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Les besoins écophysiologiques des larves d'huître creuse *Crassostrea gigas* en conditions contrôlées : effet de la température, de la nourriture et modélisation de la croissance

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1.1.1. Position systématique

L'huître creuse *Crassostrea gigas* est aussi connue sous le nom huître du Pacifique ou huître japonaise. Selon la classification phylogénétique rapportée par Ruppert et Barnes (1996), l'huître creuse *Crassostrea gigas* appartient au phylum *Mollusca* (le plus important du règne animal après *Arthropoda*), classe *Bivalvia*, ordre *Ostreoida*, famille *Ostreidae*, genre *Crassostrea* et espèce *gigas*.

1.1.2. Cycle de vie

L'huître *C. gigas* est une espèce ovipare à hermaphrodisme successif asynchrone (Buroker, 1983). Les adultes peuvent changer de sexe au cours du temps, par des raisons mal déterminées, avec un premier cycle de reproduction en tant que mâle (protandrie) (Guo et al., 1998). Au début de l'hiver, la gamétogenèse se réduit mais cette activité augmente à la fin de cette saison. Le développement de cellules germinales s'accélère au printemps pour atteindre la maturité sexuelle au début de l'été (Goulletquer, 1997). La gamétogenèse, à l'instar d'autres fonctions biologiques de l'huître, est directement liée aux changements de conditions biotiques et abiotiques (disponibilité en nourriture, température, concentration en oxygène, salinité, etc) (Chavez-Villalba et al., 2003b ; Fabioux et al., 2005 ; Enriquez-Diaz et al., sous presse). Au cours de l'été, un stimulus extérieur conduit à l'expulsion des gamètes, qui peut être partielle ou totale (Goulletquer, 1997). Les réserves énergétiques de l'huître sont

reconstituées à l'automne avant la ré-initiation d'un nouveau cycle de reproduction (Berthelin et al., 2000).

La fécondation chez *C. gigas* est externe et passe par une phase larvaire planctonique. Vingt quatre heures après fécondation, la larve de type trochophore devient une larve véligère en forme de D, appelée ainsi à cause de la forme de sa coquille. A ce stade, la larve possède une coquille de type prodissoconche, dont la taille est environ de 70 µm, ainsi qu'un velum, organe de nutrition et de locomotion. La forme de la coquille évolue au cours du développement larvaire avec l'apparition d'une extension en forme de crochet qui correspond à l'umbo. Lorsque la larve atteint une taille de 250 µm (Holiday et al., 1991), un organe sensoriel apparaît sur la coquille sous forme d'un point noir donnant à ce stade le nom de larve œillée. A l'approche de la fin de la vie planctonique, la sécrétion de la dissoconche commence ainsi que l'apparition du pied qui permet la recherche d'un substrat pour la fixation (stade pédi-véligère). Les larves d'une taille avoisinant 280 µm (Coon et al., 1990) peuvent initier la métamorphose qui se traduit entre autres par la disparition du pied et du velum (remplacé par des branchies), mettant ainsi fin à la vie planctonique pour acquérir un mode de vie benthique (post-larve ou naissain). Le premier développement gonadique peut avoir lieu au cours de la première année de vie des huîtres (Lango-Reynoso et al., 2000).

1.1.3. Répartition géographique

C. gigas est une espèce très largement répandue à travers le monde (FAO, 2008). Originaire du Japon et du Canada, cette espèce a été introduite avec succès dans de nombreuses régions du monde, en Asie sur le littoral de la Sibérie et de la Corée du Sud et du Nord, en Amérique sur la côte ouest du Pacifique (de l'Alaska à la Basse Californie et du Chili), en Europe de la Grande Bretagne et Irlande au Portugal, dans toute la Méditerranée, en

Australie et en Tasmanie. Aujourd'hui, *C. gigas* représente le mollusque le plus cultivé avec une production mondiale de près de 4,6 millions de tonnes en 2006 (FAO, 2008). Cette production s'explique par son potentiel de croissance élevé et sa grande tolérance aux variations de température (espèce eurytherme), de salinité (espèce euryhaline) et de quantité et qualité de nourriture (Grizel et Héral 1991 ; Goulletquer et al., 1999 ; Sicard et al., 2006).

1.2. Histoire de l'ostréiculture en France

Les bivalves présentent un intérêt économique comme ressource alimentaire depuis l'Antiquité. D'après Héral (1991) leur culture est très ancienne avec les premiers essais qui remontent dès l'époque romaine. Cette ostréiculture reposait alors sur l'huître plate (*Ostrea edulis*), seule espèce des côtes européennes. Cette espèce a été exploitée durant plusieurs siècles avant d'atteindre une surexploitation des bancs naturels à partir du XVIIIème siècle. Une réglementation d'exploitation a été mise en place au XIXème, parallèlement à une amélioration des techniques de captage, donnant naissance à l'ostréiculture moderne (Héral, 1991). Cependant, à partir de 1860, pour faire face à une pénurie d'huître plate, l'huître creuse portugaise *Crassostrea angulata* a été importée dans le bassin d'Arcachon. Cette espèce se répandit le long du littoral français jusqu'en 1960, puis à partir de 1970, a connu à son tour un déclin avec deux épidémies d'origine virale (Comps et al., 1976 ; Elston, 1993). En parallèle, la production de *O. edulis* a décru à cause de la surexploitation puis l'apparition de parasites dans les années 70-80 (Grizel, 1974 ; Pichot et al., 1979). Devant cette baisse d'exploitation drastique, le Comité National de la Conchyliculture (CNC) a pris la décision en 1971 d'introduire du naissain et des adultes de l'huître du Pacifique *Crassostrea gigas* du Japon et du Canada pour relancer la production ostréicole (Grizel et Héral, 1991). Progressivement, la

culture de *C. gigas* s'est développée avec succès et aujourd'hui cette espèce représente près de 95% de la production d'huître en France (FAO, 2008).

2. La Station Expérimentale d'Argenton

Avant d'aborder plus précisément les questions qui ont permis de développer ce projet de thèse, une présentation de la Station Expérimentale d'Argenton est un préalable indispensable.

La Station d'Argenton, située dans le Nord-Ouest du Finistère (Bretagne, France), est l'outil expérimental du Laboratoire de Physiologie des Invertébrés qui s'inscrit dans le Département de Physiologie Fonctionnelle des Organismes Marins rattaché au Centre de Brest, Ifremer. Lors de sa création en 2000, elle avait maintenu ses efforts de recherche dans le but de maîtriser les productions des bivalves en écloserie, tout particulièrement les processus de reproduction et l'optimisation du développement larvaire.

Actuellement, la Station d'Argenton est plus diversifiée et elle est impliquée d'une façon générale dans trois actions ou projet de recherche : (1) Action Velyger : Analyse des causes de variabilité du captage sur les côtes françaises ; (2) Action Generic : Modélisation de la croissance des bivalves marins d'intérêt aquacole ; et (3) Projet Soja : Sécurisation et Obtention des Juvéniles de quAlité. C'est dans ce dernier projet que s'est inséré ce travail de thèse.

Pour l'ensemble de ces travaux et particulièrement pour les études expérimentales de cette thèse, la culture de microalgues représente une partie importante des activités de la Station et à ce titre, quelques précisions méritent d'être apportées. Il faut ainsi préciser que la recherche d'un aliment alternatif aux microalgues vivantes utilisées en écloserie a fait l'objet de nombreuses recherches (Robert et Trintignac, 1997 ; Knauer et Southgate, 1999),

cependant la production de microalgues fraîches constitue toujours la seule source reproductible de nutrition pour le bon fonctionnement d'une écloserie.

La Station d'Argenton maintient trente cinq espèces ou clones de microalgues dont cinq sont produites régulièrement pour servir de nourriture aux bivalves à différents stades de développement (larves, juvéniles et géniteurs). La plupart des microalgues sont originaires de la Culture Collection of Algae and Protozoa CCAP (Grande Bretagne). Elles sont cultivées selon la méthode dite des volumes croissants (Helm et al., 1979), consistant à passer progressivement d'un petit récipient (150 ml ici) à des containers de volume intermédiaire (2-6 l) pour initier les cultures en grand volume (300 l).

Toutes les cultures de microalgues sont réalisées avec de l'eau de mer de salinité 35 psu et préalablement préfiltrée, sur un filtre automatique à lamelles (Splin-Klin Arkal) de porosité 25 µm puis sur poche en polypropylène de 5 µm (type PBF-012). A l'entrée de la salle d'algue, l'eau de mer est thermorégulée à 22-24 °C et à nouveau filtrée à 1 µm sur poche en polypropylène puis sur trois cartouches Nexis à 1 µm installées en cascade et finalement traitée aux ultraviolet (TP 100 Katadyn puissance 75 W) pour son utilisation en culture en grand volume. L'eau de mer est stérilisée à l'autoclave (Lequeux capacité de 165 et 370 l pour les petits et moyens volumes) à la température de 120 °C sous une pression de 1 bar pendant 45 min.

L'air est produit à partir de trois surpresseurs (deux en fonction et le troisième en secours automatique), à palette sèche en graphite à raison de 416 l min^{-1} (Pico-DLT 25 Rietschle). Sa pression est maintenue à 1,8 bars. Il est ensuite filtré sur cartouche à un seuil de 0,01 µm (Deltech 816).

Le milieu de Conway est utilisé comme source de sels nutritifs pour l'ensemble des cultures de microalgues, avec un supplément de silicates pour les diatomées (Walne, 1974). Les souches (non destinées à la production régulière de la Station) sont repiquées toutes les

deux semaines et conservées axéniquement dans les erlenmeyers (250 ml), fermées par des bouchons en silicosen (autorisant un échange gazeux avec l'extérieur), sous faible éclairage (30 µmoles photons m^{-2} s^{-1}) et maintenues à une température de 19 ± 1 °C.

Les cultures de microalgues en petits volumes (150 ml) pour la production régulière sont maintenues dans des conditions analogues mais sont repiquées plus souvent (hebdomadaire). Trois générations sont conservées ainsi pour pallier d'éventuels incidents. Une seule génération servira à démarrer la production.

Ces volumes sont éclairés en permanence sur toute leur surface par des néons de type Blanc industrie de 58 W (dotés de ballast électronique limitant le dégagement de chaleur) pour permettre un éclairage de 205 µmoles photons m^{-2} s^{-1}. Les cultures sont fermées par des bouchons en versilic traversés par deux cannes en verre, une longue plongeant jusqu'au fond de la culture pour l'adjonction d'un mélange air/CO_2 et une courte permettant l'échappement gazeux. Pour stabiliser le pH 1-2 % de CO_2 sont apportés et le brassage des cultures est donc assuré par un débit de 0,2 à 0,6 l min^{-1} pour 2 l de volume de culture et 0,8 à 1 l min^{-1} pour celui de 6 l. La température de la salle est comprise entre 20-22 °C.

Finalement, la culture en grand volume (300 l) comprend quatre lignes de onze cylindres chacune en PMMA de 5 mm, transparent thermosoudé sur une embase conique de même matière. Un ensemble de tubes fluorescents appariés et protégés par des tubes en PVC transparents, dotés de bagues étanches et de ballasts électroniques, illumine la culture sur une seule paroi à 28 000 lux. La température de la salle est comprise aussi entre 20-22 °C. L'embase des cylindres est dotée d'une arrivée d'air enrichi en CO_2 (1-2 %) à un débit de 3 l min^{-1}. La température, le débit de CO_2 et la concentration cellulaire de l'ensemble des cultures sont contrôlés quotidiennement.

Une autre activité importante de la Station Expérimentale d'Argenton, pour la présente étude, est le conditionnement de géniteurs. Celui-ci a été opéré à partir d'huîtres âgées de

deux ans, en provenance du bassin d'Arcachon et placées en poche à l'Aber Benoît (Bretagne nord) pendant deux mois. Ce conditionnement dure ainsi 45 jours à 19 °C avec un apport nutritionnel quotidien équivalent à 6 % du poids sec moyen des bivalves (poids sec d'algues/poids sec de la chair) (Utting et Millican, 1997) rapporté au poids sec d'*Isochrysis affinis galbana* (clone T-ISO, souche CCAP 927/14), *Chaetoceros gracilis* (souche UTEXLB2658) et *Skeletonema marinoï* (souche CCAP1077/3).

Finalement, en ce qui concerne l'élevage larvaire, la Station d'Argenton a investi ses efforts de recherche dans le domaine de la nutrition, dans l'optimisation des nouvelles techniques d'élevage et la détermination des besoins écophysiologiques ; plusieurs de ces avances sont exposées au cours de différents chapitres de thèse.

3. Contexte de l'étude

La production ostréicole française est principalement issue du captage naturel du naissain à partir de deux bassins de production, les bassins d'Arcachon et de Marennes-Oléron. Certains étés, ce captage peut concerner des zones situées plus au nord comme la baie de Bourgneuf (Glize, 1999) et la rade de Brest (Pouvreau, 2008). Le captage représenterait 5,6 milliards de naissains d'huîtres en 2008 (Le Roux, 2009). Cependant, la fourniture du naissain passe également par des méthodes d'élevage en milieu contrôlé via l'écloserie. En 2008, un total de 1,4 milliards de naissains provenait d'écloseries commerciales localisées en Vendée, Charente-Maritime et Manche (Le Roux, 2009). L'utilisation de naissain d'écloserie est en pleine expansion, d'une part, à cause des très fortes irrégularités de captage interannuelles dans les bassins de production avec des écarts d'abondance de naissain allant de 45 à 20 000 individus par tuile (Auby et al., 2008). D'autre part, il y a un intérêt croissant en faveur des nouveaux produits ostréicoles, comme les huîtres triploïdes qui poussent plus rapidement,

montrent une faible fécondité et une plus grande résistance aux mortalités estivales (Samain et McCombie, 2007). L'accompagnement de ces activités est une des préoccupations d'Ifremer car toute ostréiculture repose sur un approvisionnement régulier de naissain de qualité.

Or, si une zootechnie adaptée a été mise en place pour la production de naissain dans la plupart des écloseries commerciales actuelles, de nombreuses connaissances fondamentales sur les larves de cette espèce restent à acquérir pour sécuriser les rendements des élevages larvaires et maîtriser pleinement les procédés. En effet, les écloseries de bivalves utilisent des techniques qui n'ont pas sensiblement évolué depuis les années 1960 et 1970 avec les travaux des pionniers dans le domaine (Loosanoff et Davis, 1963 ; Walne, 1965 et 1974 ; Breese et Malouf, 1975). Cependant, les méthodes d'élevage sont variables d'une écloserie à une autre (type de microalgues, conditions de culture, température, système et densité d'élevage, etc.) et l'analyse du suivi des productions en écloseries commerciales reste délicate (Robert et Gérard, 1999). En plus, cette variabilité des performances est biaisée par une sélection systématique lors du tri par tamisage, tous les deux à trois jours, des larves à fort taux de croissance pour homogénéiser la production en écloserie (Laing et Earl, 1998).

La Station Expérimentale d'Argenton a donc développé des travaux expérimentaux visant à acquérir ces données. Afin de disposer de gamètes matures toute l'année, les premières études ont été dédiées à la reproduction de *C. gigas* pour laquelle une très forte plasticité était relevée et donc une adaptation assez large aux paramètres du milieu. Parmi ceux-ci la température et la nourriture s'avéraient être les principaux facteurs à contrôler pour assurer un bon conditionnement (Chávez-Villalba et al., 2001, 2003a ; Enriquez-Diaz, 2004 ; Cannuel et Beninger, 2005 ; Fabioux et al., 2005). De plus, il a été clairement démontré chez *C. gigas* qu'une part essentielle de l'énergie accumulée dans des ovocytes est issue des réserves initiales et/ou de l'alimentation des géniteurs (Gérard et al., 1997 ; Utting et Millican, 1997). Cependant, il n'y a pas d'interférence entre ces réserves initiales et la croissance

larvaire ultérieure lorsque l'apport exogène de microalgues est a priori équilibré (régime plurispécifique). Ainsi des géniteurs pourvus de réserves mais non alimentés pendant le conditionnement sont capables de produire des ovocytes de qualité (récupération par scarification), le développement larvaire ultérieur n'étant nullement affecté et ce à plusieurs périodes de l'année (Chávez-Villalba et al., 2003a). Donc, l'empreinte du conditionnement sur le développement futur de la larve d'huître est limitée chez *C. gigas* sauf dans des cas extrêmes (absence de réserve initiale et absence d'apport nutritionnel au cours du conditionnement) entraînant une carence forte en de nombreux éléments nutritionnels.

Dans ces conditions (interférence limitée), il devenait plus aisé de chercher à optimiser le développement larvaire. Celui-ci est schématiquement représenté en Fig. 1.

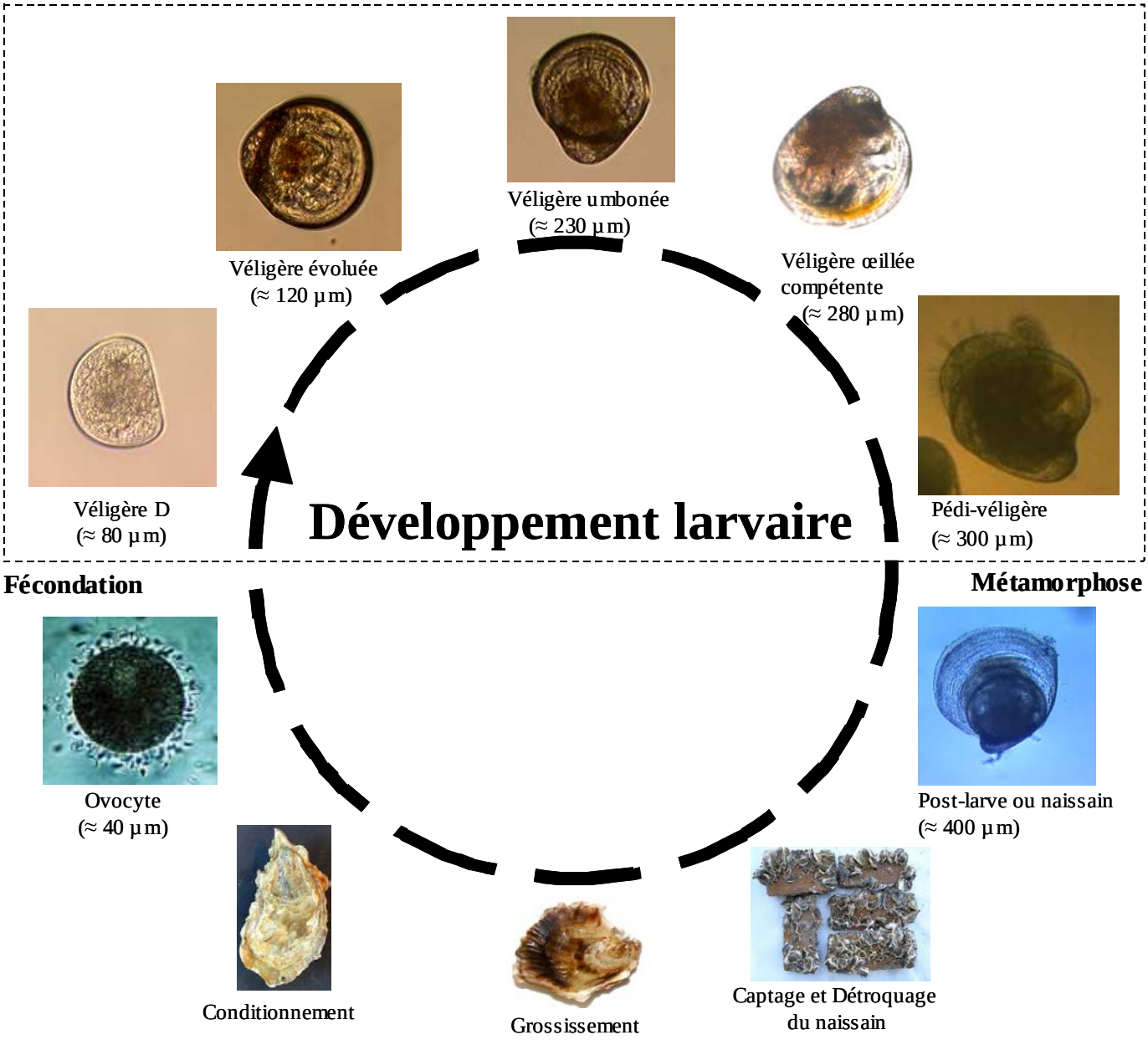

Fig. 1. Les grandes étapes de l'élevage de l'huître creuse *Crassostrea gigas*. Le développement larvaire (étape étudiée au cours de cette thèse) est encadré sur le schéma.

Depuis les premiers travaux relatifs aux techniques d'élevage de mollusques bivalves, avec le principal ouvrage publié par Loosanoff et Davis (1963), l'étude des effets du milieu naturel sur la physiologie a pris une place importante dans la recherche. En effet, l'écophysiologie est un champ restreint de la physiologie, qui étudie l'effet des facteurs externes sur les fonctions normales d'un être vivant. De ce fait, des travaux ont ainsi été consacrés à l'influence des conditions environnementales sur la croissance et la survie larvaire chez différentes espèces de bivalves (Nascimento, 1980 ; Robert et al., 1988 ; Lemos et al., 1994 ; Baldwin et Newell, 1995 ; Devakie et Ali, 2000 ; Manoj et Appukuttan, 2003 ; Dove et O'Connor, 2007). En ce qui concerne les études menées sur *Crassostrea gigas*, il se trouve que la disponibilité en nourriture et la température sont considérées comme les facteurs exerçant un effet prépondérant sur le développement larvaire (Helm et Millican, 1977 ; Gerdes, 1983 ; His et al., 1989 ; Abdel-Hamid et al., 1992). D'autre part, la salinité (15-40 psu) est décrite comme un facteur ayant peu d'influence dans la survie et la croissance des larves de bivalves en général (Lemos et al., 1994 ; Tan et Wong, 1996 ; Dove et O'Connor, 2007) tout comme chez *C. gigas* (Helm et Millican, 1977 ; Nell et Holliday, 1988 ; His et al., 1989).

Malgré ces travaux sur l'effet de ces facteurs du milieu sur la physiologie des larves de *C. gigas*, les connaissances restent encore partielles. Une telle divergence peut être expliquée, d'une part, par les différences de conditions expérimentales employées (système d'élevage, outil de mesure, etc.). En effet, l'étude écophysiologique d'un organisme marin repose sur un suivi minutieux des paramètres hydrobiologiques. Cependant, la plupart des travaux en écophysiologie larvaire ont été réalisés en système clos ou dans des enceintes avec un nombre restreint d'animaux et toujours au cours d'une brève période expérimentale. Ainsi chez des larves de l'huître creuse cette durée n'excède pas 10 jours (Helm et Millican, 1977 ;

Nascimento, 1980 ; His et al., 1989 ; Abdel-Hamid et al., 1992), alors que l'aboutissement du développement larvaire de cette espèce n'est acquis qu'au bout de 21 jours à 25 °C.

Or, l'influence de la nourriture sur les taux d'ingestion doit être étudiée dans un système de flux ouvert où l'activité d'ingestion des larves peut être mesurée dans des conditions constantes tout au cours de leur développement afin d'éviter les biais liés à la baisse de concentration en phytoplancton en système clos (Gerdes, 1983a ; Beiras et al., 1994 ; Riisgard, 2001). En outre, l'utilisation d'un système en flux ouvert évite le transfert et la manipulation de larves lors des mesures réduisant ainsi le stress des organismes.

Afin de lever ces contraintes, nous nous sommes intéressés à la mise en place d'un système en flux ouvert adapté à l'élevage larvaire de *C. gigas* et susceptible d'appréhender, de façon plus cohérente, la réponse physiologique des larves à leur milieu tout au long de leur développement. L'approche choisie a été d'utiliser la technologie du type MAREL développée par Ifremer (Blain et al., 2004). Le fonctionnement de ce système permet le suivi horaire des différents paramètres hydrobiologiques, *i.e.* température (°C), salinité (psu), fluorescence (FFU) et pH, dans chacun des bacs expérimentaux. Les données sont enregistrées et envoyées sur un serveur toutes les heures, et consultables sur le réseau Ifremer d'où elles sont extraites pour les traiter. L'ensemble de ces mesures nous permet de surveiller l'état général des élevages et le bon respect des conditions expérimentales.

D'autre part, afin de comprendre l'implication de chacun des facteurs et leurs interactions sur la physiologie des larves, l'approche modélisation semble un outil approprié. En effet, pour évaluer la croissance d'un organisme, il est nécessaire de passer par un modèle de croissance, ce qui implique l'étude de la physiologie, et plus précisément l'étude des paramètres environnementaux susceptibles d'influencer les fonctions physiologiques impliquées dans la croissance (Pouvreau et al., 2006). En effet, de nombreux modèles ont été développés au cours de vingt dernières années, afin d'expliquer la croissance de bivalves face

à leur environnement, *i.e.* la température et la disponibilité en nourriture (Ross et Nisbet, 1990 ; Barillé et al., 1997 ; Scholten et Smaal, 1998 ; Pouvreau et al., 2000 ; Hawkins et al., 2002). Au même moment, la modélisation de la croissance de l'huître creuse a connu une approche de plus en plus détaillée au cours de cette période (Bacher et al., 1991 ; Raillard et al., 1993 ; Barillé et al., 1997 ; Gangnery et al., 2003) mais principalement chez le stade adulte. Ces premiers travaux ont fait appel à des formulations empiriques pour des processus physiologiques aussi importants que la filtration, l'excrétion, l'absorption ou la respiration basés sur le potentiel de croissance ou « Scope for Growth » (SFG) (Bayne, 1976).

En ce qui concerne cette espèce, un seul modèle a été développé au niveau larvaire (Bochenek et al., 2001), qui lui aussi repose sur le concept du SFG. Ce seul modèle larvaire, utilisé sur d'autres études (Powell et al., 2002; Hofmann et al., 2004), reste relativement complexe (nombre important de paramètres à déterminer) et manque de validations sur la base de données expérimentales. De ce fait, nous nous sommes intéressés à l'amélioration d'un modèle de croissance chez la larve de l'huître creuse *C. gigas*.

Dans un premier temps, le présent travail de thèse devait déboucher sur la modélisation de la croissance larvaire de l'huître creuse dans l'esprit du SFG. Les modèles construits sur cette notion reposent sur une équation de bilan bioénergétique intégrant dans le temps les différentes fonctions physiologiques, notamment la nutrition et la respiration. Cependant, les modèles qui sont basés sur le concept de SFG ne détaillent pas les principes d'allocations énergétiques au sein de l'organisme. De plus, ils reposent sur des lois allométriques empiriques, *i.e.* les coefficients allométriques ne sont pas fixés et peuvent varier entre les espèces. Un autre problème inhérent au SFG est la prise en compte de la respiration comme une perte d'énergie dans le bilan bioénergétique pour l'organisme. Cette notion reste assez confuse car la respiration englobe la somme totale de nombreux processus (digestion, assimilation, croissance et maintenance) (Kooijman, 2000). La respiration est donc considérée

comme l'oxydation de composants biochimiques et l'énergie libérée est allouée dans la synthèse de nouveaux composants pour des différents processus chez un organisme. De ce fait, l'interprétation de la respiration reste assez complexe.

Nous nous sommes donc tournés vers un autre modèle bioénergétique. Le modèle de croissance proposé dans la présente étude est basé sur le budget d'énergie dynamique « modèle DEB » issu de la théorie « DEB » (Dynamic Energy Budget theory) développé par Koiijman (2000). Cette théorie bioénergétique assez récente fournit une trame générale afin de décrire les flux et les allocations énergétiques à travers un organisme vivant, tout au cours de son cycle de vie. Cette approche consiste à synthétiser l'ensemble des processus énergétiques clés : acquisition (ingestion et assimilation), stockage (dynamique des réserves), utilisation (maintenance, croissance, développement et reproduction). Le modèle de type DEB, n'étant pas spécifique, il possède la particularité d'être applicable à tous les organismes vivants (bactéries, plantes, animaux) et à tous les stades de vie (*i.e.* de la larve à l'adulte) d'une même espèce. En conséquence, l'un des principaux intérêts de cette théorie est son aspect générique. La seule différence intra et interspécifique réside dans la valeur des paramètres du modèle DEB. C'est ainsi que nous nous sommes intéressés à appliquer le modèle DEB développé chez l'huître creuse *C. gigas* dans sa version adulte et largement validée (Ren et Ross, 2001 ; Bacher et Gangnery, 2006 ; Pouvreau et al., 2006 ; Ren et Schiel, 2008 ; Bourles et al., sous presse) dans une version adaptée à la larve (stade véligère D à la métamorphose) et ce pour la première fois.

Comme mentionné, les modèles de type DEB intègrent la dynamique de l'ensemble des flux énergétiques d'allocation et réserve d'un organisme sur le rôle fondamental des ratios surface/volume. Le schéma conceptuel de la figure 2 résume les échanges d'énergie selon la théorie DEB (Koiijman, 2000). D'une part, la capture de nourriture dépend hyperboliquement de la densité de nourriture. D'autre part, l'assimilation d'énergie, provenant de l'ingestion de

la nourriture, est proportionnelle à la surface de l'organisme et cette énergie arrive directement dans un compartiment de réserves. Elle est aussi dépendant du type de nourriture ingéré.

Ensuite, la dynamique des réserves dépend de l'entrée d'énergie (flux d'assimilation) et de la sortie de cette énergie pour le métabolisme (flux d'utilisation). Ce flux d'utilisation suit la règle suivante : une fraction fixe de l'énergie de réserves (κ) est allouée à la maintenance somatique plus la croissance du soma (tissus) ; le reste d'énergie (1-κ) est destiné à l'investissement supplémentaire lié au développement (stade juvénile) ou à la reproduction (stade adulte), et à leur maintenance. La théorie DEB donne toujours la priorité à la maintenance sur la croissance ou le développement. A noter aussi que la théorie définit un juvénile comme un stade chez un organisme qui n'investit pas encore dans la reproduction mais dans son développement. En se basant sur cette définition spécifique, le stade « larve » est donc considéré comme un « juvénile » dans la théorie DEB. Le flux d'énergie (1-κ) est donc différencié pour une larve par le fait que l'allocation de l'énergie est utilisée pour le besoin supplémentaire lié aux développements de nouveaux organes ou de nouvelles fonctions chez la larve (tache ocellaire, pied, système enzymatique ou immunologique) afin de devenir un juvénile et ensuite un adulte.

Le modèle DEB est donc défini par trois variables d'état : une variable d'énergie de réserves (E) par où transite toute l'énergie allouée à l'organisme, une variable de structure (E_V) qui décrit l'allocation d'énergie pour la croissance et une variable d'énergie (E_R) qui décrit le développement de l'organisme au cours de son stade larvaire puis son effort de reproduction à travers son stade adulte. Les variables forçantes dans le modèle sont la nourriture disponible dans le milieu et la température de l'eau puisque la vitesse de tout processus physiologique est soumise à son effet.

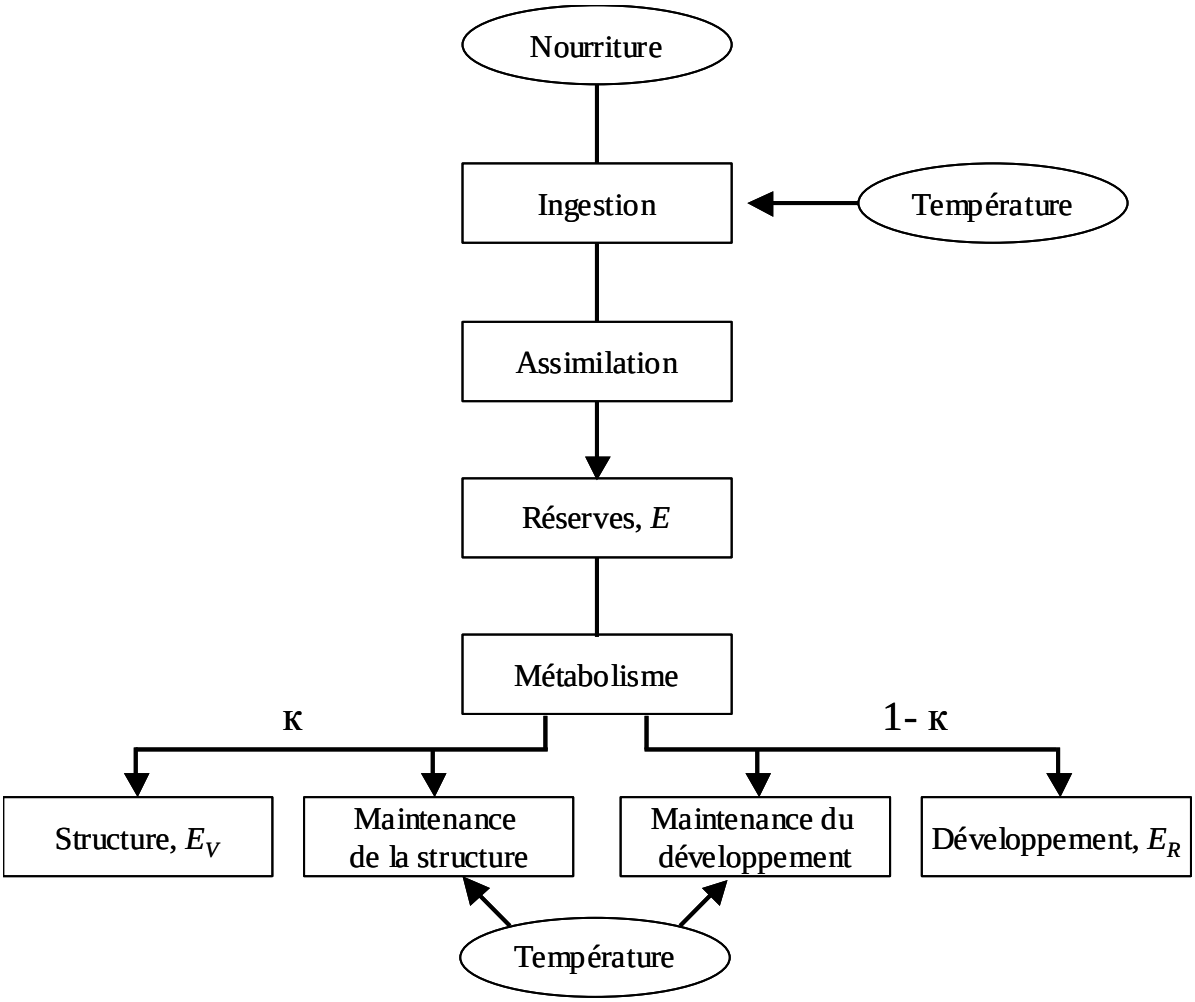

Fig. 2. Schéma simplifié résumant le flux d'énergie chez la larve de *C. gigas* sur la base de la théorie DEB (Koiijman, 2000).

4. Plan de la thèse

Cette thèse présente l'étude des éléments nécessaires à l'établissement des besoins écophysiologiques des larves d'huître creuse *C. gigas* en conditions contrôlées. Ce travail portera également sur la modélisation de la croissance des larves en écloserie. Il permettra, d'une part, de cerner les grands mécanismes de l'écophysiologie larvaire expliquant la croissance et, d'autre part, de comprendre l'influence et le poids de chacun des facteurs de l'environnement sur celle-ci. Dans ce contexte, ce modèle a pour objectif principal d'apporter de nouvelles connaissances scientifiques sur la physiologie tout au cours du développement larvaire, pouvant permettre d'évaluer les performances attendues des larves en fonction des facteurs environnementaux (température, nourriture…). Par ailleurs, tout ou partie de ces travaux pourront être utilisés pour mieux appréhender le développement larvaire de l'huître creuse en écloserie ainsi qu'en milieu naturel dans les zones de reproduction. A terme, tous ces éléments seront favorables à une amélioration de la production ostréicole.

Cette étude est regroupée en quatre chapitres et à la fin une conclusion générale avec une synthèse et des perspectives. Les chapitres 1, 2, 3 et 4 sont écrits en anglais sous forme d'articles scientifiques afin de faciliter leur soumission pour publication. Les trois premiers sont déjà publiés et le dernier vient d'être soumis.

Le **premier chapitre** traite, tout d'abord, de l'étude qualitative de la consommation des larves exposées à différents régimes de microalgues de type monospécifique, bispécifique et trispécifique tout au cours de leur développement ainsi que les performances larvaires basées sur la croissance, la survie et la métamorphose. Le régime le plus performant issu de la première partie a été évalué par la suite dans différentes proportions pour affiner cette approche qualitative sur la consommation et les performances larvaires. Enfin, une étude biochimique a été effectuée en s'attachant à l'évolution des teneurs des acides gras totaux et

stérols au cours du cycle larvaire pour dégager une relation entre les différentes compositions du régime le plus performant et leur incidence sur la métamorphose en prenant le facteur taille comme paramètre de compétence. Un tel régime constituera un préalable pour les expériences au laboratoire dans les chapitres suivants.

Le **second chapitre** porte sur la mise au point d'un système d'élevage larvaire en flux ouvert issu d'une série d'expériences. Cet outil en flux ouvert a été équipé d'un système d'acquisition de données en continu capable de réaliser des mesures des paramètres hydrobiologiques (température, salinité, fluorescence et pH) dans le but de fournir des données expérimentales pour l'étude en écophysiologie larvaire. Cet outil a été nommé SILO pour Système d'Instrumentation des Larves en flux Ouvert. Finalement, deux expériences ont été réalisées pour valider le fonctionnement de SILO en mettant en évidence les effets de la température sur la croissance et l'ingestion des larves.

L'objectif du **troisième chapitre** est de préciser l'influence de la densité du phytoplancton et de la température, en utilisant SILO, sur l'ingestion, la croissance et la métamorphose des larves tout au cours de leur développement (véligère D à la post larve). Pour ce faire, nous avons établi des conditions expérimentales constantes avec une large gamme de nourriture autour de la larve (12, 20 et 40 cellules μl^{-1}) et de température (17, 22, 25, 27 et 32 °C).

Le **quatrième chapitre** se rapporte à la construction d'un modèle de croissance des larves basé sur la théorie du « Dynamic Energy Budget » (DEB). D'une part, nous détaillons les méthodes et données utilisées pour estimer les paramètres du modèle bioénergétique DEB et, d'autre part, nous décrivons la calibration, la simulation et la validation du modèle pour mettre en évidence les interactions entre l'environnement et la croissance de la larve.

Dans la **conclusion générale**, nous reprenons les principaux résultats obtenus au cours de cette thèse afin de montrer les avancées réalisées dans la connaissance de la physiologie

des larves de l'huître creuse *C. gigas* et comment ces résultats apportent de nouvelles améliorations dans les pratiques d'élevage larvaire en écloserie. Finalement, en s'appuyant sur nos différents résultats, nous proposons certaines perspectives à ce travail.

CHAPITRE I

Influence of phytoplankton diet mixtures on microalgae consumption, larval development and settlement of the Pacific oyster *Crassostrea gigas* (Thunberg)

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Abstract

Microalgae commonly used as feed for bivalves, *Pavlova lutheri* (P), *Isochrysis affinis galbana* (T) and *Chaetoceros calcitrans* forma *pumilum* (Cp), were fed to Pacific oyster *Crassostrea gigas* to assess their nutritional value for larval development and metamorphosis during two experiments. Monospecific, bispecific and trispecific diets were firstly evaluated during 3 weeks from D larvae to young postlarvae. Then bispecific diets, based on different T and Cp proportions, were performed during a similar period. Concurrently, ingestion was studied through the whole larval and postlarval development for each diet and/or diet mixture. Because lipids are assumed to be a key nutrient for bivalves, biochemical analysis was undertaken on the second set of trials focused on fatty acids and sterols. Compared to the other diet mixtures (mono and plurispecific diet) TCp induced the best larval growth performance (13.2 µm day^{-1}), a high larval survival (98%) but did not result in higher metamorphosis (72%). In contrast, monospecific diet P was the poorest produced for larvae with low growth and low survival. When varying T and Cp proportions, best larval developments were induced with 25T/75Cp and 50T/50Cp, though quite similar to that obtained with 75T/25Cp. In contrast, unbalanced diets (95T/5Cp and 95Cp/5T) led to low larval performances. In addition, grazing experiences showed preferential uptake of microalgae with P<PT<<T<<Cp<<TCp=PCp=PTCp. For mixed diets a low daily consumption (<10 000 microalgae per larvae) was noted during the first week followed by a second phase (next 8-10 days) with a sharp increase and regular intake, reaching 90 000 microalgae per larvae per day. Finally a marked drop (40 000 microalgae per larvae) was observed at the beginning of metamorphosis from days 20 to 21. Principal component analysis between main fatty acids (19) and sterols (7) detected in larvae and postlarvae was used to discriminate profiles according to diets and/or metamorphosis competence. The correlation circle representation showed that the 26 variables are well explained by these combined variables (78%) with a repartition along the first principal component according to diets with a gradient from 5T/95Cp to 95T/5Cp. In contrast, postlarvae and larvae were discriminated on the second principal component while no relationships were found between competent and incompetent larvae.

Keywords: *Crassostrea gigas*, Larvae, Growth, Metamorphosis, Grazing, Lipids

1. Introduction

French oyster farming is dominated by the cupped *Crassostrea gigas* with 107 390tons *vs.* 1650 tons of the flat oyster *Ostrea edulis* (AGRESTE, 2005). This major production used to depend on natural spat collection but there is an increased reliance on hatchery spat in France from 5–10% in 2000 to over 30% in 2003 (Scheffer et al., 2003). Now, despite an indisputable know-how in mollusc hatchery some biological aspects are still unknown and beyond them bivalve feeding requirements are poorly understood (Knauer and Southgate, 1999; Volkman and Brown, 2006). Bivalves, unlike larvae fish and crustaceans, are fed microalgae directly. Consequently, bivalve development is closely related to the quantity and quality of phytoplankton available. To be used as food for bivalve larvae, microalgae must exhibit some specific characteristics such as an adequate size for its ingestion (less than 10 µm with an optimal range of 2 to 5 µm), no thick cell wall for its digestion, a good food value (adequate biochemical composition) and, for practical and economical reasons, must be relatively easy to be bulk produced (Robert and Trintignac, 1997). Because a mixed algal diet increases the chances of achieving a balanced diet, microalgae are generally supplied in plurispecific rations for bivalves without a clear knowledge of their need in essential components (Muller-Feuga et al., 2003a).

However, significant larval production in hatchery relies on this empirical feeding method. Indeed, each experimental or commercial hatchery has its own microalgae mixture, which can change throughout larval development (Coutteau and Sorgeloos, 1992). Despite its diversity, a combination of at least a Haptophyceae and a Bacillariophyceae is often used (Robert and Gérard, 1999).

The empirical diet used for C. gigas larvae in the experimental hatchery of Argenton (North Brittany) was derived from that applied routinely for the scallop *Pecten maximus*

(Robert et al., 1994). This mixture, named PTCp, consists of two prymnesiophytes, *Pavlova lutheri* (Droop) and *Isochrysis affinis galbana* (Green), clone t. Iso, together with the diatom *Chaetoceros calcitrans* forma *pumilum* (Takano). While the role of each microalga on larval development and metamorphosis was known for *P. maximus* (Delaunay et al., 1993) such basic information was unavailable for *C. gigas* in our larval rearing context and the literature did not report complete data throughout the whole larval stage. Moreover, larval development performances have been widely studied from a biochemical angle (see Brown et al., 1997; Knauer and Southgate, 1999). However, that sole approach is insufficient to define the appropriate diets for bivalves. Indeed, the literature shows the nutritional key roles of two essential fatty acids, eicosapentaenoic (20:5*n*−3, EPA) and docosahexaenoic (22:6*n*−3, DHA) acids. However, though *P. lutheri* is relatively well balanced in both fatty acids, it does not support good growth for *C. gigas* larvae (Ponis et al., 2003), despite a suitable size for early stages (Robert et al., 2004). On the other hand, few studies on microalgae ingestion by bivalve larvae have been reported and were essentially targeted on cell size (Walne, 1963; Bayne, 1965; Riisgard and Randlov, 1981; Sprung, 1984a). However, Gerdes (1983a) showed a consumption increase by larvae when using mixed *vs.* a single diet. To our knowledge, such information has never been confirmed and the objective of the present work was to study effective consumption by veligers exposed to different monospecific, bispecific and trispecific diets in relation to *C. gigas* larval development performance based on growth, survival and metamorphosis. Such quantitative and qualitative diet constituted a prerequisite for future work dealing with improvement of *C. gigas* larval and/or postlarval development such as research of new microalgae (Ponis et al., 2006) and/or physiological indices (Ben Kheder, 2007).

2. Materials and methods

2.1. Microalgae

Five microalgae were used for broodstock conditioning and larval development. Many confusions in the identity of specific microalgae have been reported in the past and a target study was dedicated to clarify the situation. Growth, size, biochemistry profile, biomolecular and cytofluorimetry prints have been achieved for 15 microalgae used in Argenton hatchery as food for bivalves (Robert et al., 2004). Because such detailed information is now available, we only specify that microalgae used in the present study were originated from the Culture Collection of Algae and Protozoa (GB) such as *I. affinis galbana* (CCAP 927/14), *P. lutheri* (CCAP 931/1), *C. calcitrans* forma *pumilum* (CCAP 1010/05), *Skeletonema costatum* (CCAP1077/3), and that of the University of Texas (US) for *C. gracilis* (UTEX LB 2658). Microalgae used for larvae exhibited similar size in their exponential phase of growth (40 to 45 μm^3 equivalent to 4.2 to 4.4 µm diameter) as well as similar dry individual weight (18 to 20 pg: Robert et al., 2004) and mixed feeding ration was accordingly based on a ratio 1:1.

2.2. Broodstock conditioning and larval development

Broodstock were conditioned at 19°C, in a flow through system, with a daily mixed diet of 6% *I. affinis galbana, C. gracilis* and *S. costatum* per mg oyster (dry algal weight/dry meat weight).

Six weeks later, gametes from 3 males and 6 females, obtained by scarification, were fertilized on the basis of 50 spermatozoa per oocyte and 2h later 40 embryos ml^{-1} were stocked in 150-l culture tanks for further development. Two-day-old D larvae were then

distributed in 30-l cylindro-conical tanks at the density of 5 larvae ml^{-1}, in 1-µm filtered seawater at 25 °C and 34‰ salinity. Aeration was maintained throughout the whole larval life at 0.5 l min^{-1} to avoid diatoms and debris deposition promoting bacterial source of infection. Seawater renewal and tank cleaning occurred three times a week with no antibiotic addition. Larvae were fed daily on different sole or mixed diets, however at equal quantity and adjusted to increasing larval demand (Table I.1). At each draining, larvae were retained by a 40-µm nylon mesh-based sieve during the first week and then on 60 µm. Few or no larvae were lost during mesh aperture change (necessary to avoid sieve clogging) and accordingly, larval performances reported in the present work relate to the whole population. Larval length and survival were estimated on days 2, 7 or 9, 14 or 16 and 19 by use of image analysis technique (WinImager 2.0 and Imaq Vision Builder 6.0 software for images capture and treatment, respectively).

Table I.1

Evolution of daily food ration (total number of algal cells per microliter) related with larval and/or postlarval age during both experiments from days 2 to 21 (from fertilization). During the second set of trials, metamorphosis extended until day 26 with a daily mean ration maintained at 150 cells μl^{-1}.

Age of larvae (days)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Experiment 1	25	25	25	45	45	45	60	60	60	60	60	100	100	100	120	120	120	120	120	120
Experiment 2	25	25	25	30	50	50	50	80	80	80	120	120	120	120	150	150	150	150	150	150

2.3. Metamorphosis

In *C. gigas* metamorphosis occurs when larvae are ≥280 µm (Coon et al., 1990; Utting and Spencer, 1991). The number of pediveligers ready to set (competent larvae: presence of an eyespot) was estimated prior to a selective grading on a 225-µm aperture sieve mesh. Then, after counting, the largest larvae were transferred in 30-l tanks in presence of 15 cm diameter plastic disks, used as collectors, representing a removable setting surface of 2400–3200 cm^2. During metamorphosis rearing techniques were similar to those applied for earlier larval stages. Six to seven days later, experiments ended and settlement was evaluated by counting precisely the number of remaining pediveligers while an estimation of spat attached to collectors and tank walls were performed to check data validity.

The influence of diets on larval performances was assessed using one-way ANOVA while significant differences ($P \leq 0.05$) between treatments were compared using the Scheffé's pairwise multiple comparison test (Statview 5.0). Survival, yield, competency and metamorphosis data were previously transformed (arcsin [square (x/100)]) before analysis.

2.4. Grazing

For each nutritional condition, phytoplankton consumption was evaluated concurrently to establish potential correlation between larval development measures and algal cells ingestion. Microalgae used for larvae had similar cell size and were delivered, in different mixed diets, on an equal number of cells per µl of the culture tank volume. As larval development proceeds, biomass of larvae is ever increasing and food ration was adapted to support maximum larval growth. Such demand was previously established in 150-l cultures using bispecific diet and such results were applied in the present work as a guide (theoretical

demand: Table I.1). PTCp and 50T/50Cp were the reference diets, detailed below, during experiments 1 and 2, respectively, and when food limitation was detected in the controls, daily rations were adjusted accordingly and applied to the other nutritional conditions. Grazing was studied from D larvae to pediveligers including metamorphosis for all diets. Cell consumption was measured with an electronic particle counter ZM equipped with a 100-µm aperture tube. Ten minutes after food delivery and homogenisation, enriched seawater was sampled in triplicate in each tank through a 40-µm mesh sieve, to measure initial cell concentration. An additional sample was taken the following day (18 to 20 h later) and food uptake evaluated. Because larval survival was checked regularly, a value for the mean grazing per larvae per unit of time was established for all diets.

2.5. Experimental trials

Two sets of trials were carried out in April and October 2004. During the first set of experiments, the influence of monospecific diets (P=*P. lutheri*, T=t. Iso and Cp=*C. calcitrans* f. *pumilum*), and bispecific diets (PT, PCp and TCp) on larval growth, survival and metamorphosis were studied. Positive controls consisted in trispecific diet PTCp while unfed larvae were the negative controls. Apart from starved larvae maintained in 5-l beakers, larval rearing was operated in 30-l tanks, in triplicate, across 8 treatments. The second set of experiments dealt with the effects of bispecific diets mixed in different proportions, on larval development and settlement. Unfed larvae were used as negative controls while five nutritional mixtures, 5T/95Cp, 25T/75Cp, 50T/50Cp, 75T/25Cp and 95T/5Cp were applied, in triplicate.

2.6. Biochemical analysis

The deficiency in fatty acids (EPA or DHA) of monospecific diets and its effects on *C. gigas* larval development are well documented (Waldock and Nascimento, 1979; Thompson and Harrison, 1992; Thompson et al., 1996) and therefore such biochemical analysis was not undertaken in trial 1. In contrast, the effects of mixed diet based on different proportions of prymnesiophyte/bacillariophyte have not been reported in the literature to our knowledge. Because lipids are assumed to be key nutrient for bivalves, biochemical analysis performed on the second set of trials focused on fatty acids and sterols. 10^5 eggs, 5×10^5 D larvae, 10^5 pediveligers and 2000 spat were sampled for each nutritional combination at days 0, 2, 19 and 26, respectively. Prior to metamorphosis, larvae were graded and analysis performed on the group of competent larvae (C) as well as the incompetent (NC) fraction (not retained on 225-µm nylon mesh). Concurrently 3- to 7-day-old microalgae, used as feed for larvae during that experimental set, were collected twice a week, corresponding to seven different batches of culture per species. Biochemical analyses were performed on such biomass during the whole larval and early spat developments. Samples were collected on GF/C glass fibre filters and extracted in chloroform–methanol according to Folch et al. (1957). The neutral and polar lipids were separated in a silica gel microcolumn (30 mm × 5 mm; Kieselgel Merck, 70–230 µm mesh), previously heated to 450 °C and deactivated with 5% water as described by Soudant et al. (1995), and analyzed following the method described by Marty et al. (1992). Collected fractions were used both for fatty acid (Marty et al., 1992) and sterol analyses (Soudant et al., 1998b).

3. Results

3.1 Larval development and metamorphosis

TCp was the best diet leading to high survival (98%, Table I.2) and growth (13.2 µm d^{-1}, Fig. I.1). Larvae fed exclusively *C. calcitrans* f. *pumilum* exhibited an acceptable growth however with lower survival on day 16 (76%) and characterized by a high intertank variability (CV= 26%). In contrast, feeding larvae with *P. lutheri* led to a poor development, low growth and low survival, similar to starved larvae performances (Table I.2).

Table I.2

Mean shell length (S.D.) and survival (S.D.) of *Crassostrea gigas* larvae fed on different mono- or pluri-specific diets, with P = *Pavlova lutheri*, T = *Isochrysis affinis galbana* (clone t. Iso) and Cp = *Chaetoceros calcitrans* forma *pumilum*, on days 9 and 16. Values with same letters are not significant at $P>0.05$. Initial larval shell length on D2 = 78.37 µm (4.05).

	Length (µm) (D9)	**Survival (%) (D9)**	**Length (µm) (D16)**	**Survival (%) (D16)**
TCp	166.13 (23.99) [c]	99.83 (0.12) [a]	263.51 (49.56) [g]	98.33 (1.32) [a]
PCp	148.60 (20.88) [a]	99.47 (0.35) [a]	244.71 (44.78) [a]	98.84 (0.13) [a]
PTCp	154.53 (20.35) [a]	99.29 (0.42) [a]	239.91 (52.77) [a]	95.40 (2.09) [d]
Cp	149.30 (17.68) [a]	99.80 (0.15) [a]	219.00 (33.43) [b]	76.19 (20.16) [b]
PT	126.49 (23.21) [d]	99.88 (0.15) [a]	181.52 (53.10) [c]	81.81 (7.70) [b]
T	110.19 (17.04) [e]	99.22 (0.58) [a]	162.98 (41.40) [d]	85.98 (7.88) [b]
P	94.68 (13.56) [b]	99.67 (0.41) [a]	101.88 (18.57) [e]	29.23 (7.02) [c]
Unfed	89.44 (6.62) [b]	99.15 (0.25) [a]	81.86 (7.53) [f]	33.67 (3.78) [c]

While the number of pediveligers competent to metamorphosis (greater than 280 µm) was highest on day 16 for TCp diet (70%) metamorphosis, recorded 1 week later, was similar to the other mixed diets (72–77%)–except PT–, that never led to competent larvae (Table I.3). In contrast, larvae fed solely on *C. calcitrans* forma *pumilum* exhibited lower larval competence on day 16 (18%, Table I.3) and lower metamorphosis (54%, Table I.3) which was also characterized by an appreciable intertank variability (CV= 19%) In contrast, the larvae fed the other diets did not show any competence on day 16. Despite a prolonging of their rearing a similar situation occurred on day 22. The integration of the whole rearing performances was represented by the final yield (Table I.3) corresponding to the number of postlarvae/initial number of D larvae (D22/D2). Similar overall rearing efficiencies, 25–30%, were noted for PTCp and TCp diets while the use of *C. calcitrans* forma *pumilum* as feed throughout the whole larval process led to only 7% (Table I.3).

Table I.3

Mean number (S.D.) of pediveligers ready to set at the end of rearing period (D16), mean metamorphosis (S.D.) 6 days later (D22) and final harvest yield of *Crassostrea gigas* fed on different mono- or pluri-specific diets with P = *Pavlova lutheri*, T = *Isochrysis affinis galbana* (clone t. Iso) and Cp = *Chaetoceros calcitrans* forma *pumilum.* Values with same letters are not significant at $P>0.05$.

	Morphological competence (%) (D16)	**Metamorphosis (%) (D22/D16)**	**Final yield (%) (D22/D2)**
PTCp	58.50 (13.43) [a]	77.36 (1.58) [a]	29.30 (9.30) [a]
PCp	47.66 (8.50) [b]	76.49 (1.10) [a]	16.60 (4.60) [b]
TCp	69.00 (12.12) [c]	72.02 (0.89) [a]	25.30 (8.60) [a]
Cp	18.36 (11.53) [d]	54.13 (10.05) [b]	7.18 (6.59) [c]

When varying proportions from 5% to 95% of each microalgae in bispecific diets TCp, high growth, 12.6 µm day−1, high survival, 99% (Table I.4), high larval competence and metamorphosis (78% and 87.5%, respectively, Table I.5) were obtained with 50T/50Cp. Similar performances were reported with 25T/75Cp diet (Tables 4 and 5) while a 10-µm difference in length was noted for 75T/25Cp diet from day 7 (Table I.4) leading to lower competence on day 19, 53%, with however a negligible effect on metamorphosis, 83% (Table I.5). Unbalanced diets, 95T/5Cp and 5T/95Cp produced lower larval growth from week 1 or 2, respectively (Table I.4), lower survival, 60–75% (Table I.4) and lower larval competence, 17–28% (Table I.5). A high intratank heterogeneity of larvae fed with an excess of t. Iso (95T/5Cp) was noted with two subpopulations, the first with a normal development and the second with poor growth. The situation was totally different when larvae were fed with a deficiency in t. Iso (5T/95Cp) where a high variability also occurred, but at an inter tank level. Because metamorphosis competence was lower than 50% on day 19, no measure on settlement was performed for these unbalanced diets. In this second set of experiments, unfed larvae had similar performances to those recorded previously with negligible growth 0.4 µm day^{-1} and low survival, 30% (Table I.4).

Table I.4

Mean shell length (S.D.) and survival (S.D.) of *Crassostrea gigas* larvae fed on different proportions of *Isochrysis affinis galbana* (T) and *Chaetoceros calcitrans* forma *pumilum* (Cp) in bispecific diets TCp on days 7, 14 and 19. Values with same letters are not significant at $P>0.05$. Initial larval shell length on D2 = 78.60 µm (6.02).

Diet	Length (µm) (D7)	Survival (%) (D7)	Length (µm) (D14)	Survival (%) (D14)	Length (µm) (D19)	Survival (%) (D19)
5T – 95Cp	107.01 (13.38) [a]	99.71 (0.26) [a]	162.90 (24.26) [e]	69.00 (18.24) [a]	250.11 (46.38) [e]	61.33 (23.13) [a]
25T – 75Cp	114.71 (13.98) [b]	99.75 (0.15) [a]	217.97 (36.10) [a]	98.48 (1.01) [b]	290.46 (36.60) [a]	98.45 (0.53) [b]
50T – 50Cp	115.30 (13.84) [b]	99.70 (0.28) [a]	222.01 (32.71) [a]	99.04 (0.07) [b]	293.45 (38.28) [a]	98.87 (0.84) [b]
75T – 25Cp	106.82 (15.18) [a]	99.58 (0.33) [a]	210.18 (39.60) [b]	97.89 (0.60) [b]	280.57 (48.13) [b]	97.53 (2.15) [b]
95T – 5Cp	90.73 (9.67) [c]	88.93 (8.30) [b]	137.31 (47.70) [c]	76.57 (3.95) [a]	217.32 (68.43) [c]	75.24 (7.45) [a]
Unfed	86.08 (6.24) [d]	99.35 (0.18) [a]	85.56 (6.21) [d]	89.45 (4.56) [c]	85.76 (7.03) [d]	29.21 (16.34) [c]

Table I.5

Mean number of pediveligers ready to set (S.D.) at the end of rearing period (D19), metamorphosis (S.D.) and final yield (S.D.) 7 days later (D26) of *Crassostrea gigas* fed on different proportions of *Isochrysis affinis galbana* (T) and *Chaetoceros calcitrans* forma *pumilum* (Cp) in bispecific diets TCp. Values with same letters are no significant at $P>0.05$.

* No data.

	Morphological competence (%) (D19)	Metamorphosis (%) (D26/D19)	Final yield (%) (D26/D2)
5T – 95Cp	17.51 (13.46) [b]	*	*
25T – 75Cp	75.93 (3.64) [a]	85.88 (9.60) [a]	54.30 (8.30) [a]
50T – 50Cp	78.15 (5.15) [a]	87.45 (7.11) [a]	54.60 (9.60) [a]
75T – 25Cp	53.05 (4.30) [c]	83.28 (3.60) [b]	47.60 (8.00) [b]
95T – 5Cp	28.34 (7.03) [b]	*	*

3.2 Larval grazing

For experiment 1, grazing was determined for all diets, throughout larval development from days 6 to 15, and during metamorphosis up to day 22 for three of them (PCp, TCp and PTCp). Such a study, performed for each condition in triplicate and expressed as the number of cells eaten per day per larva, showed a preferential uptake of microalgae with P<PT<<T<<Cp<<TCp=PCp=PTCp. When solely fed *P. lutheri*, a *C. gigas* larva only consumed 1000 to 3000 per day from days 6 to 15 (Fig. I.1a). Associated with *I. affinis galbana*, uptake of such haptophytes mixture (PT) was weakly improved with a daily consumption of 1800 to 5000 for a similar period (Fig. I.1b). With a grazing of 1000 to 15 000 cells per larva per day, a better ingestion of t. Iso was paradoxically observed when larvae were fed on that single diet (Fig. I.1c). Over a similar period, 7000 to 65 000 cells of *C. calcitrans* forma *pumilum* were removed, however with high intertank variations (Fig. I.1d). When this diatom was mixed with t. Iso or *P. lutheri* a higher consumption was recorded as well as excellent pattern reproductivity. Indeed from days 6 to 15, larvae fed bispecific diets grazed daily 8000 to 35 000 microalgae to reach 85 000 on day 21. Food uptake decreased during metamorphosis (Fig. I.1e). The addition of another haptophyte (P) to these bispecific diets did not increase consumption (data not reported) and grazing patterns were remarkably similar as those reported in Fig. I.1e.

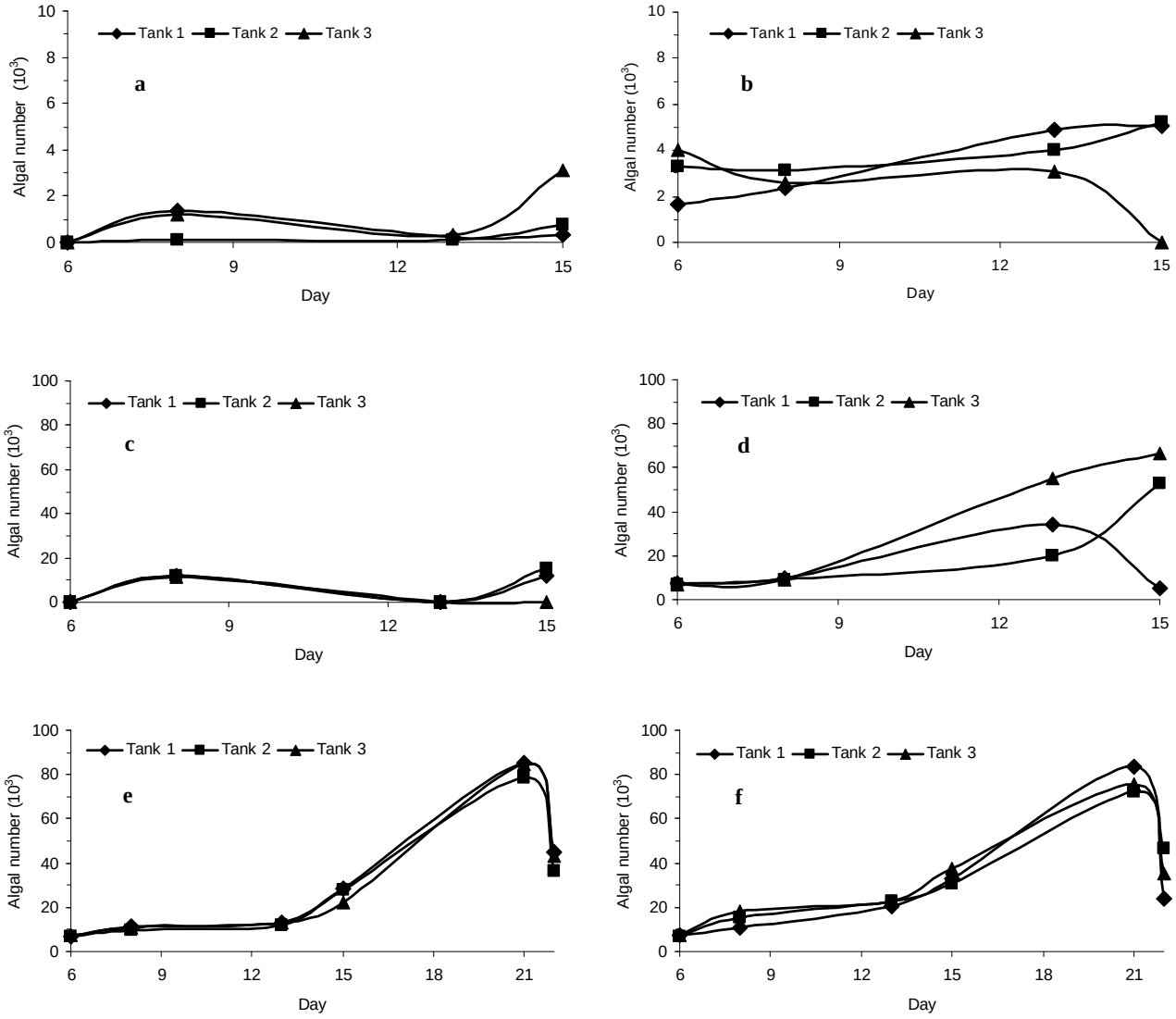

Fig. I.1. Evolution of grazing per day per larvae of *C. gigas* fed on different mono- or pluri-specific diets: (a) *P. lutheri* (P); (b) *P. lutheri* + t. Iso (PT); (c) t. Iso (T); (d) *C. calcitrans* f. *pumilum* (Cp); (e) *P. lutheri* + *C. calcitrans* f. *pumilum* (PCp); (f) t. Iso + *C. calcitrans* f. *pumilum* (TCp). Each condition was run in triplicate. From Day 6 to 16 this consumption was exclusively related to larvae while during metamorphosis newly settled postlarvae also contributed. Note that y-axis scale ranged from 0 to 10 for (a)–(b) graphs while it ranged from 0 to 100 for (c), (d), (e) and (f) graphs. Note also that the *x*-axis (time) corresponds to different sizes of larvae in the different treatments.

A thorough study performed during the second set of experiments with bispecific diets containing different proportions of T and Cp (apart from the unbalanced diets 95T/5Cp and 5T/95Cp) led to the identification of three phases. The first phase was characterised by a low consumption during the first ten days with a daily removal of 10 000 to 20 000 microalgae per larva (Fig. I.2). Then, up to day 20, an increased food uptake was recorded with values as high as 90 000 cells day^{-1} followed by a sharp 50% decrease in consumption during metamorphosis. Grazing recovered progressively until day 24 (60 000 microalgae per larva, Fig. I.2).

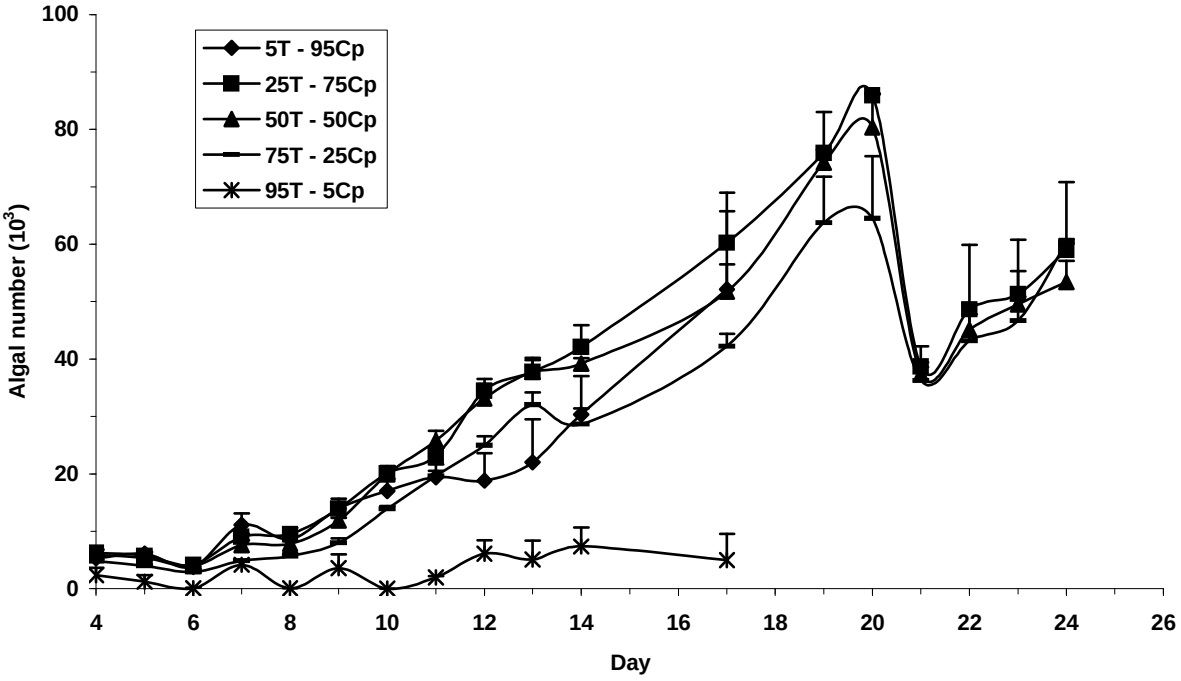

Fig. I.2. Evolution from days 4 to 24 of daily grazing per larvae of *C. gigas* fed on different proportions of *I. affinis galbana* (T) and *C. calcitrans* forma *pumilum* (Cp). Each condition was run in triplicate. From Day 4 to 19 this consumption was exclusively related to larvae while during metamorphosis newly settled postlarvae also contributed.

3.3 Biochemistry analysis

Fatty acid (FA) and sterol composition of both microalgae *I. affinis galbana* and *C. calcitrans* forma *pumilum*, of 19-day-old larvae and new settled postlarvae (≤5 day old) were determined. The diatom *C. calcitrans* forma *pumilum* was rich in 16:1*n*−7, 20:5*n*−3, cholesterol, 24-methylencholesterol and isofucosterol while t. Iso contained 14:0, 18:2*n*−6, 18:4*n*−3, 22:6*n*−3 and brassicasterol (Table I.6). The biochemical composition of 19-day-old larvae and 5-day-old postlarvae was clearly related to diet composition. For instance, brassicasterol, derived from t. Iso, was found at 55% in the larvae fed 95T/5Cp but only at 3.4% in the larvae fed 5T/95Cp (Table I.6). Similarly 20:5(*n*−3) and 24-methylencholesterol reflected the relative quantity of *C. calcitrans* forma *pumilum* eaten by larvae (Table I.6). Principal component analysis between main fatty acids (19) and sterols (7) detected in larvae and postlarvae was used to discriminate profiles according to diets and/or metamorphosis competence. Four components with eigenvalues higher than 1.0 explained 92% of the variability in the original data. The first principal component (PC1) explained 54.2% of the combined variance while 23.3% was explained by the second component (PC2). A correlation circle was plotted using PC1 and PC2 (Fig. I.3). This representation showed that the 26 variables are well explained by these combined variables (78%) with a repartition along PC1 according to diets with a gradient from 5T/95Cp to 95T/5Cp. In contrast, postlarvae and larvae were discriminated on PC2 while no relations were found between competent and incompetent larvae (Fig. I.3).

Table I.6. Total fatty acids and sterols composition (%) of *I. affinis galbana* (T), *C. calcitrans* forma *pumilum* (Cp), *Crassostrea gigas* eggs, 2- and 19-day-old larvae fed on different proportions of T and Cp in bispecific diets. Both microalgae were sampled twice a week during the whole second set of feeding experiments while on day 19 biochemical composition was performed on two types of larvae: incompetent (NC≤320 µm) and competent larvae (C≥320 µm). PL corresponding to post larvae at day 26. * No data.

Fatty acids	Cp	T	Eggs	D2	5T/95Cp		25T/75Cp			50T/50Cp			75T/25Cp			95T/5Cp	
					D19		D19		D26	D19		D26	D19		D26	D19	
					NC	C	NC	C	PL	NC	C	PL	NC	C	PL	NC	C
14:0	9.07	22.06	5.71	4.07	7.58	7.83	6.80	8.06	5.17	5.64	6.95	4.36	6.46	7.17	3.44	5.79	5.30
16:0	12.85	13.45	22.51	20.58	8.83	9.82	10.50	9.44	10.99	9.99	10.71	11.12	10.16	10.33	10.50	10.49	10.74
18:0	0.78	0.85	3.09	3.58	1.63	2.23	1.79	1.78	2.79	2.09	1.96	3.00	1.87	2.14	2.97	2.34	2.49
16:1(*n*-7)	28.17	1.71	4.39	4.37	11.07	11.61	9.35	11.03	8.91	5.66	7.66	6.44	5.38	5.03	2.42	3.45	2.95
20:1(*n*-7)	*	*	0.94	1.30	2.93	2.87	1.45	2.58	3.33	2.05	1.53	3.07	1.08	1.14	3.45	1.36	1.46
18:2(*n*-6)	0.41	3.79	1.43	1.14	0.89	0.79	2.26	2.07	2.43	3.47	3.66	4.23	5.89	6.40	5.96	7.65	7.56
18:3(*n*-6)	*	0.08	0.17	0.12	0.16	0.10	0.27	0.28	0.16	0.40	0.42	0.24	0.71	0.67	0.35	0.77	0.74
18:3(*n*-3)	0.30	3.60	3.41	2.50	0.38	0.33	1.40	1.27	0.90	2.22	2.36	1.48	3.98	4.45	2.06	5.34	4.82
18:4(*n*-3)	1.56	11.56	7.59	5.07	0.99	0.99	2.73	2.77	1.28	4.34	4.78	2.04	7.69	8.44	2.77	9.72	9.28
20:2i	*	*	0.09	0.15	0.56	0.55	0.39	0.28	0.56	0.37	0.34	0.54	0.24	0.25	0.44	0.20	0.25
20:4(*n*-6)	0.14	0.09	1.33	2.04	1.77	1.24	1.28	1.19	1.70	1.54	1.23	1.73	1.29	1.25	2.01	1.31	1.30
20:5(*n*-3)	26.91	0.38	14.73	12.15	21.34	20.74	17.33	17.24	14.61	11.26	12.21	9.75	6.88	6.91	6.09	4.41	4.29
21:5(*n*-3)	*	*	1.08	1.07	0.48	0.52	0.50	0.40	0.35	0.51	0.45	0.40	0.46	0.44	0.60	0.50	0.60
22:2i	*	*	0.40	0.72	0.13	0.10	0.27	0.19	0.36	0.57	0.44	0.50	0.49	0.44	1.04	0.49	0.63
22:2j	*	*	1.93	3.88	3.67	3.45	3.46	2.93	4.39	4.29	3.23	3.74	2.40	2.24	3.64	2.13	2.54
22:5(*n*-6)	*	2.00	0.23	0.31	0.34	0.25	0.90	0.70	1.07	1.74	1.42	1.81	2.08	2.06	2.53	2.34	2.34
22:5(*n*-3)	0.05	0.23	1.08	1.18	0.84	0.79	0.70	0.50	0.76	0.55	0.49	0.59	0.41	0.40	0.52	0.45	0.42
22:6(*n*-3)	1.70	10.15	9.38	11.43	4.12	3.19	7.24	5.72	8.15	12.19	10.50	12.34	13.95	14.10	15.52	16.17	15.53
18:0dma	*	*	3.50	4.59	4.75	3.97	4.70	3.88	4.37	7.08	5.11	4.38	4.89	3.77	5.44	3.97	5.25
Sterols																	
TdehydroCholesterol	*	*	7.31	6.44	0.62	0.37	0.25	0.25	0.47	0.24	0.16	0.41	0.30	0.22	0.40	0.43	0.40
Cholesterol	28.23	7.99	29.51	28.82	32.61	36.29	33.39	34.33	43.60	28.38	29.48	40.13	27.95	18.75	26.32	13.05	13.21
Brassicasterol	*	92.01	17.85	16.89	3.36	2.36	14.01	13.03	15.21	32.38	30.09	32.83	35.34	55.37	49.85	55.81	54.88
Desmosterol	*	*	9.96	10.13	11.43	11.18	8.85	9.20	5.37	7.93	8.04	3.72	9.10	6.00	3.73	9.87	10.62
Campesterol	1.39	*	1.91	2.06	3.34	2.09	1.87	1.76	2.24	1.46	1.52	1.78	1.24	1.10	1.56	0.12	2.18
24MeCholesterol	48.12	*	16.74	19.13	33.71	36.67	33.68	33.52	22.32	25.25	25.44	14.09	18.81	14.67	8.42	4.36	3.96
Isofucosterol	17.3	*	8.44	6.51	5.07	5.8	4.66	4.81	3.59	2.55	3.16	2.22	2.66	1.56	1.08	0.78	0.57

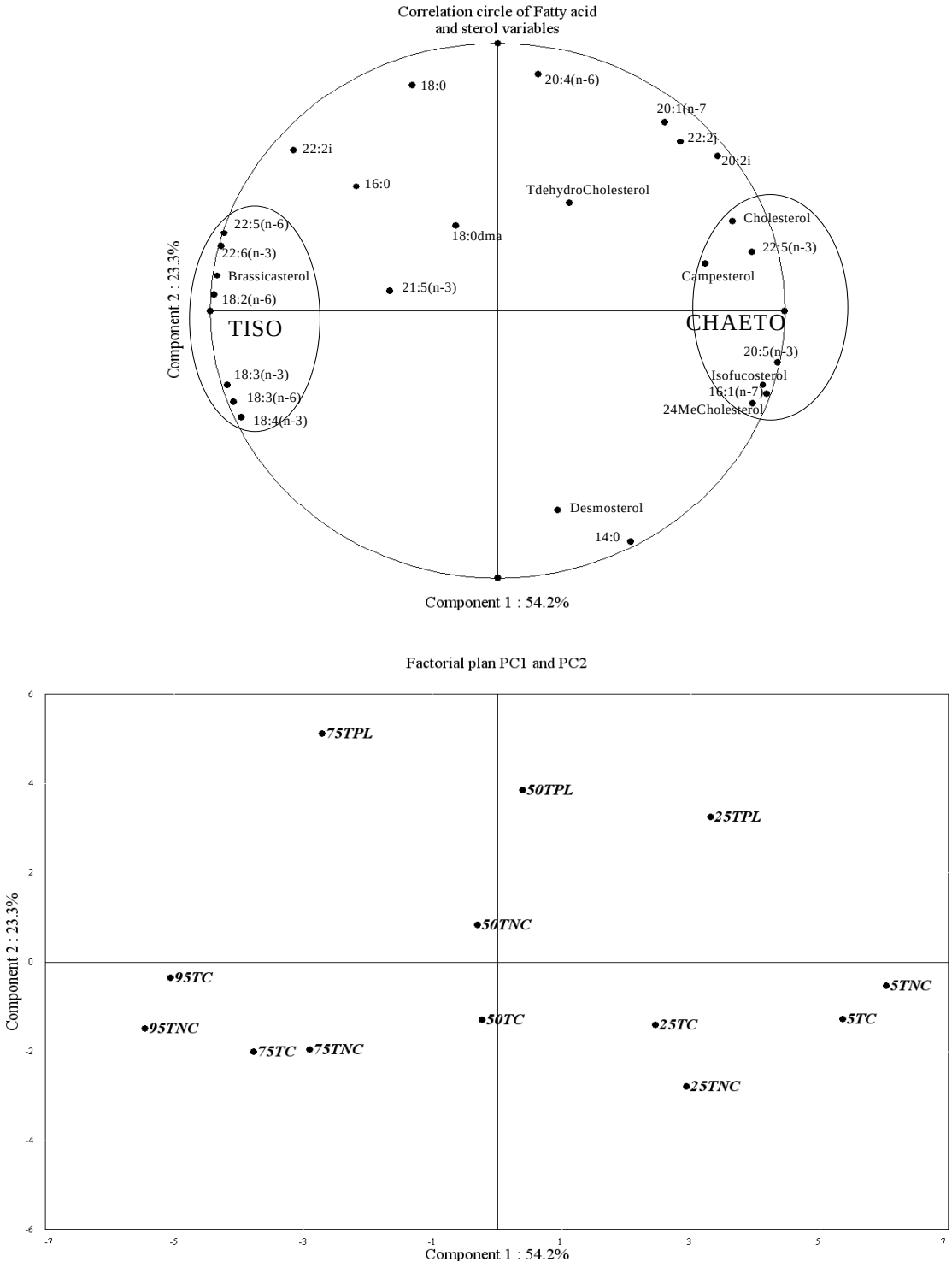

Fig. I.3. Correlation circle of fatty acids and sterols in larvae (competent=C; incompetent=NC) and postlarvae (PL) of *C. gigas* fed different proportions of *I. affinis galbana* (TISO or T) and *C. calcitrans* forma *pumilum* (CHAETO): 5T=5T/95Cp; 25T=25T/75Cp; 50T=50T/50Cp, 75T=75T/25Cp; 95T=95T/5Cp. 50TNC means incompetent larvae fed 50T/50Cp; 75TPL means postlarvae fed 75T/25Cp.

4. Discussion

Production of bivalves in hatcheries is undoubtedly related to the quality and the quantity of the supplied microalgae (Helm and Bourne, 2004). Moreover, with the present techniques used, the phytoplankton production can represent 30% to 40% of the spat cost in hatcheries (Helm, 1990; Coutteau and Sorgeloos, 1992; Borowitzka, 1999). The choice of the cultivated microalgae to be used as feed is accordingly an important one.

The influence of microalgae biochemical composition on larval and juvenile development has been widely reported in bivalves (Webb and Chu, 1983; Brown et al., 1997; Robert and Trintignac, 1997; Knauer and Southgate, 1999; Muller-Feuga et al., 2003a, b; Volkman and Brown, 2006) and it is now well known that 20:5n−3 and 22:6n−3 are both essential fatty acids for oysters as previously shown by Langdon and Waldock (1981). Nevertheless, the exclusive use of *Thallasiosira pseudonana* (Thompson and Harrison, 1992) or *Chaetoceros gracilis* (Thompson et al., 1993) both rich in 20:5*n*−3, led to inconsistent results without ensuring better performances in *C. gigas* larval growth. Similarly, in the present study, the use of the sole diet *C. calcitrans* forma *pumilum* (Cp), rich in the same fatty acid resulted in a high interspecific larval survival variability. In contrast, moderate levels of 20:5*n*−3 (EPA) and 22:6*n*−3 (DHA) led to good rearing performances because when a mixed diet (TCp) was used (from 25% to 75% of each of its component) little or no difference in larval growth occurred. Assuming that on day 19 competent larvae reflected the end of the larval cycle as well as the cumulative effect of each diet throughout the whole larval life, we can estimate that good larval developments were obtained when larvae contained 7%<EPA<17% and 7%<DHA<14% (Table I.6). Such results are consistent with those of Thompson and Harrison (1992) who reported that good larval performances were associated to an EPA content in diets of 6% to 18%. On the other hand, EPA and DHA within each

corresponding diet were similar between competent and incompetent larva, this criteria being based on adequate size and the presence of eyespot. This means that, in our conditions, all the larvae had the ability to go through metamorphosis when anatomical criteria would be attained and that it was just of question of time. Or, these specific biochemical components are not the only appropriate keys for explaining metamorphosis.

On the other hand, several fatty acids, mainly 20:1*n*−7, 22:2i and 22:2j, were present in the oyster (larvae and spat) while undetected in microalgae. Ackman and Hooper (1973) explained that 20:1*n*−7 could come from an elongation process of 16:1*n*−7. This fatty acid was particularly abundant here in Cp. Besides, 22:2i and 22:2j have been identified as non-methylene interrupted (NMID) 22 carbon fatty acids whose physiological significance are unknown. Because these fatty acids were present in the starved larvae several days later a structural role rather than a storage function has been considered by some authors (Thompson and Harrison, 1992).

Nevertheless, the nutritional value of microalgae for bivalve larvae depends, above all else, on the cell size and/or the presence of short spines allowing its accessibility and ingestion (Robert and Trintignac, 1997) while the nature and thickness of the cell wall will explain its digestibility, some species being ingested but poorly digested (Babinchak and Ukeless, 1979; Robert, 1998). The three microalgae used in the present study are known to have similar sizes and fine cell walls (Chrétiennot-Dinet et al., 1986) but, despite their similarity, a preferential consumption was shown according to the microalgal species and type of diet. In the first set of feeding trials which evaluated monospecific diets, a preference was found for *C. calcitrans* forma *pumilum* with an uptake of 10 000 to 66 000 cells day^{-1} $larva^{-1}$ at the beginning of rearing (first 12 days) then with *I. affinis galbana* whose consumption did not exceed 15 000 cells during the same period. *P. lutheri* was also ingested at a very low level, approximately ten times less than *I. affinis galbana*. Larvae fed bispecific PT showed a

weak and constant grazing behaviour throughout the whole rearing period, somewhat higher than that noted with the sole *P. lutheri* but lower than recorded with *I. affinis galbana*. Moreover, a clear preference towards the assemblages composed of flagellates (P or T) and Cp was observed. Indeed, PCp and TCp uptakes were high, similar to that recorded on trispecific diet PTCp. This means that, with the microalgae used here, there was no advantage in term of consumption to supply an association of three algae compared to two.

During the second set of experiments, the diet containing the greatest amount of diatom (5T/95Cp) exhibited an equivalent consumption to that detected on the sole *C. calcitrans* forma *pumilum* during the first set of experiments. Similar behaviour occurred with the richest haptophyte diet 95T/5Cp compared to T alone. Lastly, for the well-balanced diet 50T/50Cp in experiment 2, similar uptake as TCp in experiment 1 was reported showing a good reproducibility between experiments despite different breeding seasons, *i.e.* spring and fall.

Diets composed exclusively with Cp or with this alga present in high proportion in the assemblage, 5T/95Cp, showed large interspecific differences that might be explained by some harmfulness for larvae. A similar phenomenon has been already reported on copepods (Ianora et al., 2003) where diatoms exhibited teratogenic effects in certain conditions. Many complex lipids are readily hydrolysed to yield free fatty acids and several studies have suggested that specific free acids can be toxic (Volkman and Brown, 2006). The formation of free fatty acids due to autolysis on cell rupture seems to be a particular problem with *S. costatum* (Berge et al., 1995) and whether *C. calcitrans* forma *pumilum* has similar properties needs to be validated.

On the other hand, in the assemblages (except PT weakly ingested) the species selectivity disappeared, at least in our scale sampling, generally 24h and the patterns of the consumption for larvae fed PCp and TCp were similar.

A good intertank reproducibility in the grazing results allowed us to clearly identify three periods. The first starting from D larvae (D2 ≥70 µm) to early hinged larvae (D8 ≥120 µm) was characterized by a low but progressive phytoplankton consumption with values from 600 to 4000 cells day^{-1} $larva^{-1}$. This period represents the beginning of the larval life where larval metabolism is partially sustained by reserves. The second phase related to hinged to eyed competent larvae. During this period grazing increases considerably passing from 6000 to 25 000 cells day^{-1} $larva^{-1}$ between 10th and 15th day with 80 000 to 90 000 cells day^{-1} $larva^{-1}$ the 21st day, while larvae grew actively ≈15 µm day^{-1} *vs.* 8 µm day^{-1}. This period was very well marked during the two experimental series, though somewhat less pronounced for 75T/25Cp. Similar consumption has been already reported by Utting and Spencer (1991) on *C. gigas* larvae from 120 to 300 µm fed on *I. affinis galbana*. A third period was noted for larvae ≥320 µm during which ingestion decreased suddenly. This size is known to be critical for oyster larvae because they are normally competent to metamorphosis and ready to settle (Gerdes, 1983a; Helm and Bourne, 2004). Indeed, during metamorphosis the velum degenerates while the gills are not yet functional (Cannuel and Beninger, 2006) and accordingly filtration capacity is reduced (Baker and Mann, 1994). Finally, a fourth period during the second set of experiments was detected from the 21st day corresponding to the resumption of feeding uptake, however remaining slightly lower than that preceding metamorphosis. This resumption occurred 24h later which is in agreement with the results of Baker and Mann (1994) who reported that, except for only a few hours during the settler phase, feeding was possible throughout *Crassostrea virginica* metamorphosis. These marked differences during evolution of phytoplankton consumption clearly reflected larval development. The qualitative influence of the different diet is conspicuous at the end of the first week of rearing but strengthened beyond. Larvae fed solely *P. lutheri* showed a weak ingestion, leading to low growth (1.7 µm day^{-1}) and survival (29%) at the end of the

experiment confirming previous work on the same species (Ponis et al., 2003). In contrast, *P. lutheri* has been successfully used with scallop larvae such as *P. maximus* (Delaunay et al., 1993), *P. fumatus* (Heasman et al., 1995), *Mimachlamys asperrima* (O'Connor and Heasman, 1997). A moderate growth (≈6.0 µm day−*1*) characterized *C. gigas* larval development solely fed t. Iso. Moreover, survival remained high at the end of 3 weeks of rearing (86%) and confirmed previous data on the same species (Helm and Laing, 1987). Compared to performances recorded with *I. affinis galbana*, addition of *P. lutheri* improved slightly larval growth (7.4 µm day^{-1}) but not survival (82%). *I. affinis galbana* utilization is accordingly preferential for *C. gigas* larvae. However, *P. lutheri* might be maintained in the hatchery as an alternative of *I. affinis galbana* in case of culture collapse. In contrast, *C. calcitrans* forma *pumilum* led to good growth (10 µm day^{-1}) and it is thus an essential constituent for *C. gigas* larval growth, although insufficient as a sole diet. Indeed, its sole use led to a high survival variability, low larval competence and metamorphosis.

Both bispecific diets TCp and PCp led to similar growth, survival and competence. However, T exhibited higher food value than P when used as single diet, and TCp was accordingly preferred. Between different mixtures, 50T/50Cp appeared to be the best for larval development and metamorphosis while unbalanced rations (5T/95Cp or 95T/5Cp) are not advised due to a negative effect on growth and survival.

Excellent reproducibility of larval development performances between both sets of experiments has to be noted. Growth of larvae solely fed t. Iso (experiment 1) or mixtures rich in this haptophyte (95T/5Cp: experiment 2) were very similar (6 *vs.* 8 µm day^{-1}) while similar growth (10 µm day^{-1}) were recorded when larvae were fed *C. calcitrans* forma *pumilum* (experiment 1) and mixtures rich in this diatom (5T/95Cp: experiment 2), or with the diet TCp (experiment 1) and 50T/50Cp (experiment 2) with a daily growth of 13 µm day^{-1}. Moreover, in both sets of experiments starved larvae growth was nil or insignificant confirming the

absence of other exogenous feed components which gives confidence to the results reported here.

5. Conclusion

This study clearly shows than a bispecific diet based on the Haptophyte *I. affinis galbana* (T) and the diatom *C. calcitrans* forma *pumilum* (Cp) in a proportion of one to one fits well to *C. gigas* larval demand and allows good development from 2-day-old D larvae to 1-week-old postlarvae. The addition of *P. lutheri* (P) to TCp did not support any advantages in term of larval performance and metamorphosis, while the role of the diatom *C. calcitrans* forma *pumilum* in *C. gigas* growth is essential, contributing 75% of larval growth. Moreover, *C. gigas* larval performances are clearly related to feeding behaviour, with a low ingestion of *P. lutheri*, a high ingestion of *C. calcitrans* forma *pumilum*, and a moderate ingestion of t. Iso. In presence of bispecific diets (except PT with almost a nil uptake), grazing largely increased with a high reproductivity of the pattern in all tanks.

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CHAPITRE II

A flow-through rearing system for ecophysiological studies of Pacific oyster *Crassostrea gigas* larvae

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Abstract

While literature is relatively abundant on adult shellfish, ecophysiological studies at the larval stage are scarce because of both technical difficulties and inadequate methodology. A tool dedicated to provide basic information for larval ecophysiology was accordingly developed. Two steps were followed: first a flow-through method of *Crassostrea gigas* larval culture was perfected during a set of experiments in which rearing systems and larval density were assessed. Then a continuous hydrobiological data recorder was adapted with modifications to comply with our experimental aim. SILO (Système d'Instrumentation des Larves en flux Ouvert) allowed the successive acquisition of hydrobiological parameters in ten 150 l larval tanks in which larvae were reared using flow-through techniques under controlled environmental conditions. An automated system enabled sequential measurement of hydrobiological parameters from one larval culture to the next. A chamber system contained several probes for measurement of temperature, salinity, pH and fluorescence. The electronic system allowed real time acquisition, storage and transfer of data. SILO was successful as a larval rearing device, reducing larval disturbance that could result from transfer and handling for measurement. It was also efficient as a tool to provide basic information for larval ecophysiology research. The effect of temperature (17, 22, 25, 27, 32°C) on growth and ingestion of microalgae was studied to test SILO. Maximum growth occurred at 27 °C (16 ± 2 µm d^{-1}) and 32 °C (15 ± 3 µm d^{-1}) whereas larvae reared at 22 and 25°C showed lower development (8.6 ± 3.1 and 11.7 ± 2.5 µm d^{-1}, respectively). Moreover, metamorphosis exhibited no significant difference at 27 or 32 °C with 87.5 ± 7.1 and 85.9 ± 9.6% respectively, while at 22 or 25 °C lower metamorphosis was recorded with 55.2 ± 8.3 and 57.6 ± 9.6%, respectively. The lowest temperature (17 °C) strongly inhibited ingestion whereas the highest temperature (32 °C) stimulated maximum feeding activity over the whole larval rearing period.

Keywords: Flow-through; *Crassostrea gigas*; Bivalve larvae; Ecophysiology; Temperature

1. Introduction

The Pacific oyster (*Crassostrea gigas* Thunberg) is cultured in many countries, depending substantially on juvenile collection from the natural environment (Helm et al., 2004). However, spat production in hatcheries is increasing due to the high seasonality and variation in recruitment from year to year of this activity (Robert and Gérard, 1999). In the last decade French hatchery spat production of *C. gigas* has regularly increased from 10% to 20–25% (Ponis et al., 2006b). However, the demand for triploid oysters suddenly boosted hatchery spat production, estimated nowadays to 50% (Robert, unpublished data). Despite an indisputable know-how in commercial mollusc hatcheries, some biological aspects (*e.g.* control of sex ratio, estimation of gamete quality, larval feeding requirements) are still unknown because knowledge has been mainly achieved by an empirical approach. Optimisation in the hatchery process is accordingly necessary and involves a better understanding of molluscan physiological requirements. A major goal in hatchery production is to improve larval and post-larval survival by attempting to maximize larval growth and success at metamorphosis. For this reason, feeding has been considered as the most important aspect in hatchery operations focusing mainly on the type of the microalgae used as feed (Brown et al., 1998; Knuckey et al., 2002; Ponis et al., 2006a). In contrast, *C. gigas* larval rearing systems did not receive particular attention.

In the Ifremer experimental mollusc hatcheries, techniques for bivalves larval culture followed a standard practice protocol (Helm et al., 2004), involving a static system with full exchange of water, three times a week, and feeding once a day. This method employed by Rico-Villa et al. (2006) led to satisfactory results in terms of growth (13-14 µm day^{-1} at 25 °C and 5 larvae ml^{-1}) and final yield (45-55%). However, due to frequent renewal of seawater and tank cleaning, the labour demand is high. A strategy which would considerably reduce

these constraints is the development of simplified larval rearing techniques using flow-through systems for bivalve larvae (Malouf and Breese, 1977; Southgate and Ito, 1998; Magnesen et al., 2006).

In addition, the improvement of rearing methods should allow a new approach for the study of various environmental factors affecting larval physiology. Indeed many studies on *C. gigas* larvae have generally been focused on assessing larval growth, development, metamorphosis and settlement by varying one or two environmental combined factors (Helm and Millican, 1977; His et al., 1989; Abdel-Hamid et al., 1992), while only limited studies have involved the quantification of physiological functions such as ingestion (Gerdes, 1983a; Baldwin and Newell, 1995) or respiration (Gerdes, 1983b; Mona et al., 1993; Goulletquer et al., 2004). Moreover, most measurement practices in larval physiology have been based on the transfer and handling of larvae from rearing tanks to measurement chambers. This procedure can induce stress for the tested organisms and might have some repercussions on measured data. Flow-through system should provide required and constant experimental conditions with reduced larval handling.

For these reasons, a tool dedicated to provide basic information for larval ecophysiology was searched, developed and thoroughly tested at Ifremer, resulting in SILO (Système d'Instrumentation des Larves en flux Ouvert). This technology consisted of a flow-through rearing system allowing the maintenance of a constant flow of phytoplankton enriched seawater at desired temperature conditions, as well as, a tracking system consisting in a continuous hydrobiological parameters recorder. Moreover, SILO paid careful attention to avoid disturbance of the larvae during the whole rearing period, thus allowing the collection of valid measurements throughout larval and metamorphosis stages.

The present study describes (1) a flow-through system of *C. gigas* larval culture based on preliminary tests in which rearing device and larval stocking density were assessed, (2) an

autonomous hydrobiological measurement system capable of monitoring continuously key parameters during the whole larval period (±3 weeks), and (3) the whole device, corresponding to the combination of both systems, to study the effects of temperature on growth and ingestion of microalgae by larvae.

2. Materials and methods

All larval experiments were run on two-day-old D shaped larvae obtained from *Crassostrea gigas* broodstock maintained in cultivated area in Aber Benoît, western Brittany (France). The oysters were then conditioned at 19°C in the Ifremer experimental hatchery located at Argenton, Brittany (France) according to a well settled method consisting of a flow-through system enriched with phytoplankton mixture. The daily mixed diet consisted of *Isochrysis affinis galbana* (T-ISO), *Chaetoceros gracilis* and *Skeletonema marinoï* (1:1:1 in dry weight) at a ratio of 6% of the oyster dry weight.

Six weeks later, gametes from 3 males and 6 females, obtained by stripping, were mixed on the basis of 50 spermatozoa per oocyte in 5 l beakers. Forty embryos ml^{-1} were transferred to complete development to D-stage over two days in 150-l tanks filled with 1 µm filtered seawater at 25 °C and salinity of 34.

2.1. Preliminary larval rearing studies

Initial experiments were run to get basic data on larval performance (growth, survival and competence) in a flow-through rearing device. Experiment 1 consisted of comparing a static system (S) and a flow-through system (F) both in 150-l cylindrical tanks at 5 larvae ml^{-1} while Experiment 2 reported the effects of three stocking densities (5, 50 and 100 larvae ml^{-1})

in a F rearing system on larval performance. Experimental conditions run in two replicates were similar for both rearing systems (S and F): 1 µm filtered seawater, 25 °C, mean salinity of 34.5, aeration of 0.5 l min^{-1}. Larvae were fed a bispecific diet of T-ISO and *C. calcitrans* forma *pumilum* (1:1 in cells) because this mixture has been reported to be particularly efficient (Rico-Villa et al., 2006). In the S system, larvae were fed once a day and feed quantity adjusted as the larvae grew while tanks were drained and cleaned three times a week. In the F system a continuous phytoplankton enriched seawater flow was provided at 18 l h^{-1}. A submerged "banjo" sieve was attached to the outflow pipe and was cleaned daily to avoid clogging and overflowing.

Each experiment ended when ≥50% of the whole population as eyed larvae after draining the total tank volume. This criterion was used to estimate the percentage of morphological competence for metamorphosis. Larval size was assessed by measuring the initial and final shell length of 200 individuals using image analysis (WinImager 2.0 and Imaq Vision Builder 6.0 software for image capture and analysis, respectively). Final survival was estimated by subtracting total cumulated mortalities (number of empty shells) from the number of larvae initially stocked.

2.2. Flow-through rearing system design

In the Argenton hatchery, coarse seawater filtration consists of a 55 µm polyvinyl chloride (PVC) disc filter system (Arkal Spin Klin, Israel). Before its distribution in the experimental room, incoming seawater passed through a 30-l cartridge equipped with a 1 µm cotton bag filter and was then supplied to each outlet of experimental tanks at the appropriate temperature. Water temperature was continuously controlled by means of thermo-regulated

automatic valves type EA20 (+GF+, Switzerland) connected to digital controllers type PE65 (+GF+).

A diagram of our flow-through rearing system is shown in Fig. II.1a. The complete system consisted of a set of ten 150-l cylindro-conical fibreglass tanks. Each experimental tank inlet was equipped with a flowmeter type SK52 (+GF+, max. flow 100 l h^{-1}). However, when the experimental design was complete (ten 150-l tanks), it was difficult to maintain a minimum flow lower than 40 l h^{-1} and accordingly a continuous seawater flow of 0.66 l min^{-1} ± 0.11 l min^{-1} was provided in each experimental tank (including phytoplankton supplied directly in the seawater line). This flow allowed a complete water exchange in each tank every 4 hours equivalent to 6.4 ± 0.5 renewals over a 24 h period. Continuous outflow drained from the top side of the tank after passing through a totally submerged rectangular sieve attached to the outflow pipe. The sieve was fitted on both sides with a nylon mesh screen, offering a total surface area of 1050 cm^2, thus reducing the suction pressure across its surface and preventing larvae being stuck against the sieve or discharged with the water flow. Moreover, mesh sieve sizes ranged from 40, 60 and 80 µm as larvae grew throughout the experiment. The sole technical intervention consisted of daily sieve cleaning to avoid clogging and overflowing. Seawater in tanks was aerated from the main hatchery airline and was fitted to the bottom of each tank to maximise circulation of water to the whole tank. Moreover, air bubbles helped prevent larvae from sticking to the outflow sieve and reduced the settling of debris on the bottom of tanks during the rearing period, thus limiting a potential source of bacterial contamination.

Outflow from each tank discharged into a secondary tank named “reservoir” of 70-l, in which seawater was pumped from the bottom side for hydrobiological survey. Pumped seawater passed through a sieve to avoid large debris entering the measurement system. This reservoir was used to avoid any disturbance directly to larval culture when seawater was

sampled for hydrobiological survey. Finally, the overflow discharged from the side of the reservoir to waste (Fig. II.1a).

2.3. Description of hydrobiological tracking system

Seawater from reservoir was continuously pumped to the measurement system for hydrobiological survey. The measurement system, using the Ifremer MAREL technology (Blain et al., 2004), allowed the real time data acquisition, transfer and storage to a central database during the entire larval rearing period. Moreover, it enabled alternate pumping from one experimental treatment to the next with an automated pump and valve system.

This technology consisted of a hydraulic circuit equipped with temperature, salinity, fluorescence and pH sensors, all fitted in a unique measurement chamber with a volume of 2.5 l (Fig. II.1b). The internal circuit and sensors were cleaned between each sampling using an automatic water pump (Jabsco PAR-MAX2, range 0 to 8.7 l min^{-1}) and *in situ* chlorination of seawater to avoid fouling in the chamber. From each reservoir, 64 ± 2 l was sampled at a flow of 3.2 ± 0.1 l min^{-1} (Fig. II.1b) and pumping lasted 20 minutes with 19 min for measurement chamber rinsing and stabilization of measurements. Data acquisition, transfer and storage, with suppression of extreme values and averaging the three remaining values, occurred during the last minute. The sampling cycle provided a total time of 200 min for a complete cycle of 10 rearing tanks. Each tank was accordingly surveyed 6 to 7 times per day.

The electronic system for measurement and control was a multitask processor, which managed actuation of valves and pump, the real time data acquisition, transfer and storage. Intranet transmission system was used for a bi-directional communication with the measurement system at any time. Data transfer and storage to the central database, located in Ifremer Brest, occurred each hour.

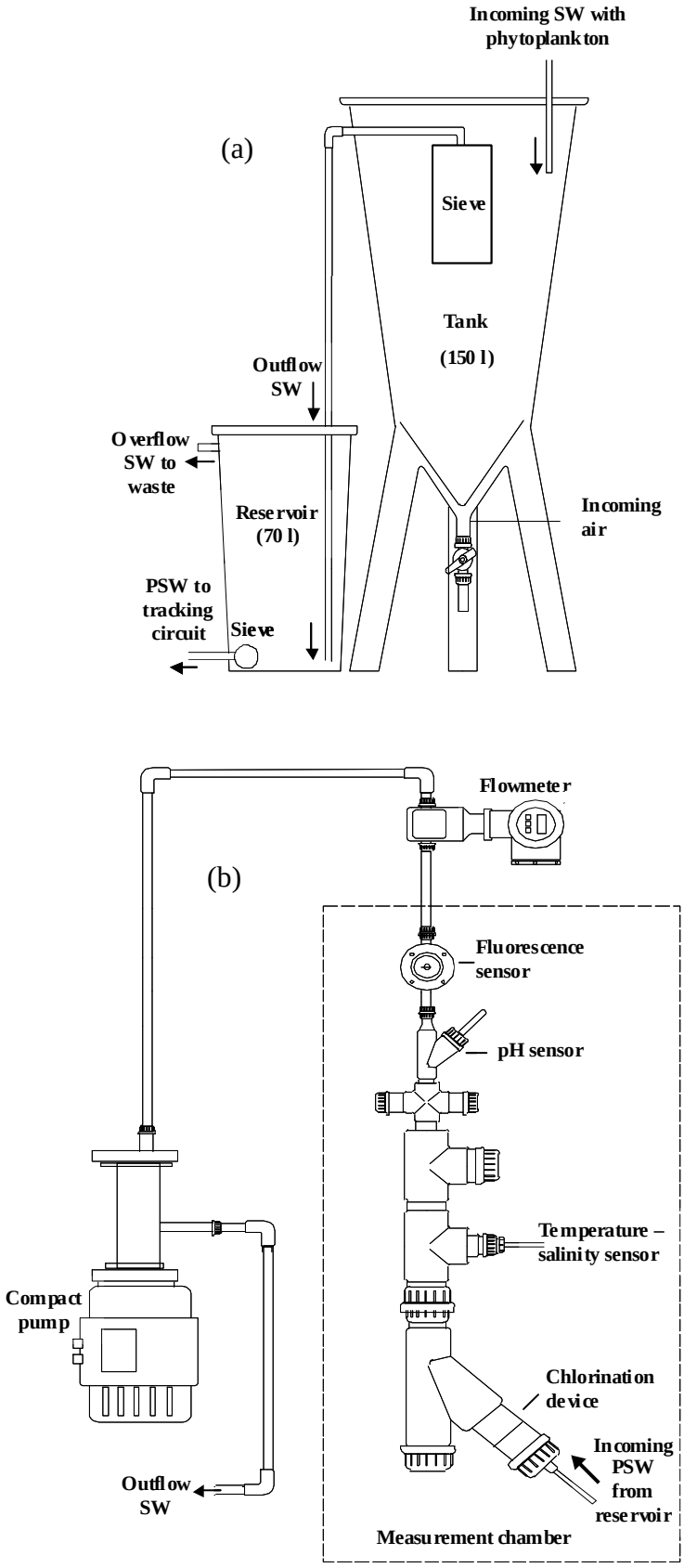

Fig. II.1. Details of: (a) flow-through rearing system and (b) hydrobiological tracking system. SW = seawater; PSW = pumped seawater.

The physicochemical sensors were calibrated at Ifremer, Brest, before experimentation. The salinity-temperature sensor (CLS 52, Endress+Hauser, E+H) measured conductivity (range 0 to 600 mS cm^{-1}) and temperature of seawater (-35 to 250 °C). The pH sensor (range 6.5 to 8.5) was equipped with an electrode CPS 11 (E+H). The fluorescence sensor (Sea Point SFC fluorimeter, Oceano instrument Ltd., range 0 to 150 FFU) had an excitation wavelength of 470 nm and emission wavelength of 685 nm. Consequently fluorescence data were measured in Fluorescein Fluorescence Units (FFU).

2.4. Experiments with SILO

The combination of the flow-through rearing system and the measurement device resulted in a tool designed for ecophysiological studies and was named SILO (Système d'Instrumentation des Larves en flux Ouvert: Fig. II.2). This technology allowed the maintenance of a constant flow of phytoplankton enriched seawater and appropriate temperature conditions, as well as, a continuous hydrobiological parameters recorder for ecophysiological measurements. Moreover, by using a secondary reservoir, SILO avoided any disturbance to larval rearing tank. To assess the feasibility of SILO, a set of experiments was carried out by measuring the effects of temperature on growth, metamorphosis and ingestion of *C. gigas* larvae. Owing to technical procedures and complexity of rearing, the replications were carried out in time (June 2005 and July 2006).

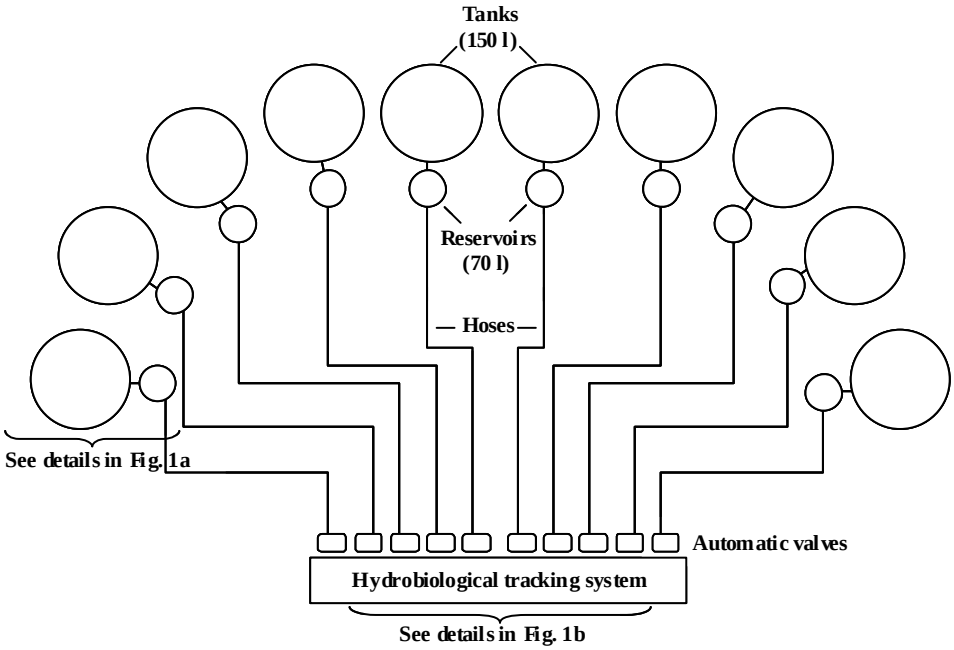

Fig. II.2. Overview of SILO in the experimental room: ten tanks (150 l) with ten reservoirs (70 l) connected to measurement tracking system for hydrobiological parameters survey.

Larvae were reared at a density of 30 larvae ml^{-1} in 1 µm filtered seawater at a mean salinity of 34.5 and at 0.5 l min^{-1} aeration. Feeding during larval and settling stages depended on larval size. It began accordingly with the supply of microalgae at 40 cells $µl^{-1}$ and ended with supply of 200 cells $µl^{-1}$. Ration consisted of a bispecific diet (1:1 in cells) of T-ISO and *C. calcitrans* forma *pumilum* (Cp, week 1) or *C. gracilis* (Cg, from week 2). Cg was used when it suited the size of the larvae (≥120 µm). Moreover, this latter diatom was produced efficiently, in batch culture in 300-l cylinders allowing satisfactory cellular densities (4–5 millions ml^{-1}), for its use in the flow-through rearing system with a high demand for food.

Temperature was maintained and continuously regulated at five different levels: 17, 22, 25, 27 and 32°C in the same experimental room. Each rearing condition consisted of a test tank containing larvae and food and a control tank with only constant flow of enriched

phytoplankton seawater, both at the desired experimental temperature. D-stage larvae, obtained 48 h after fertilization at 25 °C were then reared at the appropriate condition by progressive 2 °C step increase or decrease each 4h. Low larval mortalities (≤5%) were recorded on days 2 and 7, which corresponded for this latter to the first draining of the total test tank volume (see below).

In order to evaluate larval ingestion rate (IR), fluorescence data were analysed from pumped reservoir seawater (test and control) at each different temperature. Data collected by the fluorimeter were sent to a data acquisition system. Six to seven fluorescence recordings per day and per tank were standardized to phytoplankton density, expressed in number of cells μl^{-1}, by means of an electronic particle counter (Multisizer III) equipped with a 100-µm aperture tube. IR was estimated following the equation: $IR = [(C_c - C_t) * f] / nb$, where C_c and C_t are the phytoplankton densities (number of cells μl^{-1}) in the control and test tanks respectively, f is the water flow through each tank and nb is the number of larvae in test tank. IR data was averaged on a daily basis and expressed in cells per larvae and per day.

In each test tank, larvae were sampled (1 ml) once a day to observe larval development throughout the experiment while three precise samples (100 µl) were made each 2-3 days to measure larval shell length at each temperature. This sampling was operated from a 5 l seawater sample collected in each larval test tank and by concentrating larvae in a 1 l graduated cylinder. The remaining larvae were put back to the test tank. Shell length data were acquired following similar techniques previously described (image analysis). Moreover, once a week, the test tank was drained to achieve total number of larvae remaining and IR was accordingly adjusted. By daily observation of larval development, the occurrence of competent larvae was precisely detected for each experimental condition. When ≥50% of the population were competent, plastic disks (15 cm diameter) were placed in test tanks and used as collectors. After four days, metamorphosis was evaluated by counting the number of

remaining larvae and subtracting them from the total number of larvae initially stocked. An estimation of spat attached to collectors and tank walls was performed to confirm results.

2.5. Statistical analysis

Normality and homoscedasticity were initially evaluated with a Kolmogorov–Smirnov and Cochran test, respectively. For preliminary studies, mean (SD; n = 2 replicate tanks per rearing system and larval stocking density, respectively) of shell length, survival and competence were analysed using one-way analysis of variance (ANOVA) while significant differences (P<0.05) between treatments were determined using the Scheffé's pairwise multiple comparison test (Statview 5.0). For experiments with SILO, experimental trials in time (June 2005 and July 2006) were used as replicates (n = 2). Data of growth rate and metamorphosis were analysed using one-way ANOVA while pairwise differences between treatments were detected by Scheffé's multiple comparison test (P<0.05). Data expressed in percentage were transformed (arcsin [square (x / 100)]) before statistical analysis.

3. Results

3.1. Preliminary larval rearing studies

Initial larval shell length, on day 2, was 81.8 ± 0.1 µm and 79.8 ± 0.3 µm for experiments 1 and 2 respectively. On day 18, mean shell length in F system was 295.8 ± 45.0 µm and was significantly higher than in S system with 262.5 ± 58.4 µm (>86%: Table II.1). Moreover, on day 18, competence was found to be significantly higher in F system (71% *vs.* 55% in S system) but survival was similar (Table II.1). Lastly, a fractionation of the larval

population was observed in the static system leading to size heterogeneity from the second week of rearing, which increased thereafter (CV= 12, 21, 29% on day 7, 14 and 18, respectively).

Table II.1

Effects of rearing system (Experiment 1) on mean (SD; n = 2) shell length, survival and competence on day 18. Larvae were reared in a flow-through (F) or standard static (S) techniques. Values within the same column with a common superscript letter are not significant at P>0.05.

Rearing system	Shell length (µm)	Survival (%)	Morphological competence (%)
F	295.8 (45.0) [a]	85.7 (2.2) [a]	70.7 (3.2) [a]
S	262.5 (58.4) [b]	88.7 (1.1) [a]	54.5 (7.3) [b]

No significant differences in shell length (302.7 ± 33.6 µm to 308.1 ± 36.2 µm) and competence (82 to 84%) were detected at densities of 50 and 100 larvae ml^{-1} on day 16 in a flow-through system (Table II.2). In contrast, larval performances recorded at 5 larvae ml^{-1} on day 16 revealed a significant lower shell length (296 ± 30 µm) and competence (72%: Table II.2). Mean survival was high in all treatments (≥90%: Table II.2).

Table II.2

Effects of larval stocking density in a 150 l flow-through rearing system (Experiment 2) on mean (SD; n = 2) shell length, survival and competence on day 16. Values within the same column with a common superscript letter are not significant at P>0.05.

Larval density (ml^{-1})	Shell length (µm)	Survival (%)	Morphological competence (%)
5	296.0 (30.0) [a]	92.3 (1.3) [a]	72.2 (4.3) [a]
50	308.1 (36.2) [b]	92.8 (2.1) [a]	84.5 (5.3) [b]
100	302.7 (33.6) [b]	90.4 (2.0) [a]	82.7 (4.2) [b]

3.2. Hydrobiological environment survey

In this study, the hydrobiological environment was controlled in experimental tanks for phytoplankton concentration and temperature. Other parameters, such as pH and salinity, were recorded but generally followed the variations of the seawater from the natural surroundings. Since environmental conditions can influence significantly the physiology of larvae, we report here the data acquisition concerning seawater temperature, salinity, pH and phytoplankton density during a complete period of larval rearing.

There were no major fluctuations in temperature in test and control tanks during 35 consecutive days and the average seawater temperature was 31.8 ± 0.1 °C; 27.2 ± 0.2 °C; 25.2 ± 0.2 °C; 22.0 ± 0.2 °C and 17.4 ± 0.1 °C for 32, 27, 25, 22 and 17 °C desired in the experimental design. There were no major fluctuations in salinity and pH throughout the experimental rearing period with a mean value of 34.0 ± 0.5 psu and pH = 8.20 ± 0.01.

For the fluorescence survey, data expressed in Fluorescein Fluorescence Units (FFU) were standardized and used to calculate larval ingestion rate. For example, at 32 °C fluorescence in the control tank showed increasing values, according to demand and related to larval size, up to a maximum value on day 11 followed by a decrease during metamorphosis whereas in the test tank fluorescence showed constant values (Fig. II.3a). At 17 °C, fluorescence in control and test tanks showed similar values during the first two weeks; then from day 16 a clear separation occurred between values recorded in test and control tanks because of a noticeable increase of larval ingestion (Fig. II.3b). Whatever the temperature, the test tanks exhibited a constant value, equivalent to 30 cells μl^{-1}, corresponding to the desired phytoplankton concentration during the whole rearing period. At both temperatures, fluorescence values were lower during the first six days because of a difference in diet composition based on the mixture T-ISO + *C. calcitrans* forma *pumilum* in week 1. In week 2, *C. gracilis* substituted *C. calcitrans* forma *pumilum* (Figs. II.3a and b).

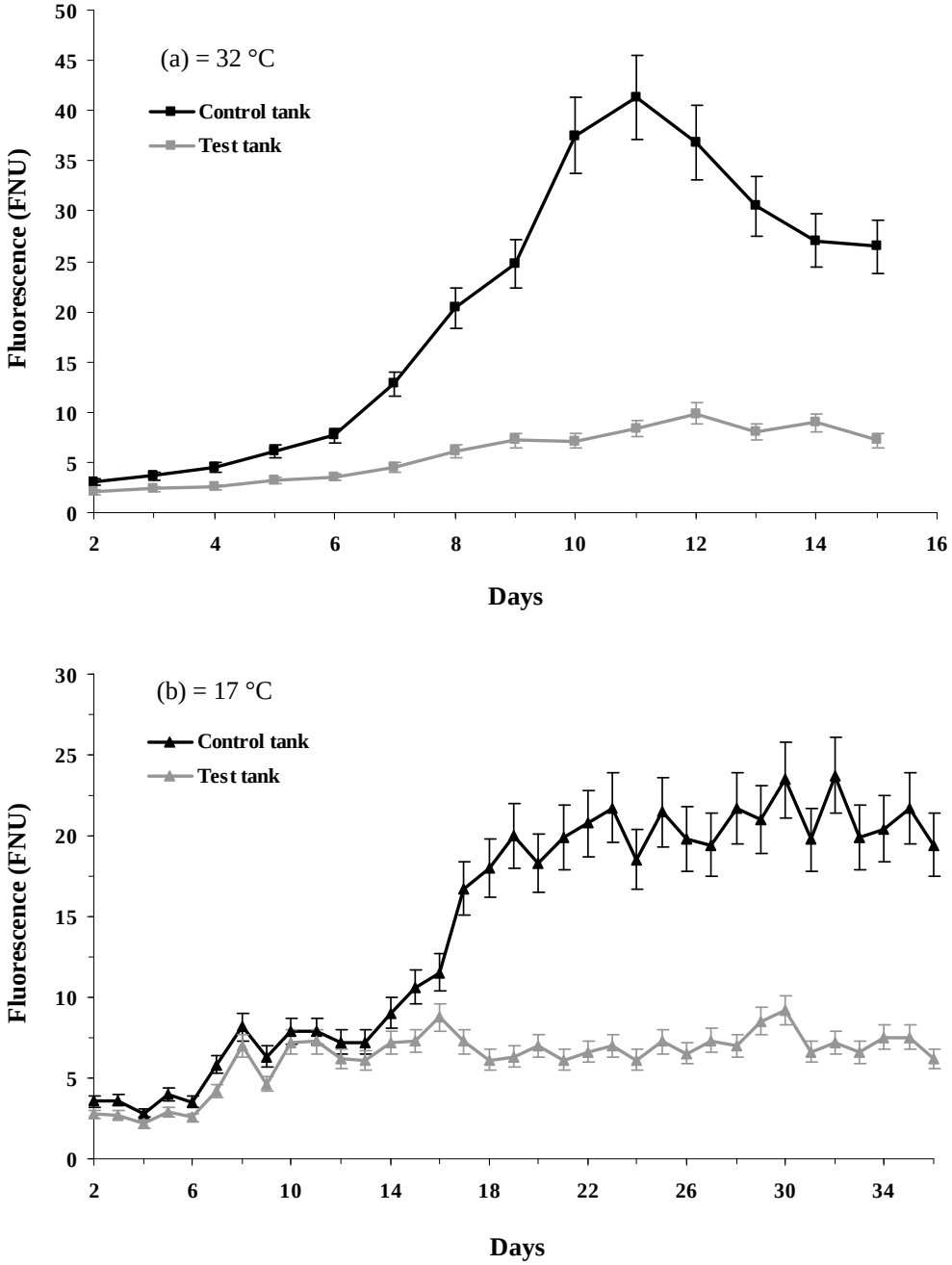

Fig. II.3. Fluorescence data (Fluorescein Fluorescence Units, FFU) recorded in SILO at 32 °C (a) and 17 °C (b) in control tanks (black line) and test tanks (grey line) during the larval rearing period. Each point represents a daily mean ± SD (*n* = 6–7 data). Note that y-axis scale ranged from 0 to 50 for (a) while it ranged from 0 to 30 for (b). Note also that the *x*-axis (time) corresponds to a longer rearing period at 17 °C (35 days *vs*. 15 days at 32 °C).

3.3. Experiments with SILO

No significant differences were detected in terms of growth rates between larvae reared at 27 and 32 °C with 16.3 ± 2.4 and 15.5 ± 3.1 µm d^{-1} respectively (Table II.3). Metamorphosis followed a similar trend with 87.5 ± 7.1 and 85.9 ± 9.6% respectively (Table II.3). Moreover, metamorphosis occurred in less than 15 days with the first competent larvae detected on day 11. Compared to the higher temperatures, larvae reared at 22 and 25 °C exhibited significantly lower growth rates (8.6 ± 3.1 and 11.7 ± 2.5 µm d^{-1}, respectively) and metamorphosis (55.2 ± 8.3 and 57.6 ± 9.6%, respectively: Table II.3). While no differences in growth rates were found at 22 and 25 °C, metamorphosis was delayed at 22 °C on day 25 (*vs.* day 20 at 25°C). At 17 °C, larvae showed significantly lower growth rates (4.8 ± 1.5 µm d^{-1}) resulting in low metamorphosis (15.8 ± 2.6%) on day 35 (Table II.3) despite high survival (70%).

Table II.3

Effects of temperature on growth and metamorphosis (mean ± SD; *n* = 2 replicate experimental trials in time) of larvae reared in SILO. Day of occurrence of competent larvae is reported for each temperature condition. Values within the same column with a common superscript letter are not significant at *P*>0.05.

Temperature (°C)	**Growth (µm d^{-1})**	**Occurrence of competence (day)**	**Metamorphosis (%)**
17	4.81 (1.53) [a]	31	15.88 (2.60) [a]
22	8.64 (2.97) [b]	21	55.20 (8.30) [b]
25	11.73 (2.48) [b]	16	57.60 (9.60) [b]
27	16.26 (2.37) [c]	11	87.45 (7.11) [c]
32	15.50 (3.11) [c]	11	85.88 (9.60) [c]

Algae ingestion by larvae increased with temperature and three phases for larvae reared between 22 to 32 °C can be distinguished (Fig. II.4). At 27 and 32 °C, a slight consumption (4000 to 20 000 cells per larvae per day) occurred during the first week followed by a sharp increase up to day 11 with values as high as 45 000 to 65 000 cells followed by a decrease ranging from 25 000 to 35 000 cells during metamorphosis. Larval ingestion at 22 and 25 °C showed similar response with 4000 to 8000 cells per day consumed during the first week, followed by an increased uptake of 35 000 cells up to day 11 with a small decrease on day 12 and recovery soon after. At 17 °C larvae exhibited poor ingestion over the whole rearing period with a consumption ranging from 1000 to 5000 cells per day (Fig. II.4).

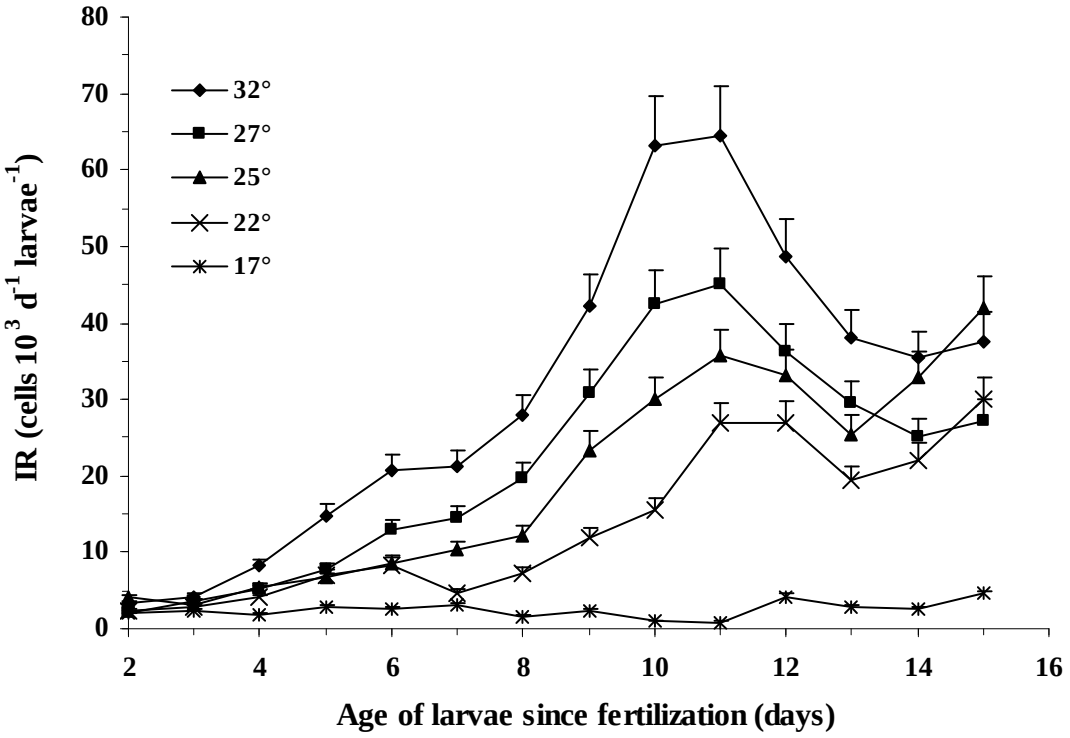

Fig. II.4. Ingestion rate (IR) expressed in cells 10^3 per day per larvae reared at five different temperatures through the whole larval life. Each point represents daily mean + SD (n = 6–7 data) at each temperature.

4. Discussion

The dedicated tool described in the present paper was designed to provide information for larval ecophysiology study. This system, which included a flow-through rearing system coupled to a tracking device, was developed over the course of three years. A number of preliminary studies were conducted to get basic data on flow-through rearing system (*e.g.* water flow and circulation, mesh size sieve and constant supply of algal food).

Significant growth and competence improvements were obtained in the flow-through system compared to static system. The lower larval performances recorded in the latter should be related to underfeeding because no adjustment of phytoplankton supply was made at night, larvae being fed once a day in the morning. Moreover, in the static system a fractionation of the larval population was observed from the second week of rearing with smaller larvae, which may be inhibited by larger ones. This heterogeneity, increasing with time, may be related to the way the larvae were fed. In the static system food became limited quite rapidly after some hours (Robert, unpublished data) and there was probably a trophic competition between larvae inducing such intra population size differences. On the other hand, the use of a flow-through system with a constant water exchange and a daily cleaning of the sieve facilitated the circulation in the whole tank and removal of catabolic products promoting favourable conditions for larval development. Moreover, as recommended in the literature (Southgate and Ito, 1998; Magnesen et al., 2006; Sarkis et al., 2006) extreme care was taken in maintaining a constant flow of phytoplankton over the course of the rearing to ensure an optimal food provision to larvae. This led to rapid shell growth and were associated with an earlier and homogenous metamorphosis for the whole larval population.

According to the present study, it was clearly demonstrated that high stocking densities can be used in the larval culture of *C. gigas*. In contrast to standard static methods

used in our laboratory, with densities of 1 to 10 larvae ml^{-1}, it became possible to initiate larval cultures with stocking densities of up to 100 larvae ml^{-1}. This was possible because of the continuous renewal strategy, which maintained constant quality of water in cultures by eliminating larval metabolites and allowed permanent food supply. Nevertheless, the significant performance improvement recorded here in the flow-through system with high stocking densities contrast with those reported by Malouf and Breese (1977) for *C. virginica* larvae, Andersen et al. (2000) and Sarkis et al. (2006) for scallop larvae (*Pecten maximus* and *Argopecten gibbus*). These authors found that an increase in larval stocking density resulted in inhibitory effect on growth and competence, which may be explained by low water flow renewal and insufficient food ration in their rearing systems. For C. gigas larval densities may undoubtedly be increased to 300 larvae ml^{-1} because preliminary trials run during two weeks in our laboratory showed similar growth performances compared to 5 larvae ml^{-1} (14.2 *vs.* 14.5 µm d^{-1}). Nevertheless, the definition of optimal larval stocking density in such flow-through rearing system was not the aim of the present contribution but sufficient biomass was needed to induce phytoplankton consumption detectable by the fluorescence probe. The present sets of data underline however the interest of flow through rearing system concept for commercial hatcheries.

Results from preliminary flow-through larval rearing led us to select 150 l as a reference volume at a density of 30 larvae ml^{-1}. This density was chosen as a good compromise between high larval performances, sufficient larval biomass to allow interpretable ecophysiology data recording and phytoplankton availability in the hatchery. Thus to allow a set of recording in SILO, a daily production of 100–150 l phytoplankton was needed. On the other hand, these initial results reported that a flow rate ≥20 l h^{-1} was suitable, and 40 l h^{-1} was chosen to prevent a draining of the compact pump and accordingly the loss of data during the continuous process of acquisition.

In its present form, the application of an automated hydrobiological data recorder system led to good results from the managerial and biological point of view. This device showed that the five seawater temperatures desired were effectively constant throughout the whole rearing period thus maintaining stable experimental conditions. Similarly, fluorescence data were consistent with measurements made with an electronic particle coulter counter (Multisizer III) to evaluate the recorder system accuracy and to standardize the required units for larval ingestion. Another feature of the system was the chlorination *in situ* which removed fouling organisms, resulting in clean equipment and stable measurements during the complete hydrobiological survey experiments.

This work showed the tolerance of *C. gigas* larvae to temperature in a range of 17 to 32 °C during its whole larval development (from D-stage to young post-larvae). Optimal larval growth and metamorphosis were recorded at highest temperatures, which is in agreement with previous observations in the literature (Helm and Millican, 1977; Henderson, 1982; His et al., 1989). However, larval production is currently run at 25-28°C in most commercial hatcheries. In these conditions, satisfying larval performances are generally achieved but an improvement in larval stocking density and larval cycle production turnover might be expected when using a flow through larval rearing system.

Grazing results allowed clear identification of three periods previously reported for *C. gigas* larvae (Gerdes, 1983a; Rico-Villa et al., 2006). In summary, a low but progressive consumption was noted from D-stage veliger to early hinged larvae (young umboned larvae) when larval metabolism is partially sustained by reserves (His and Seaman, 1992; Laing and Earl, 1998). During the second phase, microalgae ingestion increased sharply from hinged to eyed competent larvae, until a sudden decrease was noted prior to metamorphosis. These three periods were clearly distinguishable for larvae reared at 27 and 32 °C leading to a total rearing period of 15 days. In contrast, at 22 and 25 °C larvae exhibited the first two periods

but on day 12 a lesser decrease occurred. This brief reduction may be related to a transient poor quality of microalgae. Indeed, feeding activity at both temperatures recovered the next day and allowed to continue larval development and achieve high metamorphosis (>50%). In the meantime, ingestion also decreased at 27 and 32 °C but this event interfered with the decrease of ingestion occurring prior to metamorphosis without affecting final yield (>85%).

Increased ingestion rate at higher temperatures suggests a biological strategy for larvae to satisfy energetic requirements (Newell and Branch, 1980) and appears to be related to an increase in cilia beat for feeding and a high metabolic regulation (Loosanoff and Davis, 1963; Bayne and Newell, 1983; Beiras et al., 1994). On the other hand, at the lowest temperature (17 °C) larvae showed a constant but weak ingestion resulting in slow growth despite food availability. The incapability of larvae to grow at 17°C could be due to inability to assimilated ingested food at low temperature (Manoj and Appukuttan, 2003). However, larvae were quite tolerant because high survival (70%) was recorded on day 35 while only 15% of metamorphosis was achieved. Therefore, 17 °C could be considered as an inadequate temperature from a commercial standpoint.

The use of the flow-through rearing method is beneficial because this technique reduces labour demand, allows a high larval stocking density, reduces space requirements, eases larval handling, and improves larval development and metamorphosis. It was also demonstrated that its assemblage with a tracking system allows an efficient and continuous hydrobiological survey providing basic information for larval ecophysiology study in terms of growth, survival, competence, metamorphosis and ingestion of *C. gigas* larvae under different environmental parameters. Moreover, the relatively high sampling frequency gives confidence to the ingestion measurements reported by such a device.

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CHAPITRE III

Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*

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Abstract

Ingestion, growth and metamorphosis of Pacific oyster, *Crassostrea gigas*, larvae were studied under controlled conditions of food density and temperature using a combination of a flow-through rearing system and a hydrobiological monitoring device. In a first experiment larvae were exposed to three different phytoplankton densities (12, 20 and 40 cells μl^{-1}) while in a second trial larvae were reared at five different temperatures (17, 22, 25, 27 and 32°C). Both food concentration and temperature significantly affected the larval physiology throughout the entire development from D-veliger to young spat. Larvae survived over a wide range of both environmental parameters with high survival at the end of experiments. The feeding functional response provided the maximal ingestion rate (50 000 cells $larva^{-1}$ day^{-1}) which occurred at an algal density of 20 cells μl^{-1} surrounding the larvae and 25 °C. At the highest temperature (32 °C), maximal growth and metamorphosis performances were reached in less than 2 weeks while the lowest temperature (17 °C) consistently inhibited ingestion and growth over the entire larval period. The estimate of the Arrhenius temperature (T_A) was 11000 K for *C. gigas* larvae. Larval development could be divided on the basis of feeding activity into an initial mixotrophic period with a lower and constant ingestion over the first days (from D- stage to early umbonate larva of ≈110 µm length) followed by an exotrophic phase characterized by a sharp increase in ingestion (umbonate to eyed of ≈ 300 µm length) and, finally, a third period for larvae ≥300 µm during which ingestion decreased suddenly because of metamorphosis. Optimum larval development and settlement of the oyster *C. gigas* occurred at 27 °C and an increasing food supply as the larvae were growing. A food density of ≥20 cells μl^{-1} of T-ISO + CP or CG (1:1 cells number) in the culture water was required to maximise growth and metamorphosis success.

Keywords: *Crassostrea gigas*; Bivalve larvae; Ecophysiology; Growth; Food; Temperature

1. Introduction

The Pacific oyster *Crassostrea gigas* (Thunberg) is the major commercial marine bivalve in French aquaculture and, to fulfil oyster farmers demand, spat production in hatcheries has become widespread (Ponis et al., 2006). The major objective in hatchery production is to improve larval survival by maximising larval growth and success at metamorphosis in the newly settled seed. Accordingly, the effects of environmental factors on larval physiology of *C. gigas* have to be investigated and specified. Among them, phytoplankton supply and temperature have been recurrently studied and considered as key factors affecting the physiological processes of oyster larvae of the genus *Crassostrea* (Helm and Millican, 1977; Gerdes, 1983a; His et al., 1989; Abdel-Hamid et al., 1992; Mona et al., 1993; Lemos et al., 1994; Baldwin and Newell, 1995; Devakie and Ali, 2000).

Despite many studies on bivalve larval physiology, conflicting data have been reported. Great differences in experimental conditions can explain such divergence. Indeed, the influence of food levels on larval ingestion rates should be studied in a flow-through system where larval feeding activity can be measured under constant conditions over the whole rearing period (from D-stage to settlement). The use of a flow-through system avoids transfer and handling of larvae for measurements thus reducing stress on the organisms. Moreover, most ecophysiological studies on *C. gigas* larvae referred to a narrow experimental period of 7 to 10 d (Helm and Millican, 1977; Nascimento, 1980; Nell and Holliday, 1988; His et al., 1989; Abdel-Hamid et al., 1992; Mona et al., 1993). In contrast, the present work reports on physiological responses acquired over the complete larval period.

To provide basic information for larval ecophysiology research, we developed a flow-through rearing system allowing a constant flow of phytoplankton enriched seawater at

desired temperatures as well as a hydrobiological data tracking system. This device avoids disturbance of the larvae throughout the rearing period and thus allows valid measurements.

The aim of this study was to investigate the effects of temperature and phytoplankton density on 1) feeding activity from D-stage to settlement 2) growth, and 3) metamorphosis of the Pacific oyster *Crassostrea gigas* larvae reared under constant experimental conditions.

2. Materials and methods

2.1. Rearing and hydrobiological tracking system description

Broodstock *C. gigas* were transferred from western Brittany (France) into the Ifremer experimental hatchery at Argenton (Brittany, France) and conditioned at 19°C for six weeks. Larvae used in all experiments were produced from gametes that were obtained by gonad stripping (3 males and 6 females). The fertilised eggs were incubated in 150 l tanks using a static system and 1 µm filtered seawater at 25°C and a salinity of 34. Development to D-stage was complete after 2 days.

Experiments were carried out in a flow-through culture system (Rico-Villa et al., 2008) to maintain algal density and constant temperature conditions, as well as, to allow continuous hydrobiological data recording from culture tanks. The system consists of a set of 150 l cylindro-conical tanks. Outflow from each tank was discharged into a secondary tank from which seawater was pumped for the hydrobiological survey, and this reservoir was used to avoid any disturbance of the larval culture when seawater was sampled for environmental monitoring. An automated system directed tank outflows through a chamber that contained probes for measurements of temperature, salinity, pH and fluorescence. The electronic system allowed real time data acquisition, activation of floodgates and pump, storage and data

transfer to a central database throughout the larval rearing period. The system required 200 min for a complete sampling cycle, and thus, each tank was surveyed 6 to 7 times per day. In this study, phytoplankton concentration and temperature were controlled in rearing tanks, while parameters, such as pH and salinity, were recorded but generally followed the variations of the seawater from the natural environment.

2.2. Effects of food density

Two day old D larvae were placed in the flow-through tanks, at a density of 30 larvae ml^{-1}, in aerated (0.5 l min^{-1}) 1 µm filtered seawater, at a mean salinity of 34.5. Water temperature was maintained at 25 °C. Each experimental treatment consisted of two replicate tanks of larvae and an unstocked control tank with only constant flow of phytoplankton-enriched seawater.

Ration consisted of a binary diet (1:1 in cell numbers) of *Isochrysis affinis galbana* (T-ISO) and *Chaetoceros calcitrans* forma *pumilum* (CP, week 1) or *C. gracilis* (CG, from week 2) when the larvae were ≥110 µm in size. Three phytoplankton densities were calculated to supply more phytoplankton than would be consumed by larvae allowing a permanent availability of remaining phytoplankton concentration of 12, 20 and 40 cells μl^{-1} around the larvae and equivalent to T-ISO + CP or CG in 1:1 in cell numbers (Table III.1). An additional tank was continuously supplied with 1-µm filtered seawater at 25 °C to measure the phytoplankton coming in from the environment despite seawater filtration. Tanks were drained and cleaned once a week to determine larval survival.

Table III.1

Daily food ration (cells μl^{-1}) related to larval age over the whole rearing period during the experiment on food concentration effects. This supply concentration allows a permanent phytoplankton density of 12, 20 and 40 cells μl^{-1} around the larvae respectively (1:1 in cells number of T-ISO + CP or CG). See Fig. III.3 for correspondence between larval age and larval size for each phytoplankton density.

Age of larvae (day)	**2-5**	**6**	**7-9**	**10**	**11-12**	**13-15**	**16-17**	**18-19**	**20-21**
12 cells μl^{-1}	30	40	50	70	80	100	150	120	100
20 cells μl^{-1}	60	70	100	130	160	260	260	200	170
40 cells μl^{-1}	90	100	140	160	210	300	300	220	200

The feeding behaviour of *C. gigas* larvae can be divided into three different phases: mixotrophic, exotrophic and metamorphosis periods (Gerdes, 1983a; Rico-Villa et al., 2006). To obtain the ingestion rate (IR) for larvae throughout these feeding periods, fluorescence values were analysed from the pumped seawater of each tank (test and control) at each phytoplankton concentration. Water samples of bispecific diet in control tanks were measured using an electronic particle counter (Multisizer III, equipped with a 100-µm aperture tube) and expressed as cells μl^{-1}. These measurements were standardized to phytoplankton fluorescence values also recorded in control tanks to obtain a regression equation. This precaution was taken to remove any uncertainty of water samples contaminated by larval faeces in test tanks which should be analysed with a Multisizer counter but undetected using fluorescence measurements. IR was estimated following the equation: $IR = [(C_c - C_t) * f\ ] / nb$, where C_c and C_t are the phytoplankton densities (number of cells μl^{-1}) in the control and test tanks respectively, f is the water flow through each tank ($\mu l\ d^{-1}$) and nb is the number of

larvae in the test tank. IR data was averaged and expressed as cells larvae^{-1} d^{-1}. Moreover, IR at different algal cell densities was standardized to plot the relationship between food levels and IR in order to evaluate the feeding functional response of *C. gigas* larvae.

A 1 ml sample was taken from each test tank to observe the larval development. A 5 l sample was taken every 2-3 days from each larval tank to measure shell length (µm) and estimate larval growth. Shell length data were acquired using image analysis (WinImager 2.0 and Imaq Vision Builder 6.0 software for image capture and analysis, respectively). The total number of larvae was determined once a week when tanks were drained and the IR was corrected for larvae. When ≥50% of the population in a tank were eyed, plastic disks (15 cm diameter) were placed in the tank as collectors. After four days, the percentage of metamorphosis was evaluated by counting the number of remaining larvae and subtracting from the total number of larvae initially stocked. An estimation of spat attached to collectors and tank walls was performed to confirm results.

2.3. Effects of temperature

A second set of experiments was carried out to test the influence of temperature on ingestion, growth and metamorphosis performance of *C. gigas* larvae. Two day old D larvae were distributed in 150-l flow-through tanks at a density of 30 larvae ml^{-1} in 1 µm filtered seawater at a mean salinity of 34.5 and at 0.5 l min^{-1} aeration.

As in experiment 1, larvae were fed a bispecific diet (1:1 in cells numbers) of T-ISO and CP (week 1) or CG (from week 2). Feeding depended on larval size (or biomass), beginning with a daily supply of 40 cells µl^{-1} and ending with 200 cells µl^{-1}of microalgae. This ration was calculated to provide more phytoplankton than would be consumed by larvae

allowing a permanent availability of phytoplankton of 30 cells μl^{-1} of T-ISO + CP or CG around the larvae in order to sustain larval growth.

Five different temperatures were tested: 17, 22, 25, 27 and 32 °C. Each experimental condition consisted of a test tank with larvae and a control tank with only constant flow of enriched phytoplankton seawater. This experiment was conducted twice to provide replication. D-stage larvae, initially reared at 25 °C, were adjusted to each temperature at a rate 0.5 °C h^{-1} and then allowed to acclimate for 1 day. Owing to a relative short larval life, a brief period for temperature acclimation was applied. At the beginning of the experiment larval survival was estimated and revaluated a week later to secure our approach. Shell length (µm), growth rate (µm d^{1}), metamorphosis (%) and ingestion rate (cells $larvae^{-1}$ d^{-1}) were calculated as described above (Section 2.2) and the maximal ingestion rate reported here corresponded to mixotrophic and exotrophic periods at each temperature. The Arrhenius temperature (T_A) was used to describe the effect of temperature on larval growth rates within the range of temperature (Kooijman, 2000). The Arrhenius temperature was estimated by means of a linear regression of the natural logarithmic of larval growth rates against the inverse absolute temperatures (17, 22, 25, 27 and 32 °C) to obtain the slope T_A of the linear graph.

2.4. Statistical analysis

The results of two temperature effect trial were pooled (n = 2) for the purpose of statistical analysis to diminish errors associated with the repetitions in time. Percentage data was arcsine transformed prior to statistical analysis. Normality and homoscedasticity were tested using a Kolmogorov–Smirnov and Cochran test, respectively. One-way ANOVA was used to test the effects of temperature or food density and when significant differences were

detected among means, a post hoc Scheffé's pairwise multiple comparison test was performed. The Statview® 5.0 software package was used to perform these analyses. The significance level was set at 5%.

3. Results

3.1. Hydrobiological environment survey

For both experiments seawater temperatures were stable and the averages remained within 0.2 °C of the desired experimental temperature. No fluorescence was recorded (0 FNU) in the additional tank. Seawater salinity and pH throughout the experiments were 34.0 ± 0.5 psu and pH = 8.20 ± 0.01 respectively.

3.2. Effects of food density

At all food concentrations, mortality was very low throughout the experiment (≤10%). Increasing food density increased larval ingestion (Fig. III.1). Larvae surrounded by phytoplankton at 20 and 40 cells μl^{-1} (1:1 cells of T-ISO + CP or CG) showed three phases in their feeding activity throughout larval development. Low algal consumption (4000 to 8000 cells $larvae^{-1}$ d^{-1}) characterised the first 5 d and corresponded to the mixotrophic period. Throughout the exotrophic period, ingestion increased markedly up to day 14 with values as high as 45 000 to 55 000 cells $larvae^{-1}$. Then a drop in phytoplankton ingestion occurred during metamorphosis. Larvae surrounded by 12 cells μl^{-1} displayed a low ingestion over the whole rearing period with a maximum ingestion of 17 000 cells $larvae^{-1}$ d^{-1} (Fig. III.1).

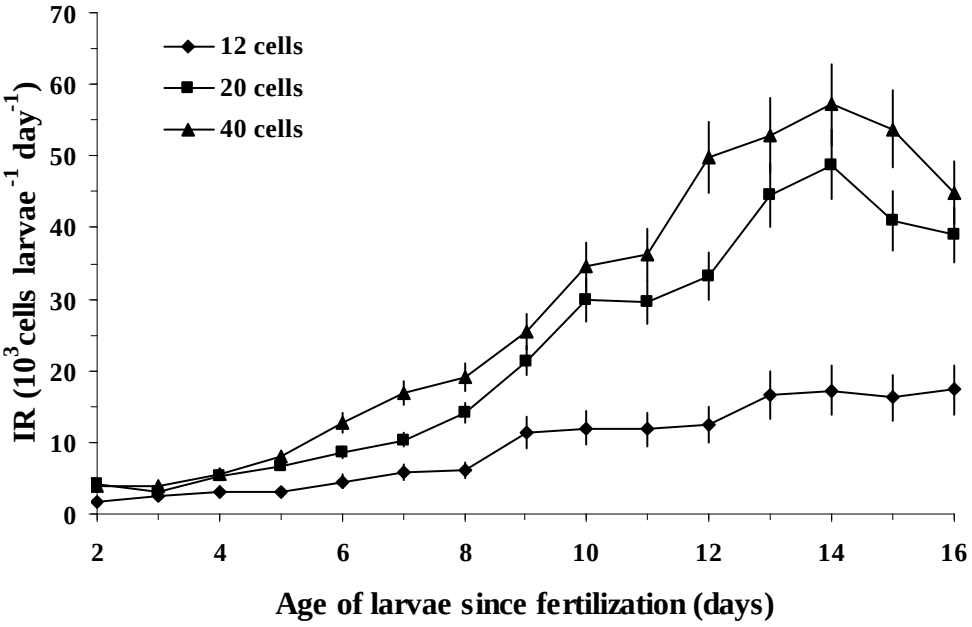

Fig. III.1. Ingestion rate (IR in 10^3 cells $larvae^{-1}$ day^{-1}) of *C. gigas* larvae, reared at 25°C, surrounded by three different phytoplankton densities. Each point represents daily mean ± SD (*n* = 6–7 measurements).

When plotting ingestion rate against food density a hyperbolic functional response was obtained (Fig. III.2). Indeed the ingestion rate was linearly correlated to increasing food density up to a maximum of 25 cells μl^{-1} corresponding to a maximal ingestion rate of 50 000 cells $larvae^{-1}$ d^{-1}. This value remained rather constant when food density increased up to 45 cells μl^{-1}.

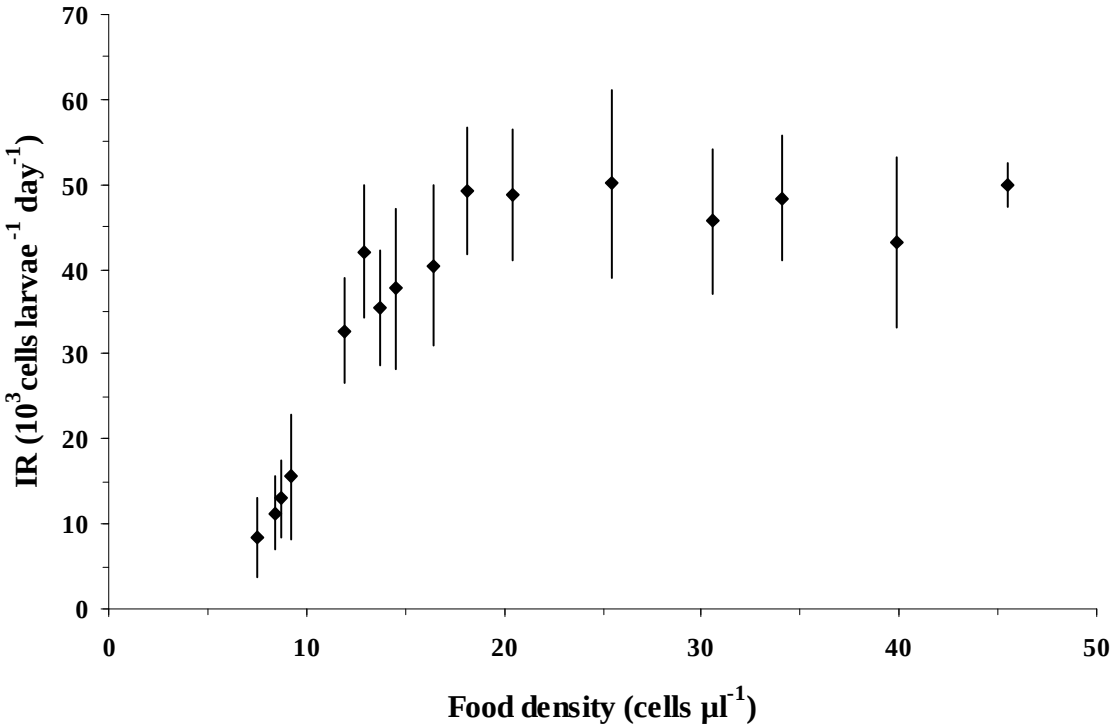

Fig. III.2. Functional response (10^3 cells larvae^{-1} day^{-1}) of *C. gigas* larvae, reared at 25°C, surrounded by different phytoplankton densities (7.5 – 45 cells µl^{-1}). Values are means (n = 10) ± SD.

Because a straight line relationship exists during the exotrophic period between larval growth (length) and food density the plotted slope was equivalent to the larval growth rates (Fig. III.3). Overall larval growth increased with increasing algal density. For all treatments, the correlation coefficient value of fitted regression lines was high ($r^2 \geq 0.97$). A constant availability of 20 and 40 cells µl^{-1} around the larvae allowed the highest growth rates with no significant differences between both values (17.07 ± 2.52 and 19.55 ± 2.63 µm d^{-1}, respectively) while a concentration of 12 cells µl^{-1} around the larvae led to the lowest growth rates (11.87 ± 1.97 µm d^{-1}), significantly different from the others (Fig. III.3).

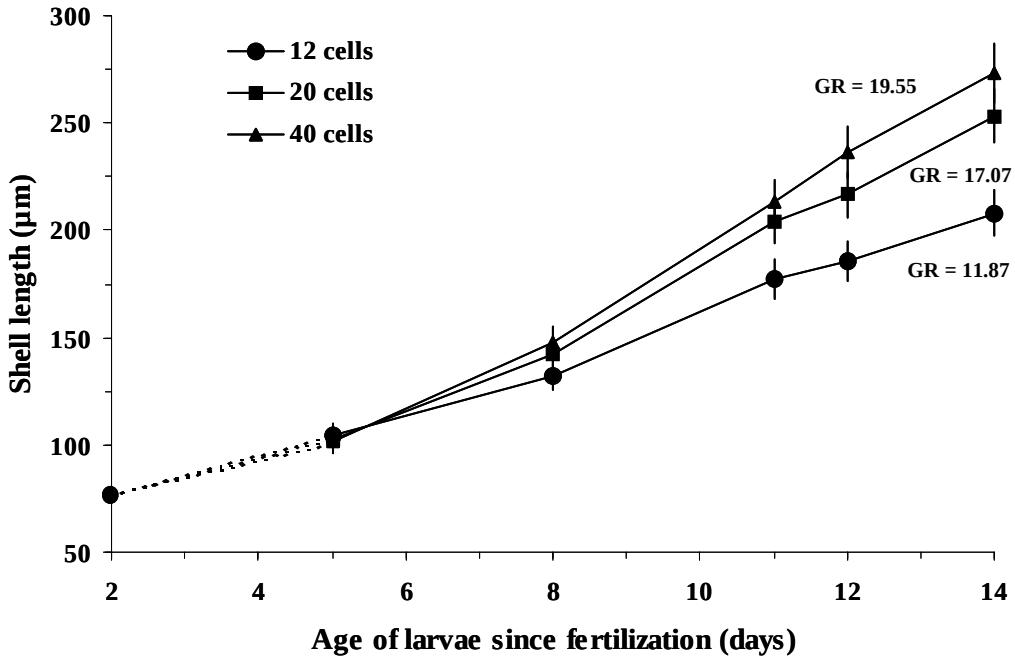

Fig. III.3. Larval growth (mean ± SD; $n \geq 200$) of *C. gigas* larvae, reared at 25°C, surrounded by three different phytoplankton densities (cells μl^{-1}). Dashed lines correspond to the mixotrophic period while plain lines represent the exotrophic phase. GR = Larval growth rates ($\mu m\ day^{-1}$) were estimated for the exotrophic period only (day 5-14).

In all treatments the first competent eyed larvae were observed on day 16. Food density affected metamorphosis. A supply of 12 cells μl^{-1} around larvae produced significantly lower metamorphosis (25.5 ± 5.3%) while larvae supplied 20 and 40 cells μl^{-1} metamorphosed at 44 (± 5.4%) and 40 (± 2.1%) respectively with no significant difference between treatments.

3.3. Effects of temperature

On day 2, at the end of the temperature acclimation period, low larval mortalities (≤2%) were recorded and confirmed on day 7 after the first draining of the test tank volume. At the end of the experiment mortality was ≤10% within the temperature range from 22 to 32 °C and ≤20% at 17 °C.

Temperature strongly influenced larval feeding activity. As a general pattern, the consumption by larvae was low during the first days of rearing corresponding to the mixotrophic period for D-stage larvae. When larvae became umbonate, ingestion increased and peaked for eyed during the exotrophic period. Thereafter, ingestion decreased to metamorphosis. According to this feeding pattern, there were differences in the maximal ingestion rate reached by larvae during mixotrophic and exotrophic periods at each temperature (Table III.2). The mixotrophic period lasted 5 d for larvae reared from 22 and 32 °C. Over this period there were significant differences in maximal ingestion rate and a test of pairwise multiple comparison pointed out two homogenous groups: one for larvae reared at 27 and 32 °C with 7700 and 8200 cells larvae^{-1} d^{-1} respectively and a second one for those reared at 22 and 25 °C with 4000 and 5400 cells larvae^{-1} d^{-1} respectively. With regard to the lowest temperature, the mixotrophic period extended to 10 d at 17 °C with a maximal ingestion rate of 3100 cells larvae^{-1} d^{-1}. The exotrophic period varied with temperature. It extended to day 12 at 27 and 32 °C and was characterized by the highest ingestion rates (55 200 and 64 500 cells larvae^{-1} d^{-1}, respectively). At 25 °C, the exotrophic period extended to day 16 with a maximal ingestion rate of 41 900 cells larvae^{-1} d^{-1} while at 22 °C this value reached 51 500 cells larvae^{-1} d^{-1}, albeit 5 d later, on day 21. Lastly, larvae reared at 17 °C exhibited the lowest ingestion over the exotrophic period with a maximal ingestion rate of 38 300 cells larvae^{-1} d^{-1}, on day 32.

Table III.2

Effects of temperature on the maximal ingestion rate (mean ± SD; n = 6–7 measurements) expressed in 10^3 cells $larvae^{-1}$ day^{-1} and recorded during mixotrophic and exotrophic periods. Numbers in brackets below indicate the duration in days of each feeding period. Larvae were surrounded by 30 cells μl^{-1} of T-ISO + CP or CG (1:1 in cells number). Different superscript letters indicate a significant difference (P<0.05).

Temperature (°C)	Mixotrophic period	Exotrophic period
17	3.1 ± 0.2 [a] (2–10)	38.3 ± 0.3 [a] (11–32)
22	4.0 ± 0.2 [b] (2–5)	51.5 ± 0.8 [b] (6–21)
25	5.4 ± 0.4 [b] (2–5)	41.9 ± 0.6 [c] (6–16)
27	7.7 ± 0.3 [c] (2–5)	55.2 ± 0.6 [d] (6–12)
32	8.2 ± 0.4 [c] (2–5)	64.5 ± 0.9 [d] (6–12)

Based on feeding activity, larvae exhibited a high ingestion rate during the exotrophic period and, accordingly, a more precise linear relationship with growth could be achieved when only larvae from 100 µm up to 280 µm were considered. Thus, for each temperature, we expressed larval growth during exotrophic period (Fig. III.4) during which a linear relation with temperature was found with high correlation coefficient values of fitted regression lines (r^2 ≥0.95). Larval growth (µm d^{-1} ± SD) increased with increasing water temperatures (F=385.67; df 4; P<0.0001): 5.95 ± 1.53, 11.41 ± 2.17, 15.99 ± 2.48, 19.31 ± 2.37, 19.95 ± 3.11 at 17, 22, 25, 27 and 32 °C respectively (Fig. III.4). Nevertheless, no significant differences between 27 and 32 °C were found by pairwise test comparison despite an artefact

on day 13 with a slight decrease of larval growth at 32 °C, probably due to early settlement of the largest larvae. In contrast, pairwise test comparison revealed significant differences in larval growth at 22 and 25 °C while lowest growth occurred at 17 °C. When growth data were reanalysed at different temperatures, as an Arrhenius plot, the linear relationship was fitted and the estimate of the Arrhenius temperature (T_A) was 11 000 ± 2500 K (r^2 = 0.97).

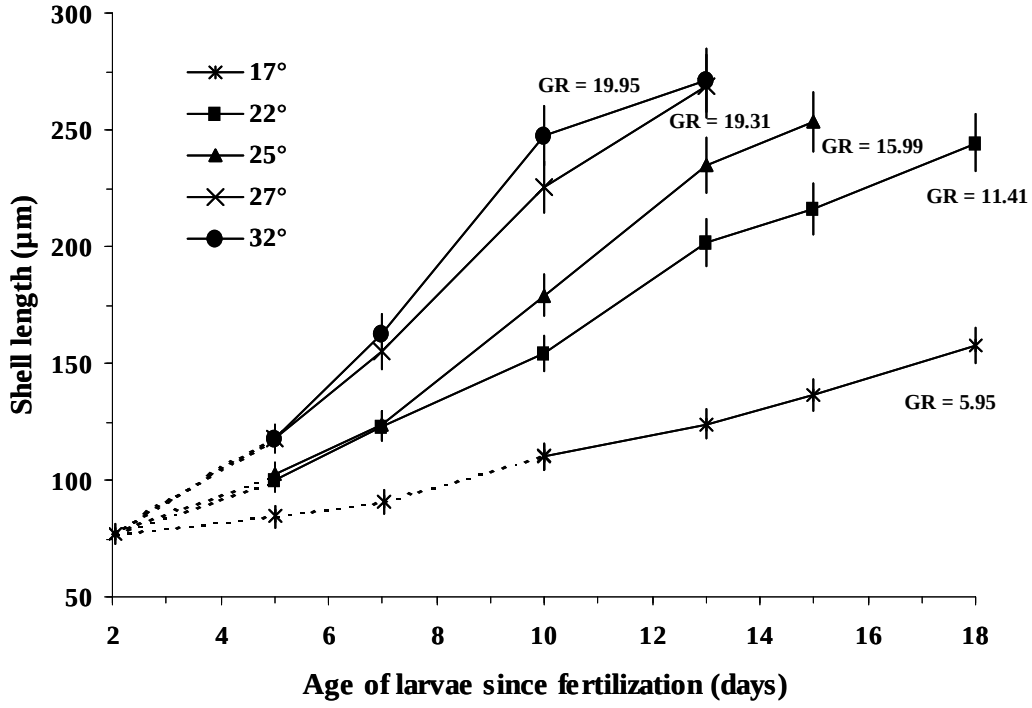

Fig. III.4. Larval growth (mean ± SD; $n \geq 200$) of *C. gigas*, surrounded by 30 cells μl^{-1} and reared at five temperatures. Dashed lines correspond to the mixotrophic period while plain lines represent the exotrophic phase. GR = Larval growth rates ($\mu m\ day^{-1}$) were calculated for the exotrophic period only.

Success at metamorphosis followed a similar trend as for growth with highest settlement at 27 and 32 °C (87 and 86% respectively) achieved in less than 15 d with detection of eyed larvae from day 11. Larvae reared at 22 and 25 °C led to similar settlement

(55 ± 8 and 58 ± 10%, respectively) but delayed at 22°C on day 25 (*vs.* day 20 at 25 °C). The temperature of 17 °C led to the lowest settlement (16 ± 3%), which only occurred on day 35.

4. Discussion

4.1. Effects of food density

This study suggests that larval algal consumption varies with larval size in order to sustain growth and metamorphosis. Food ration remained within a broad range of a phytoplankton supply from 30 cells μl^{-1} to 300 cells μl^{-1} throughout larval rearing in order to maintain a fixed algal density around the larvae by means of a flow-through rearing system. In these conditions, our experiments were accordingly carried out under constant food level and allowed the set up of a long-term physiological response (larval ingestion rate).

Larvae surrounded by 20 and 40 cells μl^{-1} exhibited three feeding phases during development. A low but progressive increase in consumption was noted from D-stage veliger to early umbonate larvae (phase 1). During phase 2, microalgae ingestion increased sharply from umbonate to eyed larvae, until a sudden decrease was noted approaching metamorphosis (phase 3). The feeding pattern during the mixotrophic phase may be related to the incomplete development of the digestive system in larvae. Gallager (1988) stated that young bivalve larvae (≤100 µm length shell) have a narrow oesophagus and a gut volume that may limit the size and number of particles ingested. During this phase, metabolism is supported by the reserve material provided in the eggs (Bayne, 1983).

In this study, *C. gigas* larvae started the exotrophic stage (phase 2) with an ingestion rate increase when they measured ≈100-110 µm. This ingestion capability may be linked to the development of the velar ciliary tracts (Strathmann, 1978) allowing the larva to trap

particles, transport them through the food groove and ingest them in an efficient manner. This feeding behaviour shows that larval rearing for the first few days may occur with a low food supply but when larvae initiate strict exogenous feeding (from days 5 to 6 in the present study) the amount of food becomes an essential factor in their successful development. One aim for the larvae during the exotrophic stage is the accumulation of sufficient reserves to allow them to meet the energy demands during the process of metamorphosis (phase 3) and ensure their capacity for survival (Haws et al., 1993). In this way, the viability of larvae, before metamorphosis, depends on both accumulation and utilisation of energy substrates, mainly related to catabolism of proteins and lipids (Bartlett, 1979; Laing and Earl, 1998; Moran and Manahan, 2004). When the larvae undergo the process of metamorphosis, their feeding pattern is altered because behavioural and morphological processes take place. Indeed, movement and feeding are inhibited because the velum is absorbed and replaced with the branchia (Cannuel and Beninger, 2006). Moreover, late pediveliger larvae increase crawling behaviour in search of a suitable substratum for metamorphosis. Therefore, a temporary stop in the ingestion is not surprising and because this event is not synchronous in the whole larval population the mean ingestion rate decreased at the end of the larval life.

Our results show that *C. gigas* ingestion activity was also linearly related to food density up to a threshold level above which ingestion remains fairly constant. This feeding behaviour can be compared to food densities to assess the functional response (Holling, 1959). An alternative pattern to describe the relationship between food density and larval ingestion rates in this study appeared to be consistent with a hyperbolic function. This function model establishing that ingestion rate is food dependant up to a plateau has also been reported for *C. virginica* (Baldwin and Newell, 1995).

In the present study ingestion rate increased parallel to food density up to a saturation level. Such a pattern is in agreement with other studies on bivalve larvae exposed to a

microalgae supply gradient. Nevertheless, the saturation level is species specific. Gallager (1988) for *Mercenaria mercenaria*, MacDonald (1988) for *Patinopecten yessoensis* and Baldwin and Newell (1995) for *C. virginica* obtained such saturation of ingestion at relatively low concentrations of food ranging from 10 to 40 cells μl^{-1} with the same microalgae T-ISO. In contrast, Crisp et al. (1985) for *Ostrea edulis* and Pérez-Camacho et al. (1994) for *R. decussatus* reported that such saturation occurred at higher concentrations from 200 to 250 cells μl^{-1} using *Pavlova lutheri* or *Isochrysis galbana* as food supply. Differences in food level saturation between species suggest that larvae may differ in energy acquisition and utilization. Moreover, these variations should also be related to rearing conditions (*e.g.* flow-through system or larval density). Nevertheless, our results show that saturation for *C. gigas* larvae occurred at higher concentrations equivalent to a residual phytoplankton of 20 cells μl^{-1}. At the same time, food supply was increased in a range of 60 to 260 cells μl^{-1} as larvae grew to avoid competition for food between larvae and maximise increase in biomass through the whole larval rearing (Table III.1). Considering this feature, maximal ingestion was reached on day 14 for a supply of 260 cells μl^{-1}.

The functional response demonstrated that *C. gigas* larvae achieved the maximal ingestion rate for 50 000 cells $larvae^{-1}$ day^{-1}. In a similar context, Baldwin and Newell (1995) reported that maximal ingestion rate of *C. virginica* larvae, expressed in cell volume concentration, was equal to 2.2×10^6 μm^3 d^{-1} $larva^{-1}$ of 200 µm length when fed natural phytoplankton. When our data are converted in cell volume, based on measurement of bispecific microalgae sample (mean value of 48 μm^3) we obtained a similar maximal ingestion rate of 2.4×10^6 μm^3 $larva^{-1}$ d^{-1}. This similarity suggests that larvae can control their feeding patterns and respond in this way to reach their maximal ingestion as long as nutritional quality is achieved because natural phytoplankton might be nutritionally comparable to cultured food (bispecific diet) used in the present study.

On the other hand, the growth rate was directly correlated to the amount of food delivered as larvae grew. This study points out that the maximal growth rate occurred when larvae were surrounded by 40 cells μl^{-1} but did not differ statistically when larvae were surrounded by 20 cells μl^{-1}. In this way, the food ration meeting this requirement corresponds to maximal ingestion rate, which was achieved from 20 cells μl^{-1}. To corroborate the feeding behaviour of *C. gigas* larvae it would be interesting to experiment on lower ($\leq$5 cells μl^{-1}) and higher ($\geq$50 cells μl^{-1}) phytoplankton densities surrounding larvae despite present evidence.

On the other hand, metamorphosis performances have been reported here because this is important from an aquaculture standpoint. In the present work, larvae settled at their convenience without either size selection or metamorphosis induction. When phytoplankton concentrations were maintained at 20 and 40 cells μl^{-1}, 40 to 44% of newly settled spat were detected 5 d after the onset of metamorphosis but it has to be considered that the remaining population of larvae was competent to metamorphose and mortality was $\leq$10% at the end of the experiment.

4.2. Effects of temperature

The results of the present study indicated that temperature greatly influenced ingestion, growth and metamorphosis of *C. gigas* larvae. Increased ingestion was recorded as temperature increased resulting in higher growth throughout larval development. Similar observations were reported by Beiras et al. (1994) on *Ruditapes decussatus* larvae. Our work however is the first report of feeding activity over an entire larval life cycle including metamorphosis of *C. gigas* over a range of temperatures (17 to 32 °C).

Gerdes (1983a) was the first author to report in detail ecophysiological data on *C. gigas* larvae, however working with a single temperature (25 °C) and using a different

technical approach (closed rearing system). Moreover, the food supply was constant during his experiments and thus he did not take into account larval biomass increase. Lastly his results were tainted with high variability of ingestion measurements which may be explained by variations in food concentration surrounding the larvae. Under these conditions it is not surprising that our results diverged from those reported by Gerdes (1983a) who found higher ingestion rates for larvae.

In our work a sustained response in ingestion activity within a wide range of temperature was possible by using an efficient flow-through rearing system, which supplied food in an abundant and reliable way to allow an adequate food uptake. A feeding response to temperature range, even at higher temperatures, was achievable because, in the one hand, food was provided in abundance to supply more microalgae than larvae would feed. On the other hand, the integrity of phytoplankton cells used in the present study when exposed to a wide range of temperatures (17 to 32°C) guaranteed the availability of the food delivered to the larvae. Lastly such a technical approach allowed a constant seawater renewal during the whole experimental period, thus avoiding deposition of algal cells at high temperatures and accordingly the occurrence of bacteria bloom due to larval metabolites and degradation of phytoplankton. This has been reported to be a recurrent problem in previous studies on bivalve larvae (Helm and Millican, 1977; Hrs-Brenko, 1981; Lemos et al., 1994). The ingestion activity increase with temperature suggests a thermal acclimation strategy for larvae to fulfil their energetic requirements. This biological strategy may be related to an enhancement in cilia activity for feeding and catalytic activity of digestive enzymes to offset thermal stress and regulate the metabolism of larvae.

This study showed undoubtedly the high tolerance of *C. gigas* larvae (17 to 32 °C) expressed with a high value of Arrhenius temperature (11 000 K). Previous studies on others bivalve larvae reported an Arrhenius relationship of 8460 K for *Mytilus edulis* (Sprung, 1984b

cited in Kooijman, 2000) and 7596 K for *Macoma balthica* (Bos et al., 2006). Although these experiments were carried out within a smaller range of temperatures (7 to 17 °C) they showed a lower thermal tolerance for these species. Our data suggest an ability to adjust physiological rates to confront environmental temperature change within the range of relevant temperatures and upper and lower non lethal margins. Moreover, this broad tolerance range of temperature might explain the cosmopolitan distribution of *C. gigas* among subtropical and temperate areas (Goulletquer et al., 1999; Langdon et al., 2003; Sicard et al., 2006) and its progressive geographical extension in new environments (Diederich et al., 2005) since their larvae are able to tolerate temperate (17 °C) to warm (32 °C) seawater temperatures.

Results from the present study confirmed that *C. gigas* larval growth increases markedly with increasing temperature as previously reported in the literature (Helm and Millican, 1977; His et al., 1989; Abdel-Hamid et al., 1992). *C. gigas* larvae proved to be an eurythermic stage within a wide temperature range as demonstrated in adult stage (Sicard et al., 2006), but with a marked thermo-dependency between 22 and 32 °C. Within this range, the optimal temperature expressed as maximum growth rates occurred at 32 °C but did not differ significantly at 27 °C. Moreover, at both temperatures low mortalities (≤10%) were displayed at the end of the experiments. Therefore the present data showed that a physiological response was achievable even at the highest temperature, which demonstrated that 32 °C is not the upper thermal limit for *C. gigas* during its larval stage. On that count our results are conflicting with those of Helm and Millican (1977) who reported high mortalities (65%) at 32°C. This sensibility to such a temperature has been also reported for *C. gigas* juveniles (Flores-Vergara et al., 2004) and adults (Bougrier et al., 1995) with mortalities ≥40%. Complementary experiments should be accordingly carried out to determine the upper thermal boundary of *C. gigas* larvae with temperature ≥33 °C. Thus, growth of *C. virginica* larvae is reduced at 33 °C and massive death occurred at 35 °C (Davis and Calabrese, 1964).

The lowest temperature applied in the present study (17 °C) caused no other apparent damage than a significant reduction of larval growth, mortalities being ≤20% after one month of rearing. The failure of the larvae to grow at low temperature may be due to their inability to digest ingested microalgae despite food availability (Manoj and Appukuttan, 2003). His et al. (1989) reported that *C. gigas* larvae can survive at 15°C but their experiments lasted only one week. The present study suggests that at such a temperature the larvae may still have been in the mixotrophic phase at the end of their experiment and therefore it is not surprising that survival was high.

Concerning metamorphosis, a positive relation can be drawn with increase in temperature, which is in agreement with previous observations on *Saccostrea glomerata* (Dove and O'Connor, 2007) with an optimal temperature at 30°C. According to Bayne (1983), larvae delay metamorphosis at low temperatures for longer periods than at high temperatures and the duration of the free swimming larval period is accordingly prolonged. Our data showed that larvae reared at 17 °C initiated settlement on day 31 but only a small fraction of the population was concerned (16%). This result demonstrates that 17 °C could be considered as an impractical temperature for *C. gigas* commercial hatchery from an economic standpoint. In contrast, 27 °C led to the best growth (19.31 µm d^{-1}) and highest settlement (87%) in less than two weeks (no significant differences at 32 °C). This temperature is accordingly recommended for Pacific oyster hatchery production but great caution is required to fulfil the larval feeding requirements and to limit bacterial proliferation. The use of flow-through rearing techniques should overcome both constraints. Lastly, in such systems, *C. gigas* larval performances are reproducible because for similar food supply (20 cells. μl^{-1} around larvae) and temperature (25 °C) growth and settlement in exp. 1 were 17 µm d^{-1} and 44 ± 5% *vs*. 16 µm d^{-1} and 58 ± 10% in exp. 2.

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CHAPITRE IV

Dynamic Energy Budget (DEB) growth model for

Pacific oyster larvae, *Crassostrea gigas*

soumis à Aquaculture

Benjamin RICO-VILLA, Ismaël BERNARD, René ROBERT, Stéphane POUVREAU

Abstract

Dynamic Energy Budget (DEB) theory aims to quantify the energetic framework of an individual organism as a dynamic model, from the uptake of food to its utilisation in metabolic processes (maintenance, growth, development and reproduction). The purpose of the present paper is to extend the existing DEB model for adult Pacific oyster *Crassostrea gigas* to the larval life stage of this species. We present the application of generic DEB theory to oyster larvae, with the formulation of the specific assumptions based on the characteristics of this stage. The model depends on seawater temperature and food density, as forcing variables, followed throughout the whole larval development. We calculated DEB parameter values for larvae by means of laboratory experiments specifically designed to collect datasets on ingestion and growth at different levels of phytoplankton density and temperature. The DEB model developed here showed good growth simulations and provided an extensive description of the energetic needs of *C. gigas* during its larval stage. It was demonstrated that, at 27 °C, a food density of 1000 $\mu m^3\ \mu l^{-1}$ must be maintained around larvae throughout larval development to maximise growth and metamorphosis success. Timing of metamorphosis decreases exponentially with increasing temperature. The model is not species specific, however, and can thus be applied to other bivalve larvae.

Keywords: Dynamic Energy Budget; *Crassostrea gigas*; Bivalve larvae; Ecophysiology; Growth

1. Introduction

Bivalve molluscs (e.g. oysters, mussels, clams and scallops) represent a significant proportion of world aquaculture production (Helm et al., 2004), in which the most important commercial marine bivalve is the Pacific oyster, *Crassostrea gigas* (FAO, 2008). Traditionally, bivalve culture in France relies on the collection of juveniles from the natural environment but, due to irregular recruitment (spatfall) and the high demand for triploid oysters, spat production in hatcheries has been increasing rapidly over the last five years. In this context, a better understanding of the biology of *C. gigas* larvae is required because the larval stage is critical for hatchery management.

The growth and survival of bivalve larvae is determined by complex interactions between their physiology and the environmental conditions. Among the diversity of influencing factors, temperature and food concentration are considered to be the primary environmental factors affecting physiological processes in oyster larvae (Robert et al., 1988; His et al., 1989; Lemos et al., 1994; Dove and O'Connor, 2007). Quantitative study of the effects of these environmental factors and their interactions on larval growth can be aided by numerical models. An existing model for *C. virginica* larvae (Dekshenieks et al., 1993) explains growth and development as function of temperature, food concentration, salinity and turbidity. Nevertheless, this work does not examine the energetic functioning of larvae at the level of basic physiology (ingestion and maintenance processes). In *C. gigas*, a net production-type model (Bochenek et al., 2001; Powell et al., 2002; Hofmann et al., 2004) was developed to examine the biochemical influence of changes in food quality and quantity on larval development. Although this approach is more appropriate for modelling larval growth, this model lacks valid experimental measurements made for the whole larval development over a wide range of environmental factors. In addition, this model is based on the Scope for

Growth concept (Bayne, 1976), which is empirically-based and free-formulated through allometric relationships (Bourles et al., in press) and assumes that assimilated energy is immediately available for maintenance while the rest is converted into structural components (growth) or storage material (reserves) (Bochenek et al., 2001).

In the present paper, a more mechanistic energetic model is proposed based on the Dynamic Energy Budget (DEB) theory (Kooijman, 2000). The recent development of DEB theory offers a general framework for examining the energetics of an organism in a systematic way. This theory describes the rates at which an individual organism assimilates energy from food uptake and stores this energy as reserves for allocation to the physiological functions of maintenance, growth, development and reproduction. In addition, DEB theory offers attractive improvements for energetic modelling based on simple assumptions that describe energy flow according to physiological processes that species have in common. This generic aspect allows different species to be compared, as the only interspecies difference will lie in the DEB parameter values. Most parameters can be estimated in the laboratory under controlled environmental conditions, using temperature and food density as forcing variables. Recently, practical applications of DEB theory have been validated for energetics in *C. gigas* adults in natural surroundings (Bacher and Gangnery, 2006; Ren and Schiel, 2008) as well as in controlled conditions (Pouvreau et al., 2006; Bourles et al., in press). Based on this last existing DEB model, the present study aimed to apply DEB theory to simulate growth of *C. gigas* at larval stage in hatchery. To our knowledge, this is the first model that would be capable of representing the energetic needs of a bivalve species throughout the whole larval life cycle.

The first part of this paper presents some basic concepts of the DEB theory and assumptions made for *C. gigas* larvae. The second part consists of the estimation of DEB parameters values for oyster larvae from experimental data on ingestion and growth collected

under precisely controlled conditions of phytoplankton density and temperature. The final part of the paper focuses on calibration and simulation with the larval DEB model using our own experimental datasets, and its validation based on the input of "external" data collected in our experimental facilities by means of other independent trials.

2. Materials and methods

2.1. Concepts and DEB model formulation for the larval stage

In DEB theory, an organism is partitioned into three main body components: 1) structural biovolume or somatic tissue; 2) stored energy reserve; and 3) gonads and/or stored energy reserves allocated to maturity and reproduction (Kooijman, 2000). Moreover, in the theory, three life stages are distinguished: 1) embryos, which neither feed nor reproduce; 2) juveniles, which feed but do not reproduce; and 3) adults which both feed and reproduce. DEB theory describes a larva as a juvenile because larvae feed and their resources are not yet allocated to reproduction, but to other developmental processes. Therefore, energy from food is stored directly as reserves and is then directed towards growth, development and their maintenance. The DEB model was accordingly simplified for the larval stage, compared with the adult stage (Pouvreau et al., 2006), due to the absence of reproduction.

The general framework of the DEB model for a larva is represented in Fig. IV.1, and parameters of different equations of the model are given in Table IV.1. DEB theory is described below and the equations used were issued or adapted from the DEB model validated for adult *C. gigas* (Pouvreau et al., 2006). The notation and symbols used are from Kooijman (2000) and faithfully follow the rules: quantities that are expressed per unit of

structural biovolume have square brackets, [ ]; quantities per unit of biosurface have braces, { }; and rates, which have dots above them, indicate the dimension over time.

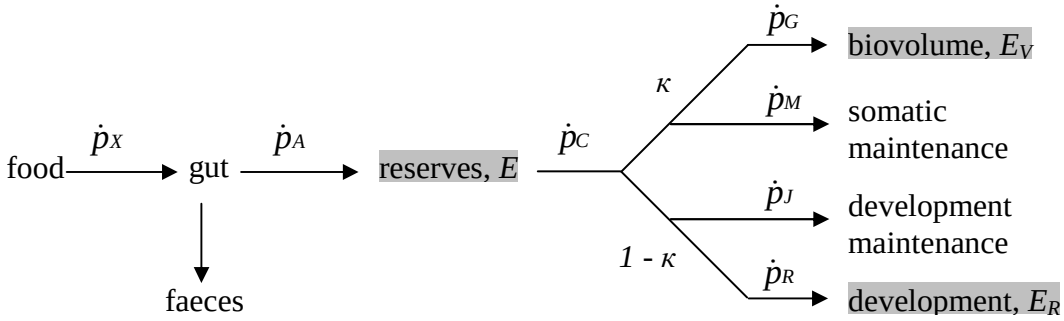

Fig. IV.1. Schematic representation of the energy flow through *Crassostrea gigas* larvae according to the DEB model (Kooijman, 2000). The three state variables are highlighted in grey.

According to DEB theory, a larva can be described by three state variables: the energy in the reserve, *E*, the structural body volume, *V*, and the amount of energy invested into development to reach juvenile stage, E_R. The ingestion rate, $\dot{J}x$ ($\mu m^3\ d^{-1}$), is proportional to the biosurface of a larva, $V^{2/3}$ (μm^2), and relies on available food density, X (phytoplankton expressed in $\mu m^3\ \mu l^{-1}$), in the environment:

$$\dot{J}_X = \left\{\dot{J}_{Xm}\right\} \cdot f \cdot V^{2/3} \qquad (1)$$

where $\left\{\dot{J}_{Xm}\right\}$ is the maximum ingestion rate per unit of biosurface (expressed in $\mu m^3\ d^{-1}\ \mu m^{-2}$), X_K is the half saturation coefficient ($\mu m^3\ \mu l^{-1}$) and *f* is the feeding function response, which can vary between 0 and 1 (dimensionless).

In the present study the feeding functional response, *f*, for *C. gigas* larvae appears to be sigmoid (or "S-shaped"), according to the type 3 Holling (1959) functional response,

$f=\frac{X^2}{X^2+X_K^2}$, which contrasts with the type 2 hyperbolic function, $f=\frac{X}{X+X_K}$, used for a *C. gigas* adult. This choice of a type 3 functional response was made because it agrees with previous observations for other oyster larvae such as those of *Crassostrea virginica* (Baldwin and Newell, 1995). In summary, the type 3 response differs from type 2 because the sigmoid model exhibits a point of inflection in the curved variation of $\dot{J}x$ with X when food is scarce (Gentleman et al., 2003).

The ingested food is converted into assimilates, which are added to the reserve, *E*, with constant assimilation efficiency (*ae*, dimensionless) and according to a food energy conversion (μ_X, J µm^{-3}) before allocation. Consequently the assimilation rate, $\dot{p}_A$, can be described in Eq. 1 as:

$$\dot{p}_A = ae \cdot \mu_X \cdot \dot{J}_X = ae \cdot \mu_X \cdot \left\{\dot{J}_{Xm}\right\} \cdot f \cdot V^{2/3} = \left\{\dot{p}_{Am}\right\} \cdot f \cdot V^{2/3} \quad (2)$$

where $\left\{\dot{p}_{Am}\right\}$ is the maximum surface-area-specific assimilation rate expressed in J d^{-1} cm^{-2}. The assimilation efficiency was assumed to be 0.4 (Walne, 1965; Sprung, 1984) and the food energy conversion was found to be equal to 4.5 × 10^{-9} J µm^{-3} of microalgae (Brown, 1991; Brown and Robert, 2002).

The dynamics of the energy in the reserve, *E*, follow the energy conservation law and can be described as:

$$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C \quad (3)$$

Energy is mobilised from this reserve pool to fuel the organism's activities at a rate called the catabolic power or utilisation rate, $\dot{p}_C$:

$$\dot{p}_C = \frac{E/V}{[E_G] + \kappa \cdot E/V}\left(\frac{[E_G]\cdot\{\dot{p}_{Am}\}\cdot V^{2/3}}{[E_m]} + [\dot{p}_M]\cdot V\right) \quad (4)$$

An extended explanation of Eq. 4 can be found in Kooijman (2000). In summary, E/V corresponds to the energy density in an organism (J cm^{-3}), which may vary between 0 and the maximum energy density, $[E_m]$ (J cm^{-3}), according to the food density in the environment. The mobilized reserve is described by the allocation rule where the parameter κ corresponds to a fixed fraction of utilisation rate, $\dot{p}_C$, spent on maintenance of somatic tissue, as absolute priority, and structural growth for larvae; the rest $(1-\kappa)$ goes to development and its maintenance during the larval stage. $[\dot{p}_M]$ is a parameter that denotes the volume-specific maintenance rate (J d^{-1} cm^{-3}). $[E_G]$ (J cm^{-3}) indicates the volume-specific costs for structural growth, E_V, with $\dot{p}_G$ as the flow of energy from the reserve allocated to this process. The fraction of catabolic power allocated to $\dot{p}_M$ and $\dot{p}_G$ can be defined as:

$$\frac{dE_V}{dt} = \dot{p}_G = \kappa \cdot \dot{p}_C - \dot{p}_M \quad (5)$$

Maintenance costs, $\dot{p}_M$, are scaled with volume and correspond to all processes that keep the organism alive. For example, for an oyster larva, this consists of the concentration gradients across membranes, osmoregulation, continuous shell production and mucociliary activity (Strathmann, 1979). Somatic maintenance has priority over growth; hence growth ceases when the fraction κ of the mobilised reserve is equal to, or lower than, somatic maintenance demands. The energy of the reserve flow allocated to maintenance costs, $\dot{p}_M$, can be expressed as:

$$\dot{p}_M = [\dot{p}_M] \cdot V \quad (6)$$

where $[\dot{p}_M]$ is the volume-specific maintenance rate (J d^{-1} μm^{-3}) and V the body volume (μm^3). Then, the dynamics for the structural body volume, V, are expressed in Eq. 5.

For larvae, DEB theory states that a fixed proportion $(1-\kappa)$ of the energy is spent on development and maintenance of this increase in complexity. This means that larvae have to become more complex for the transition to spat/juvenile stage. DEB theory states that stage transition from larva to juvenile occurs if such complexity reaches a threshold value. Growth and development are parallel processes in the DEB model. As a result, in the same way as for somatic allocation, the energy allocated to development plus its maintenance is equal to fixed proportion $(1-\kappa)\cdot \dot{p}_C$, which split into two fluxes denoted $\dot{p}_R$ and $\dot{p}_J$, respectively, and expressed in J d^{-1}. This remaining fraction can be defined as:

$$\frac{dE_R}{dt} = \dot{p}_R = (1-\kappa)\cdot \dot{p}_C - \dot{p}_J \quad (7)$$

This second flux for maintenance costs of development, $\dot{p}_J$, corresponds to the product of the energy allocated to development $(1-\kappa)/\kappa$, proportional to V and $[\dot{p}_M]$:

$$\dot{p}_J = V \cdot [\dot{p}_M] \cdot \left(\frac{1-\kappa}{\kappa}\right) \quad (8)$$

DEB theory introduces a shape coefficient, which relates length measurement, L, to structural body volume, V, for an organism. This parameter is dimensionless and indicates

that length has a specific relationship with volume in each species. During its development, an oyster larva does not have the same shape as an adult. Indeed, the shell only acquires its characteristic shape after metamorphosis. We therefore used a specific shape coefficient, δ_M, to convert shell length into volume for larvae. As a first approximation, wet weights can be converted into physical volumes representing larval structure and reserves. Therefore, the shape coefficient can be expressed as the "minimal envelope" without reserves, and be estimated by using a calibration curve with the form:

$$V = (\delta_M L)^3 \tag{9}$$

Physiological rates, such as ingestion and maintenance, depend on body temperature. This relation is usually well described by an Arrhenius-type equation within a species-specific tolerance range between upper and lower temperature boundaries (Kooijman, 2000). According to the Arrhenius relationship, this dependency can be expressed as:

$$\dot{k}(T) = \dot{k}_1 \cdot \exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\} \tag{10}$$

where $\dot{k}(T)$ is the value of the physiological rate that depends upon an ambient temperature T (in Kelvin), $\dot{k}_1$ is the value of the physiological rate at a reference temperature T_1 of 298 K (25 °C in this study), and T_A is the so-called Arrhenius temperature (in Kelvin). To take into account a tolerance range with temperature boundaries, the basic formula described above becomes:

$$\dot{k}(T) = \dot{k}_1 \cdot \exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\} \cdot \left(1 + \exp\left\{\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right\} + \exp\left\{\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right\}\right) \tag{11}$$

where T_L and T_H relate to the lower and upper boundaries and T_{AL} and T_{AH} are the Arrhenius temperatures for the rate of decrease at each boundary. All dimension temperatures are given in K . For *C. gigas* larvae, T_L and T_H were estimated from the literature and these values were initially fixed at 13°C and 32°C (Abdel-Hamid et al., 1992; Helm and Millican, 1977, respectively). As a first approximation, T_{AL} and T_{AH} values were both fixed at 75 000 K (van der Veer et al., 2006).

2.2. Specific additional assumptions for the larval stage

There are a number of specific characteristics of larval feeding behaviour that first needed to be stated in the model. Larval life begins with a low feeding phase (mixotrophic period) that lasts for 5 or 6 days for *C. gigas* larvae. The reserves contained in the embryo itself are consumed during this period (Gallager, 1988). Therefore, we established in the model that age at first feeding, a_b, occurs at 5.5 d when larvae reach a size of about 100 µm. After this mixotrophic period, exotrophic feeding begins, during which a marked increase in ingestion rate is observed (Gerdes, 1983; Rico-Villa et al., 2008). This assumption about feeding periods was applied over a wide range of temperature (17 to 32 °C) based on experimental data (Rico-Villa et al., 2009). During the exotrophic period larvae aim to accumulate sufficient substrate reserves to allow them to meet the energy demands they will face during metamorphosis, and thus ensure their survival (Haws et al., 1993). Also, a temporary halt in ingestion is expected in pediveliger larvae to allow them to complete new structures for benthic life (Gerdes, 1983; Rico-Villa et al., 2008). This feeding pattern is unsecured because late pediveliger larvae increase the energy that they use by crawling in search of a suitable substratum for settlement.

To take into account larval metamorphosis in the model, we assumed that some larvae achieved competence size earlier (>280 µm: Coon et al., 1990), underwent metamorphosis, and consequently disappeared from the population of larvae on which we were measuring mean size. As a result, we hypothesised that this precocity for some larvae is the reason for the slight bend in the curves of larval growth at the end of larval rearing experiments. To validate this hypothesis, we calculated the probability of metamorphosis of a larva as the cumulated normal distribution centred around 300 µm (S.D. 20 µm) length at metamorphosis, L_{mt}. This hypothesis also allowed the estimation of the new mean size of the population of larvae that had not yet metamorphosed.

2.3. Experimental protocol for parameters estimation

Fertilisation and larval culture were carried out at the Argenton experimental hatchery (Ifremer, France) as detailed by Rico-Villa et al. (2006). Briefly, gametes were obtained by the gonad-stripping technique on adult oysters previously conditioned at 19 °C. Embryo cultures were maintained at 25°C and salinity at 34 psu in 150 l rearing tanks of aerated 1 µm-filtered sea water for further development to the D-stage. All larval experiments were run starting with two-day-old D-stage larvae obtained by this method, and feeding supply was expressed in cell biovolume ($\mu m^3\ \mu l^{-1}$).

Three independent experiments were run on oyster larvae:

In experiment A, body wet mass of larvae was assessed according to length to determine the shape coefficient, δ_M .

In experiment B, the effects of temperature on larval growth were established to estimate the Arrhenius temperature, T_A.

In experiment C, larval ingestion was evaluated at different food density levels to calculate maximum surface area-specific ingestion rate, $\{\dot{J}_{Xm}\}$, and half saturation coefficient, X_K.

2.3.1. Experiment A: Shape coefficient (δ_M) determination

The shape coefficient parameter can be determined from the relationship between shell length and total body weight mass data for *C. gigas* larvae. Results from Gerdes (1983) and His and Maurer (1988) were combined with our own experimental data and expressed in dry tissue weight (DW). Our own dataset was acquired under the following conditions: larvae were reared in 150 l tanks at 5 larvae ml^{-1}, at 25 °C and salinity 34 psu. Larvae were fed daily *Isochrysis affinis galbana* (T-ISO: strain CCAP 927/14) and *Chaetoceros calcitrans* forma *pumilum* (strain CCAP 1010/05) at a 1:1 cell biovolume. Quantity of food supplied during larval and settlement stages depended on biomass increase, but a phytoplankton level of 1400 $\mu m^3\ \mu l^{-1}$ around the larvae was continuously maintained by progressively increasing the daily food ration at the entry to the rearing system, as detailed in Rico-Villa et al. (2008). Dry tissue weight and shell length were determined following methodologies described by His and Maurer (1988) and Rico-Villa et al. (2006), respectively, throughout the whole of larval development in these experiments. We recalculated DW data assuming that 80 % of total body weight mass corresponds to water. The shape coefficient, δ_M, was determined according to a non linear regression of Eq. 9 between the total body weight mass (ng) against the shell length (µm). Since the measured total body weight mass of larvae may include reserves as well as structure, the "minimal envelope" was estimated as the shape curve, fitting by eyes, just beneath the observed data points.

2.3.2. Experiment B: Arrhenius temperature (T_A) determination

The Arrhenius parameter T_A was calculated from laboratory datasets on larval growth recorded over a wide range of temperature. Moreover, results from His et al. (1989) were used to enhance the precision of parameters related to temperature. For our own experimental data, larvae were reared in a flow-through system to maintain a constant flow of algal cells and stable temperature conditions, and allow continuous hydrobiological data recording for each tank (Rico-Villa et al., 2008). Larvae were reared at a density of 30 larvae ml^{-1}, at five different temperatures: 17, 22, 25, 27 and 32 °C. Daily food ration consisted of a bispecific diet (1:1 in cell biovolume) of T-ISO and *C. calcitrans* forma *pumilum* (week 1) or *C. gracilis* (from week 2: strain UTEX LB2658). Food ration was adjusted as the larvae grew, allowing 1400 μm^3 μl^{-1} of phytoplankton to always be available around the larvae. Each set of experimental conditions consisted of a test tank with larvae and food supply, and a control tank with only a constant flow of phytoplankton (no larvae), both at a defined temperature. D-stage larvae, initially reared at 25 °C, were acclimated over one day at each temperature before the experiment began. Larval growth rate ($\mu m\ d^{-1}$) was estimated using shell length data assessed during the exotrophic period where larvae fed exclusively on phytoplankton and exhibited a linear shell length increase. In the present work, data were standardized to a value of 1 for a reference temperature of 298 K (25 °C). To estimate the Arrhenius parameter, we used growth rate data as function of temperature and a Newton-Raphsen algorithm within the DEBtool package developed by Kooijman and written in Matlab® software. This package is freely downloadable (http://www.bio.vu.nl/thb/deb/deblab/).

2.3.3. Experiment C: Maximum surface area-specific ingestion rate $\{\dot{J}_{Xm}\}$ *and the half saturation coefficient* X_K *determination*

Values of these parameters were estimated using trials on maximum food uptake in relation to body volume/size at different phytoplankton density levels and at a constant temperature within the optimal temperature range for *C. gigas* larvae. Larvae were reared under culture conditions similar to those described above, except that temperature was maintained at 25 °C and it was food supply that was varied. Larvae were thus continuously fed at several phytoplankton densities (expressed in cell biovolume) providing food availabilities of 70, 280, 450, 960, 1000, 1900, 2100 and 3300 $\mu m^3\ \mu l^{-1}$ around the larvae. The lowest value, 70 $\mu m^3\ \mu l^{-1}$, considered as minimal amount of particles, was that found in tank seawater after 1 µm filtration when no phytoplankton was added. Food densities from 200 to 500 $\mu m^3\ \mu l^{-1}$ were considered as low diets; 700-1000 $\mu m^3\ \mu l^{-1}$ as restricted diets, and 2000 to 3250 $\mu m^3\ \mu l^{-1}$ as *ad libitum* feeding. Shell length was measured during the larval experiments using an image analysis technique. To obtain the ingestion rate for larvae, each tank water outflow was analysed for fluorescence measurements. Six to seven fluorescence recordings per day and per tank were standardized to phytoplankton density, expressed in biovolume ($\mu m^3\ \mu l^{-1}$), by means of a regression equation between fluorescence and biovolume concentration. The ingestion rate (IR) was estimated as follows:

$$IR = [(C_C - C_L) * FR] / V^{2/3} \qquad (12)$$

where IR is expressed in μm^3 per day and per unit structural surface ($\mu m^3\ d^{-1}\ \mu m^{-2}$), C_C is the phytoplankton density ($\mu m^3\ l^{-1}$) in the control tank, C_L is the phytoplankton density ($\mu m^3\ l^{-1}$) in the larval rearing tank, FR is the flow rate through each tank ($l\ d^{-1}$) and $V^{2/3}$ is the

structural surface of the larva (μm^2). IR by larvae at different algal cell densities was standardized to plot the relationship between food levels and IR in order to evaluate the feeding functionresponse of *C. gigas* larvae and calculate feeding parameters from Eq. 1.

2.4. Model simulation and validation

Three state variables of the larval DEB model were run on Matlab® software using the DEBtool package; these state variables represent energy in reserves (E), energy in structure (E_V) and energy in development (E_R) according to their respective equations (Eqs. 3, 5 and 7). Other parameter values used in this study were taken from Pouvreau et al. (2006).

The energy content of an egg, E_0, was around 6×10^{-4} J based on experimental measurements of proteins, lipids and carbohydrates, which are considered to be the main energy substrates. However, since the model was run from day 2 (D-stage), the energy content in the structural volume of a larva, V_2, was calculated at around 2.5×10^{-4} J. An initial approximation of the energy content in reserves, E_2, was also assumed to be around 2.5×10^{-4} J. The remainder of the total egg energy content, E_0, corresponded to dissipation due to energy conversion. To verify this assumption, we made test runs of the model with these different values of initial energy content and they did not have any notable effect on the simulations.

The forcing variables used to run the model were the seawater temperature (within the tolerance range) and the food density expressed in phytoplankton cell biovolume ($\mu m^3\ \mu l^{-1}$), which corresponded to the controlled parameters in the experiments we made for this study. The state variable E_V was transformed into length, L, according to:

$$L = \left(E_V / [E_G]\right)^{1/3} / \delta_M \qquad (13)$$

Finally, growth data from other larval cultures ("external data") were used to validate our DEB model. In these cultures, larvae were reared at 25°C, salinity 34 psu and continuously fed a bispecific diet of T-ISO and *C. calcitrans* forma *pumilum* (1:1 in cell biovolume) with a constant phytoplankton availability of 1400 µm^3 µl^{-1} around the larvae. Shell length was measured during larval cultures using image analysis.

2.5. Statistical analysis

For all simulations in these experiments, the goodness of fit between observations (X) and predictions of the model (Y) was tested and compared according to the following parameters. The coefficient of determination (R^2), which is the square of the coefficient of correlation, gives the strength of the linear relationship between observations and simulations; the slope of the regression (b) indicates the presence of a systematic bias in the model; the difference between the mean of the simulated data and the observed mean ($d = \overline{Y} - \overline{X}$) quantifies this bias; lastly, the residual standard deviation (*rsd*) gives an indication of the dispersion of simulation errors.

3. Results

3.1. Parameters estimates

The calculated value of the shape coefficient, δ_M, for *C. gigas* larvae was 0.64 (Fig. IV.2; n = 77). Throughout larval development, the somatic wet weight increased slowly from D-stage (70 µm length size: 120 ng) to early umbone larva stage (110 µm: 350-500 ng).

Subsequently, somatic wet mass was higher from umbone stage (160 µm: 1400-1600 ng) to eyed pediveliger stage (≥ 280 µm: 5500-7500 ng).

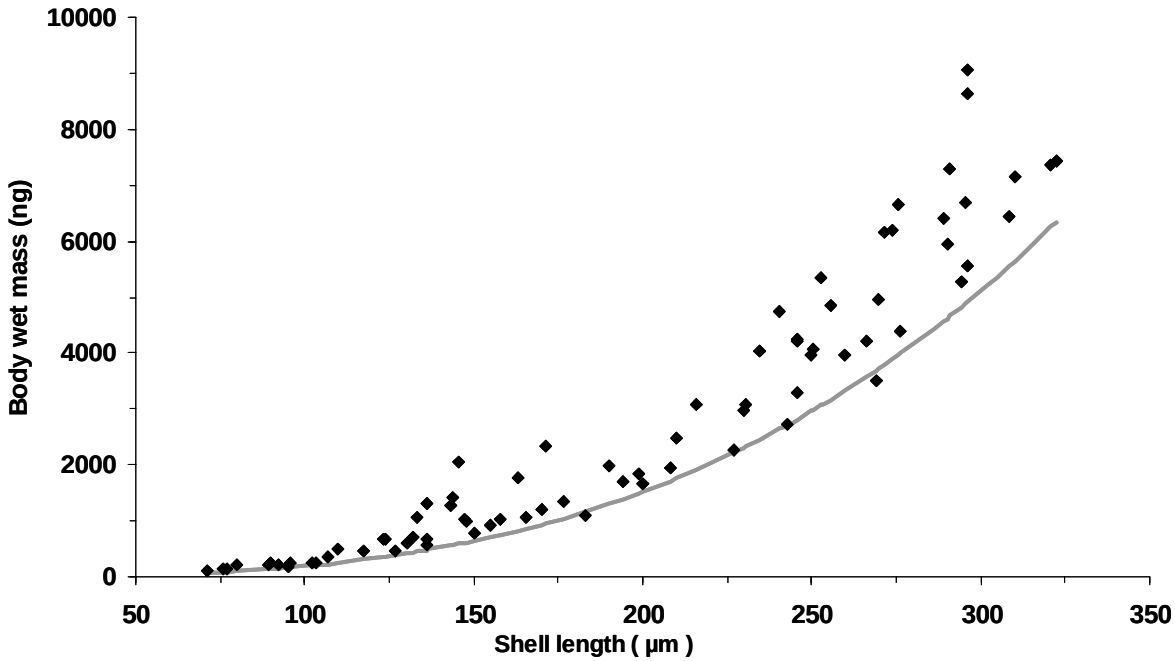

Fig. IV.2. Shell length (µm) and somatic wet mass (ng) relationships for *C. gigas* larvae reared at 25 °C. Shape coefficient value 0.64 (n = 77). Observed wet weights (dots) include structure and reserves; continuous curve (fitted by eye) represents the "minimal envelope" between length, L, and structural body volume, V, without reserves.

A routine in the DEBtool package assessed an adjustment of Eq. 11 to calculate the different parameters of the Arrhenius equation (Fig. IV.3). The lower, T_L, and upper, T_H, boundaries of the temperature tolerance range were fixed at 285 and 306 K, respectively (Fig. IV.3). The Arrhenius temperature, T_A, (± S.E.), which corresponds to the slope of the increase with this adjustment, was estimated as 11 000 ± 2500 K for *C. gigas* larvae. The Arrhenius temperature of the rate of decrease at the lower temperature boundary, T_{AL}, and at the upper boundary, T_{AH}, were both fixed at 75 000 K.

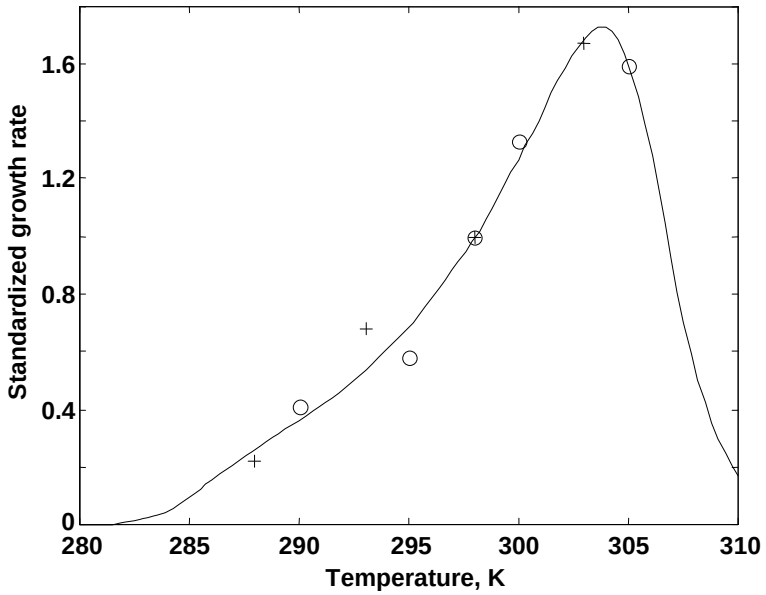

Fig. IV.3. Effect of temperature on standardized growth rate of *C. gigas* larvae. Continuous curve shows the adjustment of Eq. 11 to data sets. Crosses represent data sets from literature (His et al., 1989) and circles represent our own experimental data sets.

Feeding patterns of *C. gigas* larvae at different phytoplankton density levels appeared to be consistent with a type 3 Holling functional response: a sigmoidal or "S-shaped" curve (R^2 = 0.938: Fig. IV.4). The relationship between food density, X, and ingestion rate, $\dot{J}x$, gave an estimation of the maximum surface area-specific ingestion rate, $\{\dot{J}_{Xm}\}$, of 137 ± 3 $\mu m^3\ d^{-1}\ \mu m^{-2}$ and a half saturation coefficient, X_K, of 600 ± 50 $\mu m^3\ \mu l^{-1}$ for larvae reared at 25 °C (Fig. IV.4). These parameter values are listed in Table IV.1, together with all the other DEB parameter values for *C. gigas* larvae.

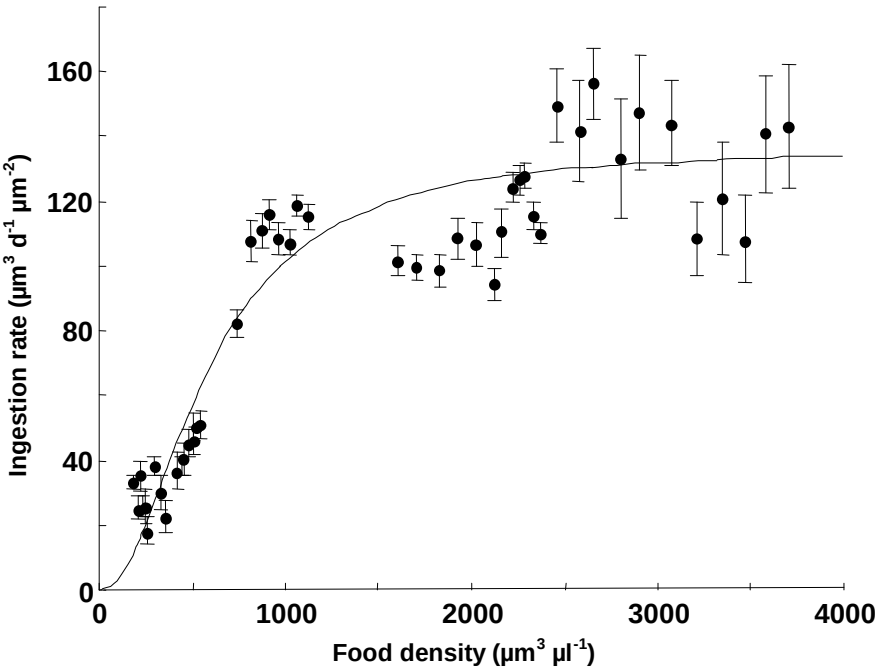

Fig. IV.4. The ingestion rate, $\dot{J}x$, of *C. gigas* larvae, reared at 25°C, as a function of food density, X. The curve is the sigmoid functional response type 3 with maximum ingestion rate $\{\dot{J}_{Xm}\}$ = 137 ± 3 µm^3 d^{-1} µm^{-2} and half saturation coefficient X_K = 600 ± 50 µm^3 µl^{-1}. Values are means (n = 10) ± standard deviation.

3.2. Model simulation and validation

All simulations were initialised by introducing larval length data from day 2. They ended when the larvae reached the size of 300 µm at which process of metamorphosis occurred.

Table IV.1.

DEB parameters values used and determined for the growth model of *C. gigas* larvae with symbol, unit, value and reference. * denotes parameters of physiological rates at 25 °C.

Parameters	**Symbol**	**Units**	**Value**	**References**
Primary parameters				
Maximum surface-area-specific ingestion rate	$\{\dot{J}_{Xm}\}$	$\mu m^3\ d^{-1}\ \mu m^{-2}$	137	This study *
Half saturation coefficient	X_K	$\mu m^3\ \mu l^{-1}$	600	This study
Assimilation efficiency	ae	-	0.4	Walne (1965) Sprung (1984)
Maximum surface-area-specific assimilation rate	$\{\dot{p}_{Am}\}$	$J\ d^{-1}\ cm^{-2}$	25	This study *
Arrhenius temperature	T_A	K	11 000	This study
Volume-specific maintenance costs	$[\dot{p}_M]$	$J\ d^{-1}\ cm^{-3}$	24	Pouvreau et al. (2006) *
Maximum energy density	$[E_m]$	$J\ cm^{-3}$	2295	Pouvreau et al. (2006)
Volume-specific costs for structural growth	$[E_G]$	$J\ cm^{-3}$	1900	Pouvreau et al. (2006)
Fraction of $\dot{p}_C$ spent on maintenance plus growth	κ	-	0.45	Pouvreau et al. (2006)
Maintenance rate coefficient	$\dot{k}_M$	d	0.0126	Pouvreau et al. (2006)
Energy conductance	$\dot{v}$	$cm\ d^{-1}$	0.183	van der Veer et al. (2006) *
Shape coefficient	δ_M	-	0.64	This study
Additional parameters				
Reference temperature	T_1	K	298	This study
Lower boundary of tolerance range	T_L	K	285	Abdel-Hamid et al. (1992)
Upper boundary of tolerance range	T_H	K	306	This study
Rate of decrease at lower boundary	T_{AL}	K	75 000	van der Veer et al. (2006)
Rate of decrease at upper boundary	T_{AH}	K	75 000	van der Veer et al. (2006)
Energy content of food	μ_X	$J\ \mu m^{-3}$	4.5×10^{-9}	Brown, (1991) Brown and Robert, (2002)
Egg energy content	E_0	J	6×10^{-4}	This study
Age at first feeding	a_b	d	5.5	This study
Length at metamorphosis	L_{mt}	μm	300	This study

3.2.1. Simulation of temperature effects

Simulations of the effects of five different temperatures on larval growth are shown in Fig. IV.5. The other environmental conditions were assumed to be optimal with a mean salinity of 34 psu and a food density of 1400 μm^3 μl^{-1} around the larvae. The model had a well matched fit with data collected on larval growth, with high coefficients of determination ($R^2 \geq 0.97$) between simulation and observed data for all temperatures (Table IV.2). Similarly, the slope of the regression was close to 1 at all temperatures, although the lowest value was recorded at 17 °C (0.812). At 17 °C there was also a higher residual standard deviation (22.54) than for the other temperatures where it was narrower (12.5 to 17.5, Table IV.2). Values for mean deviation tended to be slightly over-estimated in the simulations, particularly at 17 °C where a value of ≈18.7 was obtained (Table IV.2). The form of larval growth curves showed a clear pattern in relation to temperature, with slopes becoming sharper at higher temperatures (Fig. IV.5). This effect showed that the strong influence that temperature has on growth is intrinsically related to feeding activity during larval life. One of the main characteristics of larval development, taken into account in the specific assumptions made in this model is the mixotrophic period from D-stage to early umbone larva of ≈110 µm length, which lasted 5 d when larvae were reared between 22 and 32 °C (Figs. IV.5b to 5e). At 17 °C, in contrast, the mixotrophic period took 10 d before larvae reached ≈110 µm length (Fig. IV.5a). The exotrophic period lasted different lengths of time depending on temperature. This feeding period, from umbone larvae to eyed pediveliger of ≈300 µm length, lasted 10 d at 27 and 32 °C (Figs. IV.5d and 5e). At 22 and 25 °C the exotrophic periods lasted 13 to 16 d (Figs. IV.5b and 5c) while it lasted at least 25 days at 17 °C (Fig. IV.5a). Increased temperature enhanced larval growth, resulting in a shorter larval rearing period. When the larvae reached ≈300 µm shell length, we assumed that they were competent to undergo

metamorphosis. Therefore, simulations showed that timing of metamorphosis decreased with increasing temperature. Metamorphosis was achieved in less than 15 d at 27 and 32 °C while at 22 and 25 °C such process occurred above 20 d. Lastly, larvae reared at 17 °C never reached theoretical competence size (300 µm). Nevertheless, 16% of settlement was recorded at 17 °C on day 35 and length measurements suggested metamorphosis at 285 µm (Rico-Villa et al., 2009). Such simulations showed that the DEB model adequately describes oyster growth during larval development under different thermal conditions.

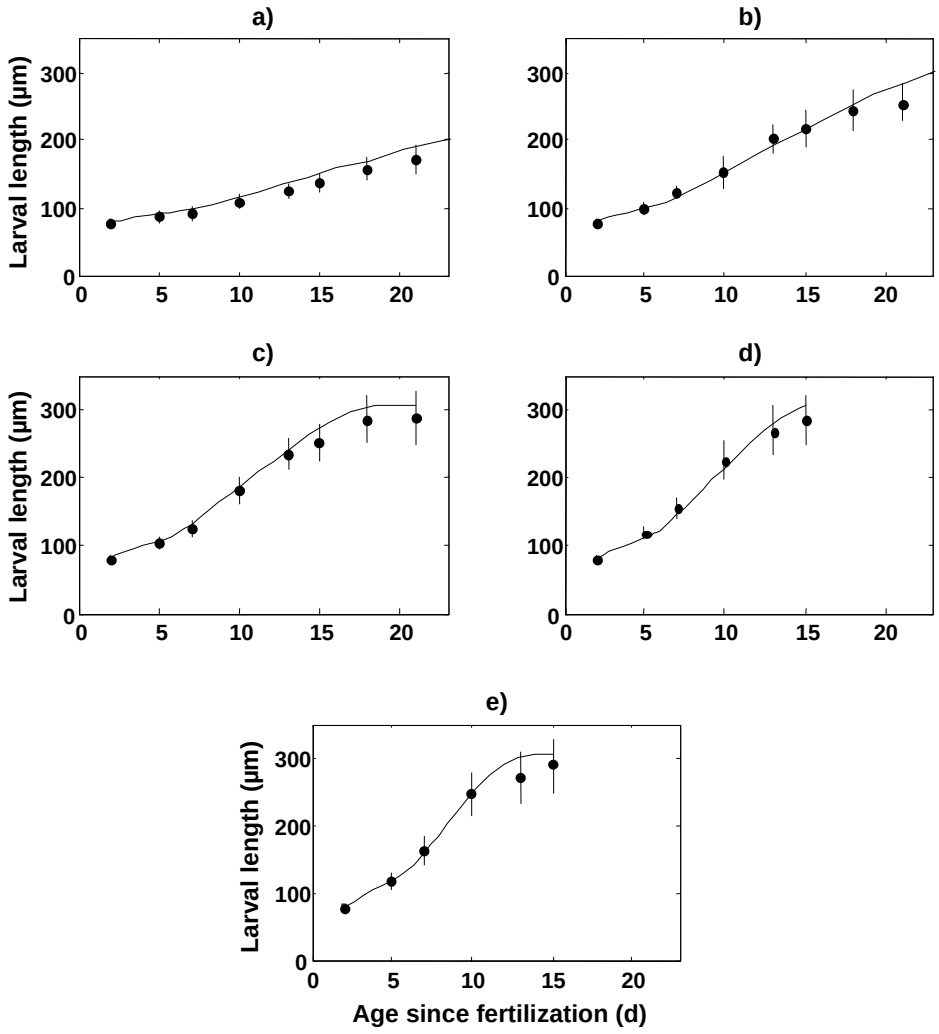

Fig. IV.5. Simulated (solid line) and observed (points) growth for *C. gigas* larvae exposed to temperature effects of 17 °C (a), 22 (b), 25 (c), 27 (d) and 32 °C (e). The other environmental conditions were optimal: 34 psu and food density of 1400 μm^3 μl^{-1} around the larvae. Mean observed values are presented with their standard deviation.

Table IV.2.
Comparison between experimental and simulated outputs for growth of *C. gigas* larvae under temperature (°C) effects by four parameters: coefficient of determination (R^2); slope of regression; residual standard deviation (rsd) and difference (d) between the mean of simulated and observed data.

Parameters	**17**	**22**	**25**	**27**	**32**
R^2	0.988	0.976	0.997	0.987	0.981
slope	0.812	0.823	0.934	0.864	0.853
rsd	22.540	16.756	13.123	12.408	17.535
d	18.696	6.127	11.605	0.280	10.168

3.2.2. Simulation of food density effects

When other environmental conditions were considered to be optimal (34 psu and 25 °C) increasing food density enhanced larval growth (Fig. IV.6). For the minimal amount of particles (70 $\mu m^3\ \mu l^{-1}$) with no added phytoplankton, the simulation fitted observed laboratory data accurately (Fig. IV.6a; Table IV.3: $R^2 = 0.95$). However, the slope of the regression was not correctly calculated due to nil larval growth at this low food level, which meant that larvae failed to undergo metamorphosis in this condition. Simulation at very low food density (280 $\mu m^3\ \mu l^{-1}$) did not accurately fit observed larval growth (Fig. IV.6b). One explanation may be that high mortalities occurred at this food level from day 10 and metamorphosis was never reached. Thus we assumed that these larvae were highly food limited and destined to die. Simulation at 450 $\mu m^3\ \mu l^{-1}$ (Fig. IV.6c) fitted experimental results slightly better than at 280 $\mu m^3\ \mu l^{-1}$ ($R^2 \geq 0.98$ *vs.* 0.61 respectively; Table IV.3). Nevertheless at both food

densities, values for mean deviation tended to be highly underestimated, especially at 280 $\mu m^3 \mu l^{-1}$, while estimates for residual standard deviation remained high (Table IV.3).

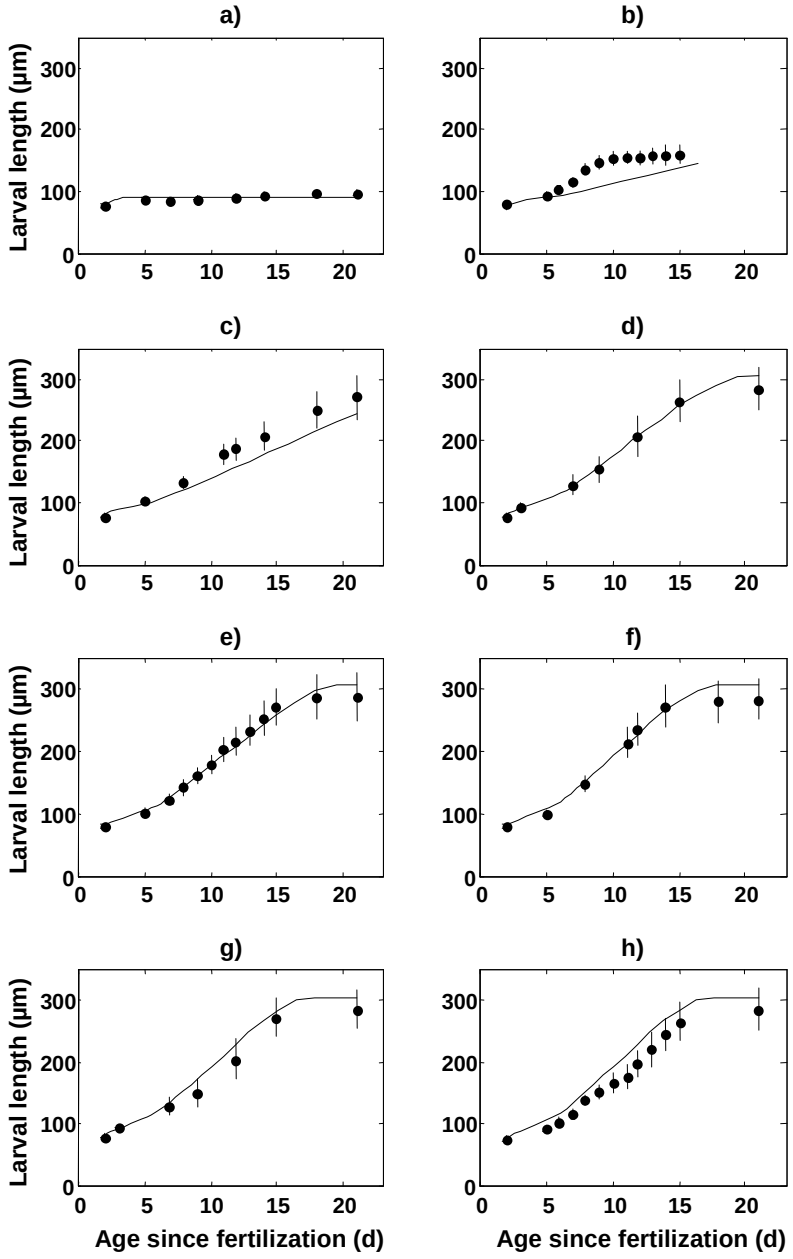

Fig. IV.6. Simulated (solid line) and observed (points) growth for *C. gigas* larvae exposed to food density effects of 70 $\mu m^3 \mu l^{-1}$ (a), 280 (b), 450 (c), 960 (d), 1000 (e), 1900 (f), 2100 (g) and 3300 $\mu m^3 \mu l^{-1}$ (h). The other environmental conditions were optimal: 34 psu and 25 °C. Mean observed values are presented with their standard deviation.

Table IV.3.

Comparison between experimental and simulated outputs for growth of *C. gigas* larvae under food density ($\mu m^3\ \mu l^{-1}$) effects by four parameters: coefficient of determination (R^2); slope of regression; residual standard deviation (rsd) and difference (d) between the mean of simulated and observed data.

Parameters	**70**	**280**	**450**	**960**	**1000**	**1900**	**2100**	**3300**
R^2	0.954	0.611	0.987	0.988	0.981	0.971	0.986	0.988
slope	-	0.840	1.145	1.019	0.981	0.932	0.911	0.937
rsd	5.439	28.768	25.187	4.825	8.695	13.556	16.315	23.753
d	-3.404	-24.606	-23.417	-1.751	-1.061	5.885	13.433	22.426

The larval growth curves obtained from the simulations using food densities from 960 to 3300 $\mu m^3\ \mu l^{-1}$ agreed accurately with the growth observed in laboratory experiments (Figs. IV.6d to 6h). The regression parameters confirmed this fit with high coefficients of determination ($R^2 \geq 0.97$) between the simulation and experimental data (Table IV.3). The slope of the regression was very close to 1 in these simulations, and values for residual standard deviation between prediction and observation were low. In contrast, mean deviation values tended to be moderately underestimated in simulations at 960 and 1000 $\mu m^3\ \mu l^{-1}$, and predictions led to slight overestimations of growth at 1900, 2100 and 3300 $\mu m^3\ \mu l^{-1}$ (Table IV.3). Overall in simulations from 960 to 3300 $\mu m^3\ \mu l^{-1}$, the mixotrophic period lasted 5 d (≈110 µm length size). Thereafter the exotrophic period, from early umbone larva to metamorphosis, did not show differences in length within simulations over this range of food density conditions. Therefore, to provide an optimal food supply that will enhance larval growth and success at metamorphosis, phytoplankton availability should be held at a minimum level of 1000 $\mu m^3\ \mu l^{-1}$ around the larvae.

3.2.3. External validation of DEB model

Finally, the DEB model was validated on growth data from four other larval cultures. These data were recorded under optimal environmental conditions: 34 psu, 25 °C and 1400 μm^3 μl^{-1} around the larvae. Comparison of the DEB model with the observed larval growth showed a correct fit with all these "external" rearing cultures (Fig. IV.7). The simulations provided high coefficients of determination ($R^2 \geq 0.99$) between prediction and observed data (Table IV.4). The other regression parameters showed a strong relationship, with the slope of the regression close to 1 and low values for residual standard deviation between simulation and observation. Values for mean deviation tended to be slightly over-predicted in the simulations (Table IV.4). In all cases, the mixotrophic period lasted 5 d, corresponding to a larval length size around 110 µm. Thereafter, the exotrophic period from early umbone to pediveliger larva did not show differences in length or success at metamorphosis among these four simulations.

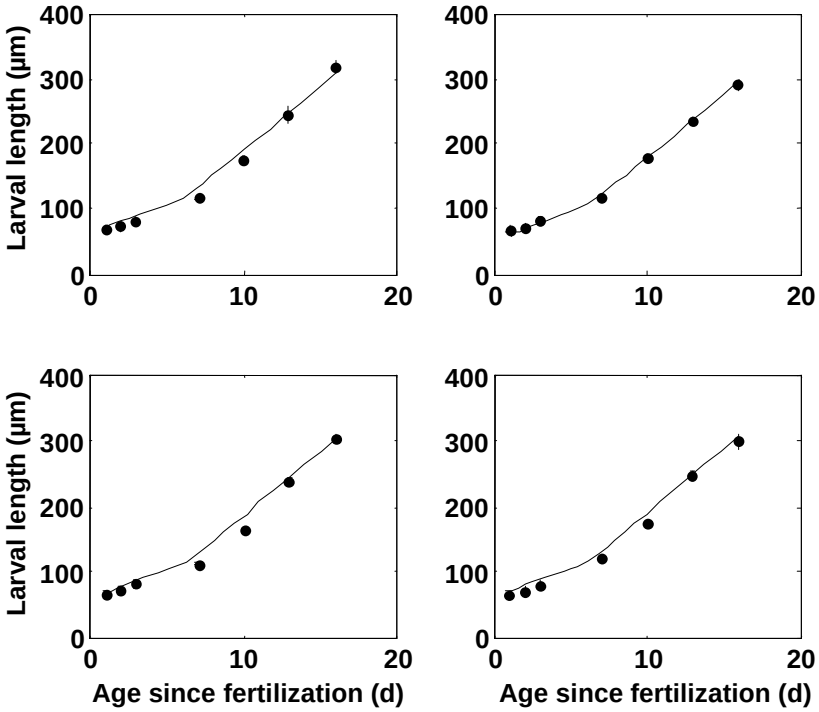

Fig. IV.7. Simulated (solid line) and observed (points) growth for *C. gigas* larvae reared in "external" data. The environmental conditions were kept as optimal: 34 psu, 25 °C and 1400 $\mu m^3\ \mu l^{-1}$ around the larvae. Mean observed values are presented with their standard deviation.

Table IV.4.

Comparison between experimental and simulated outputs for growth of four external larval cultures of *C. gigas* larvae by four parameters: coefficient of determination (R^2); slope of regression; residual standard deviation (rsd) and difference (d) between the mean of simulated and observed data.

Parameters	**1**	**2**	**3**	**4**
R^2	0.996	1.000	0.993	0.998
slope	1.071	0.968	1.031	1.016
rsd	10.475	11.950	13.685	8.594
d	6.495	11.522	11.347	7.655

4. Discussion

For several decades, bioenergetic modelling approaches have mainly focused on the adult stage of Pacific oyster *C. gigas* (Barillé et al., 1997; Ren and Ross, 2001; Gangnery et al., 2003; Pouvreau et al., 2006). With respect to the oyster larval stage, Dekshenieks et al. (1993) built a first model for *C. virginica* larvae, which illustrated the effects of environmental variables on growth and development. However, laboratory measurements were not available to cover growth rates during the whole of larval development and so the authors applied a recurrent interpolation for larvae above 250 µm. In addition, *C. virginica* experimental measurements were insufficient to establish relationships over a wide range of environmental conditions, *e.g.* temperature effects on ingestion rate. For *C. gigas* larvae, a model has been proposed to simulate growth and survival (Bochenek et al., 2001), which has since been used in other studies (Powell et al., 2002; Hofmann et al., 2004). This model, based on biochemical principles, succeeds in simulating some of the basic characteristics of *C. gigas* larvae. Nevertheless, the model has the major limitation of only being valid over a narrow range of environmental conditions, since most simulations were run at the optimal temperature of 25 °C°. Further experimental data is therefore needed for validation of this model. In contrast, our present model has been validated throughout the entire development of *C. gigas* larvae and over a wide range of environmental conditions. Moreover, the biochemical model of Bochenek et al. (2001) is based on the scope for growth (SFG) concept (Bayne, 1976). The idea behind the SFG concept is that the energy equivalent of respiration is directly subtracted from the energy derived from assimilated food, the remainder being available for growth. The fundamental difference in DEB theory, as Kooijman (2000) explains, is that respiration rates encompass metabolic costs as well as growth costs. Therefore, respiration is a composite of many physiological processes and, in this context,

cannot be subtracted from food as in the SFG concept. Moreover, respiration rates are measured over short periods during which the actual growth of the body is negligible. Since the DEB model does not use respiration rates as a primary variable, interpretation problems of this kind are inexistent. As this variable is optional in the DEB theory, respiration is sometimes used just to test the model.

The choice of a DEB model in the present study was primarily motivated by the simplicity of several aspects of this type of model, including a relatively short list of assumptions, which can be considered "mechanistic". A powerful quality of DEB theory is that the model is generic, *i.e.* intra and interspecies differences can be handled within the same model framework (Kooijman, 2000). The only difference between such different applications of the model lays in the parameter values. The present study applies this principle. Some of the parameters were obtained from specific experiments on larvae, while others were taken from the existing adult oyster model developed by Pouvreau et al. (2006). The resulting version of the DEB model for oyster larvae was then applied and validated to demonstrate its capacity to reliably reproduce oyster larval growth compared with results from several ecophysiological experiments. The results provide insights into how seawater temperature and food density, as governing environmental factors, affect growth throughout larval development from the D-stage to pediveliger larvae. One major problem when using DEB theory lays in the estimation of the DEB parameters. In our study, the proven agreement between the simulation results and experimental observations shows that most of the estimates we used can be considered as fairly reliable.

With regard to DEB parameters governing food consumption, our results showed that the relationship between food density and ingestion rates for *C. gigas* larvae appeared to be consistent with a sigmoidal function (type 3) (Holling, 1959). This function assumes that ingestion rate exhibits a curved response to increasing food levels, subsequently followed by a

plateau (Gentleman et al., 2003). These observations in ingestion rate may indicate developmental plasticity for adaptation of larvae to low or high food densities (Strathmann et al., 1993). Nevertheless, the present type 3 feeding response does not follow the assumptions proposed in DEB theory, where ingestion rate generally follows a type 2 functional response to food density. More precise laboratory experiments are therefore required to improve our understanding of the feeding response of *C. gigas* larvae and to confirm or refute our choice of a type 3 curve. However, this type 3 functional response was chosen because it has been successfully applied to feeding response of *C. virginica* larvae (Baldwin and Newell, 1995). These authors demonstrated that a sigmoid feeding response seemed to be adequate to quantitatively reproduce ingestion rate, using phyplankton biovolume (μm^3 μl^{-1}) as a food quantifier. This last feature of feeding behaviour was in agreement with our study on *C. gigas* larvae.

With regard to DEB parameters related to temperature effects, results showed that *C. gigas* larvae exhibit a high value of Arrhenius temperature, T_A, (11 000 K) over a wide thermal range. Arrhenius temperature is typically high for species that naturally experience small temperature changes, such as those that occur in pelagic species or in pelagic life stages (*e.g.* planktotrophic larvae, like oysters). The present study confirms that larval growth increases markedly with increasing temperature, as previously reported (Helm and Millican, 1977; His et al., 1989; Abdel-Hamid et al., 1992). Another trend observed in the temperature simulations was the shortening of larval life. This trend is already documented for oyster larvae (Davis and Calabrese, 1964), mainly at the warmer temperatures (below lethal temperature). *C. gigas* larvae therefore proved to be eurythermic but had major thermo-dependency between 22 and 32 °C. Future laboratory experiments should include temperatures, above and below the boundaries tested here, to confirm our T_A and threshold values and its validation in the model.

As mentioned above, one of the most attractive aspects of the DEB theory is the ability to compare different species by looking at the differences or similarities in DEB parameter values (van der Veer et al., 2006). A previous study concerning the DEB theory applied to the larval stage in *Macoma balthica* (Bos et al., 2006) can be compared with our own. For example, the T_A estimate for *M. balthica* is 7596 K over a range of temperature from cold to temperate values (7 to 17 °C), though it was 11 000 K for *C. gigas* over a wider range of temperature (17 to 32 °C). This broad range of temperature tolerance might explain the wide distribution of *C. gigas* among temperate and subtropical areas (Goulletquer et al., 1999; Langdon et al., 2003; Sicard et al., 2006) and its progressive geographical extension into new environments (Diederich et al., 2005; Brandt et al., 2008), since its larvae are able to tolerate temperate (17 °C) to warm (32 °C) seawater temperatures. Another example concerns comparison of DEB parameter values related to food consumption (*i.e.* $\{\dot{J}_{Xm}\}$, X_K and $\{\dot{p}_{Am}\}$) between the larvae of these two species. Estimates of $\{\dot{J}_{Xm}\}$ are in the same range of value for *M. balthica* (95 µm^3 d^{-1} µm^{-2}) and *C. gigas* (35 µm^3 d^{-1} µm^{-2}) at 15 °C. A similar trend may also be found for the values of X_K, which were 310 and 600 µm^3 µl^{-1} for *M. balthica* and *C. gigas*, respectively. This similarity may be related to feeding patterns controlled by the cilia that enable both bivalve species to reach maximal ingestion by catching food with the velum. In contrast a major difference was observed in estimate of $\{\dot{p}_{Am}\}$, with 104 J d^{-1} cm^{-2} for *M. balthica* and 6.5 J d^{-1} cm^{-2} for *C. gigas* at 15 °C. This difference suggests that larvae of these species may differ in energy assimilation and utilization (Sprung, 1984c). However, according to van der Veer et al. (2006), it remains unclear how certain interspecies variation in parameter values occurs, though these may be related to variability in environmental conditions, genetic variability or phenotypic plasticity. However, in the present comparison, differences between estimated parameters were probably linked to the energy budget calculations for *M. balthica* larvae, which were only realized on individuals of 200 µm length,

while in the field larvae may range from 50 to 400 µm (Bos et al., 2006). Therefore, the estimation procedure itself and the reduced number of data used to determine the parameters are sources of uncertainty in the energy budget of *M. balthica* larvae.

One other important quality of DEB theory is that the generic aspect makes it possible to compare the bioenergetics of different life stages within a single species. One example concerns the temperature dependence in the optimal range, T_A, which appears to be different between larvae and adults of *C. gigas* (11 000 and 5800 K, respectively). As mentioned, T_A has a high value when the species experiences only small temperature changes, like these that normally occur during larval life. Larvae are present only after spawning events in summer when seawater temperature is maximal. In contrast, an adult oyster experiences a wider range of temperature (3 to 32 °C) as it lives throughout the year in seashore areas, adult oysters therefore exhibit a lower T_A value than larvae.

Finally, all simulations were run including the success for a larva to metamorphose defined as the moment when it attains a shell length of ≥300 µm, as a first approximation of the trigger for metamorphosis. However, knowledge on this issue is limited and a full explanation of how factors control metamorphosis is lacking (Coon et al., 1990; Berias and Widdows, 1995). Further investigations are therefore needed to find another suitable trigger/indicator for metamorphosis, apart from shell length, to verify this first assumption and model metamorphosis.

In conclusion, the DEB model for *Crassostrea gigas* larvae has demonstrated its accuracy for simulating growth, development and metamorphosis under controlled conditions of seawater temperature and food density. Therefore, the model can be used to improve culture conditions in hatchery or knowledge of ecological requirements in surroundings. The estimation of DEB parameters was made using specifically designed experiments for these two key environmental factors. The extension of the DEB model from oyster adult to larva

presented in this paper supports the attractive aspect of a generic model applied to the Pacific oyster *C. gigas*. The next step will be to investigate the feasibility of a unique DEB model to simulate growth from larvae to adult. Thus to encompass the whole life cycle of *C. gigas*, further studies must focus on post larval and juvenile stages. However, this bioenergetic model is not species-specific and can thus be applied to other bivalve larvae. The model could also form a valuable part of a field ecosystem model to assess the influence of environmental factors on Pacific oyster culture and reproduction.

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CONCLUSION - SYNTHESE

1. Synthèse

L'objectif principal des travaux présentés dans cette thèse concernait l'amélioration des connaissances fondamentales sur les besoins écophysiologiques des larves de l'huître creuse *Crassostrea gigas*. Elle présente néanmoins un intérêt pratique pour la filière conchylicole puisqu'elle autorise une meilleure maîtrise des performances des élevages larvaires et, en conséquence, elle participe à la fiabilité de l'approvisionnement en naissain issu des écloseries commerciales.

Dans un premier temps pour l'ensemble des expériences en écophysiologie des larves, une phase préalable était nécessaire (Chapitre I) afin de définir un régime de microalgues qualitativement adapté dans le but de subvenir aux besoins nutritionnels tout au cours du développement larvaire (stade D véligère à de jeunes post-larves). Une première approche des besoins quantitatifs a aussi pu être déterminée via une étude de la consommation larvaire tout au long du cycle larvaire.

Dans le cadre de la première phase de ce travail, les résultats obtenus ont clairement démontré que l'apport de *Pavlova lutheri* seule ou en complément d'*Isochrysis affinis galbana* (clone T-ISO) n'a aucune influence bénéfique, ni sur la croissance, ni sur la survie, ni sur la métamorphose chez *C. gigas*. De telles mauvaises performances ont été aussi relevées avec l'utilisation d'autres pavlovophytes sur des larves de *C. gigas* (Ponis et al., 2006c). Par conséquent, il n'y a aucun intérêt à utiliser cette espèce en écloserie pour la production des larves d'huître creuse. La seule raison de le faire réside dans la contamination ou perte d'*I. affinis galbana*, car son utilisation en mélange avec *Chaetoceros calcitrans* forma *pumilum* (Cp) conduit à de très bonnes performances larvaires. A l'inverse, *C. calcitrans* forma *pumilum* possède une valeur nutritionnelle intéressante puisqu'elle assure à elle seule de bonnes performances de la croissance larvaire. C'est donc un composant essentiel du

développement larvaire de l'huître *C. gigas* mais elle ne couvre pas l'ensemble des besoins nécessaire à la métamorphose. De plus, cette espèce peut présenter dans certaines occasions une fragilité se traduisant par sa moindre ingestion ayant des répercutions immédiates sur le développement larvaire. Un phénomène similaire a été rapporté antérieurement (Ianora et al., 2003) où des diatomées présentaient des effets nocifs. De ce fait, son association avec *I. affinis galbana* (T) est donc incontournable. Les meilleures performances sont ainsi notées pour les combinaisons de ces deux microalgues dans les proportions 25T/75Cp et 50T/50Cp : croissance larvaire moyenne (12,6 µm j^{-1}), survie élevée en fin d'élevage (98 %) et forte métamorphose (86 %). Ces résultats sont en accord avec ceux de Helm et Laing (1987) et Utting et Spencer (1991) qui recommandent l'utilisation d'un mélange bispécifique de microalgues, associant une flagellée et une diatomée, pour les larves de l'huître creuse dès leur plus jeune âge.

Cette étude a également montré une consommation préférentielle de microalgues avec une forte relation sur les performances larvaires avec P<PT<<T<<Cp<< TCp=PCp=PTCp sans qu'une réponse claire puisse être apportée sur cette sélectivité. En effet, les trois espèces de microalgues utilisées présentent deux critères essentiels pour être ingérées et digérées par les larves et, de ce fait, être utilisées en écloserie : une taille cellulaire adéquate (2-5 µm) et une paroi fine, pour *I. affinis galbana* et *P. lutheri* ou une thèque fine pour *C. calcitrans* forma *pumilum* (Robert et Trintignac, 1997). Ainsi sur le plan morphologique aucune différence majeure ne différencie ces espèces en tant que proie potentielle pour des jeunes larves d'huître. Cependant, des travaux récents tendent à démontrer l'importance qualitative de la sélection de particules par les organes d'alimentation de l'huître creuse à son stade adulte (Beninger et al., 2008a, 2008b). Il n'est donc pas impossible qu'un tel comportement existe chez *C. gigas* dès son stade larvaire. Il expliquerait ainsi les consommations préférentielles liées à la notion d'espèce (Cp>T>>P).

De plus, l'évolution de l'ingestion au cours du développement larvaire sur la base des régimes combinés associant une diatomée et au moins une haptophycée (PCp, TCp et PTCp) est similaire. Elle a permis de dégager les trois phases connues chez les larves d'huître creuse, conformément aux résultats de Gerdes (1983a) acquis à 25 °C mais à partir d'une seule combinaison nutritionnelle : une période de mixotrophie caractérisée par une faible consommation journalière (<10 000 microalgues par larve) pendant la première semaine puisqu'elles se nourrissent encore partiellement sur leurs réserves d'origine. Il ensuit une période d'exotrophie où les larves dépendent intégralement de la nourriture extérieure et l'ingestion augmente alors très fortement, atteignant 70–80 000 microalgues par larve par jour. Finalement une période dite de métamorphose où la consommation diminue à cause de la perte du velum en attendant le développement des branchies du naissain. Ces différentes périodes d'ingestion seront discutées plus en détail dans la partie concernant le Chapitre III.

Le régime plus performant (TCp), ayant été appliqué en différant les proportions, a subi une analyse de composantes principales d'acides gras totaux (19) et stérols (7) afin de regrouper les cohortes entre les différentes proportions du régime TCp et/ou la compétence (larves) – la métamorphose (post-larves). Un cercle de corrélation a montré que ces 26 variables sont bien représentées dans le plan 1-2 et ces deux premiers axes expliquent à eux seuls une forte proportion de la variance (78 %). Les observations des différentes proportions de TCp entre les larves et les post-larves se répartissent en deux groupes. Les post-larves ont été distinguées dans un groupe sur la deuxième composante principale tandis qu'un autre groupe se réparti le long de la première composante principale selon le gradient de 5T/95Cp à 95T/5Cp pour les larves non compétentes (<250 µm) et compétentes (œillées >250 µm). Toutefois aucune autre relation n'a pu être dégagée entre les profils biochimiques, ce qui se traduit par le fait que les larves de plus petite taille (<250 µm) sont aussi aptes que les plus

grosses sur le plan biochimique à se métamorphoser en possédant déjà les réserves suffisantes ou du moins les éléments considérés comme essentiels (Soudant, 1995).

L'intérêt d'utiliser un régime bispécifique de microalgues en associant une Haptophycée et une Bacillariophycée se dégage nettement dans ce travail. Cette association systématique permet en fin de compte une stabilité en acides gras polyinsaturés comme l'eicosapentaenoique 20:5(n-3) (AEP) et le docosahexaenoique 22:6(n-3) (ADH) dans les proportions 25T/75Cp, 50T/50Cp et 75T/25Cp. L'implication du 20:5(n-3) et du 22:6(n-3) est reconnue pour être essentiels au développement larvaire des bivalves (Soudant et al., 1998a ; Robert et al., 2001 ; Brown et Robert, 2002 ; Ponis et al., 2008). Cependant, cet équilibre en acides gras n'explique pas à lui seul la bonne performance du régime bispécifique TCp dans ces trois proportions. Néanmoins, une tendance se dégage pour les autres proportions (5T/95Cp et 95T/5Cp) conformément aux travaux de Soudant (1995) et Soudant et al., (1998a). Un régime plus riche en 20:5(n-3) et cholestérol a permis une meilleure croissance (250 µm à J19 pour les larves nourries avec la proportion 5T/95Cp). Par contre, avec un régime plus riche en 22:6(n-3), les larves présenteraient une faible croissance (217 µm à J19 avec la proportion 95T/5Cp).

Cette étude sur les besoins nutritionnels au cours du développement larvaire de *C. gigas* a permis de répondre à la première partie de cette thèse qui consistait à adapter un régime performant de microalgues afin de subvenir à la croissance et la métamorphose chez les larves de *C. gigas*. Puis au cours du Chapitre II nous avons mis au point une technique d'élevage larvaire en flux ouvert couplé à un outil capable d'effectuer les mesures nécessaires à des études d'écophysiologie larvaire. En effet, la mise en place d'un suivi minutieux des données hydrobiologiques est rare en écophysiologie larvaire tant à cause des difficultés techniques qu'à la méthodologie employée (Riisgard, 2001).

Pour contourner ces difficultés, nous avons adopté l'approche largement utilisée chez l'adulte de *C. gigas* (Bourles, 2004 ; Pouvreau et al., 2006 ; Le Moullac et al., 2007) qui permet de mesurer d'une façon automatique et fréquente les paramètres hydrobiologiques (température, salinité, pH, fluorescence, etc) grâce à un outil d'acquisition en continu du type MAREL. Ce système dispose à la fois d'une facilité de stockage, centralisation et traitement de données permettant d'appréhender la réponse des animaux à leur milieu d'une façon plus cohérente. Cet outil étant opérationnel rapidement, notre approche a également consisté à réaliser ces mesures directement à partir des bacs d'élevage en mettant au point un système d'élevage en flux ouvert propice au développement larvaire. Ainsi, ce système dénommé SILO (Système d'Instrumentation des Larves en flux Ouvert) a pu être développé. Là aussi, il est à noter que ce travail a fortement bénéficié des avancées des ingénieurs du Département de Technologie des Systèmes Instrumentaux (TSI), Ifremer dans le domaine de l'acquisition des données hydrobiologiques ainsi que celle de l'équipe de la Station d'Argenton dans la mise au point de nouvelles techniques d'élevage dont le flux ouvert a été utilisé ici. Grâce à ce système, les conditions expérimentales ont donc pu être maintenues d'une façon rigoureuse avec, par exemple, le contrôle de la température de l'eau d'élevage et le suivi de la concentration de microalgues dans les bacs. Le système d'élevage en flux ouvert et l'outil d'acquisition de données en continu a permis la mise en place des expérimentations sur les différents niveaux trophiques et/ou de température tout au cours d'un élevage larvaire. De plus, ce système permettait la forte réduction des interventions liées aux pratiques d'élevage en séquentiel (vidange, nettoyage des bacs, remplissage tous les 2-3 jours) ainsi qu'à celles autrefois employées en écophysiologie comme le transfert des larves des bacs d'élevage vers les enceintes de mesure.

En outre, le système en flux ouvert développé dans cette étude permet l'utilisation de fortes densités larvaires (jusqu'à 100 larves ml^{-1}) bien que la densité larvaire utilisée dans

l'ensemble des expériences en écophysiologie était de 30 larves ml^{-1}. Cette densité était choisie car considérée comme un bon compromis entre une biomasse larvaire suffisante pour des données d'écophysiologie interprétables (supérieur au bruit de fond généré) et la capacité de production en microalgues de la Station d'Argenton (maximum 1,2 m^3 j^{-1}). Toutefois par comparaison, cette densité larvaire reste supérieure à celle employée en écloserie 5 – 10 larves ml^{-1} pour *C. gigas* (Helm et al., 2004) ainsi que la densité en milieu naturel qui peut atteindre un pic de 0.06 larves ml^{-1} (60 larves dm^{-3}) dans les mois d'été au bassin de Marennes – Olérons (http://www.creaa.fr/).

Un autre avantage de cette nouvelle approche avec SILO concernait la disponibilité d'un nombre suffisant de bacs d'élevage pour permettre de dupliquer le nombre de conditions afin de vérifier la reproductibilité des mesures relevées par l'outil d'acquisition pour chaque condition expérimentale. Enfin, la constance entre tous les bacs de certains paramètres hydrobiologiques comme la température (lorsqu'il y a une seule) ou la salinité lors les expériences avec SILO témoignent d'une zootechnie rigoureuse et de l'absence d'effet bac.

Cet ensemble d'avantages fait, qu'à notre connaissance, notre approche technologique est pionnière dans le domaine de l'écophysiologie avec de nombreuses mesures réalisées tout au long d'un cycle complet de vie chez la larve d'un bivalve (stade D véligère à la métamorphose). Cet outil reste néanmoins à améliorer surtout au niveau de dimensionnement du système d'élevage en flux ouvert car à ce jour nous utilisons des bacs d'élevage de 150 L comme des enceintes expérimentales qui sont encombrants et nécessitent beaucoup de matériel biologique. Dans ce contexte, une suggestion est envisagée dans les perspectives.

Les travaux sur l'écophysiologie larvaire réalisés au cours de cette thèse (Chapitre III) ont porté sur l'effet de la densité du phytoplancton et de la température de l'eau de mer car ce sont les deux paramètres essentiels agissant sur la croissance et la survie des larves ainsi que sur la métamorphose chez *C. gigas* (Helm et Millican, 1977 ; His et al., 1989 ; Abdel-Hamid

et al., 1992 ; Auby et Maurer, 2004). En effet, ces études ont montré le rôle clé de la température et la disponibilité de nourriture sur le développement larvaire et, à l'inverse, l'effet négligeable de la salinité sur le développement larvaire (Nell et Hollyday, 1988 ; His et al., 1989). Cependant, ces travaux étaient limités dans le temps (7 à 10 jours) et en conséquence les données de survie en fin de cycle larvaire et de métamorphose étaient absentes. Par contre, les résultats obtenus au cours de ce chapitre couvrent tous ces aspects et permettent ainsi d'approfondir les connaissances sur l'effet de ces paramètres environnementaux sur le développement complet des larves de *C. gigas*.

En ce qui concerne le facteur nutritionnel, nous avons utilisé le régime bispécifique de microalgues déterminé au début de ce travail associant une Haptophycée (*I. affinis galbana*, T-ISO) et une Bacillariophycée (*C. calcitrans* forma *pumilum*, CP, ou *C. gracilis*, CG) en proportion équivalente 1:1. Ce régime de microalgues a été utilisé pour l'ensemble des expériences dans cette étude. La densité phytoplanctonique de ce régime a été exprimée soit à l'aide d'un compteur de particules en nombre total de cellules par microlitre d'eau de mer (cellules μl^{-1}), pour la plupart des expériences en écophysiologie, soit en volume cellulaire de microalgues par microlitre d'eau de mer ($\mu m^3\ \mu l^{-1}$) pour la partie modélisation.

Un autre point important concernant l'aspect nourriture résidait dans la détermination de l'apport du phytoplancton à l'entrée du système d'élevage en flux ouvert. En effet, cet apport augmentait en fonction de la taille larvaire (biomasse). Cette façon d'opérer permettant ainsi de maintenir une densité optimale de phytoplancton autour de la larve tout au long du développement pour les expériences en écophysiologie. Par cette approche, un apport de microalgues de 60 cellules μl^{-1} doit être injecté à l'entrée du système d'élevage en flux ouvert en début de vie larvaire (stade D véligère). Il doit être augmenté progressivement jusqu'à 200 cellules μl^{-1} pour des larves compétentes proches de la métamorphose. De plus, cette augmentation de phytoplancton au cours du développement larvaire a été établie pour

permettre un apport de phytoplancton légèrement supérieur à la capacité de consommation des larves se traduisant alors par une disponibilité permanente de 20 cellules de microalgues par µl autour des larves. En effet, 20 cellules μl^{-1} de T-ISO + CP (ou CG) est définie comme la densité de phytoplancton nécessaire pour autoriser la meilleure croissance et survie et maximiser la métamorphose en fin d'élevage. Cette valeur a été déterminée en se basant sur la courbe de réponse fonctionnelle pour définir le taux d'ingestion maximal entre les différentes densités de microalgues testées (12, 20 et 40 cellules μl^{-1}) à 25 °C (notre température standard). Les résultats de la présente partie montrent que le taux d'ingestion maximal est atteint à partir d'une densité de 20 cellules μl^{-1} autour des larves. Ce taux reste sensiblement identique à une densité supérieure (40 cellules μl^{-1}).

Ces résultats sur l'ingestion en fonction des différentes densités à une température constante (25 °C) ont permis de dégager l'intérêt de l'adaptation en apport de phytoplancton à l'entrée du système d'élevage en flux ouvert tout au cours du développement larvaire. Un apport bas en microalgues doit être opéré les premiers jours d'élevage (5 à 6 jours). Ceci est conforme aux résultats de Soudant (1995) et Ben Kheder (2007) qui expliquent que l'accumulation de réserves à partir de la nourriture exogène ne peut avoir lieu qu'après cinq jours environ de la première prise de nourriture, bien que les larves commencent à se nourrir dès le stade D (Lucas et Rangel, 1983 ; His et Seaman, 1992). Par contre, lorsque les larves atteignent une taille de ≈110 µm, la ration de microalgues devient un facteur essentiel au développement et il est donc nécessaire de l'augmenter et ce en relation avec la taille des larves, tout en maintenant une densité de phytoplancton de 20 cellules μl^{-1} (équivalent à 1000 μm^3 μl^{-1}) selon nos résultats. Cette densité autour des larves permet ainsi de couvrir les besoins nutritionnels dans le but de maximiser la croissance (17 µm j^{-1}) et la métamorphose (44 %) en fin d'élevage. En effet, une densité de phytoplancton de 40 cellules μl^{-1} autour des larves n'engendre pas des meilleures performances (croissance, 19 µm j^{-1} et métamorphose,

40 %) que 20 cellules μl^{-1}. Par contre, une densité de phytoplancton de 12 cellules μl^{-1} autour des larves est considérée comme limitante car elle génère des moindres performances de croissance (12 $\mu m\ j^{-1}$) et de métamorphose (26 %).

D'autre part, l'influence de la température a été abordée via une démarche descriptive et quantitative qui permettait de cerner les réponses de l'ingestion, la croissance et la métamorphose des larves face à un environnement variant en température. Pour parvenir à examiner ces aspects, l'approche a consisté à élever au même moment des larves sous une large gamme de température : 17, 22, 25, 27, 32 °C.

Les résultats acquis au cours de cette partie révèlent que l'augmentation de la température dans cette gamme provoque l'accroissement des besoins énergétiques des larves et influence directement l'ingestion, la croissance et la métamorphose. De telles observations ont été rapportées uniquement sur l'ingestion et la croissance chez des larves de *Rudipates decussatus* (Beiras et al., 1994). Notre travail est original car les réponses physiologiques comme la croissance et l'ingestion ont été mesurées et quantifiées en fonction d'un large éventail de températures et ce tout au cours du développement larvaire chez *C. gigas* en établissant en plus un bilan de la métamorphose.

Cette étude montre bien les effets insignifiants de la plus faible température expérimentale (17 °C) rattachée à une possible incapacité des larves à digérer efficacement les microalgues (Manoj et Appukuttan, 2003). Cette température provoque alors un ralentissement de l'ingestion et de la croissance (6 $\mu m\ j^{-1}$) qui prolongent la vie larvaire à 35 jours *vs.* 20 jours à 25 °C (température proche de celle appliquée en conditions contrôlées dans de nombreuses écloseries commerciales) avec 16 $\mu m\ j^{-1}$ et 60 % de larves fixées. A l'inverse, à 32 °C, la durée de la vie larvaire liée à une croissance rapide (20 $\mu m\ j^{-1}$) était de moins de deux semaines avec une amélioration nette de la métamorphose (86 %). Néanmoins, cette température n'engendre pas de différence significative sur les performances larvaires

enregistrées à 27 °C (croissance, 19,5 µm j^{-1} et métamorphose, 87 %) associées par ailleurs à de très faibles mortalités (<10%) à ces deux fortes températures. Ces résultats sont en désaccord avec ceux de Helm et Millican (1977) chez *C. gigas*, puisque à 32 °C ils enregistrent des fortes mortalités (>65%) mais ils expliquent ceci par le fait d'un faible apport alimentaire aux larves. Notre travail contourne ce problème par les favorables conditions d'élevage employées.

De plus, l'étude détaillée de l'activité nutritionnelle aux cinq températures tout au cours du développement larvaire a permis de diviser cette activité en trois grandes périodes. Pendant la phase mixotrophe, les larves consomment faiblement (<8000 cellules $larve^{-1}$ j^{-1}) au cours des cinq premiers jours d'élevage pour des températures comprises entre 22 à 32 °C et pendant dix jours à 17°C. Ceci caractérise bien le début de la vie larvaire où le métabolisme est maintenu en partie par les réserves provenant des œufs (Bayne, 1983) bien que les larves soient capables d'ingérer des microalgues à partir du stade D (Lucas et Rangel, 1983 ; His et Seaman, 1992). Malgré cette capacité précoce d'ingestion, leur système digestif n'est pas encore complètement développé (Gerdes, 1983a). De telles observations ont été rapportées par Gallager (1988), qui mentionne que des larves <100 µm présentent un œsophage étroit pouvant limiter la taille et le nombre de particules ingérées. Ultérieurement, une forte consommation de microalgues se met en place lorsque les larves atteignent une taille de ≈110 µm entrant ainsi dans leur période exotrophe pour permettre l'accumulation de réserves nécessaires à la métamorphose. Ceci prouve que l'influence de la nourriture ne prend le relais qu'à partir de cette taille où les larves montrent une meilleure capacité d'ingestion liée au développement complet de voies ciliées du velum (Strathmann, 1978). Cette phase exotrophe se caractérise par une prise de nourriture pouvant atteindre 40 000 à 65 000 cellules $larve^{-1}$ j^{-1} en fonction de la température. Enfin, au cours de la métamorphose, une diminution de cette consommation est notée. Elle est due à la réabsorption du velum (l'organe d'alimentation et

nage chez la larve) qui sera remplacé à terme par la branchie chez le naissain (Cannuel et Beninger, 2006).

Dans la dernière partie de ce travail (Chapitre IV), des expériences complémentaires en écophysiologie ont été réalisées pour aboutir à un modèle bioénergétique de croissance des larves de l'huître creuse basé sur la théorie du « Dynamic Energy Budget » (DEB) (Kooijman, 2000).

En effet, pour donner une vision intégrative et simplifiée des processus physiologiques des larves et leur environnement nous avons fait appel à cette théorie et ses outils de modélisation bioénergétique. Comme mentionné dans l'introduction, l'un des grands intérêts de la théorie DEB est son aspect générique, c'est à dire la particularité d'être applicable à toutes les espèces vivantes et à tous les stades de vie d'une même espèce. La seule différence réside dans la valeur des paramètres. Sur la base de cette caractéristique, nous nous sommes intéressés à appliquer le modèle DEB développé chez l'adulte de l'huître creuse *C. gigas* dans une version adaptée chez la larve et ce pour la première fois.

La contribution de cette partie de thèse a porté sur la détermination des paramètres DEB propres à la larve concernant les caractéristiques de forme à ce stade, d'ingestion et d'assimilation de la nourriture ainsi que celles du préférendum thermique de la larve. Faisant la somme des données acquises à partir des nombreuses expériences d'écophysiologie au cours des chapitres précédents, le modèle DEB développé dans ce dernier chapitre permet donc de comprendre, de quantifier et de prévoir les variations de performances des larves en fonction de l'évolution de leur environnement d'élevage, en particulier la température de l'eau de mer et la disponibilité de nourriture (microalgues).

D'une part, nous avons considéré la croissance pendant le développement larvaire en prenant en compte les différentes périodes liées à l'activité nutritionnelle des larves : mixotrophie et exotrophie. Pour cela, nous avons formulé certaines hypothèses pour mieux

représenter les mécanismes sous-jacents de ces périodes nutritionnelles. Nous établissons une durée de 5 à 6 jours pour la période mixotrophe où les larves consomment faiblement les microalgues de par le développement incomplet de leur système digestif (Gerdes, 1983a ; Gallager, 1988). Nous proposons donc dans le modèle un âge de 5,5 jours à partir duquel se met en place le relais nutritionnel strictement exogène (Soudant, 1995 ; Ben Kheder, 2007). Ce relais se réalise à une taille larvaire de ≈110 µm dans le modèle basé sur les expériences en écophysiologie. A partir de cette longueur, la consommation augmente en conséquence et les larves entrent dans leur période exotrophe pendant laquelle la croissance larvaire présente une relation linéaire (Beiras et al., 1994). Finalement, nous proposons une taille larvaire de 300 µm à partir de laquelle une larve est compétente pour opérer sa métamorphose (Coon et al., 1990). Dans ce travail, cette dernière taille était à l'origine du pallier observé sur les courbes des simulations de la croissance des larves.

Or, nous avons considéré différentes conditions environnementales de température et densité de phytoplancton autour des larves pour reproduire la variabilité observée dans les données de croissance larvaire lors des simulations. Ce travail nous permet donc de mieux comprendre l'effet de ces conditions sur la durée de l'élevage et de mieux expliquer la biologie de ce stade.

Plus la température est élevée dans la gamme étudiée, moins la durée de la vie larvaire est longue. Les larves ont donc une croissance plus rapide qui en résulte dans un resserrement de la vie larvaire et en conséquence les larves atteignent la métamorphose plus rapidement par rapport à l'étalement du développement larvaire aux températures plus basses.

Les larves de *C. gigas* ne présentent pas la même valeur de la température d'Arrhenius que l'adulte (11 000 *vs.* 5800 K, respectivement), ceci lié à leur préférendum thermique. En effet, le développement larvaire s'active rapidement en cas de températures fortes (27-32 °C) mais se réduit particulièrement à faibles températures (17 °C) d'après les expériences dans

cette étude. Une valeur de la température d'Arrhenius plus élevée s'explique dès lors que l'espèce expérimente des changements de température plus instantanés, comme ceux qui arrivent normalement pendant la vie larvaire. Les larves d'huître sont présentes seulement après la ponte estivale où la température d'eau de mer est maximale. Au contraire, l'adulte expérimente une gamme beaucoup plus large de température (8 à 37 °C) (Sicard et al., 2006) au cours de l'année.

L'étude de l'ingestion des larves de *C. gigas* suggère que la réponse fonctionnelle sigmoïde (Holling type III) serait plus appropriée par rapport à celle de l'adulte de type II (van der Veer et al., 2006). Ceci peut être expliqué par une activité de recherche de nourriture qui baisse lorsque la concentration en proie devient très faible conformément aux observations de Hassell et al. (1977) et Gentleman et al. (2003). En outre, une telle observation a déjà été opérée sur les larves de l'huître américaine, *C. virginica* (Baldwin et Newell, 1995). L'analyse de ces différentes réponses fonctionnelles devra être réalisée pour trouver la fonction appropriée par exemple dans l'évolution de la théorie DEB (Kooijman, 2000).

D'autre part, les résultats collectés lors de simulation en fonction des différents niveaux de phytoplancton ont également permis de dégager certaines informations concernant un besoin minimum de 1000 μm^3 μl^{-1} autour des larves nécessaire à la survie jusqu'à la métamorphose. Dans notre travail, les larves qui survivent jusqu'à la métamorphose sont les larves qui ont eu suffisamment de nourriture pour satisfaire les processus métaboliques. Nous nous sommes basés sur l'interprétation que cette condition détermine la quantité d'énergie qu'une larve peut effectivement mettre en réserve pour la métamorphose. En conditions limitantes de phytoplancton, cette énergie peut en effet être mobilisée pour la survie jusqu'à une limite de tolérance qui une fois franchit s'exprime par la mortalité des larves.

2. Perspectives

Ce travail apporte des éclaircissements sur les besoins écophysiologiques des larves d'huître *Crassostrea gigas* en milieu contrôlé. Cependant, à l'issue de cette thèse, plusieurs axes de recherche peuvent être intensifiés.

La recherche d'un régime de microalgues performant mérite d'être approfondie pour élucider l'impact de l'assemblage TCp dans les différentes proportions et leur composition biochimique sur les performances du développement larvaire. Compte tenu de ces résultats, un suivi biochimique plus fin permettrait de dégager probablement une relation plus claire au cours du développement larvaire (Soudant et al., 1998a, 1998b) en incluant davantage de points d'échantillonnage tout au cours du développement larvaire avec le même régime de microalgues utilisé dans ce travail. Par ailleurs, l'étude de la qualité biochimique des microalgues en fonction de leurs conditions de culture pourraient permettre d'apporter une réponse quant au degré de flexibilité des microalgues aux variations des paramètres du milieu et à leur impact ultérieur sur le développement larvaire.

En outre, il serait intéressant de cibler une étude au stade larvaire afin de mieux appréhender et démontrer l'importance qualitative de la sélection de particules par les organes d'alimentation, mis en évidence chez l'adulte de *C. gigas* (Beninger et al., 2008a, 2008b) et *C. virginica* (Pales-Espinosa et al., 2008)

D'autre part, l'équipement d'un système d'élevage couplé à l'outil automatique d'acquisition de données hydrobiologiques (SILO) a permis la mise en place d'expérimentations fines d'écophysiologie larvaire. Or, à partir de cette technologie, la Station Expérimentale d'Argenton a développé des petits systèmes d'élevages des larves en flux ouvert en volume de 5 L. Cette nouvelle approche déjà utilisée sur des travaux de sélection familiale de bivalves (King et al., 2005). Ces techniques sont déjà en utilisation dans

la Station afin d'étendre la recherche au cours de cette thèse sur des espèces de faible fécondité (*Ostrea edulis*). Toutefois, des études complémentaires seraient nécessaires pour accoupler efficacement un outil automatique d'acquisition en continu à ces petits volumes et développer des expériences d'écophysiologie sur des nouvelles espèces de bivalves.

Il apparaît de façon assez claire que les conditions de nourriture et de température que nous avons envisagées au cours de cette étude sont importantes en terme de gestion des rendements de production en écloserie. Cependant, certaines questions restent à résoudre. Bien que 20 cellules μl^{-1} aient été défini comme la densité permanente de phytoplancton nécessaire autour des larves pour maximiser la croissance et la métamorphose, il serait intéressant d'envisager un affinement sur la réponse fonctionnelle de consommation. En effet, pour confirmer cette information des études supplémentaires seraient nécessaires afin de appréhender cette densité de phytoplancton en testant des concentrations plus basses ($\leq$5 cellules μl^{-1}) et plus hautes ($\geq$50 cellules μl^{-1}) autour des larves.

Les résultats obtenus concernant l'effet de la température ont montré que 32 °C engendre les meilleures performances larvaires de croissance et de métamorphose bien que le protocole expérimental l'envisageait comme la limite de tolérance selon des études précédentes sur des larves de *C. gigas* (Helm et Millican, 1977). Néanmoins, cette performance s'accompagne, d'une part, d'un développement optimal de toute la population larvaire et, d'autre part, s'explique par des conditions d'élevage très favorables lors des expériences dans l'ensemble de températures testées. Il serait donc important d'envisager des études complémentaires dans le but de définir les valeurs des performances larvaires qui approchent les limites supérieures de tolérance de température pour les larves de *C. gigas*. Par exemple, Davis et Calabrese (1964) ont rapporté que la croissance des larves de *C. virginica* est réduite à 33 °C et des mortalités massives apparaissent à 35 °C.

D'autre part, le travail présenté ici a permis d'adapter le modèle DEB de l'huître creuse de son stade adulte à la larve. De l'ensemble des expériences de simulation, nous obtenons des résultats concernant l'effet des conditions environnementales de température et nourriture sur la reproduction de la croissance des larves par le modèle. Néanmoins, des interrogations subsistent sur la valeur énergétique de la nourriture (μ_X) dans le modèle issue d'expériences de calorimétrie de la littérature. L'approche calorimétrique totale ne rend en effet pas forcément compte de l'énergie biologiquement disponible dans le phytoplancton utilisée par la larve. Une nouvelle approche serait l'étude des éléments essentiels de la nourriture et leur rôle énergétique dans l'évolution de la théorie DEB.

L'élaboration du présent modèle est difficile de part la complexité des transformations qui s'opèrent en fin de phase larvaire. Ainsi, le déclenchement de la métamorphose dans notre modèle repose sur la taille permettant une bonne description mais l'ampleur des changements que la larve subit et le peu d'informations de validation sur cette période de changements ne permettent pas d'introduire les mécanismes réellement explicatifs dans le modèle. Pour mieux expliquer la métamorphose dans le cadre de la théorie DEB (Pecquerie, 2007), il faudrait indexer les passages de la phase larvaire à la métamorphose sur la variable d'état « développement » qui décrit l'allocation de l'énergie que la larve pourrait utiliser pour sa complexité « métamorphoser » et devenir un juvénile.

Par ailleurs due à la propriété générique de la théorie DEB, le modèle de croissance développé dans le présent travail chez les larves de *C. gigas* commence à être appliqué à d'autres espèces de bivalves comme *Pinctada margaritifera* pour évaluer l'effet des facteurs environnementaux sur la croissance des larves de l'huître perlière (Thomas et al., 2009).

De la même façon dans la présente étude, le modèle DEB a été développé à partir d'expériences en conditions contrôlées en laboratoire visant à étudier l'influence de la température et la disponibilité en nourriture sur la croissance des larves de *C. gigas*.

Toutefois, le modèle DEB commence à être appliqué en milieu naturel. Ainsi, les premières prédictions de croissance du modèle *in situ* sont en accord avec les estimations en conditions contrôlées et le modèle permet donc de bien évaluer la croissance dans le milieu naturel (Bernard, 2009 com. pers.).

Finalement, après cette première version du modèle DEB chez la larve, un effort d'intégration entre le modèle adulte et le modèle larvaire reste à faire pour construire un seul modèle DEB du cycle de vie de l'huître creuse *C. gigas* (Bernard, 2009 com. pers.). Cet unique modèle devra intégrer les phases post-larvaire et juvénile et pour cela des études complémentaires doivent être réalisées dans le but d'expliquer la transition de forme entre la larve (coquille ovoïde) et le juvénile (coquille allongée).

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TITRE : Les besoins écophysiologiques des larves d'huître creuse *Crassostrea gigas* en conditions contrôlées

Résumé
La production de naissain de l'huître creuse *Crassostrea gigas* passe de plus en plus par des méthodes d'élevage en milieu contrôlé en écloserie. Le développement larvaire et le succès à la métamorphose étant considérés comme critiques en écloserie, des recherches approfondies ont donc été conduites chez cette espèce. Tout d'abord, un régime composé d'une Haptophycée : *Isochrysis affinis galbana* (clone T-ISO) et d'une Bacillariophycée : *Chaetoceros calcitrans* forma *pumilum* a démontré qu'il pouvait subvenir efficacement aux besoins nutritionnels tout au cours du développement larvaire tout sur le plan qualitatif que quantitatif. Ensuite, un système d'élevage larvaire en flux ouvert a été mis au point et couplé à un outil d'acquisition automatique de données hydrobiologiques afin de réaliser des expérimentations fines d'écophysiologie intégrant différents niveaux trophiques et/ou de température tout au cours d'un élevage larvaire. L'ingestion, la croissance et la métamorphose sont optimales aux températures les plus élevées (27 et 32 °C) et à partir d'une densité de phytoplancton (20 cellules μl^{-1}) autour des larves. La dernière partie a porté sur l'établissement d'un modèle bioénergétique de croissance sous la théorie Dynamic Energy Budget (DEB) afin de comprendre l'effet des conditions environnementales sur la croissance larvaire. Cette théorie décrit les flux d'énergie au travers de la larve, de l'assimilation à l'allocation vers la croissance, le développement et la maintenance. Le modèle DEB développé permet d'étendre la description de la croissance de l'huître creuse sur la durée d'élevage larvaire. Nous proposons alors que la forte croissance des larves s'explique par les conditions favorables de températures élevées et d'un minima de nourriture (1000 μm^3 μl^{-1}) autour de larves. Ce premier modèle DEB de la phase larvaire d'un bivalve constitue une voie prometteuse pour un développement plus poussé afin de construire un seul modèle du cycle de vie de *C. gigas* de la larve à l'adulte.

Mots clés : *Crassostrea gigas*, Larve, Croissance, Ecophysiologie, Dynamic Energy Budget

TITLE : Ecophysiological needs for Pacific oyster larvae, *Crassostrea gigas*, under controlled conditions

Abstract
Nowadays, spat production of Pacific oyster *Crassostrea gigas* relies on rearing techniques under controlled conditions in hatchery. In this context, an extensive research in aquaculture is necessary because larval development and metamorphosis are critical for hatchery management. Firstly, a diet consisted in a Haptophyceae: *Isochrysis affinis galbana* (clone T-ISO) and a Bacillariophyceae: *Chaetoceros calcitrans* forma *pumilum* demonstrated its efficiency to supply the nutritional needs throughout the whole larval rearing. Then, a flow-through rearing system was developed and coupled with a continuous hydrobiological data recorder to provide basic information for ecophysiology research through different trophic levels and/or temperature throughout larval development. Ingestion, growth and metamorphosis are found to be optimal at the highest temperatures (27 and 32 °C) and for a phytoplankton density (20 cells μl^{-1}) around larvae. Finally, a bioenergetic model of growth was built under Dynamic Energy Budget (DEB) theory to understand the effect of the environmental conditions on the larval growth. This theory describes the rates at which a larvae assimilates energy from food and stores this energy as reserves for allocation to growth, development and maintenance. The DEB model allows to extend the growth description of oyster throughout larval rearing. It was proposed that high temperatures and a food density of 1000 μm^3 μl^{-1} must be maintained around larvae throughout larval development to maximise growth. This first DEB model through the whole larval phase of a bivalve constitute a promising way to encompass the whole life cycle of *C. gigas* from larvae to adult.

Keywords: *Crassostrea gigas*, Larvae, Growth, Ecophysiology, Dynamic Energy Budget

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