texas, Marine Lab. C.R. ARNOLD) by ADAM in 1985, the first batch of juveniles (4 g body weight) was raised in net floating cages and fed a commercial pelleted food (European Seabass : 50 % protein). This first trial was to demonstrate the ability of red drum to be reared in caribbean environment. In four months they averaged a weight of 200 g and the survival was close to 50 %. A second trial, with fingerlings from the IFREMER hatchery, was conducted in 1987. Three gram fingerlings reached an average weight of 280g in 6 months fed a 54 % protein diet and a weight of 205 g fed a 37 % protein pelleted food. The conversion rates were about 2.2 :1 and 3.7 :1 for the two foods respectively. Survival averaged in both case 60 %. The best growth was oberved, on an experimental farm, where three grams fingerlings

averaged 240 g in a 4 months grow-out phase; the conversion rate was 1.6 :1 with a 54 % protein commercial food.

From the first batch, 40 fishes were isolated as broodstock. Their average weight at the end of 1988 was 5-6 kg. Spermiant males were noticed since the beginning of 1987. IFREMER has just completed a controlled environment building for maturation studies and the aim for 1989 will be to demonstrate the feasibility of inducing red drum to spawn by manipulating temperature and photoperiod cycles in Martinique.

Since 1987, studies are underway to refine intensive larval rearing techniques. While waiting for local spawns, eggs have been imported from Texas (C.R. Arnold) and Florida (Florida Department of Natural Resources, D.E. Roberts). In two months larval rearing best overall survival is closed to 20 %. More than 30 000, 2 months old fingerlings have been produced by this method.

Discussion

In Martinique, the red fish does not suffer winter conditions and the growth never slows down. So it grows a little faster than observed in Texas (Arnold *et al.*, 1987), in Louisiana (Wilson, 1987) or in South Carolina (Hopkins, 1987; Stokes *et al.*, 1987). The conversion rate are satisfying with a high protein rate food (54 %) and even inferior to those expected by Hopkins (1987) (1.6 :1 VS 1.8 :1 expected). With a 37 % protein food and 42 % animal protein, feed conversion rate reached 3.8 : 1 and are very close to the results on trout food (38 % protein), 4.2 : 1 (Stokes et *al.*, 1987). This diet might be far from optimal in promoting good growth of red drum in tropical water. After six months, experimental fishes fed on the low protein food have presented general anemia symptoms (Gallet, pers. comm.). The change of food to a 54 % protein ration (84 % animal protein) immediately stopped immediately these symptoms. Davis (1987) proposed (30-35 %) proteins in the feed with at least half animal protein. It does not seem to be enough under tropical conditions. Red drum seems particularly suitable to cage culture and the improvement that could be done, might be the formulation of a really specific pelleted food. No other pathology has been noted during the grow-out phase.

The necessity to produce fingerlings is evident for development. At present broodstock is ready to start a maturation cycle established from US works (Colura, 1974; Arnold, 1978; Roberts *et al.*, 1978; Arnold *et al.*, 1987; Roberts, 1987). The objective is to adapt in Martinique a reliable technology to spawn red drum.

For larval rearing it was not reasonable to develop the extensive technique (Colura *et al.*, 1976, McCarthy *et al.*, 1986) in such a small island. That is why many attempts have been carried out to determine intensive larval rearing techniques (Soletchnik *et al.*, in press). For two years, rearing management, feeding schemes and control of pathogens have been greatly improved. The most difficult phase stands between 15 to 30 days when cannibalism and most pathologies occur.

THE RED TILAPIA : *Oreochromis* sp.

Results

When ADAM introduced the red Tilapia in 1986 many experiments were carried out on different strains. Finally, the Florida strain was retained as the most suitable one for further fresh or sea water aquaculture projects.

Fish were grown in different facilities in fresh or seawater. Net cages and tanks have been compared (table 5).

All fish were fed a 35 % protein pellet at 3 % of the body weight. Results were very similar (table 5), but the best results were obtained in sea water cages. Unfortunately, parasitism (Monogeneous parasite *Noebenedenia melleni*) was responsible for high mortalities. MASOTEN is particularly efficient in oral treatment to treat this pathology.

Tab. 5. — Growth results from 1g fingerlings for red tilapia reared in different systems

	Fresh water		Sea water	
	1 15 m^3 tank	2 35 m^3 tank	3 35 m^3 tank	4 30 m^3 net cage
Flow rate – new water – recirculated water	 200 % / hour 0	 1 % / hour 15 % / hour	 2 % / hour 15 % / hour	 1000 % / hour 0
Aeration	No	Venturi syst. (Air O_2)	Venturi syst. (Air O_2)	No
Final stocking density (kg/m^3)	90	15 (50 expected)	15	18
Survival rate (%)	95	97	96	71
Food conversion rate	1.2	1.7	1.6	2
Pathologies	No	No	No	Parasitism
Mean weight (g) at day 40 100 170	 28 103 305	 22 112 318	 18 126 335	 25 118 348

Broodstock is reared in brachishwater (19 ppt). Sex-ratio is 4 females per 1 male. Fish density is 10 per m^2. Spawners are caught every week and sexed. Fertilized eggs are removed from females that incubate them of in the mouth. Each female can produce 200 to 500 eggs every 3 weeks. A 5 weeks rest period is practised every ten weeks, then broodstock is stored in small cages. Eggs are incubated in Zoug bottles. Hatching rate is close to 100 %.

Newly hatched larvae are reared in small net bags in broodstock tanks. Larvae immediately accept artificial diet. Stocking density can reach 600 larvae per litre during the first 2 weeks, 50/l at day 30 (1 g fry) and 1-2/l at day 60 (15 g to 20 g fingerlings). Survival rate averages more than 80 % in routine.

Discussion

Several species of tilapia are euryhaline but their adaptation ability to sea water is different. LIAO *et al.* (1983) in experimental rearings in Taiwan showed that red tilapia (Taiwanese strain) could be raised in fresh, brackish or sea water. Watanabe *et al.*, (1987) obtained similar results with the red Florida hybrid in Bahamas. In mixed sex attempts, growth in sea water was better than in fresh water. This was also noticed in Martinique.

Food Conversion Rates are different though freshwater gives the best results probably because of existing natural food in fresh water tanks.

Precited authors and Coche (1982) noticed that tilapia reared in saltwater are more susceptible to parasitical attacks and diseases. Such pathologies occured in Martinique and confirmed this higher susceptibility to infestations.

As for reproduction and early stages, in agreement with Watanabe *et al.* (1987) a higher fecundity in brachishwater (19 ppt) than in fresh or sea water and a better growth of larvae and fingerlings in brachishwater has been observed. Larval survival rate can reach 98 %.

CONCLUSION

Although the rearing technologies have to be further developed and refined, these species have already shown good potential for aquaculture in Martinique. They all have good growth rates fed with commercial pellets, in cages. But it is necessary to succeed in completing the egg-to-egg cycle to develop finfish aquaculture.

These species stand at present time at different levels of research and development. For the palometa, reproduction, and larval rearing are still being studied.

The production of red drum fingerlings under intensive conditions is close to being mastered. Red drum is already raised in cages in 3 different experimental sites of experiments. But works will now be focused on spawning broodstock by manipulating environmental conditions. The egg-to-egg cycle of the red hybrid has been completed in Martinique. Development has started and several projects were initiated in 1988, both in fresh or seawater. At this time, the expected production is 50 tonnes in 1989, and 150 t in 1990.

The red hybrid and the red drum are very attractive for development purposes and should soon become an important economical activity.

Palometa has a lower priority as an aquaculture candidate as it is rather difficult to rear. But a lot of local tropical species have not been yet studied and might be interesting for aquaculture...

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The culture of *Hoplosternum littorale*: state of the art and perspectives

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Abstract — ***Hoplosternum littorale*** *is a siluriform fish of very high commercial value in some countries of South America. Some biological characteristics as easy spawning in confinement and good tolerance of low oxygen level make it easy to cultivate.*

Aquatic oxygen uptake does not allow to sustain standard metabolism, so it is considered as an obligated air breather. Routine metabolism and growth cannot be completed without air access.

A circadian rythm for feeding behaviour is observed. Most voluntary food intake occurs during the night. The sunset is a directive factor even in case of time lag. So, a night feeding schedule should be used in practise.

Spawning occurs during the rainy seasons in nests built by the male on the water surface. The seasonal fecundity is very high with more than 25000 large size (1.4 mm in diameter) eggs for a hundred gram female. The larvae are quite large (6-7 mm length) and can be reared in standing water on complete diet with a good survival rate (70 %) but poor growth. Newly hatched fry transferred in ponds give a better growth — 12 g in one month — but with only few survivors.

As initial rearing conditions seem to be determinant for sex ratio, promising perspectives are open for a high proportion of male, if not monosex, culture. This is of interest because females reduce their growth rate early when they reach sexual maturity.

INTRODUCTION

Hoplosternum littorale is a Callichthyid armoured catfish of wide distribution in northern South America. Its preferential biotopes are swamps and marshes. As many fishes inhabit oxygen deficient biotopes, it is an air breather. A special adaptation of large intestine epithelia enables it to use atmospheric oxygen.

It has a very high commercial value in countries like French Guiana, Suriname, Guyana, and Trinidad. For some of the market the needs are

covered by importations from Brazil and Venezuela where there are small scale fisheries on abundant floodplains.

The data on its biology, even scarce, and the success obtained on the first attempts in rearing (Singh, 1978; Machado-Allison, 1987), indicate that it should be able to be cultivated. Thus, there is an opportunity to develop its culture, even if its economical impact seems to be limited. The aim of this paper is to present the state of the art on this field, summarizing the works we have done during the last few years.

CULTURE PRACTISES

Age and season for spawning

Both male and female reach sexual maturity during the first year, and sometimes as early as 6 to 7 months if they are old enough at the rainy season which is the normal spawning season. Sexual dimorphism, other than size, becomes visible only just before or during spawning period (Winemiller, 1987). The most characteristic differences are the thickening of both fin and spiny ray of pectorals for males.

Spawning behaviour

Spawning occurs in floating nests built by the male on the water surface with froth and vegetals fragments. The different stages of reproductive behaviour : pair formation, nest building, egg laying, fertilisation, and care of the eggs have been well described by Gauthier et *al.* (1988). The most peculiar steps are the oral milt collection by the female and its transfer to the foam bed before laying. The male maintains the nest until hatching adding bubbles, and protects the eggs by vigorously attacking any intruder.

Fecundity

The number of eggs counted in nests collected from the wild or in ponds ranges from 3100 to 51500 (our observations), from 5600 to 55300 (Machado-Allison and Zaret, 1984), and from 2000 to 22800 (Singh, 1978), with respective average numbers 14700, 17100, and 10200. This indicates that there are multiple layings in a nest. Indeed, during a complete spawning sequence, a 100 g. female layes from 600 to 10000 eggs as observed in aquaria or in small tanks. This amount corresponds well to the observations of Machado-Allison and Zaret (1984) who counted an average of 4500 mature ovules in females ready to spawn. These authors noticed also the presence of non mature ovules. Successive spawnings — up to 9 — are effectively observed in tanks. In a 1000 m^2 pond stocked with 4 males and 12 females (97 g. body weight), we counted 290 000 eggs in the 17 nests controlled (total nest number : 21) during a 50 days period of observation. This indicates a seasonal fecundity higher than 250000 eggs per kg body weight. This is to be considered as very high taking into account the large size (1.4 mm in diameter, and 3.5 mg in weight) of the eggs.

Broodstock management

Due to multiple spawnings in a single nest, as well as the territorial behaviour of the males during the successive steps of nest care, spawning ponds can be stocked with more females than males. We use a satisfactory ratio of 3 to 1, even if we don't know the optimal value. Small ponds of 500 to 1000 m2 are used but at very low density (20 individuals) as, even if not quantified until now, it appears clearly that high stocking rates contraries both nest elaboration and egg number. There seems to be intraspecific disturbances since the presence of other species as *Myleus rhomboidalis* and young *Plagioscion squamossissimus* perturbs less the reproduction. The needs for space for all stages of spawning are not so large, as normal spawnings are observed in half m2 aquaria (200 litres). Such a method of reproduction in aquaria is very useful when one wants to get eggs on a precise date. If the fish are previously kept in separated tank, the nest building starts 24 hours after the partners are brought together.

Incubation and hatching

Nest building takes place during the night but laying is often delayed until the following night. So it is not easy to control how long the incubation lasts. But in most cases we observed hatching about 60 hours after laying, when the temperature averaged 30°C for water and ranged from 23 to 32°C for the air. Fertility is about 100 %, in the same way as hatching rate. For the ulterior easy management of the larvae, it is desirable to conduct the end of incubation out of the nest and then collect larvae free of grass fragments.

The most useful technology we found is to draw the aggregated egg cake from the nest early in the morning of the third day after the appearance of the nest, and to end incubation of the whole mass standing on nylon wires at a few centimetres above the surface of the water. This method runs well in more than 80 percent of the trials and draws more than 85 percent hatching rate.

Larvae and fry culture

When hatched in ponds, the fry resilience is very low : after a month only few fingerlings — some per thousand to some percent — can be recovered in the spawning pond. A predation by the parents cannot be implicated as we observe the same poor results when a fish free pond is stocked with a hatching nest. At that time we don't know the reasons of such a dramatic mortality. We can own to wide daily pH variations : in French Guiana the natural waters and the soil are demineralized so they do not have any buffer capacity, and pH can fluctuate 2.5 points according to photosynthesis intensity. Other aggression as predation should be suspected, for example there are in some ponds more than one Odonate larvae per mó and these predators can eat more than 30 fish larvae per day. A trophic deficiency can be also suspected due to water poorness in fertilizers, even if manure is added.

But, in all cases, direct pond transfer of newly hatched fry gives very good growth rates, reaching 12 g body weight in one month.

In order to avoid this problem of survival, and to screen responsible factors, rearing in tanks constitutes one way : predators are easy to control, and pH remains constant. More, this technique can be done easily as at hatching *H. littorale* weights 2 mg and is 6 to 7 mm long.

Singh (1978) succeeded for the first time to produce fingerlings indoor when using live food, mainly periphyton. In our trials, in standing water tanks of 300-500 litres capacity, with a half water renewal once a week, the survival rate at 30-40 days ranges from 28 to 80 percent using trout crumbles as only one food. At this time these values are very encouraging in terms of survival rate. Unfortunately, the concomitent growth is very low, as the mean weight does not exceed 500 mg. This do not constitute a redhibitory handicap, because the ulterior growth in ponds follows a similar pattern to that observed with a direct fry rearing in ponds.

Growth in ponds

Growth potentiality in ponds appears very high as the mean weight increases twofold every month. *H. littorale* reaches, in this way, 50 g at the end of third month. Beyond it is observed a differential growth rate according to the sex. It still goes on until 150 g for the males, whereas it goes slower for females which reach a ceiling of about 100 g. Early sexual maturity seems to explain one part of these limitations, and maximum sizes round about 300 and 200 g respectively.

This fish needs to be reared in very low density (0.2 fish/m2) to reveal such potentialities even when a feeding rate of 3.5 % body weight per day is used. We do not know the main responsible parameter for such a low growth rate in higher densities, a crowding factor as well as bad feeding practises can be suspected.

NICTHEMERAL CYCLES

Feeding behaviour

In the wild, *H. littorale* is known for its nocturnal behaviour. Thus some experiments have been conducted on nicthemeral cycles for feeding and respiration.

Voluntary feeding has been studied using demand feeders based on a mechanical push of a swich driving an electric feeder. A circadian cycle is observed with a pronounced peak of nocturnal trophic activity very pronounced from 2 to 5 am. During these 3 hours, fish feed themself 40 per cent of total daily intake which oscillates around 3.5 % body weight. A lighter feeding peak is also observed just after the light is turned off. The same behaviour is noticed when the dark-light cycle is advanced, and this is true for both the first and the last day of photophase changes. Then it appears that the light-dark change constitutes the synchronizer.

This night trophic behaviour seems to be suspected as one among explanatory factors for the weak growth observed both in aquaria and in ponds at high densities when we use the normal feeding schedule during day-light.

Respiratory balances

The obligatory nature of air breathing for this fish has been studied holding it in oxygen saturated water without any surfacing abitily. A total mortality is observed within the first month. It begins early as it reaches 40 % at the end of the first hour.

In such condition *H. littorale* cannot compensate the air oxygen respiration by increasing aquatic oxygen uptake which values are 230 mg/kg BW./hour in « normoxic » situation. Aquatic respiration is hardly exhausted as it only increases until 250 mg/kg BW./hour when fish is not able to surface. Metabolism becomes anaerobic as simultaneously carbon dioxide excretion (which in every case is always aquatic) stands at 450 mg/kg BW./hour. This corresponds to a respiratory coefficient of 1.23. Such a phenomenon is known on some other fishes held in anoxic conditions.

In normal conditions, aquatic oxygen uptake remains constant (230 mg/kg BW./hour) and does not show nor circadian nor postprandial variations. Air breathing appears to be the only way to cover oxygen requirements following on locomotory activity, food intake and metabolisation. According to this, surfacing intensities vary cyclicaly with two night peaks, the first at dusk following the increases of locomotory activity, and the second at the end of the night following the food intake.

Then air breathing appears to be not only an adaptative behaviour making life easier in low oxygen waters, but an obligatory mechanism.

SEX RATIO PROBLEM

In the wild, a normal (50/50) sex ratio is observed with *H. littorale* (Singh, 1978). The compilation of successive trials done in our laboratory for different purposes brings us to wonder about sex ratio flexibility in *H. littorale.* When the larvae are reared directly in ponds, with few days (1 to 5) maintenance in concrete tanks before pond stocking, the observed sex ratio is normal. The pond management consists of feeding it daily with trout starter meal on the basis of 2 to 6 kg per hectare; whether this food is used directly or as manure has to be checked.

When larvae are kept for 20 to 40 days in plastic or concrete tanks, with trout crumbles or starter meal as main food, the ulterior sex-ratio is very unbalanced. Recorded values range from few to 25 percent of males.

At the present this constitutes a major inconvenient as we produce a large proportion of low growing females instead of fast growing males. A reverse high percentage of males should be more advisable.

The question of sex determinism in this fish species arises. If there is a genetic determinism, this means that phenotipic sexes can be oriented

by the initial rearing conditions. At this time we do not know if the main factors are the holding conditions or if there is a direct effect of the food or both.

But if the hypothesis of the production of neo-females genetically male fish is proved to be correct, some promising fields are open. One can hope for a high proportion, if not total, of males at the F2, when breeding such neo-females. Naturally the successive steps of this hypothesis still have to be tested.

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IV. FINFISH

IV.2. CONSTITUTION OF BROODSTOCK, REPRODUCTION IN CAPTIVITY

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Hormone-induced spawning of cultured tropical finfishes

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Abstract — *Commercially important tropical freshwater and marine finfishes are commonly spawned with pituitary homogenate, human chorionic gonadotropin (HCG) and semi-purified fish gonadotropins. These preparations are often administered in two doses, a lower priming dose followed a few hours later by a higher resolving dose. Interval between the first and second injections may vary from 3 - 24 hours depending on the species. Variable doses are used even for the same species and may be due to variable potencies of the gonadotropin preparations.*

Synthetic analogues of luteinizing hormone-releasing hormone (LHRHa) are becoming widely used for inducing ovulation and spawning in a variety of teleosts. For marine species such as milkfish, mullet, sea bass, and rabbitfish, a single LHRHa injection or pellet implant appears to be effective. Multiple spawnings of sea bass have also been obtained following a single injection or pellet implant of a high dose of LHRHa. In a number of freshwater fishes such as the cyprinids, LHRHa alone however has limited efficacy. Standardized methods using LHRHa together with the dopamine antagonists pimozide, domperidone and reserpine have been developed for various species of carps. The technique may also be applicable for spawning marine teleosts that may not respond to LHRHa alone or where a high dose of the peptide is required.

Although natural spawning is the preferred method for breeding cultivated fish, induced spawning may be necessary to control timing and synchrony of egg production for practical reasons.

INTRODUCTION

There are more than 80 species of freshwater and marine fish cultured in Asia. Of these, about 50 species are cultured in tropical South and Southeast Asia (Rabanal, 1988). For most of the cultured species, wild fry from natural sources is insufficient to supply the requirements for culture. Moreover, the fry supply is dependent on the season, and fluctuates with environmental and climatic conditions.

Overfishing, pollution and various human activities have caused the destruction of natural spawning and fry grounds contributing largely to

the reduction in fry catch. Of the numerous species under cultivation only a few are bred in captivity. For those species that spontaneously breed under captive condition, the time of spawning is often not predictable. Problems such as viability of naturally spawned eggs and technical difficulties in egg and larvae collection are constraints to mass-scale fry production.

Most of the information on the physiological processes involved in the hormonal control of fish reproduction are derived from studies on a few species notably the salmonids, goldfish, and common carp. Although the basic biological principles arising from studies on these fish apply to tropical species, the diversity of fish cultured in the tropics present numerous problems. This paper summarizes the recent developments in induced breeding of important species cultured in Southeast Asia.

PRACTISES IN INDUCED BREEDING

Induced breeding of captive fish may be approached in two ways, hormonal and environmental. For most of the tropical cultured fish, the specific environmental cues that trigger ovulation and spawning have not been identified. Asian fish breeders however have succesfully developed methods that stimulate spawning conditions in a few freshwater species. Environmental manipulation to induce ovulation and spawning in fish has been reviewed by Lam (1983) and Lam and Munro (1987). Specific examples of tropical fish where environmental cues are known and used to stimulate breeding activities are cited in Lam (1985).

Induction of spawning using hormones provides a direct control over the final stages of the reproductive cycle in teleosts. Hormonal induction of spawning has been the subject of many recent reviews (Harvey and Hoar, 1979; Lam, 1982, 1985; Donaldson and Hunter, 1983; Crim et *al.*, 1987; Abraham, 1988). The physiological mechanisms involved in the final stages of oocyte maturation, ovulation and egg release have been thoroughly reviewed (Fostier and Jalabert, 1982; Goetz, 1983). Harvey and Hoar (1979) and Davy and Chouinard (1980) discuss traditional practises followed in induced spawning of tropical fish.

Ovulation and spawning in teleosts as in other vertebrates are controlled by several interacting factors. Environmental stimuli are translated by the brain into neural signals which result in the release of gonadotropin releasing hormone (GnRH) and/or inhibition of the release of gonadotropin release inhibiting factor (GnRIF) causing the pituitary to secrete gonadotropins (GtH) (Peter, 1982; 1983a; Peter et *al.*, 1986; Lin and Peter, 1986). When a certain GtH level is reached, vitellogenic oocytes undergo the process of final oocyte maturation : the germinal vesicle migrates to the periphery; theca and granulosa cells of the follicle are stimulated to secrete a maturation-inducing steroid (MIS); and the MIS induces germinal vesicle breakdown (GVBD) (Nagahama, 1983; Fostier and Jalabert, 1983; Goetz, 1983).

Evidence from studies on goldfish, salmonids and other species point to 17-alpha, 20-beta progesterone (17α, 20βP) as the MIS (Nagahama,1983; Scott and Canario, 1987) although other related progestogens have been

identified (Scott and Canario, 1987). Corticosteroids have also been implicated (Goetz, 1983; Fostier and Jalabert, 1983) but may play only a supportive role (Jalabert, 1976). An additional role for 17α, 20βP and other progestogens as reproductive pheromones particularly in those species where very high levels are found, has been suggested (Scott and Canario, 1987).

Hypophysation and Human chorionic gonadotropin (HCG) administration.

Traditional methods of induced breeding involve the injection either intramuscularly or intraperitoneally of crude pituitary extracts. The pituitary extract is usually administered to the female in two doses, a stimulating dose followed after a variable time interval by a second resolving dose. Fresh or preserved pituitary glands from mature fish of the same species (homoplastic) or from other, usually related species (heteroplastic) are used. In some cases, glands from immature fish have been used but higher doses are required (Harvey and Hoar, 1979). Females that do not respond after the second injection are injected a third or even more doses. Injecting multiple doses however has seldom been successful and females regress probably as a result of stress from excessive handling. Males are injected the same or half the dose given to the female usually at the time the second or resolving dose is administered. Doses are given as fresh or dry weights of pituitary gland per unit body weight of the broodfish or in dose units, defined as the ratio of the body weight of the donor and the body weight of the recipient. Standardization of hypophysation is difficult since the potency of the pituitary extract depends on the age, sex and state of maturity of the donor. Method of collection and the technique used to preserve the pituitary also vary. Species specificity of gonadotropins has been demonstrated (Fontaine et *al*, 1972; Varikul and Sritongsook, 1981) and is an important factor to consider. The supply of pituitary glands is a problem and although crude or partially purified pituitary extracts with assayed gonadotropin potency is commercially available, the cost for the Asian fish breeder is prohibitive.

The problems of standardization and cost of hormone preparations are partly solved with the use of mammalian gonadotropin preparations. Two are available in purified form, human chorionic gonadotropin (HCG) and pregnant male serum (PMS). The dosage used varies widely between species and may be related to how closely HCG and PMS resemble the endogenous gonadotropin in each species (Lam, 1982). HCG has been successfully used in most species bred in Southeast Asia (examples are given in the succeeding sections). HCG is available and convenient to use although still expensive. The possibility that injecting HCG and pituitary extracts for several consecutive years to the same broodstock may result in the development of an immune response has been pointed out (Lam, 1982; Billard et *al* 1987).

Luteinizing hormone-releasing hormone (LHRH)

LHRH, a hypothalamic decapeptide and its synthetic analogues have been shown to stimulate gonadotropin secretion in teleosts (Crim et *al.*, 1987; Peter 1983a and b; Lin and Peter, 1986). The effectiveness of LHRH analogues in inducing ovulation and spawning of cultured fish was first

demonstrated in various species of carps by Chinese researchers (Anon 1977). The practise however was not widely adopted because consistent results were not obtained.

Recent studies have demonstrated the presence of a gonadotropin-release inhibiting factor in goldfish. Further evidences identify GRIF to be the catecholamine dopamine (for review, see Peter et *al.*, 1986). GRIF's inhibitory effect on GtH release is blocked by administration of dopamine receptor antagonists such as pimozide or metoclopramide (Chang et *al.*, 1984; Sokolowska et *al.*,1984, 1985; Peter et *al.*, 1985). Administration of dopamine antagonists potentiates LHRHa mediated GtH release in goldfish (Sokolowska et *al.*, 1984, 1985) and common carp (Billard et *al.*, 1983; Lin et *al.*, 1986) and to a lesser extent in coho salmon (Van Der Kraak et *al.*, 1986) and African catfish (de Leeuw et *al.*, 1985 a and b). LHRHa injected together with pimozide or other dopamine antagonists is highly effective in inducing ovulation in these species. The use of LHRHa alone or together with dopamine antagonists in spawning various species of cultured fish was recently reviewed by Crim et *al.*, (1987).

CATFISHES

Catfishes are a favorite food fish in Southeast Asia. They have high tolerance to crowding and adverse environmental conditions hence are easy to culture. Of the three species cultivated, *Clarias batrachus* and *C. macrocephalus* are extensively cultured in the region. Commercial culture of *Pangasius sutchi*, the riverine catfish is limited to Thailand. Only *C. batrachus* spawns in captivity.

Tab. 1. — Hormone induced spawning of *Clarias macrocephalus*

Hormone	Dose	% Spawned	Time to Ovulation/ Spawning (h)	Reference[b]
PG, dose unit[a] (Clarias sp.)	1.5-2.0	50-100	13-14	(1)
PG, dose unit (P. sutchi)	4-6	62.5-83	13-14	(1)
HCG, IU/kg	3000-4500	75-90	—	(2)
HCG, IU/fish	450-500	58	4	(1)
LHRHa, ug/kg	10-30	25-87.5	15	(1)
LHRHa, ug/kg	20	70	16-18	(3)

[a] dose unit is defined as the ratio : body weight of donor/body weight of recipient
b (1) Thalathiah et *al.* (1988); (2) Carreon et *al.* (1976); (3) Ngamvongchon et *al.* (1988).

Catfishes are spawned mainly by hypophysation or HCG injection and recent trials using LHRHa have also proved to be successful (Table 1). For *C. macrocephalus*, a single injection of 0.0026 - 0.0039 mg/fish or 1.5 - 2 dose units pituitary gland extract (PG) is effective (Tongsanga et *al.*, 1963; Thalathiah et *al.*, 1986). Doses of HCG reported to be effective are 450 - 500 IU/fish (Carreon et *al.*, 1976) and 3 000 - 4 500 IU/kg (Thala-

thiah et *al.*, 1988). LHRHa at doses of 10, 20 and 30μg/kg was tried, the most effective being 20 μg/kg (Thalathiah et *al*, 1988). A similar result was obtained by Ngamvongchon et *al.* (1988). *C. macrocephalus* spawns from 13 - 16 hours after hypophysation or HCG injection and 15 - 18 hours when LHRHa is used.

Although *C. batrachus* breeds naturally in ponds, induced spawning of this species has been reported (Sundararaj and Goswami, 1969; Devaraj et *al.*, 1972; Zonneveld et *al.*, 1988; Wembiao et *al.*, 1988). Doses of HCG and PMS effective for spawning *C. batrachus* are 250 - 375 IU/fish (cited by Shehadeh, 1975). In Southern China, 500 - 800 IU/kg HCG and a combination of 500 IU/kg HCG + 1 mg/kg carp pituitary are commonly used (Wembiao et *al.*, 1988). Males are injected half the dose used for females. The broodfish are returned to the pond or tanks and allowed to spawn naturally. Of several criteria used by Zonneveld et *al.* (1988) to standardize methods for spawning *C. batrachus*, the stripping response (weight of stripped ovary/ weight of stripped ovary × number of larvae) and working fecundity (number of larvae per 100 g female body weight) were the most reliable. Based on these criteria, a single intramuscular injection of 6 mg/kg of carp pituitary extract and a stripping time of 17 hours at 25 °C gave the best result (Zonneveld et *al.*, 1988). There has been no report of induced spawning trials using LHRHa although Wembiao et *al.*(1988) indicated that LHRHa had no effect on *C. batrachus* even when a maximum dose was applied.

Of the catfishes, *Pangasius sutchi* is relatively difficult to spawn, requiring two to three injections of pituitary extract, HCG or LHRHa. If two injections of HCG+PG are given, doses of 300 IU/kg for the first (stimulating) injection and 500 IU/kg for the second (resolving) injection combined with 1 - 2 doses units of PG gave consistent results (83-100 % spawning). The time interval between stimulating and resolving doses is 8 hours (Thalathiah et *al.*,1988). Sometimes a priming dose of 100 IU/kg HCG or 1 dose unit PG is given followed 24 hours later by the stimulating injection. Acetone-dried pituitary extract (CPE) at doses of 3-4 mg/kg body weight for stimulating and 6-12 mg/kg body weight for resolving injection is also used in place of PG. When LHRHa is used alone, the most effective protocol is 20 μg/kg for the first injection followed 8 hours later by 50 μg/kg as a resolving dose. When combined with PG, highest spawning rate (83 %) is obtained using 20 μg/kg LHRHa + 1 - 1.5 dose units as the first injection followed by 30 μg/kg + 1 - 2 dose units PG as a resolving injection (Thalathiah and Abu-Fauzi, 1986). Ovulation occurs 10 - 12 hours after the last HCG or LHRHa injection.

CHINESE CARPS

The Chinese carps commonly cultured in Southeast Asia include the common carp (*Cyprinus carpio*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), and grass carp (*Ctenopharyngodon idella*). These fishes with the exception of common carp generally have to be artificially spawned. Traditional practises for spawning Chinese carps involve the use of fresh or preserved pituitary glands from the same or

other species. HCG in combination with pituitary glands is also extensively used. Common practises in induced spawning carps in Southeast Asia are found in Harvey and Hoar (1979) and May et *al.* (1984).

Tab. 2. — Induced spawning of bighead carp (*A. nobilis*), silver carp (*H. molitrix*), and grass carp (*C. idella*) by hypophysation and HCG injection

Hormone[a]	Injection First	Injection Second	Injection Third	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)	Reference[b]
Bighead Carp (*Aristichthys nobilis*)							
PG	1	2		5-6	5-6	100 (4)	(1)
HCG	200	1500-1800		6	6	100 (4)	(1)
	100-200	700-1000		6-8	6	?	(2)
CPE	2-4	10-20		5-6	6-8	?	(2)
HCG +	0-50	250	—	24,6	?	80-90 (10)	(3)
CPE	—	—	4				
Silver Carp (*Hypophthalmichthys molitrix*)							
PG	1	2		5-6	6	100 (4)	(1)
PG, mg/kg	0.67	1.33		6	?	60-70(10)	(3)
CPE	5	10		6	?	70(10)	(3)
	2-4	16-30		4-5	4-6	?	(2)
HCG	200	1500-1800		6	6	10 (4)	(1)
	220	1350		12	?	?	(4)
	100-200	700-1000		5-6	6-8	?	(2)
Grass carp (*Ctenopharyngodon idella*)							
CPE, mg/fish	4	5		12	6	86.6 (15)	(5)
CPE	1-2	6-12		5-6	?	?	(2)
HCG +	220	1800	—	12, 24	6	?	(4)
PG, mg/fish	—	—	2.5				
HCG +	50	0-250	—		6		
CPE	—	1	4-6	24,6		60-80	(3)

[a] except where indicated hormone dosages are : PG, carp pituitary gland in dose unit; CPE, acetone-dried carp pituitary, mg/kg; HCG, human chorionic gonadotropin, IU/kg.

[b] (1) (Ngamvongchon et *al.*, 1988); (2) (Peter et *al.*, 1988); (3) (Thalathiah et *al.*, 1988) (4) (Ali and Hossain, 1984); (5) (Kumarasini and Seneviratne, 1988).

Similar protocols are used for spawning the different species of carps by hypophysation and HCG administration (Table 2). Carp pituitary gland (PG)is given at 1 dose unit as stimulating injection followed by 2 dose units as resolving injection for bighead and silver carp. When acetone dried carp pituitary (CPE) is used, a stimulating injection of 2-4 mg/kg is followed by 10-20 mg/kg or 16-30 mg/kg as resolving doses for bighead and silver carp respectively. For grass carp a stimulating dose of 4 doses units PG or 1-2 mg/kg CPE followed by 5 doses units PG or 6-12 mg/kg CPE as resolving dose is commonly used. The resolving injection is usually given six hours after the stimulating injection and stripping is carried out six hours after the second injection. Different combinations of HCG, CPE or PG were reported to give good results (Ali and Hossain, 1984; Thalathiah et *al.*, 1988).

LHRHa has been succesfully used for induction of ovulation and spawning of cultured carps in China (Anon., 1977). Information from studies on the neuroendocrine regulation of ovulation and spawning in goldfish and common carp, have been applied in field trials using LHRH analogues and dopamine antagonists to induce spawning of various species of Chinese carps. Standardized spawning methods have now been developed and are currently popularized as the LinPe method (Peter et *al.*, 1987, 1988). The LinPe method involves administering a single injection of [D-Ala^6-Pro^{9}NHEt-LHRH] (LHRHa)or [D-Arg^6-Pro^{9}NHEt-LHRH] (sGnRHa) together with one of the dopamine antagonists, pimozide, domperidone or reserpine. The required doses of LHRHa and dopamine antagonist differ for the different species and range between 10 - 100 µg/kg for LHRHa, 1 - 15 mg/kg for domperidone and 1 - 10 mg/kg for pimozide. The combination of domperidone and sGnRHa is more potent than

Tab. 3. — Induced spawning of bighead carp (*A. nobilis*), silver carp (*H. molitrix*), and grass carp (*C. idella*) using LHRHa alone or combined with HCG and domperidone (DOM)

Hormone[a]	Injection First	Second	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)	Reference[b]
Bighead carp (*Aristhichthys nobilis*)						
LHRHa	10			18-20	100 (4)	(1)
	5	15	18-20	4-8	100 (4)	(1)
LHRHa + DOM	50 + 5			8-12	?	(2)
LHRHa + DOM	7.5 + 1.5	67.5 + 13.5	12	6-14	75 (4)	(3)
LHRHa, ug/fish	15-20	—	12	?	100(3)	(4)
+ HCG	—	300-800				
LHRHa	—	10	5-6	6-8	?	(2)
+ HCG	100-200	400				
Silver carp (*Hypophthalmichthys molitrix*)						
LHRHa	5	15	18-20	4-8	100 (4)	(1)
LHRHa + DOM	50 + 5			8-12		(2)
sGnRHa + DOM	10 + 5			8-12		(2)
LHRHa	20	—	12	6-14	100 (3)	(3)
+ HCG	—	1500				
Grass carp (*Ctenopharyngodon idella*)						
LHRHa	20	50	12	?	100 (12)	(4)
+ CPE	—	0.5-1.5				
LHRHa	10	10	5-6	6-8	?	(2)
+ CPE	—	2-4				
LHRHa + DOM	10 + 5			8-12	?	(2)

[a] LHRHa, ug/kg (except where indicated); DOM, domperidone, mg/kg; CPE, carp pituitary extract, mg/kg; HCG, human chorionic gonadotropin, IU/kg.
[b] (1) (Ngamvongchon et *al.*, 1988); (2) (Peter et *al.*, 1988); (3) Fermin, A. (unpublished) (4) (Kumarasini and Seneviratne, 1988).

pimozide + LHRHa for spawning the same species of carp (Peter et *al.*, 1988). Ovulation in the three species occurs from 8 - 12 hours after the injection.

The use of dopamine antagonists for potentiating the effect of LHRHa on induction of ovulation and spawning in Chinese carps has not been applied in other Southeast Asian countries. A preliminary trial with bighead and silver carp reared to maturity in cages in a freshwater lake in the Philippines had promising results although the doses used were higher (75 μg/kg LHRHa + 15 mg/kg domperidone)(Fermin, A. personal communication) compared to those used in China (50 μg/kg LHRHa + 5mg/kg domperidone). LHRHa alone or in combination with HCG or PG however has been tried in several institutions (Table 3) with good results. A single injection of 10 μg/kg was reported to be as effective for spawning bighead carp as two injections (5 μg/kg for priming and 15 μg/kg for resolving injection) administered 18-20 hours apart Table 7, Ngamvongchon et *al.*, 1988). The time required from the first injection for ovulation or stripping appears to be longer (18-28 hours) when LHRHa is used alone compared to the combined treatment of LHRHa + domperidone (8-12 hours) or LHRHa + HCG (11-14 hours). Needless to say, the Linpe method is obviously a convenient and reliable technique for spawning Chinese carps and it should be validated in other countries to simplify and lower the cost of producing carp fry.

INDIAN MAJOR CARPS

The Indian major carps, rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigal*) and catla (*Catla catla*) have been introduced in many Asian countries. In their natural habitat, Indian carps spawn once a year during the monsoon season. Increased rainfall and lower water temperatures are believed to be the factors which trigger final gonadal maturation. In countries where these species are introduced, they are usually spawned by hypophysation (May et *al.*, 1984; Harvey and Hoar, 1979). Pituitary gland from common carp or from the same species is given as a single or two injections spaced 6 hours apart. Dosages used in different countries vary slightly : from 7 - 14 mg/kg PG injected once or as two injections, 1/3 the dose as a priming injection followed by 2/3 the dose as a resolving injection. A single injection of 250 IU/kg HCG together with 6 mg/kg CPE is also effective for spawning rohu, catla and mrigal (Thalathiah et *al.*, 1988). Males are injected the same or one-half the dose used for females given at the same time as the last injection. Results using HCG and PG are relatively consistent. Efforts to further optimize dosages or try new spawning agents such as LHRHa have not been done perhaps because Indian major carps are easy to spawn and the hormone dosages required are low.

OTHER CYPRINIDS

Other freshwater fishes that have been succesfully spawned by hormone induction are the barb, *Probarbus jullieni*, puntius carp, *Puntius*

gonionotus and the sultan fish, *Leptobarbus hoevenii.* A single injection of 3-12 mg/kg SG-G100 or 3-6 mg/kg CPE is effective for *P.goniomotus, P. jullieni* and *L. hoevenii* are spawned with a stimulating injection of 250 IU/kg HCG together with 1 mg/kg CPE followed 6 hours later by 4 mg/kg CPE (Thalathiah et *al.*, 1988).

Tab. 4. — Hormone protocols for induced spawning of grouper species

Hormone[a]	Injection First	Second	Third	Interval (h)	Time to Ovulation/ Spawning (h)	% Spawned (No. of females)
I. *Epinephelus akaara* (Tseng and Ho, 1979)						
HCG, IU/ fish	1000	500		24	14-23	100 (8)
II. *E. fario* (Kuo et *al.*, 1988)						
HCG, IU/Kg	1000	1000		24	24-48	80 (5)
	1000	1000	1000	24	24	50 (4)
III. *E. salmoides* (Kungvankij et *al.*, 1988)						
A. HCG, IU/fish	500	500-1000	0-500	12-24	12-15	100 (10)
+ CPG, *ug*/kg	3	0-3	0-3			
B. LHRHa, *ug*/kg	10	10	10	12	12-15	100 (2)
IV. *E. tauvina* (Chen et *al.*, 1977)						
A. HCG, IU/kg	500	500		12.5-15	?	?
B. HCG, IU/kg	500-1000	500-1000	0-500	10-13	?	66-100 (15)
+ SPE, mg/kg	0-10	0-10	0-15			
V. *E. salmonoides* (Huang et *al.*, 1986)						
HCG, IU/kg	1000	1000	0-1000	24	11-24	?
+ PG (*E. salmo-noides*)	0-1	1				

GROUPERS

Several species of groupers are commercially cultured in Southeast Asia. Fry or juveniles are obtained from natural source but are scarce. At present, hatchery production of grouper fry is at the experimental stage and only few reports on induced breeding of groupers are available. At the Kuwait Institute for Scientific Research, *Epinephelus tauvina* broodstock reared from juveniles spawn naturally in concrete tanks between April and July (Hussain et *al.*, 1975). In Southeast Asia there has been no report

of natural spawnings of groupers although this may occur. The few reports on induced spawning of grouper species include those of Tseng and Ho (1979), Chen et *al.* (1977), Huang et *al.* (1986), Kungvankij et *al.* (1986a), and Kuo et *al.* (1988).

In species where induced spawning has been attempted, all were spawned with HCG alone (*E. akaara*, Tseng and Ho, 1979; *E. fario*, Kuo et *al.*, 1988), in combination with salmon pituitary extract (*E. tauvina*, Chen et *al.*, 1977) or pituitary gland from the same species (*E. salmonoides*, Huang et *al.*, 1986) (Table 4). For *E. salmoides*, 3 injections of 10 ug/kg LHRHa are as effective as HCG (500 IU/fish) combined with 3 ug/kg CPE (Kungvankij et *al.*, 1986a). Males were given one to two injections of half the dose given to females at the same time as the females. Females are usually stripped 10 - 24 hours after the last injection.

Although the various combinations and doses of the hormones used have been effective, methods for spawning the different species of groupers have not been standardized mainly because of the limited number of broodstock. Fertilization rates reported for the various species, whether eggs were artificially fertilized or naturally spawned were generally low and may be related to the scarcity of ripe males or infertile males. Groupers are protogynous fishes and male broodstock are difficult to collect. Sex-inversed male *E. fario* obtained after six months of oral administration of methyl testosterone to mature females were not functional (Kuo et *al.*, 1988). Further work to determine the appropriate stage of egg development, time interval between injections and appropriate time for stripping ovulated eggs need to be done. For example, a low response rate was obtained even after three injections, if female *E. fario* had oocyte diameters less than 0.50 mm (Kuo et *al.*, 1988). The low fertilization rates reported for *E. salmonoides* (Huang et *al.*, 1986) may also be a result of inappropriate time employed for stripping the females.

MULLET

Mullet is widely distributed and is a good source of cheap animal protein but its culture is not as extensively practised in Southeast Asia as that of other species. This may be due to difficulty in identifying the fry of fast growing species like *Mugil cephalus* from other less desirable mullet species. Most of the information on mullet breeding are results of research done in Taiwan and at the Oceanic Institute in Hawaii. These studies have been reviewed (Liao, 1975; Nash and Shehadeh, 1980). Lee and Tamaru (1988) also provide an update of their recent work at Oceanic Institute.

The mullet, *Mugil cephalus* matures in captivity but has never been reported to spawn naturally. Among the marine species being bred, *M. cephalus* is probably one of the most difficult to induce spawning, requiring at least two injections of high doses of hormones. Various hormone protocols have been tried and these are summarized in Table 5. Gravid females will respond to hormone induction when oocyte diameter is at least 0.60 mm but preferably larger than 0.65 mm. The amount of gonadotropin required to complete final maturation is ontaneous proportional to the initial egg size (Nash and Shehadeh, 1975; Kuo, 1982).

Two injections are given at 24 hours interval and spawning occurs around 12 - 15 hours after the second injection but may be delayed until 45 or 50 hours (Table 5). The different protocols tried using sGTH, CPH and HCG were reported to give high ovulation rates but SGTH gave more predictable results (Nash and Shehadeh, 1980).

Tab. 5. — Hormone protocols for spawning mullet

Hormone[a]	Injection		Time to Ovulation/ Spawning (h)	% Ovulation	% Fertilization	Reference[b]
	First	Second				
PG +	1-2	1.5-30	16-45	15.8-53	62-100	(1)
Synahorin	10-20	20-30				
Vitamin E	100-200	0-200				
SGtH	3.1-9.1	4.6-16.8	12-25	100	53-98	(2)
	5.1-7.0	10.2-14	10-12.5		83-98	(3)
CPH (mg/ fish)	20	40	10-15.3	75	24-73	(4)
HCG	12000-19600	16400 50000	11.8-21	100	45-98	(2)
SGtH + HCG	6.7-9	33300-44900	12-13		94-96	(2)
CPH + HCG	50-66.7	30000-33000	9.5-11.7		77-95	(2)
SGtH + DOC	4.8-7.8	50-110	11.5-23		76-91	(2)
CPH + DOC	50-71	95-157	10.2-20.3		68-92	(2)
LHRHa	101-278	202-484	13-24	68.4	0-95	(5)
CPH + LHRHa	16.53-51.86	165-536	11.5-50.5	94.1	27.5-99.7	(5)

[a] PG - Pituitary gland/fish; Synahorin - RU (Rabbit Units)/fish; Vitamin E - mg/fish SGtH - SG-G100, mg/kg; CPH - carp pituitary extract, mg/kg (except where indicated); HCG- human chorionic gonadotropin, IU/kg; DOC - deoxycorticosterone, mg/kg; LHRHa, ug/kg

[b] (1) (Liao, 1975); (2) (Nash and Shehadeh, 1980); (3) (Kuo, 1982); (4) (Lee et al., 1988); (5) (Lee et al., 1987).

In efforts to optimize spawning procedures and reduce the cost of larvae produced in the hatchery, Lee et *al.* (1987, 1988) compared various strategies using CPE, HCG and LHRHa. Based on the price of the hormones and assuming : 1)that the males were in the same maturation condition, 2) that average fecundity is one million eggs/kg, and 3) that hatch rate is 50 %, the most cost effective protocol for spawning mullet is CPH (20 mg/fish) as a priming injection and 200 *u*g LHRHa as a resolving injection. The estimated cost for producing mullet larvae in the hatchery using this strategy was $0.97 - 1.17 per 100 000 larvae. This is a small fraction of the cost when HCG is used alone ($78.60) or even when HCG is replaced by CPH as a priming dose ($57.20/kg fish) (Lee et *al.*, 1987).

RABBITFISH

Herbivorous fishes like the rabbitfish are good candidate species for aquaculture especially in developing countries where cheap sources of protein are needed. Of the different species of rabbitfishes, *Siganus guttatus* and *S. canaliculatus* are especialy suitable for culture because of their high tolerance to environmental factors, handling and crowding (Carumbana and Luchavez, 1979). *Siganus canaliculatus, S. rivulatus* and *S. argenteus* have been spawned with single or multiple injections of HCG (Lam, 1982). The doses used for these species are similar and range from 250 to 300 IU/kg body weight. Multiple injections are given at 24 hours interval. Female *S. canaliculatus* are stripped 5-10 hours after the last injection (Soh and Lam, 1973) while *S. rivulatus* and *S. argenteus* spawn 36 hours after a single injection (Popper et *al.*, 1979). *S. guttatus* females having eggs with diameters of 0.46 mm will spawn after one injection of 2000 IU/kg HCG while those with smaller eggs (0.43 mm or less) require multiple injections or will not spawn (Juario et *al.*, 1985; Duray and Juario, 1988).

Captive *Siganus guttatus* spawn every month a few days after the first quarter moon throughout the year (Hara et *al.*,1986). However, only a few females appear to spawn each time. Harvey et *al.* (1986) however, reports that when implanted with LHRHa silastic pellets (D-Nal(2)6 LHRH, 6.7 mg/pellet) ten days before the expected spawning, eight of ten pellet-implanted females spawned on the ninth day, one day earlier than control females and all spawned within two days. Sham-operated females spawned over a period of four days beginning on the tenth day after treatment. Spawning on the succeeding month was also advanced in the LHRHa-implanted group and was relatively synchronized.

MILKFISH

Of the finfishes cultured in Southeast Asia, milkfish (*Chanos chanos*) accounts for over half of total fish production from aquaculture and is the single most important species being cultured in the region (Rabanal, 1988). Research on the artificial propagation of this fish has been recently reviewed (Lam, 1984; Kuo, 1985; Kelley and Lee, 1986; Marte, 1987). Table 6 gives a summary of successfull attempts at spawning wild and captive milkfish using salmon or carp pituitary homogenate (SPH or CPH) together with HCG. The hormone dosages, number of injections and time interval between injections varied. Females that were induced to spawn had oocyte diameters greater than 0.66 mm (Lam, 1984). Females were stripped from 4-24 hours after the last injection. Mullet pituitary together with HCG has also been used to spawn milkfish (Liao and Chen, 1984; Lin, 1984). In these early experiments, only one female spawned « slow » pellets following a single injection of 1000 IU/kg HCG.

Synthetic analogues of LHRH have been succesfully used to spawn captive milkfish broodstock (Table 7). LHRHa and D-Ala6-sGnRHa were equally effective when administered as a single injection, cholesterol-pellet

Tab. 6. — Summary of succesfull spawning attempts in wild and captive milkfish using Salmon pituitary homogenate (SPH), carp pituitary homogenate (CPH) and human chorionic gonadotropin (HCG)

Hormone	Dose-range SPH/CPH, mg/kg HCG, IU/kg	No. of Injections	Interval (h)	No. of Fish Spawned
SPH + HCG	42-100* 2,800-10,000*	2-4	8.2-24	8
CPH + HCG	5.6-14.4 430-5,714	2-5	9	4
HCG	1,000-1,429	1		7

* Total dose
Source : (Lam, 1984); (Liao and Chen, 1984); (Kuo, 1985).

implant or osmotic pump implant (Marte et *al.*, 1987). The pellets contained 100 *u*g of the analogue in a matrix of 100 % cholesterol (specific dose = 20.6-35.7 *u*g/kg body weight), the osmotic pump released 10-16 *u*g LHRHa /day (estimated actual dose = 2.8-4.4 *u*g/kg) and injection was given at 10 *u*g/kg. Lee et *al.* (1986 a,b) also succesfully spawned tank-reared broodstock with a single pellet implant or injection of 200-250 ug LHRHa. D-Arg6-sGnRHa was as effective as D-Ala6-sGnRH and D-Ala6LHRH, and HCG was comparable if not more effective than the analogues for spawning milkfish (Marte et *al.*, 1988). Spontaneous spaw-

Tab. 7. — Induced spawning of milkfish with analogous of luteinizing hormone - releasing hormone

Analogous	Mode of Administration	Dose ug/kg	Total dose ug/fish	No. of fish spawned	References**
LHRHa	Pellet implant	20.6-30.8	100	4/10	(1,2)
	Injection	10	34.5-62.5	5/7	
	Osmotic pump implant	58-92*	330	2/3	
	Pellet implant	41.7 ± 3.3	200-250	9/17	(3)
	Injection	58.7 ± 9.3	250	10/33	
D-Ala6-sGmRHa	Pellet implant	19.2-26.3	100	3/3	(1)
	Osmotic pump implant	65-69*	330	2/3	
D-Arg6-sGnRHa	Pellet implant	21.5-35.7	100	1/7	(2)
	Injection	24-34	100	4/4	

* Release rate from the osmotic pump is 10-16 ug/day hence actual dose is estimated at 2.8 and 4.4 ug/kg.
** (1) (Marte et *al.*, 1987); (2) (Marte et *al.*, 1988); (3) (Lee et *al.*, 1986); (Kelley and Lee, 1986).

ning occurred 16-32 hours after treatment with the analogues or HCG. LHRHa is cheaper to use as a spawning agent than HCG, however, cost-effectiveness of this hormone will have to be assesed in terms of viable spawns and number of fry produced from induced-spawned females. Reported fertilization rates after LHRHa treatment were highly variable and ranged from 20-88 % (Marte et *al.*, 1987) and 14-99 % (Kelley and Lee, 1986).

SEABASS

The seabass *Lates calcarifer* is a popular foodfish in Southeast Asia. Techniques for culture of sea bass were first developed in Thailand in the early 1970's (Wongsomnuk and Manevonk, 1973). Seabass culture has since been popularized in Thailand and is now bred routinely in many government and private hatcheries by either environmental or hormonal means (Maneewong, 1986).

Natural spawning of seabass appears to be periodic. Ripe spawners are usually collected at around the full moon and from 18 :00 to 22 :00 hr at the time of the rising tide (Kungvankij et *al*, 1986b). Natural spawning of cage-reared broodstock also occurs at this time (personal observation). Induced spawning is thus normally done during this period and injections timed such that spawning occurs between 18 :00 - 22 :00 hr.

The widely practised method for induced spawning of seabass involves injecting wild spawners or mature captive broodstock with either Puberogen,Pregnyl, HCG or HCG + pituitary gland of Chinese carps or sea bass (Kungvankij, 1986; Kungvankij et *al.*, 1986b; Maneewong, 1986). Mature females are injected once or twice with 50 - 200 IU/kg body weight Puberogen. A double dose is given as a second injection if two injections are administered. Males receive 20 - 50 IU/kg body weight. Spawning occurs 36 hours after a single injection or within 12-15 hours after the second injection. The doses used for HCG and carp pituitary extract are 150 - 1000 IU/kg body weight and 2-3 mg/kg respectively following the same injection protocol as for Puberogen.

Various methods of LHRHa administration for spawning seabass have been tried with interesting results. One or two injections of from 10 to 75 *u*g LHRHa/kg body weight induced two successive spawnings at 24 hours interval (Lim et *al.*, 1986; Nacario, 1987; Nacario and Sherwood, 1986). Pelleted LHRHa in a cholesterol matrix at doses ranging from 9-23 *u*g/kg induced spawning 72 hours after implantation (Harvey et *al.*, 1985). Implantation of an osmotic pump designed to deliver a continous release of 9 *u*g of the analogue per day at 26 °C induced up to five consecutive spawnings in individual females (Nacario, 1986; Nacario and Sherwood, 1986; Almendras et *al.*, 1988). Up to four consecutive spawnings were also obtained after daily injections of 60-100 *u*g LHRHa (Almendras et *al.*, 1988). Similarly, multiple spawnings were induced after a single implantation of « quick-release » or « slow-release » LHRHa cholesterol pellets. The pellets contained 100 ug LHRHa in a matrix consisting of different proportions of cholesterol and cellulose. « Quick » pellets had 80 % cholesterol whereas « slow » pellets had either 95 % or 100 % cholesterol.

Garcia (in press) has determined the minimum dose of LHRHa in cholesterol pellet that will induce the maximum spawning rate (total number of spawnings per fish over four days multiplied by 100) to be between 37.5-75.0 *u*g/kg body weight. Total number of eggs produced by females implanted with the graded doses of LHRHa did not differ. The most number of eggs was spawned on the first day progressively decreasing during succeeding spawning days. Fertilization rate was significantly lower in fish implanted with the high LHRHa dose (300 *u*g/kg). Hatching rate although not significantly different in fish implanted with increasing doses was also lower in females implanted with the high LHRHa dose. A graded spawning response to LHRHa injection was also demonstrated (Garcia, submitted). At similar doses (50-100 *u*g/kg) up to three consecutive spawnings were obtained following a single LHRHa injection, the lower doses (1-10 *u*g/kg) induced at most 2 spawnings.

Seabass will spawn from 30-36 hours after hypophysation (Kungvankij et *al.* 1986), LHRHa injection (Nacario, 1987) or LHRHa implantation (Almendras et *al.*, 1988; Garcia, in press and submitted) but may be as long as 72 hours after a single pellet implantation (Harvey et *al.*, 1985) In these experiments, oocyte diameter of mature females was usually less than 0.50 mm. The response time however was shortened to 8-9 hours in females with oocyte diameter greater than 0.50 mm (Garcia submitted).

The results of these experiments demonstrate that administering LHRHa via pellet implants is an alternative method for spawning fish. The method may prove to be more cost-effective and convenient especially for batch or sequential spawners like seabass.

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Maturation and spawning of tropical and subtropical marine finfish by environmental manipulations

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Abstract — *Circannual, diel, and lunal periodic mechanisms in naturally reproducing stocks of tropical and subtropical fishes are reviewed. Manipulations of biological clocks, used to control reproduction in captive broodstocks, are detailed. Experiments to induce controlled maturation and spawning in fishes either test efficacy of changing environmental conditions (regimes), or constant environmental conditions (controlled). Data has been generated in experimental/scientific studies, as well as applied research. Generally, photoperiod and temperature are the major controlling factors in gonadogenesis. Each parameter seasonally affects various stages of the reproductive cycle. Condensed and protracted regimes can alter the natural spawning season of captive broodstocks by advancing or delaying maturation and spawning. Controlled long and short photoperiods, and cool and warm water temperatures, initiate various cytological changes in the gonads of marine fish, respectively. These changes are different for different species. Effects of controlled environmental regimes on serum steroids, gametogenesis, spawning, fry performance and egg quality are summarized for selected members of* ***Centropomidae, Serranidae,*** *and* ***Sciaenidae.***

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Effect of broodstock diets on reproduction of fish

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Abstract — *Nutrition is known to have a profound effect upon gonadal growth and fecundity, however until recently, little information was available on broodstock nutrition of fish. The purpose of this review is, therefore, to summarize and discuss recent advances in knowledge of broodstock nutrition and to indicate the necessity for further research on detailed nutritional requirements of broodstock.*

The red sea bream ***(Pagrus major),*** *an important marine culture species, is mass produced in hatcheries in Japan and is used as an example of the evolving research on this topic. The quality of the diet fed to broodstock has been shown to exert a major effect on fecundity and egg quality. Data from protein, lipid, vitamin and mineral trials are reviewed. Protein concentration in the eggs is proportional to the amount of protein included in the diet. Vitamin A and E are also found to be incorporated in the eggs together with lipids. Related to this finding, vitamin E, phospholipids and astaxanthin have been shown to be critical elements related to egg quality in this fish. Parameters such as fecundity, egg quality (buoyancy, presence of one or more oil globules), hatching rate and presence of larval deformities were monitored.*

Suggestions are made regarding the effects of time of initiation of feeding and duration of feeding but more research is needed on these topics. Initial results suggest that diets given shortly before spawning have a profound effect on reproduction. More work is needed on establishing the nutrient requirements of this species coupled with identification of the critical parameters to be monitored as an indication of reproductive success. Further collaborative research between nutritionists and reproductive physiologists is suggested.

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The sexuality of cultured hermaphroditic fish species : analysis of morphological and endocrinological features in a protogynous hermaphrodite, *Epinephelus microdon*, as a basis for further research to control reproduction in the grouper

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Abstract — *The polynesian grouper, **Epinephelus microdon**, was chosen as an experimental model in order to investigate about factors controlling sexuality in groupers.*

232 fish were captured in the wild and sacrificed, 124 of these fish were analyzed for sex steroids. Sex was determined by histological observation. Female and male fish were respectively found mostly in the smaller and the larger weight range whereas fish with intersexual gonads were observed in a large intermediate range (0.5 to 1.9 kg). However, some large females could also be observed. The maximum proportion of mature fish was observed in may 86, but interannual variations could occur. Plasma and gonadal concentrations of testosterone, 11β-hydroxyandrostenedione and estradiol exhibited some variations depending on sex and maturity stage. The most striking result is the similarity between the endocrine steroid pattern of intersexual individuals and immature females, characterized by a high ratio 11β-hydroxyandrostenedione/11-ketotestosterone.

50 fish captured in the wild and individually identified were maintained in captivity during one year and submitted to periodic blood samplings and gonadal surgical biopsies. Protogynous sex-inversion was observed in 3 cases, whereas 3 cases of sex-reversion (from male into female) and 2 cases of inversion immediately followed by reversion were also observed. Stable females and males exhibited sex-specific plasma profiles of estradiol and 11ketotestosterone. The occurence and the type of sexual change is hypothesized to be related to the initial sex ratio in tanks and to be partly regulated by social factors.

INTRODUCTION

Various cultured fish species of potential interest for aquaculture are successive hermaphrodites. Some are protandric, such as the seabass or barramundi, *Lates calcarifer* (Moore, 1979; Davis, 1982) and the sea bream *Sparus aurata* (D'Ancona, 1941, 1949, Zohar et *al.*, 1978), some others are protogynous, such as the grouper *Epinephelus tauvina* (Tan and Tan, 1974). This kind of sexuality raises several problems for broodstocks management, concerning the prediction of natural sex inversion (age and/or size) and the artificial control of sex. Steroid treatments have been used successfully in several gonochoric species in order to control sex in fish culture (reviewed by Hunter and Donaldson, 1983), but they appear to pose problem in amphisexual fish. According to Reinboth (1987), there has been no example that a steroid could promote sex-inversion from male to female in a protandric species. Although some success has been obtained in protogynous species, many problems still remain in order to optimize treatments. In groupers for example, sex-inversion has been attempted through the empirical administration of androgens. The results were negative with testoterone implants in *Thalasoma bifasciatum* (Kramer et *al.*, 1988) but they may appear encouraging with synthetic androgens in the food, since spermiating males can be obtained after oral application of methyltestosterone during several months in *E. tauvina* (Chen et *al.*, 1977), in *Mycteroperca microlepis* (Roberts and Schlieder, 1983) and in the blue-spotted grouper, *E. fario* (Kuo et *al.*, 1988). However, artificially sex-inversed males can spontaneouly reverse into females after cessation of the androgenic treatment (Chen, 1979; Roberts and Schlieder, 1983), or fertilization attempts using the sexually reversed males can be unsuccessful (Kuo et *al.*, 1988). Such drawbacks are not really surprising, taking into account past conflicting experiments performed in various ambisexual fish (see Reinboth, 1970). So far, only few physiological proofs have been supporting the hypothesis by Yamamoto (1969), according to which the natural inducers of gonadal differentiation in teleosts would be steroids (Van den Hurk and Slof, 1981; Nakamura and Nagahama, 1985; Baroiller et *al.*, 1988). As underlined by Reinboth (1988), steroid hormones are still supposed to play a major role in sex-inversion, but a cause and effect relationship has not yet been established. Obviously, more studies are required in order to characterize possible specific steroid fluctuations announcing or accompanying sex-inversion at both endocrinological and gonadal levels.

However, experimentation in the above-mentionned cultured species may encounter limitations due to the large size of the fish. Therefore, a smaller sized polynesian species, *E. microdon*, available in the wild (Tuamotu archipelago), was chosen as an experimental model. The first step, reported here, was to describe some general morphological and endocrinological features of each sexual phase in fish either sacrificed just after capture or maintained in tanks and submitted to periodic sampling of blood and gonad by surgical biopsy. In the latter animals, several inversions (from female to male) and reversions (from male to female) were observed during captivity. A further detailed individual endocrinological and histological analysis of these phenomenons will be published separately.

MATERIAL AND METHODS

Groupers from the species *Epinephelus microdon* were caught with line in Tikehau atoll (Tuamotu archipelago), kept for 1 to 5 days within a net cage in the lagoon, then transported by boat (22 hours) within small oxygenated sea water tanks (100 kg fish/m^3) to the Pacific Oceanic Center (C.O.P.) in Tahiti (French Polynesia) where they were either sacrificed or placed in rearing tanks (30 m^3) for further experiments.

Each sacrificed fish was killed by a knock on the head. A 5 ml blood sample was taken from a caudal vessel using a syringe previously rinced with a solution of sodium heparinate (1000 UI/ml in NaCl 0.9 %), cooled at 4°C and centrifuged (10 mn at 3000 g). The supernatant plasma was kept frozen (−20°C) untill subsequent steroid assays. Various body measurements were taken : weight (whole or eviscerated body, gonad, liver), standard length, lengths between the nose and the operculum, the dorsal and the pelvian fins, and the body maximal perimeter. A piece of gonad in median position was put into Bouin-Hollande fixative and further processed for histological examination. Rest of the gonads was kept frozen (−20°C) for steroid assays.

Fifty fish captured between February and May 1986 were individually tagged and kept for at least one year in tanks supplied with sea water from the lagoon, in order to follow possible sex changes in different situations of initial sex ratio. They were fed with a mixture of 50 % fish meal and 50 % crushed fresh fish (bonito). Every two months, these fish were anaesthetized (phenoxy-ethanol, 0.06 % in sea water) and submitted to the following operations :

— body external measurements and blood sampling (2 ml) for plasma preparation in the same way as for sacrificed fish,

— surgical sampling (biopsy) of a small piece of gonad (2-5 mm^3), processed for further histological observation. The piece of gonad was taken alternatively in each gonad after a short incision (2-4 cm) in the abdominal wall. Stitches were removed one week after operation.

After each operation the fish were treated during one hour in a solution of formalin (100 ppm) and malachite green (3 ppm) in sea water.

— On the first sampling time, a rapid and approximative determination of the gonadal sex was performed on a squash of fresh gonadal tissue taken by aspiration with a catheter introduced through the genital papillae and observed under microscope at a low magnification (× 20). On the basis of this rough sex-determination, the fish were distributed into 3 tanks, in order to reach 3 different « expected » sex ratios (50, 100 and 25 % females, respectively).

— On the final sampling time (usually after more than one year), the fish were sacrificed, following the same protocol as for wild animals.

Fixed gonad samples were embedded in paraffin, sectionned 4 µm thick, and colored with Regaud hematoxylin, orange G and anilin blue (Gabe, 1968).

Frozen samples for steroid assays were processed as follows :

— plasma samples were extracted twice in 50/50 cyclohexane/ethyl acetate,

— the whole gonad (when weighing less than 2 g) or a 2 g sample was first homogenized in 1 ml saline solution (NaCl, 0.9 %), before addition of recovery tracers (tritiated estradiol and testosterone). After addition of 4 ml ethanol, the sample was homogenized again, then submitted (twice during 15 s.) to ultra-sonic sounds (Branson sonifier B-12, 20000 Hz), and centrifuged 15 min. at 3000 g. After recovery of the supernatant, the pellet was extracted once more in aqueous ethanol (80 %), re-centrifugated, and the two supernatants were pooled. This ethanolic preparation was partially evaporated, then extracted 3 times with 5 ml dichloromethane. The last organic extract was dried and solubilized in 0.2 ml ethanol. Steroids were separated by chromatography on sephadex column (0.5 cm diameter, 14 cm height) using dichloromethane/methanol 95/5 eluent. Elution volumes are recovered in the following succession :

  — fraction 1 (1 ml) : discarded,
  — fraction 2 (0.8 ml) : androstenedione,
  — fraction 3 (1.2 ml) : testosterone (T), 11-ketotestosterone (11KT), 11βOH-androstenedione (11βOH δ 4),
  — fraction 4 (1.4 ml) : estrone (E1),
  — fraction 5 (3.1 ml) : estradiol-17β (E2).

Before assay, fractions 3 and 5 from gonadal samples extracts and plasma samples were recovered respectively with 0.9, 0.5, and 0.8 ml phosphate buffer (0.01 M, pH 7.25) with 0.1 % gelatine. Then, steroid assays were performed as described by Fostier et *al.* (1978, 1982). 11βhydroxy-androstenedione antibody was a gift from Prof. R. Reinboth (Mainz Univ.)

RESULTS

Wild fish

Sex determination in immature or intersexual fish was performed on histological sections using classical criteria already described in the grouper (Bruslé and Bruslé, 1975; Abu-Hakima, 1988). Moreover, in order to establish possible relationships between endocrinological and histological observations, we established a simplified scale of gonadal maturity (table 1).

The weight repartition of each sex in the whole sampled population is shown in fig. 1 (no fish weighing less than 0.3 Kg could be caught by our technic of capture). Most female fish were found in the range between 0.3 and 1.7 Kg, but some large female were also observed. Male fish occured in the range 0.5-2.3 Kg, and intersexual fish between 0.5 and 1.9 Kg.

The maturity stages of sampled groupers revealed very heterogeneous at any sampling time. Nevertheless, the proportion of mature animals (stage 4) in the successive samples exhibited a significant variation throughout the year, with a maximum in May 86 (Fig. 2). However the proportion

of mature fish appeared significantly different in February 86 and in February 87, showing in addition, that some interannual variations could occur.

The mean plasma concentrations of 11ketotestosterone, testosterone and estradiol are presented in Fig. 3. For each sex, data were grouped

Tab. 1. — Scale of gonadal maturity in the grouper *Epinephelus microdon*

Sex	Index	Histological criterion (germinal cells)
male	1	Only spermatogonia
	2	from spermatogonia to spermatocytes
	3	from gonia to spermatids (some spermatozoa)
	4	mostly spermatozoa (spermiation)
	5	general atresia
female	1	oogonia and basophilic oocytes
	2	previtellogenetic oocytes
	3	early vitellogenesis (yolk < 50 % ovarian volume)
	4	**late vitellogenesis (yolk > 50 % ovarian volume)**
	5	**general atresia**

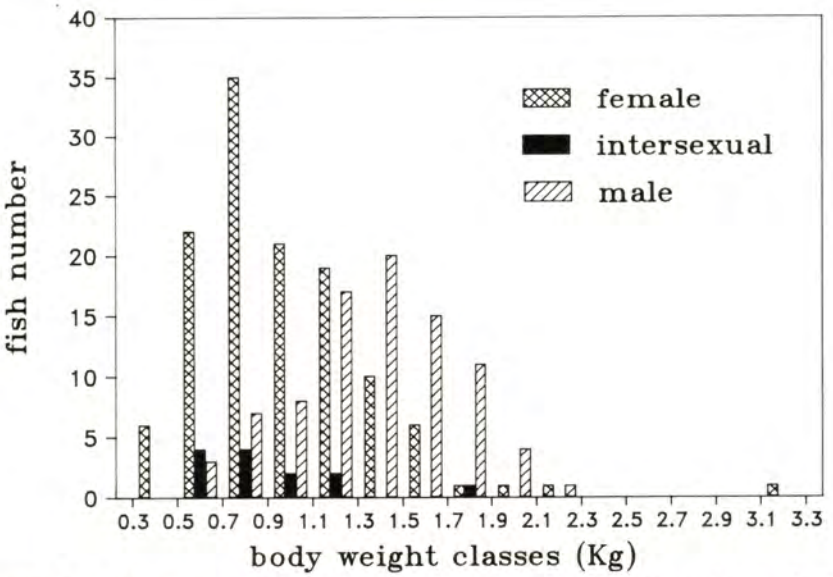

Figure 1. — Weight repartition of each sex type in the whole sampled population of wild *Epinephelus microdon.*

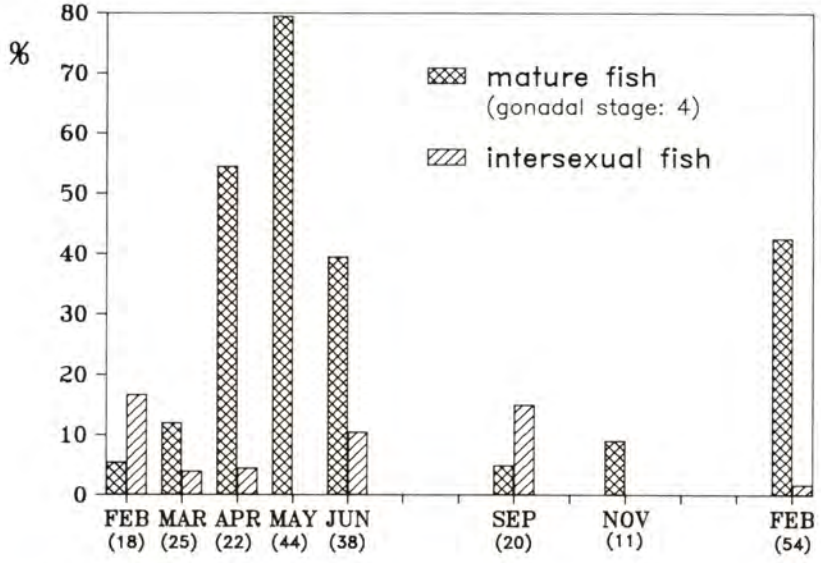

Figure 2. — Percentages of mature fish (males and females at gonadal stage 4) and fish with intersexual gonads in the various samples of wild *E. microdon.* Numbers between brackets represent the total number of fish captures in each sample.

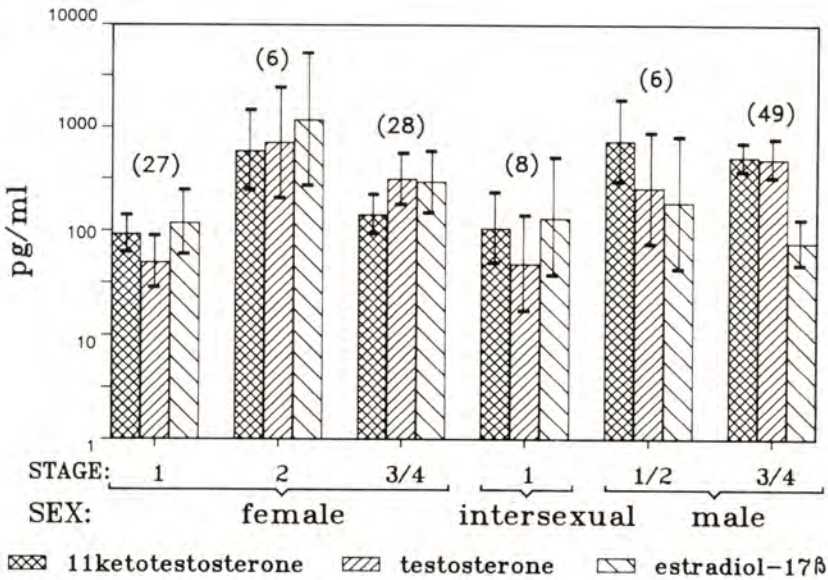

Figure 3. — Mean concentrations of 11-ketotestosterone, testosterone and estradiol in the plasma of female, intersexual and male fish at various stage of gonadal maturity. Vertical bars represent the confidence limits of the mean at the probability level p = 95 %. Numbers between brackets represent the total number of fish at each stage for each sex type.

together according to maturity stages and independently of sampling times. Moreover data concerning successive maturity stages were also grouped together when not significantly different (vitellogenetic female, stages 3 and 4; males stages 1 and 2, 3 and 4). Plasma concentrations of the three steroids assayed are the lowest and very similar in both immature females and intersexual males. Interestingly, all categories of fish exhibit noticeable levels of E2, including males and intersexual fish. Only previtellogenetic females (stage 2) present E2 levels significantly higher than other fish categories. As a whole, differences between sexes appear very small at the plasmatic level, with a tendancy for an inversion in the ratio E2/androgens between females and males.

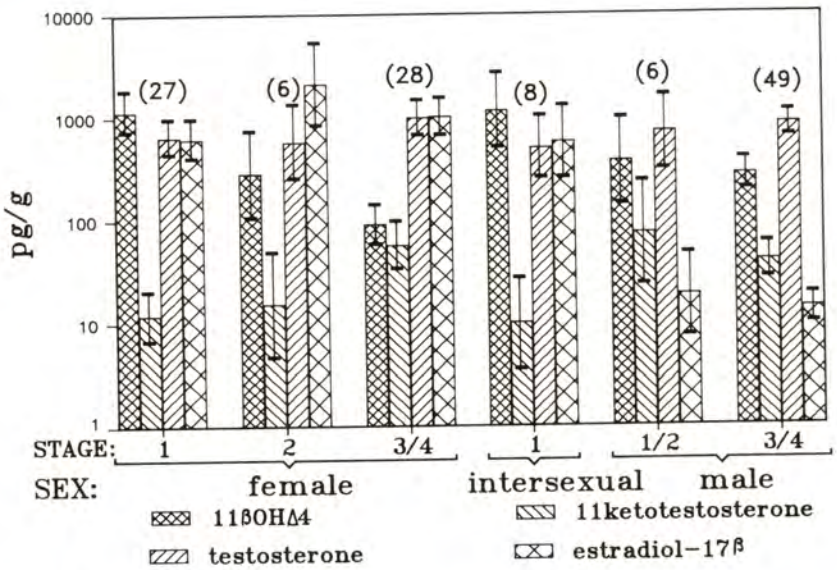

Figure 4. — Mean concentrations of 11β-hydoxyandrostenedione, 11ketotestosterone, testosterone and estradiol in the gonads of female, intersexual and male fish at various stage of gonadal maturity. Vertical bars and numbers between brackets like in fig. 3.

The mean gonadal concentrations of 11βOHδ4, testosterone, and 11ketotestosterone are shown in Fig. 4. Data are grouped together as above. The most striking features are :

— the mean concentrations of each steroid assayed look very similar in immature females and in intersexual fish, with high levels of 11β OH β 4, testosterone and estradiol, and low levels of 11ketotestosterone,

— although present at much lower concentrations than in females, E2 is also still present at noticeable levels in male gonads.

Captive fish

After histological control, it appeared that some of the rough sex determinations performed on a squash from a gonadal sample taken by

aspiration through a catheter, were wrong. Therefore, the actual sex ratio at the beginning of the experiment is somewhat different from the sex ratio expected initially in the different tanks (Fig. 5). However, the final sex ratio observed when the fish were sacrificed after one year, at the end of the experiment, showed interesting changes compared to the actual initial values (the assumption about the reality of these changes, some of which were quite unexpected, was based on the histological observation of the successive samples of gonads taken by surgical biopsy every two months on each individual, and of the final sampling of the whole gonad, at the end of the experiment) :

- in tank Nr 1, where males were in large excess, 3 reversions (change from males into females) occured, leading to a final sex ratio close to 50 %/50 %,
- in tank Nr 2, where the sex-ratio between males and females was initially equilibrated, no changes were observed in the final sex ratio. However, it appeared from the histological observation of successive individual gonadal samples, that one inversion and one reversion had occured during the experiment,
- in tank Nr 3, in which females were in excess, 2 inversions and one reversion occured.

The different kinds of sexual evolution during the experiment are summarized in Fig. 6.

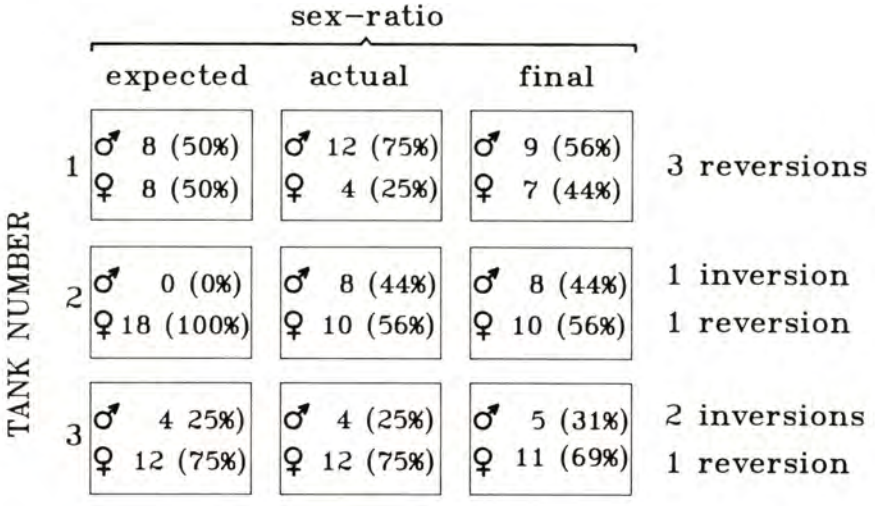

Figure 5. — Evolution of sex ratio in captive groupers *E. microdon* placed during 14 months in 3 tanks. The « expected » sex ratio was an initial estimation obtained by the observation of squashes from fresh gonadal samples taken with a supple catheter at the beginning of the experiment. The « actual » sex ratio was obtained after histological observation of the first gonadal sample, at the beginning of the experiment. The « final » sex ratio was obtained by histological observation of the gonads of sacrificed fish, at the end of the experiment. The occurence of so-called « inversions » and « reversions » was established by the histological observation of successive gonadal samples from each fish.

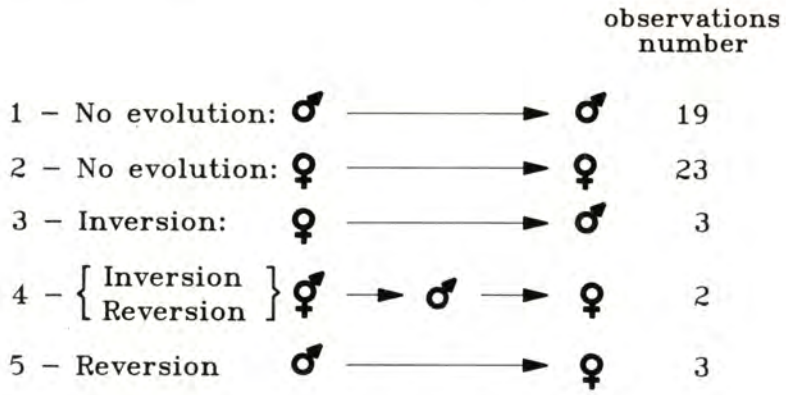

Figure 6. — Schema of the different kinds of sexual evolution observed in captive groupers during the experiment.

The mean evolution of plasma levels of 11ketotestosterone, testosterone and estradiol in « stable » females and males (exhibiting a stable gonadal sex-phenotype throughout the experiment) are respectively shown in Fig. 7 and 8. Besides the evolution of these steroid profiles throughout the year, partially linked to the evolution of gonadal maturity, the most striking feature lies in the differences between males and females concerning the ratio between estradiol and 11ketotestosterone. Whereas females are characterized by high levels of estradiol and low levels of

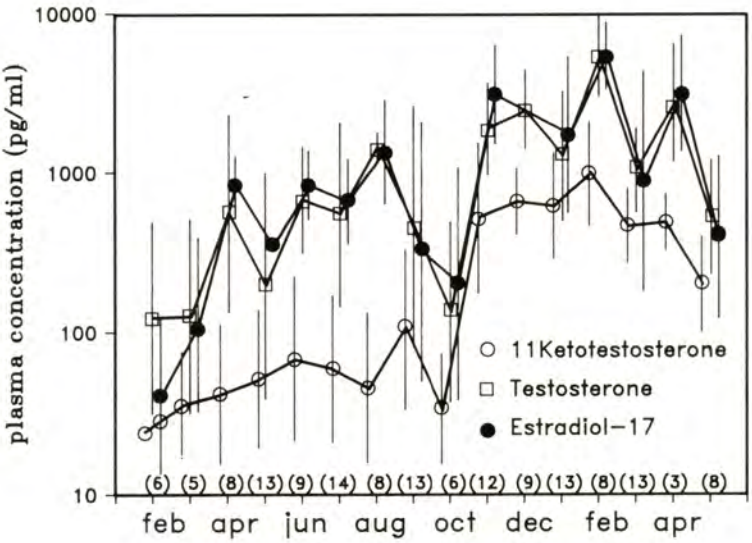

Figure 7. — Evolution of the mean concentrations of 11-ketotestosterone, testosterone and estradiol in the plasma of sexually stable captive females. Vertical bars represent the standard deviation of the mean. Numbers between brackets represent the number of sampled fish at each sampling time.

11ketotestosterone, males are characterized by an opposite situation. Testosterone levels keep in the same order of magnitude in both sexes.

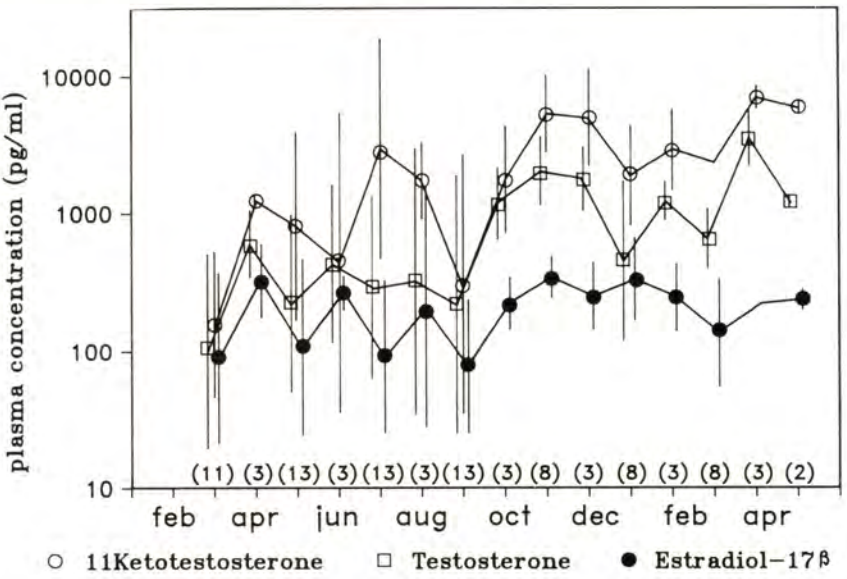

Figure 8. — Same as fig. 7 in sexually stable males.

DISCUSSION

Reproduction in the polynesian grouper, *Epinephelus microdon*, is associated with migratory and gathering phenomenons (for example in the pass of Tikehau atoll, as observed in May 86), like in other grouper species (Johannes, 1978, Colin et *al.*, 1987, Smith, 1972). In May, our samples contained only such fish, thus confirming the seasonal character of the phenomenons. But they were also probably biased to the detriment of immature or intersexual animals. Preferential spawning seasons have been mentionned in other groupers (Moe, 1969, Bruslé, 1976, Johannes, 1978, Bouain, 1980, Chen et *al.*, 1980, Moore and Labisky 1984, Loubens, 1980). However, fully mature *Epinephelus microdon* were caught all year long, which is in agreement with the data of Loubens (1980). Moreover, the seasonnal frequency of mature fish may exhibit interannual variations, as shown by the comparison of the proportion of mature fish in February 86 and in February 87.

The proterogynous character of a species may be deduced from the analysis of the size structure of each sex within the whole population (Sadovy and Shapiro, 1987). In groupers, secondary sexual characters are generally lacking (with possible exceptions such as in *Centropistes striatus*, according to Lavenda, 1949), thus often requiring a direct or even an histological observation of the gonads. Such analysis of the size structure of the population have been performed by considering the repartition of

weight (Bruslé and Bruslé, 1975 a,b, 1976; Barnabé, 1974), body length (Moe, 1969; Bouain, 1980; Bruslé and Bruslé, 1975 a,b, 1976; Colin et *al.*, 1987; Thompson and Munro, 1978), and age (Moe, 1969; Chen et *al.*, 1980; McErlean and Smith, 1964; Moore and Labisky, 1984). The wide overlapping between size criteria of each sex show a great variability in the time of sex-inversion as already underlined by Bruslé (1982), Thompson and Munro (1978) and Moe (1969). In The case of *Epinephelus microdon*, we tried to use various morphometric measurements, but we could not find any criterion better than weight, in order to discriminate between sexes.

Finally, we observed the occurrence of very large females of *Epinephelus microdon*. This phenomenon was also observed in other grouper species, and it was suggested that such large females could have escaped to the process of sex inversion which would not be a general rule. Without eliminating such a possibility, our own results showing that reversions from male to female can occur in captivity in *E. microdon* also suggest that the same kind of sex reversion could occur in the wild.

According to our results, and from an endocrinological point of view, sexual inversion in *Epinephelus microdon* looks like a return to the immature female stage, before the development of male gametogenesis. However, this hypothesis would require to be verified in the case of other steroids than those assayed in the present study. For example, 5-reduced androgens might be important in the steroid biosynthesis pathways of protogynous species (*Monopterus albus* : Yeung et *al.*, 1985; *Coris julis* : Reinboth and Becker, 1984; *Spicara maena* : Reinboth, 1979).

The comparison between the various sexual stages shows no qualitative difference. Such an ubiquity of androgens and estradiol has already be mentionned in other ambisexual fish (Yeung and Chan, 1987 a,b; Shapiro et *al.*, in press). Besides, the levels range found in our data like in others, appear very low compared with those reported in gonochoric species such as salmonids for instance (Fostier et *al.*, 1983).

Considering our results on the gonadal concentrations of steroids (Fig. 4), the intersexual state appears to be characterized by a decrease of 11-ketotestosterone and a rise of 11β-hydroxy-androstenedione. A possible role for 11β-hydroxylation in the male sexual determination has already been suggested in gonochoric species (Van den Hurk and Slof, 1981; Baroiller et al., 1988). Most endocrinological studies on the sexual steroids in ambisexual fish have been dealing with gonadal steroidogenesis *in vitro* (Reinboth, 1979; Chan and Yeung, 1983; Fostier et *al.*, 1983), and only few data are available about plasma levels (Idler et *al.*, 1976; Chan et al., 1975; Yeung and Chan, 1987a,b; Shapiro et *al.*, in press); Moreover, no measurements were performed so far, to our knowledge, in the gonadal tissue itself. Most of our own assays in sacrificed *Epinephelus microdon* were carried out both on plasma and gonadal samples (Fig. 3 and 4). The steroid concentrations measured in each case are not stricly comparable since plasma levels are not only determined by the gonadal synthesis. Plasma levels are regulated by the gonadal secretion rate, the synthesis and metabolism of other peripheral steroids, and the metabolic clearance rate. Nevertheless, a similar evolution as a function of maturity stages can be observed both in gonads and in

plasma in the case of estradiol, which also appears as the best discriminating steroid between males and females. Besides, a similarity between the steroid patterns of intersexual fish and immature females can be observed either at the plasma or at the gonadal level. From our data, the rise in 11β-hydroxy-androstenedione level could be related to a decrease in 11β-oxydoreductase activity leading to 11-ketotestosterone. Such a fact, in addition to the further decrease in estradiol levels in males (due to a lowered aromatase activity ?), may be important from a practical point of view. The stimulation of a particular physiological endocrine pattern in order to promote sexual inversion may require more cues than increasing the level of only one specific steroid. For that purpose, the use of specific steroidogenesis blockers may provide useful tools, in addition to the administration of exogenous hormones, in order to modulate the ratio between various hormones.

Our data on the evolution of sex ratio in captive fish, showing several occurences of protogynous sex inversion, comfirm the ambisexual character of the polynesian grouper, *Epinephelus microdon*. This species can therefore be considered as protogynous like other grouper species in which sufficient data are available, taking also into account the weight repartition of sampled wild fish among which no male could be found below a certain weight. However, our data also bring the proofs that reversions from male to female are possible and occur as frequently as protogynous inversions, in the conditions of captivity. To our knowledge, this is the first time such a data is observed in any grouper species. Considering the evolution of sex ratio in the different tanks as a function of the initial ratio, it must be noticed that the sexual changes which occured contributed to some readjustment towards a ratio close to 1 male/1 female, when the initial ratio was unbalanced. Although these changes cannot be considered as significant, considering the number of inversions and reversions, the total number of fish, and the absence of repetitions in the experiment, they bring some support to the hypothesis that social factors could participate in the determinism of sexual changes in the grouper as well as in other serranids (see Shapiro, 1986, for review).

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Morphological and cytological aspects of sex inversion in a protogynous hermaphrodite, *Epinephelus microdon* (*Teleostei, Serranidae*)

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Abstract — *The sexuality of the grouper **Epinephelus microdon**, caught by diving in the area of Tahiti Island, from January 1986 to March 1987, is investigated. Small specimens are females, males occur among the largest fishes, revealing the protogyny of **Epinephelus microdon** as in other Epinephelinae (Smith, 1959). Nineteen fishes among 233 samples studied are in sex inversion. The change of sex (from female to male) occurs during the post-spawning time and extends throughout the sexual rest period (from June to September) in fishes which are about 1 kg in weight. Using light and electron microscopic criteria, the sex inversion of **Epinephelus microdon** is revealed, in one hand by the identification in the ovary of some seminiferous cysts including spermatocytes or spermatids, scattered in the parietal part of the ovarian lamellae, and, on the other hand by a massive spermatogonial proliferation which develops throughout the gonad according to a centripetal orientation and which is followed by an early spermatogenic activity. These findings are hypothesized to be two consecutive events. Before and during the spermatogonial proliferation, ultrastructural investigations have allowed us to detect the presence of numerous primordial germ cells (PGCs), showing a mitotic activity. PGCs, characterized by irregular outlines and a high electron density, are both undifferentiated (high nucleus to cell ratio, abundant ribosomes, scarce membrane organelles) and bipotential cells (similar ultrastructural features in females and in males). These early germ cells are involved, as well as spermatogonia (which arise from them), in the testicular ontogenesis which takes place, in the ovary, during the sex inversion.*

RESULTS AND DISCUSSION

Hermaphroditism and gonadal sex-inversion in Teleosts have been reviewed by Atz (1964) and Reinboth (1970, 1983). In the family of Serranids, two patterns of hermaphroditism have been identified

(Smith, 1959) : synchronous hermaphroditism and protogynous hermaphroditism, which seem especially common in *Epinephelinae*, as noted by Smith (1959).

The present study deals with the sexuality of the grouper *Epinephelus microdon*, caught by diving in the area around the Tuamotus (French Polynesia), from February 1986 to February 1987. Sexual maturity was variable according to the individuals and occured from March to September. The spawning time in 1986 was from the end of April to the end of May, whereas in 1987 it began in February. The females have oval-shaped ovaries, hollow organs in which ovarian lamellae are perpendicular to the major axis. The testes of the males are hollow organs too, with lamellae in which spermatogenesis can be discerned. Such a pattern of the testes is unusual in Teleosts, but is classically described in groupers (Mc Erlean and Smith, 1964; Smith, 1964; Liem, 1968; Reinboth, 1968, 1970; Bruslé, 1982) and suggests a protogyny in *E. microdon.* Furthermore, as small specimens are females and the larger specimens are males, the protogynous hermaphroditism of *E. microdon* seems to be corroborated.

Among 232 groupers examined, 13 fish undergoing sex-inversion were identified. Sex-change, from female to male, occurs during the post spawning time and continues throughout the sexual rest period, from June to September, in fish which are about 1 kg in weight. In February-March, it is possible to find some sex-inverting groupers with gonads displaying male germ cells close to oocytes in the early stages of vitellogenesis.

Using light and electron microscopic data the first cytological indication of sex-inversion is the occurence of seminiferous cysts scattered in the parietal part of the ovarian lamellae. Spermatocytes or spermatids are found in cysts, this being the first event of spermatogenic activity in the ovary. The gonads of some sex-inverting *E. microdon* show another pattern in the shape of massive spermatogonial proliferation (spermatogonia being clustered in nests) which develops throughout the ovary according to a centripetal orientation and which is shortly followed by spermatogenic activity. These findings are assumed to be two consecutive events. Seminiferous cysts have been described in previous studies (Smith, 1959, 1964; Reinboth, 1967; Bruslé and Bruslé, 1975; De Mousac, 1986; Abu-Hakima, 1987), but spermatogonial proliferation has not been detected, either because it escaped the attention of the researchers or because *E. Microdon* shows a different pattern.

Before and during spermatogonial proliferation, ultrastructural investigations enabled the presence of numerous primordial germ cells (PGCs) to be detected. These early germ cells, previously identified in Mugilids (Bruslé, 1980, 1989), Serranids (Bruslé, 1983) and Labrids (Bruslé, 1987), have the same general ultrastructural features in *E. microdon* as those described in these families. PGCs are characterized by their irregular outlines and high electron density, and are conspicuously different from spermatogonia (regular outlines, low electron density, endowed with more membrane organelles). PGCs have the following features : undifferentiated cells (high nucleus to cell ratio, abundant ribosomes, few membrane organelles); bipotential cells (similar ultrastructural features in males and females) which differentiate into oogonia or spermatogonia, as revealed

by intermediate steps. The presence of PGCs, established in all fish at each stage of the sexual cycle, shows that these early germ cells constitute a permanent germ stock in the gonad. In sex-inverting fish, PGCs are easily identified because they are more numerous than in males or females (especially during spermatogonial proliferation) and because they divide. These early germ cells are therefore involved, like spermatogonia which arise from them, in the testicular ontogenesis which takes place in the ovary during sex-inversion. The participation of PGCs as bipotential cells, being at the origin of the male germ line, has been previously detected in the protogynous hermaphrodite, *Coris julis* (Bruslé, 1987) during the sex change. Several authors have put forward the hypothesis of undifferentiated cells being involved during sex-inversion, but they failed to detect them (undifferentiated cells : Duchac, 1981; Bentivegna and Rasotto, 1983; undifferentiated gonia : Reinboth, 1962, 1967, 1970; Mc Pherson, 1977; bipotential gonocytes : Yeung and Chan, 1987).

Since Smith (1959), some investigators have believed that the identification of degenerative processes inside a gonadal tissue reveals sex-inversion (Moe, 1969; Lissia Frau et *al.*, 1977; Shapiro, 1977; De Mousac, 1986; Garrat, 1986; Abu-Hakima, 1987). However, the evolution and intensity of this degeneration are often too difficult to evaluate to be really significant. In sex-inverting *E. microdon*, it is possible to detect in addition to some atretic oocytes, a large number of eosinophilic granulocytes. Quite often in phagocytotic acitivity, these immune cells characterized by their homogenous, dense granules and endowed with enzymes suggest a true degeneration of the female tissue.

In conclusion, the protogynous hermaphroditism of *E. microdon* is established from our morphological and cytological investigations. The cytological modalities of sex-inversion are characterized by the occurence of seminiferous cysts scattered in the ovarian wall probably followed by massive spermatogonial proliferation in which primordial germ cells, undifferentiated and bipotential cells, are involved.

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Enzymatic polymorphism study as help for constitution of initial broodstock for a new cultivated finfish species

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Abstract — ***Leporinus friderici*** *is a South American promising species for fish culture. In French Guiana the « morphological species », **L. friderici**, **L. granti**, **L. lebaili** and **L. affinis steyermarki** are found. Their identification is sometimes difficult on usual criterion. To constitute an initial broodstock of **L. friderici** from local wild populations, the genetic structure of these species was studied. Fifteen enzyme systems, representing twenty loci, were screened.*

The biological status of the morphological species was investigated. Diagnostic loci for unequivocal species identification and marked genetic differences indicate that these species are reproductively isolated and therefore constitute true biological species.

*On an other hand, two **L. friderici** groups of populations are distinct in French Guiana. In spite of geographical closeness, a genetic distance of 0.016 separates West populations (Sinnamary, Iracoubo and Mana rivers) from east populations (Oyapock, Approuague and Comtè rivers). The western group possesses the allele Me-1 (300) with high frequency (0.022 to 0.26) but not the allele Ldh-2 (130). The eastern group possesses the allele Ldh-2 (130) with high frequency (0.29 to 0.49) but not the allele Me-1 (300). Furthermore, the heterozygoty of the west populations ($0.04 < h < 0.07$) is twice that of those of the east ($0.10 < H < 0.11$).*

*The interspecific differences and the interpopulational differences of **L. friderici** will allow us to establish pure broodstock. Now we have a guide for selecting genetically different populations for comparisons of breeding and rearing performances.*

INTRODUCTION

Given the aquacultural potential of freshwater fish in French Guiana, *L. friderici* has been selected for experimental breeding on the basis of biological criteria (Boujard et *al.*, 1988). Establishing a reproductive stock from wild populations requires knowledge of the biological status of the species and its population structure. The artificial mixture of genetically different populations, or different species, may lead to the appearance of undesirable phenomena such as segregation, hypofertility, and premature death of embryos.

In French Guiana *L. friderici* is integrated in a taxonomic complex that Gèry (1977) qualified as « the worst chinese puzzles of characoïdes systematics ». Juveniles of this species have similar morphologies and markings to those of *L. granti*, *L. lebailli*, and *L. affinis. steyermarki.* While adult fishes differ in coloration and in some morphomeristic characters, their correct identification can be difficult as well.

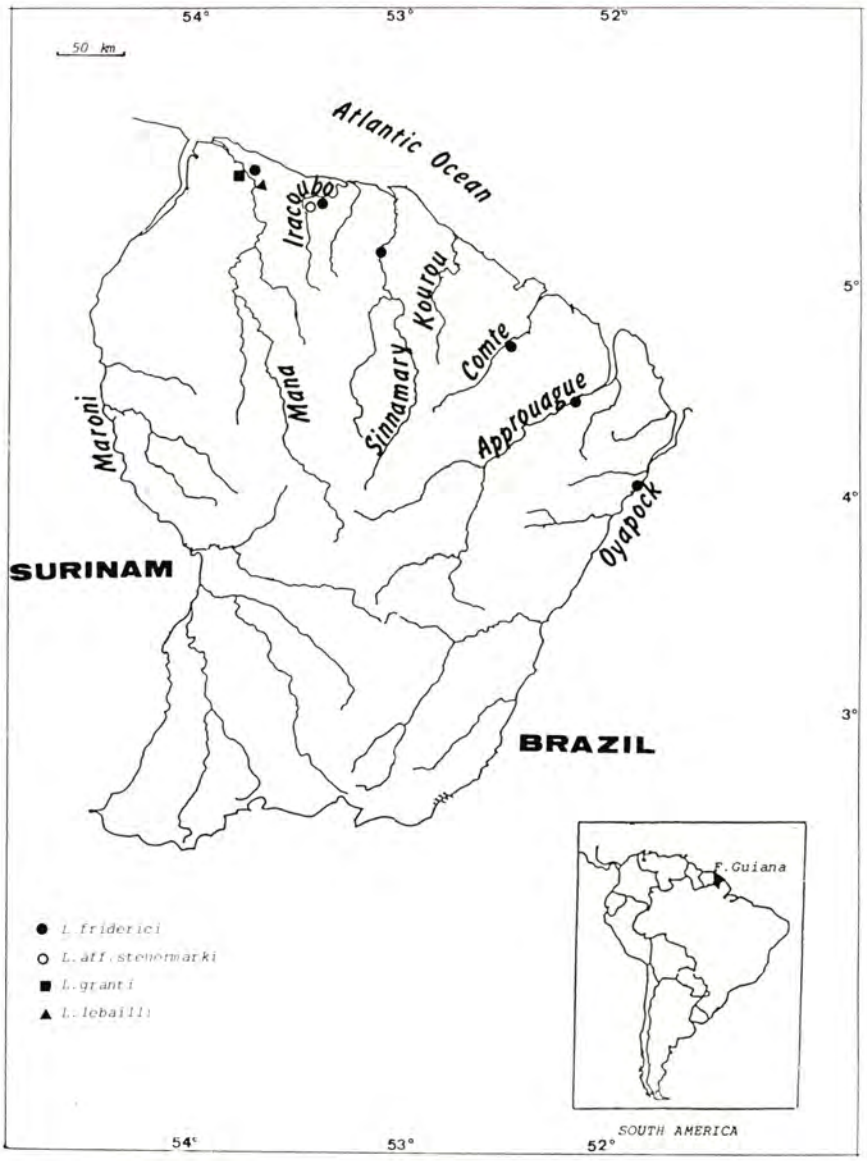

Fig. 1. — Sampling points of *L. friderici, L. granti, L. lebaili* and *L. aff. steyermarki* in French Guiana considered in this study.

In order to provide a guide for the establishment of initial broodstocks, diagnostic genetic variants using protein enzymatic electrophoresis were identified. In this way the reproductive isolation of the different species of *Leporinus* complex was verified. Then the genetic differenciation among six populations of *L. friderici* was described.

MATERIALS AND METHODS

Samples

The samples were collected between January 1987 and June 1988, in the six principal river basins of French Guiana. Adult fish were caught with 30-60 mm net trammel while juveniles were obtained by poisoning.

Fish were identified with morphomeristic characters according to the keys established by Gèry (1977) and Le Bail et *al.*, (1984) and labelled according to the nomenclature of Gèry et *al.*, (1988).

Genetic analysis

Starch-gel electrophoresis of muscle and liver tissues was employed to examine enzymatic variability. Gels were developed according to Guyomard and Krieg (1982) and Pasteur et al., (1987). The electromorphs, indicating alleles and characteristics of each enzymatic system, were numbered in order to increase electrophoretic mobility.

RESULTS

Twenty one putative loci common to the four species were examined. No difference in genetic determinism of species was found in the 15 systems studied. Hardy-Weinberg equilibrium among genotype frequencies was found for each locus, indicating that the population in each case is likely to be panmictic.

Interspecific differenciation

The loci for which there exists one or more alleles, of which the total frequencies equal 1 in one group and 0 in another (alternative alleles), are considered as diagnostic loci of species. The number of such diagnostic loci, separating each species from the others varies from 2 (*L. granti/L. lebailli*) to 9 (*L. granti/L. aff. steyermarki*). Four diagnostic loci (20 %) distinguish *L. friderici* from the other species. The proportion of these diagnostic loci are given in table 1.

The genetic variability in each population is estimated by the proportion of polymorphic loci (P), and by the rate of observed heterozygosity (H). A locus is considered as polymorphic when the common allele has a frequency lower than 0.95. Among the four species (P) varies

from 0.21 (*L. granti*) to 0.33 (*L. friderici*). (H) which indicates the mean frequences of heterozygotes in a population varies from 0.08 (*L. granti*) to 0.12 (*L. friderici*).

Tab. 1. — Diagnostic loci for separating each species from the others, and the corresponding percentage. In each species, P = proportion of polymorphic loci (a locus is considered polymorphic when the incidence of the most frequently occuring allele is less than 0.95), H = mean observed heterozygosity in a population (I = Iracoubo river, M = Mana river).

	L. lebaili	*L. affinis steyermarki*	*L. granti*
L. friderici (I) H = 0.12 P = 0.33 (M) H = 0.11 P = 0.29	Aat −1 Est Fum Mdh −2 20 %	Agp Ldh −1 Ldh −1 Me −1 19 %	Aat −1 Fum Mdh −2 Pmi 20 %
L. granti H = 0.08 P = 0.21	Pmi Sod 10 %	Aat −1 Ldh −2 Agp Mdh −2 Est Me −1 Fum Pmi Ldh −1 45 %	
L. aff. steyermarki H = 0.12 P = 0.27	Aat −1 Me −1 Agp Ldh −1 Est Fum Mdh −2 35 %		
L. lebaili H = 0.11 P = 0.26			

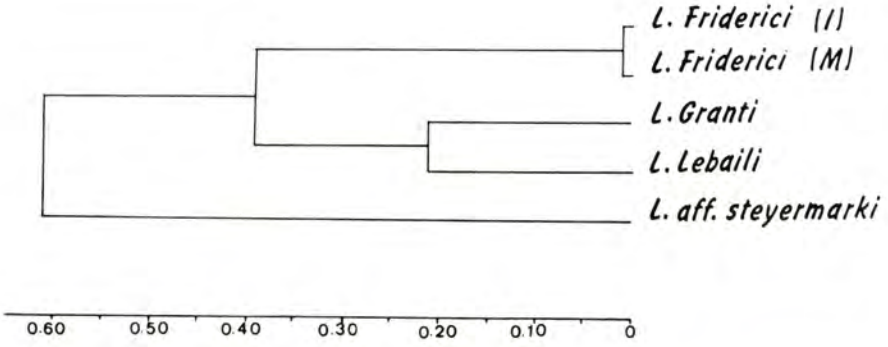

Fig. 2. — Dendrogram (UPGMA) deducted from the Nei genetic distances between the four species, *L. friderici, L. granti, L. lebaili* and *L. aff. steyermarki.*

The Nei (1971) genetic distances between *L. friderici* and the other species are always superior to 0.35.

Intraspecific differenciation

Two distinguishable blocks of *L. friderici* populations are emphasized. The western block, including populations from the Mana, Iracoubo and Sinnamary rivers, possesses the allele Me-1 (300) with a high frequency (0.22 to O.26), but not the allele Ldh- 2 (130). In contrast, the eastern block, including populations from the Comté, Approuague and Oyapock rivers, possesses the allele Ldh-2 (130) with high frequency (0.29 to 0.49) but not Me-1 (300). The heterozygosity rate between populations doubles from east ($0.04 > H > 0.07$) to west ($0.10 > H > 0.11$), and the proportion of polymorphic loci varies from 9.5 % (Approuague, Comté) to 33 % (Iracoubo). The calculated genetic distance between the blocks is 0.016.

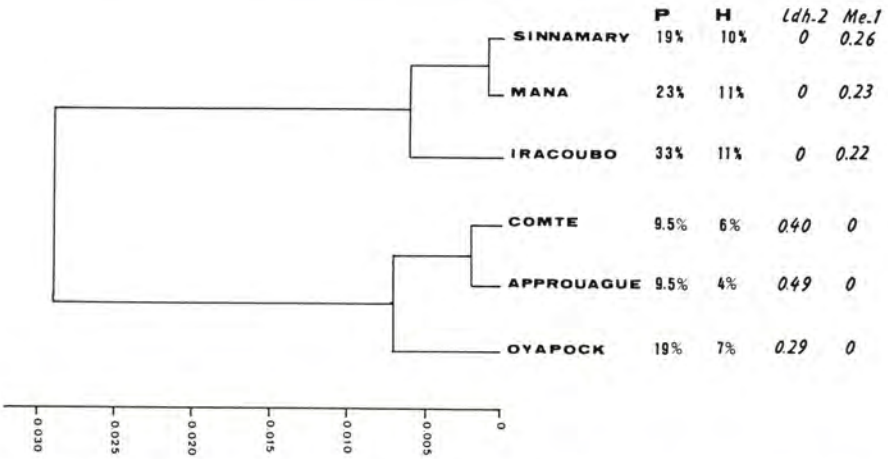

Fig. 3. — Dendrogram (UPGMA) deducted from the Nei distances between the *L. friderici* populations. For each population is indicated : P = polymorphic proportion of loci, H = mean observed heterozygosity, and the frequencies for the Ldh-2 (130), and Me-1 (300) alleles.

DISCUSSION

This study demonstrates the presence of loci with alternative alleles among sympatric populations of *L. friderici* with *L. granti*, *L. lebailli*, and *L. affinis steyermarki*. This indicates that there is not any heterozygote resulting from hybridation and clearly proves the existence of an isolation in reproduction. Thus the *L. friderici* species studied is true biological species.

Given this, broodstocks of *L. friderici* can be established without the risk of introducing wild hybrids or using misidentified species. The management of *Leporinus* populations crossing within the same species,

or interspecific hybridations, should be done taking into consideration genetic markers of crossed forms in order to identify the exact genetic characteristics of successive generations.The usefulness of isozyme and protein in identifying species and their hybrids was demonstrated by Macaranas et *al.* (1986) with *Tilapia* sp.

The genetic variability in *L. friderici* will allow a genetic improvement programme. The mean genetic intrapopulation variability reaches 0.12 (Iracoubo population); this is a high value compared to those reported for bred fish. For example, the average heterozygozity among twenty populations of masu salmon (*Oncorhynchus masou*) was 0.05 (Okasaki, 1986), and the average heterozygosity among 6 populations of brown trout (*Salmo trutta*) was 0.09 (Guyomard, 1982).

The genetic structure of *L. friderici* into blocks could be considered as a help for screening populations in the management of the broodstocks. In a genetic improvement programme based on the comparison of the rearing performances, testing populations of both blocks seems more judicious than working in the dark.

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Constitution of aquacultural stocks : genetic aspects

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Abstract — *Before reaching of production level justifying an elaborated genetic improvment program, the new aquaculture species deserve a minimal strategy to avoid the consequences of uncontroled genetic phenomena. Three main aspects will be discussed :*

- *initial constitution of the broodstock : how to collect a sufficient genetic variability without encountering the problems resulting from the mixture of differenciated populations ?,*
- *maintenance of the broodstock : the regular introduction of new animals in the broodstock is a classical way to avoid inbreeding but his cost/benefit ratio has to be discussed in the case of aquaculture. An alternative option (multiple closed gene pools) is proposed to minimize the effect of inbreeding,*
- *selection of the broodstock : mass selection is often feasible selection for tropical species but his efficiency is an object of controversy. We present a modified mass selection procedure (PROSPER), which should maximise the genetic progress.*

Developing new aquacultural species for consumption or restocking generally implies the creation and maintenance of spawner stocks in captivity. The involvement of genetics in this field is generally considered in terms of *improvement of performances* by selection, crossing or various manipulations. On the other hand, the necessity of installing a *genetic stock management* even in the absence of clearly identified improvement objectives is often ignored. We therefore considered that it was necessary to develop this topic in particular by showing how various aspects of genetics may intervene in the creation of aquacultural stocks.

A distinction will be made between two cases :

- sampling from only one source population,
- creation of heterogeneous stocks from several populations.

ONE SOURCE POPULATION

In many cases stocks should be constituted from only one source population. For instance in the case of particular sanitary guarantees on

one population, the will to save a given population with a view to restocking (the cases of the salmon of the Allier or the sturgeon of the Gironde) or the deliberate choice of a population based on performances proved to be satisfying.

Then it is a question of collecting the largest possible fraction of the population's genetic variability. Empirically, it is obvious that the sample should represent a large number. However, in genetic terms the notion of sample size may be notably different from that of numerical size.

1. Definition of genetic size

Usually the introduction is made at the level of fertilized eggs or larvae. If N is the numerical size of this sample, the share of genetic variance of the sample relative to that of the source population may be estimated by using the following equation (Kimura and Crow, 1963; Lacava and Hughes, 1984) :

$$\frac{V_E}{V_T} = 1 - \frac{1}{2\ N_e} \qquad (1)$$

where N_e is the sample's genetic size (or effective number). It is obtained by calculating the effective number of spawners for each sex :

$$N_{em} = \frac{N_m\ K_m - 2}{K_m - 1 + \frac{V_m}{K_m}} \qquad (2)$$

$$N_{ef} = \frac{N_f\ K_f - 2}{K_f - 1 + \frac{V_F}{K_f}} \qquad (3)$$

— N_{em} and N_{ef} are the effective numbers of male and female spawners, N_m and N_f being the real numbers of male and female spawners used.

— K_m and K_f represent the average number of progeny per male and female spawner. Thus, if the sample includes a total of N individuals, we obtain

$$K_m = \frac{N}{N_m} \qquad (4)$$

$$K_f = \frac{N}{N_f} \qquad (5)$$

V_m and V_f are the variances of these numbers of progeny between male and female spawners.

Finally, the effective number of the sample is obtained by :

$$\frac{4}{N_e} = \frac{1}{N_{em}} + \frac{1}{N_{ef}} \qquad (6)$$

Combining the different relations, the equation can also be written :

$$\frac{4}{N_e} = \frac{(K_m + \frac{V_m}{K_m}) + (K_f + \frac{V_f}{K_f}) - 2}{N - 2} \quad (7)$$

2. Variation factors

Therefore three parameters are going to play an important role in this equation to maximize N_e at a given value of N.

— *The number of spawners used* : for an introduced sample of a given size, this number may in fact vary considerably in aquatic species with a high fertility. A single couple may give rise to a sample of several thousand juveniles. Therefore, it is advisable to attach much importance to this parameter whose role is more essential than that of the numerical size of the introduced sample. Thus, assuming that each spawner contributes to the sample in the same way, we obtain (see further on) $V/K = 1$, where :

$$N_e = \frac{4(N-2)}{K_m + K_f} \quad (8)$$

For a sample of 10,000 individuals originating from 4 males and 4 females, $K_m = K_f = 2{,}500$, hence $N_e \approx 8$ whereas for a sample of only 100 individuals originating from 5 males and 5 females, $K_m = K_f = 20$, hence $N_e = 9.8$.

— *The sex ratio in the stock of spawners used* : Fig. 1 illustrates this aspect. It seems that if a relatively small number of spawners is sufficient when the sex ratio is equilibrated, an imbalance requires use of a much larger number. Thus, the collected variability is lower with 5 females and 95 males (i.e. 100 spawners) than with 10 females and 10 males, i.e. only 20 spawners (97.2 compared to 97.5 %). Usually, the number of females really constitutes a limiting factor owing either to the difficulty in capturing the animals at the right time or to the will not to reduce too much the reproductive potential of the source population. However, using a large number of males gives only a partial compensation : equation (6) shows that whatever the number N_m of males used and the sample size, *the genetic size will always be smaller than 4 times the number of females used.*

— *The variance of family size*, in connection with the contribution of each spawner to the constitution of a sample of juveniles. In the theoretical case where all the spawners contribute equally to the constitution of the new generation, the sample constitution can be compared to a random sampling described by the Poisson distribution interestadly characterized by a variance equal to its mean. Equation (8) can thus be used. However, this hypothesis seems little realistic in aquatic species. In particular, the quality

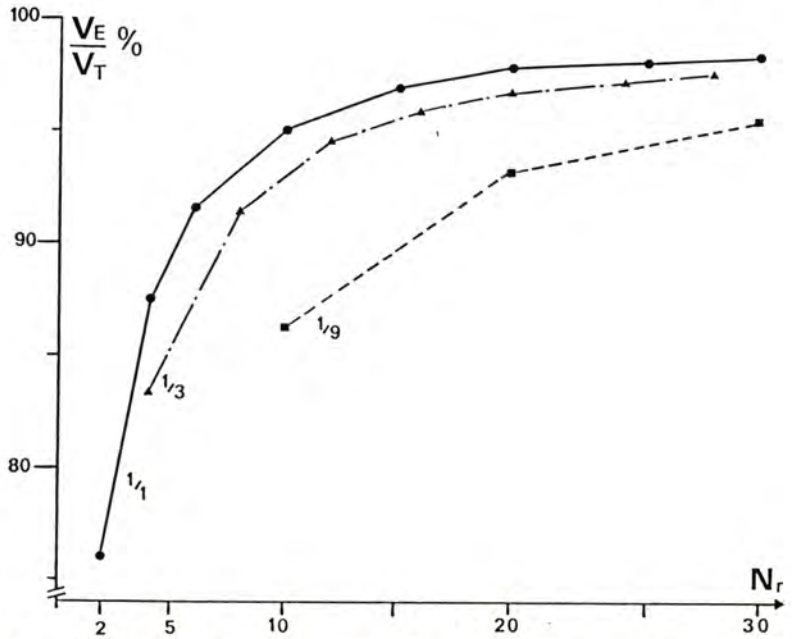


Fig. 1. — Part of the total genetic variance of the initial population (in %) collected in a sample resulting from a group of N_r spawners with various sex ratios.
1/1 1 male for 1 female
1/3 3 males for 1 female
1/9 9 males for 1 female

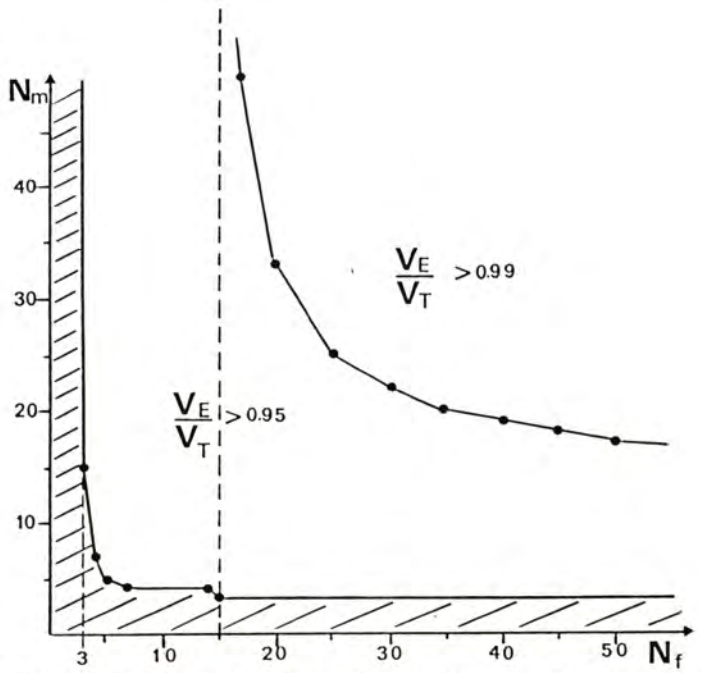


Fig. 2. — Number of male (N_m) and female (N_f) spawners required to collect at least 95 or 99 % of the total genetic variance of the source population : for a number of males larger than 50, the minimal number of females are respectively 3 and 15.

of collected ova may be different from one female to another and fertilization and hatching rates may vary considerably. Thus, the contributions of each female to the sample are different and the ratio V/K may be much higher than 1 which results in a marked decrease in the female effective number (Table 1, case C). In order to minimize this phenomenon, it is therefore desirable to keep the various spawnings separated during the whole period where large variations in survival linked to the female may show up (embryonic development, hatching and vesicular resorption and if possible beginning of exogenous feed intake). At the end of this stage, regrouping is possible by taking in each group :

- equal numbers if each spawning was fertilized by the same number of males,
- numbers proportional to their genetic stock if the spawnings were fertilized by mixtures of sperms from different males

Thus, if three spawnings A, B, and C, fertilized by 5, 10 and 20 males, respectively, are available, the genetic stock of these spawnings ($N_e \approx 4\ N_m/(N_m+1)$) is 3.20, 3.64 and 3.81, respectively. Therefore, for instance 320 alevins originating from spawning A, 364 originating from spawning B and 381 from C will be regrouped.

Tab. 1. — Effect of the variance of family size on the value of the effective number :

$$N_e = \frac{N - 2}{K - 1 + V/K} \quad \text{(see the text)}$$

Female	1	2	3	4	5	6	7	8	9	10	N	V/K	N_e
A	9	5	4	7	6	3	1	4	5	6	50	0.88	9.83
B	5	5	5	5	5	5	5	5	5	5	50	0	12
C	27	25	10	8	4	2	1	1	1	1	80	11.3	4.37
D	27	25	10	8	4	2	—	—	—	—	76	7.57	3.95
E	12	10	10	8	4	2	1	1	1	1	50	3.64	6.28
F	8	8	8	8	4	2	1	1	1	1	42	2.47	7.05

A is the case of a random distribution with the same probability of occurence for each female
B the effective number is slightly higher in the case of perfect equality of family size
C when the occurence of the females are very unequal, the effective size can be highly reduced
D, E, F see figure 3

This practise may seem to be paradoxical because it leads to adjusting the sampling on the group having exhibited the lowest survival rate instead of regrouping all the survivors from the various spawnings. This strategy of *truncation from the top* (Table 1, E and F) is, however, to be recommended, as the effective number is much more sensitive to family size variance than to their average number (Fig. 3).

On the other hand, it may be useful to distribute the sampling effort over time. This may allow to collect a larger genetic stock, especially when the number of females constitutes a limiting factor, and to take into account possible « heterogeneities » of the spawner population. All the spawners present at the same place or mature at the same time may represent only a fraction of total variability even if their number is high, especially in migratory populations exhibiting a precise « homing » (coming back to the place of birth). Therefore, sampling should rather be distributed over the spawning period or repeated during several years whether consecutive or not.

3. Conclusion

If we go back to equation (1), obviously, by respecting the precautions defined above it is rather easy to collect a large fraction of the genetic variability of the source population (Fig 2.) : if the introduction of 95 %

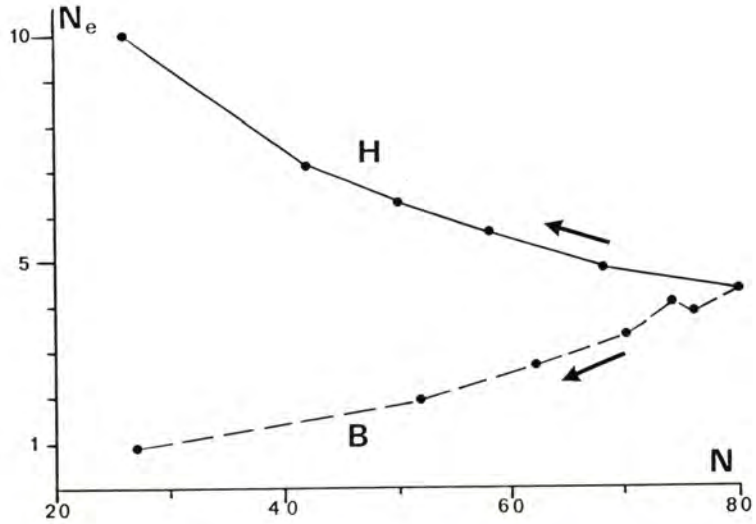


Fig. 3. — Effect of two alternative strategies for equalizing family size. The initial situation is case C (Table 1).
H : reduction of the size of the more numerous families (case E and F). The effective size N_e *increase.*
B : elimination of the less numerous families (case D). The effective size N_e *decrease.*

initial variability is fixed as a « standard » objective, this end may for instance be attained with a sample of 100 individuals originating from 3 females, each being fertilized by 5 different males. With 15 females also being fertilized by 5 males the loss of variability is only 1 %. Therefore, these principles should be widely disseminated among hatchery managers.

SEVERAL SOURCE POPULATIONS

It is often possible to identify several natural or cultured populations within a species and a more complex strategy has to be adopted in order to create a spawner stock. Should only one population be chosen (and in this case which one ?) or on the contrary, should a « synthetic » population be created by mixing many of these populations ? In most of the cases, information available on these populations is limited to historical data which are often vague (concerning the origin of culture populations, possible exchanges between populations), or in situ biological observations (growth, morphology, reproduction period) whose interpretation in terms of genetic differentiation is problematic. In this case it is therefore necessary to *determine more accurately the organization of intra- and inter-population genetic variability* in order to define a sampling strategy.

1. Description methods

Two types of approaches may be considered.

— The first consists in introducing the different populations in one and the same place in order to undertake the *evaluation of their performances.* Whereas the principle of this method is satisfying it has the disadvantage of being long and expensive :it requires the setting up of specific progeny testing equipment making it possible to raise several groups under similar conditions and a protocol concerning at least two generations in order also to be able to study the crossings between populations.

[The observation of performance differences between two populations introduced in the same environment can only be interpreted in genetic terms after having evaluated the maternal effects of each population. Therefore, it is generally necessary to create a protocol in two steps : the first (« parallel cultures ») allows to regroup the populations, to carry out a first evaluation of performances and to obtain sexual maturation of the animals under similar conditions; the second (diallel progeny testing) allows to analyse the maternal effects, genetic effects of each population and crossing effects (heterosis) by intra- and inter-population crossings].

Therefore it is intended for species whose aquacultural development is already large enough to justify such an investment (salmonids, carps...). In the case of poorly developed species, this approach can only be applied a posteriori on a restricted number of selected populations according to other criteria (see further on).

In any case it is important to recall that the principles defined in the former paragraph are applied even in the case of a transitory introduction of a population for progeny testing purposes. The sample must exhibit a sufficient variability in order to be representative of the population. It is shown that for a

sample of size N and effective number N_e, the precision ± (in %) obtained on the genetic value of the original population equals :

$$\sigma = C\ V\ (\frac{h^2}{N_e} + \frac{2 - h^2}{2N})\ 1/2$$

C V is the observed variation coefficient of the considered trait, h^2 is the heritability of this trait (proportion of the observed variance linked to the genetic variance between individuals).

Thus, for a trait like growth performance having a variation coefficient of some 30 % and a heritability value of 0.2, at least 100 individuals originating from 5 males and 5 females will be needed to estimate the genetic value with 5 % accuracy, which is a low value. In order to obtain a higher accuracy, the effective number N_e will generally constitute the limiting factor. It will be necessary to attain at least $N_e = 60$ (for instance 30 males and 30 females) in order that the accuracy reachs 2 % even by measuring 1000 individuals.

— The second consists in considering traits with a simple genetic determinism which are not sensitive to direct environmental action. This is especially the case of the chromosome number and structure (karyotype) and of protein polymorphism shown by electrophoresis, or more recently of nucleic acid polymorphism. The study of protein polymorphism has been particularly developed in aquatic organisms the last ten years and it seems useful to discuss the results in relation to the constitution of aquacultural stocks.

2. Results

Fig. 4 and 5 taken from Guyomard (1989) indicate an example of data obtained from a study on protein polymorphism. Three aspects can be taken into consideration.

— *The differentiation between populations* : the genetic distance between populations can be estimated by various measures where Nei's index is often used (1978). The populations are represented on a dendrogramme (Fig. 4) regrouping them according to their genetic resemblance. In the present example, three big groups can be identified : the first one, which is clearly separated, is made up of two natural Corsican populations; the second one regroups almost all the other Mediterranean populations. The third constitutes the « Atlantic group » in which all hatchery populations are scattered.

— *The genetic intra-population variation* measured by the mean rate of heterozygous individuals for all the genes studied. In this case (Fig. 5) the values are rather spread, ranging from 0 to 10.6 %. The variability is strong for hatchery populations, slightly lower for populations of the Atlantic group and much lower for Mediterranean populations, especially after correction of restocking effects by hatchery populations.

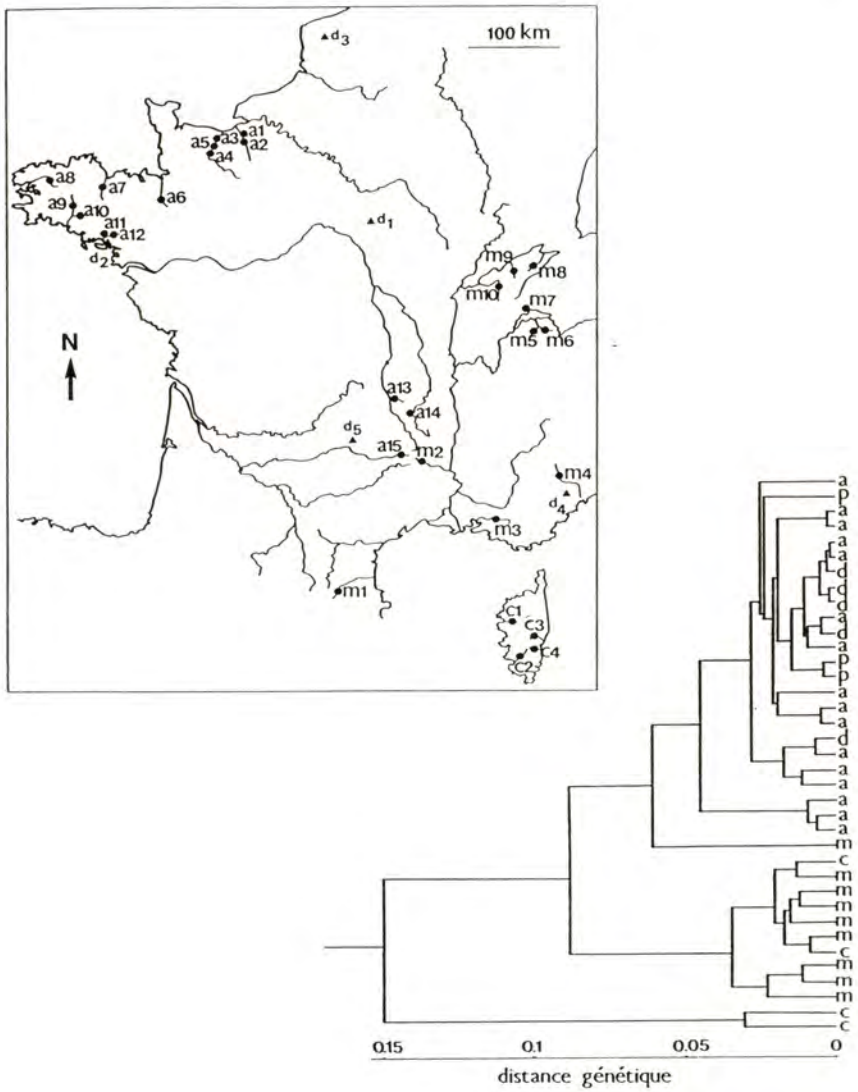


Fig. 4. — Genetic differentiation between french populations of brown trout *Salmo trutta* (from Guyomard, 1989).
a. wild populations from the Manche and atlantic drainage areas
m. wild populations from mediterranean drainage area
d. domestic populations
c. wild populations from Corsica
p. population from Polish origin (Baltic sea).

The combination of these two pieces of information makes it possible to give a general opinion on the differentiation rate of the studied populations, namely the relationship between the average genetic inter-population variation and total genetic variation of the species obtained by regrouping all these popu-

lations. In this example, this rate is high, exceeding 50 % for all the 47 populations studied.

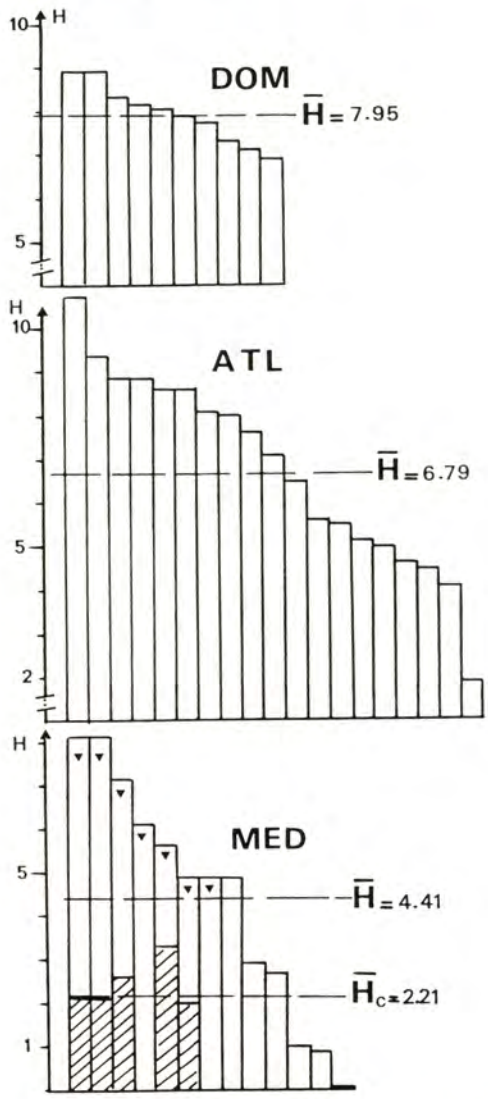


Fig. 5. — Distribution of heterozygosity (%) among 42 french populations of brown trout *Salmo trutta* (46 enzymatic loci).

DOM 10 domestic populations
ATL 19 wild populations from the Manche and atlantic drainage areas
MED 13 wild populations from the mediterranean drainage area.

— Rivers with a restocking with domestic populations

— Corrected value of heterozygosity to eliminate the effect of restocking.

Fig. 6, 7 and 8 illustrate various situations met in salmonids and allow to draw several general conclusions.

— *The variability within natural populations usually exceeds 50 % of the total variability of the species.* Therefore, it is advisable to give up definitively a conception according to which a local population should be made up of identical individuals different from those of another population. On the contrary, with certain exceptions each population represents an important pool of genetic variability.

The variability within hatchery stocks is not systematically lower than that observed in natural populations (Table 2). Some cases of a marked decrease in variability may be observed whereas some stocks may exhibit a high variability possibly linked to their heterogenous origin.

Tab. 2. — Comparison of intrapopulation heterozygosity in wild and domestic populations of salmonids

Species		Wild populations	Domestic populations	References
Salmo clarkii	H	2.2 (1, USA)	2.7 (1, USA)	Allendorf and Leary, 1988
Salmo gairdneri	H	31.0 (1, USA)	18.0 (9, USA) (8.6 - 29.5)	*in* Guyomard, 1981*
	H R		31.2 (6, FRANCE) (24.1 - 34.8)	
Salmo salar	H R	2.8 (9, SWEDEN) (2.2 - 3.5)	2.2 (9, SWEDEN) (1.5 - 3.1)	Stahl, 1983
	H	21.8 (1, IRELAND)	17.3 (1, IRELAND)	Cross and King, 1983*
Salmo trutta	H R	6.79 (19, West EUROPE) (1.8 - 10.8)	7.94 (5, FRANCE)	Guyomard, 1983
	H R	4.41 (13, South FRANCE) (0.0 - 8.2)		

* heterozygosity is estimated with a set of polymorphic loci and thus overestimated.
The table gives the mean value of heterozygosity (H), the number and origin of the populations and the extremes values observed in those populations.

The genetic differentiations observed between populations do not always confirm the hypothesis based on ecoethological observations. Thus, in salmonids a great attention is often paid to the migratory or sedentary character of the populations or their way of life (lacustrine or river populations) and sometimes so much that various ecotypes are considered as subspecies. The case of the arctic char (*Salvelinus alpinus*) perfectly shows (Fig. 7 B) that these ecological differentiations seem to be genetically minimal as compared to the geographic differentiation between populations of the same ecotype. Inversely, the case of the cutthroat trout (*Salmo clarkii*) illustrates the necessity sometimes of managing ecologically similar populations as different subspecies (Fig. 7 A). The results obtained with the brown trout (Fig. 3) lead to similar conclusions.

— *the genetic structure of the species may vary to a wide extent even between similar species* (Fig. 8) and therefore a particular study is necessary

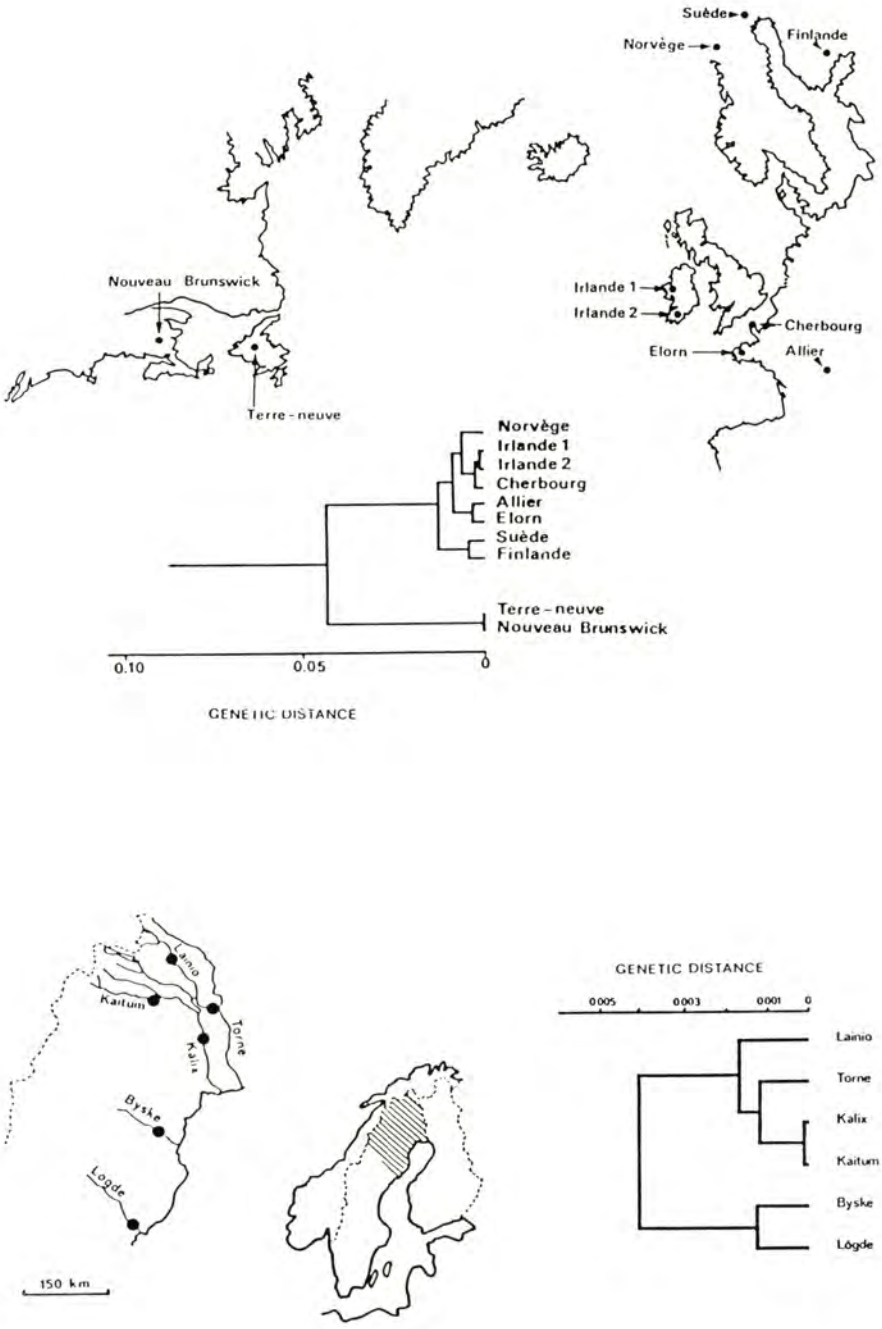
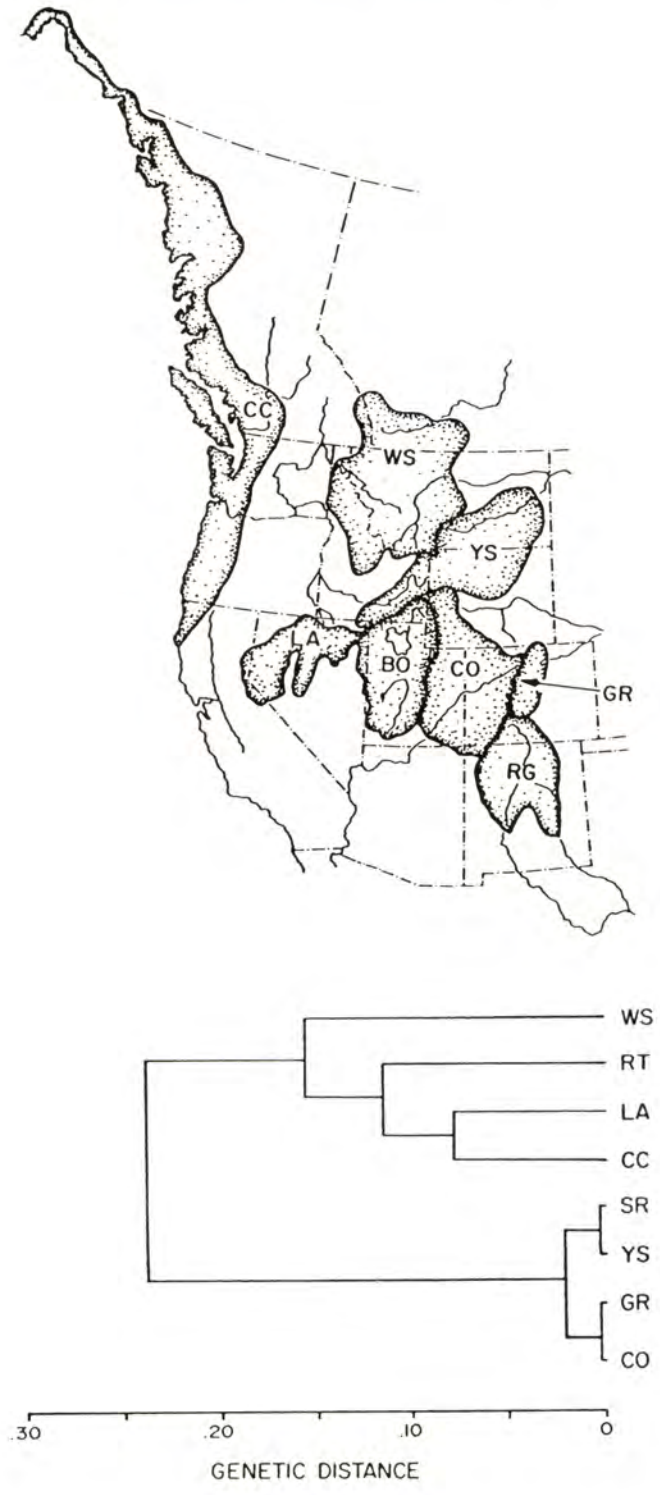


Fig. 6. — Genetic differentiation between populations of Atlantic Salmon from different european and canadian rivers (above) and within a small Swedish area (below).
From Guyomard (1987) and Stahl (1981).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GENETIC DISTANCE

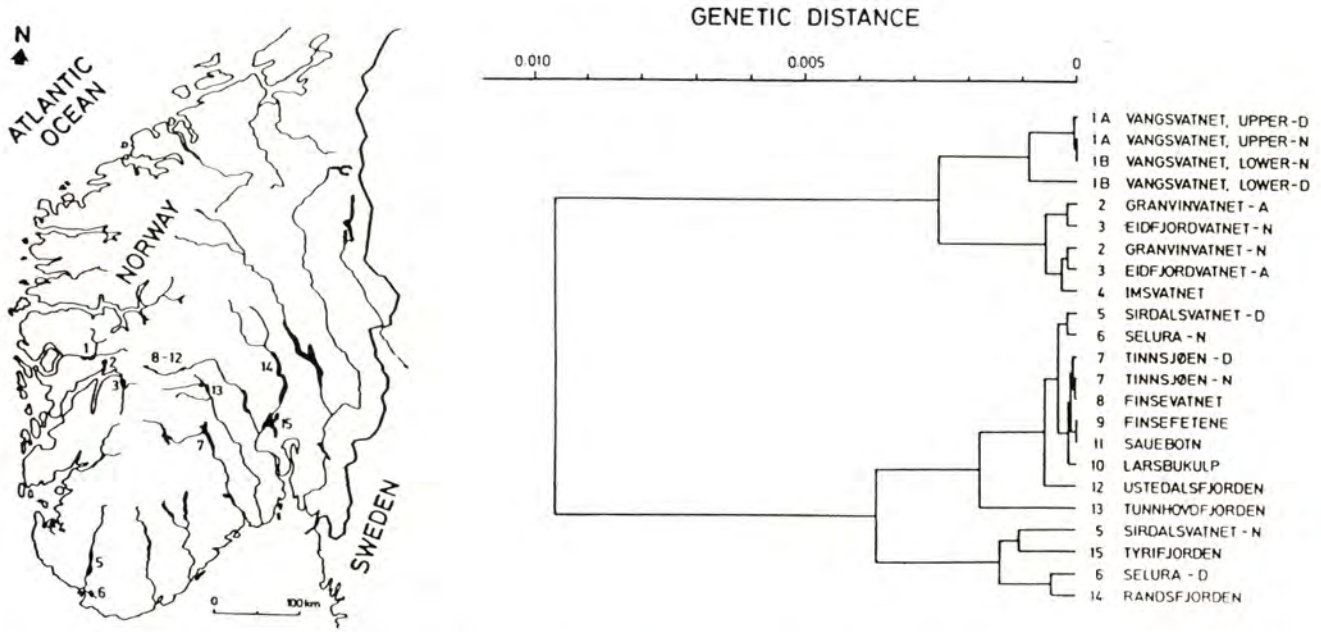

Fig. 7. — Two examples of genetic differentiation between populations of Salmonids.

above : *Salmo clarkii* (cutthroat trout) in the western part of the USA. The level of differentiation is high and the distinction of a new species (WS) is proposed (Allendorf and Leary, 1988).

below : *Salvelinus alpinus* (arctic char) is the south of Norway. The level of differentiation is low, especially between dwarf (d) and normal ecotypes inhabiting the same lake and considered before as « sibling » species. (Mindar et al., 1988).

each time. Thus, among 54 populations of a pacific salmon *(Oncorhynchus keta)* the differentiation rate is only 2 % (98 % intra-population variation). On the other hand, this rate reaches 70 % in cutthroat trout (*Salmo clarkii*) whereas it is only 16 % in a very similar species, the rainbow trout (*Salmo gairdneri*).

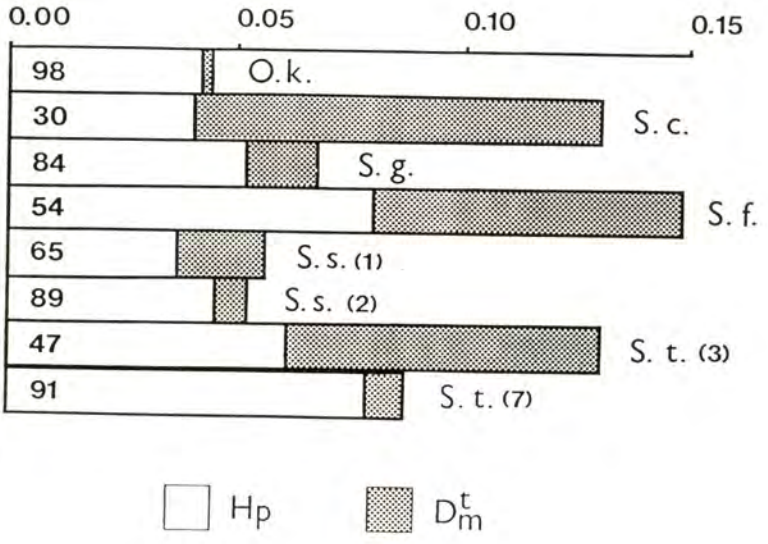

Fig. 8. — Decomposition of the genetic variability of different species of salmonids into two components :

H_p is the mean within population variability, D_m^t is the mean between populations variability. The left value is the level of differentiation

$$\frac{D_m^t}{D^t + H_p} \ (\%)$$

OK *Onchorynchus keta*, 54 populations, 22 loci
Sc *Salmo clarkii*, 24 populations, 35 loci
Sg *Salmo gairdneri*, 38 populations, 16 loci
Sf *Salvelinus fontinalis*
Ss *Salmo salar* (1) 10 populations, 32 loci
(2) 6 atlantic european populations, 32 loci
St *Salmo trutta* (3) 37 european populations, 46 loci
(7) 11
(Guyomard, 1989).

3. Interpretation and applications

How can these data be taken into account in the constitution of a spawner stock ? Generally, the advantage of constituting a stock exhibiting a large genetic variability from the beginning is justified by two types of arguments.

— *In the short term, there may be a certain relationship between the genetic variability of a stock and its culture performances.* Although they are not systematic such relationships were revealed several times in aquacultural species. Some examples will be given hereafter. Thus, a study of the length of time of embryonic development in 6 natural populations of rainbow trout (*Salmo gairdneri*) conducted by Ferguson *et al.* (1985) showed a positive relationship between the average heterozygosity of the strain and its growth rate (Fig. 9). The deviation is approximately 4 days (10 %) when the level of heterozygosity increases from 4 to 8 %. The same variations were found in this species when the relationship between individual heterozygosity measured by enzymatic markers and early hatching was studied within a population (Danzmann *et al.*, 1984). In the same way in molluscs marked relationships between growth rate and individual heterozygosity have been shown several times (Zouros and Foltz, 1987 a review). Fig. 10 illustrates this phenomenon.

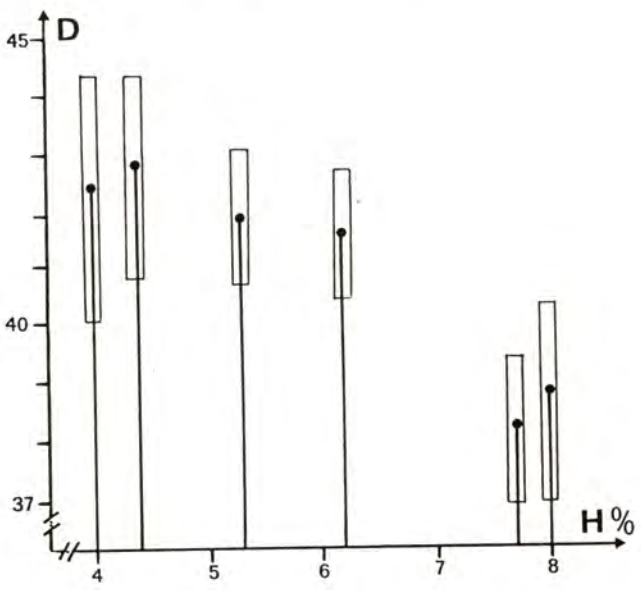

Fig. 9. — Relationship between the duration of embryonic development D (in days) and the mean heterozygosity H in 6 populations of rainbow trout *Salmo gairdneri*. The rectangle gives the standard deviation around the mean (from Ferguson et *al.*, 1985).

The heterozygosity effect may also be revealed on the variability of the trait instead of on its average value. A better « developmental stability » (developmental stability or genetic homeostasis, Lerner, 1954) would result in a lower variance of traits in heterozygous. The already mentioned study of Ferguson *et al.* (1985) (Fig. 9) especially showed that the hatching period was shorter in the most heterozygous populations. In the same way, Mitton (1978) showed in a poeciliidae, *Fundulus heteroclitus*, that

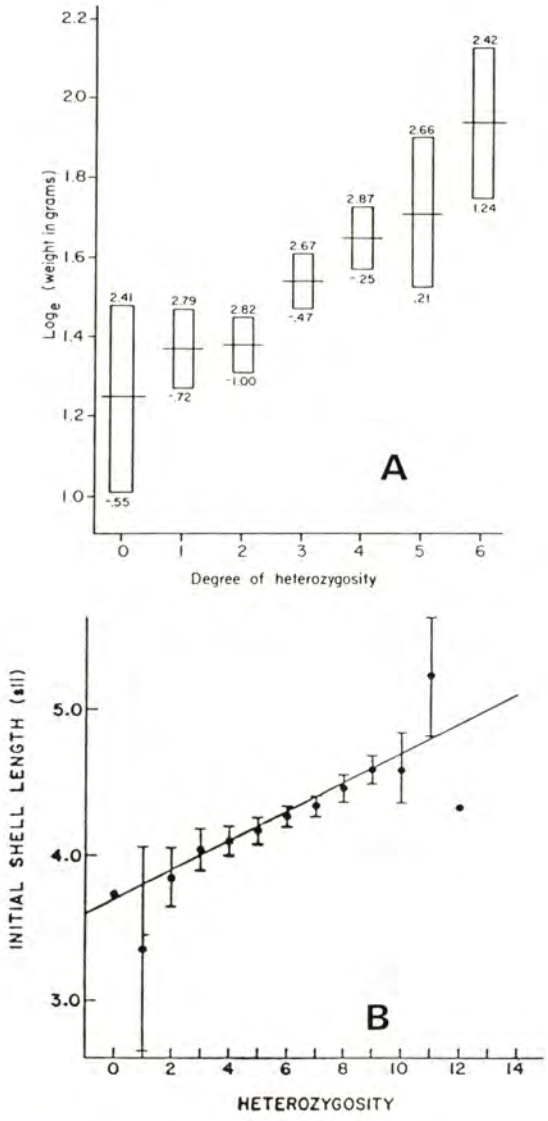

Fig. 10. — Two examples of positive relationship between individual heterozygosity (estimated by the number of loci which are heterozygous in a given individual) and growth potential in Molluscs.

A. weight of individuals at 1 year of age in the american oyster *Crassostrea virginica* (Foltz et *al.*, 1983)

b. lenght of the shell at settlement in *Mulinia lateralis* (Koehn et *al.*, 1988).

the inter-individual variance of meristic characters (number of scales, number of fin rays) was also generally lower in heterozygous individuals. This notion was developed in particular in vertebrates by studies on bilateral dissymmetry of these traits :

based on the counting of even external traits (number of fin rays, branchiospines, scales on the lateral line) it is possible to characterize each individual or each population by a dissymmetry index supposed to measure its « developmental stability » and connect this index with biochemical measures of heterozygosity. Several examples (Fig. 11) illustrate the relevance of this by

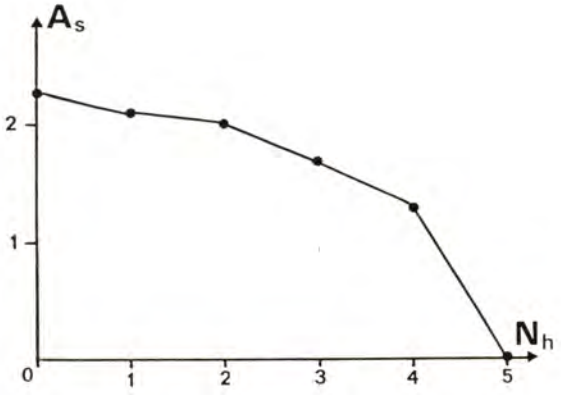

Fig. 11 A. — Individual relationship between number of heterozygous loci Nh and mean number of assymetric characters A_s in a rainbow trout population (from Leary et *al.*, 1983).

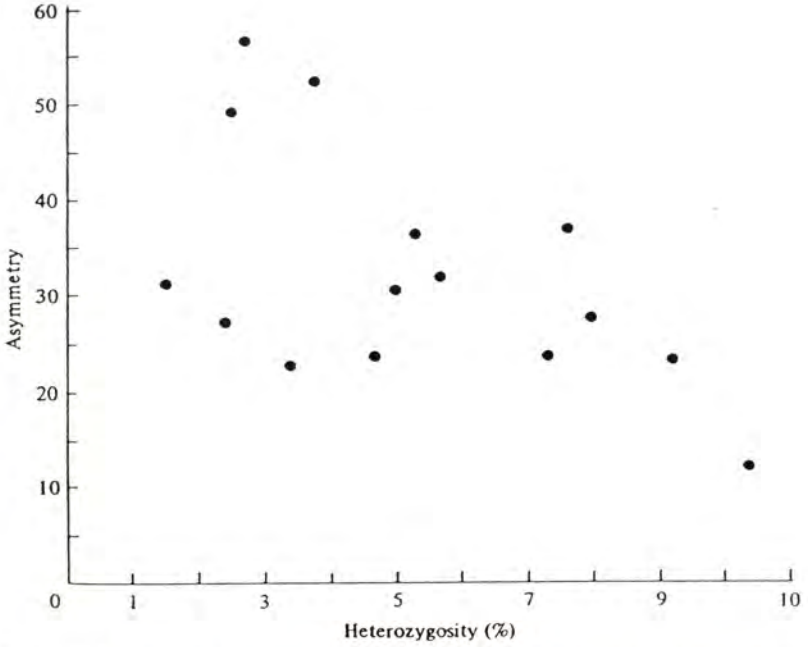

Fig. 11 B. — Relationship between mean heterozygosity and a symmetry coefficient among 14 populations of the lizard *Uta stansburiana* (from Soulé, 1979).

showing an increase in the dissymmetry index when the heterozygosity decreases (Leary et al., 1983, 1984, 1985a).

However, such approaches cannot replace a direct evaluation of performances of individuals or populations : these relationships are not systematic [see especially Mc Andrew *et al.* (1986) in plaice *Pleuronectes platessa*, Beacham and Withler (1985) in pink salmon *Oncorhynchus gorbuscha* for negative results], they only involve some traits and the correlations observed between performance and heterozygosity when they are significant, generally only explain a small part (10 to 30 %) of the total variability of the trait. Therefore, a direct improvement of the traits should not be replaced by methods aiming at maximizing heterozygosity. However, these illustrations seem sufficient to justify *a priori* selection of populations exhibiting a high heterozygosity.

— *In the long term, the response capacity of a population to an environmental change is classically linked to its level of genetic variability.* But domestication, with or without an additional selection of certain traits, constitutes an important « adaptation test » whose final result may partially depend on the population's initial genetic diversity. Carrying out experiments in this field is not possible in the case of aquacultural species but Fig. 12 gives an example of such evolutions in *Drosophila.*

In these conditions the constitution of a stock based on the regrouping of a large number of natural populations appears to be a solution to recommend systematically. However, a considerable modification of this proposal seems to be necessary.

- *In the case of a species with a low differentiation rate*, selection of a population with a high heterozygosity makes it possible to collect a large fraction of the total variability. When heterozygosity varies considerably between populations it is even possible to show that the choice of the population with the highest heterozygosity rate leads to a larger genetic variability than that of a synthetic population regrouping systematically all available populations. Therefore, regrouping two or three of the most heterozygous populations is sufficient. Regrouping live animals is often much more problematic than sampling intended for biochemical analysis. In this approach a progeny testing of performances of some interesting populations may constitute a supplementary selection criterion.
- *In the case of species with a high differentiation rate*, the creation of synthetic populations may have negative consequences in the short or long term which should be evaluated. Beforehand, a comment on the notion of high differentiation rate is probably necessary. As a matter of fact, a simple model known by the name of « molecular clock » (Nei, 1975) makes it possible to relate the genetic distance between two populations and the age of their differentiation. Thus, in the example given in Fig. 6, the average genetic distances between Swedish salmon populations of some 0.3 % are likely to correspond to an isolation of some 15,000 years. Therefore, one should be very prudent towards the possible

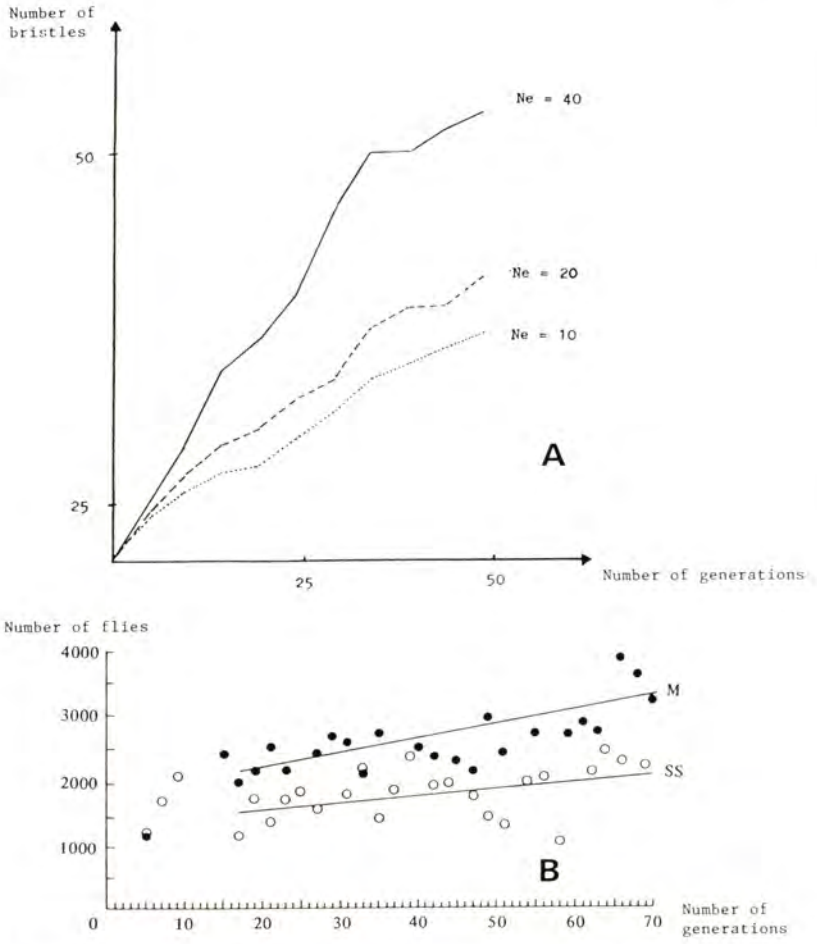

Fig. 12. — Two examples of relationship between genetic variability and « evolutive capacity » in drosophilae.

A. Long term response to selection for the number of sternopleural bristles. N_e is the effective size of the selected population at each generation.

B. Demographic evolution of two experimental populations : SS is a single strain population, M a « synthetic » strain resulting from a mixing of different strains. The average rate of increase in population size is about twice larger in the M population.
(from Ayala, 1965 in Frankel and Soulé, 1981).

disorder created by the regrouping of such populations. However, several examples lead to examine the validity of this approach. In particular experiments aiming at introducing salmonids on the Kerguelen islands conducted for some forty years with a single initial population have led to stocks with the same present differentiation levels (Fig. 13; Guyomard, 1984). Therefore, it seems difficult to concede a major evolutive significance to differentiation levels of about 1 to 2 % unless they are supported,

in the case of some enzyme systems, by marker alleles specific of certain populations.

Thus, in a study on two populations of brown trout coexisting in the same lake and having a relatively low genetic distance (2.5 %), Allendorf *et al.* (1976) were able to show that for an enzyme system (LDH 1) each population had a specific allele (common to all the individuals of one population and absent in the other) which certified the absence of crossings between these two populations. If such a phenomenon should be taken into account in terms of view management of natural populations it should not be interpreted as a genetic incompatibility between the two stocks. Spawner isolation may be the result of various ecoethological phenomena (for instance difference of spawning periods or zones) and only an experimental study would allow to draw conclusions on the possibility of obtaining viable progeny by crossing these populations.

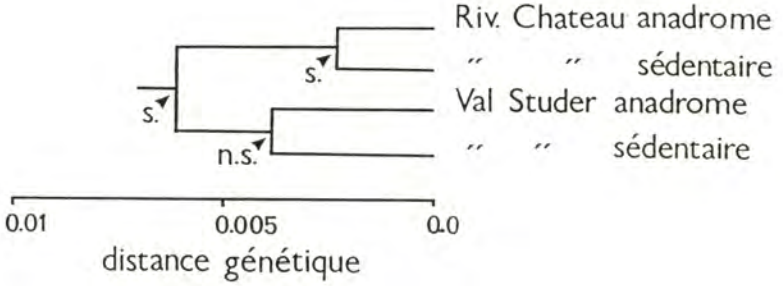

Fig. 13. — Ecological and genetic differentiation between brown trout populations of Kerguelen Islands (Indian Ocean) resulting from the same introduction around 1950.

For higher rates of differentiation (5 to 10 %) there are two possible consequences of the mixing of populations.

— *First generation individuals originating from the crossing between the populations may have a reduced viability.* This is known for interspecific hybrids (see especially Chevassus, 1983). On the other hand, examples in the case of intraspecific crossings are less numerous and not systematic (see in particular Frankel and Soule, 1981, p. 152 for a discussion) and tests carried out in particular between Atlantic and Mediterranean groups of brown trout (differentiation of about 10 %) do not seem to lead to such phenomena. In the same way after restockings in cutthroat trout (Fig. 7), the presence in nature of hybrids between the different populations, and even with a closely related species, rainbow trout, indicates that the viability of these hybrids is not substantially lower even if more accurate measures show slight depressions for some traits (Leary *et al.* 1985; Ferguson *et al.* 1988). Inversely, these crossings may reveal interesting performances, i.e. the heterosis effect (hybrid vigor) in particular illustrated in carp (Wohlfarth *et al.* 1975) and in American catfish (Plomb *et al,.* 1975). Therefore, the relationships between differentiation and performances of crossbreds should be considered according to a

probability curve model (« optimum outcrossing distance », Bateson, 1978) which should be standardized in each case.

— *Second generation individuals may have viability problems even when the first generation reveals to be viable* or even more performing than the parental populations. This phenomenon may in particular be the result of the occurrence of chromosomal abnormalities when the chromosomes of two populations present a certain number of structural differences. In the extreme case first generation individuals may be viable but sterile, and no second generation individual is produced. A gradual disappearance of the less viable individuals as affected by natural or artificial selection can be expected, but some theoretical models illustrate the possibility of establishing a long lasting depression of the fertility of individuals originating from such crossings. Once again examples must be taken outside aquatic species (Frankel and Soule, 1981, p. 152 a review), but they illustrate the necessity of taking this problem into account.

4. Concrete proposals

Because of the different previously mentioned arguments the manager is thus confronted with a possible contradiction between the advantage of regrouping a large genetic variability and the risk of having to face negative phenomena related to the regrouping of incompatible genetic units. Moreover, the relatively discreet and deferred character of these phenomena (advent in second generation) is likely to make their diagnosis difficult without their impact being negligible. Remaining in the case of limited experimental possibilities it seems advisable to adopt a triple strategy.

— *The constitution of a « reference population »* based on one population or a regrouping of extremely similar populations ($d < 0.01$). The proximity being moreover confirmed by geographical or historical considerations. This or these populations should be selected among those exhibiting a high level of heterozygosity.

— *The constitution of an « experimental synthetic population »* based on a larger selection of slightly different populations.

The maintenance of these two units will be similar and their survival, growth and fertility performances should be evaluated for at least two or three generations especially if the domestication involves a voluntary selection of certain traits. At the end of this period, it will be possible to eliminate one of the two units in particular if their performances differ markedly. On the other hand, if the performances are similar, conserving the two units, if possible in two different hatcheries, will constitute a precious security and possibly allows to develop original management and improvement strategies.

— *The experimental study of crossings between populations representative of strongly differentiated groups.* Taking into account the necessity of continuing this study to the second generation it can

only involve a restricted number of « couples » and shall be conducted at the same time as the other two operations. It may, however, contribute to detecting the effects of heterosis or to identifying original characteristics for subsequent use.

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IV. FINFISH

IV.3. LARVICULTURE, PRODUCTION OF JUVENILES

ADVANCES IN TROPICAL AQUACULTURE Tahiti, Feb. 20 - March 4, 1989
AQUACOP IFREMER Actes de Colloque 9 pp. 595-607

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Production of live prey for marine fish larvae

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Abstract — *Tropical marine fish larvae vary in their requirements for live planktonic food. Selection of live prey species for culture depends on larval size and larval tolerance of water quality. This report describes some of the cultured prey species, and their uses and limits as effective food for fish larvae. Methods are presented for the culture of phytoplankton, rotifers, copepods, and other live feeds. Difficulties in rearing certain species of marine fish are compared to their dietary requirements.*

Feeding methods are more complex for larvae from smaller eggs, more pristine environments, and pelagic versus demersal embryonic environments. Copepods are the natural diet of almost all first feeding marine fish larvae, and can be cultured in unlimited quantities. The nutritional profile of a cultured copepod, ***Euterpina acutifrons*** *surpasses all other live larval foods. The smallest and most sensitive reef fish larvae tested will consume nauplii of* ***E. acutifrons****, but survival is poor due to a toxicity effect.*

Dependable phytoplankton culture is the key to successful larval rearing. Sterilization, inoculation, nutrient enrichment, species selection, and facility design are discussed.

Rotifer production depends on temperature and algal species. About 2.5 % of larval rearing volume is sufficient rotifer production volume for densely stocked, fast-growing fish larvae. Algal enrichment is the safest method when rotifers are used for marine fish larvae, and yields two-fold growth of rotifers to 100-150 rotifers/ml.

Copepods are cultured to densities of 20-50 copepodites/ml (0.1 g/l.), with population growth rates of 15 %/day. Algal species and water quality maintenance determine copepod growth rate and harvestable biomass.

INTRODUCTION

Marine fish larvae are more difficult to raise than anadromous fishes or invertebrates. Much of this difficulty can be attributed to complex requirements through a relatively long hatchery phase (see Figure 1). Species become more difficult to raise when their eggs are smaller or less numerous, or when artificial spawn induction is required. Length of time in the hatchery phase, and number of food species required increases the chances for making fatal errors or introducing catastrophic disease, and larval tolerance to organic pollutants makes a big difference in the amount of care needed to achieve high yields.

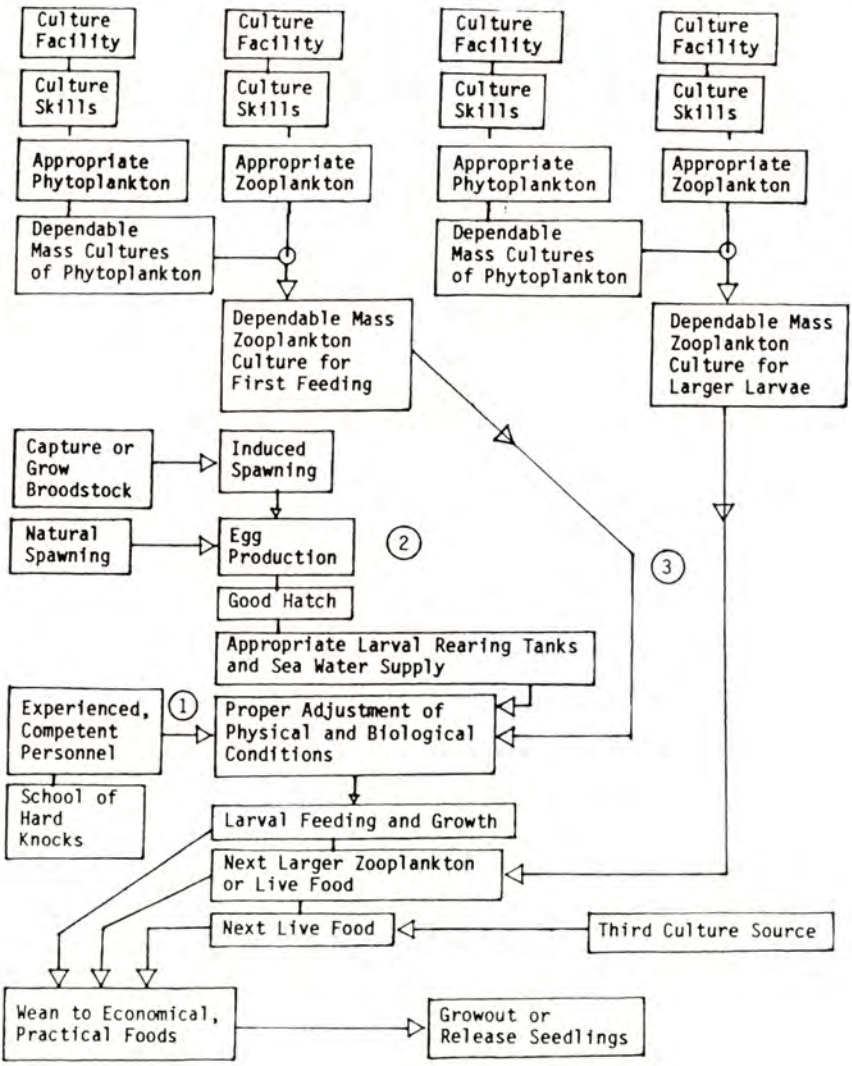

Fig. 1. — Necessary components of successful marine fish seedling production.

OUR EXPERIENCES WITH VARIOUS LARVAE

Because of its large inventory of tropical marine fishes, the Waikiki Aquarium frequently has the opportunity to attempt to rear many types of larvae. Some of our attempts succeed, and some fail. Those larvae that survive our manipulations fall into one of the following descriptions : eggs larger than 0.9 mm; larvae hatch from demersal nest, ready to feed; larvae found naturally in stressful environments (i.e., tidepools, mangrove swamps, nutrient-rich waters, etc.). Some larvae survive first feeding

without problems but seem to suffer from nutritional inadequacy after 2-3 weeks (eg. reef shrimps.). Most larvae of tropical reef fishes hatch from pelagic eggs in offshore waters, are less than 750μ diameter, and fail to survive first feeding in our hatchery. These larvae do not ingest rotifers, *Brachionus plicatilis*, probably because of size. The dinoflagellate, *Gymnodinium spendens*, has been used successfully for anchovy larvae (Lasker et *al.*, 1970). Our preliminary tests with this small (20 × 30 × 40 μ) flagellate show ingestion by *Scorpaenid larvae* (1.3 mm TL), but poor survival. Further tests may be more successful. Although the smallest of these larvae consume nauplii of our cultured copepod, *Euterpina acutifrons*, they usually become weak and cease feeding within 1 day. This particular problem is common to larger larvae from pristine environments, namely the mahimahi, *Coryphaena hippurus*. Thus we are using mahimahi as a model to develop methods of using cultured plankton for feeding tropical marine fish larvae.

There are other approaches to first feeding of delicate larvae : Victor Oiestad (Kvenseth and Oiestad, 1984) promotes natural zooplankton blooms in large natural and artificial impoundments, then stocks hatched larvae, with good survival. Similarly, redfish larvae are stocked in Texas ponds, and Rabbitfish larvae are stocked in mangrove ponds. Stocking extensive « natural » environments is successful, but difficult to control and analyze. We are hopeful that the study of plankton cultures, using mahimahi as a model, will help us understand the nutritional and environmental requirements of larvae, so that we can improve hatchery yields.

MAHIMAHI AS A MODEL

Mahimahi spawn without artificial inducement, and their larvae are larger (4.5-5.0 mm at hatch) and grow faster than most tropical marine fishes. However, their need for several different live diets, and their sensitivity to pollutants (they normally spend their larval phase in pristine open ocean water) call for stringent technology. Therefore, mahimahi are a sensitive model for testing live feeds, and they have the advantage of being available from natural spawns year round.

Successful hatchery culture of the mahimahi *Coryphaena hippurus* depends on meeting relatively fastidious dietary and environmental requirements. Yolk sac larvae require upwelling sufficient to counteract downward migration. First feeding larvae prefer copepodites (electivity index, e = + 0.52) and nauplii (e = + 0.20) of the cultured copepod *Euterpina acutifrons*, but survive significantly better on the rotifer *Brachionus plicatilis* (e = + 0.15). Thus, for first feeding larvae, food preference (Figure 2) is not a valid indicator of optimal diet. Some first feeding mahimahi larvae ingest newly hatched brine shrimp *Artemia franciscana* (e = - 0.25), but larval survival is poor if brine shrimp are used as a sole diet.

Food preference and optimum diet composition change as larvae grow. From the seventh to 20th post-hatch day, larval survival and growth rates are significantly higher when larvae are fed cultured copepods rather

than rotifers or brine shrimp. Fry occasionally begin accepting nonliving foods by day 20. From first feeding through day 20, larvae gain 20 % body weight per day with a food conversion efficiency of 0.3 at ambient Hawaii temperatures (23 to 26°C) (Kraul et *al.*, 1989).

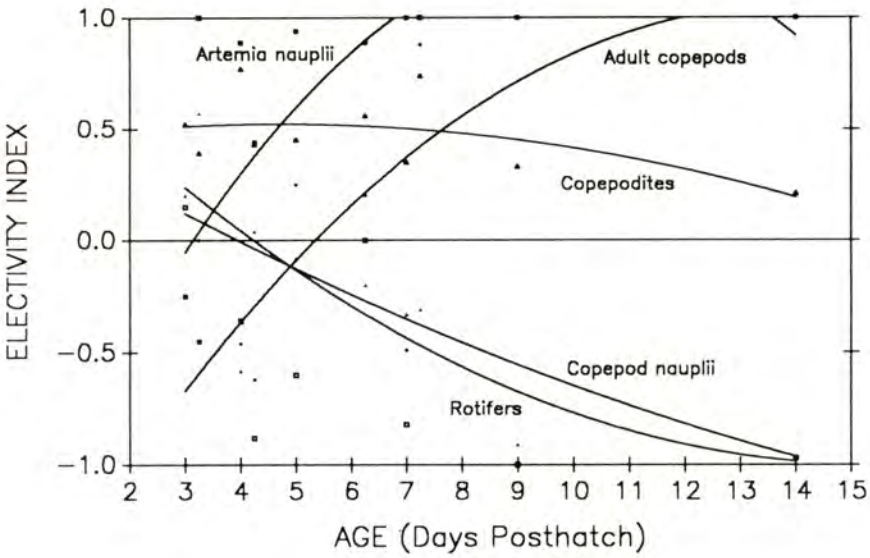

Fig. 2. — Food preference of larval Mahimahi *(Coryphaena hippurus)*.

Mahimahi larvae appeared to select food based on size and some other visual quality : (optical reflectivity, motion). When rearing water became supersaturated with oxygen, newly feeding larvae ingested gas bubbles of appropriate size until their guts were full of gas. This phenomenon has also been observed in mullet (Kraul, 1983) and other fishes. Copepods were selected over rotifers of equal width. Movement and light refraction differed in these two prey species. Nevertheless, larvae consumed any appropriately sized live food items when they were sufficiently abundant.

Lower survival on copepods during the first week was not a nutritional problem since larval growth rates were not significantly different on any of the diets offered. Survival of starved larvae was greater than survival of copepod-fed larvae by day 4 (Figure 3). Carnivorous attacks on the larvae by *E. acutifrons* were not observed. The first feeding larvae's deleterious reaction to copepods may have been caused by a toxin or microorganism associated with copepod culture medium. Older larvae were able to tolerate copepod « poisoning », perhaps through the development of a functional immune system, and derived better nutrition from copepods than from brine shrimp. Improved larval survival on a copepod diet has been demonstrated for mullet (Kraul, 1983).

There is a tank size effect at first feeding. Growth was comparable in large (4,000 l.) and small (200 l.) tanks through day 5 but was signifi-

cantly greater in the large tank by day 9 (7.92 +/− 0.39 mm total length for large tank larvae with all foods versus 6.48 +/− 0.34 mm for copepod-fed and 6.64 +/− 0.30 mm for rotifer-fed larvae in small tanks).

Tab. 1. — Daily feeding rations for 20000 Mahimahi larvae. Based on starting weight of 0.7 mg, growing 25 %/day, FCE = 0.3

		Number of food items × 1 million			
DAY	GRAMS OF FOOD	≠R($\times 10^6$)	≠C($\times 10^6$)	BS(g) cysts to start for	≠H($\times 10^5$)
2	1.1	6			
3	1.3	7.3			
4	1.6	9.1		3d BS :	
5	2.0	11.4			
6	2.4	14.2	1.0		
7	3.1	17.8*	1.3*	3*	
8	3.8	22.3	1.6	4	
9	4.8	27.8	2.0	5	
10	6.0	34.8	2.4	6	
11	7.5		3.1	7	
12	9.3		3.8	9	
13	12		4.8	11	
14	15		6.0	14	
15	18		7.5	18	
16	23		9.3	22	
17	28		11.6	28	
18	36		14.6	35	.4
19	44		18.2	44	.5
20	56		22.7	55	.6
21	69		28.4	68	.8
22	87		35.5	85	1.0
23	108			107	1.2
24	136			133	1.5
25	169			167	1.9
26	212			208	2.4
27	265			260	3.0
28	331			325	3.7
29	414			406	4.7
30	517			508	5.8

* Quantity in each column fulfills the entire food ration. In practice, one food type is decreased while the next type is introduced, and the food ration is provided by the sum of the two types.

Nutrition has been shown to be a factor with the types of food reported here. Watanabe et *al.* (1978a, b) found that *Tigriopus* sp. copepods had a high proportion of essential fatty acids regardless of their medium, whereas rotifers (and *Artemia*) were not as nutritious unless their culture medium was optimum. Enrichment of rotifers and brine shrimp with essential fatty acids is currently being studied, using the nutritional profiles of copepods as a standard.

Heavy mortality during the transition to nonliving foods has been observed in other species (Bromley and Howell, 1983), and stressful energy expenditures during the metamorphic transitions in these and other species (Corbin, 1977) may explain the importance of diet in improving survival. Our current studies show that the use of newly hatched mahimahi larvae as a food (starting at day 18, when PLs are over 15 mm TL) greatly

improves postlarval survival through weaning. Once PLs are weaned onto squid (with a vitamin supplement), they suffer few mortalities. Development of a commercially practical diet is in progress.

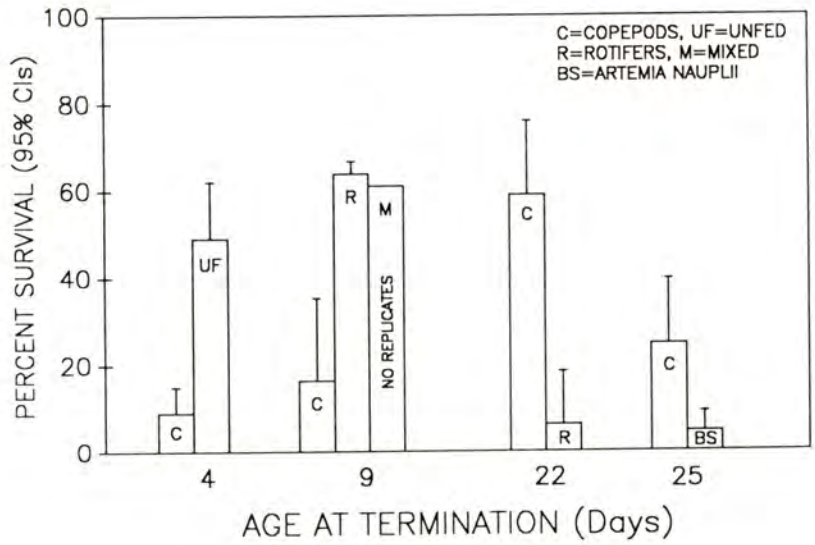

Fig. 3. — Effect of diet on larval mahimahi survival.

Summary of Mahimahi as a Model

Rotifers, copepods, and brine shrimp were accepted in varying degrees as a first food by larval mahimahi. Best survival was obtained by feeding rotifers days 2 to 8, copepods day 6 to 21, brine shrimp day 10 to 25, and newly hatched mahimahi larvae after day 18. Mahimahi larvae increased their weight at least 20 % per day through day 21, and had a food conversion efficiency of 0.27 to 0.33. Total live plankton consumption through 21 days was 1,000 rotifers plus 1,333 copepodities plus 2,500 brine shrimp nauplii per larva. From day 18 through 40, each PL will consume about 8,000 newly hatched mahimahi larvae.

PLANKTON CULTURE METHODS

A. Algae culture

Microscopic, single-celled algae (phytoplankton) is cultured at the Waikiki Aquarium for use as a food for filter feeders such as the *Tridacna* clam and various corals, and for feeding microinvertebrates such as brine shrimp *(Artemia)*, rotifers, and copepods. Microinvertebrates are used for feeding larval and juvenile fishes and corals. We culture several families of phytoplankton; the techniques for their culture are almost identical.

Some of the species we use are *Tetraselmis chuii, Chaetoceros gracilis,* and *Gymnodinium splendens.* To keep track of which species is in which container, each species is given a code of the first two letters of the genus name, i.e. TE, CH, GY.

Procedures

1) Clean the containers. Bottles should be acid cleaned and flushed. Large vats should be scrubbed lightly and rinsed out. The object here is to remove most of the surface-bound organic matter so that the chlorine sterilization (next step) is more effective. Be sure to fill acid cleaned bottles completely with tap water to remove acid vapors.
2) Fill containers with seawater, and add 1.0 ml of Clorox (5.25 % sodium hypochlorite) for every litre of seawater. Let the containers sit unaerated away from strong light overnight. If water is needed sooner, add 5 ml of Clorox per litre and let stand at least 2 hours. The object here is to maintain a chlorine residual of > 10 ppm overnight. Residual chlorine is affected by organic load, so more will be needed in dirtier water. A swimming pool test kit is adequate for measuring residual. Strong light and aeration will disperse chlorine and reduce its strength.
3) Neutralize the chlorinated seawater by adding 1.0 ml of sodium thiosulfate solution (1N, 250 g Na2S2O3.5H2O per litre of distilled water) for every 4.0 ml of Clorox that was added earlier. Neutralization is complete when water is mixed (i.e. turn on air). Airstones are not recommended for algae cultures because they take too long to clean. A plastic pipette or piece of lead on an airhose will do. If airstones are used, soak them in full-strength Clorox between batches and rinse them thoroughly before use.
4) Add nutrients to the sterile seawater. Our « F/2 » nutrient stocks (Guillard, 1975) are made up at 500 times final concentration (Appendix B). Thus, 2 ml of nutrient should be added to each litre of sterile seawater. Nutrients are kept in a plastic bottle in the refrigerator. Normally, 2 l. bottles get 4 ml of F/2; 100 l « buckets » get 200 ml of F/2, etc.
5) Add algae inoculant. It is best to check microscopically and record cell counts and health occasionally. You can usually tell by color if a 2 l culture is healthy and dense enough to use as inoculant for 100, 200, or 600 l cultures. Note the date, inoculant source, inoculant quantity, and algal species on a piece of tape (or directly on glass bottles) whenever a new culture is started. This procedure will help you to determine how well a culture is doing, and is good for inventory control.

B. Rotifers

Much literature is available on the culture of *Brachionus plicatilis* (Theilacker and Mc Master, 1971; Snell and Carrillo, 1984). At Waikiki Aquarium, rotifers reproduce parthenogenically about 100 % per day,

feeding exclusively on *Tetraselmis chuii* (TE). At densities above 100 R/ml, we replenish 50 % of rotifer water with undiluted TE daily, to maintain 100 % rotifer growth.

The keys to using rotifers successfully are : use only heathy phytoplankton of good nutritional value; clean the rotifer container frequently, especially before using the rotifers as a larval food; keep the harvesting screen (we use 60ñ nytex) submerged to avoid irreversible drying; monitor rotifer density and change water before density decreases.

C. Copepods

The harpacticoid copepod, *Euterpina acutifrons*, is a nearly ideal food for larval marine fishes due to its size, trophic ecology, nutritional value, culturability, and (most importantly) acceptability by pelagic marine fish larvae. There are few literature references to successful copepod culture. Suggested readings include Theilacker and Kimball (1984); and Zurlini et *al.* (1978). Culture techniques are easy, using the following suggestions.

1) Do not use an algae that gets too slimy and settles heavily. You will want to aerate sufficiently to suspend the algae, but not so much that you interupt sexual coupling. Slimy surfaces will trap nauplii. *Chaetoceros gracilis* ($4 \times 4 \times 5$ µ) works well. You may get faster growth and a higher fecundity if a dinoflagellate or other flagellated green phytoplankter is present. Normal growth rates at the Waikiki Aquarium are 10-15 % per day (up to 100 fold increase in 8 days), with harvest densities of 20 to 50 adults copepods per ml.
2) Partial shading helps, if cultures are outdoors. Keep the cultures in a growth phase, and change them over to a clean container every few weeks.
3) Inoculate with 1-10 % of your harvest. I like to keep the density above 1 per ml so I can count them with a 1 ml. pipette, but they will grow fast at densities of 1 per litre. Algal densities of 5×10^4 to 2×10^5 cells per ml will give good growth rates. You can approximate these densities as visibilities of 7 to 10 cm.
4) Do not let rotifers enter the system. They will usually outproduce the copepods. It is difficult to keep these two zooplankters separate with screens because their sizes overlap. If you do get a rotifer takeover, isolate 10-100 gravid female copepods and start over in 2 to 40 l of new medium (check them in a microscope to make sure there are no rotifers).
5) There are 6 naupliar stages, and 6 copepodite stages, including the adult. Size is $50 \times 50 \times 70$ µ (N1) to $150 \times 175 \times 700$ µ (C6). I use a 37 µ screen to harvest N1 & N2, and a 100 µ screen for copepodites. Generation time is about 8 to 11 days under best conditions, at temperatures of 24-26°C. If you stock your rearing container with all sizes of copepods, new nauplii will be produced by the adults to replace those consumed by the fish larvae.

D. Brine shrimp

For our purposes, *Artemia* spp. are not cultured, but merely hatched, fed, and used as nauplii. Other review at this workshop discuss brine shrimp in detail.

E. Other plankton

If postlarval marine fishes will not accept nonliving foods after metamorphosis, they can sometimes be fed grown out brine shrimp. However, we find that brine shrimp are nutritionally deficient for mahimahi postlarvae. It is possible that new enrichment technology will allow the use of juvenile and adult Artemia as a food for postlarval fishes. At the Aquarium, we use newly hatched mahimahi larvae as a food for postlarval mahimahi when they are larger than 15 m TL. Hatchling mahimahi are not cultured, but merely hatched, rinsed, treated with Prefuran, and added to the rearing tank as a live food. Growth and survival are excellent using hatchlings, and we almost always have enough for mass cultures.

Other larger plankton is generally difficult to culture, or of low yield. Cladocerans (Takami et *al.* 1978) and calanoid copepods may be used on a limited scale.

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APPENDIX I

FOOD REQUIREMENTS FOR REARING 20,000 MAHI-MAHI LARVAE IN 4000 LITERS

A. CULTURE CAPACITIES

1. *ROTIFERS*

MINIMUM DENSITY & QUANTITY = > 2/ml and > 200R/larva

a) 2/ml × 4 × 10^6 ml = 8 million rotifers = 400 R/larva
b) 200 R/larva × 20000 larva = 4 million rotifers

CULTURE & BACKUP :

a) 8 million = 40 R/ml × 200 litres = feed for day 2.
b) 20 million = 100R/ml × 200l = suggested minimum preparation.
c) Tank ≠ 1 = 100 R/ml × 150 l = 15 million on day 2.
   Tank ≠ 2 = 50 R/ml × 150 l = 7.5 million on day 2.
d) 1 million R = 0.18 g (our wet weights).

2. *COPEPODS*

a) 1 million copepodites (> 100 μ screen size) = > 2.3 g.
b) Day 6 feed = 1 million C = 50 l × 20 C/ml.
c) Use Table 1 after day 6, using 3 l weight samples, rather than ≠ C/ml.

3. *HATCHLING (H) mahimahi = 0.9 mg*

On day 18, 36 g of food can be provided with 40000 newly hatched mahimahi larvae.

4. *LARVAL WEIGHT GAIN ANALYSIS*

a) Rotifers/first feeding : Assume larva growth = 0.7 mg × 20000 larvae × 25 % weight gain/day × 0.3
   FCE = 1.05 g food on day 2 = 5.8 million Rotifers. Use table 1 for subsequent days.
b) Copepods : Use weights, not numbers, unless scale is not available.

APPENDIX II
ALGAL NUTRIENT (F/2) PREPARATION

Algal nutrients are prepared by adding 4 major chemicals, 5 trace elements, 3 vitamins, and 1 optional chemical (silicate : we already have plenty in our water) to de-ionized or distilled water. This « F/2 » stock is stored in the refrigerator and added to the sterilized seawater at 2 ml per litre to give the final concentrations listed below.

ELEMENT (AW)	FINAL CONC.	SOURCE (FW)	AMOUNT OF SOURCE PER 3.5L STOCK
N (14)	12 mg/l	NaNO3 (85)	128 g
P (31)	1.2 mg/l	KH2PO4 (136)	9.2 g
EDTA (292)	4.3 mg/l	Na2EDTA.2H2O (372)	9.7 g
Fe (56)	0.65 mg/l	FeCl3 (162)	3.4 g
Trace elements	(see below)		1.8 ml @ × 5
Vitamins	(see below)		17.5 ml

TRACE ELEMENTS are easier to measure as dilute stock solutions. A single pipette can be used to add each of these to the F/2 stock. Each of these 100 ml bottles is enough for about 100000 l of algae culture. These bottles do not have to be refrigerated. Keep each trace element in a separate 100 ml. bottle.

ELEMENT (AW)	FINAL CONC.	SOURCE (FW)	AMOUNT OF SOURCE PER 100 ML STOCK
Cu (64)	2.5 ug/l	CuCl2.2H2O (170)	0.66 g
Zn (65)	5.0 ug/l	ZnCl2 (136)	1.05 g
Mn (55)	50.0 ug/l	MnCl2.4H2O (198)	18.00 g
Co (59)	5.0 ug/l	Co(NO3)2.6H2O (291)	2.47 g
Mo (96)	3.0 ug/l	Na2MoO4.2H2O (242)	0.76 g

VITAMINS B12 and biotin should be kept frozen, if possible, at 100 mg per 50 ml distilled water. To prepare vitamin stocks, add 16 mg (8 ml) of thawed, stirred vitamins to 300 ml distilled water. Also add 6.6 g Thiamine HCL (vitamin B1). Store this solution in the refrigerator and use 17.5 ml per 3.5 l. F/2 stock.

Final concentrations using these doses are :

B1 = 2.2 × 10-4 g/l;
B12 = 5.4 × 10-7 g/l;
Biotin = 5.4 × 10-7 g/l

SILICATES may be added if needed to diatom cultures at a final concentration of 4.3 mg/l. 17.4 g of Na2SiO3. 9 H 2O in 400 ml of distilled water can be used as a source (1 ml/l) of enrichment for small cultures. For large cultures, it is easier to weigh out 4.35 g of silicate per 100 L of culture. Hawaiian waters have abundant silicate, so it is not added.

BORON in the form of boric acid is sometimes used in µg/l quantities for seawater sources which lack boron. Hawaiian waters are not limited by Boron, so it is not added.

SUBSTITUTIONS :

You can compute the grams (g) of any source mineral needed with this formula :

$$\frac{\text{(final conc.) (500 ×) (FW) (3.5 l)}}{\text{(AW) (moles of element per mole of source)}} = \text{g of mineral added to 3.5 litres of F/2 stock}$$

i.e., for N : $\frac{\text{(12 mg/l) (500) (85 g/mole) (3.5 L)}}{\text{(14 g/mole) (1 mole/mole) (1000 mg/g)}}$

= 128 g of NaNO3

Substitutions can also be made by the following proportion :

Amount of new source

$$= \frac{\text{(FW new source) (amount of old source)}}{\text{(FW old source)}}$$

as long as the new element has the same number of moles of the important element as the old source.

SOME SUBSTITUTIONS :

CuSO4 (FW = 160)	0.62 g/100 ml stock
CoCl2.6H2O (FW = 238)	2.02 g/100 ml stock

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Enrichment of live foods

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Abstract — *Live foods such rotifers and Artemia are still indispensable for mass propagation of larval fish. At present, more than 20 fish and crustacean species are mass produced in Japanese governmental fish farming centres and private hatcheries. Fry are either released into the coastal areas or used for commercial cultivation. This culture technology is rapidly developing with new species being introduced each year and larval rearing is increasingly identified as the number one constraint. Artemia is used most extensively, particularly for the mass production of Kuruma prawn,* ***Penaeus japonicus.***

The dietary value of Artemia nauplii for fish and shrimp larvae is dependent on the geographical origin of the particular strain. This dietary value is mainly controlled by the essential fatty acid (EFA) composition. However, this can be manipulated and the direct and indirect methods of enrichment used in Japan are reviewed. The principal factor for fish is n-3 highly unsaturated fatty acids (n-3 HUFA) such as 20 :5n3 and 22 :6n3. Differences in rates of incorporation of n-3 HUFA into live food and their nutritional quality will be discussed from the viewpoint of the different lipid classes such as methyl esters and triglycerides. Results have shown that the enrichment in terms of n-3 HUFA incorporation into live feed by both the direct and indirect methods is very effective in improving the dietary value of rotifers and Artemia nauplii.

ADVANCES IN TROPICAL AQUACULTURE, Tahiti, Feb. 20 - March 4, 1989.
AQUACOP, IFREMER, Actes de Colloque **9** pp. 611-623.

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Effects of nutritional enhancement of live food organisms on growth and survival of Barramundi/Seabass *Lates calcarifer* (Bloch) larvae

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Abstract — *Larvae of barramundi (**Lates calcarifer** Bloch) reared intensively in some Australian hatcheries have suffered periodic high mortalities which have been ascribed to nutritional deficiencies in the live food organisms used, particularly deficiencies of polyunsaturated fatty acids. Barramundi larvae were reared in an experimental system and fed on four diets, representing combinations of supplemented and unsupplemented rotifers **(Brachionus plicatilis)** and supplemented and unsupplemented brine shrimp **(Artemia salina).** Supplementation of rotifers and brine shrimp with a commercially available microencapsulated diet increased the levels of several polyunsaturated fatty acids in the food organisms. Two test diets (both using freshly hatched brine shrimp) produced near total mortality by day 30, while the other two (using supplemented brine shrimp) produced negligible mortality over the same period. Supplementation of rotifers and brine shrimp resulted in significantly larger larvae at day 22 compared with larvae fed on unsupplemented rotifers and newly hatched brine shrimp. The enhancement of the fatty acid composition of live food organisms used in intensive rearing is discussed with respect to the observed effects of different diets on growth and survival of barramundi larvae.*

INTRODUCTION

Barramundi (*Lates calcarifer*) is the premier sportfish of northern Australia. It supports an important commercial wildstock fishery and is a major component of the recreational fishery of Queensland, the Northern Territory and Western Australia. Because barramundi are catadromous, this species cannot support self-maintaining populations in freshwater

impoundments, which are important recreational fishing areas throughout eastern Australia. For this reason, there is considerable public demand for barramundi to be stocked into impoundments, as well as into rivers and streams where existing barramundi populations are believed to be in decline. In addition, there is increasing interest in this species for aquaculture, due to the consistently high prices demanded by barramundi on the Australian market.

The Northern Fisheries Research Centre (N.F.R.C.) in Cairns has designed a small-scale hatchery to develop techniques for rearing barramundi. Many of the hatchery techniques developed at N.F.R.C. are now in use at commercial hatcheries in Queensland. These commercial hatcheries are primarily involved in aquaculture of « plate size » barramundi (c. 500 g) for the restaurant trade, but most also produce barramundi fry for stocking.

Barramundi reared at N.F.R.C. have periodically suffered severe mortalities (up to 90 % in some batches) at around 12 to 14 days after hatching. The symptoms involved a stress response where the larvae swam erratically following any disturbance, followed by « fainting », then death or recovery. Histological examination of the larvae showed extensive vacuolation of the brain and spinal cord and accumulation of excessive fat deposits in the liver (Rodgers and Barlow 1987).

Commercial barramundi hatcheries in northern Queensland have reported frequent occurrences of a similar mortality syndrome. Larval mortality problems are adversely affecting production from Australian barramundi hatcheries and are a significant threat to the establishment of a viable barramundi aquaculture industry in Australia.

From a survey of available literature on intensive rearing of marine fish larvae, Rodgers and Barlow (1987) tentatively ascribed the observed symptoms to nutritional deficiencies (particularly of polyunsaturated fatty acids) in the live food organisms used to feed the barramundi larvae (rotifers *Brachionus plicatilis* and brine shrimp *Artemia salina*).

Following the adoption of supplementary feeding techniques for rotifers and brine shrimp using a microencapsulated diet known to be high in polyunsaturated fatty acids (Frippak « CAR 1 » and « Booster ») during the 1987-88 season, this syndrome has not recurred at N.F.R.C. (Rimmer et *al.*, 1988).

In order to investigate the nutritional basis of the mortality syndrome, a series of experiments was conducted at N.F.R.C. to test the effects of nutritional enhancement of live food organisms on growth and survival of barramundi larvae reared under intensive conditions. This paper presents the results of the initial experiments which compared four diets, based on the use of supplemented and unsupplemented rotifers and brine shrimp in combination.

MATERIALS AND METHODS

Barramundi larvae used in these experiments were reared from fertilised eggs obtained from wild spawning barramundi at Weipa in the north-eastern Gulf of Carpentaria in northern Queensland. Larvae were

air-freighted to Cairns, and arrived at N.F.R.C. around the time of hatching (12-17 hours after fertilization at 28-30°C). Although the term « larva » is used throughout this paper to describe the early life history stages of barramundi, the larger fish used in these experiments are properly termed « juvenile » (Kendall et *al.*, 1984). Barramundi larvae metamorphose at 8-12 mm in length (MacKinnon, 1987; Russell, 1987). For convenience, the term « larva » has been used to describe the fish used in these experiments.

LARVAL FEEDING SCHEDULE

Barramundi larvae reared intensively at N.F.R.C. were fed rotifers at 10-20/ml from day 2 (where hatching is designated as day 1) to day 14, and brine shrimp at 2/ml from day 8, increasing to 5/ml by day 12 and continuing at 5/ml until day 18.

Rotifers were reared outdoors on algae (usually *Chlorella* species) together with small quantities of yeast. Rotifers were harvested daily in the morning and fed to the barramundi larvae that afternoon and the following morning. Rotifers to be fed in the afternoon were supplemented with Frippak « Booster » in aerated 3 litre plastic soft drink bottles for 4 hours at 1.0 g dry weight of microcapsules per litre of water with a rotifer concentration of 4 million rotifers per litre. Rotifers to be fed the next morning were supplemented for 20 to 22 hours at 0.2 g of microcapsules per litre with a rotifer concentration of 2 million rotifers per litre.

Brine shrimp nauplii (« Aquarium Products » brand) were harvested daily and starved for 24 hours to ensure that the yolk was absorbed before they were offered to the barramundi larvae or were supplemented. Brine shrimp starved for 24 hours were fed to barramundi larvae from day 8 to day 12; from day 13 to day 18 the larvae were fed on supplemented brine shrimp. Supplementary feeding of brine shrimp took place in a 50 litre glass aquarium. Microcapsules were added at 0.3 g/l and brine shrimp were maintained at densities of 0.5-1.0 million per litre.

EXPERIMENTAL LARVAL REARING UNIT

The experimental larval rearing unit used in these experiments comprised 22 individual chambers each of 2 litres capacity. The design of the unit ensured that fish in each chamber were subjected to identical conditions of water quality, temperature and light. Water from a header tank gravity-fed to the rearing chambers, from which it drained via small nylon screens (63, 120 or 200 microns aperture) which could be changed to permit the retention of various sized organisms. The water from the rearing chambers collected in a sump and was pumped back to the header tank via a biological filter. Temperature was maintained at 29 ± 1°C by air-conditioning the room and heating water in the header tank. Lighting was provided by fluorescent lamps at an intensity of 400 lux at the water surface. Water quality parameters (temperature, pH, salinity, ammonia, nitrite and nitrate) were monitored daily.

EXPERIMENTAL DESIGN

For the experiments described here, 16 chambers were used to test 4 replicates of 4 diets in a block design. The diets tested were :

Diet 1 : Rotifers reared on *Chlorella* only; newly hatched brine shrimp.

Diet 2 : Rotifers reared on *Chlorella* then supplemented with Frippak « Booster »; newly hatched brine shrimp.

Diet 3 : Rotifers reared on *Chlorella* only; brine shrimp starved for 24 h (day 8 to day 12), then brine shrimp starved for 24 hours before supplementation with Frippak « Booster » (from day 13 on).

Diet 4 : Rotifers reared on *Chlorella* then supplemented with Frippak « Booster »; brine shrimp starved for 24 hours (day 8 to day 12), then brine shrimp starved for 24 h before supplementation with Frippak « Booster » (from day 13 on).

These diets were designed to represent the original (unsupplemented) diet in use when the mortality syndrome was first encountered at N.F.R.C. and enhanced (supplemented) diets during both the rotifer and brine shrimp feeding phases of the larval rearing period. The larvae were fed twice daily to ensure that they had constant access to freshly supplemented food organisms. Before each feed, approximately 90 % of the water from each chamber was siphoned through a large surface area screen (200 or 400 microns) to remove food organisms while retaining barramundi larvae.

The experiment was run twice, once to determine daily mortality patterns (N1) and again to investigate the effects of the different diets on growth (N2). The same procedures and experimental design were used in both experiments. The density of hatched larvae was estimated volumetrically; 545 larvae were introduced to each chamber for experiment N1 and 133 for N2. Mortalities were monitored daily by counting dead larvae, although in practice, mortalities could only be accurately estimated after about day 10, when larvae were large enough to leave visible corpses. Experiment N1 was terminated at day 30 when cumulative mortalities in two treatments were at or near 100 %. Experiment N2 was terminated at day 22, when all survivors were preserved in 10 % formalin for later measurement of total length (TL); this measurement was used to compare growth between treatments.

HISTOLOGY

Samples of dead and live moribund larvae were taken for histological examination at irregular intervals. Most of the larvae sampled live at day 22 in experiment N2 (a total of 389 larvae) were used for histological examination. Specimens for histology were preserved in 10 % formalin, processed using conventional wax embedding techniques, sectioned and stained with haematoxylin and eosin.

FATTY ACID ANALYSES

Samples for fatty acid analysis were sieved to remove small particles (particularly algal cells and microcapsules) and then extracted with chloroform/methanol (2 :1 v/v) using the modified methods of Folch et *al.* (1957) and Bligh and Dyer (1959) and stored under nitrogen at −25°C until analysed.

The excess solvent was removed using a rotary evaporator and the lipid residue taken up in a minimum of hexane. The base- catalysed transesterification procedure of Christopherson and Glass (1969) was used to prepare the fatty acid methyl esters from the lipid solution. The esters were separated by gas- liquid chromatography on a Shimadzu R1-A with a 2.1 m × 3 mm i.d. glass column packed with 15 % OV-275 on 100/120 Chromosorb PAW-DMCS. The column oven was temperature programmed from 190° to 220°C increasing at 2°C/min and the carrier gas (nitrogen) flow rate was 65 mL/min.

The peaks were identified and quantified on a Shimadzu RPR-G1 GC processor calibrated using the methyl esters of authentic triacyloglycerol standards supplied by Sigma (Sigma Chemical Co., St Louis, MO, USA). A comparison was also made with a standard methylated cod liver oil sample supplied by R. Johns of the University of Melbourne.

RESULTS

Nutritional Enhancement of Live Food Organisms

The effects of supplementary feeding with Frippak « Booster » microencapsulated diet on the fatty acid composition of rotifers and brine shrimp are shown in Figures 1 and 2. The fatty acid composition of barramundi eggs (which Dendrinos and Thorpe (1987) suggested reflects the optimal fatty acid composition of the larval diet) is also shown for comparison.

The fatty acid composition of the rotifers fed to barramundi larvae did not closely match the composition of the egg yolk (Fig. 1). Rotifers were found to contain lower levels of 16 :0 than egg yolk, but higher levels of 16 :1. Unsupplemented rotifers were deficient in four fatty acids which were found in barramundi egg yolk : 18 :2, 22 :4n-6, 22 :5n-6 and 22 :6n-3. Supplementation increased the levels of 22 :6n-3 to about 3 % which is still well below the level found in barramundi egg yolk (17 %). Supplemented rotifers were still lacking 22 :4n-6 and 22 :5n-6 which were present in barramundi egg yolk. Levels of 20 :5n-3 in unsupplemented rotifers (11-12 %) were much greater than those found in barramundi egg yolk (4 %) and supplementation of rotifers only provided a slight increase in 20 :5n-3 to 13 %.

The fatty acid composition of brine shrimp did not closely match the fatty acid composition of barramundi egg yolk. Brine shrimp had lower levels of all the saturated fatty acids than was found in the egg yolk, but higher levels of 18 :1, 18 :2 and 18 :3 (Fig. 2). The fatty acids 20 :0, 20 :4n-3

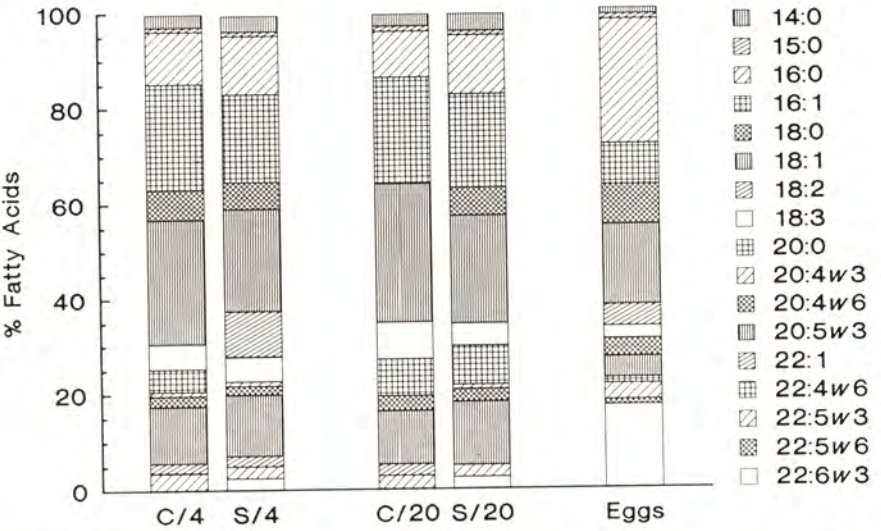

Fig. 1. — Fatty acid composition of rotifers used in diets 1-4. C/4 : not supplemented, used 4 hours after harvest (p.m. feed, diets 1 and 3); S/4 : supplemented at 1.0 g microcapsules/litre for 4 hours after harvest (p.m. feed, diets 2 and 4); C/20 : not supplemented, used 20 hours after harvest (a.m. feed, diets 1 and 3); S/20 : supplemented at 0.2 g microcapsules/litre for 20 h after harvest (a.m. feed, diets 2 and 4). Eggs : egg yolk from eggs stripped from spawning barramundi at Weipa, Queensland.

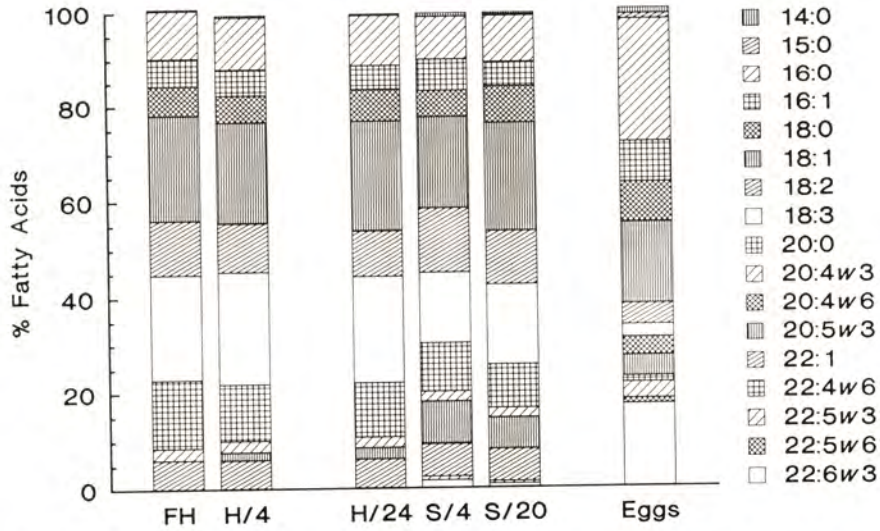

Fig. 2. — Fatty acid composition of brine shrimp used in diets 1-4. FH : freshly hatched brine shrimp (a.m. feed, diets 1 and 2); H/4 : brine shrimp harvested about 4 hours after hatching (p.m. feed, diets 1 and 2); H/24 : brine shrimp harvested about 24 hours after hatching (a.m. and p.m. feeds, diets 3 and 4, day 8 to day 12); S/4 : brine shrimp harvested about 24 hours after hatching, then supplemented at 0.3 g microcapsules/litre for 4 hours (p.m. feed, diets 3 and 4, day 13+); S/20 : brine shrimp harvested about 24 hours after hatching, then supplemented at 0.3 g microcapsules/litre for 20 hours (a.m. feed, diets 3 and 4, day 13+). Eggs : egg yolk from eggs stripped from spawning barramundi from Weipa, Queensland.

and 22 :1 were present in brine shrimp but not in barramundi egg yolk. Freshly hatched brine shrimp were deficient in 5 fatty acids found in barramundi egg yolk : 20 :4n-6, 22 :4n-6, 22 :5n-3, 22 :5n-6 and 22 :6n-3. Brine shrimp supplemented with microcapsules showed increased levels of 20 :5n-3 (7-9 %), 22 :5n-3 (1 %) and 22 :6n-3 (1-2 %) (Fig. 2), but still lacked 20 :4n-6, 22 :4n-6 and 22 :5n-6 which were present in barramundi egg yolk.

Water quality

Water temperature ranged from 28-30°C; pH from 7.8-8.0; salinity from 27-32 g/l; ammonia from 0-0.2 mg/l total NH3; nitrite from 0-0.2 mg/l; nitrate was constant at about 20 mg/l.

Survival

The four test diets showed dramatically different effects on survival of barramundi larvae. Larvae fed on diets 1 and 2 began showing stress symptoms (pale colouration, erratic swimming followed by « fainting ») on day 18. Large-scale mortalities began on day 20 (N1) or day 21 (N2), with mortality tapering off after 5 or 6 days (Fig. 3 and 4). In comparison, larvae fed on diets 3 and 4 had negligible mortalities over the same period.

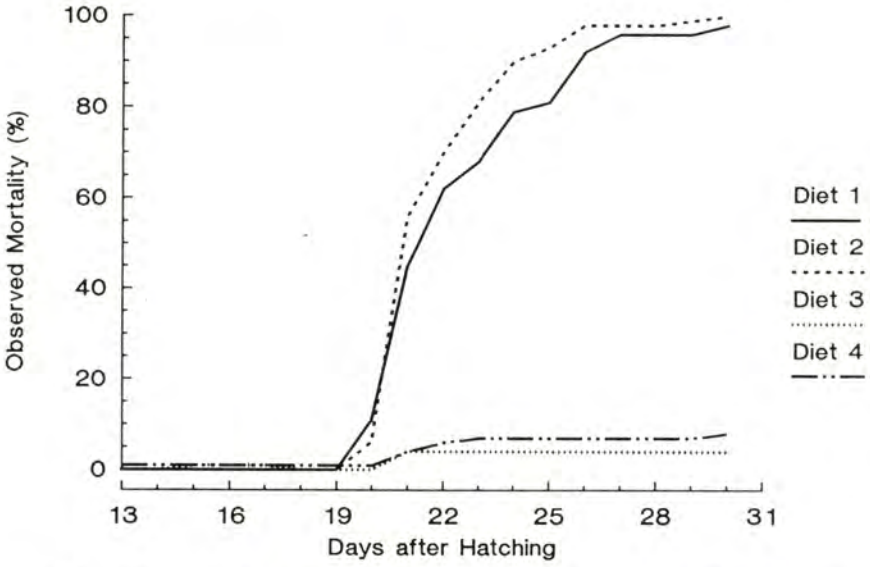

Fig. 3. — Cumulative mortality of barramundi larvae in experiment N1 from day 13.

Growth

The four test diets produced significantly different growth rates in barramundi larvae. Larvae fed on diets 3 and 4 were significantly larger

at day 22 than those fed on diets 1 and 2 (ANOVA, $P < 0.01$). Larvae fed on diets 1 and 2 averaged 8.29 mm TL and 8.69 mm TL respectively at day 22, while larvae fed on diets 3 and 4 averaged 9.98 mm TL and 10.48 mm TL respectively at day 22 (Fig. 5).

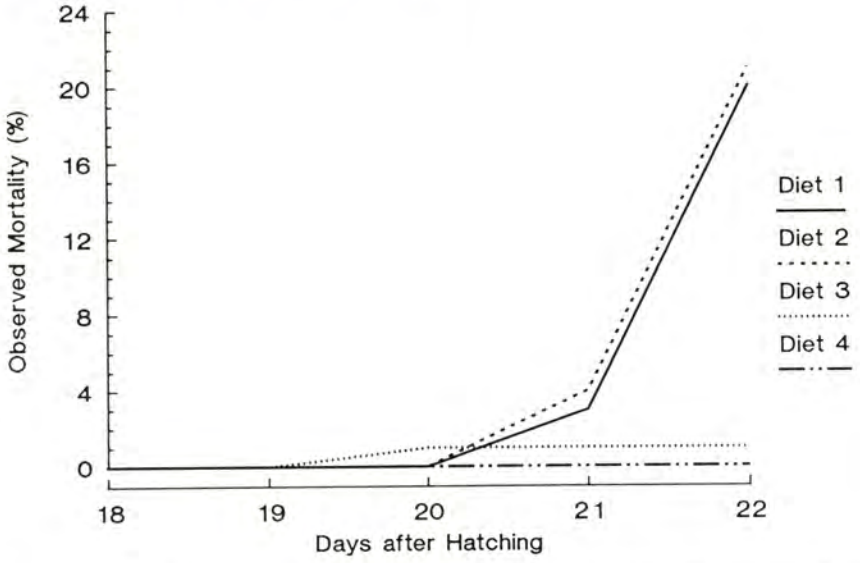

Fig. 4. — Cumulative mortality of barramundi larvae in experiment N2 (terminated at day 22).

When the effects of unsupplemented rotifers (used in diets 1 and 3) and supplemented rotifers (used in diets 2 and 4) were analysed, the results indicate that barramundi larvae fed on supplemented rotifers were significantly larger at day 22 than those fed on unsupplemented rotifers (ANOVA, $P < 0.01$). Larvae fed on unsupplemented rotifers averaged 9.11 mm TL while those fed on supplemented rotifers averaged 9.54 mm TL at day 22 (Fig. 6).

Similarly, barramundi larvae fed on starved and supplemented brine shrimp (used in diets 3 and 4) were significantly larger at day 22 than those fed on newly hatched brine shrimp (used in diets 1 and 2) (ANOVA, $P < 0.01$). Larvae fed on newly hatched brine shrimp averaged 8.47 mm TL while those fed on starved and supplemented brine shrimp averaged 10.20 mm TL at day 22 (Fig. 7).

Histology

Larvae fed on diets 3 and 4 showed no abnormal pathology. Several larvae fed on diets 1 and 2 showed some minor vacuolation of the spinal cord at day 22.

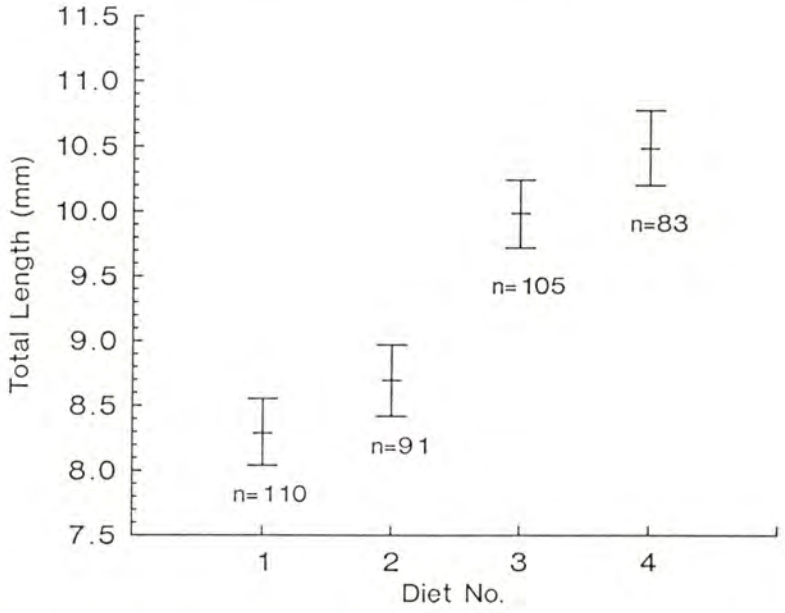

Fig. 5. — Total length of barramundi larvae at day 22, fed on four test diets (see text for details). Means and 95 % confidence limits shown; numbers below bars represent sample sizes.

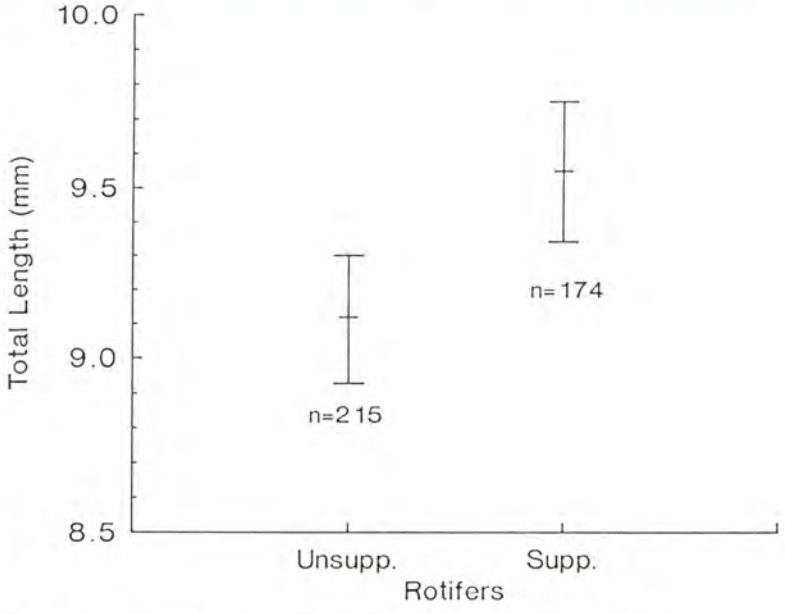

Fig. 6. — Total length of barramundi at day 22, fed on unsupplemented rotifers (diets 1 and 3) and supplemented rotifers (diets 2 and 4). Means and 95 % confidence limits shown; numbers below bars represent sample sizes. freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4). Means and 95 % confidence limits shown; numbers below bars represent sample sizes.

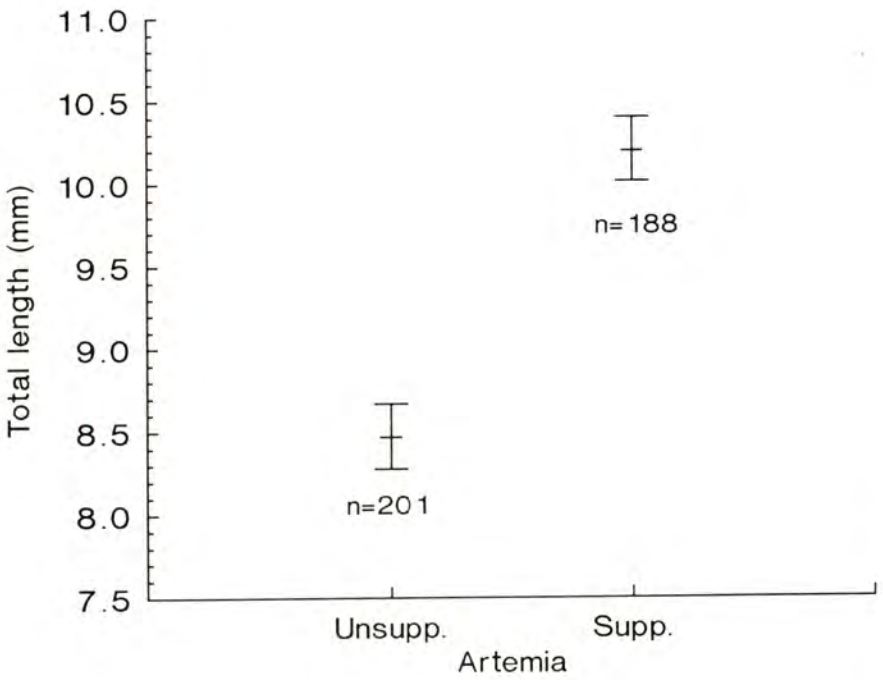

Fig. 7. — Total length of barramundi larae at day 22, fed on freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4). Means and 95 % confidence limits shown; numbers below bars represent sample sizes.

Tab. 1. — Presence (+) or absence (−) of polyunsaturated fatty acids found in barramundi egg yolk in the four dietary treatments used in experiments N1 and N2

Fatty Acid	Rotifers		Brine Shrimp	
	Unsupp.	Supp.	Newly hatched	Supp.
18 :2	−	+	+	+
20 :4n-6	+	+	−	−
20 :5n-3	+	+	+	+
22 :4n-6	−	−	−	−
22 :5n-3	+	+	−	+
22 :5n-6	−	−	−	−
22 :6n-3	−	+	−	+

DISCUSSION

Numerous studies have shown the importance of fatty acids, particularly long chain fatty acids of the n-3 series, in the diet of marine fish larvae. The essential fatty acids for marine fish are generally considered to be the C20 and C22 unsaturated fatty acids (Watanabe et *al.*, 1983; New, 1986), although survival in some species may also be limited by the levels of C18 fatty acids in the diet (Dendrinos and Thorpe, 1987).

Microencapsulated diets provide a convenient method of enhancing the fatty acid composition of live food organisms without the disadvantages inherent with other techniques, such as the degradation of water quality that may accompany the use of oil-based supplements (Rodgers and Barlow, 1987; Rimmer et *al.*, 1988). Several studies (Walford and Lam, 1987; Ahmed and Jones, 1988; Budd, 1988) have investigated the relationship between microcapsule concentration and supplementation duration for enhancing the polyunsaturated fatty acid composition of rotifers and brine shrimp using a microencapsulated diet. Generally, short periods of supplementation (4-8 hours) have been found to be most effective in increasing the levels of polyunsaturated fatty acids (Walford and Lam, 1987; Ahmed and Jones, 1988; Budd, 1988).

In the present study, levels of 22 :6n-3 were maintained for up to 20 hours supplementation at a level comparable with that found after 4 hours supplementation (Fig. 1 and 2).

Dendrinos and Thorpe (1987) suggested that the requirements of Dover sole (*Solea solea*) larvae for 20 :5n-3 and 22 :6n-3 fatty acids during early larval development could be met by the small quantities in the food used, and that after this period these requirements could be met by elongation and desaturation of shorter-chain fatty acids such as 18 :3n-3. If this hypothesis is adopted for barramundi larvae, the presence or absence of a particular fatty acid may be more important than the precise proportions of each fatty acid in the organism.

The C14, C16 and C18 saturated and monounsaturated fatty acids found in barramundi egg yolk were also found in the live food organisms fed to the larvae (Fig. 1 and 2). However, many of the polyunsaturated fatty acids found in barramundi egg yolk were absent from the live food organisms used in the test diets; these are summarised in Table 1.

The major difference in the fatty acid composition of supplemented and unsupplemented rotifers was the presence of 18 :2 and 22 :6n-3 in supplemented rotifers (Fig. 1). Barramundi larvae fed rotifers deficient in these two fatty acids showed similar survival to those fed rotifers with enhanced levels of 18 :2 and 22 :6n-3, suggesting that these fatty acids are not essential for survival of barramundi larvae. In addition, the absence of 18 :2 and 22 :6n-3 from the rotifer diet did not predispose the larvae to the effects of the mortality syndrome seen at day 20+ (Fig. 3).

The main differences between the fatty acid composition of diets 1 and 2 (freshly hatched brine shrimp) and diets 3 and 4 (starved and

supplemented brine shrimp) were the presence of 22 :5n-3 and 22 :6n-3 and the increased levels of 20 :5n-3 in the supplemented brine shrimp used in diets 3 and 4 (Fig. 2). Unsupplemented brine shrimp were found to lack 22 :5n-3 and 22 :6n-3; newly hatched brine shrimp lacked 20 :5n-3 but brine shrimp used about 4 hours after hatching had a low concentration of 20 :5,n-3 (2 %). However, since a deficiency of 22 :6n-3 did not adversely affect survival of barramundi during the rotifer feeding phase, it is unlikely that such a deficiency would cause extensive mortalities during the brine shrimp feeding phase, when larval organogenesis is well advanced and the metabolic functions of the larvae are presumably more competent (Kendall et *al.*, 1984; Dendrinos and Thorpe, 1987). The deficiency of 22 :5n-3 and the low level of 20 :5n-3 in newly hatched brine shrimp may have caused, or contributed to, the mortalities seen in fish reared on diets 1 and 2. Alternatively, the overall deficiency of C20 and C22 polyunsaturated fatty acids in newly hatched brine shrimp may have influenced survival of barramundi larvae fed on these diets.

Supplementation of both rotifers and brine shrimp also improved growth rates. The difference in the mean size at day 22 of fish fed on diet 1 (8.29 mm TL) and those fed on diet 4 (10.48 mm TL) is substantial and would offer real advantages to hatcheries using intensive culture techniques by reducing the length of time the fish are in the hatchery. Faster growth (presumably associated with better nutrition) may also contribute to better « quality » of larvae and fry.

Future research into the nutritional requirements of barramundi larvae will involve experimental investigation of the effects of varying proximate composition and amino acid composition of the live food organisms used during intensive rearing.

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Early weaning of marine fish larvae onto microdiets : constraints and perspectives

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Abstract — *Weaning small sized marine fish larvae at first feeding directly onto compound pellets is still difficult, while good results can be obtained when used in combination with live prey or when a prefeeding period on live prey is provided up to a size of about 2-3 mg. Microdiets are generally well ingested as their acceptability and visual detection may be improved using feeding activators and increasing good perception contrast. In theory, food may be processed to have a correct nutritional balance, selection of high digestible components is very important as larval digestive system is not fully functional during the first 2 or 3 weeks of life. Specific larvae requirement are partly unknown, common nutritional indices of dietary value are not usefull, and any nutritional deficiency may cause disorders, skeletal abnormalities and/or more generally growth retardation. MD use, microcapsules or microparticles, is now developing fast in Europe and Japan. The different feeding strategies usable are discussed from an economic point of view.*

INTRODUCTION

It is generally admitted that for all marine fish species with small sized larvae, live food utilization is necessary for a short time after hatching to ensure high survival and growth rates. This feeding strategy based on live prey is rather expensive, it requires manpower and expensive equipment, while the nutritional and sanitary values of prey are difficult to control. It would be economically advantageous either to use live prey substitutes directly at first feeding, or at least to minimize the duration of this period in the absence of a suitable artificial diet usable directly at first feeding.

During the last two decades, many attempts have been carried out at a laboratory scale on the early weaning of most marine fish larvae concerned by aquaculture programs, with relative by poor success in comparison with fresh water fish species such as Coregonids (Gatesoupe et *al.*, 1977; Kanazawa et *al.*, 1982; Dabrowski, 1984; Zitzow et Millard, 1988). Now, the interest of microcapsules and microparticles in

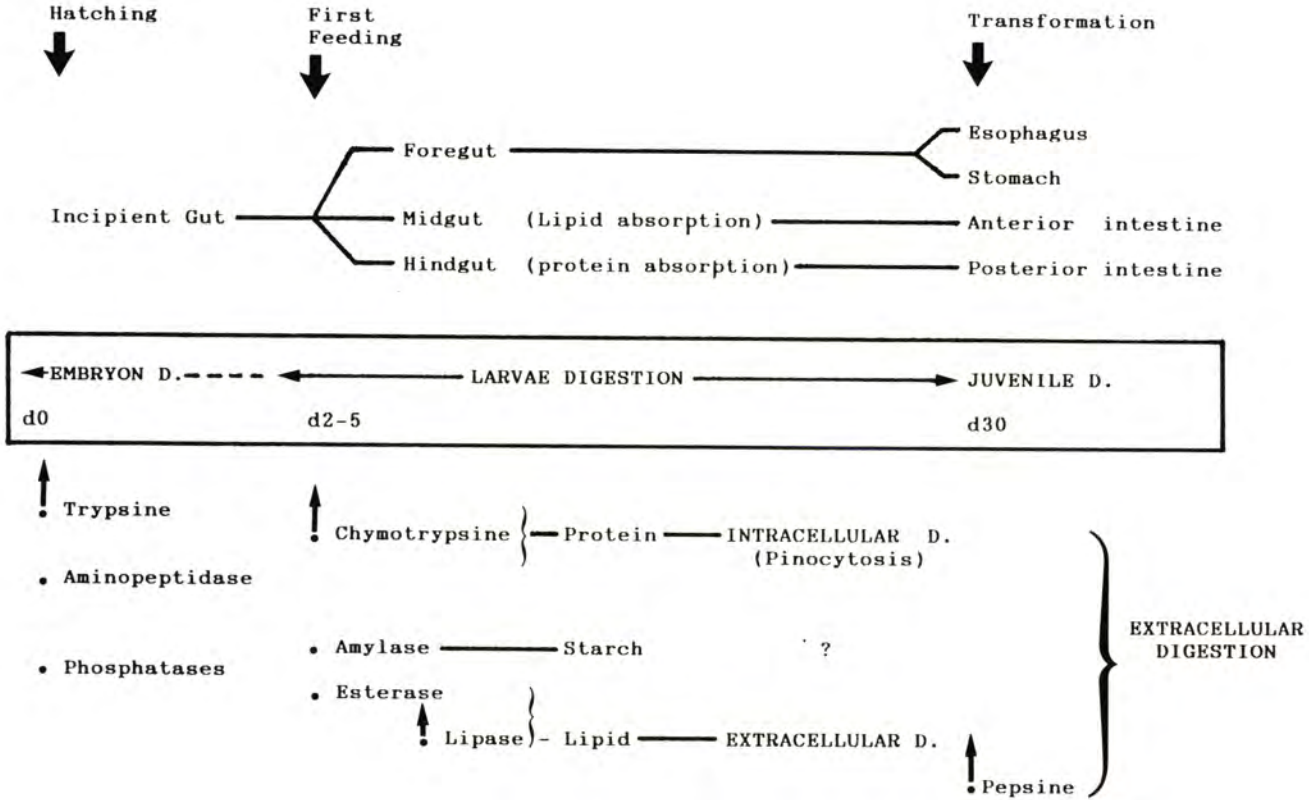

Fig. 1. — Diagramm of digestive tract development in a typical stomachless larvae.

marine fish farming is growing up in Europe and Japan and rapid progresses are expected as in Penaeid crustaceans (Kanazawa, 1981).

The purpose of this paper is (1) to draw the attention to the fact that larvae with an uncompletly developed metabolic system are not as easy to wean as juveniles, (2) to sum up the main requirements of a suitable microdiet (MD) with respect to larval feeding behaviour and nutritional requirements, (3) to compare different types of MD potentially suitable for young stages, and (4) to discuss the main feeding strategies usable in marine fish early weaning, predator species being exclusively concerned.

PECULARITIES OF DIGESTIVE PHYSIOLOGY IN LARVAE

The most important changes in the digestive tract during ontogenesis are summed up for a typical marine fish larva lacking a morphological and fonctional stomach until juvenile stage (Fontaine, 1981; Govoni et *al.*, 1986; Cousin et *al.*, 1987).

Morphological aspects

At hatching, the digestive tract is a straight tube closed at the mouth and histologically undifferenciated along its length (Fig. 1). It remains quite unchanged from mouth opening until the completion of yolk absorbtion, then becomes segmented into a buccopharynx, foregut, midgut and hindgut. The larval period ends with the development of a stomach with gastric glands and pyloric caeca. The liver and the pancreas are formed at hatching and are functional at first feeding.

Digestive enzymes

At first feeding, the digestive system appears not fully functional with an amylolytic and a proteolytic activity : amylase, trypsine, chymotrypsine and aminopeptidases should provide a good starch and protein digestion. Lipid digestion during embryonic and larval stages still remain unclear : in most species, a lipase activity is detected quite late, while esterases are present at first feeding. Alcaline and acid phosphatases are observed very early; if their role is not well known, they are probably involved in yolk lipid and oil globule utilization. A pepsine activity (gastric gland secretion) is detected very late (generally not before 1 month posthatching).

Enzyme activities are observed to be generally low at first feeding and each enzyme develops independently during ontogenesis. Larvae can partly control their enzymes production (trypsine at least) in response to feeding (Hjelmeland et *al.*, 1988). Exogenous enzymes of live preys may play an important role in initiating larvae digestion, but apparently they are destroyed soon after.

Digestive mechanisms

In larvae, digestion and absorbtion mechanisms are concentrated in the mid-gut and hind-gut but they are not well known. Lipids are digested

to fatty acids and monoglycerid in the mid-gut lumen, then absorbed in the mid-gut epithelium and after an intracellular resynthesis to lipids they are deposited in large lipid droplets for a short time. Proteins are digested in the epithelial cells of the hind-gut after a predominant pinocytotic absorption of macromolecules along the intramicrovillous plasma membrane. To our knowledge, carbohydrate absorption has not yet been studied in marine fish larvae.

In juvenile stages, when the stomach becomes functional, secretions of gastric glands facilitate the complete hydrolysis of proteins, in the mid and hind-gut, into peptides and amino acids through the action of pancreatic and intestinal enzymes : trypsine, chymotrypsine and amino-peptidases.

In larvae, digestion efficiency seems relatively low as the digestive system is not fully functionnal and that the digestive tract is short and transit rapid compared to juveniles : in sea bass larvae, live prey pass through the fore-gut within seconds, through the anterior midgut within minutes and remain in the posterior mid-gut and hind-gut for a few hours, so defaecation begins less than one hour after first live prey has been eaten.

FEEDING BEHAVIOUR

Some problems in early weaning may be due to a bad knowledge with respect of feeding and/or social behaviour of larvae. Feeding behaviour may be divided in an appetite phase : detection-identification of the potential prey (alert), then location (search) and a consummatory phase subdivided into a bite, test and ingestion phase. According to Mackie and Mitchell considerations (in Cowey et *al.*, 1985), smell plays an important role in the alert phase, then larvae approach the food guided by chemical and/or visual stimuli or sounds. Close to the food an incitant invokes initiation of feeding. Taste (taste buds in the mouth) nearly always plays a role in the test phase. If food feels right (size, texture, roughness), it is ingested and continuation of feeding is promoted by a stimulant. There are different ways to improve the detection, perception and palatability of microdiets.

Feeding activators

The attractiveness of food made from fresh materials such as squid, molluscs or fish flesh is generally sufficient. When powdered ingredients are exclusively used, incorporation of appetizers is recommended to stimulate feeding. Previously natural appetizers were used with respect to the feeding habits of wild fish under natural conditions. Now chemical substances identified as feeding activators for a lot of marine fish are in current use in larvae food formulation (Mackie and Mitchell, in Cowey et *al.*, 1985). In juveniles they belong to a fairly small group of chemical substances : L amino acids, glycine betaine, inosine or inosine 5 'monophosphate. Glycine betaine and inosine are the most efficient feeding activators in sole juveniles, while it is inosine in turbot (Person-Le Ruyet et *al.*, 1985). Practical levels of specific feeding activators are 1 % of the

diet in turbot juveniles during 5 to 10 d., 5 % or more in sole during all weaning period and 2 % in sea bass (complete mixture of L amino acids) for a few time, but they are probably overestimated.

Feeding activators have not been studied in larvae, but as their role is potentially more important than in juveniles, it is advisable to use at least juvenile optimum levels known. If no specific information is available, Mackie and Mitchell's complete mixture (L amino acids plus inosine and glycine betaine) may be used first.

Food location by sight

Most larvae locate food mainly visually, mechanical disturbance of the ear or lateral line by vibrations seem to play a minor role with inert food. Vision in marine fish larvae has been studied by Blaxter (1980) and Neave (1984). They are not very efficient hunters at first feeding, their ability to catch prey is progressively enhanced during ontogenesis once eyes developed. In turbot (*Scophthalmus maximus*) larvae, visual behavioural acuity is very low at hatching but increases from 6-7° at first feeding (d.3) up to 11° at early metamorphosis (d.15). In most species, past first feeding, light threshold for feeding averages about 10-1 lux, equivalent for late dusk or early dawn (0.4 µW/cm-2 at the surface).

Whether or not a potential prey is visible most of all depends on the contrast between the prey and its background. Contrast perception may be easily manipulated by changing prey and tank walls colour and illumination conditions. Feeding efficiency may be improved in staining MD red, using canthaxantine, to match *Artemia* nauplii, colour, then particles selection by colour is avoided. On the other hand, additional artificial light after dusk or before dawn to prolong the feeding day has beneficial effects on growth and generally on survival but not necessarily on food conversion. Scarce is the data concerning optimum light intensity, colour and distribution for larvae, while it is advisable to use either a continuous soft illumination or preferably, as regards to physiological rhythms, long daylength (18 L/6 D).

Physical characteristics and food availability

It may be partly possible to copy the motility of live prey both by controlling the density of MD and their sedimental speed. Good results are generally obtained using sligthly hydroscopic particles which float at the surface for a few minutes before sinking slowly in the tank to be definitely lost. Some help may also be found in a correct control of water supply, water direction and intensity, and in good food distribution. Small quantities of dry MD may be easily supplied each 10 or 15 minutes using automatic feeders. It is an advantageous way to control food availability, to reduce larvae fasting and avoid or limit social disturbances, cannibalism for example.

Little is known on larvae food texture preference. Sole (*Solea solea*) and turbot larvae are relatively independant of food palatability compared to juveniles which prefer soft texture diets. The use of particles as round

as possible (as microcapsules) is recommended, with MBD a good selection of components of adapted size and/or the elimination of any kind of detrital materials such as chitin by a proper sewage is necessary.

NUTRITIONAL REQUIREMENT

The unsuitability of MD may be partly linked to the incomplete knowledge of larvae nutritional requirement. MD formulation is based both on the nutritional requirements of juveniles of the same species or group and on the composition of larvae natural foods, zooplankton. Some practical recommendations for MD formulation are made here, detailed informations on fish nutrition could be found in Fontaine (1981) and Cowey et *al.*'s (1985) books.

Main sources of energy

Some data are available on the best balance between the main energy sources : proteins, lipids and carbohydrates, as on the basal energy requirement of larvae. Dietary protein levels as high as 55-60 % are most often used for several reasons : since larvae grow fast, they are supposed to have a high protein demand, high quality proteins are observed to promote growth in juveniles and larvae natural diets are rich in proteins. Lipids are the most energy rich class of nutrients in addition to supplying the essential fatty acids. As in young juveniles, limits above which growth rate decreases are relatively high (around 12 % in sea bass, 9 % in gilthead sea bream (*Sparus aurata*) and 10 % in red sea bream (*Chrysophrys major*), in MD, lipid levels are generally increased to 15-20 %. Inspite of the fact that most marine fish are said to have a limited ability to digest carbohydrates, some digestible forms tend to be more and more included in MD at levels ranging from 10 to 20 % of the diet.

Amino acid requirement

There are 10 essential amino acids (EAA) : arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophane; cystine and tyrosine being considered as semi-EAA. There is a general similarity in quantitative AA requirements between species, and a good correlation between the AA requirement pattern and the muscle and/or eggs AA pattern (Cowey and Luquet, 1983; Wilson and Poe, 1985); so the EAA profile of fish eggs may serve as a reference in formulating diets.

To overcome any EAA deficiency, natural protein sources are commonly selected according to their AA composition (AA availability values and digestibility coefficients of different proteins may be found in NRC tables) and deficient proteins may be supplemented with synthetic AA.

In practise, as long as high value protein diet such as fish meal (AA composition close to the fish one) are used, the EAA requirement is more

or less covered when protein requirement is met (dietary protein levels are most often overestimated). Whereas it is advisable to pay attention to methionine levels, for the european sea bass, methionine requirement is 1 % of the diet (Alliot et *al.*, 1985).

Essential fatty acid requirement (EFA)

Due to a limited ability to chain-elongate and desaturate linolenic acid, marine fish larvae have a general dietary requirement for 20 :5 n-3 and 22 :6 n-3 (EFA), C18 : n-3 being much less efficient. Excess of n-6 series FA may have detrimental effects. Whereas in some species, as in turbot, 20 :4 n-6 PUFA seems to be required in high quantities (Bell et al., 1985).

In practise, the n-3 EFA requirement of most marine fish larvae (dietary levels range from 0.5 to 1 % of 20 :5 n-3 plus 22 :6 n-3) are met when diets provide a high lipid level of marine origin. As fish are susceptible to peroxidative problems, attention must be paid to oxydation risks of fish oils unless stabilized by synthetic or natural antioxydants.

On the other hand, to be as near as possible to the PUFA pattern of wild zooplankton, high levels of phospholipids are recommended. They are more easily emulsified than triglycerides in larvae gut and facilitate the absorption of FA and cholesterol. A positive effect of dietary phospholipids (supplied by 1-2 % lecithin sources) has been observed on red sea bream and ayu (*Plecoglossus altivelis*) culture by Kanazawa et *al.*, 1983.

Vitamin and mineral requirement

Vitamin dietary levels given in classical tables for Salmonids, are in theory usable for larvae provided that losses during processing, storage and sea water leaching are not excessive. In the best storage conditions (cold and dry conditions), a good preservation of vitamins is only guaranteed for 6 months except for vitamin C. In practise, to overcome any vitamin shortage or degradation, MD dietary vitamin supply is increased in comparison with juveniles, more specially for vitamins C and E. The tendancy is either to use dietary vitamin C levels as high as 2 to 3 % (as 20-80 % losses are recorded during food processing) or when possible to supplement MD just before use. To limit the risks of peroxydation, MD are also overfortified in vitamin E, minimum dietary levels used : 30 to 50 IU per Kg dry diet.

In marine fish the major part of minerals are absorbed from sea water. Whereas compound diets may require a mineral supplementation even if they contain a high proportion of fish meal, a relatively good source of dietary mineral. The most important point to consider is probably phosphorus supplementation, with soluble salts such as mono or disodium phosphate, as it is found in insufficient quantities in sea water. The dietary requirement of available phosphorus is about 0.6 % in red sea bream. Little is known about calcium and iron potential demand, while trace metals have been shown to be beneficial in larvae by Robin et *al.*, 1987.

DIFFERENT TYPES OF MICROPARTICLES

As aforesaid to be suitable for larvae, MD should have a high acceptability, a correct stability in sea water, and preferably they should be easily graded to an adapted size (under 150-200 μm for rotifer substitutes and from 150 to 250 μm for artemia substitutes) and usable in automatic feeders. Consequently, wet pastes or gels and moist pellets (40-60 % and 20-40 % water content respectively) may not be considered as realistic foods. Dry MD are commonly classified in 3 or 4 types : flakes and rehydratable diets obtained by pressure cooking methods, microbound diets (MBD) obtained by binding powdered diets or a mash, microencapsulated diets (MED) obtained by encapsulating a complete diet with a membrane, and microcoated diets (MCD) obtained by coating powdered diets by specific materials.

Flakes and expanded diets

Both types are obtained through pressure cooking methods with compaction at high temperature (130-190°C) on a rotative drum for flakes or with extrusion cooking under high temperature and pressure followed by an abrupt depression. Their stability in sea water may be high and preparation of both diets requires a special machine. Extrusion cooking is a high cost technique presenting many advantages : improvement of digestibility of some components, possible destruction of antinutritional or toxic factors (avidine of hen eggs). Most of all, this MD type has a good aptitude to flottability and a very high ability to rehydratation or absorbtion of various solutions allowing some modifications of texture and attractiveness just before use and compensation of losses due to heating during food processing (Melcion et *al.*, 1983).

Microbound diets (MBD)

Different MBD types are classified according to the binder used : most often agar, carragheen and alginate, sometimes sodium polyacrylate and gelatin. Mixed to the diet ingredients, agar and kappa carraghen needed to be heated up to 100 or 85°C until they coagulate completely. Sodium alginate which is soluble in cold water is precipitated by calcium ions with, in theory, restricted losses of thermolabile substances. Raw fresh ingredients or dry powders are usable and no special equipement is required. MBD processing is quite simple and sea water stability of particles may be very high.

Microencapsulated and microcoated diets (MED and MCD)

Two main methods are used to prepare microparticles more or less well protected by a water proof wall which will be in theory easily broken down just after ingestion by enzymes, bacteria or pH variation in the gut : microencapsulation by coacervation which consists in precipiting a polymer in liquid phase around the microparticles and microcoating where

the wall is obtained by evaporation of the external phase (Teshima et *al.*, 1982).

With microencapsulation losses of soluble substances may be important and it is difficult to obtain a complete food but easy to have well graded small size particles of high stability in sea water. Capsules can be made of natural (gelatin, zein) or synthetic (nylon, polystyrene) polymers. This technique is usable at a laboratory scale and is developing at a commercial scale.

For coating, zein is most often used, sometimes a cholesterol-lecithin mixture. The coating solution may be evaporated in an oven and very small sized particles are easily obtained by atomisation. Soluble substances are spared and coating solution can contain feeding activators.

DIFFERENT FEEDING STRATEGIES

To suppress or reduce live prey utilization, different feeding strategies can be used : direct weaning at first feeding in large sized larvae, direct weaning prior to metamorphosis or progressive weaning as soon as possible for small sized larvae.

Direct weaning possibilities at first feeding : sole

Plaice (*Pleuronectes platessa*) and sole (*Solea solea*) are said to be relatively easy to wean very early. First successes, reported by Adron et *al.*, (1973) and Gatesoupe et *al.*, (1977) using either conventional particles or microcapsules were therefore associated with low survivals and poor growth. When weaning sole at first feeding onto MD is singly used, survival at metamorphosis is low, about 30 %, and time required to complete metamorphosis is increased by about 50 %. In constrast, when a 10 cl *Artemia* prefeeding is used, similar growth rate and survival may be obtained at metamorphosis with MD and live preys, while a growth retardation is always observed post metamorphosis : at d. 70 juvenile weight represents from 50 % to 80 % of the control and *Artemia* saving is about 90 % from hatching (Appelbaun et *al.*, 1985; Gatesoupe, 1983).

These weaning success differences related to larvae age can be partly explained by the fact that completely different stages are concerned : in 10 days old larvae, yolk sac and oil droplet are completely resorbed and they are approaching 3mg weight, in contrast, at first feeding (d.2), larvae are 0.3-0.4 mg weight and their digestive system is still primitive (fig.2).

Direct early weaning of old larval stages : sea bass at day 20.

As sea bass are said difficult to wean at first feeding, the strategy chosen over the past three years at IFREMER was to develop a MD suitable for 20 days old larvae for 2 main reasons : at this age the chances of success are high as larvae are about 3 mg weight (as 10 days old sole) and up to this age, live prey demand is rather limited but starts to increase sharply, so important *Artemia* sparing is expected (Fig. 3 and 4). Results obtained are reported in Table 1 and 2.

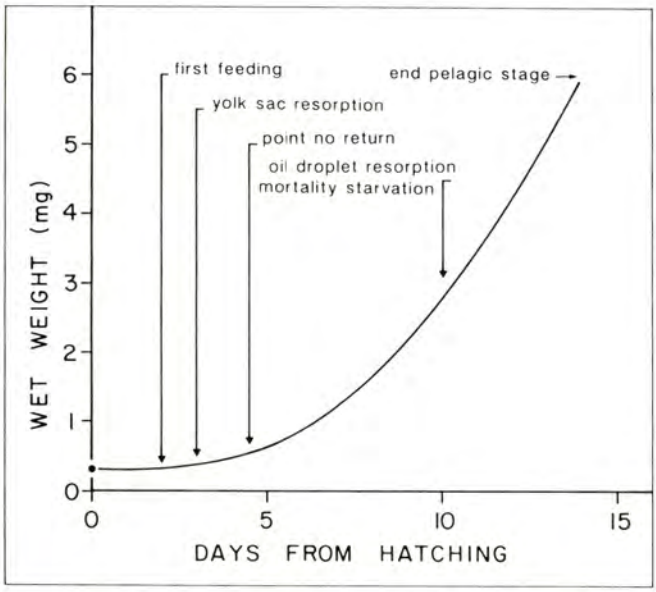

Fig. 2. — Sole larvae development and growth at 19°C.

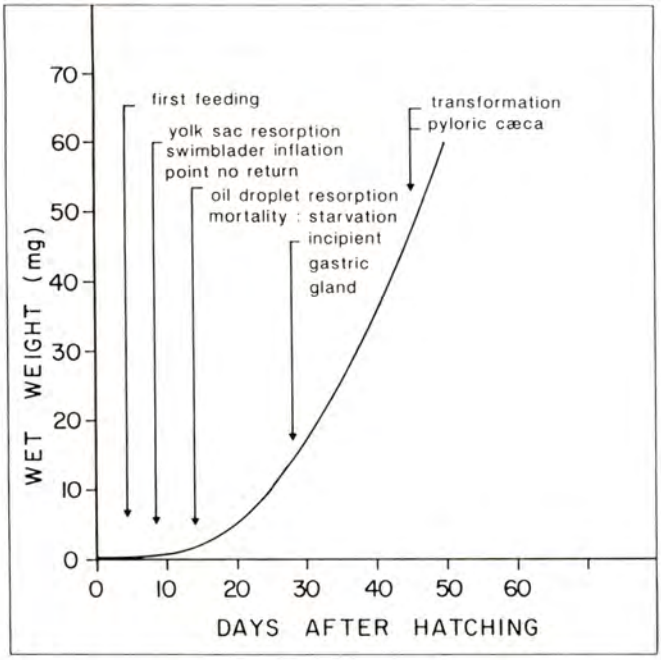

Fig. 3. — Seabass larvae development and growth at 19°C.

Tab. 1. — Composition and proximate analyses of experimental diets expressed in % of dry matter

DIET	I	II	III	IV	V
Expensed basal diet (1)	—	—	89.90	—	—
Fish autolysate	—	20.50	—	—	—
Casein	—	22.70	—	—	—
Whole squid	44.00	—	—	—	35.70
Squid mantle	—	—	—	45.20	—
Whole shrimp	20.68	—	—	—	—
Pollack filet	—	—	—	27.18	20.00
Pollack eggs	—	—	—	—	8.00
Salmon liver	—	—	—	—	12.00
Fresh hen eggs	15.00	—	—	15.00	—
Deshydrated hen eggs	—	15.00	—	—	—
Fresh yolk eggs	—	—	—	—	5.00
Soya lecithin	—	3.00	1.00	3.00	—
Cod liver oil	4.00	7.00	3.60	2.00	2.00
Precooked starch	—	10.00	—	3.00	3.00
Glucose	—	5.00	—	1.00	1.00
Sucrose	—	—	—	1.00	1.00
Soduim alginate	10.00	—	—	3.00	6.00
Carragheen	—	5.00	—	—	—
Carrophyll red R (2)	0.30	0.30	—	0.30	0.30
Vitamin premix (3)	2.00	5.00	3.48	2.00	2.00
Ascorbic acid	2.00	—	—	2.00	2.00
Cholic acid	0.02	0.02	—	0.02	—
BHT	—	—	0.01	—	—
Mineral premix (4)	2.00	4.00	—	2.00	—
Mineral premix (5)	—	—	—	—	2.00
Attractants (6)	—	2.50	2.00	—	—
Dry matter	93.8	94.5	93.7	94.8	
Protein	56.7	50.2	57.8	64.32	
Lipid	18.9	16.7	9.5	13.2	
Ash	13.6	10.3	7.5	8.8	
Ascorbic acid (7)	1260.0	—	1770.0	4880.0	

(1) Metailler et *al.* (1983).
(2) Containing 10 % canthaxantin.
(3) IFREMER vitamin premix 69 % (per kg of premix : vit.A acetate - 1 000 000 IU; vit.D_3 - 100 000 IU; alfa tocopherol acetate - 4 000 mg - vit.K_3 - 100 mg; Thiamin - 1 000 mg; Riboflavin - 2 500 mg; D Ca pantothenate - 5 000 mg; pyridoxin - 1 000 mg; Cyanocobalamin - 6 mg; Niacin - 10 000 mg; folic acid - 500 mg; Biotine - 100 mg; Meso-inositol - 100 000 mg). choline chloride 21 % - ascorbic acid 10 %.
(4) In % : Na_2HPO_4 - 90 and $FeSO_4 7H_2O$ - 10.
(5) Luquet (1971) mineral premix, (Na_2SeO_3) 0.5 ppm, ($NiCl_2$) 2 ppm, $Cr(NO_3)_3$, $9H_2O$ 2 ppm and (Na_2SeO_3) 1 ppm.
(6) In % : L-Prolin 50.3; glycin 30.6; L-alanin 9.3; L-threonin 1.5; L-serin 1.1; L-valine 1.2; DL-methionine 1.0; L-isoleucine 1.0; L-leucine 1.8; L-tyrosine 0.75 ; L-phenylalanine 1.0.
(7) mg/kg of dry matter.

From the 3 types of MD tested first (experiment 1) : alginate MBD, carragheen MBD and expanded MD, the first one appeared the most efficient : at day 40, average juveniles weight represented 50 % of the control and survival 70 %, while the level of skeletal abnormalities (like scoliosis and lordosis) was excessive. As the acceptability of alginate MBD

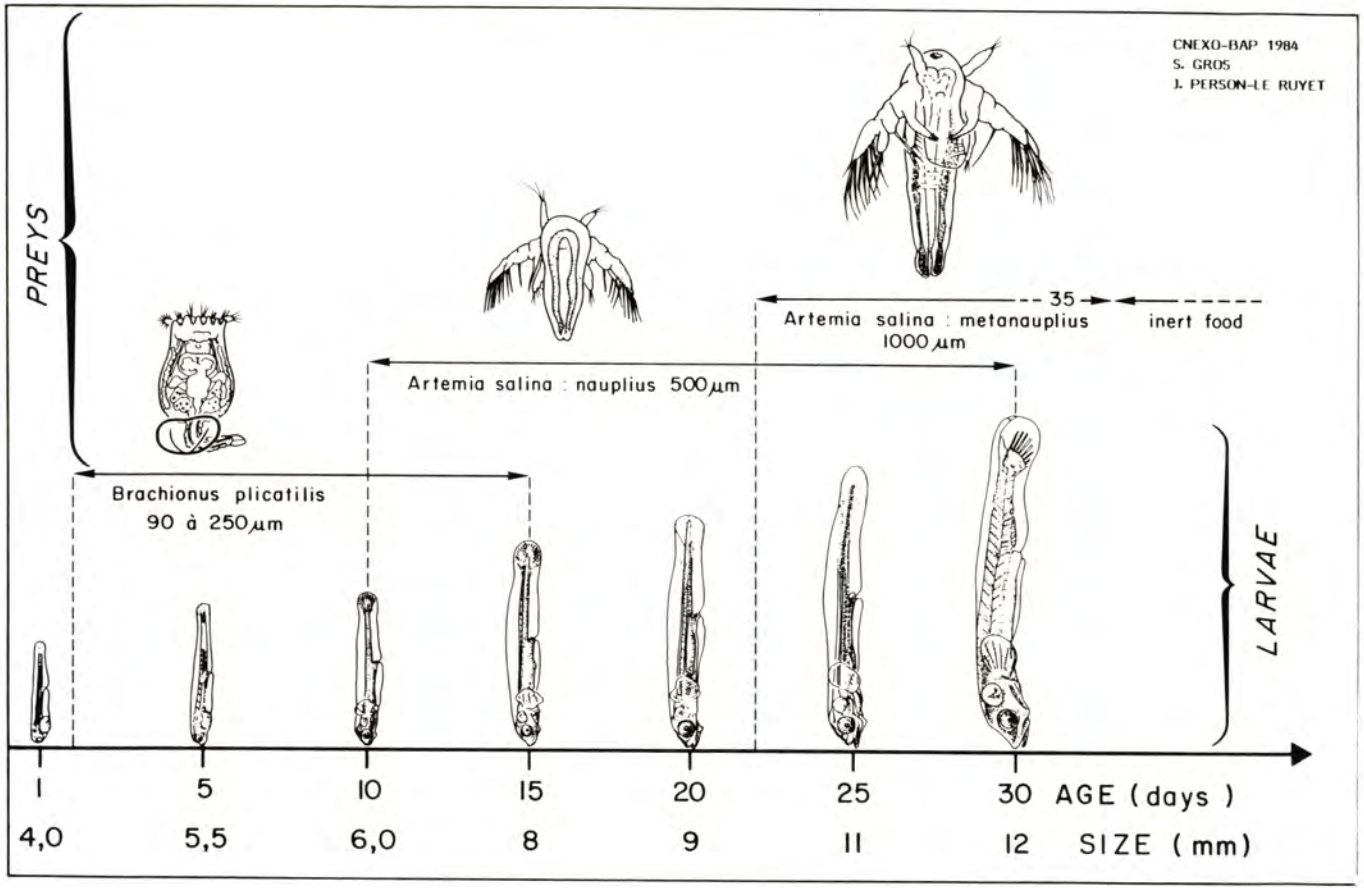

Fig. 4. — Seabass larvae development and feeding scheme at 19°C.

was very high in comparison with the other 2 diets, it was selected as a basis for the next experiments. First modifications made to diet 1 formulation concerned a reduction of lipid content and undigestible components, chitin and alginate, supposed to be excessive. General weaning results were highly improved, more particularly in experiment 3. Differences were observed between diets 1 and 4 until day 40, while at day 50, 10 days after MD were changed for a classical weaning diet (diet 3 formulation), a significant growth improvement was obtained with diet 4. The percentage of skeletal abnormalities was with both diets very low : nought with the control as previously, it was significantly improved with diet 4. As long as the initial weight range was over 3-4 mg, weaning success seemed more dependent on larval healthy state (expressed by specific growth rate from hatching and susceptibility to swimbladder stress syndrome) than on weaning age. Variations of trypsin and amylase specific activities controlled in experiment 4, during development in relation with diets and weaning age show that larvae may adapt quite well to food ingested.

Tab. 2. — General weaning conditions and results obtained at IFREMER in seabass rearing at 19°C

Exp	Initial conditions				Weaning results			
	Diet	Age	Weight	Nb lots	Age	Weight	Survival	Abnormality
1	I	23(1)	4.4(2)	2	40(1)	50(3)	66(3)	62(4)
	II		3.5	2		30	21	—
	III		3.7	2		29	83	45
2	I	23	3.4	2	40	100	72	18
	IV		3.4	2		81	92	7
3	I	21	4.4	2	50	72	100	6
	IV	21	4.1	2		72	100	0
4	I	23	4.4	2	50	73	100	30
	I	29	10.8	2		76	100	25
5	I	20	2.5	4	40	52	82	66
	V	20	2.5	4		6041	47	

(1) day post hatching.
(2) mg.
(3) as % of control.
(4) D.50 as % of control

Seabass larvae can be successfully weaned as early as day 20-23 (3-4 mg weight) onto alginate MBD which acceptability and stability in sea water are very high. As tank design is correct no signs of water pollution has ever been observed. Whereas, with MD, a growth retardation is always observed, or less soon (either after a 20 days MD period or 10 days later. In comparison with *Artemia*, with MD, at 50 days juvenile weight loss is about 30 %, while survival may be similar, up to 60-80 % or more from day 20 to day 50 post hatching. From an economic point of view, when larvae are weaned at D.23 instead of D.40, the feeding cost of a 50 days juvenile is significantly reduced, mainly by decreasing by 4 or 5 the Artemia biomass used (Fig. 5).

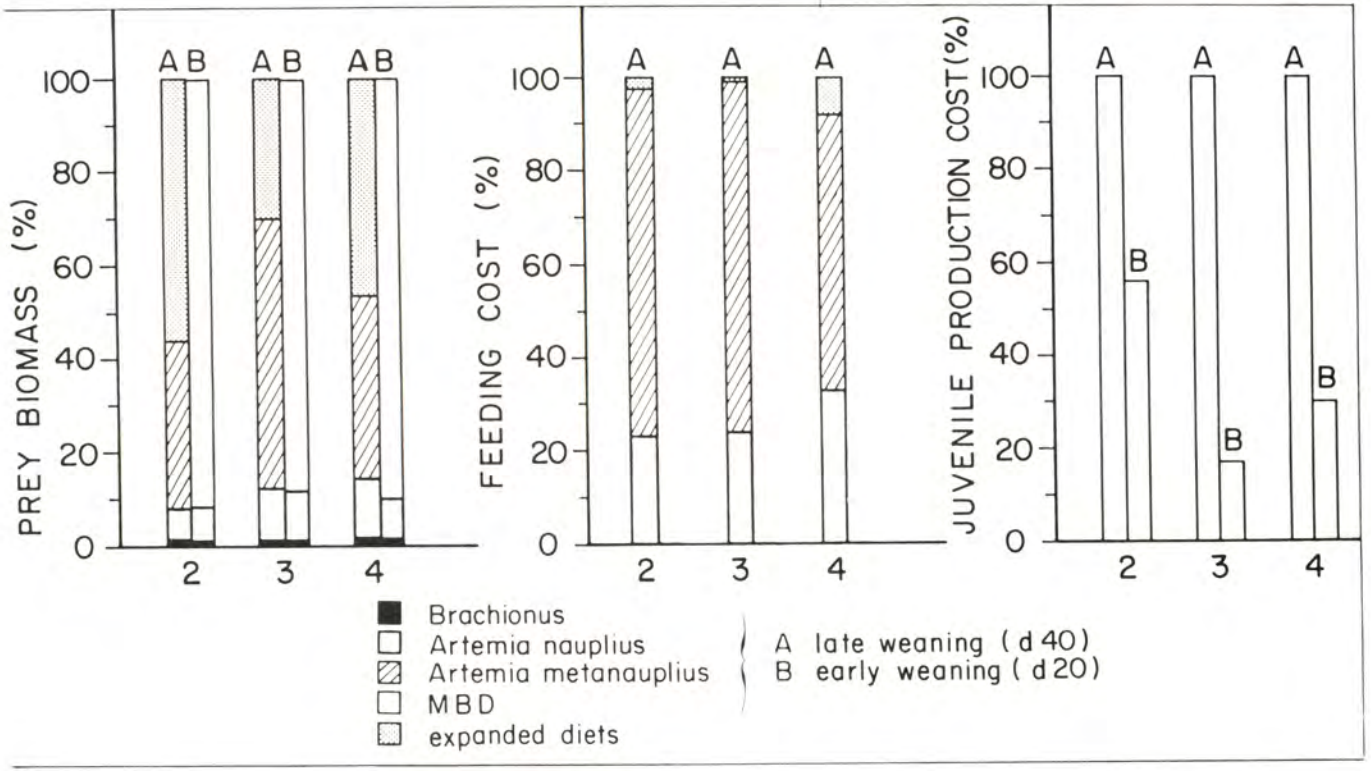

Fig. 5. — Preys requirement and relative feeding cost of a 50 d. juvenile according to weaning age.

Both the lower nutritional value of MD compared to live prey and the occurrence of skeletal abnormalities in some experiments, cannot be clearly explained through all the analyses and other controls performed on diets and larvae. Food intake is high and near a maximum 2 days post hatching as more than 80 % of larvae are observed to feed correctly on diet 1. No sign of nutritional deficiency is evidenced and some possible causes of skeletal abnormalities may be rejected : (1) the EFA requirements are fulfilled with all diets which lipid content range is 13-19 %, and the dietary PUFA pattern on body pattern seems correctly balanced (an excess of n-6 FA series in diet 4 is well controlled by larvae); (2) a reenforcement in dietary phospholipids (by a better selection of dietary components) has not had any clear effect on juvenile quality; (3) there is a good correlation between the EAA pattern of diets 1 and 4 and eggs or larvae pattern, and apparently no shortage in methionine and tryptophane; (4) ascorbic acid levels are correct in diets and larvae, larvae contents are similar in MD batches and in controls, and in abnormal and normal larvae (Table 3).

Tab. 3. — Ascorbic acid content (in mg of wet matter) of seabass larvae in relation with age and diets (Table 1 legends)

Experiment	Diet	Ascorbic Acid (mg/WM)				Abnormality (%) d. 50
		d. 0	d. 24	d. 40	d. 50	
2	Control	—	90	57	68	0
	I	—	—	55	78(2)	18
	IV	—	—	65	74(3)	7
3	Control	—	—	62	53	0
	I	—	—	70	47	6
	IV	—	—	76	71	0
4	Control	—	—	—	48	0
	I	—	—	—	70	30
	I(1)	—	—	—	59	25
5	Control	32	—	46	—	0
	IV	—	—	55	—	66
	V	—	—	52	—	47

(1) Weaning age is d.30 instead of d.20 - 23 in all other experiments.
(2) Average level, in normal and abnormal fish, 76 and 85 respectively.
(3) Average level, in normal and abnormal fish, 80 and 54 respectively.

In contrast, some signs of disturbance in digestive physiology are pointed out : (1) after a 2 days use, evacuation rate is twice than longer with diet 1 that with *Artemia*, 7 hours instead of 3.30 hours at 19°C; (2) when larvae are fed on diet 1, basal specific activity of trypsine increases slowly, that is generally interpreted as a mechanism by which an inadequate diet (of poor digestibility here), is compensated (Hofer, in Cowey et *al.*, 1985); (3) MD food intake tends to decrease with time, as a nutritional unbalance possible consequence.

On the other hand, the relative unreliability in quality juveniles with diets 1 and 4, specially in experiment 5 suggests that with MD made mainly from fresh materials, part of problems may be due to antinutritional factors

or toxin accumulation in foods (no thiaminase in fish fillet used and eggwhite avidine destroyed by heating). As the sanitary quality of alginate MD made of fresh materials is difficult to control before and during food processing, it seems advisable to change for powdered components before developing this food technology at a production scale.

Progressive early weaning with additional live preys.

MD may be used in combination with live prey more or less early according to the adaptation age of each species to be weaned. This strategy has been applied for a long time in Japan in several species : red sea bream, japanese flounder (*Paralichthys olivaceus*), striped knifejaw (*Oplegnathus fasciatus*), while MD technology has been improved progressively. Now MCD efficiency, specially zein-MCD, is so high that no significant differences in growth and survival are observed in comparison with live foods as reported by Kanazawa et *al.* (1987). Commercial MD (Kyowa Co) are tested in Japan at a production level. According to the feeding models supplier, MD are used for sea bream after a 5 days rotifer prefeeding and in combination with rotifer and *Artemia* up to day 14, while japanese flounder are fed MD after a 25 days rotifer period and in combination with live prey up to day 40, at that age the stomach is functional (it starts to develop at day 20-25).

CONCLUSIONS

Weaning marine fish larvae at first feeding directly on compound pellets is still difficult, while good results can be obtained when used in combination with live preys or when a prefeeding period on live prey is provided up to a size of about 2-3mg. This weight range is equivalent to fresh water species such as carp (*Cyprinus carpio*) and vendace (*Coregonus albula*) at hatching, which are known to be relatively easily weaned at first feeding (Dabrowski, 1984). Most marine fish larvae are active feeders, and MD may be well accepted quite immediately. There are many ways to increase the acceptability of MD and also their visual detection. Feeding behaviour may also be affected by larvae appetite which may be partly controlled by environmental conditions such as temperature, water quality and tank design. In theory, inert food may be processed to have a correct nutritional balance. Selection of high digestible components is very important particularly because the larvae digestive system is not completely functional the first 2 or 3 weeks of life. Since growth is rapid any nutritional deficiency may cause disorders, malformation of axial cord and/or more generally growth retardation. Specific requirements of larvae are partly unknown and common nutritional indices of dietary value are not useful at that age. When known the best reference is probably the composition of wild zooplankton which differs from most artificial foods in many points : soft texture and high digestibility (no larvae overloading), large amounts of soluble proteins of high nutritive value, digestive enzymes that may activate fish zymogens, and perhaps different unknown nutrients that may be essential or that may promote larvae digestion directly or not.

As yeast and mollusc flesh or meal may contain « unknown growth factors », they are often used in food formulation in combination with preferably powdered natural components and predigested protein sources. The reasons why natural foods are more efficient than compound foods are still questions to be solved to progress surely in microdiet formulation.

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ADVANCES IN TROPICAL AQUACULTURE Tahiti Feb 20 - March 4 1989
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Marine larval fish rearing

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Abstract — *Various methods of producing juvenile finfish will be discussed. This includes the intensive production in controlled environments as well as extensive pond production. Factors important to survival such as temperature, salinity, pH, water quality, bacteria and parasites, density, food types and food density as well as information on food and food production will be given.*

ADVANCES IN TROPICAL AQUACULTURE. Tahiti. Feb. 20 - March 4. 1989.
AQUACOP. IFREMER. Actes de Colloque 9. pp. 645-659.

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Larviculture of Seabass *(Lates calcarifer)* and Grouper *(Epinephelus malabaricus)* in Thailand.

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Abstract. — *Artificial propagation of seabass was first achieved in Thailand in 1971 by stripping the ripe spawners collected from natural spawning grounds. The duration of embryonic development is approximately 17 hours at 27°C. The larvae are fed on rotifer first after which* ***Artemia*** *and minced fishes are given at 10 days old and 25 days old, respectively. The fingerlings can be transferred to nursery or culture at enclosures at 35-40 days age.*

At present, natural spawning of grouper can be obtained in captivity by environmental management. The larvae at age of 3-11 days are fed with rotifer and ***Artemia****, respectively. Then,* ***Artemia*** *mixed with minced fishes are given. At the age of 45 days, the fingerlings were fed only with minced fishes. Survival rate of fry was very low. The hatchery techniques have to be improved.*

INTRODUCTION

The culture of seabass (*Lates calcarifer*) and grouper (*Epinephelus malabaricus*) are widely developed in South East Asia. They are more popular marine food fish of high market value in the region. In the past, seabass fry were collected from the wild for stocking in ponds and cages. The artificial propagation of sea bass was first achieved in Thailand in 1971 by stripping the ripe and running spawners collected from natural spawning grounds (Ruangpanit, 1986). After that, effective hatchery techniques were largely developed allowing commercial culture of sea bass to expand rapidly along the coastal provinces of Thailand.

The induced breeding trials for grouper have been conducted in Singapore since 1977 (Chen et *al.*, 1977). Induced spawning by hormone injection has been reported successful in Singapore, Kuwait and Thailand. However, the larvae rearing techniques are still under study. Therefore, the grouper fry are still mostly collected from the wild.

INDUCTION OF SPAWNING

Seabass

Seabass broodstock would be ready to start spawning at the end of their third year when they reach 3.5 kg (Maneewongsa, 1986). The best milt is obtained from 2-4 years old, and the best eggs would be from female fish more than 3 years old. The parent fish should moved to spawning tank about 1 month before spawning season. The spawning tank would be around 80-100 tons and at least 2 m in depth. It can be rectangular or round. About 24 spawners can be kept in each tank with a ratio of male : female 1 :3.

The broodstock can be raised directly from the juvenile stage in net cages. The fingerlings would be collected from the wild or from fry spawned in the hatchery. In the past, the wild-caught adults were kept and reconditioned in the hatchery (Kungvankij, 1986).

Two major techniques of mass production of seabass fry are praticed in Thailand, induced spawning and artificial fertilization (Chen et *al.*, 1977; Kungvankij, 1986; Maneewongsa, 1986). Induced spawning techniques is mostly employed. Two methods are normally used for inducing seabass to spawn in captivity; hormonal injection and environmental manipulation. Both methods induce the fish to spawn naturally in the tank. The popular techniques for induced spawning is environmental manipulation. This is the best method for commercial scale seabass spawning because much more fish seed can be produced (Ruangpanit, 1986). The techniques are controlling the feeding at 1-2 percent of total body weight. The feeding is done once a day in the afternoon. Thirty to fifty % of tank water is changed daily.

Natural spawning in captivity takes place at the same time as natural spawning in open water (Maneewongsa, 1986). Spawning activity always occurs during spring tide 2-3 days before the new or full moon, and up to 5-6 days after the new moon at about 1800-2300 hours; (Chen F.Y. et al., 1977; Ruangpanit, 1986). The ripe males and females swim together, often turning laterally and hitting the surface of the water before spawning. They spawn continuously 3-5 days/month and spawn every month during rainy season or year round (Table 1).

Grouper

Grouper broodstock are ready to spawn at an age of approximately 3 years and 3.0-4.0 in body weight (Ruangpanit et al., 1988). The spawning techniques are similar to the techniques of seabass spawning. Induced spawning by environmental manipulation is mostly employed at present. In the past, induced spawning by hormonal injection and artificial fertilization were done.

For broodstock development, male groupers were obtained by accelerating the process of sex reversal of 3 years old females through oral application of methyltestosterone at the dosage of 1 mg/kg for a period of about 2 months (Ruangpanit et *al.*, 1988; Ratanachot et *al.*, 1986). This

is due to a protogynous hermaphrodite characteristic of *Epinephelus malabaricus.*

The parent fish can spawn naturally in captivity for several days during both lunar phases, (full moon and new moon) once a month and also every month between November and April of the following year (Table 2).

Table 1. Monthly fish egg production and hatching rate of seabass by environmental manipulation at Satul Fisheries Station in 1981 (Kungvankij, 1986).

		Tank No.	No. of egg ('000)	No. of yolk fish ('000)	Hatching rate (%)
April	25-28	5	5200	4200	80.7
May	23-27	5	6120	4710	76.9
June	22-25	5	7860	6150	78.2
July	20-24	5	11240	9450	84.1
July	23-25	4	1350	550	40.7
August	22-26	5	13510	10900	80.1
August	24-27	4	2540	1750	68.8
September	22-23	4	1730	1000	57.8
October	20-22	4	2520	1917	76.7
November	19-21	4	390	272	69.7
December	18-21	4	1700	1215	71.5
January	16-17	4	200	86	43.0
February	12-20	4	1438	1140	79.3
March	15-18	4	3770	2960	78.5
April	14-17	5	6640	4849	73.6
May	13-15	5	14000	11950	85.4
Total			80208	63140	78.72

Table 2. Monthly egg production and hatching rate of grouper by environmental manipulation at National Institute of Coastal Aquaculture (NICA). From Ruangpanit et *al.*, 1988.

		No. of eggs ('000)	No. of yolk fish ('000)	Hatching rate (%)
January	20, 24, 26, 27, 28, 29, 31	9 290	3 760	40.47
February	1, 3, 5, 6, 7, 8, 9, 12, 14, 16, 17, 18, 19, 22	12 7507	7 627	59.82
March	3, 4, 5, 6, 17, 18, 19, 21	11 350	7 481	65.91
April	15-21	4 900	1 149	23.07
Total		38 370	20 017	57.16

NATURE OF EGGS AND EMBRYONIC DEVELOPMENT

Seabass

The mature egg is round with its shell membrane fully distended (no spaces nor distortions), measuring about 0.8 mm in diameter. They tend

to stick together and while in groups, the eggs give a golden hue. It has one oil globule inside which measures about 0.2 mm in diameter (Tattanong et *al.*, 1988).

The milt will flow freely from a mature male spawner. It should be of good amount preferably about 10 ml and not very sticky so that it flows freely if poured from container. If the milt is examined under the microscope, the sperm can be observed to move very rapidly.

Table 3. Duration of embryonic development in seabass at 27°C. (From Tattanong and Maneewongsa, 1988)

Embryonic stage	Period	
	Hours	Minutes
(a) Fertlized egg	0	0
(b) One-cell	0	35
(c) Two-cell	0	38
(d) Four-cell	0	44
(e) Eight-cell	1	03
(f) 32-cell	2	12
(g) 64-cell	2	43
(h) 128-cell	2	55
(i) Pre-blastula	3	11
(j) Blastula	5	32
(k) Gastrula	6	30
(l) Neurula	8	32
(m) Embryo devlops head, optic lobes and tail buds	11	20
(n) Heart starts functioning, tail free, body starts to move	15	50
(o) Hatching	17	30

Table 4. Rate of absorption of yolk as shown by decreasing diameter of yolk inside the sac in seabass (Tattanong and Maneewongsa, 1988)

Diameter* of yolk in yolk sac (mm)	Time (days)
0.8800	0
0.3525	1
0.2752	2
0.1530	3**
0.0050	4
0.00	5***

* As the sac is actually elongated, this is a measurement across the longer acis from anterior to posterior

** Mouth opens

*** Yolk completely absorbed

When the milt and eggs are mixed by the dry method, fertilization takes place. There appears to be no significant changes on the egg from outside observations during the early stages. It was observed that it takes about 35 minutes after the mixing of the eggs and milt for embryonic development to begin. The approximate time and duration of various embryonic stages of seabass are enumerated in Table 3.

The period from fertilization to egg hatching can be affected by temperature. At 27°C, the eggs hatch in about 17 hours (Table 3) while at 30-32°C, the eggs hatch in 12-14 hours (Tattanong et *al.*, 1988). The newly hatched larvae is 1.5 mm in length with a big yolk sac. The yolk sac has one big oil globule at its anterior. This makes the larvae to stay in the water with head raised up at 45° to 90° angle of the water surface (Tattanong et *al.*, 1988). The body is slender and pale in colour with a loose distribution of pigments. The eyes, digestive tract, anus and caudal fins are distinctly seen but the mouth remains closed for a period of about three days. Table 4 presents the rate of absorption of the yolk as been observed under usual conditions.

Grouper

There have been no specific studies on the development of gonad in grouper in Thailand. However, induced spawning made in recent years was successful. Larval rearing has only been studied in laboratory.

When the milt and eggs are mixed by the dry method, fertilization takes place. The fertilized eggs are about 0.82 mm in diameter. It was observed that it took about 40 minutes after fertilization for embryonic development (Ratanachot et *al.*, 1986).

The approximate duration of the various embryonic stages of grouper were 19 hours at water temperature, 25-32°C and water salinity 31-33 ppt. (Table 5).

The mechanism of hatching of the grouper embryo has not been studied in detail. The newly hatched larva is about 2.50 mm in length with a yolk sac. They have a free tail fin and can move freely (Ruangpanit et *al.*, 1988; Ratanachot et *al.*, 1986).

DEVELOPMENT OF LARVAE, FRY AND JUVENILES

Seabass

The newly hatched larvae have a free tail fin and can move very freely. The larvae tend to confine at about 0.5 m below the water surface,often near areas of water that has aeration or slight movement (Ruangpanit, 1986).

The mouth opens when the larvae are about 3 days old and the yolk has almost completely absorbed. This is a sign that the fry can start feeding. Up to seven days old, the larvae are pale in colour. From seven days to metamorphosis, at 18-20 days, they appear dark with distinct vertical stripes on parts of the body. After 18-20 days, the larvae again assume a pale brownish colour. This time the vertical stripes can be more clearly distinguished. There are three stripes, one at the caudal peduncle, another at the level between the spinous dorsal fin and the soft dorsal fin, and a third over the head, all of which are particularly distinct.

In one month, the larvae metamorphose into the fry stage which has the appearance very close to the parent fish. The fry measures 1.5-2.0 cm.

These further grow and develop into juveniles after the third to fifth month when they attain 8-15 cm (Ruangpanit, 1988).

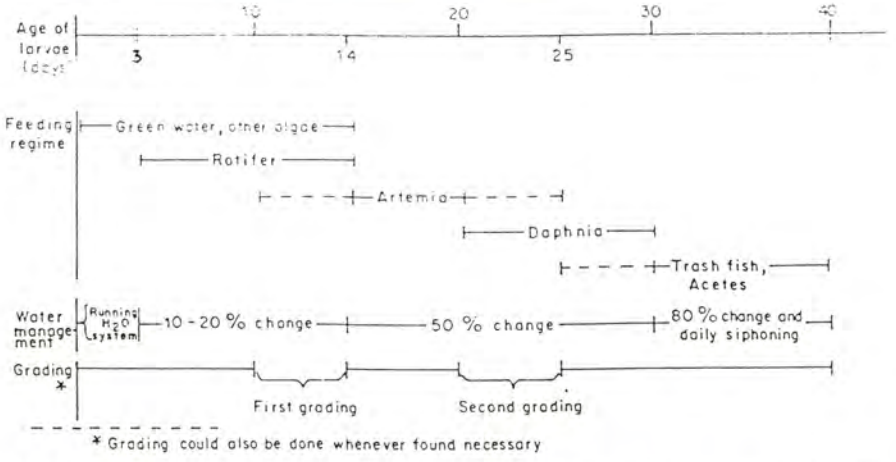

Fig. 1. — Chart showing management method for seabass nursery tank within the first 40 days period.

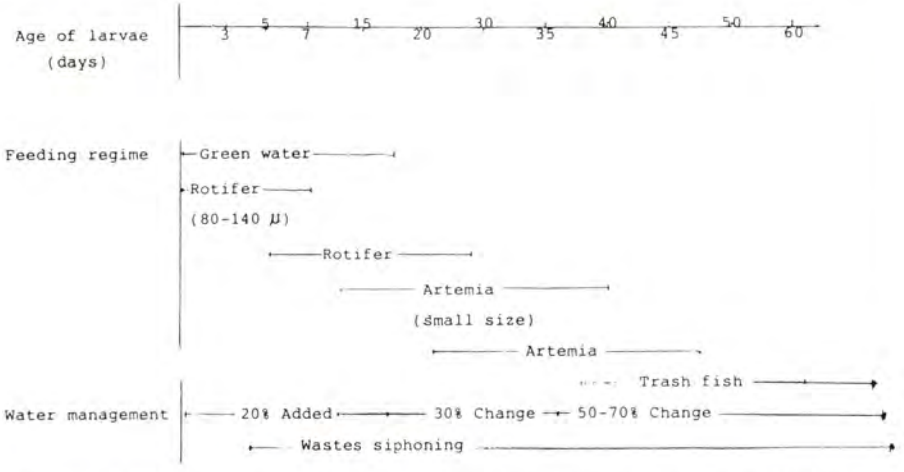

Fig. 2. — Chart showing management method for grouper nursery tank within the first 60 days period.

Grouper

As mentioned previously, the hatching mechanics, embryonic and larvae development have not been studied in details in Thailand. However, some details of larvae and fry development were observed. The larvae start to feed at the age of 3 days. The spines of the larvae were formed at the age of 10 days or length of 3.72 mm (Ratanachot et *al.*, 1986). The fry looks like the parent fish at the age of 40 days, length of 35 mm. (Table 6).

Table 5. Duration of embryonic development in grouper at 25-32°C and salinity, 31-33 ppt (from Ratanchot and Pakdee, 1986).

Stage	Duration hours	minutes	Size (mm)
Fertilized egg	—	—	0.82
2-cell	—	40	—
4-cell	—	50	—
8-cell	1	03	—
16-cell	1	12	—
32-cell	1	27	—
64-cell	1	52	—
128-cell	3	06	—
Morula	4	06	—
Blastula	5	06	—
Gastrula	7	47	—
Form Notochord	9	13	—
Form mouth	15	27	—
Form fin	16	22	—
Heart beat visible	17	09	—
Newly hatched larva	19	45	1.756

Table 6. Duration of larval development in grouper at 25-32°C, and salinity 31-33ppt (From Ratanachot and Pakdee, 1986).

Stage	Duration day	Size mm	Remarks
Larvae	2	2.50	
—	3	2.62	Start to feed
—	5	2.86	Black colour on abdomen
—	7	3.10	
—	10	3.72	Dorsal spines formed
—	15	4.98	
—	20	17.20	Dorsal spines developed to the tail and colour spot appeared on the body
—	25	19.80	Mucous tissue cover on dorsal spine
—	30	23.00	Appearance of dark stripes
—	40	35.00	Metamorphosis completed (appearance similar to sarent fish)

LARVAE REARING AND MANAGEMENT

Seabass

As described previously, natural spawning in controlled tanks takes place at the same time as natural spawning in open water which is from the beginning of April to the end of September. The spawning in other periods must be induced by hormone injection. After spawning, fertilized eggs float to the surface and unfertilized eggs sink.

Egg collection and incubation. Eggs in spawning tanks can be collected and transferred to incubation tanks by either of the two following procedures (Maneewongsa, 1986; Ruangpanit, 1988) :

— the spawning tanks are supplied with continuous flow of sea

water after spawning. The overflowing water should carry the eggs into a small tank containing a plankton net (200 microns mesh size). Eggs are collected and transferred to incubation tanks the following morning,

— the eggs are collected from spawning tanks using a fine mesh (200 microns) seine net the morning after spawning.

Table 7. The amount of rotifer fed to seabass larvae/day (stocking 40 larvae/litre in 30-litre containers).

Rep. No.	Age of larvae (days)							
	2	3	4	5	6	7	8	9
1	369	164	410	820	820	1 066	984	984
2	278	556	648	648	1 204	1 019	1 019	833
3	369	553	560	560	876	1 107	1 245	1 199
4	478	318	398	398	955	955	1 115	1 035

Table 8. The average growth (T.L. mm) of seabass reared at different densities (From Maneewongsa and Ruangpanit, 1984).

Age (days)	No. larvae/litre			
	50	80	110	140
1	1.24	1.24	1.24	1.24
5	2.99	2.91	2.71	2.93
8	3.31	3.24	3.16	3.29
11	4.58	3.93	4.10	3.91
13	5.16	4.51	4.27	4.19

Table 9. Survival rate of grouper larvae rearing at NICA (1988). (From Rungpanit et *al.*, 1988).

Date	Newly hatched larvae No.	Fingerlings at 50 days old No.	Length (cm)	Survival rate (%)
20-1-88	630,000	7,644	3.3-5.0	1.2
24-1-88	250,000	6,691	3.7-5.1	2.6
26-1-88	200,000	6,274	3.7-5.2	3.1
28-1-88	540,000	23,869	3.5-5.0	4.4
9-2-88	320,000	2,869	3.7-5.1	0.9
16-2-88	350,000	3,183		
Total	2,290,000	50,530	3.5-5.2	2.2

Eggs are collected with fine mesh net and placed in incubation tanks at 100-200 eggs/l passing the eggs through 1 mm mesh screen in order to remove floating algae and other debris that have adhered to the egg. The optimum salinity for hatching appears to be between 20 and 30 ppt.

Larvae rearing. The rearing tanks commonly used in Thailand are made of concrete, either outdoors and indoors (Ruangpanit, 1986). Volume ranges from 5 to 25 tonnes. The process of larvae rearing is divided into two steps (Maneewongsa, 1986; Ruangpanit, 1988). The first step or primary rearing extends from hatching to a larval size of 4-6 mm in total length or 10-15 days after hatching; second step or secondary rearing covers the period of 6-15 mm length size or age 16-35 days after hatching.

In the primary rearing, the stocking density for newly hatched larvae in rearing tanks is 50-100 larvae/l. The bottom of the larval rearing tanks should be cleaned every day. The sea water should be changed and replaced every day at the rate of 30-50 percent in the morning. Good quality sea water with a salinity 30-31 ppt (Maneewongsa, 1986; Ruangpanit, 1988), is required for larvae rearing. If possible, a running water system should be used in the rearing tank for 2 days before feeding.

Feeding with rotifer, *Brachionus plicatilis* starts on the second day after hatching, corresponding with the formation of mouth opening which has been generally observed in the afternoon of the second day. The rotifer density of 10-20 pieces/ml was required as shown in Table 7. Rotifers should be given in adequate amounts up to the age of 15 days.

Green algae, either from *Tetraselmis* or *Chlorella* sp. should be added daily to maintain a density 8-10 x 103 or 3-4 x 104 cells/ml, respectively. The algae serve a dual purpose as a direct food to rotifer and a water conditioner in the rearing tank. Brine shrimp (*Artemia* sp.) nauplii were used to feed larvae together with rotifers from age 8 days. The survival rate was between 60 and 85 percent at densities from 50 to 140 fish/l. The growth rate is 4.19-5.16 mm in total length. (Table 8)

For the secondary rearing, when the fish larvae reached 15 days old they are transferred to another tank. The brine shrimp nauplii can be used for feeding. The stocking density would be reduced to about 10-30 larvae/l. Addition ground fish meat can be fed with *Artemia* nauplii when the fry reach 11-15 mm, or about 25-30 days old. The sea water in the nursing tank must be changed daily at approximately 50 percent. The survival rate of larvae are 77.7, 87.7 and 90 percent at stocking densities of 10, 20 and 30 larvae/l.

Grading techniques. Cannibalistic behaviour of seabass fry can be observed after the fry completes metamorphosis, when they are about 15 days old (15 mm in total length). To maintain a uniform size and minimize the mortality of the fry, grading of fry to size groups at regular and frequent intervals must be done. After the first size grading at around 12-15 days old, size grading should be done every 3-5 days (Maneswongsa, 1986; Ruangpanit, 1988). The material usually used for grading consists of plastic containers punched at the bottom with holes of 2, 3.5, 5, 6 and 7 mm in diameter. Fish are placed in the plastic containers which are floated in the newly prepared larvae nursing tank. The small fish can pass through the hole to the new tank. The remaining fish in the plastic containers are transferred into another tank and likewise graded with the use of a plastic container with larger holes.

Growth. By nursing seabass with enough food and appropriate techniques as described previously, they should attain a length of 1.2 cm in 30 days (Maneewongsa, 1986; Ruangpanit, 1986). The normal growth within the first 30 days is shown in table 9.

Grouper

Natural spawning in controlled takes place from November to the end of April in the following year. As described previously, the techniques of larval rearing of grouper fry have not been studied. The larval rearing techniques of sea bass fry are applied. However, the techniques have to be improved to obtain better survival rates of fry. At present, the hatchery techniques are employed as follows :

Egg collection and incubation. After spawning, fertilized eggs, also float to the pond water surface while unfertilized eggs tend to sink. The eggs can be collected by similar method for sea bass egg collection. At present, eggs are mostly collected and transferred to incubation tanks the following morning by using a fine mesh (200 microns) seine net (Ratanachot et *al.*, 1986; Ruangpanit, 1988).

The eggs are washed of floating algae by passing through 1 mm mesh screen. Fertilized eggs are then dipped in 1 ppm of acriflavine solution for 1-2 minutes to get rid of bacteria and other microscopic organisms. Unfertilized eggs and waste materials are also removed by siphoning from the bottom of the nursing tank. The water salinity for hatching appears to be about 30 ppt.

Larvae rearing. As mentioned previously, the tanks, food and feeding and other techniques are similar to those employed in sea bass fry rearing. The process can be divided into two steps : primary rearing, which extends from hatching to a larval size of 5.0 mm or 15-20 days after hatching; and secondary rearing which covers the period up to 3.5-5.0 cms size or 20-50 days after hatching.

— At primary rearing the stocking density for newly hatched larvae in rearing tank is 10-60 larvae/l (Ruangpanit et *al.*, 1988). The air supply should be provided very slowly keeping about 4-5 air stones in the tank of 26 tonnes. The live food organisms must be fed for the first 30 days after hatching. Three days after hatching, the fry are fed with small size rotifers (80-140 microns). At this point the completion of mouth opening has been observed. The normal sized rotifer would be used for feeding at 5-7 days, after hatching. The stocking density of rotifers in the rearing tank can be approximately 10-50 /ml. Rotifers should be given in adequate amounts up to 20 days age. Green algae *Tetraselmis* or *Chlorella* sp. should be added daily to serve as a direct food to rotifer and as water conditioner. Small size shrimp nauplii are fed to larvae together with rotifers from age 12-15 days (Ruangpanit, 1988).

Filtered sea water is stocked at a depth of 60 cm in 26 tonnes tank. Then, 20 percent sea water volume is replaced daily. The waste material is siphoned out daily from the bottom of the rearing tank after 7 days.

— At secondary rearing, brine shrimp nauplii are fed up to age 45 days. The small size brine shrimp nauplii are fed during age 12-30 days, while the normal size nauplii were fed from age 30 days to 45-50 days. Approximately 30 percent of filtered sea water is changed daily. P.V.C. pipe (1.5-2') for hiring particle were fixed in the rearing tank at level 30 cm above the bottom. Filtered sea water would be daily changed and supplied as previously.

indispensable food for marine finfish larvae. It is rich in nutrients and small enough in size for the larvae to consume.

Table 10. Normal growth rate of seabass larvae in 30 days. (From Tattanong and Maneewongsa, 1988).

Age (days)	Total length (mm)	Remarks
Fertilized egg	0.870	Diameter of fertilized egg
0	1.51	
—	2.18	Beginning to hatch *
7	3.59	
14	4.36	
20	8.10	
30	12.05	

* Hatching is at 12th to 17th hour depending on temperature.

Table 11. Disease of Seabass and Grouper Culture in Thailand.

Size	Disease	Cause of	Mortality (%)	Treatment
3-8 days (0.5 cm)	Gas-bubbles disease	—	90-100	Formalin 25-30 ppm 24 hours
10-20 days (0.5-1.5 cm)	Blackbody	—	50	Formalin 100-200 ppm 15-20 minutes and tetracycline 25 ppm 24 hours
2.5-8 cm	Marine white-spot disease	*Cryptocarion* sp.	10-100	Formalin 30 ppm 30 minutes
1.5 inches	Kidney disease	*Vibrio* sp;	5-100	Ampicillin 50-100 ppm 5-7 days
3 inches	Columnaris disease	*Flizibacter columnaris*	60	Acriflavin 3 ppm 3 days or NaCl 3-5 %
3 inches	Fin rot and tail rot	*Aeromonas hydrophillia A. punctata*	5	Tetracycline 25 mg/kg fish or inject 6 mg/kg fish
4-7 inches	Lymphocystis	DNA Virus	—	—

Rotifer are usually reared in concrete or fibreglass tanks. The size of the culture tank ranges from 1 to 50 tonnes. The tanks are initially filled with *Chlorella* sp. or *Tetraselmis* sp. cultured at a density of 10×10^6 and 10×10^4 cells/ml respectively. Rotifers are added at a density 10-20/ml, reaching 80-100/ml in 5 days. There are then ready for harvesting by siphoning the water from the rotifer culture tanks through 63 microns mesh bags, leaving half of the original column to serve as a starter for the next batch. Then phytoplankton (100,000 cells/ml) are added to the rotifer culture tank to the same level in order to grow the next batch of rotifer. Dry baker's yeast is added as a supplementary food at the rate of 0.5 g/million rotifer when water in the rotifer culture tanks became clear. Each rotifer culture tank was used for culturing for 7-10 days, then cleaned and reused.

Juvenile rearing. When the fry are 50 days old or 3.5-50 cms length they are transferred to another tank (Ruangpanit et *al.*, 1988). The ground fish meat can be fed at age 45 days with *Artemia* nauplii. Filtered sea water is totally changed and supplied every day. The semi moist compound diet is given three times a day.

Growth. Growth of grouper fry which survived in hatcheries are shown in Table 6. The fry attain a length of 6.25 cm in 60 days.

As mentioned previously, the rearing techniques are still under study in Thailand. At present, grouper fingerlings collected from the wild are the only source. Only one crop of fry survived from NICA hatcheries in 1988. The average survival rate of 50 days old grouper fry was 2.2 percent (Table 10).

LIFE FOOD ORGANISM CULTURE

The culture of aquatic micro-organisms as food for larvae of fish is very important. The success of the production of fish fry depends on a constant supply of food.

Phytoplankton culture

Algae species used in seabass hatcheries in Thailand are *Chlorella* sp. and *Tetrasemis* sp. The first stage of the phytoplankton culture is conducted in the algal room, except for large-scale culture which is done outdoors. It is necessary to maintain pure stocks of algae throughout the year.

Mass production of phytoplankton started at a 1-1 scale. The scale of culture is then gradually increased to a volume ratio of 1 :10. The average culture cycle in unicellular algal mass production is 3-5 days. Cell density is 1-2 million cells/ml for *Chlorella* sp. and 80,000-120,000/ml for *Tetraselmis* sp. (Maneswong, 1986). The measurement of cell density by means of a blood cell counter is used only at the first stage culture. For the estimation of cell density in the large-scale culture, the measurement of transparency of green water is employed by using a white disc 15 cm in diameter. The culture of *Tetraselmis* sp. in Thailand is more successful than with *Chlorella* sp. which is usually contamined with a blue-green algae.

Tetraselmis sp. can be cultured in natural sea water between 15 and 36 ppt and grown at temperatures between 15 and 33°C under natural light conditions. Because the temperature of the outdoor tank for culturing phytoplankton was between 26 and 33°C, *Tetraselmis* was suitable for culturing for feeding rotifer in Thailand. The culture media for these algae are as follows : ammonium sulfate 100 g/tank; superphosphate 15 g/tank; urea 8 g/tank.

Rotifer culture

There are many species belonging to Rotifera, but the most suitable for mass culture appears to be *Brachionus plicatilis.* It is an important and

DISEASE AND PREVENTION

Many diseases have been identified in nursing of seabass and grouper fry in Thailand. The parasites found in grouper fry included protozoa and trematodes (Table 11). Diseases caused by *Cryptocaryon* sp., *Vibrio* sp. and *Flexbaxter columnaris* were also found in sea bass fry. Among these, white spot is most commonly observed during hatchery operations. Some treatment methods have been studied. The preventive measure is to grow strong fry, which can withstand pathogenic agents, through proper control of water quality, provision of clean rearing tanks, provision of fresh, high-quality feed in proper quantity, and appropriated stocking density of fry.

Table 12. The economics of seabass hatchery in Thailand (Kungvankij et *al.*, 1987).

Item		Value	
A. Income			
Newly hatched larvae (1 day old)	10 M. (1,000/2 US$)	20,000	
0.5 cm larvae (15 days old)	2 M. (1,000/6 US$)	12,000	
2.5 cm larvae (40-50 days old)	2 M. (1,000/100 US$)	200,000	
Sub-total A		**232,000**	
B. Fixed Cost			
Land cost (10,000 18 % interest)		1,800	(1.2 %)
Hatchery construction (50,000 10 % depreciation)		5,000	(3.5 %)
Equipment (20,000 20 % depreciation)		5,000	(2.6 %)
Interest (200,000 16 %)		36,000	(23.7 %)
Property tax (1.5 %)		150	(0.1 %)
Sales tax (1 %)		2,320	(1.5 %)
Sub-total B		**49,270**	
C. Operating Cost			
Broodstock		2,500	(1.6 %)
Broodstock feed		2,000	(1.3 %)
Artemia cyst		40,000	(26.3 %)
Hormone		2,000	(1.3 %)
Chemical/fetilizer		2,000	(1.3 %)
Larval feed		5,000	(3.3 %)
Electricity (12,000/tonth)		14,400	(9.5 %)
Fuel and oil		1,000	(0.7 %)
Labor chief technician	400 x 12 = 4,800		
	technician 3 x 300 x 12 = 10,800		
	workers 2 x 100 x 12 = 7,200	22,800	(15 %)
Materials and supply		5,000	(3.3 %)
Maintenance		4,000	(2.6 %)
Sundry		2,000	(1.3 %)
Sub-total C		**102,700**	
D. Total Cost (B + C)		151,390	
Net operation cost (A-C)		127,300	
E. Net income (A-B-C)		78,030	
F. Income over total cost			51.34 %

Table 13. The following hatchery facilities used in Thailand.

Stage	facility	Stocking density	Unit volume (t)	Size, Shape construction material
Adults	spawning tank	1 fish/5 ton	50-150	square 5 x 5 x 2 m circular 2 m depth concrete with aeration
Eggs	incubation tank	100-200 e./l.	0.5-2	conical, circular, fibreglass, concrete
Larvae	larvae rearing tank	20-50 l./l.	5-25	circular, rectangular concrete
Natural food	starter tank	0.5-1	circular, fibreglass	
Phytoplankton	algal culture tank	10-50	rectangular concrete	
Zooplankton	rotifer culture tank	10-50	rectangular concrete	

THE ECONOMICS OF SEABASS SEED PRODUCTION

Seabass seed production and culture have been developed over the past 15 years. The technology has helped to expand and to develop the industry into a promising enterprise.

The spawners can be collected from the culture area. Spawning is induced by hormone injection and water ambient manipulation. Larval rearing techniques developed in Thailand have been successful and the technology can be and has been transferred to private hatcheries and fishfarmers (Tookwinas, 1988).

The financial analysis of seabass hatchery is shown in Table 13. A hatchery in Thailand producing larvae in excess can dispose of newly hatched larvae to farmers or to other hatcheries (Kungvankij et *al.*, 1987). The farmers can operate their own backyard hatchery to rear seabass fry to nursery stage. Therefore, it becomes convenient and economical to operate a seabass hatchery. Table 12 shows that income from seabass hatchery is 51.34 percent over total cost.

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Intensive larval rearing trials of red Drum *(Sciaenops ocellata)* in Martinique (F.W.I)

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Abstract. — *The red drum* ***Sciaenops ocellata*** *(Linneaus, 1766) was introduced in Martinique (F.W.I) from the United States (Texas and Florida). Thirteen intensive larval rearing attempts, from 7 batches of imported eggs, have been conducted for two years. From newly hatched larvae through metamorphosis, larvae were reared in cylindroconical tanks (300 and 1000 l). Post larvae were weaned and raised up to 1-4 g fingerlings in raceways (1800 l). Mean stocking density ranged 50-75, 6-23 and 1.5-2.5 larvae per litre for phases 1, 2 and 3, 4 respectively of the rearing. For larval rearing attemps 3, 4, 8 and 11, survival rates were 6, 16, 17 and 5 percent respectively. 30000 two month old fingerlings were produced. Growth of the larvae was widely related to the mean stocking density. Larvae were fed rotifers, followed by brine shrimp nauplii, weaned onto squid and shrimp meat before they were finally fed on commercial pellet (55-60 % protein). During the grow-out phase, the food conversion rate ranged from 1.1 :1 to 1.8 :1 and final stocking density fluctuated between 0.7 and 5.5 kg/m^3*

INTRODUCTION

The red drum, *Sciaenops ocellatus* (Linneaus, 1766), was selected in 1987 as a potential candidate for culture in Martinique (French West Indies). This exotic species from the Gulf of Mexico was previously introduced in Martinique in June 1985.

In the United States, because of the commercial significance of this species, several studies have been conducted from many years. The main objective is to preserve natural resources for recreational and professional fishing activity. Artificial propagation (maturation and spawning) under controlled conditions (temperature and photoperiod) have been studied (Arnold, 1978; Roberts et *al.*, 1978; Arnold et *al.*, 1979). Larvae are reared on extensive culture systems in ponds (Colura et *al.*, 1976; McCarty et *al.*, 1986). Growth of red drum fingerlings in impoundments was reported by Theiling and Loyacano (1976).

In the Caribbean area, small islands such as Martinique do not provide enough land for extensive culture. That is the reason a reliable technique for intensive rearing has to be set up. As broodstock is not yet available in Martinique, eggs and larvae were imported from the USA (Texas and Florida) to initiate the programme.

From 13 larval rearing trials carried out since 1987 in Martinique, four rearings (attemps 3, 4, 8 and 11) were conducted with efforts focused on improving larval survival. This paper presents the results and observations made during the course of the study and contributes to preliminary information for the establishment of a reliable technique for fingerling production under intensive rearing system.

MATERIAL

Eggs were imported from USA. Seven batches were shipped from April 1987 to October 1988 : 4 from the University of Texas (C.R. ARNOLD., April, May and June 1987, May 1988) and 3 from the Department of Natural Resources in Florida (D. Roberts., October 1987, June, October 1988).

Cylindroconical fiberglass tanks, 300 litres and 1000 litres and raceways 1800 litres were used. During the rearing, water was supplied through sand filters.

Larvae were fed rotifers, followed by artemia, weaned onto squid and shrimp meat before they finally fed on commercial pellets (55-60 % protein).

Artemia collectors, size graders, slow defrosting distributors, demand feeders (run 3 and 4), automatic feeders (vibrating distributors -run 8- or feeding trays -run 11-) were also used during the rearings.

From 13 larval rearing trials, run 1 was preliminary. Attempts 2, 7, 9, 10, 13 were strictly devoted to experimental purposes. Trials 5 and 6 had failed prior to metaporphosis. Runs 3, 4, 8, and 11 led to a consequent production of fingerlings for experimental grow-out purposes. The following results principally concern these latest runs.

METHODS

Rearing period was separated in four phases, previously determined by both biological and technical contrainsts (transfer of population, change in food items...) and also by specific operations necessary for larval rearing control (sampling, enumeration, screening...) (Fig. 1). Fish were screened at the end of the second phase (30 to 34 days old) onto 3 mm size grader for the trial 3 and onto 1.5-2.0 mm size grader for the trials 8 and 11. The length of the different phases (1, 2, 3, 4) are about 2, 2, 1 and 3 weeks respectively. The growth of larvae was recorded by measuring the individual length of batches of 15-60 larvae at 3-7 day intervals. The survival rates were based upon the enumerations between each phase of the rearing, and the dead larvae removed from the tanks during cleaning operations.

Larvae were fed live prey (rotifers and nauplii of brine shrimp) 3-4 times a day; one part directly in the rearing tanks, and one part in a continuous feeding system to delay the period of food supply. Weaning in raceways needed the use of slow defrosting distributors for froozen shrimp and squid and later, automatic distributors for dry pellets. During the fourth phase of rearing, vitamins are daily added to the first distribution of food.

RESULTS

Transportation

Six shipments were carried out since May 1987. Live larvae ranged from 11 000 to 238 000, according to the time of transportation (9 to 19 hours) and the density of larvae (1600 to 5500 eggs or larvae per litre). Survival rate fluctuated between 60 to 98 %, excepted for one batch, where an accident reduced the survival to 14-19 %. Four shipments are considered in this study (Table 1). A 12 hour trip led to 95 % survival rate with a density of 5000 eggs per litre. A 19 hour trip reduced the survival to about 60 percent with almost the same density.

Table 1. Origine of the larvae and results of shipments.

Origine of the batch	Shipment length (hours)	Mean density of eggs or larvae (n/litre)	Final number of larvae	Survival %	Attempt reference
Texas	19	5 500	51 000	60	3
Texas	19	2 500	48 000	94	4
Florida	12	5 000	79 000	95	8
Florida	16	5 100	61 000	72	11

Growth

Feeding scheme during the rearing was almost similar. Change of food item is mostly dependant of the growth and associated to a precise size of the larvae. Larvae were fed rotifers (10 to 55 ind./ml/day) from day 15-20, nauplii of *Artemia* (2 to 35 ind./ml/day) from day 12-17 to day 32-33, fresh meat (squid and shrimp) from day 28-35 to day 35-40 and then on dry pellet from day 28-35 to the end of the rearing.

Fig. 2-5 present the growth curves of the different attempts. Two months old larvae reached quite different sizes. Mean standard length ranged from 32 to 64 mm (batch 11 and 3 respectively).

Daily growth drastically increased from phase 1 to phases 3-4. Phase 1 ranged from 0.13 to 0.27 mm; phase 2 from 0.39 to 0.77 mm and phase 3-4 from 0.63 to 1.45 mm (Table 2). During the first grow-out (phase 4), feeding rate decreased from 43 % to 5 % from the beginning to the end of the period. Food conversion rate ranged from 1.1 :1 to 1.8 :1. Final stocking density reached 5.5 kg/m3 (Table 3).

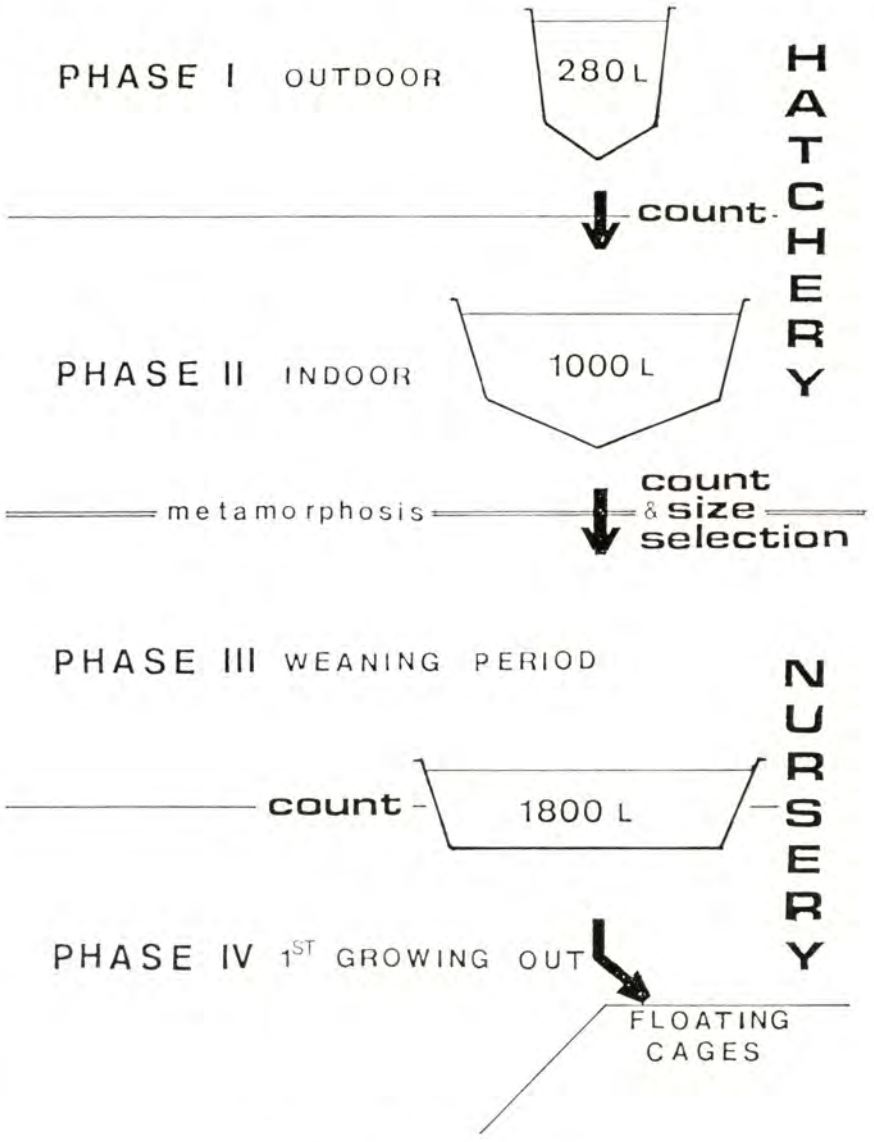

Fig. 1. — Rearing of Red Drum.

Environmental parameters were monitored regularly. Temperature ranged from 25 to 29.5°C and salinity from 32 to 35 ppt. The rate of water exchange fluctuated from 10 to 100 percent per hour. Light was natural (1000-20 000 lux and 11-13 hours daylight) during the first, third and fourth phase of the rearing and artificial during the second phase with an increase of the photoperiod to 14-16 hours daylight. Ammonia concentration, regularly monitored never exceeded 0.1-0.2 mg/l.

Table 2. Daily growth rate (mm) (Groups A-H).

Phase	Run	3	4	8	11
1		0.27	0.14	0.15	0.13
2		0.77	0.50	0.39	0.45
3-4		1.41(A) 1.30(B)	1.45(C)	0.94(D) 1.04(E) 1.19(F)	0.63(G) 0.69(H)

Table 3. Nutrition rate and food conversion rate of the red drum during the first grow out. (phase 4).

RUN	3	4	8	11
Period (day-day)	40-67	36-75	54-75	47-60
Daily nutrition rate (%)	14.1-7.0	42.7-5.1	20.5-8.7	20.4-8.2
Initial mean weight (g)	0.41	0.10	0.38	0.23
Final mean weight (g)	5.66	5.74	2.55	0.71
Final stocking density (kg/m^3)	4.80	5.48	3.79	0.69
Food conversion efficiency	1.11	1.48	1.82	1.78

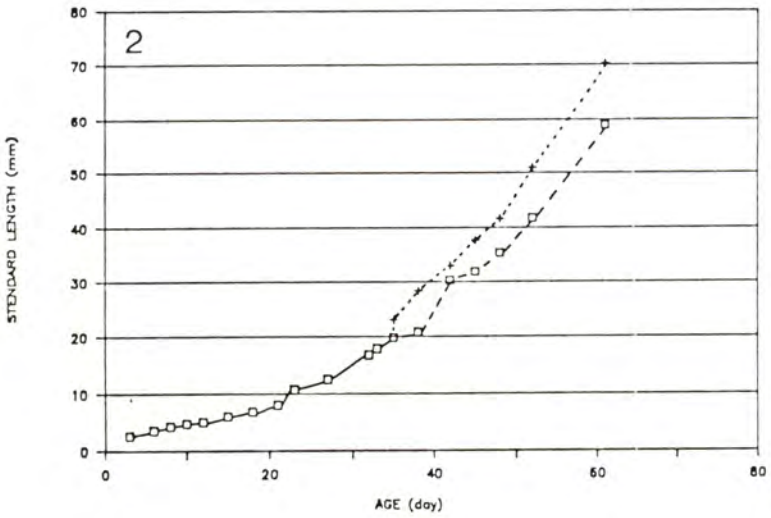

Fig. 2. — Growth (SL) of Red drum from newly hatched larvae through fingerling (Day 61). Trial 3.
(— — Group A. ..... Group B).

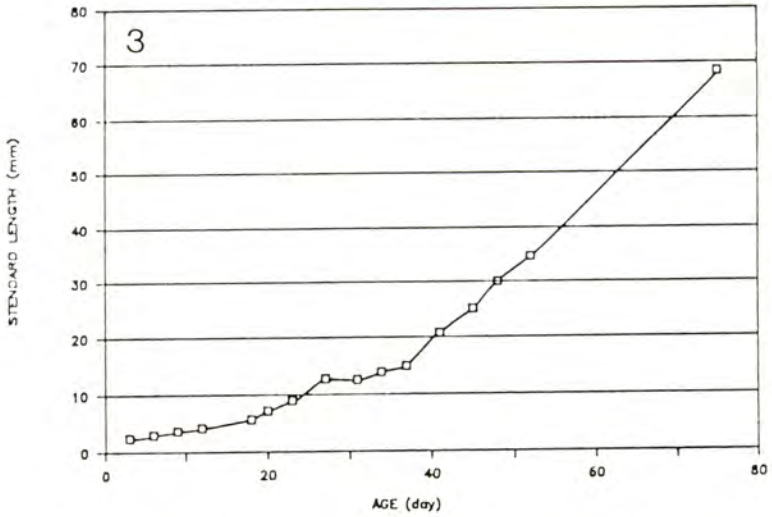

Fig. 3. — Growth (SL) of Red drum from Newly hatched larvae through fingerling (Day 75). Trial 4. (——— Group C).

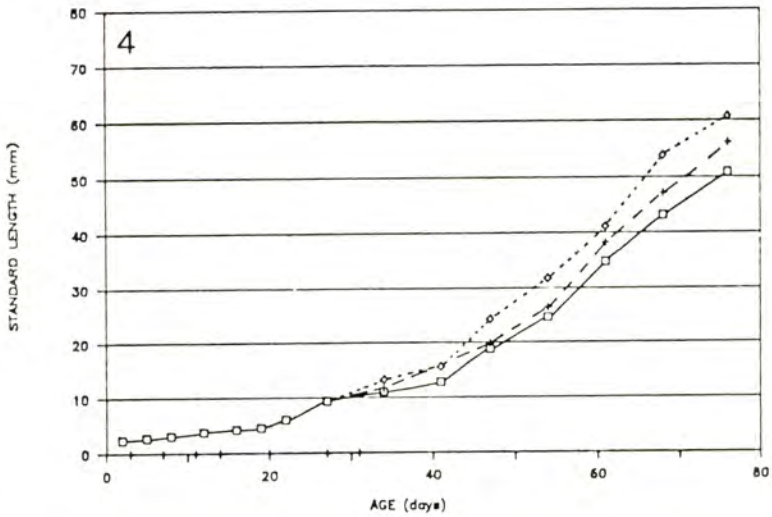

Fig. 4. — Growth (SL) of Red drum from newly hatched larvae through fingerling (Day 76). Trial 8.
(——— Group D; — — Groupe E; ..... Groupe F).

Survival

Runs 3, 4, 8, 11 produced respectively 3 300, 7 200, 17 400 and 2 700, 2 months old fry with a final survival of 6.5 %, 15.0 %, 22.0 % and 5.3 % (Fig. 6). About 30 000 fry were raised during these experimental larval

Table 4. Larval survival rate of Red Drum from newly hatched larvae through fingerling.

Phase \ Run	3	4	8	11
1	62	79		
1-2	12	24	35	34
3	56	74	74	22
4	84	89	66	70
1-2-3-4	6	16	17	5

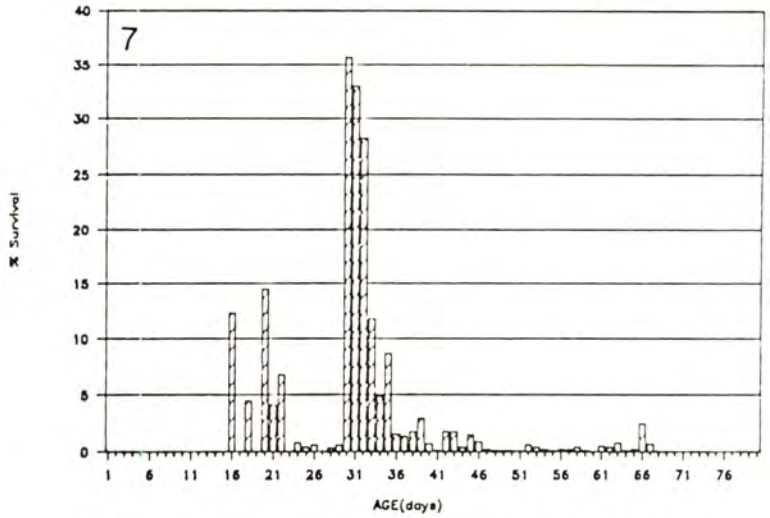

Fig. 7. — Daily rate survival of Red drum (attempt 3).

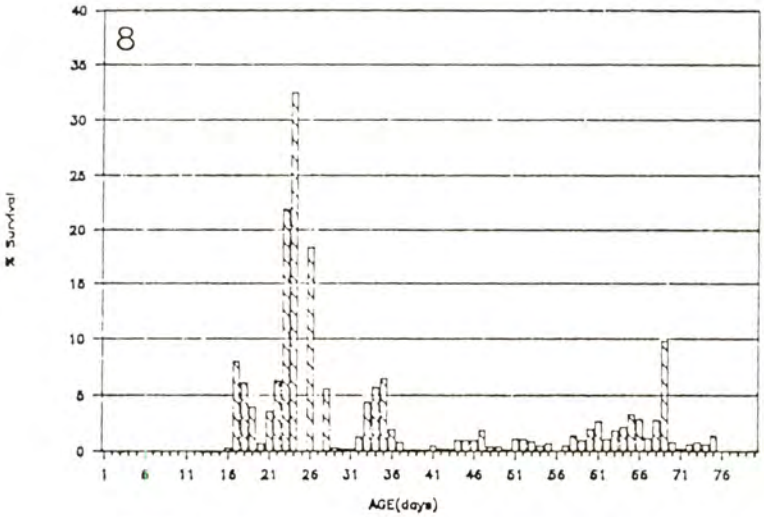

Fig. 8. — Daily rate survival of Red drum (attempt 4).

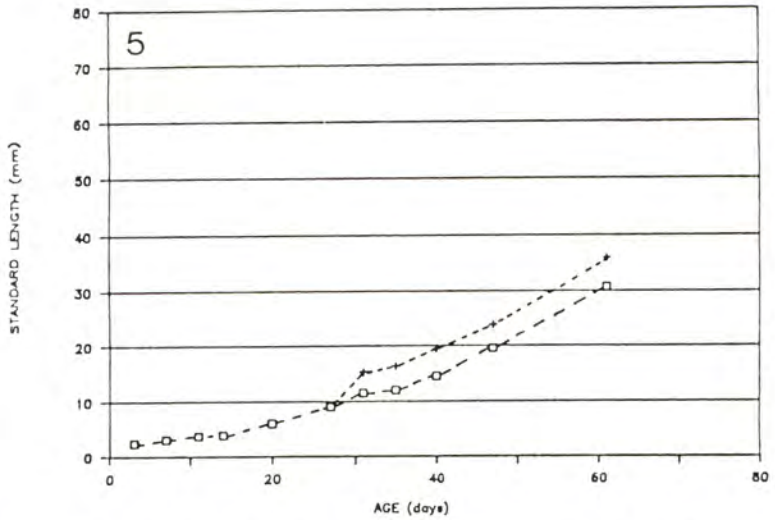

Fig. 5. — Growth (SL) of Red drum from newly hatched larvae through fingerling (Day 61). Trial 11.
(— — Group G; ..... Group H).

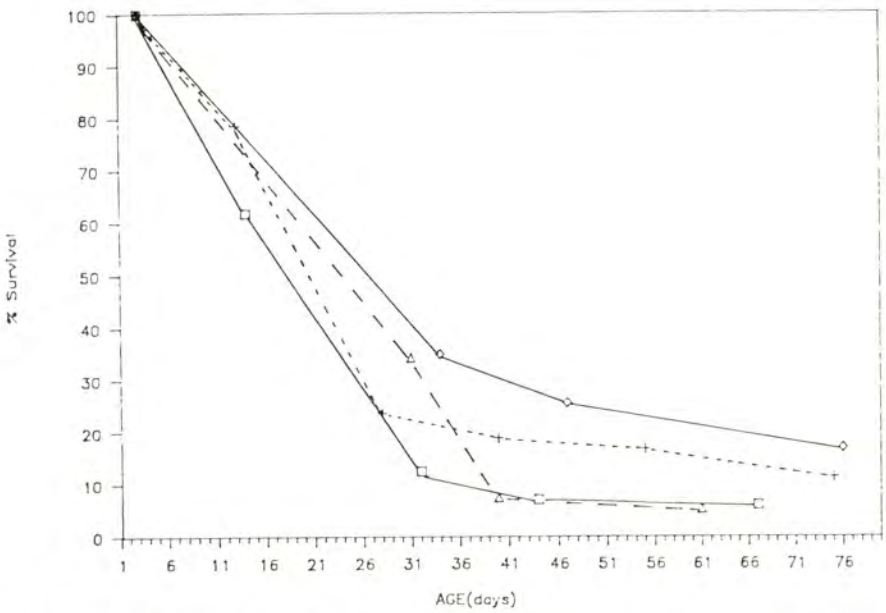

Fig. 6. — Mean percent survival of the Red drum to day (61-76) in attemps 3, 4, 8 and 11.
(— — 3; ..... 4; ..... 8; — — 11).

rearings. During the first month of rearing, survival never exceeded 35 % (attempt 8) (Table 4). The best result was obtained during the second month of the fourth rearing (66 %). Dramatical periods appeared around days 30-32 for run 3 (« unexplained mortality ») and around days 22-26 for

run 4 (microsporidia (?) infestation). An other long period of « unexplained mortality » extended from day 30 to day 41 (run 11). A technical accident was recorded on day 69 (run 4). *Amyloodinium ocellata* (Brown, 1931) infested run 3 by day 16-22 and probably gas bubble disease affected run 8 during the first grow-out stage (Fig. 7-10).

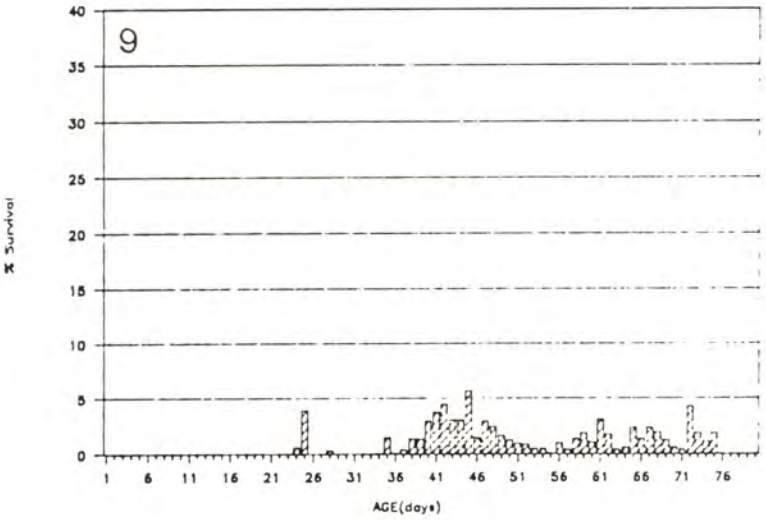

Fig. 9. — Daily rate survival of Red drum (attempt 8).

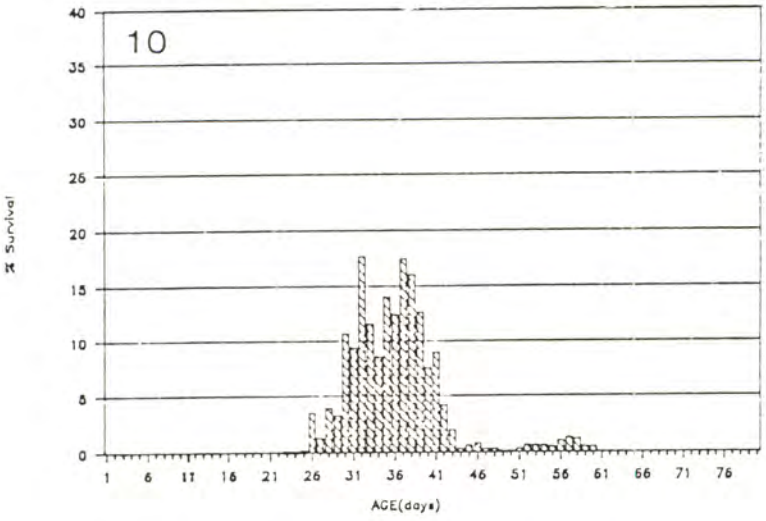

Fig. 10. — Daily rate survival of Red drum (attempt 11).

DISCUSSION

Temperature between 25-29.5°C ranged correctly in the optimum defined by Lee et *al.* (1984); Holt et *al.* (1987). Salinities of 35-38 ppt were above the natural conditions at 20-35 ppt (Simmons and Breuer, 1962 in Holt et *al.*, 1981) and above the optimal of 25-30 ppt for the larval rearing purposes (Holt et *al.*, 1987).

The growth performance of red fish larvae : 38 mm SL in four weeks at 28.5°C is similar to the result of trial 3. The succession of rotifers and brine shrimp nauplii is a classical feeding scheme for rearing fish larvae. During the first phase, a good quality food is provided by rotifers fed *Chlorella* or baker's yeast enriched with fish liver oil (Kitajima et *al.*, 1980 *in* Foscarini, 1988). Important risk was taken by feeding the second phase larvae onto the single brine shrimp nauplii. Early weaning is successfully practised on sea bream *Pagrus major* from 13 mm total length (Peter, 1980 *in* Mok 1985; Foscarini, 1988). Preliminary investigations were realised on red fish, without success. The red sea bream (*Pagrus major*), under intensive and large scale rearing is fed manually with fish meal and early stage (30-40 days old) with artificial diet 250 % of their body weight 10 times a day (during one week). Then 150 % until the end of the second month of cage culture. Hidalgo et *al.* (1988), learnt from different authors that such different species as guppy, carp, mullet, carassin, blackbass, trout, sea bass, sea bream, may be conditioned to use the demand-feeder with efficiency in few hours... For the red fish this solution should reduce drastically the feeding rate and still improve the food conversion rate. The latter ranging from 1.1 :1 to 1.8 : 1 (phase 4) is rather satisfying despite the excess of food distributed.

For many sea water species, artemia is suspected to induce mortality in rearing larvae (Bryan and Madraisau, 1977). Many causes are identified by Sorgeloos et *al.* (1980). Over the past few years, studies pointed out the importance of fatty acids to provide required quality of artemia as food for fish larvae.

Artemia deficiency in highly unsaturated fatty acid (HUFA), 20 :5 and 22 : 6n-3 is suspected to induce high mortality on rearing fish larvae (Watanabe et *al.*, 1978, 1979; Watanabe, 1979; Fujita et *al.*, 1980).

For the species : *Seriola quinqueradiata* produced in Japan, the use of nauplii of artemia is avoided when possible (Cueff, pers. comm., 1973). Mok (1985) drastically increased larval survival rate of the white sea bream, *Mylio berda* when copepod nauplii were substituted to brine shrimp nauplii. High content in linolenic acid (18 : 3 n-3) in some strains such San Fransisco Bay strain is pointed out in different works (Watanabe et *al.*, 1978; Gatesoupe et *al.*, 1983). Some of these authors suggest that the excess in linolenic acid would induce high mortality in fish larvae. In the present attempts (trials 3, 4, 8 and 11) the different strains of brine shrimp eggs have not been analysed. However, the quality of the nauplii fed as sole source for about 15 days is widely suspected. As observed on larval rearing attempts 5 and 6, other authors such as Lee and Hirano (1979) on *Sillago sihama*; Fujita (1973) on *Chrysophrys major*; Kitajima (1978, *in* Foscarini, 1988) on *Seriola quinqueradiata*, have reported similar scenaries

of mass mortality of larvae 4, 7 and 10 days after the first feeding on nauplii. If no guarantee exists on the artemia strains whatever its origin (geographical area, period of harvest...) (Sorgeloos, 1980), the feeding regime must be modified. Other food items such as copepods (Soletchnik et *al.*, in press), or enrichied metanauplii of artemia (Bryan and Madraisau, 1977) should be substituted to nauplii.

If nutritional deficiency affected some of the rearing, pathological events and environmental quality also contributed to reduce the larval survival. The first phase of the rearing, is quite a safe phase. Larvae are fed rotifers and larval survival can reach high rates (up to 80 %). The second phase is much more critical for the larvae. Cannibalism, food adequacy, pathologies (*Amyloodinium* sp.), Microsporidia (?) affected drastically the survival. The dinoflagelate *Amyloodinium ocellatum* is a well known parasite of the red drum (Paperna, 1983; Johnson, 1987). This ectoparasite is also an endemic species in Martinique and has been observed in the rearings of sea bass *Dicentrarchus labrax* in floating cages (D. Gallet, Pers. Comm., 1984). If these parasites induced high mortality on the first attempt, a prophylactic control was established with daily observation of larvae to prevent the pathogen development.

One other cause of mortality called « unexplained mortality » affected two of the rearing attempts (trials 3 and 11) few days after a freshwater treatment against the parasite *Amyloodinium ocellatum*. Investigations (environmental control, histological studies...) were unsuccessful to explain the phenomenon. One hypothesis is that the fresh water treatment at early stage would induce failure in the development of the larvae.

Excessive handling (samplings, screenings, transfers) during these experimental rearings also affected the survival rate. Squamation is an important factor which determines hardness of the larvae. Squamation is also related to change of behaviour on *Epinephelus akaara* (Fukuhara and Fushimi, 1988). Juveniles fully squamated are more tolerant to a wider range of environmental factors than larvae lacking scale protection. (Norman, 1975; Lagler et *al.*, 1982). Scale formation is completed at 10-14 mm total length for the sea bream *Pagrus major* (Fukuhara, 1976 *in* Foscarini, 1988), at 25 mm standard length for the red drum (Holt, 1987). From preliminary data about 90 % of the red drum larvae have first spot scale on caudal part of the body when standard length ranged from 10 to 11.5 mm.

Cannibalism is usually observed on larvae from day 17-20 to day 35-40. In rearing, it increased drastically at day 25-30 during the metamorphosis, when differences in size are important. Holt et *al.* (1987) suggest to rear the red fish at 1-2 larvae per litre during the second phase of the rearing. Rearing density of red sea bream must be reduced to 10-15 larvae/litre to avoid cannibalism (Giovanardi, *in* Foscarini, 1988). In phase 2, mean stocking density ranged from 6 to 23 larvae/litre. It never exceeded 3.2 larvae/litre during the second period of rearing (phases 3-4) (Table 5). Cannibalism of red fish does not seem a « biological necessity ». It is induced by environment and rearing management. Weaned fish in small rearing tanks without current and dynamic flowthrough still had a cannibalistic behaviour. Other fishes from the same population, reared the

classical way in raceway, did not present any more this character. In an other experiment, in particular conditions of rearing with abundance of artemia supply (2000 to 3500 nauplii/larvae/day) cannibalism did not occur despite of large differences in body size of the larvae (4.5 mm to 12.0 mm SL). Larvae were reared in 40 l tanks with a density of 23 larvae/litre.

Table 5. Mean stocking density of larvae (N/litre).

TRIAL	PHASE					
	1		2		3-4	
	Initial	Final	Initial	Final	Initial	Final
3	60.9	37.6	10.5	2.1	1.8	0.9
4	57.1	44.9	12.6	5.3	2.0	1.3
8	87.8	61.4	30.7	15.6	3.0	1.9
11	67.7				3.2	1.0

The swimbladder hyperinflation was observed at different stages of the larval rearing. According to different pathologists it is more or less a stress syndrome as spinal abnormalities, calculi in the urinary bladders (Johnson and Katavik, 1984) or hypertrophic gall bladder (D. Gallet, pers. comm., 1988). Johnson and Katavic (1984) called it the Swimbladder Stress Syndrome (SBSS). During the rearing of red drum, it appeared twice, at 2 to 4 day old larvae and later during the second phase of the larval rearing. Previous experiments demonstrated neither the quality of light neither the intensity were responsible of the SBSS. Secondary experiment showed brutal change in light intensity (night-day) should induce high mortality. During the unsuccessful rearings with deficient strains of nauplii of brine shrimp, the SBSS first anounced the disaster. High rate of abnormal fingerlings (pughead, spinal distortion...) in some rearings pointed out deficiency on environmental control. Such abnormalities frequently affected the gilthead Sea bream, *Sparus aurata* (Franscescon et *al.*, 1988).

CONCLUSION

This study demonstrates that the red drum can be reared from eggs to fingerling size in fiberglass tanks and pure sea water, with the common living preys rotifers and brine shrimp nauplii under tropical conditions in Martinique (F.W.I). Up to present, encouraging results have been obtained in rearing larvae under intensive conditions (20 to 200 larvae/litre along the larval rearing phase with a larval survival up to 35 % and 65 % for the first and second month of rearing respectively). However, an important effort has to be achieved to refine the zootechnical standard of such an intensive larval rearing. The high mortality is attributed to numerous

factors. An essential one is the avaibility and nutritional adequacy of live foods provided to the larvae at their different stages of growth, and principally the nutritional deficiencies of some strains of artemia nauplii, when used as single feed for larvae. A substitution or an enrichment of the nauplii would alleviate this problem. Treatments have already been set up for most of the pathological events encountered and a prophylactic control would increase the safety of the rearing. As a second step of research, there is interest to reduce the period when fishes are fed on living preys, working on early weaning with diets on microparticles...

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Larval rearing and weaning of Seabass, *Lates calcarifer* (Block), on experimental compounded diets.

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Abstract. — *Larval rearing trials of seabass larvae, either imported from Singapore or obtained from the captive broodstock of IFREMER/C.O.P. at Tahiti, were carried out since 1987, by testing different technologies originally used in South East Asia (green-water culture, sequential water changes) or developed by IFREMER for other species (clear water system, preys enrichment, continuous water renewal).*

The preliminary results reveal a high variation of survival and growth rates after the first month, which are not clearly related to one of the closen rearing technic. Best results are obtained at a starting density of 30 larvae/liter in 450 liter cylindro-conical tanks whith a survival rate superior to 50-60 %, for a final mean weight of 20 mg on day 25.

In other trials, strong mortalities are observed from day 12 to 14 with symptoms of depigmentation, over-inflation of the swimbladder and abnormal swimming behaviour followed by death. Histological observations studies reveal vacuols appearing in retinal, CNS and liver a few days before the first symptoms. Pathological researches are now under way to reduce these mortalities and explain these phenomenons. No clear relation with nutritional deficiency is demonstrated.

The weaning of seabass larvae on artificial pellet is feasible as early as the 25th day when the mean weight of fry ranges from 20 to 40 mg with alternative food sequences of live to frozen Artemia and dry pellet containing a high protein (56 %) and lipid (16 %) level. A grading sequence has been defined, which allows to reduce the cannibalism observed as soon as live preys are replaced by artificial diet. Survival rates (50 %) and growth results (mean weight between 1 and 2 g on day 80) are similar to those obtained by other research teams in Singapore and Australia, and confirm the good ability of seabass fry to accept dry pelleted feeds.

Preliminary experiments to replace live prey by artificial microparticulate diet (MD) as soon as the 15th day (mean weight inferior to 15 mg) have been conducted with encouraging results.

INTRODUCTION

Seabass, *Lates calcarifer* is an economically important foodfish in many Asian countries and in Australia. His production reached 18 099 tonnes in 1982 (FAO, 1984 in Grey, 1986), and aquaculture industry is in extension in Thailand (Sirikul et *al.*, 1988); Indonesia (Danakusuma et *al.*, 1986); Malaysia (Awang, 1986); Singapore (Cheong, 1987); Philippins (Duray et *al*, 1986) and Australia (Rimmer et *al*, 1989).

Fingerlings have been recently imported from Singapore to Tahiti to study their ability to be reared in the particular environmental conditions of French Polynesian lagoons (high temperature : 26 - 28°C; and salinity : 34 - 35 ppt), and their growth potential investigated, feeding them on a dry pelleted diet including 56 % CP and 12 % CL.

Encouraging results have already been obtained, indicating that seabass is easily adaptable to lagunal waters of Tahiti : 450 to 500g mean weight fish are obtained in one year from the eggs at a final density of 30 to 35 kg/m3, in cage with a food conversion ratio interior to 1.7 : 1 (Fuchs, 1986; Aquacop et *al.*, 1989).

The maintenance of the captive broodstock and the control of seed production are been investigated in a second step as the supply of a large number of fry will conditionne the futur development of this new aquaculture in the area.

Suitable rearing techniques have been developped several decades ago in Thailand and rapidly extended in Asia, after the successful artificial propagation achieved by Songkla Fisheries Research Station in 1971 (Maneewong, 1986).

Since that date, considerable improvements have been achieved and 50 Thai private farms have produced more than 100 millions fry in 1983-1984, mainly exported to other countries : Singapore, Hong-Kong, Indonesia and Taiwan (Ruangpanit, 1986).

Rearing techniques are based on green algae culture (*Chlorella sp, Platymonas sp*) in large rectangular concrete tanks (5 to 15 tons) where density of larvae decrease from 50 to less than 20 per litre when transferred on day 15 in nursing ponds (Ruangpanit, 1986; Kungvankij et *al.*, 1986).

The cycle of larval rearing lasts 20 to 30 days and includes the succession of following preys : green algae (D1 to D14), Rotifers (D2 to D15), *Artemia* (D10 to D20), moina or Dapnia (D20 to D30), (Maneewong, 1986; Lim et *al.*, 1986)

With this technique, survival can reach 50 to 60 % after one month, whith important variations depending on sanitary conditions, food quality and egg supply.

Weaning larvae on inert food generally starts on day 30 using minced trash fish. During this period, cannibalism is a major problem and frequent grading are practiced (every 5 to 7 days) with grading trays made of plastic basins with many holes (2.5 to 5mm diameter) to minimize mortality. With regular grading and accurate managment of the seed, survival rate is 40 to 60 % from fry to juvenile (Lim et *al.*, 1986).

More recently, Australian Department of Primary Industry, in Cairns (Q.L.) started a pilot-scale hatchery, adapting Thai research results to conditions of Australia (physical, chemical and economical). Mac Kinnon (1986) reported that using close system, clear water and prey enrichment, survival reached 20 to 30 % on day 20, unless strong mortalities were sometime observed on day 10 to 15. Weaning larvae with artificial salmon dry pellet from D25, at a length of approximately 13-15 mm, were tempted and survival and growth rate were encouraging : 90 mm (TL) and 10 g mean weight at 65 days after hatching. Recent informations reveals that a private hatchery, based south of Cairns, produced more than 200 000 fry weaned on artificial diet in 1988, half a million in 1989 (Rimmer pers. com).

Larval rearing trials were recently carried out in Tahiti with eggs imported from Singapore or locally produced by brooders maintained in cages since 1984 and 1985. Seabass green water rearing technique, originally used in South East Asian countries was compared in term of survival and growth potential to clear water rearing method developped by IFREMER on others species like European seabass and seabream (Chatain, 1986; Ronzani-Cerqueira, 1986)

A number of differents weaning trials were also conducted with 30 to 35 days old larvae held in tanks and adapted to high protein level pelleted diets. Several formulations and presentations successfully used for seabass, seabream and flatfish in France (Metailler et *al.*, 1983) were applied to seabass fry.

MATERIAL AND METHODS

Origin of the larvae

Just hatched larvae or 15 days old larvae produced at the Primary Production Department of Singapore, following the technique described by Lim (1986), were air-shipped to Tahiti. Packaging was practiced in plastic bags at a mean density of 1000 to 1500 larvae per litre, each bag being fullfilled with one volume water for 3 volumes of oxygene. In these conditions, larval survival after 24 hours was rather high although water quality was deteriorated with pH inferior to 6.5 and ammonia level superior to 9 and 10 mg/litre. Quality of the larvae was fluctuating with each shipment which made difficult the interpretation of the preliminary larval rearing tests, conducted in 1987.

More recently, first maturation and spawning of local brooders were successfully carried out and a large supply of eggs was obtained.

Broodstock maintenance and managment

Two separate seabass broodstock batches were raised from 500g mean weight fish, imported in 1984 and 1985 at the stage of larvae. They were stocked in netcages mesuring 2 x 2 x 2m deep or 4 x 2 x 3m deep at a density of 4 to 8 pieces per m3. Feeding regime was mainly a dry pelleted diet, including 55 % protein, 12 % lipid offered at 1 to 1.5 % of the body

weight per day. It was completed by a moist pelleted diet (50 % bonitos meal + 50 % fish meal) offered during the maturation season.

After 2 years, each fish was tagged and regular gonad conditions were checked. Maturation was monitored by canulation of gonads and date of sex-reversal of this hermaphrodite fish was identified.

Ripe male and female with ovocyte diameter superior to 400 microns were selected and transfered from cages to spawning tanks (10 m3 capacity) at a rate of 2 females and 4 males per tank.

Techniques of spawning, described by Lim et *al.* (1986) and Nacario (1986) were tested with one injection of 10 microgrammes of LHRH (a) or 500 UI of HCG per kg of female. After several unsuccess trials, it was observed that fertilization ratio was improved when brooders were maintained one or two months in the spawning tank before injecting. Injection was always practiced one or two day before the full moon, although no clear effect was noticed.

Natural spawning occured 36 to 40 hours after injection and the eggs were concentrated and their number and quality were investigated before transferring to the incubation unit. Each cylindro-conical incubator, 25 litres capacity, was filled with 100 to 150 000 eggs. Pourcentage of hatching was checked before transferring larvae in the rearing tanks.

Larval rearing

— *Rearing conditions*

Larval rearing trials were conducted in 80, 150 or 450 litres cylindro-conical tanks painted in grey. Each tank was supplied with mild central aeration and keep in shaded natural light condition (maximum intensity : 1000 Lux).

Seawater, pumped deep in the lagoon, was filtered through several pressure filters (maximum filtration 100 microns) and continously or partially distributed. A biological coral filter, corresponding to 10 % of the rearing volume was connected to the rearing tanks in trial 3.

Live food culture included microalgae : *Chlorella sp*, *Platymonas sp* or *Isochrysis*, mass cultured in 300l indoor or 2 m^3 outdoor fiberglass tanks at a final mean density of around 2-4 millions and 1-2 millions per ml respectively. Rotifers, *Brachionus plicatilis* were maintained in 1.5 m^3 tanks and feed on algae and baker yeast. Their density was increasing from 20 to 80-100 per ml in a 5-6 days cycle of production. Enrichment techniques, described by Gatesoupe et *al* (1983), were generally used.

Nauplii and two days old *Artemia* from different strains, were enriched with silco enrichment technique, proposed by Duray and *al.* (1986), before being fed to the larvae.

Green algae larval rearing technique, traditionnally developped in Asia (Lim, 1986; Maneewong, 1986) was compared to rearing method used for European seabass which include : clear water, continuous water flow from early stages, food quantity exactly ajusted to the consumption and Rotifers and 2 days old *Artemia* enriched with high fatty acids.

Physical parameters of water were identical with temperature increasing from 25 to 28-29°C and salinity stabilized around 30 ppt.- Desigh of experiments

The designs of 3 experiments conducted with larvae imported from Singapore are as follows :

Trial 1. 450 litres tanks. 4 treatments. No replicate. One clear water. 3 with green algae : *Chlorella* : 100 000 c/ml or *Isochrysis* : 30 000 c/ml. Initial density = 33 larvae per litre. Water exchange = 5 to 10 %/hour in continuous (Treatment 1) or 50 to 80 %/day (treatments 2-3-4). Duration : 25 days. Feeding regime : Rotifers D.5-15; Nauplii *Artemia* D.9-25; 2 days old *Artemia* D.15-25.

Trial 2. 80 litres tanks. 3 treatments. 2 replicates. One clear water. 2 with green algae : *Chlorella* : 100 000 c/ml or *Isochrysis* : 30 000 c/ml. initial density = 25 larvae per litre. Water exchange : 30 to 80 %/day in discontinuous. Duration : 25 days. Feeding regime : Rotifers D.3-15; Nauplii *Artemia* D.10-22; 2 days old *Artemia* D.18-25.

Trial 3. 450 litres tank. One treatment. 2 replicates. Clear water. Initial density : 33 larvae per litre. Water exchange : 5 to 15 %/hour. Close recirculated system. Feeding regime similar to trial 2.

Five other larval rearing trials were tempted with larvae locally produced in Tahiti : spawning n° 9 (March 1988); spawning n° 17 (November 1988); spawning n° 20 (january 1989). Clear water technique was used in 150 and 450 litres tanks as indicated in table 1.

Table 1. Experiment larval rearing trials conducted with larvae produced locally in Tahiti in 1988 - 1989, with continuous water exchange from early stage.

Trial	Spawning number	Total larvae reared	Tanks	Initial concentration /litre	Treatment	Water exchange
1	9	170 000	12 x 150 l	33	C W	C
			1 X 10 m^3	11	G A	D
2	9	130 000	9 X 450 l	32	C W	C
3	17	135 000	9 X 450 l	33	C W	C
4	20	135 000	9 X 450 l	33	C W	C
5	20	60 000	12 X 150 l	33	C W	C

C W : clear water
G A : green algae
C : continuous
D : discontinuous

Weaning larvae on pelleted diet

After the first month of rearing, larvae were transferred from rearing tanks to circular tanks (1.5 m^3 capacity) and weaned on artificial diet. During transfer, larvae were graded and dispatched into 3 differents sizes (inferior to 1.5mm; superior to 1.5 — inferior to 2; superior to 2 mm) to

minimize the rate of cannibalism appearing as soon as live preys were replaced by inert food. Graders used have been described by Vasselin (1984) for European seabass. Grading frequency was fixed every 10 to 15 days, depending on the behaviour of the fry.

Food sequency included the overlapping from live Artemia (5 days) to frozen Artemia (10 days) and dry pelleted starter diet (56 % Protein, 16 % Lipid) offered from 5th day. (see exemple in figure 1). Live and frozen food were distributed 3 times per day, and artificial diet in 8 hours, thanks to automatic feeders. Quantity of dry pellet was calculated to be in excess (20 to 30 % of the body weight per day).

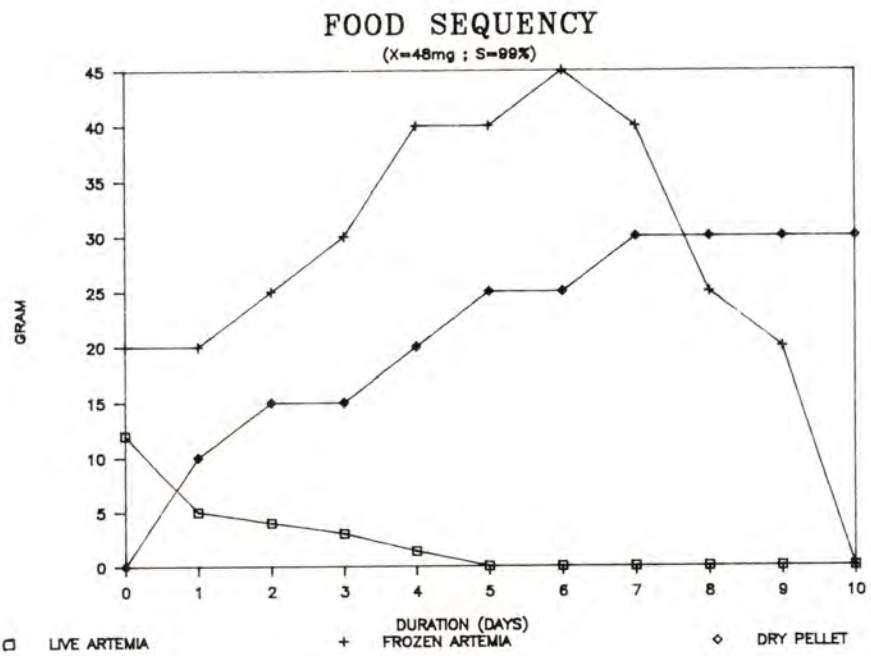


Fig. 1 — Exemple of Seabass larvae weaning with an overlap of 5 days on live Artemia and 10 days on frozen Artemia. (number : 2000 fry; initial mean weight : 48mg; age : 35 days).

Larval density was fixed between 0.5 to 1.5 larvae per litre and water quality was 27°C for temperature, 35 ppt for salinity. Water exchanges were increasing from 10 to 50 %/hour. Light intensity was low (inferior to 5000 lux) as seabass fry are sensitive to any environmental stress.

The whole population was weighed and regularly graded into different sizes varying from 1.5 to 5 mm. Grading was provided every 10 to 15 days to avoid cannibalistic behaviour and density was maintained between 0.5 to 1.5 larvae per litre.

Three tests were conducted in 1987 and 1988,following this design with 35 days old larvae issued from first larval imported from Singapore. Initial number of larvae was respectivelly 4800, 18 000 and 13 000 in trials 1-2 and 3 and duration varied from 20 to 60 days.

Weaning seabass larvae with attractive substances

Four different mixtures of chemical attractive substances, successfully tested on european seabass by Metailler et *al* (1983) were tentativelly incorpored in the weaning diet of seabass. The objective was to evaluate the effect of these substances on pellet acceptability and possible survival and growth improvement.

Rearing conditions were similar to the former. Density was fixed to 2 larvae per litre or 160 larvae, (50.55 ê 0.9 mg mean weight), in 70 litres rectangular tank.

Diet sequency included direct weaning on dry pellet, distributed 8 hours per day with a first stabulation of larvae during 6 days, feeding them with frozen Artemia.

Water quality was similar with a temperature of 27 ê 1 °C, 10 % water exchange per hour and a salinity of 35 ppt.

Six treatments and 2 replicates were tested

T.1 : control fish fed on frozen Artemia
T.2 : weaning Diet without attractive substance : Diet 1
T.3 : weaning Diet 1 plus 0.5 % glycine and betaine : Diet 2
T.4 : Diet 2 plus 1.77 % neutral amino-acids : Diet 3
T.5 : Diet 3 plus 0.37 % amino-acids : Diet 4
T.6 : Diet 4 plus 0.054 % inosine and hypoxanthine : Diet 5

Duration of the experiment was 24 days, without intermediar grading.

Early weaning of seabass larvae on microparticulate diets

Microbinded diets (MBD) similar to those described by Person-Le-Ruyet (1986-1989) and including fresh products, were tested on seabass larvae as early as the 20th day. Rearing conditions were differents to the formers with the use of 80 litres cylindro-conical tanks. Density was variable from 1.5 to 2.5 larvae per litre, and each treatment was replicated 2 times. Diet sequency included MBD distributed from the first day in continuous during 8 hours with automatic feeders. Diet quantity was ajusted to the needs (1 to 2 g per day).

From day 10, MBD was gradually replaced by former weaning diet in an overlap of 3 to 4 days. Size of particules were 125-250 M for MBD and 250-300 M for weaning diet. Live *Artemia* were given during the first 10 days of experiment; quantity was calculated to be exactly 10 % of the dose offered to the control.

Water temperature was fixed to 28°C and water exchange to 200 % per day.

Three experimental designs have been carried-out :

Trial 1 : 1 MBD formulation. 2 different binders : carragheen or alginate. 1 control with live *Artemia.* Initial larvae age : 20 days and 15.2 mg mean weight. Duration 15 days.

Trial 2 : 1 MBD formulation. 1 binder (carragheen). 1 principal ingredient (squid). 1 treatment with live *Artemia* : 10 % of control. 1

treatment with frozen *Artemia* : 10 % of control. 1 control with live *Artemia.* Initial larvae age : 21 days and 24.4 mg mean weight. Duration : 15 days.

Trial 3 : 2 MBD formulations. 1 binder (carragheen). 2 principal ingredients in the diet : mussel or squid. 1 control with live *Artemia.* Initial larvae age : 31 days and 39.9 mg mean weight. Duration : 11 days.

No grading were provided during the experiments.

RESULTS

Maturation in Captivity

Although, the complete cycle of reproduction of this imported species is still under study, first observations indicate that seabass seems to mature during the rainy season, like the local species as grouper (Debas et *al.*, 1989). Peak of maturation is observed when light duration and temperature are increasing (November to April) and sexual rest is noticed from june to October (Guiguen, pers. com.).

Seabass males reach sexual maturity at the age of 2 to 2.5 years and are generally ripe 5 to 6 months per year. First sexual reversal changes were noticed in November 1987 and February 1988 for the 2 broodstocks respectivelly imported from Singapore in 1984 and 1988. (The proportion of reversal rapidly increased in few months and a significant number of female was obtained in 1988).

More accurate observations, realized on 3 batches of 30 brooders, isolated from the second broodstock in June 1988 indicated that a minimum duration of 3.5 years was necessary to observe the first reversal and 4 years for 40 % of the population to reverse from male to female (Nedelec, pers. com.).

Spawning and hatching

Spawning trials, were conducted in November, December 1987 with the first broodstock and from February 1988 to May 1989 with the second broodstock. Males and females were always issued from the same original batch (imported in 1984 or 1985). Synthesis of results are summarized in table 2.

The preliminary trials conducted in November, December 1987 with females issued of the first broodstock (1984) were not successfull and only one female spawned 6 000 unfertilized eggs.

Between February and May 1988, 7 females spawned and 12 depositions were compted. A total of 6.39 millions eggs fertilized at a rate of 27 %, were collected. and 1.7 millions were incubated in conical incubators.

During the last spawning season, from October 1988 to March 1989, 6 females naturally spawned more than 7.8 millions eggs fertilized at 65 %.

Table 2. Results of Seabass spawning between November 1987 and May 1989.

	November December 1987	February to May 1988	October 1988 to March 1989	Total
Origine of female	Broodstock nº1	Broodstock nº2	broodstock nº2	
Number of female treated	2	9	9	20
Number of female spawning	1	7	6	14
(%)	50	78	67	70
Total number of deposition	2	12	12	26
Number of deposition with fertilized eggs	1	3	10	14
(%)	50	25	83	54
Total number of eggs (millions)	0.006	6.39	7.86	14.246
Number of fertilized eggs (millions)	0	1.72	5.13	6.82
% fertilization	0	26.9	65	48
Mean egg diameter (mm)	0.88	0.77	0.817	0.822

10 of the 12 deposition were normally fertilized and 5.1 millions eggs were transfered in incubators.

Figure 2 illustrate this regular increase in the pourcentage of fertilized eggs between 1987 and 1989.

The regular improvment in the management of broodstock can partially explained thes increasing results. The pourcentage of fertilization was very low when brooders were injected 2 to 3 days after transfer from cage to spawning tank due to stress behaviour. A minimum of 1 month of stabulation in the spawning tank is highly recommended to improve the pourcentage,as previously noticed by Lim et *al.* (1986) in Singapore.

Origin of the brooder and the choose of large females (mean weight superior to 4-6 kg) are also important factors to consider.

Finally, determination of the natural cycle of reproduction and sex-reversal period of this imported species in Tahiti, would certainly help to choose the better period of the year to successfully induce the brooders.

Larval rearing

Trial nº1

The first larval rearing trial, conducted in 1987 with larvae imported from Singapore gave encouraging results as shown in table 3.

Traditionnal S-East Asian rearing techniques using green algae and a discontinuous water exchange was successfully transfered to the local conditions of Tahiti. Survival after 25 days was superior to 50 % with a maximum of 82 % in Tank nº3, where larvae received Rotifers and *Isochrysis sp* algae. Mean weight at the end of the rearing period reached 25 to 30 mg and food intake for each produced larvae was estimated to 5-6000 Rotifers, 3000 nauplii of *Artemia* and 600 of 2 days old *Artemia.*

Table 3. Larval rearing trial nº 1 conducted in 1987 in 450 litres tanks with larvae imported from Singapore. Traditionnal S.E.Asian larval rearing techniques was compared to clear water rearing method where Rotifers and Artemia are enriched.

Tank	Treatment	Initial number	Survival (%)	Final density /litre	Final mean weight (mg)	Food intake/larvae produced Rotifer	Na Artemia	2 days old Artemia
1	Clear water	11 600	52	13	46	4 800	3 000	1 200
2	Green water (Chlorella)	11 600	56	14	29	5 100	3 700	680
3	Green water (Isochrysis)	11 600	82	21	24	6 000	2 300	410
4	Green water (Chlorella)	11 600	78	20	26	6 300	2 700	650

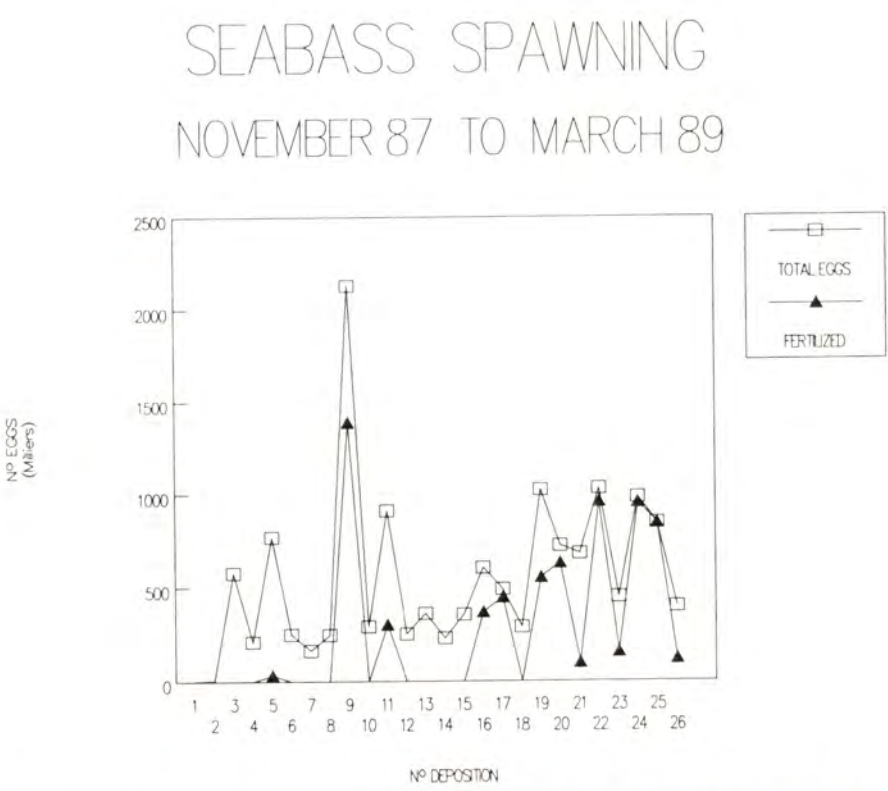


Fig. 2. — Total number of eggs and fertilized eggs obtained by spawning of induced female of Seabass (*Lates calcarifer*) in Tahiti from November 1987 to March 1989.

In comparison, the first batch, reared in clear water and fed on enriched Rotifers gave lower survival rate (52 %), but the final mean weight (46 mg on day 25) was significantly higher than in the 3 others batches. Food intake was similar but 2 days old *Artemia* were offered in larger quantity due to a higher growth speed.

Trial n°2

First experimental design was replicated in 80 litres tanks, with 2 tanks per treatments (Table 4).

When larvae were fed on Rotifers and algae (*Chlorella* or *Isochrysis*) : tank 1 to 4, survival rate was superior to 50 % (maximum 71 %) except in tank 1 where an anormal strong mortality was observed on day 15 although a high initial survival rate. Food intake was similar to trial 1 with a total of 8000 Rotifers, 3000 Nauplii of *Artemia* and 450 2 days old *Artemia* offered per larvae produced on day 25. Final mean weight reached 18 to 25 mg without clear difference when larvae received *Chlorella* or *Isochrysis* algae strains with Rotifers.

Table 4. Larval rearing trial n° 2 conducted in 1987 in 80 litres tanks with larvae imported from Singapore. Traditionnal rearing techniques are compared to clear water rearing method where Rotifers and Artemia are enriched.

Tank	Treatment	Initial number	Survival (%)	Final density /litre	Final mean weight (mg)	Food intake/larvae produced		
						Rotifer	Na Artemia	2 days old Artemia
1	Green water (Isochrysis)	2 000	14.5*	4	12.5	36 000	1 100	1 400
2	«	2 000	51.9	14	17.5	10 000	3 500	470
3	Green water (Chlorella)	2 000	63.7	16	22.8	7 800	3 000	380
4	«	2 000	70.7	18	13.1	6 900	2 700	340
5	Clear water	2 000	31	8	32.9	8 700	5 400	1 000
6	«	2 000	31	8	27.6	5 600	4 700	700

* Mortality on day 15.

In comparison, larvae reared in clear water from early stage had the same lower survival rate in the 2 replicates (31 %). Important mortality was noticed during the first 5 days of larval rearing (first feeding). Total amount of live prey was 7000 Rotifers, 5000 Nauplii of *Artemia* and 350 of 2 days old *Artemia* per larvae produced on day 25. Final mean weight was also significantly higher with a mean value of 30 mg when Rotifers were enriched.

Trial n° 3

Althoug the difficulty encountered to compare these results due to the lack of control, it has been observed that seabass larvae where able to survive when water was totally recirculated on a biological filter as previously mentionned in Australia by Mac Kinnon (1986). (Table 5).

Survival rate was 30 % after 20 days with high mortality, observed as in the former trial, from day 1 to 5. Food quantity intake was similar with around 7000 Rotifers, 3500 Nauplii of *Artemia* and 600 of 2 days old *Artemia* offered to each day 20 old larvae. Growth speed was satisfying and final mean weight varied from 14 to 17 mg.

Table 5. Larval rearing trial n°3 conducted in 450 litres tanks. Water is recirculated on a biological filter and larvae are fed on enriched Rotifers and Artemia.

Tank	Treatment	Initial number	Survival (%)	Final density /litre	Final mean weight (mg)	Food intake/larvae produced		
						Rotifer	Na Artemia	2 days old Artemia
1	Clear water and Close system	12 000	26	7	17.2	8 300	3 700	710
2		12 000	34	9	14.4	6 400	3 300	550

Larval reared trials conducted with eggs produced in Tahiti

Five trials have been conducted in 1988-1989 with eggs produced by brooders maintained in captivity since 1985. Clear water and enriched Rotifers wzere offered in each treatment. Unsuccessfull results have been obtained with extremely high mortalities occuring from day 10 to 15 (Table 6). Clinical symptoms were similar to those described by Rimmer (1989) in Australia with

+ stop feeding
+ depigmentation of the larvae
+ overinflation of the swimbladder
+ anormal swimming behaviour

Mortality generally occured 2 to 3 days after the aparition of the first symptoms and rapidly extended to the whole population. Histological studies shown clear vacuolisation in the retina and CNS. Final survival was so poor that larval rearing were stopped after 3 to 4 days.

Table 6. Larval rearing trials conducted with eggs locally produced. Rearing techniques include clear water or green algae.

Date	Number of larvae reared	Number of tanks/litre	density	Treatment mortality	Peak of survival	Final
1th Ap. 88	170 000	12x150 l	33	Clear Water	8	0 %
		1x 10 000 l	11	Green Algae	12-13	0 %
	130 000	9x450 l	32	Clear water	5-6	0 %
24th Nov. 88	135 000	9x450 l	33	Clear water	11-13	0 %
24th Jan. 89	135 000	9x450 l	33	Clear water	12-13	0 %
24th Jan. 89	60 000	12x150 l	33	Clear water +Green water	15-17	5 %

Weaning seabass on a dry pelleted diet

Weaning seabass larvae on a dry pelleted diet after an overlap of 10 days on live and frozen *Artemia* has demonstrated to be feasible. Results of the first two trials, conducted in 1987, (table 7), confirme the preliminary observations carried-out in Tahiti in 1985 (Fuchs, 1986).

Mortality (up to 30-40 %) was concentrated during the first 15 days as shown in figure 3, with a strong cannibalistic behaviour when live preys were replaced by artificial pellets. After 20 days, fry were already weaned and the number of dead larvae dicreased, and final survival, after 2 months was around 50 % in trial 2.

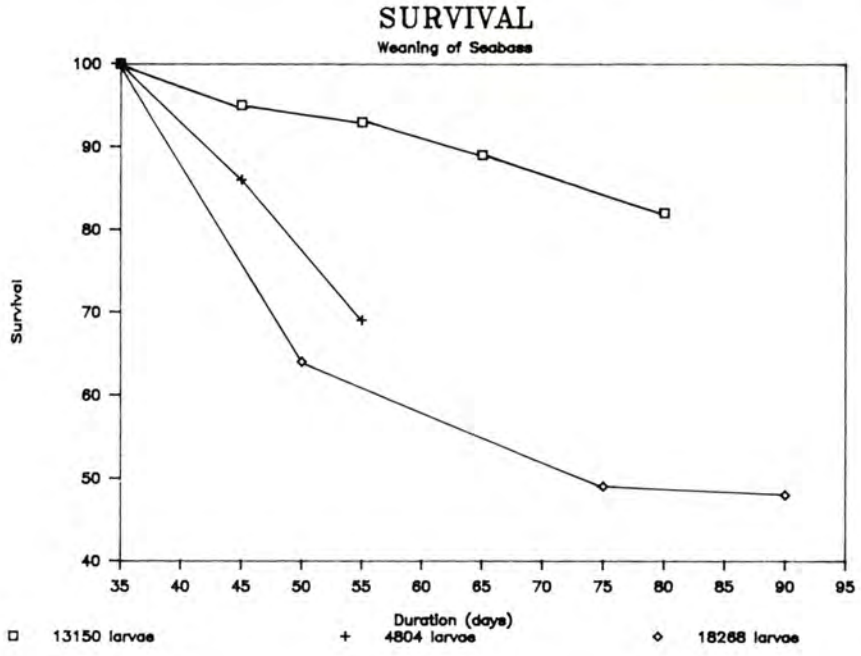
Growth speed was slow during the first period of adaptation to pellet but drastically increased to reach a final mean weight of 2 grammes at the end of the weaning period in trial 2 (see figure 3). Food efficiency was regularly increasing and final conversion ratio was 2.3 : 1 in trial 2, 4.05 : 1 in trial 1 (Table 7).

Size dispersion after 2 months was large with 67 % of the whole population distributed in grading size : 3.5 to 4 mm, 23 % between 4-5 mm and 10 % between 5 and 6 mm.

Table 7. Seabass weaning on dry pelleted diet after an overlap of 10 days on live and frozen Artemia (trial 1-2, 1987 ; trial 3, 1988).

Trial	Age (d)	Duration (d)	Number	Survival (%)	Density /litre	Rearing volume m^3	Mean weight mg	Conversion ratio
N° 1	35	0	4 800	100	0.8	6	44	—
	45	10	4 110	86	0.5	9	119	5.2 :1
1987	55	20	3 300	69	0.4	9	332	3.5 :1
	Total	20	3 300	69	0.4	9	332	4.1 :1
N° 2	35	0	18 300	100	1.8	10	84.2	—
	50	15	11 610	64	1.0	12	220	4.4 :1
1987	75	30	8 927	49	0.75	12	1 010	2.2 :1
	90	55	8 821	48	0.7	12	2 100	1.1 :1
	Total	55	8 821	48	0.7	12	2 100	2.3 :1
N° 3	35	0	13 200	100	1.8	5	47	—
	45	10	12 535	95	1.7	6	122	2.3 :1
1988	55	20	12 206	93	1.4	6	340	1.7 :1
	65	30	11 807	90	1.3	6	720	1.1 :1
	80	45	10 829	82	1.2	6	2 400	0.7 :1
	Total	45	10 829	82	1.2	6	2 400	0.7 :1

The better knowledges of seabass larvae behaviour and the regular improvment of weaning technique and tank management from 1987 to 1988 allowed to increase the performances as shown by results obtained in 1988 (Table 7). Survival at day 80 range 82 %, average mean weight increased from 47 mg to 2.4 g in 45 days and final conversion ratio reached 0.8 : 1 in as fed. Cannibalism of fingerlings was controlled by a frequent grading, every 10 days during the first month, then every 15 days (Figure 3). Size distribution was more homogeneous with 53 % of the population distributed in the range 4-5 mm, 23 % inferior to 4 mm and 24 % superior to 5 mm.



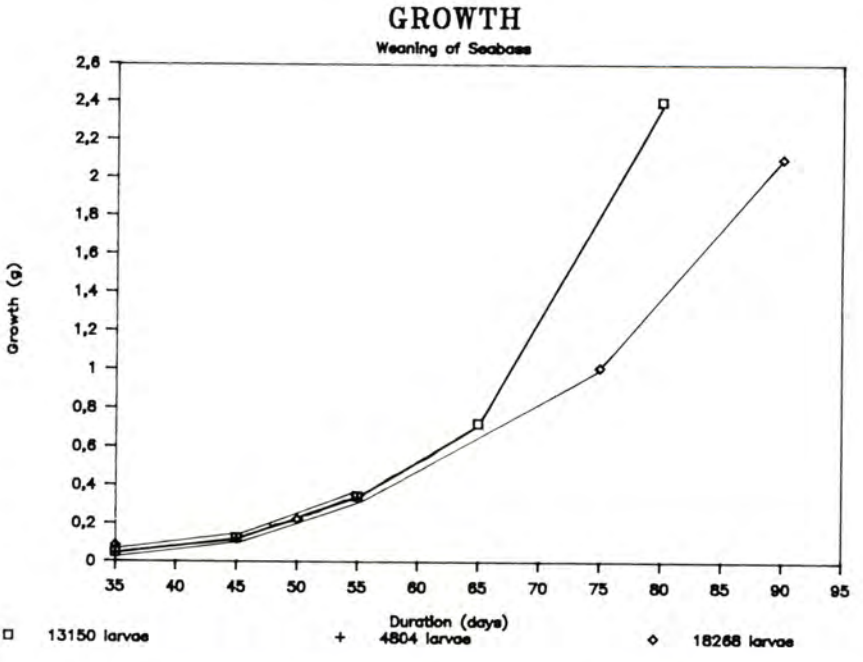


Fig. 3. — Survival and growth of Seabass fry during the weaning on dry pelleted diet (56CP 16CL). Trial 1 : 4800 larvae, Trial 2 : 18 300 larvae, Trial 3 : 13 150 larvae.

Weaning seabass larvae with attractive substances

Survival and growth results of the 6 treatments are summarized in Table 8.

When attractive chemical substances were incorporated in the weaning diet by coating, no significant difference was noticed, between treatments, after 25 days. Survival was superior to 75 % in each trial and the mean weight was increasing from 50 to 380 mg in 25 days, corresponding to a daily weight increase of 7.7 %.

In comparison, the control fish, fed on frozen *Artemia* shown a better survival rate (up to 87 %) but their growth speed was significantly lower with a final mean weight less than half of the weight obtained when larvae were fed with pellets. These results suggest that frozen Artemia would not cover the requirements of fingerlings during this phase, although survival rates are satisfying.

Table 8. Weaning of Seabass larvae on dry pelleted diet including different attractive chemical substances during a 25 days experiment (initial mean weight : 50.55 mg).

Treatment	% Survival		Final mean weight (g)		D.W.I. (%)	
	Per replicate	Mean s.	Per replicate	Mean w.	Per replicate	Mean D.W.I.
Control	91.8		176.3 ± 30.6		5.3	
frozen Artemia	82.5	87.1	186.1 ± 28.8	181	5.2	5.25
Diet 1	79.9		422.5 ± 71.3		8.5	
without Artemia	66.9	73.4	407.7 ± 75.2	415.1	8.3	8.4
Diet 2	79.6		368.8 ± 80.7		7.9	
	70.5	75.1	443.3 ± 106.8	406.1	8.7	8.3
Diet 3	71.1		442.4 ± 104.4		8.7	
	84.7	77.9	32[illegible]9 ± 46.6	382.1	7.4	8.05
Diet 4	79.0		391.9 ± 88.1		8.2	
	73.0	76	339.7 ± 61.5	365.8	7.6	7.9
Diet 5	88.6		386.0 ± 54.1		8.1	
	74.2	81.1	322.5 ± 55.8	354.2	7.4	7.8
Control		87.1		181		5.25
Artificial diet		76.7		384.7		8.09

Early weaning of seabass fry on microbinded diets

Results of preliminary early weaning tests (Table 9) clearly shown that seabass larvae were able to accept microbinded diets as early as 20 th day when offered with a few amount of live *Artemia* (10 % of control dose) during 10 days. In trial 1 and 2, survival rate, at day 35, end of the weaning period, ranged from 22 to 56 % with best results obtained when microdiets were binded with carragheen. Microdiet, incorporated alginate binder was not accepted and induced a strong mortality after 10 th day. Replacement of live *Artemia* by frozen ones (trial 2) did not affected survival as shown in table 9.

Table 9. Early weaning of Seabass larvae with microbinded diet incorporating different ingredients. 10 % of live Artemia offered to the Control are distributed during the first 10 days.

Diet	Number		Survival	Mean weight (mg)		D.W.I.	Conversion
	Initial	Final	(%)	Initial	Final	%/Day	Ratio
Trial 1. Day 20 to 35							
MBD Carragheen	200	111	56	15.2	142	15	1.5 :1
MBD Alginate	200	44	22	15.2	76.5	11	7.3 :1
CONTROL	200	163	82	15.2	142	15	1.3 :1
Trial 2. Day 21 to 36							
MBD Live Artemia	200	84	42	24.4	163	13	6.6 :1
MBD Frozen Artemia	200	89	44	24.4	178	13	5.5 :1
CONTROL	200	102	51	24.4	166	13	2.3 :1
Trial 3. Day 31 to 42							
MBD Mussel	110	95	87	39.9	95.3	8	3.1 :1
MBD Squid	110	95	87	39.9	142	12	1.7 :1
CONTROL	110	104	94	39.9	214	15	0.7 :1

Similar differences were noticed on growth performances and average mean weight increased from 15-24 mg to 150-160 mg after 15 days using caragenane against 75 mg when alginate was incorporated as binder in the diet.

Growth potential were similar when live or frozen *Artemia* where comparatively offered as complement to the MBD.

Conversion ratio also fluctuated with the differents tested diets (range 2 to 7.1 : 1) confirming the good potential of MBD incorporating squid.

In comparison, control larvae, exclusively fed on live Artemia had a higher survival rate (up to 82 % in trial 1) but final mean weights were no significantly higher than when larvae were fed on a well balanced MBD.

Results of trial 3 (Table 9), conducted with larger larvae, (31 days old, initial mean weight : 39.9 mg) indicated that the MBD formulation has a major importance. Significant better growth was obtained when squid was incorporated in large amount in the MBD, compared to mussel (140 mg again 95 mg after 15 days). Survival was very high in both case with values superior to 85 %. In this trial, results of survival and growth were improved when live *Artemia* were offered as unique food with respectively 94 % survival and final mean weight : 214 mg after 15 days.

In this trial, conversion ratio were respectively 0.7, 1.7 and 3.1 : 1 respectivelly when larvae were fed with live *Artemia*, MBD quid or MBD mussel.

DISCUSSION

Seabass, *Lates calcarifer* has been selected as one of the most promising species to develop a new aquaculture industry in French Polynesia. Successfully nursing and grow-out trials have been carried-out since 1985 with fry imported from Singapore and no particular problem has been encountered when fry were exclusively fed on a compounded diet, pelletized in Tahiti (Aquacop et *al*, 1989).

The major contraint to increase the production and really initiate a development programme was the seed production which has to be manage with appropriate feasible production techniques. The climatic conditions of French Polynesian oceanic waters (high salinity) and the difficulties encountered to supply large amount of natural fresh food to larvae and juveniles were identify as possible limiting factors to overcome.

The first observation of ripe males (in 1987) after 2.5 years of stocking in floating cage raised our dubiousness of getting maturations of seabass in Tahiti meanwhile his catadromous characteristic (Grey, 1986). One year later, sex-reversal phenomeous was identified and the first females appeared. Since that date, many induced spawning have been successfully tempted, confirming the validity of the spawning techniques, described by Nacario et al (1986), Lim et al (1986) or Duray et al (1986). Fecondity ratio of female regulary increased from very few eggs up to 600 000 per kg, fertilized at a high rate.

These encouraging results seem to indicate that maturation and egg supply are not limiting factors but many improvements are actually purchassed to propose an appropriate feasible technique.

In this objective, several axes of research are actually investigated :

- Determination of the natural cycle of reproduction of *Lates calcarifer* in the particular conditions of Tahiti and determination of the period of sex-reversal.
- Improvement of the quality of the gametes in relation with the food quality and the optimal environmental conditions (temperature, salinity...).
- Extension of the spawning season by environmental manipulations (temperature-photoperiod).
- Cryopreservation of sperm, testing existing technologies.

Larval rearing trials, carried-out with larvae imported from Singapore, demonstrated that seabass could easily be produced in Tahiti using the simple technique communly developped in many Asian countries (Maneewong et *al*, 1986 in Thaïland; Duray et *al*, 1986 in the Philippines). Interesting preliminary results have already been obtained in stagnant water with a feeding regime including algae, Rotifers and Artemia. Several contraints have nevertheless been noticed, limiting our interest to extend this method in Tahiti : survival and growth results are highly fluctuating

with the quality of algae and Rotifers offered and water exchanges and tank management are time consumming.

In comparison, the technique developped in Europe to produce European Seabass and Seabream larvae (clear water, prey enrichment and continuous water exchange), was tentativelly adapted to tropical seabass larvae with interesting preliminary results. It has been observed that the growth potentail of larvae fed on enriched preys, as recommanded by several authors (Watanabe et al, 1983; Gatesoupe et al, 1983)was significantly increased compared to larvae fed on Rotifers grown on a mixture of algae and yeast. In the preliminary larval rearing tests, survival rate was nevertheless lower than with the former technique (30 to 50 % on Day 25) due to the high mortality observed during the first feeding stage (day 5-6). The strict adjustment of the quantity of preys offered to the consumption of the larvae which is recommanded with this technique, could partially explain these mortalities. The concentration of Rotifers was not exceeding 1 per ml due to the small consumption of preys at that age and the quantity available seem to be insufficent to feed all the larvae. In comparison, Duray et *al* (1986) maintain a concentration of 10 to 15 Rotifers/ml from day 2 to 15 and Rimmer et *al* (1989) supplies Rotifers at a concentration of 20 Rotifers/ml, remove every day.

Meanwhile these difficulties, the clear water larval rearing technique presents several advantages in simplifying the tank manadgment, reducing the rearing volumes and increasing the growth speed of the larvae and is recommanded for the futur. Many attemps have nevertheless to be carried-out before providing an appropriate feasible seed production technique, adapted to the local conditions.

The strong mortalities observed from day 12 to 14 when the larvae were issued of local brooders were difficults to explain. The symptoms of depigmentation and over-inflation of the swimbladder were very similar those previously described by Mac Kinnon (1986) and Rimmer et *al* (1989) in Australia but, in our conditions, no clear relation to the former techniques or to nutritionnal deficiencies of preys was noticed.

It has been verified that the larvae, imported from singapore and reared exactly in the same conditions in clear and green water did'nt show any symptoms of mortality (survival > 50 to 60 % on day 25) in both case which suggest that the sanitary quality and the origin of the larvae in relation to the manadgment of brooders has a major importance.

Research efforts are actually focused on :

- Sanitary conditions of brooders and eggs because some authors (Owens, pers. comm.) suspect a viral disease to be responsible for seabass larvae mortalities in Australia.
- Managment of broodstock in relation with food quality and optimisation of environmental conditions.
- Quality of live preys with the use of efficient enriched products like Frippak booster, recommanded by Rimmer et *al* (1989) to limit mortalities during the *Artemia* feeding stage.
- General managment of larval rearing environment with the use of biofilter in close system which allows to stabilize the chemical and bacterial containt of rearing water.

Weaning Seabass larvae from early stage with microparticulated diets or starter diet (56 CP, 16 CL) after an overlap of several days on live or frozen *Artemia* has been demonstrated to be feasible. Encouraging results have already been obtained in adapting to *Lates calcarifer*, the knowledges on European Seabass and Seabream (Chatain, 1986; Rozani-Cerqueira, 1986; Person-Le-Ruyet, 1986-1989).

Seabass larvae are able to accepte dry pelleted particules after a few days and survival and growth after 15 to 20 days are not drastically decreased, compared to the control fish, fed on lived or frozen *Artemia* if a certain number of conditions are respected :

- In order to homogenize the population and limit the size dispersion,, a grading of larvae is recommanded before starting the weaning.
- Selection of savety larvae issued of successfull larval rearing give a higher survival and growth potential and a lower dispersion.
- An overlap from live to frozen *Artemia* in 10 days is recommanded when larvae are weaned on starter diet to facilitate the adaptation of fry to the new rearing conditions and to the feeding regime. Preliminary trials conducted with high quality and attractive microparticulate diets suggest that this overlap could probably be reduced with the improvement of the diets.
- Continuous distribution of particules with automatic feeder is also an important factor in providing diet during the all days. Traditional feeding regime, proposed by Maneewong et *al* (1986), with fine trash fish meat, distributed 2 to 3 times per day induce strong dispersion and poor final survival (20 to 30 % in general).
- Frequent grading, provided every 10 to 15 days is also recommanded although constant improvement of diet and water management would probably reduce dispersion and cannibalistic behaviour.

In conclusion, the futur of seed production of *Lates calcarifer* seems to be encouraging but the problems encountered in these very preliminary trials suggest that the technique need several adjustments and improvements to be feasible and to allow the development of a new aquaculture activity of this very promising species in French Polynesia.

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Problems related to the lack of functional swimbladder in intensive rearing of *Dicentrarchus labrax* and *Sparus auratus.*

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Abstract. — *The primordial inflation of swimbladder normally occurs at a size of 4 to 5 mm in sea bream Sparus auratus and 5 to 6 mm in sea bass* **Dicentrarchus labrax.** *However, in intensive larval rearing conditions, lack of inflation is often observed and leads to the production of up to 80 % of postlarvae whose swimbladder is not functional. They are, on the average, 20-30 % smaller in weight than normal fry. Their survival rate can also be reduced by any kind of stress such as weaning, handling or hypoxic conditions. It is suggested that the selective mortality which affects the abnormal fish results both from a decrease in predatory efficiency and an increase in energetic needs. The lack of a functional swimbladder in larvae will also lead to lordosis in older fish. This skeletal deformity appears at a size of roughly 20 mm and the lordosis angle increases afterwards. It is hypothesized that lordosis appears because those fish which cannot modulate their density, are continuously swimming in an oblique position to avoid sinking.*

Primordial inflation can be artificially inhibited by preventing the larvae from reaching the water surface, suggesting that air gulping is necessary to realize it. Systems which eliminate, from the surface, the naturally occuring oily film, significantly improve the inflation rate in reared sea bass and sea bream populations.

INTRODUCTION

Strong interest has been raised in recent years concerning the swimbladder of Teleosteans. This organ, which develops from the dorsal wall of the digestive tract, plays an important role in hydrostatic regulation, perception and sound production and respiration (for a detailed review see Harden-Jones, 1957; Steen, 1970; Love, 1981). Its morphology and physiology is well known in adult fish but little information is available on its post-embryonic development, especially in reared *Serranidae* and *Sparidae.*

The swimbladder primary inflation of the Japanese sea bream *Pagrus major* was studied by Yamashita (1966, 1982), Takashima et *al.* (1980), Kitajima et *al.* (1981) and Chatain (1982). Beside the morphological and biometrical aspects of the development, these authors also gave some indications on the relationship between the lack of inflation and fish development anomalies, in particular skeletal deformities. Doroshev and Cornacchia (1979), Bulak and Heidinger (1980), Doroshev et *al.* (1981) discussed similar results for the american sea bass *Morone saxatilis*, Giavenni and Doimi (1983), Weppe and Bonami (1983) and Johnson and Katavic (1984) for the european sea bass *Dicentrarchus labrax* and Paperna (1978) and Weppe and Bonami (1983) for the common sea bream *Sparus auratus*.

In 1982, larval rearing of *Dicentrarchus labrax* and *Sparus auratus* produced, in France, roughly 400 000 fry of which 70 to 95 % presented swimbladder anomalies. Research undertaken by IFREMER to significantly improve the larval rearing techniques of these two species led to the production, in 1988 of 6 millions normal fry. The aim of the present paper is to summarize the main steps of this research.

MORPHOLOGICAL ASPECTS OF THE DEVELOPMENT OF THE SWIMBLADDER

In sea bass and sea bream the primordial swimbladder can be observed *in vivo* by transparency as early as the third day after hatching. In both species, normal swimbladder development, from the initial vesicle, is characterized by two stages.

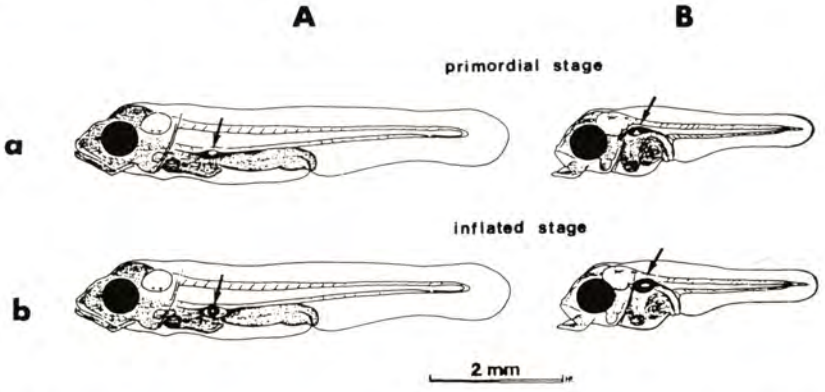

Fig. 1. — The two stages of the swimbladder (arrow) in (A) *Dicentrarchus labrax* and (B) *Sparus auratus* (a) prior and (b) after inflation (from Chatain, 1986).

The first stage, called « initial inflation », is characterized by the apparition of a light refractive bubble in the initial vesicle (Fig. 1). It occurs in 5 mm sea bass larvae (around 7 days old) and 4 mm sea bream larvae (around 5 days old). Inflation coincides with oil globule resorption (Fig. 2). When initial inflation fails, the swimbladder development is stopped at a

stage ressembling that prior to inflation and is not functional. Its size is always inferior to the size of the inflated swimbladder.

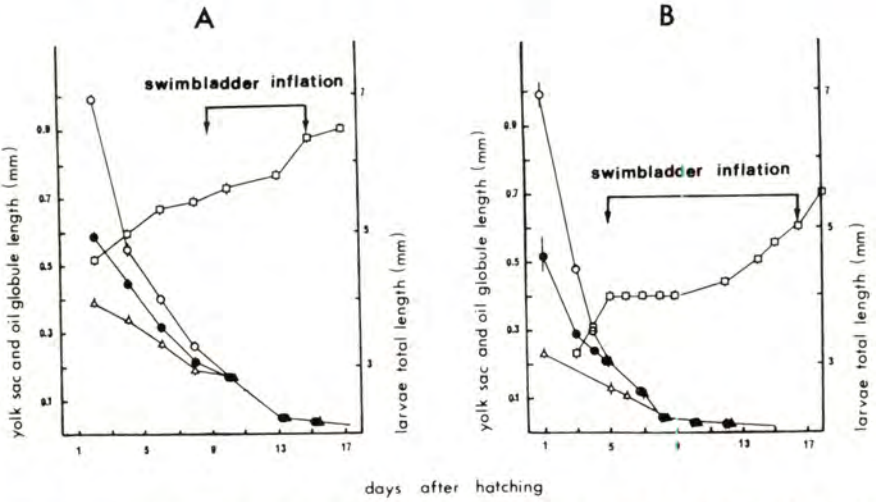

Fig. 2. — Resorption of the yolk sac [length (○) : width (●)] of the oil globule (△) and growth in length of larvae (□) of (A) *Dicentrarchus labrax* and (B) *Sparus auratus*. Mean values are represented with their confidence interval (α = 5 %) (from Chatain, 1986).

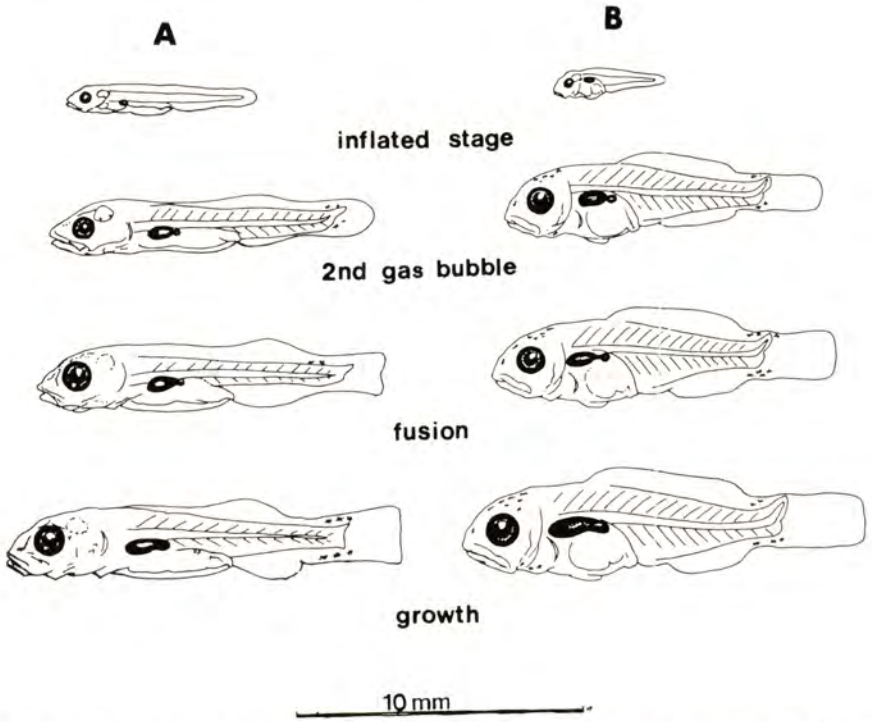

Fig. 3. — The different stages of the swimbladder expansion in (A) *Dicentrarchus labrax* and (B) *Sparus auratus* (from Chatain, 1986).

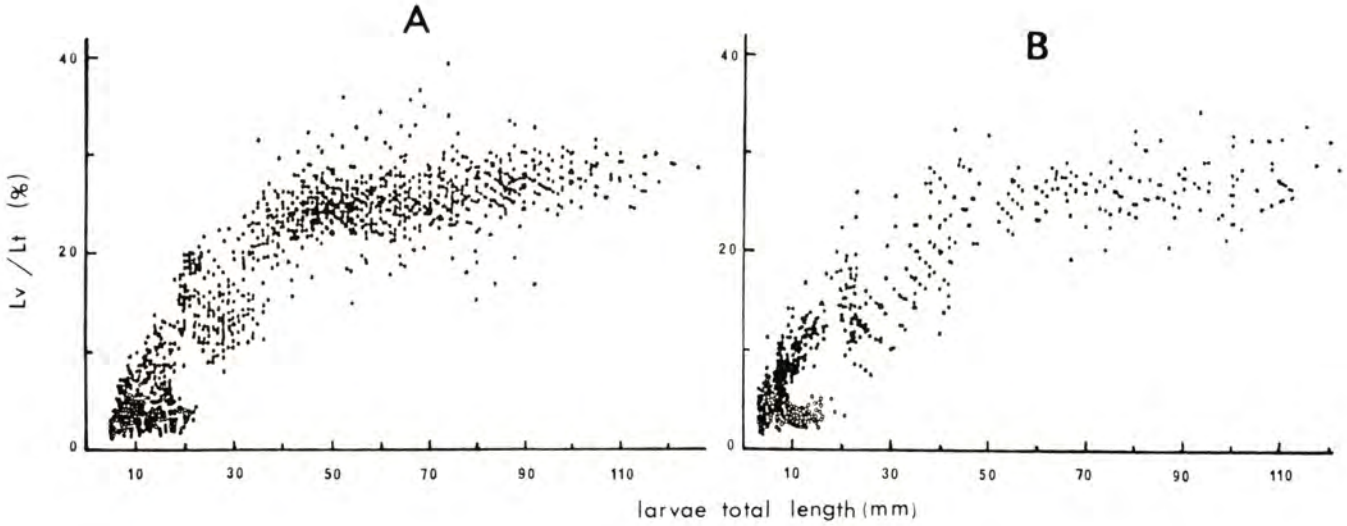

Fig. 4. — Evolution of the ratio of swimbladder length (Lv) to fish total length (Lt) versus growth in (A) *Dicentrarchus labrax* and (B) *Sparus auratus*. Individuals with (●) or without (○ a functional swimbladder (from Chatain, 1986).

The second stage corresponds to an expansion phenomenon. In larvae greater than 12 mm long, the swimbladder looks like an ellipsoidal vesicle which progressively stretches backwards during growth (Fig. 3). It becomes stable in 40-50 mm fish reaching 20 to 30 % of the total length (Fig. 4). Among larvae which fail to inflate, the swimbladder development stops and the bladder never exceeds 3-5 % of the fish length.

INFLUENCE OF THE SWIMBLADDER DEVELOPMENT ANOMALIES ON LARVAL GROWTH

In intensive larval rearing conditions, lack of inflation is often observed and leads to the production of up to 80 % of postlarvae whose swimbladder is not functional. It was showed that such postlarvae were smaller in size and weight than normal ones (Fig. 5).

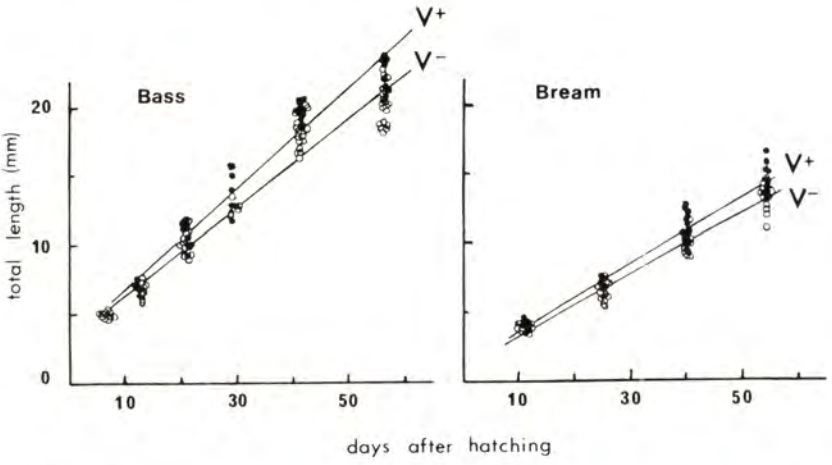

Fig. 5. — Growth in length of *Dicentrarchus labrax* and *Sparus auratus* larvae with (●,V+) or without (○,V-) a functional swimbladder (from Chatain, 1987).

The growth delay in larvae without a functional swimbladder can be observed at the age of 16 days in sea bream and 30 days in sea bass. At 60 days, the end of larval rearing, larvae in which the swimbladder did not develop normally have a body weight 23-33 % less than normal. How can we explain such an important difference in growth ?

Competition for food seems unlikely because in intensive rearing conditions, larvae are always fed in excess. The growth difference most probably lies in the higher energetic needs of abnormal larvae which present buoyancy anomalies. Such anomalies affect the swimming behaviour of the larvae as soon as the yolk sac resorbtion is completed. The development of a functional swimbladder provides the capability for hydrostatic regulation and thus the ability to overcome increasing specific gravity. The achievement of neutral buoyancy probably reduces the energetic cost of swimming and improves predatory efficiency.

INFLUENCE OF THE SWIWBLADDER DEVELOPMENT ANOMALIES ON LARVAL REARING

The larvae which have no functional swimbladder are viable but show a strongly reduced resistance to any kind of stress like handling, hypoxic conditions or weaning. In the case of weaning, the survival rate could be directly related to the proportion of normal fish initially present in the group (Fig. 6).

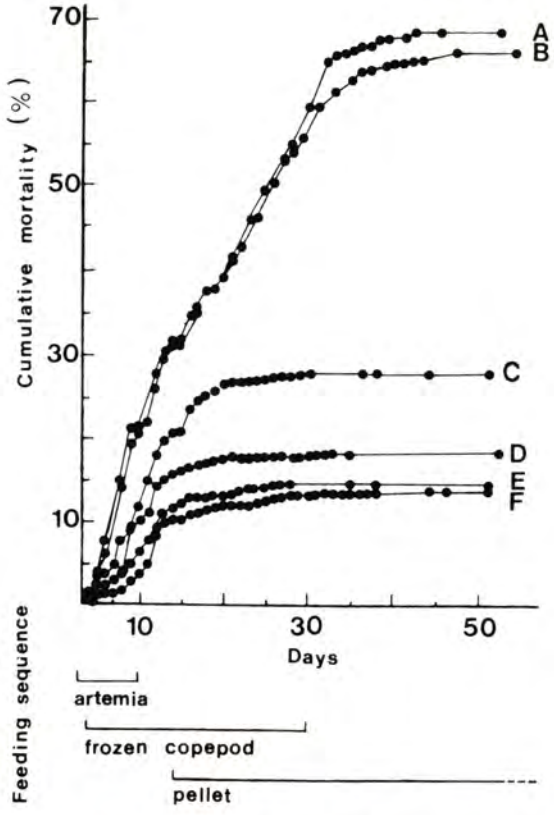

Fig. 6. — Weaning mortality in 6 groups of seabass, *Dicentrarchus labrax*, presenting, at begining of experiment, different functional swimbladder proportions : group A : 1 %; group B : 1 %; group C : 51 %; group D : 61 %; group E : 80 %; group F : 82 % (from Chatain and Dewavrin, *Aquaculture*, in press).

The radiography of dead fry revealed that 86 to 100 % of these animals had no functional swimbladder (Fig. 7). It is again suggested that the observed selective mortality results both from a decrease of predatory efficiency and higher energetic needs in abnormal fry.

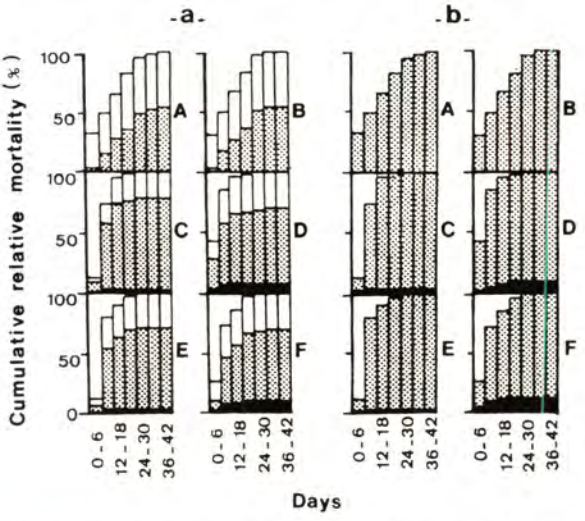

Fig. 7. — Relative cumulative mortality during the weaning of *Dicentrarchus labrax* fry with (■) and without (□ ...) functional swimbladder □ : fry whose state does not allow classification. a - gross results; b - corrected results by inclusion of undetermined fish in the two others categories (from Chatain and Dewavrin, *Aquaculture*, in press).

RELATIONSHIP BETWEEN UNINFLATED SWIWBLADDER AND LORDOTIC DEFORMITIES

In mass rearing conditions, various types of deformations commonly occur but skeletal abnormalities and especially the V shaped lordosis, are the most important (Fig. 8).

In lordotic individuals, two or three vertebrae are deformed at the curving point of the spine. They are always located behind the digestive mass. Such a skeletal deformity appears on larvae measuring roughly 20 mm both in sea bass and sea bream. At this size, the lordosis angle is small. The occurence and the angle of the lordosis will increase as the fish grow.

It was found that there was a close relationship between the occurence of lordosis and the rate of uninflated swimbladder. It is hypothesized that lordosis appears because the fish which cannot modulate their density are continuously swimming in an oblique position to avoid sinking.

IMPROVEMENT OF THE PRIMARY INFLATION RATE OF THE SWIWBLADDER

Primordial inflation can be artificially inhibited by preventing the larvae from reaching the water surface (covering the water surface with a liquid paraffin layer) suggesting that air gulping is necessary to realize it.

The low inflation rates (< 30 %) observed to date in those species under intensive rearing conditions, could therefore eventually be related to the presence of a superficial oily film which usually develops from the feed. Three systems intended to remove this natural layer were tested (Fig. 9) : a hydrojet (a) and a sprinker (b), connected to the sea water arrival, which concentrated the oily layer on the tank walls and a blower (c and d) concentrating the film in a floating trap.

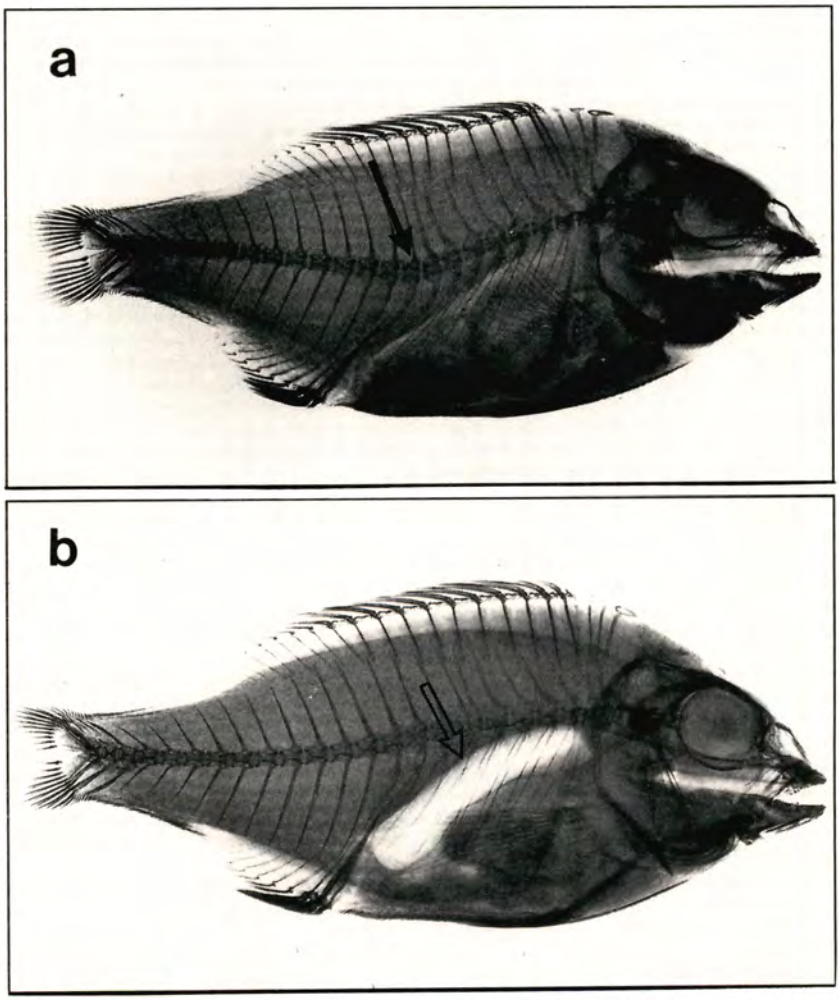

Fig. 8. — - a - sea bream juvenile without a functional swimbladder and with a lordosis (→); - b - normal juvenile with a functional swimbladder (⇒) (from Chatain, 1982).

The only system which significantly improves the inflation rate (80 %) without disturbing the growth (Fig. 10) is the blower. The two other systems have either little (sprinkler) or no effect (hydrojets) on the inflation rate (Table 1). Although they are efficient in removing the surface film, they create strong water turbulences and these probably prevent the larvae from reaching the surface in due time.

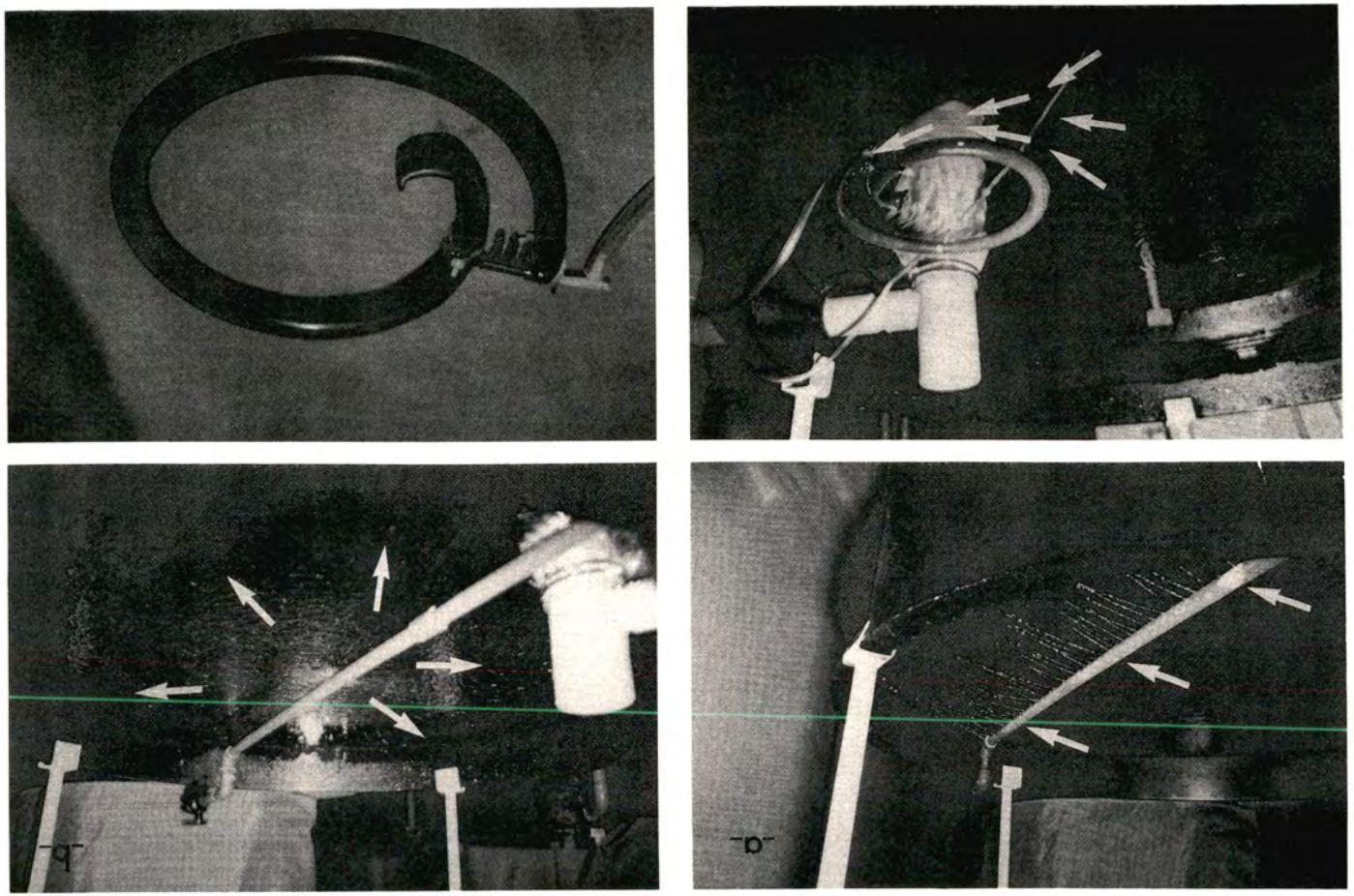

Fig. 9. — The three systems tested to remove the oily superficial layer which develops at the water surface of the rearing tanks. a : hydrojets; b : sprinkler; c and d : blower.
→ Movement of the superficial layer (from Chatain and Ounais-Guschemann, submitted to *Aquaculture*).

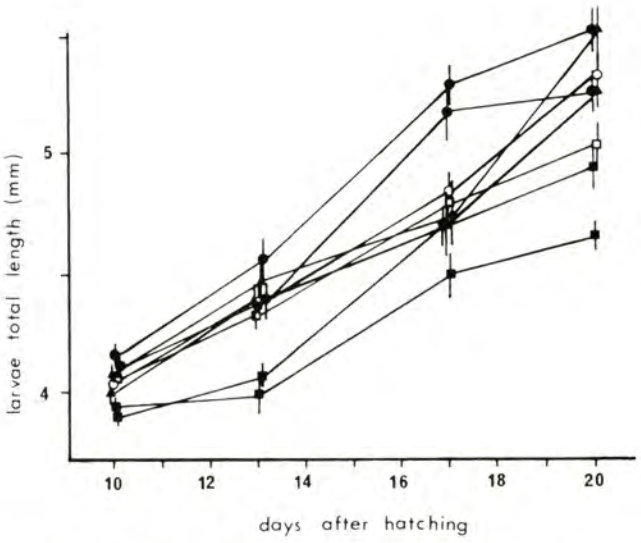

Fig. 10. — Growth in length of *Sparus auratus* larvae, within groups differing by the water surface treatment. Surface cleaned by hydrojets (■); sprinker (▲); blower (●); surface sealed by a liquid paraffin layer (□); control with uncleaned surface (○). The mean values are represented with their confidence interval (α = 5 %) (from Chatain and Ounais-Guschemann, submitted to *Aquaculture*).

Table 1. Comparison between the functional swimbladder rate (V+) obtained, 20 days after hatching, in tanks whose surface was cleaned and in the uncleaned control. U test, standard error method with angular transformation (Dagnelie, 1975). NS : not significant; * : significant at the 5 % level; ** : significant at the 1 % level (from Chatain and Ounais-Guschemann, submitted to *Aquaculture*).

CLEANING SYSTEM	HYDROJET		SPRINKLER		BLOWER		CONTROL
REPETITION	1	2	1	2	1	2	1
V+ (%)	0	13	36	44	80	81	28
U test	2.341*	2.254*	0.969NS	1.870NS	5.682**	5.769**	—

By the use of this simple system, which greatly enhanced the swimbladder inflation rate, it was possible to significantly improve the growth and survival rates of the two species and to suppress the occurence of lordosis.

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IV. FINFISH

IV.4. NURSING AND GROW-OUT

ADVANCES IN TROPICAL AQUACULTURE Tahiti, Feb. 20 - March 4, 1989
AQUACOP IFREMER Actes de Colloque 9 pp. 713-727

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Status and potential of Australian *Lates calcarifer* culture

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Abstract — ***Lates calcarifer*** *is distributed widely in northern Australia and is important to both commercial and recreational fisheries. Interest in culture of the species is nationwide but most culture activity to date has been in Queensland.*

Queensland Department of Primary Industries commenced research on hatchery production of the species in 1984 and this work is continuing. Hatchery reared fish have been stocked to reservoirs in northern Queensland and have been used in experimental grow-out trials.

Fish weaned onto formulated diets by the age of 25 days were reared to marketable size (> 500 g) in freshwater tanks. Despite suboptimal water temperatures the fish reached market size by the age of twelve months. An overall food conversion ratio of 1.1 :1 was obtained during grow-out. Taste panel analysis showed that the pellet fed fish were of good quality.

Fish stocked to reservoirs grew rapidly demonstrating growth potential of the species under favourable conditions. Mean weights in excess of 450 g were attained by six month old fish and in one reservoir year old fish averaged approximately 2.5 kg. The sensitivity of growth rates to water temperature was demonstrated in both grow-out trials and reservoir stockings.

Fish bred from geographically separated broodstocks exhibited differences in growth rates when stocked to two reservoirs with similar temperature regimes. It is suggested that these differences may be genetically influenced. Electrophoretic studies indicate the presence of a large number of distinct stocks of ***L. calcarifer*** *in Australia, raising a number of important issues for the culture industry.*

Large scale commercial grow-out is presently limited to one company which produced its first sizeable harvest estimated at 20 tonnes in 1988. The fish were reared in saltwater cages on a pellet diet. The company operates its own hatchery and has supplied fingerling fish to other pilot-scale farming operations.

Other commercial grow-out operations in the planning or pilot stages include recirculating freshwater culture systems and culture in geothermally heated freshwater. An angler pay fishing facility is also in operation.

INTRODUCTION

The barramundi, *Lates calcarifer* (Bloch), is one of the most highly regarded sport and table fish in Australia. It inhabits rivers and inshore waters over roughly 8000 km of coastline in Queensland, Northern Territory and Western Australia and is important to commercial and recreational fisheries throughout this range.

Barramundi catch by the commercial sector was about 1000 tonnes (live weight) per annum in the five years to 1983 (Grey 1987). At 1987 prices the return to fishermen would have been around $A 3.5 million per annum.

Estimates of nationwide recreational catch are lacking but angler catches account for about 30 % by weight of barramundi landed in the Northern Territory (Griffin 1989). Data from Australian Bureau of Statistics (1986) suggest that over a one year period 200000 individual anglers fished Queensland barramundi waters, most of them repeatedly. Other species are also sought in these waters but the barramundi catch by these anglers is obviously substantial (about 100 tonnes if 20 % of anglers caught one legal sized fish). Expenditure on barramundi angling probably exceeds the value of the commercial fishery several fold. Direct spending on recreational fishing in the Northern Territory, most of which is directed at barramundi, has been estimated at $A47 million per annum (Cam and Ross, 1986).

Declining catches and significant stock reductions in many areas have prompted increased research effort since the mid 1970's as summarized by Griffin (1987). Increased knowledge of barramundi biology, the desire to conserve wild populations, and the importance of recreational fisheries have directed barramundi culture research into areas not addressed in other countries farming *L. calcarifer.*

The first recorded attempts to culture Australian barramundi were pond trials with wild caught juveniles (Anon, 1955). The ponds were located in south Queensland outside the distribution of barramundi and cool winter temperatures apparently resulted in mortalities. Maclean (1972) considered commercial culture would be feasible if adequate supplies of fry and cheap food were available, and advocated hatchery breeding for conservation as well as farming. Plans for hatchery production were announced in the mid 1970's by a company which sought to stock Lake Argyle, a large reservoir in the north of Western Australia with barramundi in exchange for commercial fishing rights (Anon, 1975, 1976). The scheme did not eventuate however and the next published information came from aquarium rearing of wild caught juveniles (House, 1979).

Interest in barramundi aquaculture intensified in the early 1980's helped by reports from Australian workers of successful hatchery production in Thailand (Barlow, 1981; MacKinnon, 1983). In addition to commercial grow-out there was great interest in hatchery breeding for stocking natural and impounded waters, especially in Queensland where many dams and weirs prevent upstream migration of barramundi causing them to disappear from large areas of former habitat.

HATCHERY PRODUCTION

From 1984 to 1986 the Queensland Department of Primary Industries (Q.D.P.I.) undertook a pilot hatchery project (MacKinnon, 1987a).

Eggs and milt were obtained from ripe wild fish and by hormone induction of freshly captured fish belonging to sexually precocious stocks in the Embley River estuary near Weipa, Far North Queensland. The fish spawned on discrete localized grounds after full moon and new moon as do Southeast Asian *L. calcarifer* but spawning grounds were further upstream than in Southeast Asia reflecting different estuarine salinity regimes.

Fertilized eggs were transported by air to Cairns for transfer to the Northern Fisheries Research Centre. Larvae were hatched and reared in circular fibreglass tanks of 1.2 t capacity, described in detail by Russell et *al.* (1987). Up to 2 weeks from hatching rearing procedures were based on those used in Thailand (Chomdej, 1986). Salinity ranged from 30-34 ppt. and temperature from 26-27.5°C.

Larval development corresponded closely to that of Thai fish as described by Kosutarak and Watanabe (1984), and larval growth rate (mean length approximately 10 mm at 20 days old) equalled the fastest recorded in Thai experiments (Maneewong et *al.*, 1986).

A condition which became manifest at 12 to 14 days of age caused near total mortality in several batches of fish. First symptoms included pale coloration and abnormal swimming behaviour. The only survivors in the first trials of 1984 were a few fish transferred to fresh water soon after the mortalities began. Subsequent research indicates that this condition could result from nutritional deficiencies (Rodgers and Barlow, 1987; Rimmer et *al.*, 1988, Rimmer, 1989), while other recent evidence suggests a virus may have been implicated (L. Owens, pers. comm.).

From about 14 days after hatching, a variety of methods were used to rear the fish through to the juvenile stage. Some larvae were reared further in salt water while others transferred to fresh water at Walkamin Research Station were reared in tanks or earth ponds.

Three batches of fish were placed in separate fertilized 0.1 ha earthen fry ponds. One batch of larvae, all apparently healthy, was stocked at 16 days old (8-10 mm length), and 50 % survived the pond rearing period of 25 days. Growth was rapid and mean length of the fish at harvest was 50 mm. The other two ponds were stocked with 17 day old fish some of which showed symptoms of the nutritional disease mentioned above. Survival to harvest (20 % and 28 %) in these two ponds was lower than in the first pond but growth rate was similar, the mean length being 50 mm on harvesting at 45 days old. These results supported the notion that in Australian conditions pond rearing of advanced larvae might be as cost effective as intensive tank culture through to fingerling stage (MacKinnon, 1983).

Two batches of 15 day old fish were placed in fresh water tanks and fed pond cultured zooplankton and Artemia nauplii. Weaning onto dry formulated diets started at 20 days old and was completed by about

25 days old when the mean length of the fish was 15 mm. Conversion to pellet food at this early stage provided an alternative to the minced fish diet used in Thai hatcheries. Feeding minced fish flesh is labour intensive and carries risks of pathogen introduction, nutritional problems, and pollution of rearing tanks. These problems are minimized by feeding dry pellets.

Investigations to refine freshwater pond rearing techniques for advanced larvae are continuing. It has been found that behaviour of barramundi stocked to ponds at T.L. ì 10 mm makes them susceptible to predation by dragonfly larvae. Survival in freshwater ponds can be increased substantially by delaying stocking until fish are ì 17 mm T.L. Recent pond rearing trials have exhibited survival of over 90 % (C.Barlow, pers. comm). Other continuing Q.D.P.I. hatchery research includes larval nutrition studies (Rimmer, 1989) and induced spawning trials (Garrett and Rasmussen, 1987).

A private company, Sea Hatcheries Ltd., received government funding to investigate hatchery production and grow-out of barramundi during the period 1983-1986. Following this pilot research the company was listed on the stock market in 1986 and in 1987 it relocated from Cairns to a new complex at Mourilyan Harbour about 100 km south. During its early years of operation Sea Hatcheries obtained eggs stripping ripe wild fish and hormone induction of captive fish but recently there have also been natural spawnings of captive fish held in 100 tonne tanks. Larvae are reared in recirculating systems. Early production runs in the new hatchery suffered high mortalities which were attributed to metal toxicities. These problems were apparently overcome however periodic mortalities have continued some of which appear to be similar to mortalities experienced in QDPI hatchery research. Despite these problems Sea Hatcheries produced 220000 fingerlings in the 1987/88 summer and in the current summer it had produced more than 400000 fingerlings (D. Hallam, pers. comm.) before severe mortality problems recurred in the hatchery.

Two smaller private hatcheries have also bred barramundi recently. One of these located near Cairns produced some 2700 fingerlings, obtaining its eggs from ripe wild fish. The other, located near Bundaberg in southern Queensland, reportedly produced in excess of 100000 fingerlings through hormone induction of wild fish, initial rearing in tanks and transfer of larvae to ponds until fingerling size was attained. Seven hatchery permits exist for barramundi production in Queensland (R. Quinn, pers. comm).

Outside Queensland, the Western Australian Department of Fisheries has conducted trials on maturation of tank held fish at Wyndham in the north of that state (Morrissy, 1987) and the Northern Territory Department of Fisheries recently completed a successful nursery trial using larvae supplied by Q.D.P.I. (N. Sammy, pers. comm).

FEEDING TRIALS

Q.D.P.I. pilot hatchery research included several small scale tank trials on the culture of barramundi using dry pelleted feeds. The trials investigated effects of food composition, feeding regimes, salinity and temperature on growth and food conversion (MacKinnon, 1987, MacKinnon et *al.*, 1987 : Tucker et al., 1988, D.J. Russel pers. comm.). Barramundi weaned onto dry formulated feeds as young as 25 days old and grow out in fresh water showed excellent food conversion (FCR 1.1 :1) and reached a mean weight of 566 g at 12 months old. An expert taste panel evaluated pellet fed fish using descriptive criteria similar to those of Tucker et *al.* (1985). The panel rated overall acceptability of the fish at 7.8 on a 9-point hedonic scale, clearly indicating a high quality product suitable for the restaurant trade.

One trial carried out in fresh water compared two diets both of which contained 27 % anchovy meal and approximately 53 % crude protein but with different levels of fat (9.3 % and 12.9 %). Differences in growth rate between the two groups were nonsignificant but significantly better food conversion (FCR 0.93 :1). was obtained with the higher fat diet than with the lower fat diet (FCR 1.01 :1).

Another trial carried out in salt water compared six experimental diets all of which contained approximately 50 % crude protein but which incorporated different proportions (20, 40 or 60 %) of anchovy meal. While the best food conversion (FCR 0.89 :1) was obtained with the diet containing the most fish meal (60 %) and fat (16.9 %), a food containing only 20 % fish meal and 13.4 % fat gave results which were almost as good (FCR 1.04 :1).

The results of these two trials suggested that diets containing > 20 % high quality fish meal, 48-54 % protein, 13 % fat and 10-16 % carbohydrate will produce good growth and food conversion in juvenile barramundi larger than about 10 g.

A trial to investigate the effects of feeding frequency on growth and food conversion was carried out in fresh water tanks. Experimental fish were fed to satiation with a commercial salmon starter formula either once per day or twice per day for 42 days. There was little difference in growth rate between treatments but food conversion was more efficient and food consumption less in fish fed once daily.

On completion of the feeding frequency trial, 30 fishes were retained in freshwater tanks and fed on commercial salmon starter until they were approximately one year old. At the start of this period the fish were 181 days old (mean weight 160 g) and on conclusion 369 days old (mean weight 566 g). Mean water temperature in the trial tanks during this period was 25°C. Over the whole grow-out period average FCR was 1.13 : 1.This suggested that in commercial grow-out conditions in fresh water FCR of about 1.5 :1 could be achieved.

Growth and food conversion of fingerling fish in fresh water and salt water at three different temperatures (22°, 27°, 32°) were also trialled (D.J. Russell com. pers). There was no significant difference in growth rate between fresh water and salt water but marked differences with temperature. Daily growth rate was fastest at 32°C (5.1 %) but the greatest difference was between 27°C (4.5 %) and 22°C (2.2 %). Food conversion was best at 27°C (FCR 1.02 :1). Considering the relatively low temperatures at which the Walkamin feeding trials were carried out, the growth rates compare favourably with those of pellet feeding trials elsewhere (Chou, 1984; Fuchs, 1986) and with growth of fish on a trash fish diet (Sakaras, 1984). Growth at 25°C allowed a complete crop cycle within a year but grow-out time might be considerably reduced at higher temperatures. Effects of temperature on growth are discussed further in subsequent sections.

COMMERCIAL GROW-OUT

Virtually all barramundi grow-out operations at the present time are located in Queensland, the sole exception being a scheme to culture the species in geothermally heated water at Portland in Victoria. This operation lost its initial crop at an early age. In Queensland 13 grow-out operators hold permits endorsed for barramundi production and 8 of these list barramundi as the sole or prime culture species. Not all the endorsed permit holders are presently culturing barramundi (R. Quinn, pers. comm.).

To the present time only one company, Sea Hatcheries, is marketing farmed barramundi in any quantity. The first large harvest of plate sized fish (400-450 g) was made during 1988 and this crop was still being harvested in early February 1989. So far more than 25 tonnes i.e 60000 fish have been harvested. The company states that 180000 fish were transferred from it's hatchery to the grow-out site during the 1987/88 summer indicating a survival over the grow-out period in the order of 35 %. The company is confident that improved cage management will increase survival in future crops (D. Hallam, pers. comm). The cage grow-out section of the companies operations is located in a tidal mangrove creek system 6-8 m deep near Cardwell some 80 km south of the hatchery facility. The cage farm cost $400000 and has been designed to cater for a production level of 300 tonnes per annum. Twelve people are employed in this section of the company's operations. Mean grow-out time to market size (400-500 g) is about 10 months and production cost is around $A5/kg, more than 30 % of which represents feed costs (FCR $\approx$ 2 :1 and the pellet feed costs the company around $1/kg.).

Several small operators have made trial stockings of barramundi fingerlings purchased from Sea Hatcheries however there are few indications that any of these operations have yet reached a commercial scale. At least one pond grow-out operation is operating as a fishout facility for tourist anglers however this facility in common with several small scale pond culturists has suffered substantial mortalities some of which have been due to protozoan infections and others which are of uncertain causes.

An operation which is currently commencing pilot scale production at Buderim in southern Queensland has converted a former ginger processing factory to raise barramundi in recirculated freshwater tanks heated to 28-30°C. The company plans to produce two crops per year for a total annual production of 80 tonnes. The plant is primarily intended as a tourist venture and it is planned that most of the production will be marketed on site (A. Cockerell, pers. comm.).

RESERVOIR STOCKINGS

Some 14000 barramundi (45 days old, mean length 50 mm) produced by the Q.D.P.I. pilot hatchery project were stocked to Lake Tinaroo (surface area 3300 ha) in late December 1985 (MacKinnon and Cooper, 1987). Extremely rapid growth was exhibited by these fish and by early April 1986 gillnet samples indicated a mean length of 300 mm and mean weight of approximately 390 g. By one year after stocking the fish had reached a mean weight of more than 1.1 kg (despite total cessation of growth during the winter months from June to September) and at three years from stocking the mean weight had increased to 5.2 kg. This growth rate exceeds most growth estimates for *L. calcarifer* in Australia and Papua (Garrett and Russell, 1982; Reynolds and Moore, 1982; Davis and Kirkwood, 1984) and is very much greater than the slow growth rate calculated by Davis (1984) for the parent stock in the Embley River. Only the estimates of Dunstan (1959) exceed the growth rate of the Tinaroo fish. Further stocking of Embley River fish to Lake Tinaroo in 1987 resulted in similar growth rates to the original stocking.

Continuing Q.D.P.I. research has included the stocking of another local storage, Lake Morris, with fish originating from populations in the Cairns area. These fish have exhibited even faster growth rates than the Embley River fish stocked to Lake Tinaroo. Fish stocked to the dam at 2 months old (mean length 58 mm) had reached a mean weight of 5.2 kg (equivalent to 3 years old fish in Lake Tinaroo) at the age of 20 months. Sampling of these fish has been irregular and it remains to be seen whether the difference in growth rates between the two lakes is primarily due to different physico-chemical conditions, genetic differences or a combination of both. At least part of the growth variation probably results from a temperature differential between the lakes (see subsequent section on temperature).

Other stockings of barramundi have been made to the Clare Weir in the Burdekin River system and to the Gunpowder dam near Mt. Isa in northwestern Queensland but no information on the success of these stockings is available.

WATER TEMPERATURE

The latitudinal range of *L. calcarifer* in Australia extends from sub-equatorial conditions at Cape York (10°S) to the southernmost limit of the species in the sub-tropics at about 26°S. This range covers a wide

variety of seasonal temperature regimes which have important effects on survival, growth and reproduction. Temperature is not a limiting factor for *L. calcarifer* culture in equatorial areas but it is of prime importance to Australian culture. In spite of this importance little information exists on barramundi temperature requirements.

The effects of temperature on growth of juvenile barramundi are clearly demonstrated by Q.D.P.I. tank rearing trials. Fish held at 22°C grew at an average daily rate of 2.1 % while fish held at 27°C grew at an average daily rate of 4.5 %. (D.J. Russel, pers. comm.). These results suggest that at least part of the growth differential between fish in Lake Tinaroo and fish in Lake Morris is due to temperature effects. The two lakes are at slightly different altitudes and data from monthly water temperature sampling suggests that mean annual surface water temperatures in Lake Tinaroo ($\approx$ 25°C) are approximately 1°C cooler than those of Lake Morris ($\approx$ 26°). The tank trials with small fish together with various observations on captive fish of all sizes indicate that a 1°C temperature differential over prolonged periods could produce significant size differences in fish.

House (1979) reared Western Australian barramundi in an aquarium at 27-28°C and recorded weights approaching 10 kilos at four years old. He reported that fish were disinterested in feeding when temperatures were allowed to fall to 24°C. Fish from stocks near the southern limit of distribution were held in tanks at Q.D.P.I. Southern Fisheries Research Centre. They fed little during winter when temperatures sometimes fell to 19°C. but fed enthusiastically at temperatures at around 25°C (J. Burke, pers. comm.). Specimens from the Cairns area held in ponds at Q.D.P.I. Walkamin Research Station became very inactive during winter months when mean monthly surface water temperatures fell to 18-20°C. Embley river fish reared in freshwater tanks at around 25°C continued to grow throughout the winter but day to day variation in food consumption was obvious with minor temperature fluctuations.

Marked seasonal variation of growth rate has been noted in wild barramundi populations. Dunstan (1959) indicated a slower growth during winter months in Queensland east coast stocks and Davis and Kirkwood (1984) showed that growth of age 0+ fish was very seasonal in the Gulf of Carpentaria and Northern Territory waters. Most growth took place during summer months and seasonal growth variation was most marked in the areas with lowest winter water temperatures. Barramundi stocked to Lake Tinaroo showed very clear seasonal growth variation. Growth ceased completely between June and September when surface water temperatures in the storage ranged between 20°C-23.5°C. Fastest growth was from October to February when water temperatures were 27°-30°C.

Fragmentary observations such as those above suggest that barramundi from several stocks and over a range of sizes show similar growth responses to temperature. Below about 20-22°C little or no growth occurs and feeding is greatly reduced. A great increase in general activity and feeding with each degree of increase is noticeable at temperatures around 25°C and optimal temperatures for growth and food conversion lie above this level, probably in the range 27-30°C.

Although the general relationship described above probably holds true for most stocks there may be minor latitudinal shifts in the response to temperature. Studies of wild populations have not detected markedly slower growth rates in stocks near the southern end of the range (D.J. Russell, pers. comm.). Published information on water temperatures at the southern end of distribution is lacking but data from other sites in Queensland shows close correlation between mean monthly temperatures of inshore/estuarine surface waters and mean monthly air temperatures. Air temperatures at southern Queensland coastal centres suggest that if the southernmost stocks of barramundi had growth/temperature responses identical to those of fish stocked to Lake Tinaroo then they would only grow rapidly for 1-2 months of the year and would show little or no growth for 6-7 months. If this was true, slow growth in comparison to northern stocks would almost certainly have been detected in field studies.

Dunstan (1959) suggested that the latitudinal limits to distribution are probably determined by temperature, and mentions that minimum sea temperatures are in the vicinity of 21°C at both northern and southern extremities of the species distribution. This temperature probably has little direct relevance to survival of barramundi and is well in excess of the critical minimum temperature for survival of the species which Dunstan suggests may be in the vicinity of 15.5°C. Mortalities of wild barramundi during spells of cold weather have been reported from as far north as the tropic of Capricorn. Fish held in ponds at Walkamin Research Station have survived water temperatures as low as 13°C overnight however when these fish were handled at 16°C severe stress was obvious in all specimens and some deaths resulted. These observations suggest that Dunstan's estimate of the critical thermal minimum for survival over extended periods is reasonably accurate though perhaps slightly high.

Temperature requirements for breeding may be just as important as ability to withstand winter temperatures in determining the limit of distribution. Differences in the timing and duration of spawning are apparent throughout the latitudinal distribution of barramundi. Near the equator spawning of captive broodstock may occur throughout the year (Hussin Mat Ali, 1987) and at Songkhla in Thailand (7°N) spawning occurs for at least half of the year at water temperatures of 28-34°C (Maneewongsa and Tattanon, 1982). In the Northern Territory at 12°S Davis (1985) found larval barramundi from September to February at water temperatures of 28-35°. At Weipa in northern Cape York peninsula spawning appears to commence in September or October and is mainly finished by late November or early December (Garrett et *al.*, in prep.). Estuarine water temperatures at Weipa were over 27°C throughout the spawning season. In the Cairns/Tully region young larvae were first detected in late October when water temperatures were 26.5-28°C (Russell and Garrett, 1985) although most spawning in this area apparently occurs from December to February. Further south in the Fitzroy estuary Dunstan (1959) identified two spawning peaks in November and January and in the Burrum River very close to the southern limit of distribution spawning appears to take place between late December and February when water temperatures reach 25-27°C (J. Burke, pers. comm.). Based on limited data it seems that water temperatures above 26-27°C may be necessary for spawning of *Lates calcarifer* and it may be this factor rather

than winter water temperatures which determines the southern limit of distribution.

There is a need for formal studies on temperature responses of barramundi. It is clear that even small climatic variations between potential culture sites could have considerable impact on economics of hatchery and grow-out operations. Similarly a shift of only 1°C in growth/temperature responses between populations could have a significant impact on production costs. Studies comparing the temperature responses of genetically isolated stocks from northern and southern ends of the distribution would be especially valuable.

GENETIC CONSIDERATIONS

The existence of many genetically distinct stocks of Australian *Lates calcarifer* (Shaklee and Salini, 1985; Salini and Shaklee, 1987) creates both problems and opportunities for the culture industry. The precise number and extent of the individual discrete stocks is still unknown, much less any differences existing between them which may represent adaptation to their particular environments or differing suitability for culture.

Two possible examples of genetic differences between populations affecting aquaculture potential have been mentioned in preceding sections viz. different growth rates of Embley river fish and Cairns district fish in neighbouring reservoirs which may be due partly to genetic factors, and sexual precocity of stocks in the Weipa area which makes them particularly easy to handle and maintain as captive broodstock.

In order to protect the genetic integrity of the individual wild stocks and to reduce possible risks of reduced viability through mixing stocks, barramundi released to Queensland public waters must be bred from the stocks occuring in the locality. It is not practical to maintain separate captive brood stocks from each area to be stocked and production of fish for stocking public waters usually has involved field operations to obtain ripe fish from spawning grounds and or/near ripe fish for hormone induction. Costs and difficulties of such exercises have been considerable and in some river systems attempts to obtain fertilized eggs so far have been unsuccessful.

The restrictions on source of seed do not apply to the grow-out culture industry. Provided that risks of escape are considered negligible seed material does not necessarily have to be of local origin and most fish grown out to date are descended from stocks in the Weipa area.

GENERAL INDUSTRY PROSPECTS

In marketing terms barramundi is one of the most promising aquaculture candidates for Australian conditions. There is an existing market for the species and a long tradition of high market prices. The species is well known to most Australians and is strongly identified as Australian. Many people are surprised to learn that the species has a wide

distribution in Asia. Ever increasing prices suggest the market is presently undersupplied but it seems unlikely that production from Australian capture fisheries will increase. Because only large fish may be taken by commercial fishermen and because of the remoteness of commercial fishing operations from markets, the fish is currently marketed almost exclusively in the form of frozen fillet. Farmed barramundi would almost certainly be marketed in the form of whole plate sized fish, either chilled or frozen. Such a product will appeal particularly to the restaurant and hotel trade and because of uniform quality and freshness, should enjoy a price advantage over product from the capture fishery. Sea Hatcheries Ltd receives approximately $A15/kg for its current production. Marketing of fish in the whole form allows easy identification of the species and this also improves market appeal as widespread substitution of cheaper product for barramundi fillet has attracted adverse publicity in recent years.

The labour intensive nature of fish culture means that the Australian industry is somewhat vulnerable to competition from imports of frozen barramundi, however the ability to market a fresh chilled product of guaranteed quality will probably continue to allow the Australian product to sell at a premium.

The prospects for export of Australian cultured barramundi do not look as promising. It apparently costs Sea Hatcheries Ltd about $A5 per kg to produce plate sized fish. This is roughly equivalent to the supermarket retail price of similar product in Thailand. The premium price paid for fresh chilled product in Australia would be much harder to obtain on overseas markets where cultured barramundi would have to compete as a frozen product against barramundi produced in Asia, Nile Perch from Africa, and European Sea bass, *Dicentrarchus labrax*. Sea Hatcheries Ltd. considers there are few prospects on Asian markets but there could be opportunities in Europe where taste evaluation of the companies product was recently carried out (D. Hallam, pers. comm.).

The development of dry pellet diets has been a major factor allowing the development of the barramundi grow-out industry in Australia. Problems with cost and continuity of supply would probably preclude any large scale culture based on trash fish feeding. Further trials comparing growth and food conversion in salt and fresh water over the entire grow-out period would be valuable as some published information indicates more rapid growth in freshwater.

The prospects for hatchery production in Australia are good and are not limited to supplying the grow-out culture industry. As mentioned above there are prospects for a major expansion of the recreational fishing industry through the stocking of large public reservoirs. In Queensland alone there are over than 100 large reservoirs with individual capacities in excess of $1m^3 \times 10^6$ (MacKinnon, 1987b) and the majority of these have thermal regimes suitable for survival of barramundi.

In addition to the public pressure for the stocking of reservoirs there has been considerable public pressure for the « restocking » of river systems where barramundi populations are perceived as declining. A number of complex issues have to be considered if such programs are to be effective and they can easily be counterproductive to the conservation

of the naturally reproducing population. Q.D.P.I. is evaluating such stockings as a possible management tool however the current indications are that they should be approached with caution.

There is also potential for sales of fingerlings to owners of numerous private dams. Stocking of these small farm dams forms a significant part of the market for several hatcheries producing native freshwater fish. Judging by the enquiries made to Q.D.P.I. barramundi is a highly favoured species for farm dams.

Differing hatchery techniques might be adopted to produce fingerling fish for different purposes. At present cost savings in production of fingerling fish can be achieved by transferring advanced larvae to ponds and this may well be the best method to provide stock for farm dams, reservoirs and natural waters. The grow-out industry however will presumably require fish of a uniform size which are weaned onto artificial diets and unless methods for weaning and grading pond reared fish are developed most production for grow-out will continue to be by intensive tank culture.

The Australian hatchery industry is protected from possible import competition by quarantine regulations which prohibit the import of live fish except for an approved list of aquarium species. At the present time there seems to be little opportunity for export of hatchery product however the possible future development of *L. calcarifer* strains with superior characteristics for aquaculture could create markets overseas as well as increasing profitability of Australian grow-out operations.

General prospects for future development of Australian barramundi culture are good but experience to date shows that speculators cannot be guaranteed a quick return. Adaptability, patience and close contact between researchers and culturists are essential to realizing the full potential of the industry.

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Grow-out mariculture techniques in tropical waters : a case study of problems and solutions in Hong Kong

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Abstract — *Raft culture is practised and trash fish is being used as feed in Hong Kong. Culture space is limited and most of the culture sites are over-crowded. Food conversion ratio is poor (about 10-15), grow-out mortality is high (30-50 %) and fish kills caused by oxygen depletions, algal blooms and red tides occur frequently. Disease and water pollution are also problems. To solve some of these problems, a computer simulation model has been developed to : (a) determine the optimal stocking density, and (b) to forecast the likelihood of oxygen depletion at culture sites. The use of biological indicators for oxygen depletions and fish kills has also been proven successful. An efficient aeration system has been designed for use in periods of low oxygen values. The possibility of replacing trash fish with artificial feeds is also being studied.*

MARICULTURE PROFILE AND GROW-OUT TECHNIQUES

Marine fish culture has developed in Hong Kong over the past twenty years, and production has increased drastically from 565 tonnes in 1978 to 3000 tonnes in 1988 (valued at US$ 4 & 25 millions respectively) (Fig. 1). The high price of live fish offers great incentive for the development and proliferation of the industry. In 1988, the total mariculture area is 180 ha., and almost all of the 1800 farmers are owner operators dependent upon family workers.

Raft culture is practised in Hong Kong. The raft is built of timber (average size about 180 m2) and is supported by a number of floating units made of empty plastic drums or polystyrofoam floats. Net cages (3 × 3 × 3 m) are hung from the raft and the structure anchored to the

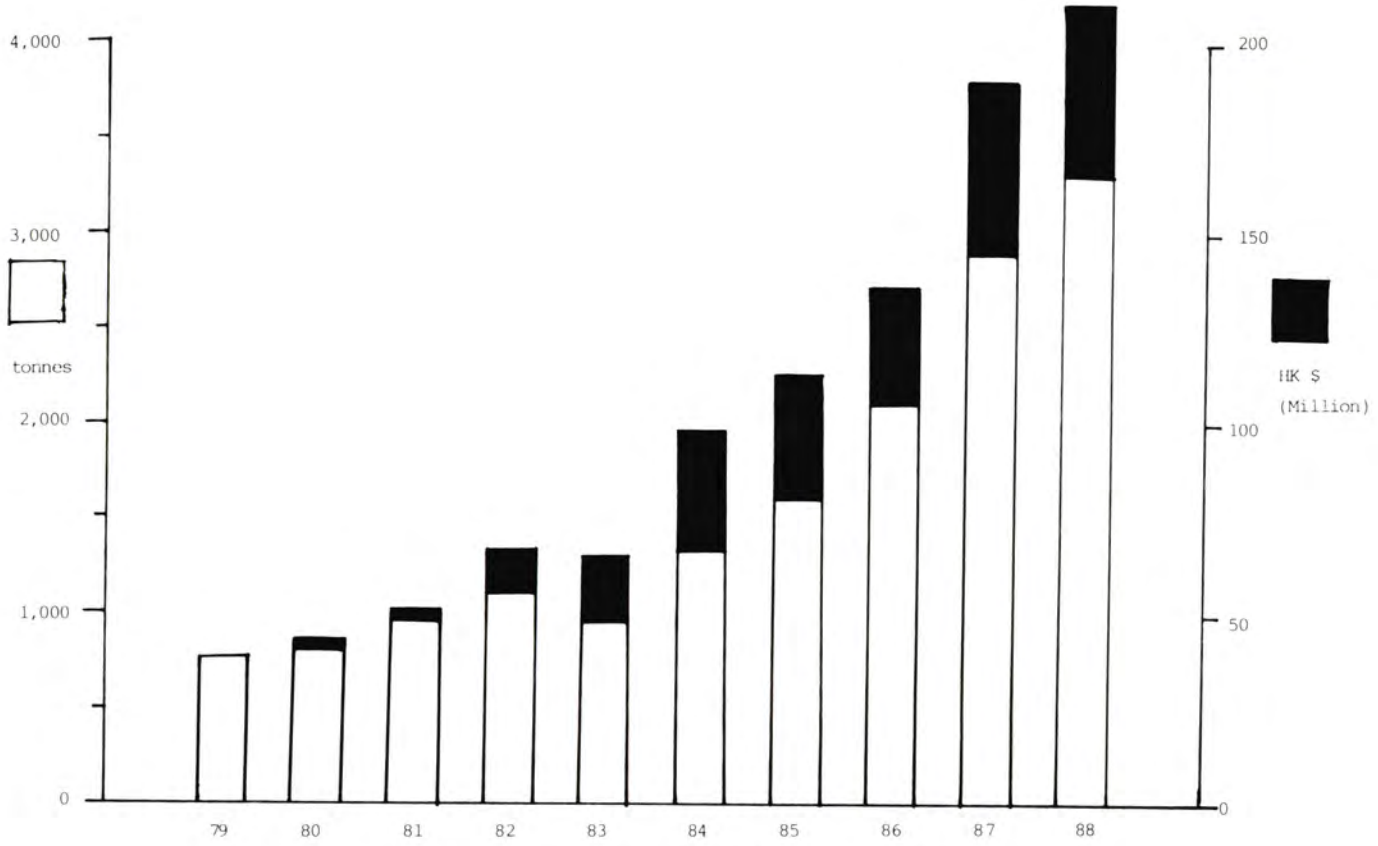

Fig. 1. — Mariculture production in Hong Kong (1979-1988).

sea bed. About 25 % of the raft area is normally set aside for transferring or holding fish during the clean up of fouling organisms from the cages.

Ten to fifteen species of marine fish are commonly cultured in Hong Kong (Table 1). A mixture of sea bream, grouper, snapper and giant perch is normally cultured in a single farm or cage and the average stocking density is 21 kg/m^2 cage. Sea bream fry are normally collected from local waters while over 80 % of grouper fry and all giant perch fry are imported from Thailand, Taiwan, China and the Philippines. Grow-out mortality is high (from 30-50 %) and fish diseases are common. These can be attributed to overcrowding, poor husbandry practises and the lack of imported fry quarantine facilities.

Tab. 1. — Major mariculture species in Hong Kong.

Serranidae	
	Epinephelus akaara
	E. tauvina
	E. awoara
Sparidae	
	Chrysophrys major
	Rhabdosarga sarba
	Mylio macrocephalus
	M. berda
	M. latus
Others	
	Lates calcarifer
	Lutjanus ruselli
	Letherinus nebulosus
	Pomadasya hasta

The cultured fish are fed daily with chopped or minced trash fish, although feeding frequency may be higher in the summer and lower in the winter. The overall food conversion ratio is poor (from 10-15). Malnutri-

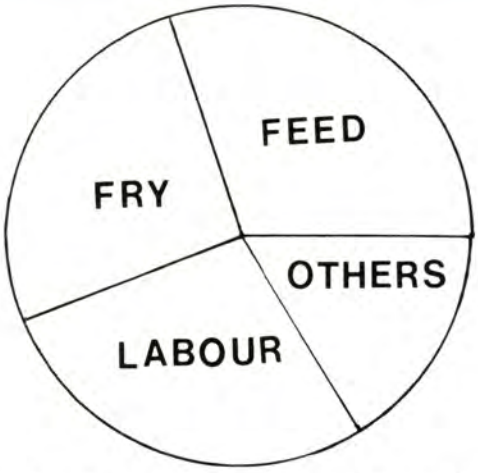

Fig. 2. — Breakdown of mariculture grow-out costs in Hong Kong.

tional syndromes are common and attributable to an unbalanced trash fish diet. Grow-out period varies from 12 to 30 months, depending on location and species cultured.

Despite the high grow-out mortality and incidence of disease, the capital return is fast (about 3 years). A breakdown of grow-out cost is shown in Figure 2. Labour, feed and fry constitute over 80 % of the fish price in roughly equal proportions.

PROBLEMS

The mariculture industry inevitably faces, and at the same time causes, considerable problems. For example, the development of fish farming is in direct conflict with other uses of the same coastal resources, e.g. reclamation, amenity, recreation and navigation. Conversely, pollution and squatter problems have developed in many culture zones. The Marine Fish Culture Ordinance was introduced in 1980 to regulate as well as to protect the mariculture industry. Under the Ordinance, 28 fish culture zones with a total area of 180 ha. have been designated. Farmers have to apply for a licence to culture fish in designated sites within these fish culture zones.

The results of a survey indicate that the major problems of the industry are, in order of priority : limitations of space, high mortality, poor growth and disease. Obviously, many of these problems are inter-related. Results of a one year water monitoring programme showed that ammonia, inorganic phosphate, nitrate and nitrite as well as phytoplankton numbers are generally higher, while dissolved oxygen levels are lower in fish culture zones (Table 2).

Tab. 2. — A general comparison of various water quality parameters at a fish culture zone (sha Tau Kok) and a control station (Kat O). Mean and range of each parameter are based on data collected over a one year study period (After Wu, 1988)

	FISH CULTURE ZONE		CONTROL	
PARAMETERS	Mean	Range	Mean	Range
Dissolved oxygen (mg 0_2 l^{-1})	5.99	4.18-7.48	6.70	5.23-8.09
Phytoplankton (cells ml^{-1})	1,829	162-6,070	203	103-389
Inorganic phosphate (µg l^{-1})	16.7	8.0-23.1	13.7	3.7-23.8
Nitrate (µg l^{-1})	20.0	5.3-60.1	5.9	3.1-10.4
Nitrite (µg l^{-1})	4.3	1.9-9.2	2.4	1.6-3.5

The bottom sediment in many of the fish culture zones is clearly enriched, as indicated by elevated percentages of organic matter (range : 6.2 - 13.3 %) compared with background levels (ranges : 1.2 - 2.7 %).

Hydrogen sulphide is present in the bottom waters and sediments of some zones. The poor water quality is mainly result from pollution with domestic sewage by the farmers, surplus fish feed, fish wastes resulting from over-stocking and by regular cleaning of fouling organisms attaching to the cages.

Pollution has also directly or indirectly caused a substantial loss to the mariculture industry. From 1976 to 1986, it was estimated that some 532 tonnes (valued at US$ 4.6 million) were lost to the industry in 106 fish kills. Of these, 36 incidents were attributed to oxygen depletions, algal boom and red tidal and another 16 incidents to direct pollution effects (Table 3). Fish kills caused by oxygen depletion is thus the most important problem facing the industry and hence forms the major target for research. Pathological infections have not been studied although they are apparently important.

Tab. 3. — Loss to the Hong Kong Mariculture industry due to various causes from 1976-1986

Cause :	No. of incidents
Pollution	
Oxygen depletions, algal blooms and red tide	38
Oil spills, toxic discharges and development	16
Pathological infections	30
Mis-management	9
Hot/cold spells	6
Unknown	7
Total	106

SOLUTION

Oxygen budget model to determine optimal stocking density

The limitations on space in Hong Kong makes it necessary to maximise the use of available fish culture zones. Conversely, over-crowding and over-stocking inevitably lead to enhanced disease transmission, a deterioration in water quality and hence fish kills. It is therefore important to determine the carrying capacity of the water body in relation to total organic loading and stock capacity, in order to optimize the culture activities in each zone. Where oxygen depletions are likely to occur, it would be of value to forecast the occurrence of oxygen depletions so that precautions may be taken to prevent fish kills. A computer simulation model has therefore been developed for the above purposes (Lee and Wu, in prep.). The model aims at predicting the depth-averaged dissolved oxygen level in a fish culture zone, by quantifying oxygen production and consumption resulting from CBOD, NBOD, sediment oxygen demand, fish

respiration, photosynthetic production, algal respiration, and surface re-aeration in the system under varying environmental conditions. Light intensity, water temperature and salinity were identified to be the major environmental forcing factors. Extensive field and laboratory work have been carried out to collect the necessary data for the development of the model, and its predictability has been tested under varying environmental conditions. In all situations tested, predicted dissolved oxygen values agreed closely with actual field measurements. The maximum stock that the water body can sustain was also calculated. The model has proven to be a useful tool for mariculture management in Hong Kong.

A biological indicator for the onset of oxygen depletion

It would be highly desirable for fish farmers to anticipate the occurrence of oxygen depletions at their farm, so that the water could be aerated to prevent fish kills. Studies on the behaviourial responses of nine species of fish to hypoxic conditions revealed that *Chrysophrys major* is far more sensitive to hypoxia than with other species, and shows abnormal behavioural responses, e.g. jumping out of water and abnormal swimming, within 20 min. when dissolved oxygen values dropped to < 1 mg O_2 l^{-1}. Fish farmers are advised to keep a small number of *C. major* in their culture cages so that the quick behaviourial hypoxic response of the species may serve as a useful and effective biological indicator for the onset of oxygen depletions at their farms (Wu, 1988).

Design of an efficient aeration system

An efficient aeration system has been designed for use by fish farmers to increase dissolved oxygen levels within a relatively short time during oxygen depletions. The system includes an oil free blower operated on gasoline (since power supply is not available on most culture rafts), and is capable of producing 0.35 m^{-3} air min^{-1} at 3 m depth. Air produced from the blower is supplied to the air diffuser through PVC tubing. The air diffuser is made of porous tube, and the aeration efficiency is greatly increased because of its large surface area and the small air bubbles produced (Fig. 3). The system has been tested *in situ*, and is able to bring dissolved oxygen levels in a volume of 9 m3 from 0.5 mg/l to 2.0 mg/l, the « safe » limit for the great majority of species, within 15 minutes. This means that as soon as the onset of oxygen depletion is discerned (as reflected by the abnormal behaviourial responses exhibited by *C. major)* the farmer would be able to bring the dissolved oxygen above the safe level within a short time.

Artificial Feed to replace trash fish

Experimental results showed that both food wastage and leaching of organic matter and nutrients can be significantly reduced when artificial feed is used instead of trash fish, particularly for extruded feed which is relatively buoyant and provide a longer feeding time in the water column. Food wastges may be further reduced if extruded feed is used in

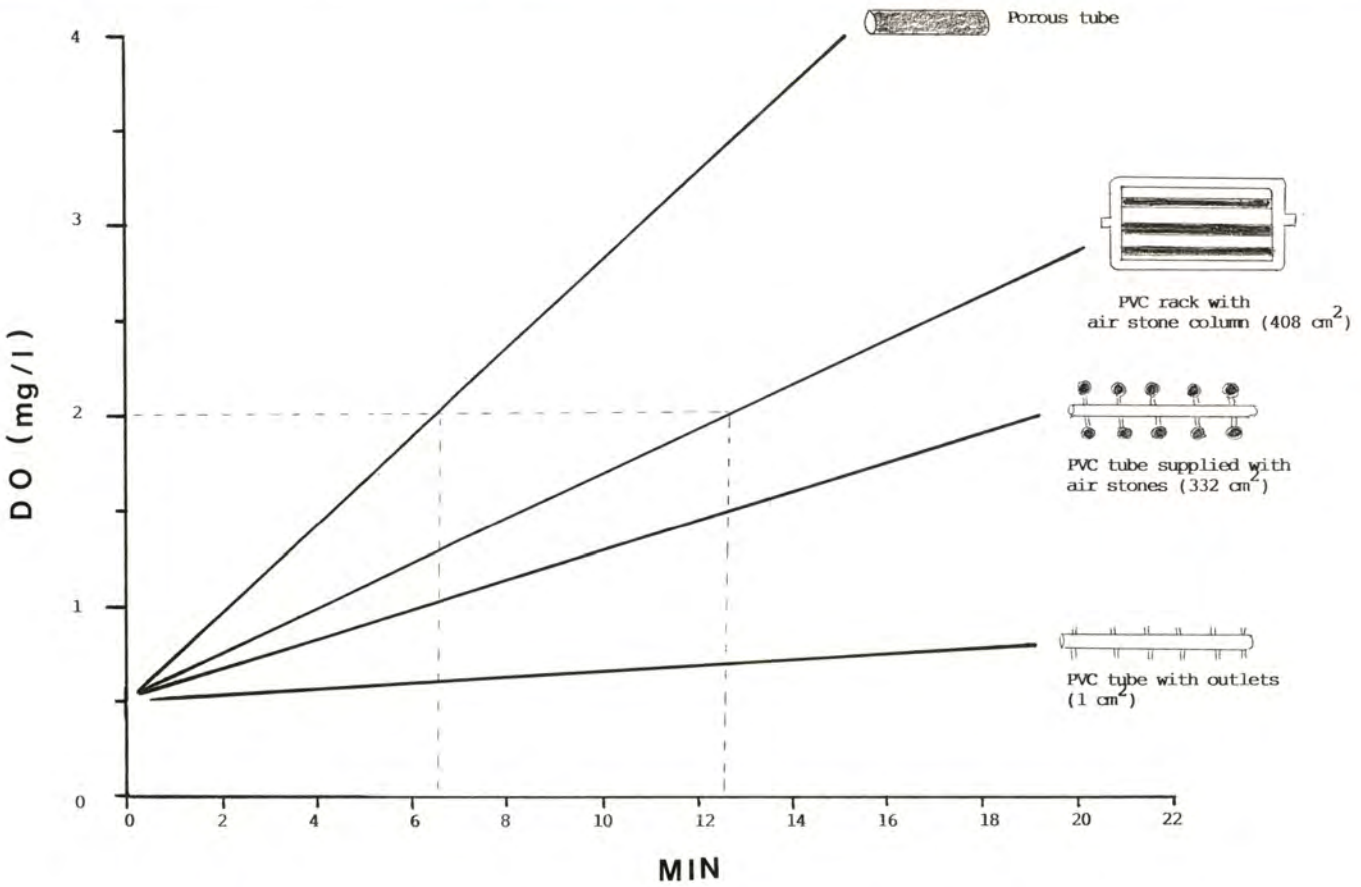

Fig. 3. — A comparison of aeration efficiencies by different types of diffusers *in situ*. Total aeration surface of the various types of diffusers is given in brachets. For detailed design of the system see text.

conjunction with auto-feeders/demand feeders. Research is now underway to study the nutritional requirements of local cultured species, with a view to replacing trash fish with suitable artificial feeds and to rectifying the malnutritional and pollution problems.

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Review of grow-out techniques under tropical conditions : experience of Thailand on Seabass *(Lates calcarifer)* and Grouper *(Epinephelus malabaricus)*

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Abstract — *Seabass and grouper have been reared in cages coastal waters in Thailand for 10 years. The fingerlings are nursed for a month in nylon nursing cages. Then, the juvenile fish are reared in culture cage which can be stationary or floating. The cage is usually 5 × 5 × 2 metres. The stocking density is around 500 to 2000 fish per cage. The survival rate is approximately 80 percent. The selling price at the culture site is 80 baht and 250 baht per kg for sea bass and grouper respectively. The culture method and economics of culture are explained.*

INTRODUCTION

Marine fish culture in Thailand has been practised in ponds and cages. Seabass can be cultured in a pond or a cage. In comparison, grouper can be cultured only in a cage. This is due to the water salinity and other habitat requirements of the species.

From the fisheries census of 1985, some 1579 families engage in marine fish culture in an area of 3698 rai (592 ha) with around 17920 cages (Table 1). Some 87 percent of the marine fish culture is in the south (Table 2). The 1985 total production of seabass was about 512 tonnes, more than the combined production of grouper and mullet (Table 3) (Anon, 1987).

SOME GENERAL CHARACTERISTICS OF SEABASS AND GROUPER

Seabass

Seabass, *Lates calcarifer* (Bloch), is widely distributed in tropical and sub-tropical areas of the Western Pacific and Indian Ocean, between longitude 50°E-160°W, latitude 24°N-35°S (Kungvankij et *al.*, 1986). It is found throughout the northern part of Asia, southward to Queensland (Australia), westward to East Africa.

Seabass is a euryhaline and catadromous species (Sirimontaporn, 1988). Sexually mature fish are found in the river mouths and lagoons where the salinity and depth range between 30-32 ppt and 10-15 m, respectively. The newly-hatched larvae (15-20 days old or 0.4-0.7 cm) are distributed along the coastline in brackishwater estuaries while the 1 cm size larvae can be found in freshwater bodies (Kungvankij et *al.*, 1986; Sirimontaporn, 1988).

Grouper

The grouper which has been cultured in Thailand is estuarine grouper (*Epinephelus malabaricus* Bloch et Schneider) (Tunvilai, 1986). It is a marine fish which is mostly distributed in coastal and marine waters, especially along coral reefs. Grouper is a protogynous hermaphrodite; it matures as a female but transforms into a male when it grows bigger and older (Chen et *al.*, 1977).

Selecting a suitable site for cage culture

Cage culture V.S. pond culture

At present, brackishwater fish are mostly cultured in cages. Cage culture is quite well developed in South East Asia. The advantages of cage culture to pond are as follows :

- cages are usually set in sites with better aquatic environmental conditions. Therefore, cages can be stocked with more fish than ponds,
- the cost of cage preparation is much cheaper than the cost of pond construction,
- during culture period, cage culture would not need water changing and pond preparation, which means cage culture operation would cost less.

Selection criteria

Criteria for selecting a suitable site for cage culture of seabass and grouper are the following (Tookwinas and Charearnrid, 1988).

- Salinity : would range from 10-32 ppt for seabass and from 20-32 ppt for grouper.
- Tide and water depth : water depth should be more than 2-3 metres.

This is due to the usual size of culture cage which is 5 m × 5 m and 2 m deep. The tidal fluctuation should allow the water depth to be at least 2 m at the low water of spring tide.

— Current and waves : area should be protected from strong winds, waves and current; an ideal area would be in protected bays, sheltered coves and inland sea.

— Water quality : the site should be relatively free from domestic, industrial and agricultural wastes and other environmental hazards.

— Water circulation : the site should have enough water circulation to improve poor water quality that could occur at some period in the culture due to the decomposition of waste materials which often accumulate at the bottom under the net-cage.

The water quality parameters which are considered of minimum range for cage culture are shown in Table 4.

CAGE PREPARATION

There are two types of cages used in seabass culture in Thailand (Tookwinas and Charearnrid, 1988) :

Floating cages

The net-cages are hung on GI pipe, wooden or bamboo frames. The cage is kept afloat by styrofoam drum, plastic carbuoy or bamboo. The most convenient dimension for a cage is a rectangular and volume of 50 m^3 (5 m × 5 m × 2 m). The cage unit is stabilized with concrete weights at each bottom corner. The cage unit has to be anchored to the bottom. The cages might be rocked a little by strong wind and current. Floating cages can be set on coastal waters where tidal fluctuation is wide.

Stationary cages

This type is fastened to wooden poles installed at its four corners. Stationary cages are usually set in shallow bays where the tidal fluctuation is low. The size is the same as the floating cages.

Cost

Floating cages are more popular than stationary cages. This is because floating cages are usually set in sites with better aquatic environmental condition such as deeper water, narrow fluctuations of water salinity, more rapid circulation and farther distance from sources of pollution. Therefore, floating cages can be stocked with more fish than stationary cages. In Thailand, the floating cages are mostly used on the west coast (Andaman sea), east side and some area in southeast, while the stationary cages are used in Songkhla lagoon and some areas in southeast.

The type of floating cages can be divided into two :
a) standard which is made of GI pipe frames,
b) ordinary which makes use of wooden frames.

The total cost is 12,700 baht per standard cage and 4,400 baht per ordinary cage. The cost per year is 4,051 baht and 1,575 baht respectively (Table 5 and 6) (1 US $ = 25 baht).

The cost of a stationary cage can be estimated by the method described in Table 5 and 6.

NURSERY

Seabass

Seabass fry and fingerlings should be reared in concrete tanks up to the size 2.5 cm or 1 inch. After that, they can be transferred for rearing in nylon net-cages until they attain 25 cm in about 2 to 3 months of culture period.

The most convenient cage design is a rectangular cage made of synthetic netting attached to wooden, GI pipe or bamboo frames. It is either a) kept afloat by styrofoam, plastic carbuoy or b) stationary by fastening to a wooden or bamboo pole at each corner. The size of cage varies from 0.9 × 2 m to 1.0 × 2.0 m and a depth of 1.0 m. The mesh size of the nylon net is 1.0 mm. However, after a month of nursing, they can be transferred to cages with nylon net with mesh size of 0.5 cm.This would allow water to pass through the cages more freely (Table 6).

The stocking density is approximately 1000 fingerlings per cage. Grading of fingerlings has to be done at least once a week during the nursery period. Stocking is done separetely for each size group. This would minimize losses from cannibalism. Fingerlings of 2.5-5.0 cm should be fed with ground trash fish at 8-10 percent of body weight daily or about 4 to 5 times a day. After that, they can be fed with finely chopped trash fish.

The net cage should be checked daily to ensure that it is not damaged by crabs of clogged with fouling organisms. The cage should be cleaned every other day by soft brushing in order to allow water circulation in the cage.

The survival rate for the nursery period would be 50 to 80 percent. This would depend on feeding, aquatic environmental conditions and the expertise of the fish farmers.

Grouper

At present, grouper fry have been collected from the wild for culture in net cage in Thailand and other countries in Southeast Asia. The fry of size of 3-4 inches, 7.5-10 cm are usually collected by fish traps set in coastal waters near mangrove areas. The fry can be normally collected the year round. However, the peak season is from May to December.

The fish farmers have to collect grouper fry or buy from the collector everyday until they have enough stock for culture. Before stocking, the fry

would be dipped in a formalin solution at a concentration of 100-250 ppm for 1 hour.

The fry can be stocked in nursery cages as mentioned previously. Stocking is done separately for each size group. This is due to the cannibalistic behaviour. First, the ground trash fish is fed 3-4 times a day. The fish farmers should give the feed slowly and watch the fish. Feeding should be stopped when the fish no longer come up to the surface; it shows that the amount of feed is enough for them. The grouper fry get used to manual feeding after one week of stocking. Then, the feeding can be done about two times a day, in the morning and afternoon. The fry can be stocked in nursery cage for about 15-30 days before transferring to marketable cages.

REARING MARKETABLE FISH

Seabass

Seabass are reared from juvenile to marketable size for another 5 to 20 months. The marketable size requirements of the seabass are between 700-900 g and 2000-3000 g (Tookwinas and Charearnrid, 1988). However, the 700-900 g fish is prefered by the local market as consumers in neighbouring countries.

Stocking density for marketable fish culture varies from 12-300/m^3 (Table 8) : depending on water quality and the environmental conditions of the culture site. Floating cages can be stocked more than stationary cages. This is because floating cages are usually set in sites with better aquatic environmental conditions such as deeper water, smaller fluctuation of water salinity, more rapid circulation and further away from sources of pollution.

Trash fish is the main feed for seabass culture. Trash fish should be fresh and clean. Trash fish used in Thailand are sardines and other small marine fish. The trash fish should be chopped and fed twice a day, in the morning and afternoon. The size must be suitable for the size of the mouth of the fish. The farmers should feed fish slowly and watch them. Feeding should be stopped when the fish no longer come up to the surface which indicates that the amount of feed is enough for them.

Food conversion rates of seabass culture in Thailand range from 4.0 to 10.0 : 1 (Tookwinas and Charearnrid, 1988). It also depends on the quality and quantity of trash fish. Normally, seabass can grow at an average of 1 kg/yr.

Survival rates for marketable fish culture are about 80-95 percent in normal culture conditions.

The cages should be checked once or twice a month to ensure that they are not damaged by fouling organisms, crabs or flotsam. The cages should be cleaned or changed every month. Therefore, fish farmers should have spare nylon net cages. Changing cages also allows the farmer to check on the number and health of the fish.

Cover nets can be used to prevent fish from jumping out especially when sea is rough. Cover nets are also used to prevent the fish from predators such as sea otter. The cover net is essentially another net panel which is placed onto the top edges of a cage.

Grouper

The fish are reared in cages until they attain marketable size in about 10-18 months (Table 9). The marketable size requirement of the grouper are between 700-900 g and 1200-1400 g. The fish are mostly exported live by air to Hong Kong and Taiwan.

Stocking density for marketable fish culture varies from 12-100/m^3, depending on water quality and the environmental conditions of the culture site.

Trash fish is also the main feed for grouper culture in Thailand. From the experiment, grouper can be fed with artificial diets easier than sea bass. Feeding and cage maintenance should be the same as in sea bass culture.

Food conversion rates (FCR) of grouper culture varies with stocking density. They range from 6.0 to 7.5 (Tanomkiat et *al.*, 1987; Sakaras and Kumpang, 1988). At a high stocking density, FCR is lower than at the low stocking density which is the same as in sea bass culture (Table 10). This is due to the following factors :

- metabolic rate is decreased at high stocking density. This is because of the fish in big group do not have to swim against a strong current in cages,
- high stocking density stimulates feeding. The feeding per fish would be better than at low stocking density (Sakaras et *al*, 1988).

FEEDS AND FEEDING

Feed is the major constraint to seabass and grouper culture. At present, trash fish is the only known feed stuff used on the first two months of culture, 10 percent of body weight is the feeding rate. After that, it can be reduced to about 5 percent of body weight (Kungvankij et *al.*, 1986). Since the supply of trash fish is insufficient and expensive, trials on moist feed or artificial diets have been conducted. The feed composition recommended for grouper is presented in Table 11. The artificial diet for seabass culture are still at an experimental stage.

WATER QUALITY AND AQUATIC ENVIRONMENT

The physico-chemical properties of coastal aquaculture and grouper cage culture in lower south of Thailand (Krabi, Trang and Satul Provinces) were surveyed between July 1980 and August 1982 in 19 survey stations. The methodology for water analysis was set along the standard line of Apha (1975), Lind (1974) and Strickland and Parsons (1969), as temperature, visibility, dissolved oxygen, pH, salinity, ammonia-nitrogen,

nitrate-nitrogen, phosphate and silicate(Tookwinas et *al.*, 1985; Tookwinas, 1988a).

The water quality then is indicated in Table 12.

The major aquaculture is Songkhla Lake for seabass culture in net cages. It was introduced in 1972 by the Department of Fisheries. In 1986, seabass production from the 300 net cages of some 115 farmers in the lake was approx. 98.5 tonnes.

Songkhla Lake is the largest lagoon in Thailand and in Southeast Asia, as well. It is located at latitude 7°08 -7°50 N and longitude 100°07 -100°37 E. Total area is approximately. 89,680 ha. The eastern side of the lake (Thale Sap Tonnok) opens into the Gulf of Thailand).

The physico-chemical parameters of water in the lake are given in Table 13.

Other culture areas were also investigated. The bottom sediment under the net cage contained a high level of waste organic matter as shown by the chemical oxygen demand (COD) value. The benthic organism found was a polychaete which can bloom in polluted condition. It can be noted that the decomposition process occurs in the bottom sediment, which consumes a lot of dissolved oxygen in the water column.

Seabass culture in Songkhla Outer lake has been going on for 8 to 10 years. The waste material from cage culture is directly deposited at the bottom. The cages are also set very close to each other so that the number of culture cages could have been more than the carrying capacity of the area. The fish farmers have stocked up to 42.8 kg/m^3. The investigations suggest that the aquatic environment at the culture site must be improved by the following measures; (Tookwinas et *al.*, 1986; Tookwinas 1988 a).

a) The maximum stocking density of fish should be 300 fish per cage (cage size 7 × 8 × 2 m).

b) The dissolved oxygen can be increased by air pump, especially at night from 0200-0800 hours.

c) For a long term improvement measure :

- the culture cages should be moved farther away from one another and from the village (about 300 m). This would avoid the effect of excretory waste materials,
- the bottom sediment should be dredged. This would decrease the decomposition of waste materials.

DISEASE AND PREVENTION

Since the cages are floating in estuarine water, there are frequent changes in water quality. As a result of environmental problems, the fish are subject to stress and their resistance to infectious diseases is lowered. Diseases may therefore result in significant losses (Chonchuenchob, 1986; Ruangpan 1988). Diagnosis and treatment of fish disease, especially for subtropical and tropical species, have not been well established. Therefore, the most important preventive measure for disease is to grow strong fish, which can withstand pathogenic agents, through proper provision of fresh,

high-quality feed, appropriate stocking density, and suitable water quality at culture site.

Numerous diseases of seabass and grouper have been reported in Thailand (Ruangpan, 1988). The causative agents of these diseases are parasitic organisms, bacteria, viroses, malnutrition and environmental stresses. Some disease and parasites associated with seabass and grouper culture are presented in Table 14 and 15.

MARKETING AND ECONOMICS

Grouper and seabass are more expensive than most other fish species. The demand is therefore rather limited. The supply for the local market is already adequate and the prospect for markets abroad is being developed by local producers (Tookwinas, 1988b). For seabass, the demand for specific processed types and various sizes of marketable fish will also influence the expansion of the industry and its foreign market.

At present, seabass is usually sold in the local markets. The product is also exported to neighbouring countries. For grouper, the live product is mostly exported to Hong Kong by air (Fig. 1).

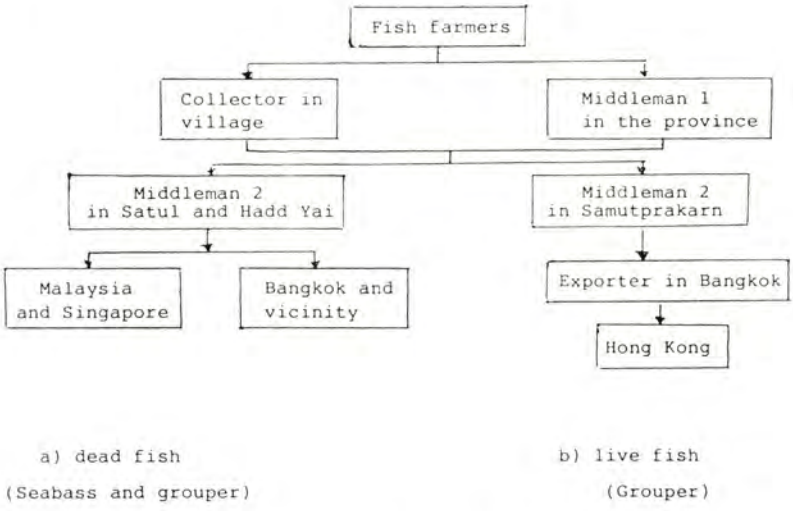

Fig. 1. — Marketing of Seabass and Grouper.

The income from grouper culture is much more higher than from seabass culture one's. At present, the demand is continuous the year round. The net income of grouper and seabass culture in normal conditions per cage and per year are 62,849 and 3,849 baht respectively (Table 16).

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Tab. 1. — Coastal aquaculture in Thailand, 1985.
(From Anon. 1987)

Type of culture	No. of families	Area (rail)*	Percent by area
1. Fish culture	1 579	3 698	1.58
— Pond	289	3 418	1.46
— Cage	1 290	280**	0.12
2. Shrimp culture	4 480	217 574	92.98
3. Crab culture	122	369	0.16
4. Oyster culture	1 170	3 924	1.67
5. Mussel culture	257	1 456	0.62
6. Cockle culture	112	6 956	2.97
7. Horse mussel culture	6	13	0.005
8. Others	3	3	0.001
Total	7 720	233 993	100

* 1 Rai = 1600 m^2
** 280 Rai = 17 920 cages (5 × 5 × 2 m)

Tab. 2. — Marine fish culture in Thailand, 1985
(From Anon. 1987)

Zone	No. of	Percent
Eastern part	94	5.9
Central part	222	7.03
Southern part	1 374	87.02
Total	1 579	100.00

Tab. 3. — Production from fish culture, 1981-1985 (tonnes)
(From Anon. 1987)

Species	1981	1982	1983	1984	1985
Seabass	215	145	1 059	473	512
Grouper	—	—	176	149	117
Mullet	—	1	—	4	—
Total	215	146	1 235	626	629

Tab. 4. — The suitable water quality for cage culture of seabass and grouper

Parameters	Ranges
pH	7.5 - 8.3
Dissolved Oxygen	4.0 - 8.0 mg/l
Salinity	10 - 32 ppt. (seabass) 20 - 32 ppt. (grouper)
Water temperature	25 - 32°C
Ammonia - nitrogen	less than 0.02 mg/l
Hydrogen sulfide	none
Current	normal

Tab. 5. — Cost of investment for standard floating cages (5 × 5 × 2 m) (Tookwinas and Charernrid, 1988)

Material	N°	Duration (yr)	Total cost (Baht)	Cost per year (Baht)
1. GI pipe frame	4	5-8	2 800	623
2. Nursing net	3	1-2	900	600
3. Marketable net				
— mesh size 2.0 cm	1	3-5	3 600	1 028
— mesh size 4.0 cm	1	3-5	2 000	500
4. Styrofoam drum	1	3-5	2 000	500
5. Other mareials	—	—	1 000	500
Total			12 700	3 751

Tab. 6. — Cost of investment for ordinary floating cages (5 × 5 × 2 m) (From Tookwinas and Charernrid, 1988)

Material	N°	Duration (yr)	Total cost (Baht)	Cost per year (Baht)
1. Wooden frames	4	2	800	400
2. Styrofoam drum	4	2-4	1 200	400
3. Nursing net	1	3-5	1 000	250
4. Marketable net	1	3-5	900	225
5. Other material	—	—	500	300
Total			4 400	1 575

Tab. 7. — The suitable netting mesh size for various size group of fish. (From Tookwinas and Charernrid, 1988)

Mesh size (cm)	size of fish (cm)
0.5	1-4
1.0	5-19
2.0	20-30
4.0	30 and up

Tab. 8. — Growth of seabass at different stocking densities in cages. (From Sakaras, 1985)

Culture period (days)	Stocking density (/m³)				
	100	150	200	250	300
30	119.7 g	115.6 g	116.7 g	117.7 g	117.8 g
60	222.7	218.4	206.6	212.4	208.1
90	309.0	306.4	294.4	293.1	285.1
120	380.0	361.2	368.0	353.0	345.7
150	448.0	420.5	418.0	410.9	379.4
180	523.4	495.8	463.3	449.9	436.5
210	573.3	569.9	551.4	527.9	505.4

Tab. 9. — Growth of grouper at different stocking densities in cages

Cultured period (days)	Stocking density	
	58/m³ *a	100/m³ *b
0	83.7	26.9
30	158.7	45.6
60	186.5	65.9
90	243.9	98.7
120	283.7	137.0
150	296.8	217.1
180	355.8	312.4
210	443.9	387.6
250	—	586.6

*a fed with trash fish
*b fed with artificial diets

Tab. 10. — Food conversion ratio (FCR) of Seabass and Grouper culture at various stocking densities

Species	Stocking density (/m³)					
	58	100	150	200	250	300
Seabass	—	7.79	5.78	5.38	5.02	4.64
Grouper	7.48	6.36	—	—	—	—

Tab. 11. — Artificial diets for grouper culture

Composition	Weight (kg)
Fish meal	75
Rice bran	20
Soy bean oil	0.5
Vitamin and mineral	0.2
Banana	20-30 pieces

Tab. 12. — Water quality in coastal aquaculture area in Krabi, Trang and Satul Provinces, lower and south of Thailand, monthly average from 19 surveyed stations, July 1980 to August 1982

Parameters	Mean	S.D.
Depth, m.	2.10	1.348
Visibility, m.	0.87	0.324
D.O., mg/l	5.24	0.495
pH	7.80	0.170
Salinity, ppt	26.10	3.923
NH_3-N, mgN/l	0.01	0.01
NO_2-N, mgN/l	0.0039	0.004
PO_4, mgPO_4/l	0.04	0.018
Si, mgSi/l	2.50	0.279

Tab. 13. — Physico-chemical properties of water in Songkhla Lake (Thale Sap Tonnak) (1984-1985)

Parameters	Mean (av. annual)	Ranges
Temperature (°C)	30.17	24.0-34.0
Turbidity (FTU/NTU)	19.34	6.90-33.50
Conductivity (mmhos/cm)	22.24	0.10-58.70
Salinity (ppt)	13.68	0-34.00
pH	7.89	6.40-8.55
Dissolved oxygen (mg/l)	7.19	3.10-9.95
COD. (mg/l)	2.46	0-9.50
Orthophosphate (mgP/l)	0.27	0-1.60
Nitrate-nitrogen (mgN/l)	0.01	0-0.09
Alkalinity (mg/l)	56.24	21.43-95.00
Acidity (mg/l)	2.79	0-5.96

Tab. 14. — Parasite of Seabass culture in Thailand

Parasite	Attacked position	Mortality (%)	Treatment
Protozoa			
Blastodimidae	Gill	—	formalin 250 ppm
Epistylis sp	Gill, body, eye, fin	5-80	30 minutes or
Henneouva sp	Gill	5-80	formalin 50 ppm 24 h
Opistonectus sp	Gill, body, skin	5-80	3-4 times/3 day
Trichodina sp	Gill, body, skin	50-100	
Helminths			
Deplectanum latesi	Gill	2-5 every day	
Crustacean			
Aega sp	Gill	5	formalin 50 ppm 3-6 h.
Gnathis sp	Gill, mouth cavity	>20 %	or dipterex 0.25-0.5 ppm
Caligus sp	Gill, mouth cavity	10-80 in small size fish	or NaCl 3-5 % for 5 mn
Lernanthropies sp	Gill		

Tab. 15. — Some parasites and diseases associated with culture of grouper in Thailand

Disease or Parasite	Attacked position	Mortality (%)	Treatment
1. Monogenetic trematode	Skin, gill		Dipterex 0.3 ppm 24-48 hrs
2. Cryptocaryon sp (Ich.)	Skin, gill		Malachite green 0.1 ppm + formalin 25 ppm, 24-48 hrs
3. Trichodina sp	Skin, gill		Formalin 250 ppm 30 '(twice)
4. Bacteria			Oxytetracycline 1.5 g/1 kg of feeds, feeding 7 days

Tab. 16. — Economic of Seabass and grouper culture per year in Thailand. (unit : Baht; 1 US$ = 25 Baht)

	Seabass	Grouper
1. Cages (standard)	4 051	4 051
Seed	2 500	10 000
Feed	7 000	7 000
Labour	3 600	3 600
Total cost	17 151	24 651
2. Production (kg)	350	350
3. Income	21 000	87 500
4. Net income	3 849	62 849
5. Net Income over cost	18.3 %	71.8 %

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Energy — Protein management by some warmwater finfishes

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Abstract — *If warmwater finfish protein requirements as level in diet is low, absolute intake per day is similar to those of coldwater species. But relation of this requirement with specific growth rate differs for strictly warmwater fishes as Tilapias. Analysis of partition between protein and non-protein energy retention shows that these species with* ***Clarias*** *make a better use of non-protein energy provided and consequently improve their protein retention. Better carbohydrate digestibility and metabolism are propunded as part of the explanation.*

INTRODUCTION

One may discuss the problem of nutrition on aquacultured fish under three broad categories : (a) physical and biological modes of feeding, (b) specific nutritional requirements, and (c) diets formulation.

In the first category, we have to consider not only the physical texture and stability of compounded pellets from which belongs the relative food consumed, but also the feeding practices for which too little attention has be done. Indeed, meal timing plays a major role in feed utilization by fish. It affects growth rate, feeding efficiency, and body composition. It is related to the existence of circadian variations of metabolism and its hormonal control (see vol. 113 of the Trans. Am. Fish. Soc., 1984).

Among nutrients, protein requirements and optimal dietary protein levels remain an apparent melting pot even if a sustained literature is available in these fields. Additionally, both problems - protein requirements and optimal dietary protein levels - are often confused by reason of interfering problems of protein to energy relations, calorigenic function of proteins, apparent protein sparing effects of fats and carbohydrates, and available energy (Cho and Kaushik, 1985).

Because of the relationship between protein and energy levels, the optimal dietary protein level varies widely between successive experiments if diet energy density or feeding rate has been changed. However, in most cases the authors cannot conclude in terms of optimal dietary protein level,

but only as optimal dietary to energy ratio (Garling and Wilson, 1976; Wang et *al.*, 1985 a,b).

The origin of the energy by itself can also act, and very heavy factorial plans have to be mounted. Consequently these types of experiments are scarce, and when they exist, a doubt often subsists (Berger and Halver, 1987).

These introductory statements have a general value, whatever the fishes, but they constitute many aspects to be taken into account to review specific nutrient requirements of warm water fishes. As a matter of fact, energetic aspects will be more extensively considered than protein requirements by itself, even if the protein cover need remains the chief purpose.

Optimal dietary protein level

The range for reported optimal dietary protein levels is quite large, but some results should be considered with cristicism, either they are unique and not corroborated, or too distant of the general tendancies. Considering only the most reliable values for growing fish, e.g. when there exist various convergent results, obtained with adequate feeding rates and diet energy levels, we may establish that there exists a general pattern when protein sources of good value are fed.

Apparent protein level needs seem to disminish when normal life temperature increases. For rainbow trout the reported values range from 35 to 45 percent of the diet, and the optimal level is close to 36 % when high fat diets are used (Takeuchi et *al.*, 1978a). The same team, thus working in the same type of approach, found that optimal protein level in diets ranges from 31 to 38 % for common carp at 22-25°C, and estimates that the optimum content of dietary protein for maximum growth is around 31 % (Takeuchi et *al*, 1979b). Summing up literature data, Luquet (1989) concludes that Tilapia species have a protein requirement of 28-35 % of the diet and that 30 % constitute a safe level for a 23-28°C normal temperature range. Channel catfish, living at around 27°C, are considered to need 28 % protein in their diet (Garling and Wilson, 1976), even if recorded values range from 22 to 36.

Daily protein requirements

When expressed in terms of daily allowances (gram of protein by kilogram of body weight per day) for optimal growth or maximal protein deposition, protein requirements appear more homogeneous. The recorded values range from 6.2 to 10.2 for rainbow trout (Takeuchi et *al.*, 1978 a,b,c), from 7.0 to 12.1 for carp (Takeuchi et *al.*, 1978b, 1979a,b), and from 7.2 to 10.8 for channel catfish (Garling and Wilson, 1976). For tilapias the data vary mostly according to the feeding rate (Luquet, 1989). When fed at 3 % B.W. per day, the data are closed (9.6 to 10.7) to that above; they increase to 15-20 with a 5-6 % feeding rate, and reach 68 with a feeding rate as high as 20 % B.W. (Winfree and Stickney, 1981). Thus, protein daily requirements of omnivorous warmwater fishes are similar or higher that for carnivorous coldwater fishes when expressed against body weight.

Tacon and Cowey (1985) have widely discussed the best way to express protein requirements. After the statement that « it may be more meaningful to express protein requirements as digestible protein energy relative to the digestible energy content of the diet », they show that protein daily allowances must also be related to the specific growth rate of fishes. They conclude that « there exists an almost linear relationship between daily protein requirement (grams of protein per kilogram body weight per day) and specific growth rate (SGR, percentage per day) of the different species examinated ». The data quoted include the Tilapia *Oreochromis niloticus*, *O. aureus*, *O. mossambicus*, and *Tilapia zilli*.

When adding other results related to the Tilapias, it seems therefore that Tilapia displays some particular trends. Two reasons may explain this, one related to experimental conditions and the other to own species characteristic such as strict stenotherm status. Needs values over the general trend are associated to feed conversion ratio over the means, involving a possible surestimation of needs. On the other hand, lower values observed indicate that Tilapia are able to maintain a higher specific growth rate without exceeding protein daily allowance. This implies a better management of available proteins.

Protein utilization

Protein utilization, usualy expressed as retained protein/protein intake ratio, constitutes a common criterion to check the synergistic interactions between major nutrient classes (protein sparing) or energetic cost of nutrient utilization (specific dynamic action = SDA). A dietary formulation low in protein entails minimal SDA, and would permit more efficient energy utilization.

Table 1 presents results of protein and energy efficiency in terms of % retained for some species. It is based on literature data selected as the most efficient in order to make a comparison of relative potentiality of these species in protein and energy utilization. In addition the body store compartiments (protein and non protein stores) are presented, as well as the percentage of non protein dietary retention. By this way, Cho and Kaushik (1985) have discussed in detail the partition of the energy and protein retention or losses in trouts under different protein to lipids diets.

The comparison between species allows us to ascertain that :

— *O. niloticus* retains more efficiently protein intake than other species. More than 55 % of energy retained is fixed as a nitrogenous form, while other values are below 50 %,

— energy retention in Tilapias, carp and *Clarias* is lower than for rainbow trout and channel catfish,

— this is mainly due to the fact that non protein energy is less retained (< 35 %) in these species,

— in carcass, most of the energy (more than 60 %) is retained as protein energy in *Clarias* and Tilapia, and as non protein energy (lipids) in trout, carp and channel catfish,

— the eurythermal carp and channel catfish appear closer to

coldwater trout than strict warmwater fishes such as Tilapia even living in warmwater.

Then Tilapia appears to be very efficient to utilize both protein and energy. For proteins this is evident insofar as the fixed/ingested ratio is high. For non protein energy, reverse reasoning must be done : less the retention is high, better is their utilization efficiency. Indeed, energy incorporation in diets is for fuel purpose for metabolism or for protein synthesis. Then, in fish cultivated for food, the fixed energy, except that bound in proteins, must be considered as misused because not burnt.

Tab. 1. — Protein and energy efficience in term of % retained for some species

	Diet Characteristics					
Species	Prot. %	Fat %	D.E.(MJ /100g(1)	N.retained % intake	E. retained % of DE intake	% of non prot E. retained
Rainbow trout	34	22	1.760	47	63	73
«	55	13	1.870	34	53	88
»	33.8	19.5	1.967	47.9	58.8	58
Carp	31.5	14.6	1.549	39.1	42.5	39
Catfish	36		1.703	35	55	65
«	24.1		1.151	49.8	62.2	83
Clarias gariepinus	50.4		2.021*	38	32*	24*
O. niloticus	31.2	5.5	1.507	62.3	51.5	35
	31.4	2.5	1.632	55	38.5	21
O. mossam	42	9.83	1.578	25.6	20.8	10

* Gross Energy basis

(1) Energy value are calculated, if not given, on proximate analysis basis. Coefficient are 22.2 CP; 38.9 EE; 17.2 NFE for gross energy and 18.8 CP; 37.7 EE; 16.7 NFE for digestible energy.

Energy availability and fate

Non protein energy supply in diets is obtained by addition of high lipids or carbohydrate compounds. Their utilization involves a good acceptability of fishes either during digestive or metabolism process.

Lipids are reported to be highly available with an emphasized digestibility coefficient of fat as ambiant temperature increases (Andrews et *al.*, 1978). Carbohydrates do not display any general performances. As a monosaccharide requires no digestion to be assimilated, a polysaccharide must first be hydrolised. The polysaccharides mainly used are starch and dextrin. With salmonids, starch digestion is depressed with increasing intake and could be largely enhanced by a precooking treatment (Bergot and Brecque, 1984). The ability of Tilapias to digest starch is not dependent on such kind of constraint, apparent digestibility of crude starch being close to lipids values (over 15 %) even with an intake as great as 6 mg/g body weight/day (Wang et *al.*, 1985a).

Energy efficiency of different digested carbohydrates must be comparable. Their utilization by *O. niloticus* is relatively homogeneous except for glucose which efficient use could be handicapped by a too fast assimilation (Anderson et *al.*, 1984). Recalculated non-protein energy retention is about the same ranging from 17 to 23 % for starch, dextrin, sucrose and glucose incorporated to diet at a rather high level, 40 %. Taking pelleting process into account, lipids could be added in diet. Rates over 15 % are well used (Takeushi et *al.*, 1978a), but unsaturated fatty acids composition must be regarded with care. Excess amount of n-3 has adverse effect on growth and lipid metabolism (Takeushi et *al.*, 1983; Robinson and Wilson, 1985). Once essential fatty acid needs are content, saturated fatty acids containing sources prevail as they dont show unfavourable propensity (Takeuchi et *al.*, 1978a; Cho et *al.*, 1985).

CONCLUSION

Warmwater fishes may use better provided protein. This is not only due to a better protein retention capacity but also to a better utilization of furnished non-protein energy. Being less retained, the protein sparing objective of this energy is more fulfilled. Better polysaccharides digestibility should play one of the leading parts of non-protein energy utilization, but the metabolic fate of nutrients has to be considered as fishes have to manage an « unconventionally » large amount of energy metabolites compared to traditionnal coldwater species.

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Nutrition of the Seabass *Lates calcarifer*

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Abstract. — *The ability of seabass to wean with dry pelleted feed was demonstrated from as early as the fry stage by the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER), Tahiti. Dietary protein and lipid requirements of seabass were also investigated from the fry to market-size stages by IFREMER. Results indicated that the seabass fingerlings required 45-50 % protein in diets made with high quality fishmeal. Dietary lipid can be reduced to 6 % without real protein-sparing effect on growth, and fish survival was not affected.*

The Primary Production Department of Singapore (PPD) studies covered the weaning of seabass from fingerlings stage onwards and the dietary protein requirement of early grow-out fish. Dietary protein requirement of early grow-out seabass was demonstrated to be between 40-50 %, at dietary lipid level of 12 %, using fishmeal protein. Nevertheless fish growth was significantly higher with trashfish feed, but apparent protein retention was significantly better with formulated feed.

INTRODUCTION

This research on the nutrition of Seabass was initiated in 1985, thanks to a cooperation between IFREMER and PPD including :

- supply of fry and fingerlings;
- exchange of nutritional data in order to evaluate some nutritional requirements of seabass;
- development of formulations;
- propose conditions of manufacturing such a feed in order to sustain future seabass operations whether in Singapore or in Tahiti.

The objectives of seabass nutrition studies conducted in Tahiti were the estimation of basic protein and lipid requirements of fry and the reduction of the cost by optimization of the formulation and selection of low cost protein sources.

Seabass fry were imported from Singapore (PPD) in 1985 for the first experiment and in 1986 for two other ones; larval rearing was conducted in Tahiti with 15 days old larvae.

Rearing conditions for fish nutrition studies were as follows : 100 litres capacity fiberglass, rectangular tank; water exchange at 10-100 % per hour and sand filtered sea water ; aeration at 8 litres/minute; light with low intensity and black plastic lid covering 3/4 of the tank; temperature between 27 and 29°C; automatic feeder for feed distribution 8 hours per day.

Definition of different terms employed in the study :

— relative growth rate in % per day

$$= \frac{\text{Ln } Wt^2 - \text{Ln } Wt^1}{\text{Number days}} \times 100$$

— conversion ratio $$= \frac{\text{Quantity of dry feed}}{\text{Weight gain}}$$

— feed efficiency ratio % $$= \frac{1}{CR} \times 10$$

— protein efficiency ratio % $$= \frac{\text{Weight gain (g)}}{\text{Ingested gain (g)}}$$

— Energy/protein ratio $$= \frac{\text{Energy (kcal)}}{\text{protein (g)}}$$

All statistical analysis were done with one way analysis of variance on total weight increase, each month at alpha risk of 5 % and a Newmann - Keuls test.

Protein requirement

Experimental design selected for protein requirement was a range of 4 protein content : 35, 40, 46 and 55 and a control at 48 %, each treatment in 2 replicates, 39 fry per tank, and an initial weight of 34,7 ê 0,6 g. Feeding levels were fixed at 3 % of body weight. Formulations were given as indicated in Table 1.

Lipid requirement

Experimental design selected for lipid requirement included 3 levels of lipids at 6, 10 and 14 % and 3 blocks with 2 replicates per treatments,

Table 1. Diet Composition for Lates Fingerlings in Protein Requirement study.

Raw materiel %	A	B	C	D	Control
Norseamink	39	45	55	58	34
Fish concentrate					20
Shrimp meal	5	5	5	5	
Meat bone meal					4
Corn	44	34	18		
Soja	2	3	10	23	10
Whole wheat					8
Wheat flour	5	5	5	5	
Yeast					10
Dried whey					4
Vitamin Rovimix					2
Alfalfa					3
Minerals mix					1.4
Guaranate	2	2	2	2	
Capelin oil	3	3	5	6	4
% As fed protein	34.4	39.5	46.7	54.5	48
Fat	8.3	8.9	10.5	12	10.6
Ash	6.5	7.3	8.6	9.7	7.9
crude energy Kcal/kg	4 600	4 700	4 900	5 100	4 900

21 fry per tank and initial weights of 20,5 ± 0,5, 25,0 ± 0,6, 30,5 ± 0,8 for blocks 1, 2 and 3 respectively. Feeding levels were fixed up at 3 % body weight. Formulations are given as indicated in Table 2.

Table 2. Lipid requirements of *Lates calcarifer* observed in a preliminary study.

Raw materials %	DIETS A	B	C
Norseamink	34	34	34
Fish concentrate	20	20	20
Meat bone meal	4	4	4
Soja concentrate	9.6	9.6	9.6
Whole wheat	12	8	4
Yeast	10	10	10
Dried whey	4	4	4
Vitamins	2	2	2
Alfalfa	3	3	3
Minerals	1.4	1.4	1.4
Capelin oil		4	8
Crude protein (1)	56.4	57.5	55.5
Fat (1)	6.8	10.6	14
Carbohydrates (1)	20	15	14
Ash (1)	9	9	9
Crude energy Kcal/Kg	4.7	4.9	5.1

(1) % as fed.

RESULTS

Protein requirement

Summary results on the comparison of five protein levels in compounded diets for sea bass fry during a 3 months experiment are given as indicated in Table 3.

Significant growth differences correlated with protein levels appeared as early as the first month of experiment. Optimum protein level was around 50 % as showed by conversion and feed efficiency ratio values. Importance of protein source quality with a mixture of fish meal and fish protein concentrated was demonstrated by best results obtained with the control. At a constant digestible energy protein ratio of 7,5 Kcal/g of protein, there were a slight accumulation of body lipids when protein content of the diet was superior to 40 %.

Table 3. Results of survival, weight gain, food conversion ratio (FCR) and food efficiency ratio (FER), comparing 5 protein levels in compounded diet for Seabass.

Diet	Crude Protein %	Survival %	Total Weight Gain (g)	Relative Growth Rate (%/day)	FCR	FER
A	34	100	77	1.3	1.8	1.8
B	40	97	100	1.5	1.5	1.8
C	46	100	120	1.6	1.4	1.6
D	54	99	124	1.7	1.4	1.4
E	48	99	138	1.8	1.3	1.8

Seabass fish appeared as a strictly carnivorous species with an optimum protein level around 50 %. No further improvement of growth was observed even with a protein level higher than 50 %.. Protein quality appeared as an important factor to be considered to enhance fish growth.

Body analysis results are indicated in Table 4.

Table 4. Summary of body analysis results on seabass fry fed at five different protein levels in the compounded diet during a 3 months experiment.

	Diets	Crude (1) Protein %	Fat (1) %	Ash (1) %
INITIAL	A.E.	14.2	3.1	3.7
	A	16.2	3.5	4.3
	B	16.3	4.1	4.2
	C	16.5	5.0	3.9
	D	16.4	6	3.9
	E	16.3	6.1	3.8

(1) % of live fish.

Lipid requirement

Weight gains, relative growth rate and food conversion ratio results on the comparison of 3 fat levels in the diet are indicated in Table 5.

Table 5. Growth results on the comparison of 3 fat levels in dry feeds for seabass fry during 120 days experiments.

Total weight gain in grams.

	% LIPID IN DIETS		
	6	10	14
Block 1 « 20 g »	114	93	140
Block 2 « 25 g »	94	125	81
Block 3 « 30 g »	161	121	110
mean	123	113	100

Results

Mean relative growth rate (%/day).

% lipid in diet	%/day
6	1.4
10	1.3
14	1.2

Mean foood conversion ratio.

% lipid in diet	FCR
6	1.7 :1
10	1.7 :1
14	1.8 :1

Fat body analysis (Table 6) did not show lipid accumulation at high fat level in the diet for 20 g average weight fish and a slight increase with other fish sizes.

Conversion ratio, feed efficiency and protein efficiency ratio were stable. Total weight increase fluctuated in a range of 100 to 120 g over 4 months but classification of diets versus growth was not strictly correlated with fat level. Survival and growth of sea bass fry were not significantly

Table 6. Body fat analysis of 3 sizes of seabass fry fed at 3 fat levels in the diet during a 4 months experiment, in % of fresh fish.

% fat in diet	Block 1	Block 2	Block 3
6	2.9	2.1	2.9
10	2.9	3.1	3.4
14	2.9	3.7	4.4

Table 7. Seabass experimental diet used at PPD. Singapore.

	%
Singapore fish meal	17
Norseamink	36
Dried mussel meal	4
Dried meal	2
Squid meal	4
Meat bone meal	6
Soya bean meal	5.5
Whole wheat	3
Vitamin mixture	1
Mineral mixture	5
Alpha starch	10
Cod liver oil	4.4
Crude protein (%)	48
Crude fat (%)	13
Ashes (%)	15
Calcium	2.8
Phosphorus	2.4
DE Kcal/kg	3 522
DE/P mg Kcal	136

Table 8. Dietary protein requirement of seabass. Results obtained at PPD (Wong and Chou, 1988).

Diet with Protein Level (%)	Growth Rate g/fish/day	FCR	Survival %
CP 30	0.3	2.0	90
CP 35	0.5	1.5	90
CP 40	0.6	1.3	95
Trash fish (control)	1.0	1.2	90

N.B. 1.6 g average initial weight of fish.

affected when fed at 3 fat levels in the diet. Preliminary conclusions on lipid requirements related to 6 % fat level brought in by capelin oil in the diet which seems sufficient to bring in energy and fatty acids to sea bass fry. Fat level higher than 6 % did not improve growth nor reduce food consumption although the digestible energy/protein ratio was.

CONCLUSION

From our results and in accordance with literature on Seabass nutrition, Chou (1984), Wong et *al* (1988), protein and lipid levels in formulated feeds should be around 50 and 6 % respectively for fish ranging from 20 to 200 g mean weight. These percentages should be confirmed for larger sized fish up to the commercial mean weight (500-600 g). Utilization of low cost protein sources is to be considered as large scale aquaculture development of this species is expected. More fundamental research is needed to specify essential amino acids and fatty acids requirements to improve and optimize feed formulations.

Feed formulation is guided towards a substitution of fish meal by soya meal in a way to reduce the cost of feed. Meanwhile it was shown that pelleted feed versus extruded feed was proved identical in terms of growth and survival rate.

Least cost formulation is already possible with a minimum of nutritional constrainsts and a right selection of quality ingredients.Extruded or pelleted feeds can sustain growth of *Lates* from 20 g up to 650 g after 180 days in floating cages at a density of 60 fry/square metre with a FCR of 1.4 to 1.0 : 1 (Fuchs, 1986).

At PPD in Singapore, similar formulations (Table 7) are achieved in order to replace trash fish by cheap extruded or pelleted feeds and similar studies are carried out on protein (Table 8) and lipid requirements of seabass. Such studies would help to sustain a reasonable cost of feed in order to convince local farmers to use pelleted feeds instead of trash fish.. Inclusion of local fish meal is necessary for economical reasons ; utilization of local mussel meal represents a good potential protein source for part of *Lates* feed. The future of pelleted feed relies entirely on a performant but economical formulation of the feed to get a chance to compete with large use of trash fish by farmers in Singapore.

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FINFISH SESSIONS

SYNTHESIS AND DISCUSSIONS

Review of Knowledge on Aquaculture for the Principal Species

Reporter : M. MACKINNON. QDPI. Australia

In opening the session, Dr Chen Foo Yan reminded us that farming marine fish is a relatively new development with most of the activity in the field being in the last twenty years. Japanese culture of yellowtail and bream gave a lead. With the exception of one or two species where numerous fry could be collected from the wild, hatchery technology had to be developed and this was a big problem initially.

L. Cheong, then, gave us an overview of a successful farming industry for seabass in South East Asia which currently produces about 2 500 tonnes of *Lates calcarifer* annually and supports more than 100 hatcheries in Thailand alone. As well as grow-out production there is considerable export of fry from the region mainly to Taiwan and Hong-Kong. Grow-out is practised in saltwater, brackishwater or freshwater ponds and in fixed or floating sea cages. Yields as high as 196 kg/m^3/annum have been obtained experimentally although most production in cages gave 30 kg/m^3/annum. Dr Cheong felt there was room for further research in this area. FCR commonly obtained on trash fish diet ranged from 3-10 : 1 with most ponds in Singapore giving an FCR of around 4.5 : 1. Labour costs had a big impact on cost of production in Singapore but were minimal in Thailand. Limitations to culture included tolerance to cold, high food costs - presently limited to small scale farming. Production in highly intensive marine raceways was mentioned as a possibility for the future.

During the discussion on this paper, it was pointed out that there could be need for further research to improve survival during the first few weeks in cages when mortalities of around 30 % are common.

R. Wu outlined the biological factor important in selecting species for culture and the main economic factors which affect profitability in relation to Hong-Kong which has considerable spatial and temporal variation in the physico-chemical culture environment. Main biological factors for selection were growth efficiency, foraging efficiency, environ-

mental tolerances, and suceptibility to disease / crowding; economic factors were price of fry, price of food and market price. Low temperatures and low dissolved oxygen were important limiting factors for aquaculture in Hong-Kong and experimental work to determine tolerance of aquaculture species to these factors was outlined. Biological and economic parameters were combined to produce a model which gives culturists a basis for choosing species and culture procedures and allows for optimal profits under variable cost conditions.

S. Kraul created a lot of interest with his talk on the two species of Mahi-Mahi which appear to be close to the perfect candidates for culture with fast growth, good food conversion, rapid maturity, high fecondity, frequent natural spawning and high market price. The main problems to be solved appear to be in the transfer of hatchery technology from the îlot scale to commercial scale, and the requirement for very clean oceanic water. Cannibalism was high in fingerling fish. Not much know about cage culture potential and probably best suited to tank culture.

In the next paper, the recent development of an aquaculture industry for *Lates calcarifer* in Australia was outlined. The importance of pre-existing commercial and recreational fisheries, the discovery of many reproductively isolated stocks, and the distribution of the species over a wide variety of climate types have been important influences on culture activity in Australia. A number of commercial hatchery and grow-out operations have commenced however only one company is producing on a large scale to date. Government research in Queensland currently concentrates on the hatchery production of fingerling fish for stocking programmes although good results have been obtained in grow-out trials using dry pelleted feeds.

The paper, by J. Fuchs and his AQUACOP colleagues described the processes through which suitable species were selected to culture in French polynesia. Species from four families of local fishes (Carangids, Coryphaenids, Serranids and Siganids) and two exotic families (Centropomidae and Cichlidae) were screaned initially as aquaculture candidates. From these, a selection was made on the basis of growth potential, adaptability to dry feed, reproduction, ease of hatchery production, resistance to disease and stress and suitability for the local market. Seabass selected for development in the short term and groupers, dolphin fish and red tilapia are considered to have a potential although there are still larval rearing or disease problems to be solved before an industry can be established. *Siganus argenteus* and *Caranx ignobilis* are considered to have low potential for culture.Discussion on this paper mentioned that the presence of ciguatera was a factor in selecting species for culture in Tahiti and this (together with problems of fry production) was the reason that lutjanids were not considered for culture in Tahiti. The risks of introducing exotic species were raised, however it was considered that *L. calcarifer* had little chance of successful reproduction in the wild in Tahiti. Nursery phase was still seen as a problem for *L. calcarifer.* There was also some general discussion on the likehood of genetic considerations becoming increasingly important in selecting stocks for aquaculture.

D. Roberts pointed out that there is no large scale marine fish culture in North or South America. Most aquaculture in the two continents is in

freshwater and their combined aquaculture production accounts for less than 5 % of total world aquaculture. In USA nearly all culture research is aimed at stocking programmes rather than commercial grow-out. Most of the marine fish farming that does occur is in the Caribbean area. Large amounts of money invested in pompano culture research over 25 years failed to produce an industry. Current interest in Snook is centred in Florida. Problems with obtaining reliable supply of eggs. General requirements of larvae and juveniles well known. Most of the requirements seem very similar to those described for *Lates calcarifer.*

C. Arnold, then, reported recent sucesses in captive spawning and fingerling production in red drum. Demand for culture of this species has been greatly increased by recent prohibitions on the commercial fishery for the species. Now able to control spawning by environmental manipulation, spawning is frequent and fecundity is high (500 000 - 1 000 000 eggs per spawning). Encouraging results have been obtained in grow-out of red drum in ponds (85 % survival, growth to 3 lb at 18 months) and in intensive tank culture. Now 8 red drum hatcheries in southern US, 2 of these equipped for grow-out.

W. Watanabe reported research by the Caribbean Marine Research Centre on red tilapia. Found excellent potential for culture of saline tolerant tilapia for application in tropical islands with limited freshwater resources. Seed production was best achieved through egg removal. Reproduction and larval rearing was very successful under intensive conditions in recirculating brackish water. Grow-out of monosex fish in seawater tanks and floating cages was also successful and in some trials fish reached 450 g in 6 months. The feasibility of greatly reducing feed costs by reducing protein levels to 20 % or perhaps even less during grow-out was demonstrated. General discussion resulting from this paper included legal aspects of marketing fish fed androgens, and the benefits of monosex culture.

The IFREMER team from Martinique then reported on the selection of suitable species for culture there. Native species were looked at initially. Lutjanid species screened initially included *L. apodus, L. analis, L. griseus* as well as Palometa. Wild caught juveniles of both these species were reared in cages. Palometa was selected as having most potential. Under grow-out conditions, Palometa gave good conversion with trash fish diet but because of its pelagic habits and small mouth it is hard to feed on a pellet diet. Difficulties obtaining eggs have prevented hatchery trials with the species. because of the problems of the native species two exotic species were imported (red drum from Texas and red Florida hybrid Tilapia). Pilot-scale grow-out of red drum is underway and commercial culture of tilapia has been commenced. Discussion on this paper included general problems of culturing carangids — pelagic behaviour, reproduction and disease.

Last paper of the session by P. Luquet reported on the aquaculture potential of the armoured catfish *Hoplosternum littorale* from tropical South America. The species commands a high market price in French Guyana and has many desirable features for culture. It is easy to spawn naturally in small tanks, has relatively large eggs and larvae and a high fecundity. Being an obligate air breather it is very tolerant to low dissolved

oxygen. The fish can be marketed at a very small size (150 g). It is suggested that initial rearing conditions may affect sex-ratio and that this could allow the development of monosex male culture.

In general discussion at the end of the days proceedings delegates from the different countries were asked to summarize prospects for fish culture.

In Hong-Kong, groupers were seen as having a lot of potential because of their high market price but larval rearing was seen as a major problem. Dr Chen questionned whether high market price should be the most important factor and pointed out that the demand for lower priced species such as mullet was very considerable in Hong-Kong.

In the Philippines production of fish to meet the severe protein shortage was seen as more important than production of high priced species. Herbivorous fish would receive the most emphasis.

In Singapore, it was suggested that private enterprise would concentrate on the higher priced species leaving the government to work on the lower priced species however limited land was a major constraint to production of lower priced fish.

In both Australia and USA high labour costs were seen as an important constraint to commercial farming and the present level of emphasis on stocking programs was thought likely to continue. Threadfin and goatfish were mentioned as possible future candidates for culture in Hawaii. In Australia, a number of marine and freshwater fishes may have biological potential for aquaculture however in most cases they are either not presently marketed or seed supplies are unavailable.

A number of speakers felt that groupers were of great importance world wide and research should be increased on them. Hybridization was suggested as a possible means of overcoming rearing difficulties in groupers.

There was also some general discussion on whether it was now time to move research effort away from established culture species or if continued refinement of techniques was still necessary for the established species. There was no general agreement on this. It was suggested however that all of the established species had major problems left to solve.

Constitution of Broodstock Reproduction in Captivity

Reporter : W. O. WATANABE. U.S.A.

The final discussion of the finfish workshop on reproduction in captivity attemted to identify major problems associated with sexual maturation and spawning for species of potential importance in tropical aquaculture.

Reproduction of Seabass, *Lates calcarifer*, in captivity can be obtained with good reliability. In Singapore, natural spawning is obtained, although single injections of HCG (500 IU/kg) or LHRH-a (10 ug/kg) may also be used to induce spawning. Similary, in Tahiti, spawning is easily obtained in tanks or cages by one to 2 injections of LHRH-a on females with oocytes > 400um diameter. Based on the observation that the first spawning is usually not fertilized, it was suggested that spawning of males may be more difficult than females.

While larval survival of *L. calcarifer* is reported to be relatively high (averaging approximately 30 %) for commercial culturists in Southeast Asia, it was suggested that egg quality and larval survival may be improved through improved broodstock nutrition.

In Singapore, female *L. calcarifer* spawners of 5-7 kg are used for breeding purposes. In Australia, some strains undergo sex inversion (male to female) at larger sizes. It was suggested that the development of a method for promoting early sex inversion can increase availability of females for spawning programs.

In the common snook, *Centropomus undecimalis*, spawning of adults collected from wild stocks can be induced with hormones although reliability is still poor and larval survival low. Use of controlled photoperiod and temperature to induce maturation and spawning has not been successful to date. It was hypothesized that this is related to stress in broodstock.

There is relatively little information on hormone induced spawning in groupers. In Tahiti, spontaneous spawning of *Epinephelus microdon* has been observed in tanks. Control of sex-inversion or reversion without hormones, through use of social factors (e.g. changing proportions of sexes in a tank), could be useful in practice. For groupers, larval survival is problematical. Trials conducted to date have been met with poor survival through fingerling stages.

Techniques for maturation and spawning of red drum (*Sciaenops ocellata*) by environmental control are well established and are being applied commercially in the U.S. No major problems exist, although reduced egg quality and fry viability after 3-4 months of spawning has been observed. It is unclear whether transfer of environmental control techniques developed in the U.S. to tropical areas such as Martinique is possible. It was suggested that this problem may be overcome by exposing fish to natural photoperiod and temperature treatment. This possibility needs further study.

The mahi-mahi, *Coryphaena hippurus*, spawns naturally year-round in tanks and domestication has been achieved by breeding over 6 generations. No problems related to maturation and spawning exist. As no sexual dimorphism occurs before a marketable size, methods for controlling sex are not required.

In areas where grow-out of red tilapia (*Oreochromis sp.*) is limited by season, such as the Bahamas, the development of methods for out-of-season spawning by environmental control will be useful to maximize the effective length of the growing season. The development of methods for synchronization of spawning can also improve availability of fry. Development of alternative methods to hormonal sex-reversal is required to improve reliability of monosex male culture, thereby minimizing the possibility of unwanted introductions. It was suggested that genetic methods may possibly be used to produce all normal male populations.

For the palometa, *Trachinotus goodei*, control of maturation and spawning is problematical, as seems to be characteristic of most Carangidae. In Martinique, broodstock are difficult to manage and are suceptible to infection by the monogenean fluke, *Neobenedenia melleni*. While fecundity is very low in the palometa, natural fecundity is unknown. More information on the natural spawning cycle is required in order to identify environmental cues for controlling reproduction.

Natural spawning of milkfish, *Chanos chanos*, has been obtained in floating cages in the Philippines and in ponds in Taiwan and in Hawaii. Spawning can also be obtained by hormone induction. Larval survival varies widely and it was suggested that this may be related to broodstock nutrition. It was emphasized, however, that broodstock nutrition should not be ignored in any species.

For mullet, *Mugil cephalus*, reliable and cost effective procedures for spawning by use of carp pituary homogenate and LHRH-a are available. Egg quality and larval survival have been problematical. While mean egg diameter is the only practical criterion available for judging readiness for hormone induction, it was suggested that an alternative criterion be developed.

Constitution of Broodstock Genetic Subsession

Reporter : B. CHEVASSUS. INRA. France

Two recommendations have been proposed :

1. — To develop at least in the public hatcheries a « genetic book » in order to collect the different parameters allowing the calculation of the genetic evolution of the stock : origin and number of the male and female spawners used at each generation, mating design, reproductive success (number of offspring) of the different females. This data could be used in particular to evaluate the loss of genetic variability and inbreeding coefficient of the population and to incriminate or not genetic factors as possible causes of some negative events (mortality, low fertility...)
2. — To develop for the new species evaluation programmes of genetic resources by *in situ* studies of natural or domestic populations. Genetic polymorphism of proteins or DNA can be used for those studies in order to evaluate the genetic variability within each population and the level of differentiation between the different populations. This information could be used in a first step for choosing stocks for aquaculture (on the basis of heterozygosity) and in a second step to develop a strain evaluation programme (introduction of the different populations in a testing station, and evaluation of performances of pure and crossbred products).

Larviculture — Production of Juveniles

Reporter : . A. RIMMER. QDPI. Australia

1. — Advantages and disadvantages of rearing larvae in extensive, semi-extensive and intensive systems

   a) High success rate (i.e. production of juveniles) can be achieved more easily using an extensive system; short-term approach.

   b) Intensive systems allow greater control over factors affecting larval growth and survival.

   c) Cost/benefits of extensive and intensive systems differ between countries.

2. — Factors affecting growth and survival of marine fish larvae

   a) Water temperature, salinity, pH, other water quality parameters

   b) Food quality and density

   c) Fish density

3. — Larval rearing problems

a) Causes	— Nutrition	Red drum
	— Disease	Barramundi/Seabass
	— Egg/larval quality	*Dicentrarchus*
	— Cannibalism	Seabream
	— Water quality (oil)	Seabass & -bream
b) Effects	— Decreased survival and growth	
	— Increased no deformities	

4. — Future research priorities

   a) Understanding larval and juvenile development — esp. grouper

   b) Nutrition

      — Composition of foods,

      — Use of live and inert foods microdiets shows promise (*Dicentrarchus, Lates*)

      — Weaning techniques for red drum and seabass (*Lates*)

Overall— Similar problems with larval rearing in different species and in different countries.

Nursing and Grow-Out

Reporter : G. CUZON. IFREMER Tahiti

Summary of interests

- Trash fish : benefits and disadvantages.
- Automatic feeders.
- Extruded/pelleted feed.
- Nutritinal requirements :
  - energy,
  - levels of proteins in feeds,
  - warmwater versus cold water fish.
- Replacement of fish meal by vegetable protein sources.
- Cost of fish feeds.

Trash fish is a kind of feed largely utilized in all South East Asian areas where floating cages are widely distributed in order to grow juveniles up to the commercial size. As in Hong Kong sometimes trash fish pollutes the water or the bottom of the sea in the area of floating cages. It is not available all year round and its quality is not constant. There is a trend to shift from fresh fish to pelleted or extruded feed. But there is a problem of nutritional balance of the feed or a question of cost.

For species like *Lates*, it looks pretty feasible to produce a good extruded or pelleted fish feed but, in some cases like in Tahiti, there is a real problem of cost of feeds. It reaches peaks due to a high level of quality fish meal from Norway or fish protein concentrate, together within yeast which seems to be a necessary ingredient in the fish feed. So the problem is how to replace such clear ingredients without decreasing the quality of the feed, or trying to replace fish meal by soybean meal at maximum level compatible with the growth of the species. Up to now a fairly high level of LTI soya bean meal seems to be adequate.

This way is fairly promising as it could solve the problem of availability of fish meal, and would help to decrease the cost of feed. But some biochemical controls on liver and blood have to be done in order to be sure that warmwater fishes like *Lates calcarifer* can grow fairly well with at least 20 % soya bean meal in the diet.

It should be highly recommendable to review fish experiments in terms of standardization as it was already proposed in Hamburg in 1974 when a working group was held to give solutions to this problem of reference diet and standard procedure.

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Le colloque « *Advances in tropical aquaculture* » qui s'est tenu à Tahiti, en 1989, a été l'occasion de faire le point sur l'aquaculture marine dans les pays du Pacifique Sud : Australie, Fiji, Hawaï, Nouvelle-Calédonie, Nouvelle-Zélande et Polynésie française; ainsi qu'une synthèse des connaissances et des recherches dans trois domaines :

— pathologie des élevages : crevettes, mollusques et poissons tropicaux,
— nutrition des crustacés : pénéides tropicales,
— sélection et les essais d'élevage des poissons tropicaux.

Outre les communications présentées lors de ce colloque, le lecteur trouvera les bibliographies qui font référence dans ces matières.

The workshop « Advances in tropical aquaculture » *held in Tahiti, in 1989, was the opportunity to review the marine aquaculture activities in the South Pacific countries : Australia, Fiji, Hawaii, New Caledonia, New Zealand and French Polynesia; as well as a synthesis of the knowledge and research in three fields :*

— *pathology of shrimps, molluscs and finfishes,*
— *nutrition of crustaceans,*
— *evaluation of candidate species for fish farming.*

Beside the communications presented during this workshop, the reader will find the bibliographical references.

IFREMER