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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.

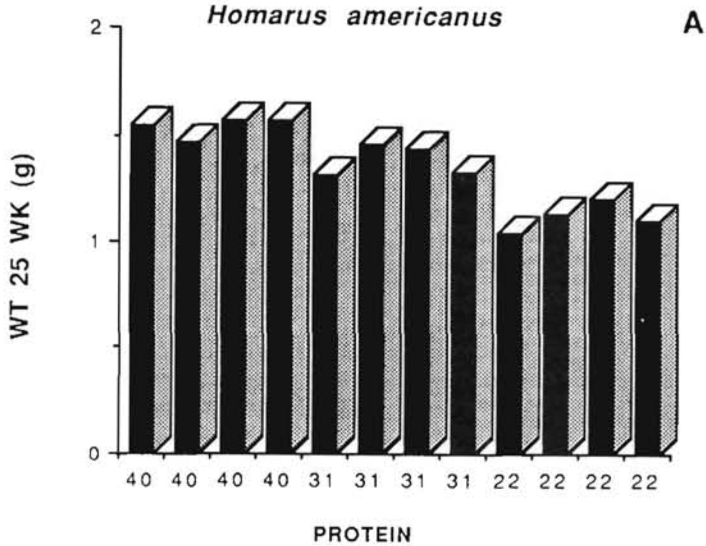
UNREFINED SRD

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



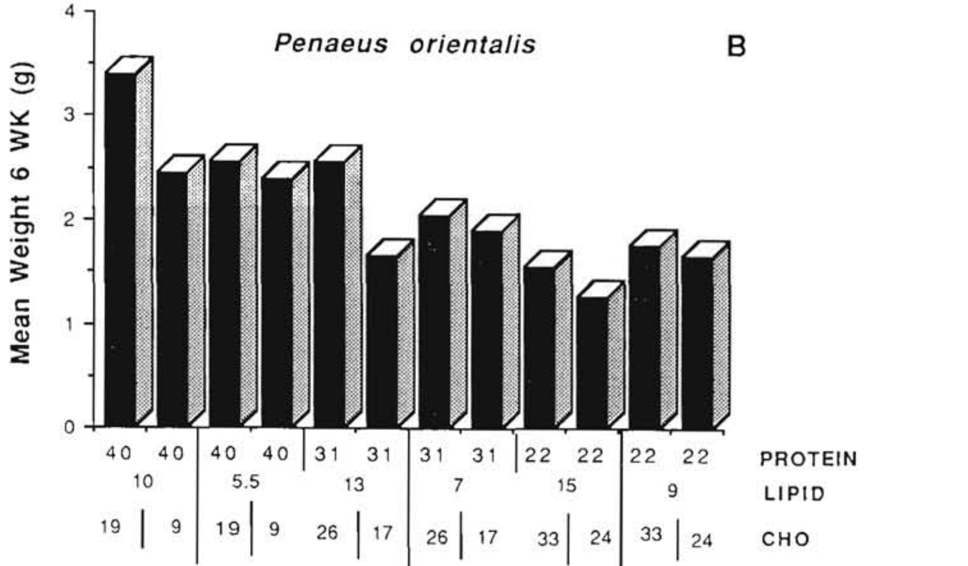


Fig. 1. — Mean final weights of (A) lobsters, *Homarus americanus* after 25 weeks and (B) prawn, *Penaeus orientalis* after 6 weeks feeding with diets varying in protein, lipid and carbohydrate.

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

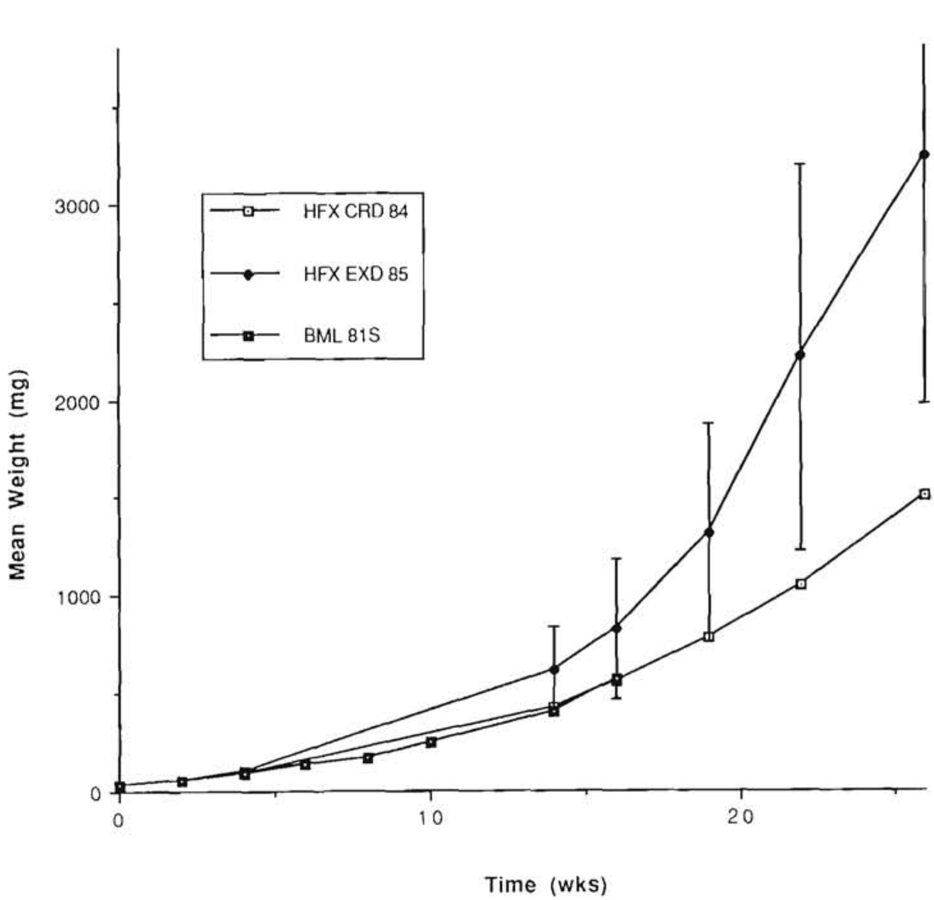


Fig. 2. — Growth curves of lobsters fed either of the purified Standard Reference Diets (BML 81 S or HFX CRD 84) for 26 weeks after reaching 4th stage, compared with growth of lobsters from the same family fed the unrefined Reference Diet (HFX EXD 85).

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 improvement 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.

American Institute of Nutrition Committee on Standards for Nutritional Studies. First report of the ad hoc Committee on Standards for Nutrional Studies. *J. Nutrit.* 1340-1348.

American Institute of Nutrition Committee on Standards for Nutritional Studies, 1980. Second report of the ad hoc Committee on Standards for Nutritional Studies. *J. Nutrit.*, **110** : 1726.

American Institute of Nutrition Committee on Nomenclature, 1987a. Nomenclature policy : Generic descriptors and trivial names for vitamins and related compounds. *J. Nutrit.*, **117** : 7-14.

American Institute of Nutrition Committee on Nomenclature, 1987b. Nomenclature policy : Abbreviations of amino acids. *J. Nutrit.*, **117** : 15.

American Institute of Nutrition Experimental Animal Nutrition Committee, 1987. Guidelines for describing diets for experimental animals. *J. Nutrit.*, **117** : 16-17.

Boghen A.D., J.D. Castell and D.E. Conklin, 1982. In search of a reference protein to replace vitamin-free casein in lobster nutrition studies. *Can. J. Zool.*, **60** : 2033-2038.

Bordner C.E., 1989. A standard Reference Diet for crustacean nutrition research. V. Growth and survival of juvenile dungeness crabs (*Cancer magister*). *J. World Aquacult. Soc.*, **20** (in press).

Bowser P.R. and R. Rosemark, 1981. Mortalities of cultured lobsters, *Homarus*, associated with a moult death syndrome. *Aquaculture*, **23** : 11-18.

Castell J.D., J.C. Kean, L.R. D'Abramo and D.E. Conklin, 1989. A Standard Reference Diet for crustacean nutrition research. I. Evaluation of two formulations. *J. World Aquaculture Soc.*, **20** (in press).

Castell J.D., J.C. Kean, D.G.C. McCann, A.D. Boghen, L.R. D'Abramo and D.E. Conklin, 1989. A Standard Reference Diet for crustacean nutrition research II. Selection of a purification procedure for production of the rock crab (*Cancer irroratus*) protein ingredient. *J. World Aquacult. Soc.*, **20** (in press).

Conklin D.E. and A.D. Beck, 1979. The World Mariculture Society Nutrition Task Force purpose, progress and problems. *In*: Proceedings of the World Nutrition and Fishfeed Technology Symposium, Hamburg, West Germany, June 20-23, 1978. Eds. J.E. Halver and K. Tiews. Heenemann Werlagsgesellschaft, Berlin. Vol. 2 : 561-564.

Cruz-Ricque, L.E, J. Guillaume, G. Cuzar 1987. Squid Protein Effect on Growth of four Penaeid Shrimp. *J; world Aquacult. Soc.*, **18** : 209-217.

Cruz-Suarez L.E., J. Guillaume , A. Van Wormhoudt, 1987. Effect of Various Levels of Squid Protein on Growth and Some Biochemical Parameters of *Penaeus japonicus* juveniles. *Bull. Jpn. Soc. Sci. Fish.*, **53** : 2083-2088.

D'Abramo L.R., C.E. Bordner, D.E. Conklin and N.A. Baum, 1981. Essentiality of dietary phosphatidylcholine for the survival of juvenile lobsters, *Homarus*. *J. Nutrit.*, **111** : 425-431.

D'Abramo L.R., L. Reed and J.M. Heinen, 1988. A culture system for nutritional studies of crustaceans. *Aquaculture*, **72** : 379-389.

EIFAC, 1980. Report of the EIFAC, IUNS, and ICES Working Group on standardization of Methodology in Fish Nutrition Research, J.D. Castell and K. Tiews (Eds). EIFAC Tech. PAP. 36 : 1-24.

Kean J.C., J.D. Castell, A.G. Boghen, L.R. D'Abramo and D.E. Conklin, 1985. A re-evaluation of the lecithin and cholesterol requirements of juvenile lobster (*Homarus americanus*) using crab protein-based diets. *Aquaculture*, **47** : 143-149.

Metailler R., 1987. Experimentation in Nutrition. In training session : Nutrition in Marine Aquaculture, Lisbon, 20-30 October 1986. Ed. A. Bruno. FAO/UNDP Mediterranean Regional Aquaculture Project, Tunis (Tunisia); 1987, 303-330.

Morrissy N.M., 1989. A standard Reference Diet for crustacean nutrition research. IV. Growth of freshwater crayfish (*Cherax tenuimanus*). *J. World Aquacult. Soc.*, **20** (in press).

New M.B., 1976a. A review of shrimp and prawn nutrition. *Aquaculture*, **21** : 101-128.

New M.B., 1976b. A review of shrimp and prawn nutrition. *Proc. 7th Ann. Meeting World Maricult. Soc.*, **7** : 277-287.

Reed L. and L.R. D'Abramo, 1989. A Standard Reference Diet for crustacean nutrition research III. Effects on weight gain and amino acid composition of whole body and tail muscle of juvenile prawn *Macrobrachium rosenbergii*. *J. World Aquacult. Soc.*, **20** (in press).

Rosenthal H., J.E. Stewart, J.D. Castell and H. Ackefors, 1986. Glossary of Aquaculture Terminology. International Commission for Exploration of the Sea, Mariculture Committee C.M. 1986/F : 34, p. 62.