Incidence de la nutrition sur la reproduction et le développement larvaire d'Ostrea edulis

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A ma fille Carolina,

à mes parents, mes frères et ma sœur

à ma belle Breizhad, Gaëlle, à Samy et à Julie

« à tous ceux qui n'ont jamais cru »

« Nous sommes tous ignorants, mais nous n'ignorons pas tous les mêmes choses »

A. Einstein

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PRESENTATIONS ET PUBLICATIONS

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INTRODUCTION GENERALE

L'huître plate est restée très longtemps la seule huître consommée en France. Goulletquer et Héral (1997) rapportent qu'au début de notre ère, elle était expédiée jusqu'à Rome et que, pendant plusieurs siècles, les gisements naturels ont été largement exploités.

Au 19e siècle, la production encore récente de l'huître plate (*Ostrea edulis*) ne pouvait satisfaire une demande croissante. Des huîtres creuses (*Crassostrea angulata*), en provenance du Portugal, furent alors introduite en France. Ainsi en 1867, un navire, « Le Morlaisien » pris dans une tempête a dû rejeter sa cargaison dans l'estuaire de la Gironde, entraînant l'implantation de cette espèce sur ces côtes. Au dessus de Nantes, un décret interdisait sa culture et l'huître plate était toujours l'espèce phare en Bretagne et en Normandie, ces régions n'ayant pas développé d'activité ostréicole à cette époque.

Jusqu'en 1920, la production ostréicole des deux espèces était équivalente. Entre 1920 et 1960 la production d'huître plate a été sévèrement impactée par une maladie inexpliquée qui a favorisé le développement de l'huître portugaise. Elle est donc restée largement dominante dans les sites conchylicoles (Goulletque et Héral, 1997). En 1971, suite à deux épizooties (maladie des branchies et Iridovirus) qui ont touché l'ensemble des gisements (Comps, 1970), l'huître portugaise a subi une importante mortalité et toute importation de cette espèce a été alors interdite. Peu de temps après, alors que la production de l'huître plate en France se situait entre 10 000 et 20 000 tonnes, *O. edulis* était à son tour affectée par une attaque parasitaire (*Marteilia*

refringens) provoquant des mortalités dans les élevages en estuaire (Grizel et al., 1994). Les cultures ont donc été transférées en eau profonde, mais un nouveau protozoaire, *Bonamia ostreae*, est apparu et a entraîné le déclin de la production d'huîtres plates qui n'est actuellement que de 1 000 – 1 500 tonnes par an (Goulletquer et Héral, 1997 ; Scheffer, 2003 ; FAO, 2011).

Ces deux épizooties ont également impacté la production européenne d'*O. edulis* au cours des quarante dernières années (FAO, 2011), en Espagne (3 000 tonnes actuellement), en Croatie (500 tonnes) et en Irlande (382 tonnes). Pour remplacer *C. angulata* du naissain d'huître japonaise *Crassostrea gigas* a été importé à partir de 1971 sur le littoral français, dont les premiers essais d'élevage ont été couronnés de succès (Grizel et Héral, 1991). Ces importations ont donc été opérées régulièrement jusqu'en 1977 tandis que des géniteurs issus de Colombie Britannique ont été introduits successivement en 1973, 1975 et 1977. A ce jour, l'ostréiculture française connaît une situation de quasi-monoculture, avec 98 % de la production constituée d'huître creuse *C. gigas* (FAO, 2011).

La diversification des espèces élevées en conchyliculture a été préconisée pour élargir le marché et minimiser les risques pathologiques qui peuvent mettre en péril l'activité professionnelle tournée traditionnellement vers la monoculture.

Parmi les différentes espèces produites en écloserie, l'huître plate reste une espèce difficile à maîtriser. Elle a, en effet, un cycle reproductif original : c'est une espèce larvipare et protandrique avec une première maturation en tant que mâle, suivi par un rythme régulier de maturation en alternant les phases femelles et mâles (Orton, 1927, 1933 ; Cole, 1942, da Silva et al., 2009).

Par ailleurs, *O. edulis* reste toujours fortement impactée par la présence de *B. ostreae*, représentant une contrainte majeure. Pourtant, des essais d'introduction et d'acclimatation d'espèces du genre *Ostrea* ont été tentés dans l'espoir de trouver une variété d'huître plate à la fois performante en croissance et résistante à la bonamiose. Les études menées sur *Ostrea chilensis* (Grizel et al., 1983), *O. denselamellosa* (Le Borgne et Le Pennec, 1983), *O. angasi* (Bougrier et al., 1986) et *O. puelchana* (Pascual et al., 1991) ne permettent pas aujourd'hui d'envisager l'élevage de ces espèces sur le littoral français.

De nombreux travaux couvrant différents domaines ont montré que la mortalité élevée due à la bonamiose se produit lorsque les huîtres sont proches de la taille commerciale (Tigé et al., 1982 ; Montes et al., 1991 ; Robert et al., 1991 ; Culloty et Mulcahy, 1996). Pour limiter les effets de ce parasite, les moyens de lutte sont relativement restreints. L'utilisation d'un traitement ou d'une vaccination n'est pas envisageable puisque son élevage se fait en milieu ouvert. De ce fait, les moyens de lutte reposent essentiellement sur une surveillance des stocks, l'utilisation de modèles prédictifs de l'évolution de la maladie et le développement d'animaux résistants/tolérants à l'infection. Ainsi au début des années 90, l'Ifremer a initié un programme de sélection génétique de l'huître plate résistante ou tolérante à *B. ostreae* (Martin et al., 1993). Des lignées ont été obtenues par pression de sélection, grâce à un protocole de purification du parasite qui a permis l'infection expérimentale des huîtres (Hervio et al., 1995). Ces lignées ont été reproduites entre elles afin d'obtenir des générations successives d'huîtres plates mises au contact de *B. ostreae* en laboratoire ou dans des zones naturelles infestées (Naciri-Graven et al., 1998).

L'utilisation de naissain sélectionné doit cependant répondre à deux contraintes majeures : cette production doit impérativement passer par écloserie et le naissain fourni doit être d'une taille compatible avec les pratiques ostréicoles en milieu ouvert.

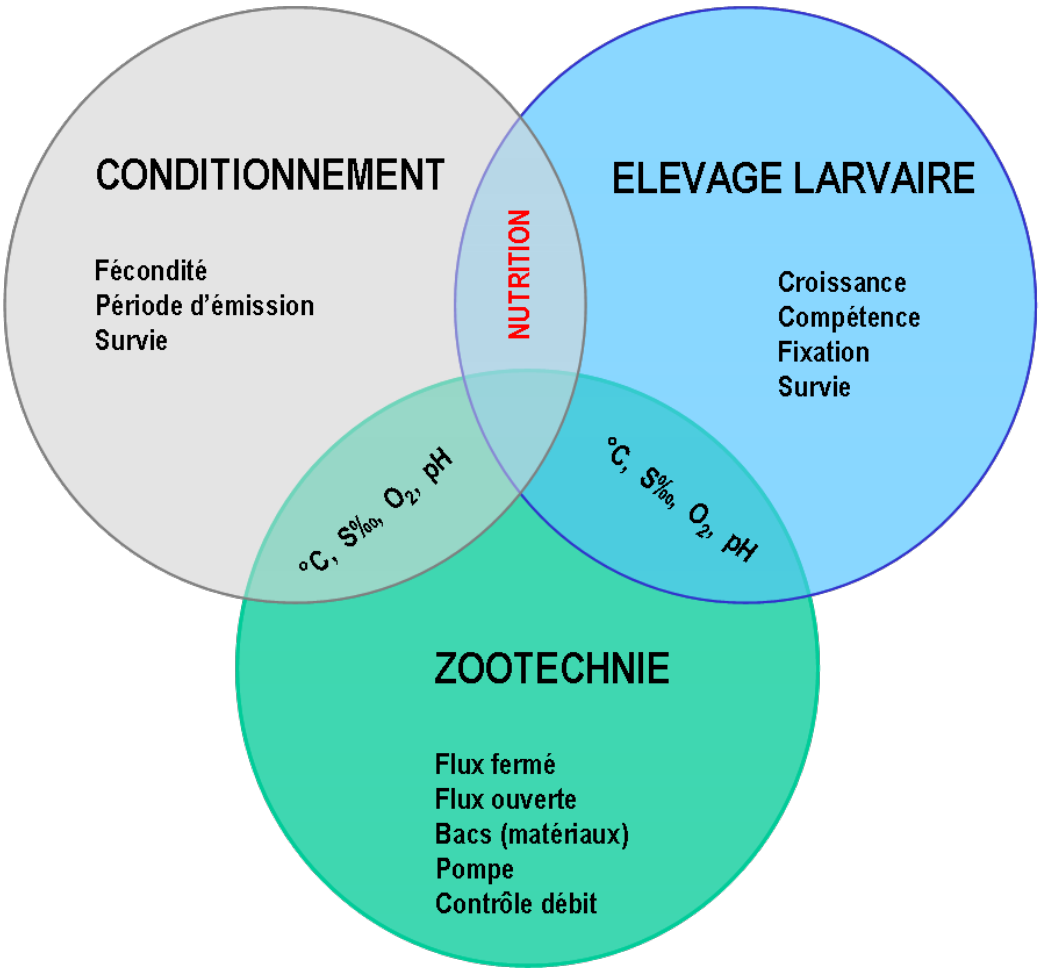

Figure I-1 : Modèle mécanistique d'interaction entre les différents compartiments en écloserie de mollusques.

Une grande partie de nos connaissances actuelles sur les techniques d'élevage de l'huître plate, en milieu contrôlé repose sur les travaux de Walne (1966, 1970) à Conway (UK) conduits dans années 70. L'apparition de la marteiliose puis de la

bonamiose dans les années 70-80, a freiné ces travaux, la priorité étant donnée à la maîtrise de l'élevage de l'huître japonaise *C. gigas* récemment introduite (Helm et Millican, 1977). Depuis, à l'exception de quelques travaux menés par Ferreiro et al. (1990), Frolov et Pankov (1992), Millican et Helm (1994), Bernsston et al. (1997), les connaissances acquises chez l'huître plate n'ont pas beaucoup évolué.

Or, le contrôle de la production des juvéniles de cette espèce passe tout d'abord par la maîtrise de la reproduction des géniteurs. Pendant le conditionnement, des facteurs physiques comme la température (Loosanoff, 1962 ; Sastry, 1966), la salinité (Muranaka et Lannan, 1984) et la nutrition (qualité et type d'alimentation) ont été décrits. Ainsi, la croissance et la survie larvaire ont été corrélées avec la teneur initiale en lipides (Gallager et Mann, 1986 ; Gallager et al., 1986, Helm et al., 1973), mais l'importance de chaque espèce de microalgue sur le développement gonadique, l'allocation biochimique et son impact ultérieur sur le développement larvaire reste à ce jour méconnu.

Dans ce contexte, il devient donc crucial de se réapproprier un savoir-faire sur les différentes techniques d'élevage en reproduction contrôlée, d'identifier les mécanismes d'interaction entre les différents compartiments d'une écloserie (Figure I-1) et d'en estimer les poids respectifs chez l'huître plate.

Parmi les principaux paramètres d'élevage, la nutrition est l'un des plus importants à maîtriser en écloserie et la plus complexe à cerner car elle intervient à la fois aux niveaux quantitatif et qualitatif par action directe ou indirecte (constitution et utilisation de réserves). Dans la présente thèse, nous nous sommes donc attachés à comprendre les effets de la nutrition chez l'huître plate tant au niveau

conditionnement (impact sur la reproduction) qu'au cours de l'élevage larvaire (impact sur le développement des phases précoces).

Pour ce faire, la présente étude s'organise en trois chapitres.

Le premier concerne la mise en évidence de l'impact de l'alimentation au cours du conditionnement de l'huître plate et son influence ultérieure sur le développement larvaire.

Le second est exclusivement consacré à l'étude du rôle de la nutrition sur la reproduction de l'huître plate, en étudiant les étapes successives conduisant à l'activation de ce compartiment biologique.

Enfin, le troisième est consacré à l'étude de l'influence de l'alimentation des géniteurs sur le développement larvaire de l'huître plate.

GENERALITES

L'huître plate (Ostrea edulis)

Répartition géographique

L'huître plate *Ostrea edulis* (Linné, 1758) est la seule huître endémique des côtes européennes, atlantiques et méditerranéennes. Elle appartient à l'embranchement des Mollusques, classe de Bivalves (ou Lamellibranches), ordre de Filibranches et famille des Ostreidae (Grassé, 1960)(Tableau I-1).

De caractère plus océanique que l'huître creuse (*Crassostrea gigas*), *O. edulis* vit dans des eaux salées et peu turbides. Les bancs et les cultures sont situés sur des zones infralittorales ou toujours immergées. Son aire de répartition naturelle couvre cependant une zone géographique beaucoup plus vaste que les côtes européennes, depuis la Norvège (65ème degré de latitude Nord) jusqu'à la baie d'Agadir au Maroc, en passant par le pourtour méditerranéen, en France, Italie, Sicile, mais aussi en Tunisie, et jusqu'en Adriatique et en Mer Noire (Ranson, 1967 ; Jaziri, 1990 ; Figure I-2).

Les huîtres du genre *Ostrea* sont des espèces hermaphrodites asynchrones, à sexualité consécutive rythmique (Marteil, 1976). Elles peuvent en effet, changer de sexe plusieurs fois au cours de la même saison de reproduction. Ce sont des animaux généralement protandriques, c'est à dire qu'elles sont mâles la première année de reproduction. Elle constitue ses gonades au printemps et prend un aspect laiteux. Elle se reproduit en été et en automne dans le milieu naturel. Les huîtres du

genre *Ostrea* présentent un cas particulier. Ces dernières, à l'encontre des autres bivalves communément cultivés, n'ont pas besoin d'être stimulées pour le déclenchement de la ponte. Ces huîtres peuvent pondre de leur plein gré durant le processus de conditionnement et porteront les larves à l'intérieur de la cavité de leur manteau pendant des périodes variables selon les espèces. Ce groupe d'huîtres est considéré comme des larvipares dites incubatrices.

Embranchement	Mollusques
Classe	Bivalves
Ordre	Filibranches
Famille	Ostreidae
Genre	Ostrea
Espèce	*Ostrea edulis*
Fécondation	Interne
Reproduction	Hermaphrodite alternant, Protandrique

Tableau I-1. Position systématique et particularités de la reproduction d'*Ostrea edulis*.

Lors de la reproduction, les mâles libèrent leurs gamètes dans l'eau qui sont aspirés par la femelle, et la fécondation a lieu dans la cavité palléale. Selon certains auteurs, l'incubation des larves peut durer de 8 à 10 jours (Korringa, 1947 ; Marteil, 1960). Les larves véligères sont expulsées dans le milieu à une taille de 160 à 200 µm. Elles mènent une vie pélagique qui, pour Marteil (1976) peut durer de 8 à 14 jours pour des températures variant de 18-20°C à 15-16°C. Puis la larve pédivéligère cherche un support pour se fixer ; elle mesure alors 260 à 290 µm (Martin et al., 1997).

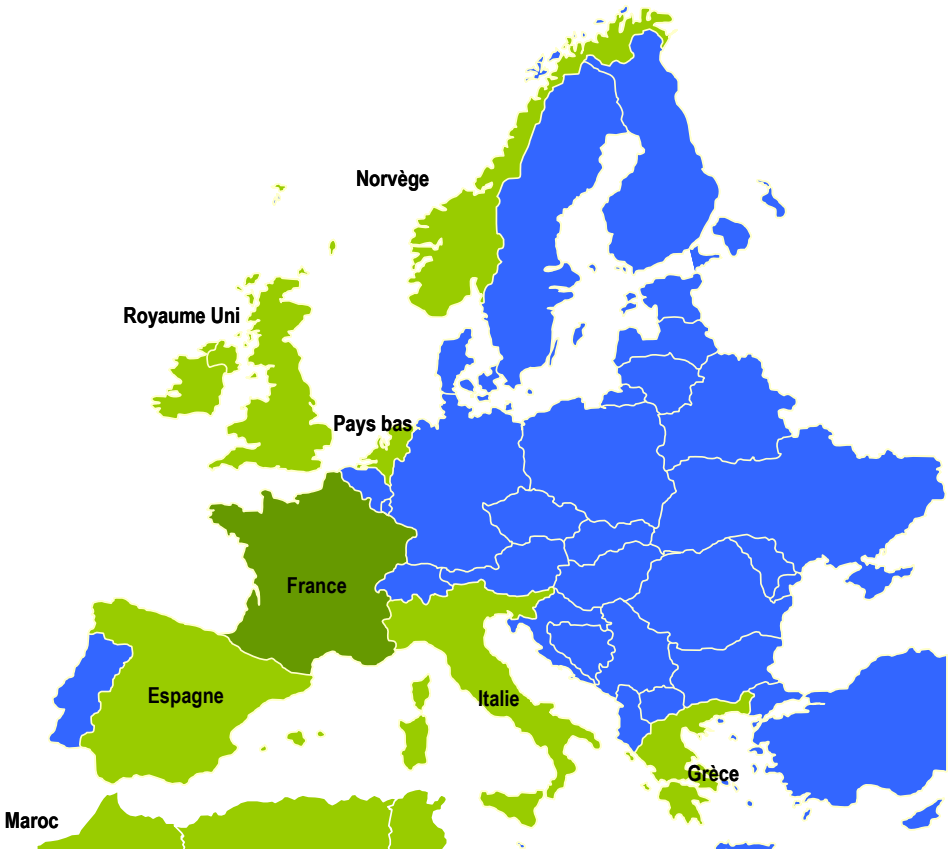

Figure I-2. Distribution géographique d'*Ostrea edulis* (en vert)

Anatomie de l'huître plate

Un ligament, élastique, corné, réunit les deux valves et joue un rôle de charnière (Figure I-3); c'est autour de cet axe que pivote l'écaillé supérieure dans les mouvements d'ouverture et de fermeture de la coquille. L'élasticité du ligament tend à éloigner la valve plate de la valve creuse tandis que le muscle adducteur tend à les rapprocher. L'ensemble du corps mou de l'huître est revêtu d'un tégument appelé manteau. C'est une feuille de tissu conjonctif contenant des muscles, des vaisseaux sanguins, des nerfs et recouverte d'un épithélium unicellulaire. On y distingue deux lobes qui, soudés l'un à l'autre sur le bord antérodorsal, forment le capuchon céphalique recouvrant la bouche et les palpes labiaux (Fig. I-3).

En revanche, les bords sont libres dans la région ventrale et suivent le contour de la coquille, l'espace libre compris entre les deux lobes du manteau est appelé cavité palléale (du latin pallium = manteau). Les branchies divisent cette cavité en deux, une partie ventrale ou chambre inhalante et une partie dorsale ou chambre exhalante (Fig. I-3).

En dehors du rôle capital que joue le manteau dans la formation de la coquille et la sécrétion du ligament, il contribue à abriter les œufs rejetés et les larves en cours d'incubation. Il stocke enfin les matériaux de réserve (glycogène et graisse) qui améliorent la condition de l'huître.

La respiration des huîtres est assurée au moyen de branchies appelées encore cténidies. Il y en a deux, chacune d'entre elles étant composée de deux lames ou lamelles formées elles-mêmes de deux feuillets, l'un direct ou descendant, l'autre

réfléchi ou ascendant ayant la forme d'un « V ». Une section transversale d'une branchie représente ainsi un «W». Chaque feuillet est constitué par une série de filaments accolés les uns aux autres, disposés en groupes ou plis qui donnent à la branchie son aspect plissé. Le nombre de filaments par pli diffère légèrement suivant les espèces et les individus ; il oscille entre 9 et 12 chez *O. edulis* (Yonge, 1960), entre 12 et 19 chez *Crassostrea angulata* (Comps, 1970), entre 11 et 17 chez *C. gigas* et entre 10 et 16 chez *C. virginica* (Galtsoff, 1964). Entre deux tubes ou filaments contigus, existent de petites fenêtres ou ostia qui permettent le passage de l'eau de la chambre inhalante vers les chambres branchiales. Ces ostias doivent laisser passer les œufs émis par l'huître femelle. L'eau filtrée sort ensuite par la chambre exhalante. Le rôle des branchies ne se limite pas à la satisfaction des besoins respiratoires, mais s'étend au domaine de la nutrition; les particules en suspension dans l'eau filtrée sont retenues à la surface des branchies, enrobées de mucus et conduites par les battements des nombreux groupes de cils vers la bouche. D'autres, les plus lourdes, tombent sur le manteau et sont rejetées sous forme de pseudofèces à partir de la chambre inhalante, les fèces véritables ou déchets issus de la digestion étant, elles, expulsées dans la chambre exhalante.

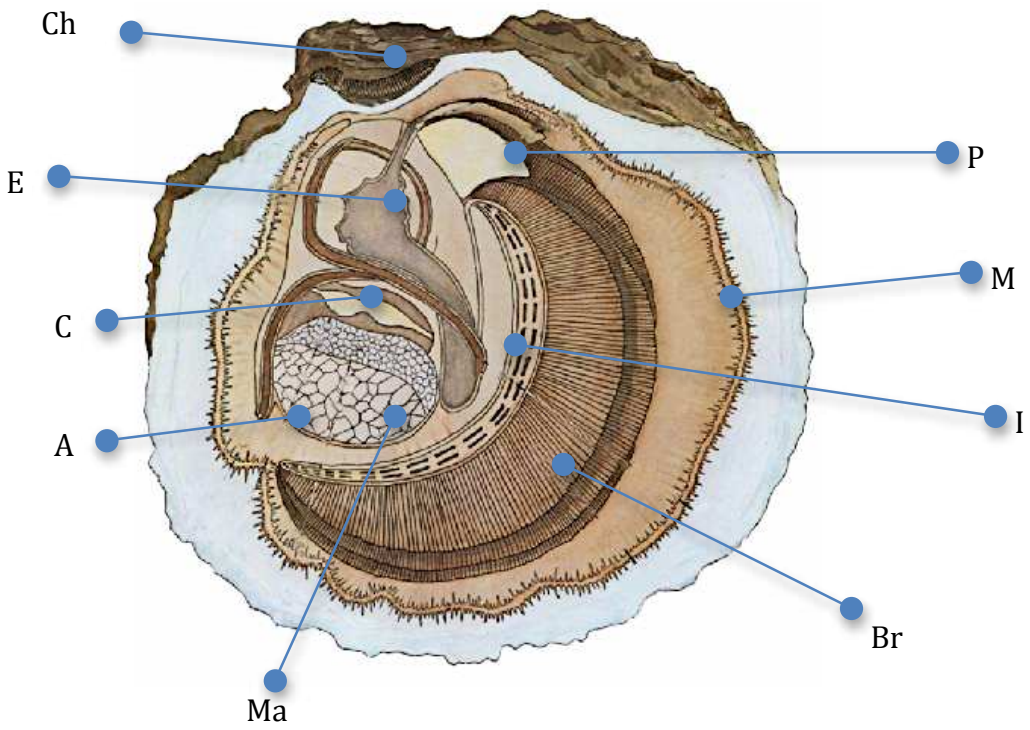

Figure I-3: Anatomie d'une huître plate *Ostrea edulis* (Linné 1758) développée : Ch- Charnière ; E- Estomac ; C- Cœur ; A- Anus ; Ma- Muscle adducteur ; Br- Branchie ; I- Intestin ; M- Manteau ; P- Palpes.

A la ponte, les ovocytes passent à travers les branchies dans la chambre inhalante de la cavité du manteau où ils se développent entièrement en larves coquillées durant une semaine ou plus. L'adulte émet les larves quand elles sont capables d'ingérer et digérer les algues. Quand les larves des huîtres sont libérées dans l'eau, elles nagent à la surface et vont se développer dans la colonne d'eau pendant 6-15 jours en fonction des conditions du milieu (température, nutrition, techniques d'élevage). A maturité, dès l'apparition d'une tache occelaire appelée œil, d'abord du côté ventral puis dorsal, la larve va chercher une surface sur laquelle se fixer et se métamorphoser.

Physiologie des bivalves marins

Filtration

La branchie est à l'origine de l'induction d'un courant d'eau qui traverse la cavité palléale grâce aux battements de cils latéraux, situés sur les filaments branchiaux. Cette circulation d'eau permet de couvrir les besoins en oxygène de l'huître, par la ventilation des surfaces respiratoires fortement vascularisées (principalement la branchie mais aussi le manteau), et d'apporter les éléments nutritifs nécessaires à son alimentation. Une fois piégées par les cils latéro-frontaux du filtre branchial, les particules en suspension sont enrobées de mucus et acheminées par des cils frontaux vers les « sillins nourriciers » qui alimentent la bouche. Un autre tri est observé à proximité de la bouche par les palpes labiaux divisés en quatre parties égales. Selon Bernard (1974), ces organes pourraient dissocier les particules du mucus avant l'ingestion et rejeter les matières indésirables vers l'extérieur, sous forme de pseudofèces (Fig. I-4).

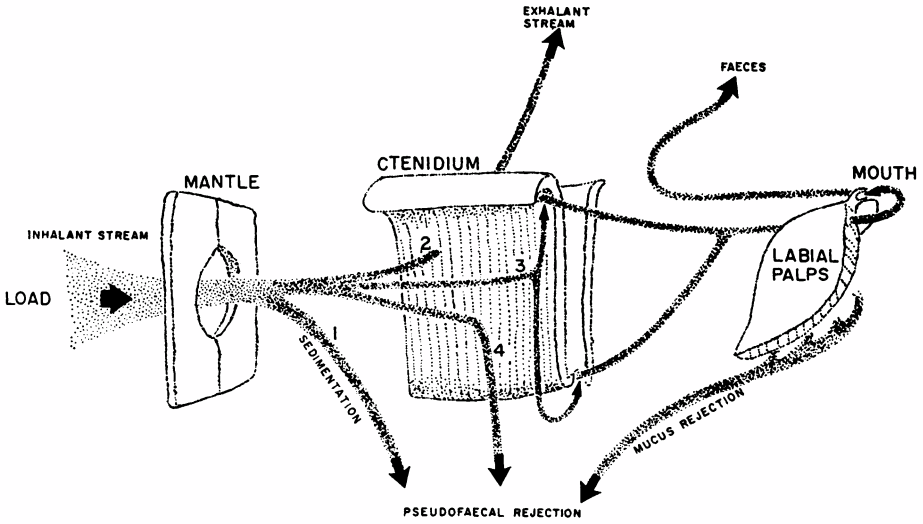

Figure I-4 : Représentation des différentes voies de déplacement des particules dans la cavité palléale de bivalves (d'après Bernard, 1974).

Nutrition

La production de gamètes étant dépendante de l'énergie dérivée de la nourriture ingérée, les huîtres, comme d'autres bivalves marins, présentent des cycles de stockage de mobilisation d'énergie étroitement liés au cycle annuel de reproduction (MacDonald et Thompson, 1986).

Si, fondamentalement, l'énergie allouée pour la reproduction trouve son origine dans les nutriments ingérés puis assimilés, les voies métaboliques menant des épithéliums digestifs aux cellules sexuelles en formation sont complexes et multiples chez les bivalves et comportent toutes des dispositifs de stockage. Pour les espèces filtreuses comme l'huître plate, l'écosystème marin, particulièrement en milieu tempéré, est caractérisé par une très haute variabilité de la disponibilité de la nourriture contre laquelle ont été sélectionnés de nombreux particularismes

physiologiques. Parfois, cette contrainte temporelle a pour conséquence de reléguer la gamétogenèse à des périodes qui ne sont pas nécessairement les plus favorables du point de vue trophique.

Mathieu et Lubet (1993) ont rapporté qu'il existe une diversité de types cellulaires de stockage de réserves chez les mollusques bivalves. Ainsi, ils montrent la présence, chez les Mytilidés, de véritables cellules nourricières, sous forme d'adipogranulocytes, réparties dans les espaces inter-acini du manteau tandis que chez les Ostréidés, les cellules conjonctives interfolliculaires jouent ce rôle. Chez les Pectinidés, aucune cellule spécifique de stockage n'est connue et de nombreux travaux ont montré le rôle primordial du muscle adducteur comme tissu de réserves (Ansell, 1974 ; Comely, 1974 ; Lubet et al., 1987 ; Bricelj et al., 1987).

Bien que le muscle représente la plus importante source de réserves pour les Pectinidés, la glande digestive joue aussi, en plus de son rôle physiologique d'assimilation de l'énergie ingérée, un rôle non négligeable dans le stockage des réserves (Sastry et Blake, 1971) avec les lipides comme principale source d'énergie (Taylor et Venn, 1979 ; Roman et Acosta, 1991 ; Mestre, 1992 ; Saout et al., 1999).

Par ailleurs, Parson et al. (1961) ont déterminé les teneurs en protéines, glucides et lipides de 11 espèces de microalgues cultivées dans des conditions identiques, et rapporté qu'il n'y avait pas de différences significatives de composition. Chu et al. (1982), Enright et al. (1986) et Whyte (1987) concluent à des résultats inverses. Cette contradiction pourrait s'expliquer par le fait que la composition proximale des microalgues est très influencée par le stade de croissance et la concentration du milieu en sels nutritifs. L'origine de la variabilité de la qualité nutritionnelle des

microalgues est par conséquent à rechercher au niveau de leur composition biochimique (acides aminés, acide gras, vitamines et oligo-éléments), dans la mesure où elles sont de taille adéquate, digestibles et non toxiques.

Il existe une importante variabilité dans la composition en acides gras des microalgues (Ackman et al., 1968 ; Volkman et al., 1989). Les acides gras sont des acides carboxyliques à chaînes hydrocarbonées de longueur et d'insaturation variables[1], présents en général sous forme estérifiée dans les principaux lipides rencontrés chez les organismes vivants. Ils ont trois rôles métaboliques différents dans le fonctionnement cellulaire. Tout d'abord, ils constituent, dans les triglycérides, une forme de stockage de l'énergie et ils représentent les principaux constituants des œufs d'invertébrés marins. Chez les mollusques, il a été montré que les lipides constituent une importante source d'énergie lors du développement précoce. Ce rôle énergétique prépondérant des lipides par rapport aux carbohydrates est maintenu pendant le développement larvaire et post-larvaire (Holland et Spencer, 1973 ; Whyte et al., 1987).

De plus, ils sont des éléments essentiels à la structure et au fonctionnement des membranes cellulaires et sub-cellulaires. Les principaux constituants sont les phospholipides, le cholestérol et les protéines. La composition en acides gras des phospholipides est un paramètre très important dans la détermination de la fluidité membranaire et de l'environnement lipidique des fonctions associées aux membranes (transport, catalyse, récepteur).

[1]Nous utiliserons la nomenclature des nutritionnistes, dans laquelle les acides gras sont nommés par la formule C:X(n-Y) où C est le nombre de carbones, X le nombre de double-liaisons et Y la position de la première double-liaison comptée à partir du méthyle terminal. Bien qu'elle soit moins précise que celle des chimistes, qui indique la configuration (cis-trans) de chacune des double-liaisons,elle permet de différencier facilement les acides gras en fonction des critères qui conditionnent leur rôle métabolique.

Enfin, ils interviennent dans la communication intra-cellulaire, directement ou par l'intermédiaire de leurs dérivés. Les acides gras polyinsaturés 20:3(n-6), 20:4(n-3) et 20:5(n-3) sont les précurseurs de très nombreux dérivés ayant un rôle hormonal.

Après la fécondation, toutes les larves de bivalves passent successivement par les mêmes stades morphologiques, dont les principaux sont la larve trochophore, la larve véligère (larve D) et la larve pédivéligère (larve umbonée dotée d'un pied).

Lorsque des régimes alimentaires monospécifiques sont utilisés, la croissance des larves et des juvéniles des bivalves est très variable et dépend des espèces de microalgues apportées (Bayne, 1965 ; Waldock et Nascimento, 1979 ; Nascimento, 1980 ; Enright et al., 1986 ; Laing et Millican, 1987). En général, le mélange de plusieurs espèces a un effet synergique sur la croissance (Epifanio, 1976) indiquant que les déficiences, différentes suivant les espèces, sont compensées.

L'étude des facteurs qui définissent les priorités d'allocation d'énergie entre la reproduction et le stockage dans les tissus somatiques est donc nécessaire pour une meilleure compréhension et un contrôle de la physiologie d'une espèce.

Dans ce contexte, la présente thèse met en évidence l'impact de l'alimentation sur le conditionnement de géniteurs d'huître plate et son influence sur le développement larvaire. Pour ce faire, dans un premier temps (Chapitre I), des géniteurs ont été conditionnés sur la base de trois régimes nutritionnels, deux monospécifiques et un bi-spécifique (*Rhodomonas salina*, *Thalassiosora weiisflogii* et *R. salina* plus *T. weissflogii*). Les larves issues de chaque régime nutritionnel ont été mises en élevage avec un régime « standard » T-Iso+*Chaetoceros gracilis,* comparé à un régime composé de la

seule *C. gracilis* afin d'apprécier le rôle des diatomées dans le développement de cette phase précoce.

Dans un deuxième temps, l'étude du rôle de la nutrition sur l'huître plate, a été abordée en deux phases : la première (chapitre II) concerne les approches physiologique, biochimique et histologique de l'impact de huit espèces de microalgues: T-Iso, *C. gracilis*, *Skeletonema marinoï, Tetraselmis suecica*, *R. salina*, *T. weissflogii*, *Thalassiosira pseudonana* et *Pavlova lutheri*. Pour chaque microalgue, l'ingestion, l'assimilation et l'absorption par les géniteurs de l'huître plate ont été étudiées afin d'évaluer leur importance relative et permettre un classement entre ces différentes sources nutritionnelles. Des échantillons des quatre principaux organes (gonade, glande digestive, muscle et branchies) ont été prélevés au début et à la fin de la période de conditionnement afin d'estimer le transfert des composants biochimiques (protéines, glucides, acides gras et stérols) de chaque microalgue vers l'animal. Une allocation spécifique de certains composés dans la gonade a ainsi été recherchée. A la fin de chaque conditionnement, une étude de la gamétogenèse via une analyse histologique a été réalisée afin d'estimer l'effet de chaque espèce de microalgue sur le développement gonadique de l'huître plate permettant ainsi de vérifier l'adéquation des différentes approches, physiologiques, biochimiques et histologique.

La deuxième partie (Chapitre III) a été consacrée à l'étude de l'alimentation des géniteurs sur le développement larvaire de l'huître plate et elle s'est intéressée à l'impact de régimes monospécifiques parentaux sur le développement ultérieur des descendants (larves).

Chapitre I

INCIDENCE DE DIFFERENTS ASSEMBLAGES SUR LA FECONDITE, CROISSANCE, SURVIE ET METAMORPHOSE DE L'HUITRE PLATE *OSTREA EDULIS* L.

Au cours des dernières décennies, la production d'huître plate *Ostrea edulis* (L.) a été affectée par l'apparition de deux maladies. La première, due à *Marteilia refringens*, a principalement touché les exploitants des zones intertidales à la fin des années 70 et la seconde, due à *Bonamia ostreae* a concerné, dès 1979, les zones sub- et intertidales. Ces deux maladies ont anéanti de manière pérenne la production d'*O. edulis*.

Parmi ces deux parasites toujours présents, *B. ostreae* représente la plus forte contrainte pour la production d'huîtres plates que ce soit en France (Grizel, 1983) ou dans la plupart des pays européens (Laing et al., 2005). Pour pallier ce problème plusieurs stratégies ont été envisagées pour relancer sa production: développement de techniques d'élevage permettant de lutter efficacement contre ce parasite, introduction d'espèces exogènes résistantes, changement radical d'espèce en élevage, développement de lignées génétiques résistantes. Toutes ces pistes ont été prospectées dont seule l'importation et l'extension de la culture d'une autre espèce d'huître, *C. gigas* a été rapidement couronnée de succès, y compris dans les zones réfractaires à son élevage à l'origine (Bretagne).

De ce fait, les autres pistes ont été progressivement abandonnées. Pourtant si les essais d'amélioration zootechniques où l'introduction d'huîtres plates exogènes n'ont pas permis de lever ces verrous biologiques (Le Borgne et Le Pennec, 1983 ;

Grizel, 1983 ; Bougrier et al., 1986, Pascual et al., 1991 ; Le Bec et al., 1991), les derniers essais de sélection génétique (4e génération de sélection) et les tests sur le terrain étaient particulièrement prometteurs (80 % de survie *vs* 36 % chez les témoins : Bédier 2004).

Ces travaux se sont heurtés néanmoins à une sérieuse difficulté, la maîtrise de la production de naissain en écloserie, technique incontournable dans ce process d'amélioration génétique. En effet, la plupart des responsables d'écloserie, qu'elle soit expérimentale ou industrielle, ont relaté des difficultés propres à cette espèce : brusque mortalité en fin de cycle larvaire ou post métamorphose (Helm et al., 2004). Parmi les différents paramètres susceptibles d'expliciter ceux-ci nous nous sommes essentiellement intéressés à la nutrition dans le présent travail, la qualité sanitaire des produits et des intrants étant étudiés en parallèle par l'équipe d'Argenton dans le cadre du projet européen SETTLE.

L'importance de ce paramètre nutritionnel sur la fécondité, la survie larvaire et le succès à la métamorphose a été abordée dans le cadre de cette première publication.

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Influence of diet assemblage on *Ostrea edulis* broodstock conditioning and subsequent larval development

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Abstract

In contrast with the Japanese oyster *Crassostrea gigas* and the Manila clam *Ruditapes philippinarum*, *Ostrea edulis* seed production in the hatchery has been reported to be erratic, with sudden and unexplained larval and post-metamorphosis mortalities. Fecundity and initial larval quality have been related to broodstock conditioning, but effects on larval development and metamorphosis remain poorly understood. In addition, molluscan larval mortalities have been often associated with bacterial contamination and flow-through techniques may help to overcome this problem. Both aspects have been considered in the present work. *O. edulis* broodstock were conditioned at 19 °C and fed three different microalgal diets. Two were single-species diets : *Rhodomonas salina* (R*s*) or *Thalassiosira weissflogii* (T*w*) and the third was a combination of both species (R*s*T*w*: 50/50 in equivalent cell volume). Mean fecundity, expressed as mean number of larvae released by oysters fed different diets, was 0.16, 0.28 and 0.39 million, respectively; whereas, mean larval size at release differed significantly from 174 to 181 µm. Moreover, when broodstock were fed combined assemblage (R*s*T*w*), larval release occurred more consistently. Larvae were subsequently fed two different diets over an 11-day period : *Chaetoceros gracilis* solely (C*g*) or a bi-specific assemblage (T: *Isochrysis affinis galbana* plus C*g*). Larval growth ranged from 5.5 to 7.4 µm d^{-1} for larvae fed C*g* and was generally higher (8.1 µm d^{-1}) in larvae fed the mixed diet TC*g*. On day 11, larval survival and competence ranged from 50 to 75% and 40 to 70% respectively, these results being closely related to broodstock nutrition. On day 18 the larval settlement ranged from 1 to 60%. When analyzing overall performance, from fecundity to settlement, best results were obtained with broodstock, fed the bi-specific diet (R*s*T*w*), which released numerous larvae over a short period with satisfactory larval development and high metamorphosis, and these larvae also fed bi-specific diet, TC*g*.

Introduction

From the 70s to the 80s two successive diseases affected *Ostrea edulis* production in Brittany (main area in France for its culture) and the population dropped from 20,000 t to 1000–1500 t y^{-1} nowadays (Buestel et al., 2009). Despite several attempts to control marteiliosis and bonamiosis in natural surroundings (Grizel, 1985) or eradicate its effects through modified husbandry (Le Bec et al., 1991; Robert et al., 1991), introduction of exotic flat oysters (e.g. *Ostrea puelchana*: Pascual et al., 1991), and genetic improvement (Naciri-Graven et al., 1999), the flat oyster population has never recovered.

This situation was quite similar for most countries in Europe (Laing et al., 2005) and, in this context, except in some limited free disease areas (e.g. Scotland, North Ireland, Norway, Denmark) flat oyster farming consists in improving oyster growth before the fateful limit of 3 years old or equivalent size and, accordingly, *O. edulis* production in Europe is constrained.

However, progress has been made in breeding for diseases resistance including new genetic tools (Lallias et al., 2009; Morga et al., 2011). Currently, a selective breeding program is, accordingly a possibility to enhance flat oyster farming. Such targeted genetic orientation, however, will not be feasible until the difficulty inherent to a lack of fully reliable methods in hatchery for this species is overcome. Indeed in the hatchery, unexplained mortalities have often been reported during larval rearing on day 8 and post-settlement (anonymous, 2004; Bédier, 2004; Laing et al., 2005). Hatchery methods are now relatively well known for many mollusks (e.g. *Crassostrea*

gigas: Utting and Spencer, 1991, *Ruditapes philippinarum*: Helm and Pellizzato, 1990, *Mercenaria mercenaria*: Castagna and Kraeuter, 1981). Despite indisputable know-how, mainly due to pioneer works (Walne, 1974), the state of the art in hatchery rearing of *O. edulis* remains clearly insufficient to support reliable seed production, probably because of a lack of updated, detailed knowledge of the biology of this species. Compared to oviparous and dioecious species such as *C. gigas* and *R. decussatus/ philippinarum*, the flat oyster is larviparous. Fecundity and initial larval quality have been related clearly to broodstock conditioning, mainly food delivery (Helm et al., 1991; Millican and Helm;, 1994; Utting and Millican, 1997) but further effects on larval development and metamorphosis are poorlyknown. Maternal effects on metamorphosis have been reported with *Ostrea chilensis* which incubates larvae for a very long period (Wilson et al., 1996); whereas in *O. edulis*, such parental effects have been shown to affect only larval growth and survival (Berntsson et al., 1997). The present work will contribute to this scope by focusing on the effects of food on *O. edulis* broodstock conditioning and subsequent larval development, including metamorphosis.

Moreover molluscan larval mortalities have often been associated with bacterial contamination, specifically to vibrios (Elston, 1984; Estes et al., 2004; Thompson et al., 2003). Widely described for Pectinidae (Lambert et al., 1999; Riquelme et al., 1996), and cupped oysters *Crassostrea spp.* (Elston and Leibovitz, 1980; Elston et al., 2008; Jeffries, 1982; Sugumar et al., 1998), vibriosis has recently been shown to affect *O. edulis* as well (Prado et al., 2005) confirming previous results on the same species (Jeffries, 1982). It is well known that scallops are very sensitive to vibrio infection (Nicolas et al., 1996) which led to the use of antibiotics, as a preventive

measure, to limit larval mortalities (Robert et al., 1995). Such practices are not sustainable, and Norwegian researchers have partly overcome this problem by developing 5000-L flow-through larval rearing technique (Magnesen et al., 2006). To study the ecophysiological requirements of mollusks with low fertility (1–2 million oocytes in *O. edulis*) vs. 10–20 in *P. maximus* in hatchery conditions (Le Pennec et al., 1998) a 5-L container was designed, and a new, flow-through larval-rearing configuration was developed for *O. edulis*.

Material and methods

Microalgae and diet composition

Four different microalgal species were used in the present study: two large species for broodstock conditioning, *Rhodomonas salina* (R_s: volumetric size ≈ 200 μm^3, dry weight 60 pg $cell^{-1}$, strain CCAP 978/24) and *Thalassiosira weissflogii* (T_w: 950 μm^3, 250 pg $cell^{-1}$, CCAP 1077/5) and two small species for larval rearing, *Isochrysis affinis galbana* (T: 40 μm^3, 12 pg $cell^{-1}$, CCAP 927/14 also named T-Iso) and *Chaetoceros gracilis* (C_g: 80 μm^3, 25 pg $cell^{-1}$, UTEX LB2658).

O. edulis broodstock conditioning was assessed using three microalgal diets in duplicate : two single-species, *R. salina* with a daily ration of 10^9cells $oyster^{-1}$or *T. weissflogii* ($0.25 \times 10^9 day^{-1} oyster^{-1}$) and the bi-specific combination of *R. salina* plus *T. weissflogii* (50/50 equivalent cell volume). Because feeding in the inhalant chamber occurs in *O. chilensis* during motherhood (Chaparro et al., 2001, 2006), 10% of T-Iso

supplemented all diets during the second month of conditioning.

O. edulis larval development was achieved using mono (*C. gracilis*) or bi-specific diets (T-Iso plus *C. gracilis*) delivered to maintain continuously 25 cells (±5) per each larva, as recommended for *C. gigas* larvae (Rico-Villa et al., 2009); whereas unfed larvae were used as a negative control. Additionally in a single experiment, a batch of larvae was also fed T-Iso to elucidate its role in larval development excluding metamorphosis.

Broodstock conditioning

Three hundred flat oysters from the same location were dredged from the Bay of Brest (Brittany, France) in March 2007. After scrubbing shells free of fouling organisms and debris before stocking in experimental structures at the Ifremer experimental station of Argenton (Brittany, France), oysters were pre-conditioned to experimental temperature (19 °C) by a daily gradual increase of 1 °C during 10 days. Seawater temperature was maintained at 19 °C by means of regulated thermal flood- gates. Thereafter 50 2 year-old oysters were distributed homogeneously (70 g ± 20 mean whole weight, 1.7 g ± 0.6 mean meat dry weight, 70 mm ± 8 mean length) in each duplicate tank (2 m length × 0.5 m width × 0.2 m depth) per experimental condition. Continuous flow of 1-µm filtered-seawater was delivered from the top at a constant flow rate of 40 L h^{-1} (40% renewal h^{-1}). Daily rations of 6% dry weight microalgae (mg) per oyster meat (g) were provided to broodstock by peristaltic pumps that mixed the algae with filtered seawater at the inlet of each

tank. All tank outlets were secured with a 100-µm mesh sieve to prevent larvae from escaping. *O. edulis* broodstock conditioning continued over a 4 month-period from February to May 2007.

Seawater at inlet and outlet of each experimental tank was sampled twice a day (morning and afternoon) and phytoplankton counts were made using an electronic particles counter (Multisizer 3 equipped with a 100-µm aperture tube). Grazing was expressed in number of cells removed from suspension oyster^{-1}d^{-1} or in µm^3 oyster $^{-1}$d^{-1}. For each experimental condition, fecundity was assessed as the number of released larvae over the considered period.

Larval rearing

When detected, expelled larvae were counted and measured for length. When a large release of larvae was recorded (≥1 million), larval rearing was set up in a dedicated flow-through cylindrical system inspired by the Cawthron design (King et al., 2005). Made from 3 mm, transparent Polymethyl methacrylate, six 5-l tanks (104 cm height and 9 cm diameter) were self-supported on a 12-mm PVC table of 150 cm length (Fig. 1a). The outlet of each tank was equipped with a 32 mm PVC pipe connected within the tank to a beveled, 100-µm sieve to prevent larvae from escaping. Each microalga was delivered by pumping from a reservoir into each inlet pipe through a 4-mm translucent, flexible line (Fig. 1b). Each larval rearing tank received from the top 1-µm-filtered phytoplankton-enriched seawater through a secondary line with flow controlled by a flow-meter (one per tank: Fig. 1c). In each

larval tank, aeration was provided from the bottom and was maintained at 0.5 L min $^{-1}$using a 4-mm PVC valve (Fig. 1). Lastly the outlet of each cylinder was connected to a 6-mm tube allowing complete tank draining. For each broodstock origin (diet) and when a sufficient number of released larvae were collected, larvae were reared in those flow through units at a density of 5 larvae ml^{-1} with seawater flow maintained at 1.3 L h^{-1}(≈ 6 renewals day^{-1}). Seawater temperature/salinity was maintained at 22 °C/34 ppt according to Robert et al. (1988). Each broodstock diet was split into treatments with three larval diets, i.e., 9 different larval conditions were assessed : BR$_s$LS, BR$_s$LC$_g$, BR$_s$LTC$_g$, BT$_w$LS, BT$_w$LC$_g$, BT$_w$LTC$_g$, BR$_s$T$_w$LS, BR$_s$T$_w$LC$_g$ and BR$_s$T$_w$LTC$_g$. Moreover a high number of released larvae, originating from broodstock fed R$_s$T$_w$, allowed to set up an additional feeding condition and accordingly a batch of larvae was fed T. Iso alone. Thus, the designation BR$_s$LS means that broodstock was fed *R. salina* and larvae from them were starved; whereas, BR$_s$T$_w$LTC$_g$ means that broodstock was fed *R. salina* plus *T. weissflogii*, and larvae from them were fed T-Iso plus *C. gracilis*.

Phytoplankton cell density was assessed twice a day at inlet and outlet of each experimental rearing tank, and adjustments were made to stabilize cell count around each larva at 25 cells (±5) or 1000 µm^3 equivalent T-Iso, values reported as an effective algal-cell density for *C. gigas* larval development (Rico-Villa et al., 2009).

In each rearing tank, larvae were collected by siphoning subsamples and larval shell length was measured on days 0 (released), 3, 7 and 11 using image analysis (Image vision Builder version 6.0). Survival was estimated on day 11 on the entire larval population collected by draining, mixing thoroughly, and counting a sub-sample

under the light micro- scope. The number of larvae ready to set (competent larvae showing presence of large eyespots and active foot) was estimated prior to a selective grading on 200-µm mesh. Pediveligers > 280 µm were distributed at an initial density varying from 0.3 to 0.7 larvae ml^{-1}in 30-L tanks containing plastic disks as cultch (settlement material) (Rico-Villa et al., 2006). They were fed the bi-specific diet (TC*g*: 50/50, v/v) thereafter, post-set, were maintained in a flow through system (9 L h^{-1}; 30% h^{-1} seawater renewal) for an additional week. The number of eyed pediveligers selected was, however, limited and we only obtained sufficient numbers of post-set for duplicate treatments fed bi-specific diets from broodstock to larvae.

Analytical procedures

Growth and cell size of microalgae cultures were estimated using a Multisizer 3, and for dry weight determinations, 50 ml of culture was harvested and centrifuged (3200 g, 10 min); pellets then were rinsed with 20 ml of 0.5 M ammonium formate, re-centrifuged and placed in pre-weighed tin capsules. Dry weight and ash were measured after heating at 80 °C (overnight) and 450 °C (4 h) respectively. Gross composition analysis followed methods of Lowry et al. (1951) for proteins, Bligh and Bligh (1959) for lipids and Dubois et al. (1956) for carbohydrates. Fatty acids were analyzed following methods described by Marty et al. (1992) with 23:0 as an internal standard. Fatty acid composition was expressed as the relative contents of the total fatty acids of the entire fraction.

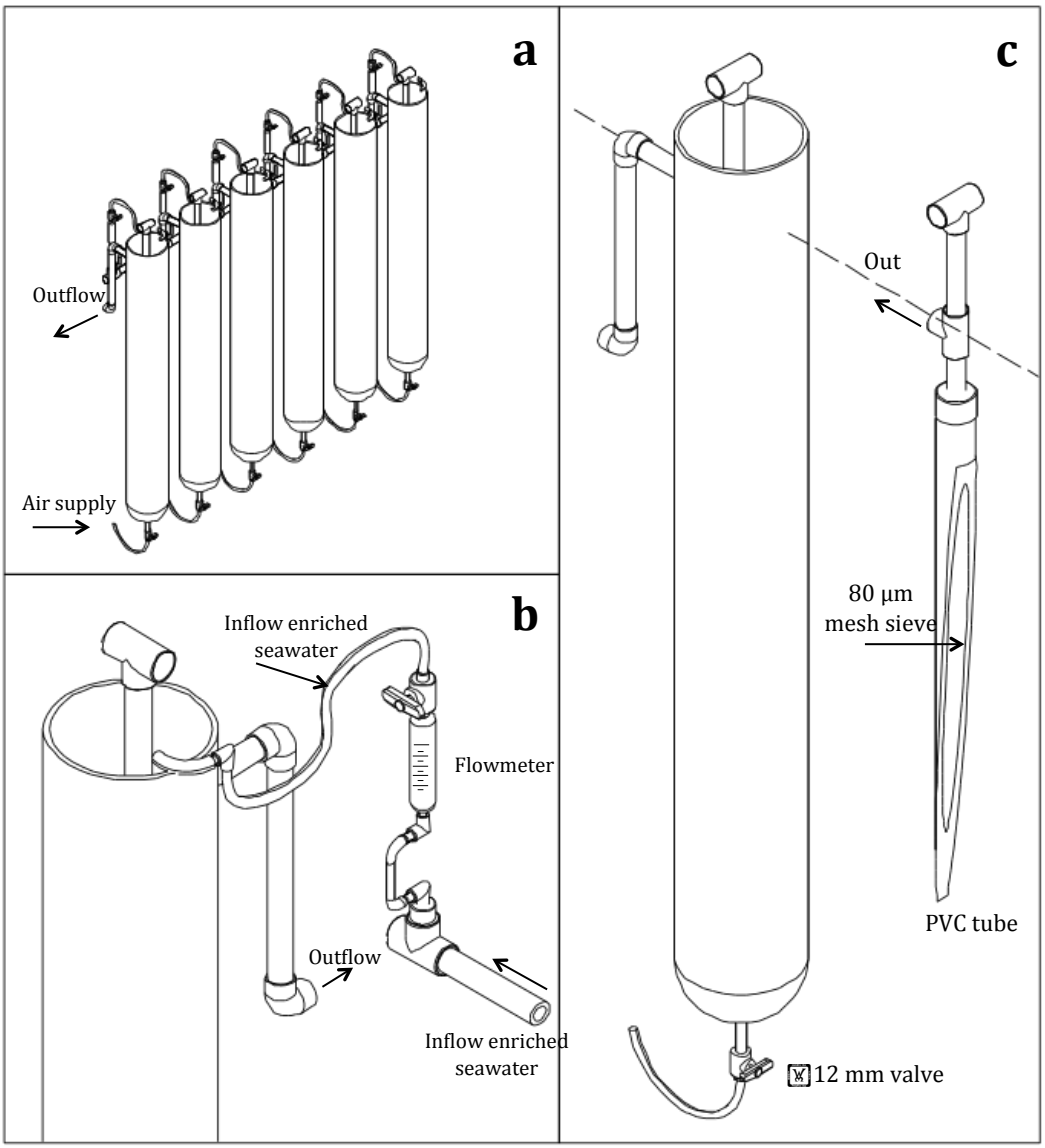

Fig. 1. (a) Overview of 5 L cylinder for *Ostrea edulis* larval rearing, (b) details of inlet and outlet of phytoplankton enriched seawater and (c) overview of mesh sieve in larval tank.

Statistical analysis

Data were analyzed using STATISTICA software (version 8.0). One-way analyses of variance (ANOVA) were used to test the effects of larval diet on larval length,

survival and competence. Two-way analysis of variance was used to test the combined effects of broodstock nutritional diets and larval diets on larval length. When needed, data transformation (arcsin[sqr(χ/100)]) to respect homogeneity of residues distribution (Sokal and Rohlf, 2001) was made. Differences of mean were assessed using a posteriori Tukey's tests.

Table 1 : Proteins, carbohydrates and total lipids, expressed in % of dry weight (±S.D., n=3), in *Ostrea edulis* released larvae originated from broodstocks fed *R. salina* (R_S), *T. weissflogii* (T_W), *R. salina* plus *T. weissflogii* (R_ST_W).
Same letter in the same line indicates no significant differences.

	R_s		T_w		R_sT_w	
Proteins	[a] 13.00	(1.73)	[a] 16.23	(1.27)	[a] 15.30	(0.53)
Carbohydrates	[b] 0.96	(0.06)	[b] 1.15	(0.10)	[b] 1.18	(0.22)
Lipids	[c] 2.88	(0.12)	[c] 4.02	(0.66)	[c] 3.80	(0.57)

Results

Broodstock conditioning

Consumption by oyster

Grazing was estimated daily. When expressed in cells number per oyster per day, grazing clearly was related to the type of food delivered during conditioning, with maximum values of 0.52×10^9 ($\pm 0.10 \times 10^9$, on day 19), 1.1×10^9 ($\pm 0.1 \times 10^9$, on day 31) and 2.2×10^9 ($\pm 0.4 \times 10^9$, on day 35) for T_w, R_sT_w, and R_s respectively. When

expressed in volume ($\mu m^3 oyster^{-1} d^{-1}$), similar trends were observed with optimal values of 120×10^9 ($\pm6.7\times10^9$, on day 19), 180×10^9 ($\pm15\times10^9$, on day 31) and 220×10^9 ($\pm 72\times10^9$, on day 35) for T_W, R_ST_W and R_S respectively. Whatever the diet, a general decrease in grazing was detected from day 35 until the end of the experiment, but this trend was difficult to quantify because of data overlap. To obtain a clearer overall view of microalgae uptake changes, data were accordingly stacked and expressed on a weekly basis (Fig. 2). With this visualization, grazing, expressed as cells number $oyster^{-1}wk^{-1}$, showed a steady decrease from week 6 until the end of the experiment for broodstock fed R_S alone or the mixture R_ST_W, with values decreasing from $1 - 1.2\times10^9$ to 0.2×10^9 (six fold decrease). In contrast, grazing seemed to be almost constant when broodstock were fed T_W (Fig. 2a); however a decline occurred when grazing was expressed as cells volume $oyster^{-1}d^{-1}$ (Fig. 2b) with values decreasing from 85×10^9 to 5×10^9 (17 fold decrease).

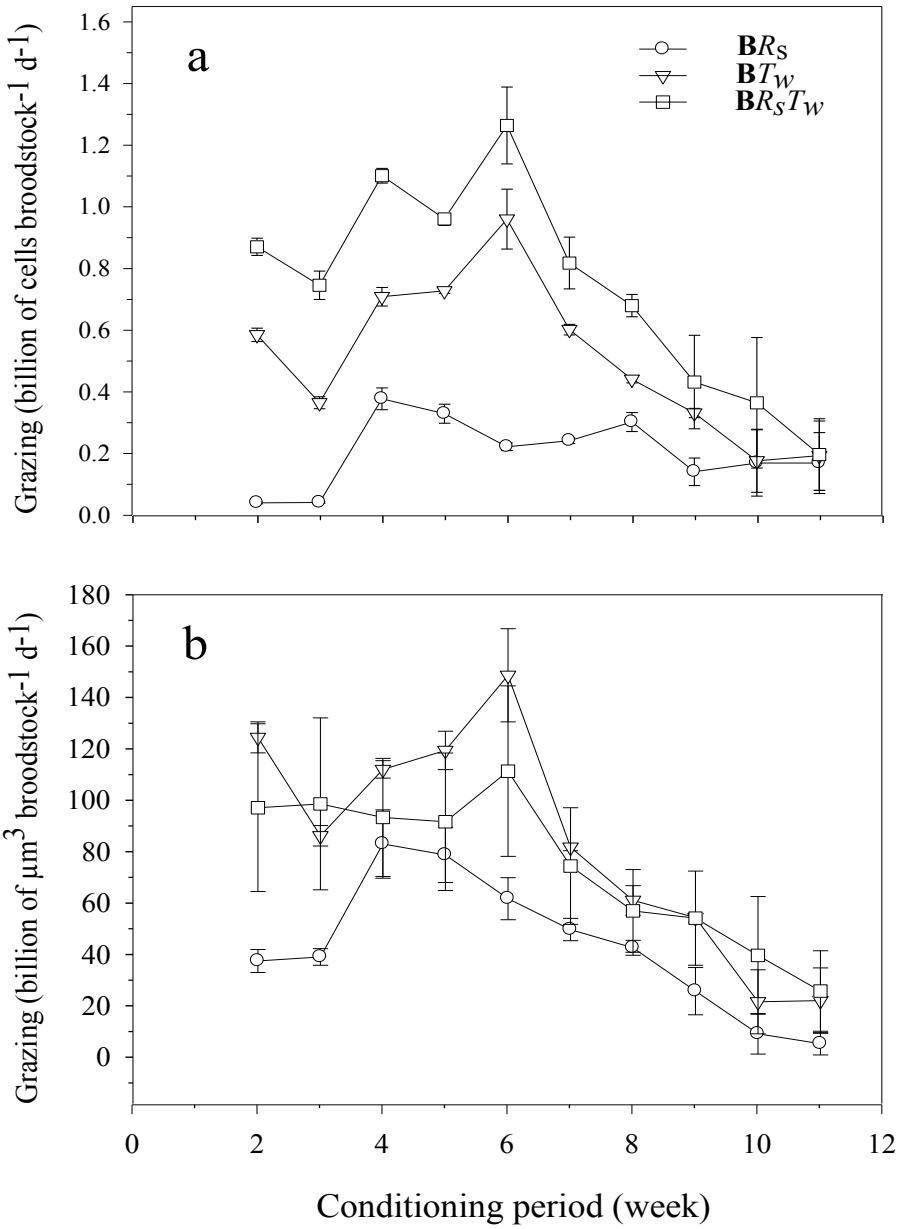

Fig. 2. Weekly consumption in *Ostrea edulis* (L.) broodstock, fed single *Rhodomonas salina* diet (BR_S), single *Thalassiosira weissflogii* diet (BTw) and bi-specific diet BR_STW, expressed as billion of cells oyster^{-1}d^{-1}(a) and billion of µm^{3}oyster^{-1}d^{-1}(b).

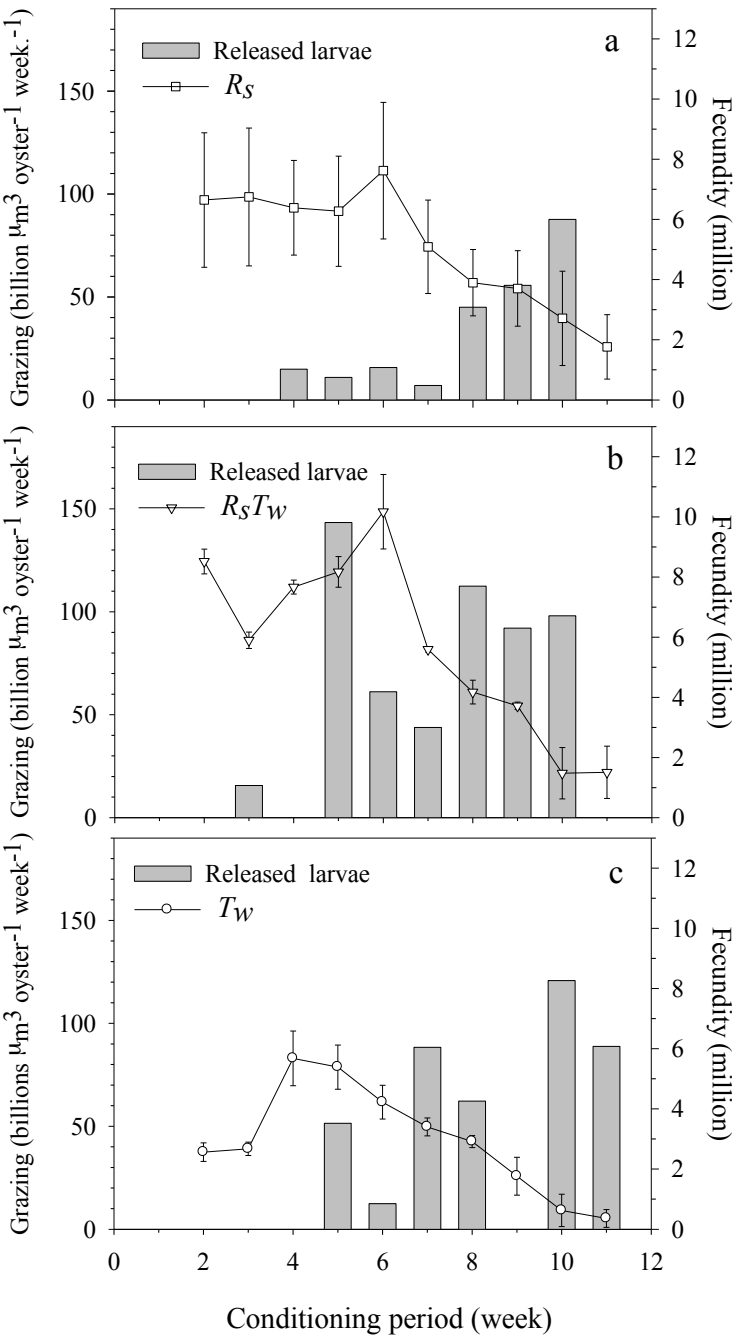

Fig. 3. Weekly evolution of grazing (billion $\mu m^3 oyster^{-1}$) and fecundity of *Ostrea edulis* (L.) broodstock fed: (a) *Rhodomonas salina* (R_S), (b) *Thalassiosira weissflogii* (T_W), (c) *R. salina* plus *T. weissflogii* ($R_S T_W$).

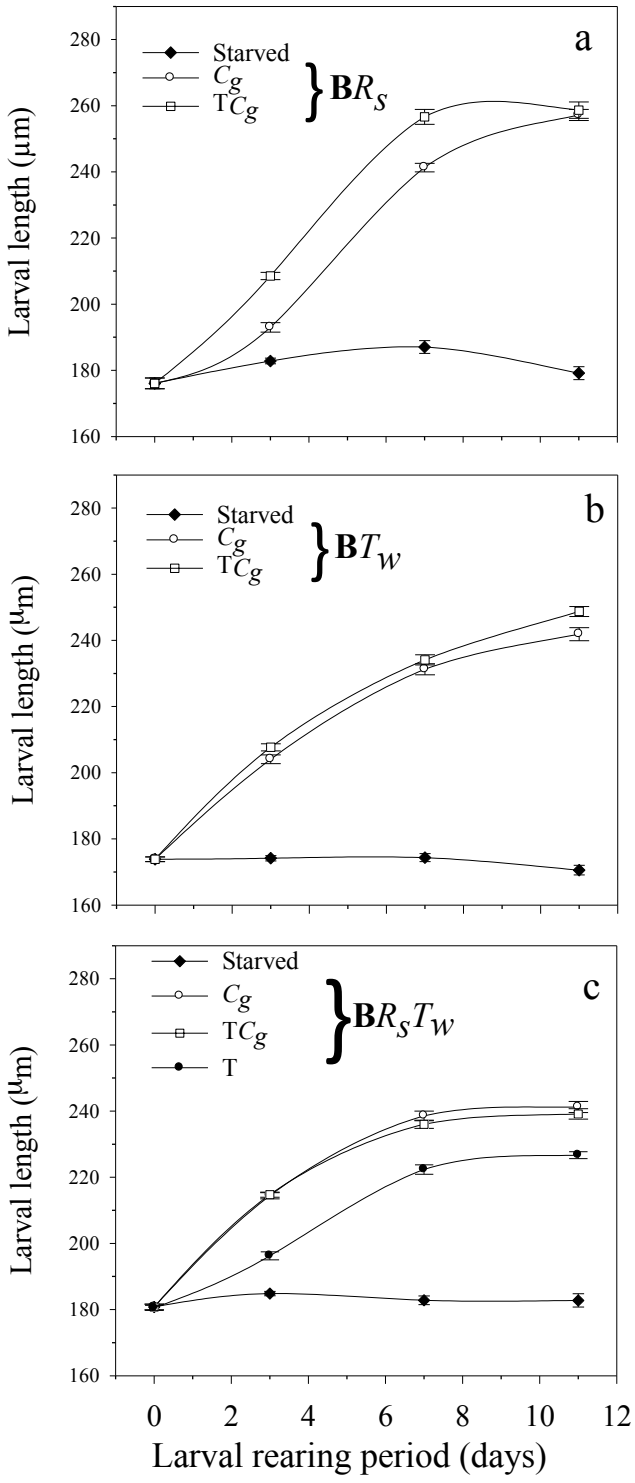

Fig. 4. Larval growth (µm) for larvae fed *Isochrysis affinis galbana* (T), *Chaetoceros gracilis* (Cg), bi-specific diet TCg and originated from *Ostrea edulis* (L.) broodstocks previously fed: (a) *Rhodomonas salina* (BR_S), (b) *Thalassiosira weissflogii* (BT_W), (c) *R. salina* plus *T. weissflogii* (BR_ST_W).

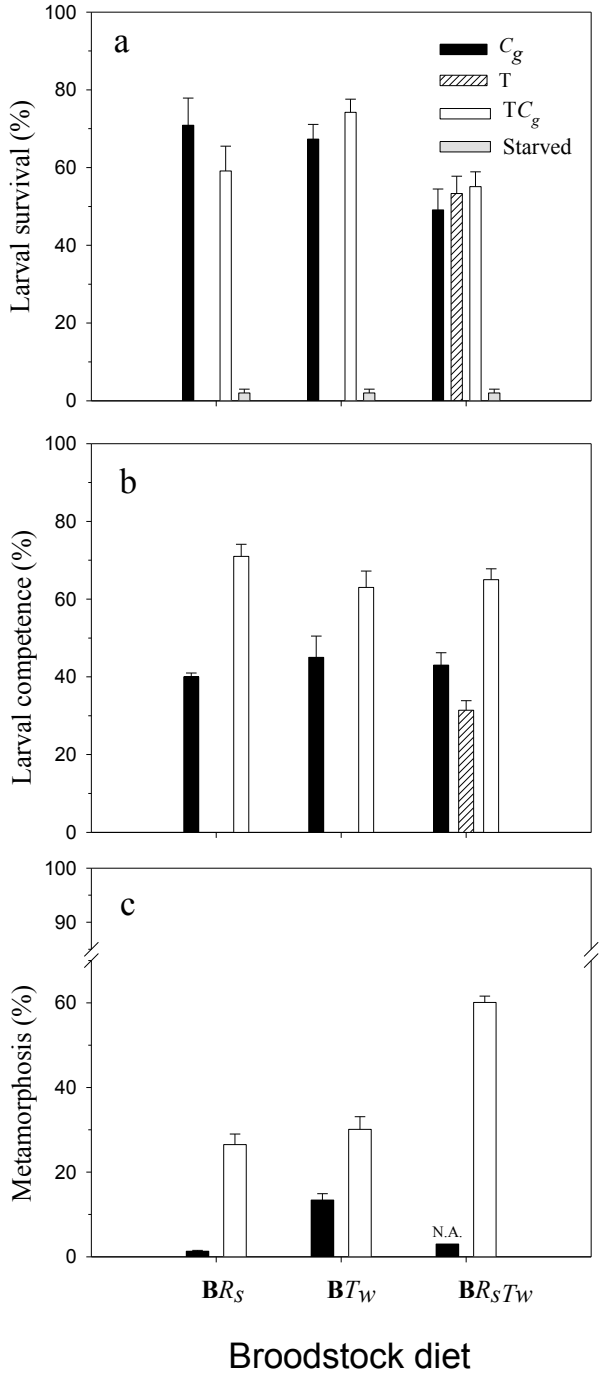

Fig. 5. Survival (a), competence (b) and metamorphosis (c) of *Ostrea edulis* (L.) larvae starved or fed *Chaetoceros gracilis* (Cg) or *Isochrysis affinis galbana* +*Chaetoceros gracilis* (TC_g), and originated from oysters fed *Rhodomonas salina* (BR_S), *Thalassiosira weissflogii* (BT_W), *R. salina*+*T. weissflogii* (BR_STW). N.A.: Data not achieved due to technical failure.

Oyster fecundity

As previously done with grazing, fecundity data were stacked and expressed on a weekly basis. For broodstock fed R_S alone, the first larval release occurred on week 4; whereas fecundity increased from 0.5–1, on week 6, to 6×10^6larvae, on week 10, simultaneously with a grazing-rate decrease (Fig. 3a). Similar relationships between larval release and grazing decrease were also found with the other feeding conditions : T_W (Fig. 3b) alone or the mixture R_ST_W (Fig. 3c). The greatest number of larvae released occurred with flat oysters fed R_ST_W with a mean number of 0.39 million ± 0.04 per oyster with precocious (week 3) and steadier expulsion. The lowest number of released larvae was recorded for broodstock fed R_S alone with a mean number of 0.16 million ± 0.007 per oyster; whereas, brooders fed T_W exhibited intermediate performances (0.28 million ± 0.12) with the last expulsion occurring on week 11 (Fig. 3b).

Larval development

Size at release and biochemical composition

Initial larval size was related to broodstock nutrition, with mean lengths of 174 µm (± 1), 176 µm (± 2) and 181 µm (± 1) for larvae originating from broodstock oysters fed R_ST_W, T_W and R_S respectively, with these differences being significant (2, 5.5, p = 0.004). Tukey's tests, how- ever, revealed significant differences only between T_W and R_ST_W. In contrast, no differences in gross biochemical composition

of newly-released larvae were recorded, with proteins varying from 13.0 to 16.2% (2, 5.1, p = 0.051), carbohydrates from − 1 to 1.2% (2, 2.1, p = 0.2) and lipids from 2.9 to 4.0% (2, 4.2, p = 0.07) (Table 1).

The algal species used differed in several aspects of fatty acid com position. *R. salina* exhibited relative high content of 20:5(n-3) with 10% and was also rich in 22:6(n-3) with 8%; whereas, 20:4(n-6) represented 3% of total fatty acids (Table 2). In contrast, *T. weissflogii* showed lower amount of 22:6(n-3) and 20:4(n-6) with 4% and 0.2% respectively, but this diatom was particularly rich in 20:5(n-3) reaching 20% of total fatty acids. On the other hand, *R. salina* was characterized by a high content of 18:2(n-6), 18:3(n-3), and 18:4(n-3) representing 18%, 12% and 14% respectively; whereas, *T. weissflogii* was poor in these three fatty acids with 1–2%. An opposite trend occurred with 16:3(n-4) and 16:1(n-4), which both were 18% in *T. weissflogii*; whereas, these fatty acids did not exceed 1% in *R. salina* (Table 2).

Despite these differences in the fatty acid composition of the diet, newly-released *O. edulis* larvae were very similar in PUFA contents with no significant differences between larvae originated from broodstock fed different diet ($p<0.05$) : Table 2.

Larval growth

Regardless of broodstock diet, larvae were released in sufficient quantity in week 8 and were reared under two different feeding conditions; unfed larvae were used as negative controls.

Table 2 : Total fatty acids composition in *R. salina*, *T. weissflogii* microalgae and *O. edulis* (L.) released larvae originated from broodstock fed *R. salina* (R_S), *T. weissflogii*+*R. salina* (T_WR_S) and *T. weissflogii* (T_W), expressed in % of total fatty acids (±S.D.; n=3).

	Microalgae				Larvae					
	R. salina		*T. weissflogii*		*Rs*		*TwRs*		*Tw*	
14:0	1.79	(0.57)	24.97	(2.81)	1.29	(0.01)	1.56	(0.12)	1.86	(0.26)
16:0	0.86	(0.31)	0.18	(0.01)	12.28	(1.14)	13.65	(0.32)	15.73	(0.97)
18:0	0.54	(0.23)	0.01	(0.00)	5.39	(0.90)	4.48	(0.18)	4.27	(0.65)
16:1(n-9)	1.12	(0.22)	0.01	(0.00)	0.29	(0.01)	0.23	(0.06)	0.19	(0.07)
16:1(n-7)	0.74	(0.36)	20.14	(3.25)	1.05	(0.12)	3.15	(0.96)	5.03	(1.32)
18:1(n-9)	1.26	(0.56)	0.01	(0.00)	1.85	(0.17)	1.77	(0.22)	2.03	(0.19)
18:1(n-7)	2.04	(0.32)	1.18	(0.08)	1.62	(0.30)	3.38	(0.53)	4.08	(0.19)
16:2(n-7)	0.01	(0.00)	1.66	(0.05)	0.18	(0.20)	0.32	(0.28)	0.38	(0.33)
16:2(n-4)	0.09	(0.09)	5.85	(0.90)	0.01	(0.01)	0.14	(0.05)	0.21	(0.09)
16:3(n-4)	0.01	(0(00)	17.95	(1.44)	0.01	(0.00)	0.05	(0.01)	0.07	(0.06)
18:2(n-6)	18.04	(18.04)	0.57	(0.01)	3.07	(0.16)	2.64	(0.57)	1.89	(0.67)
18:3(n-6)	3.76	(3.76)	0.27	(0.01)	0.2	(0.05)	0.21	(0.06)	0.11	(0.05)
18:3(n-3)	11.51	(11.51)	0.59	(0.09)	1.59	(0.12)	1.39	(0.34)	0.89	(0.41)
18:4(n-3)	13.66	(13.66)	1.54	(0.01)	1.35	(0.30)	1.36	(0.21)	1.02	(0.28)
20:4(n-6)	2.41	(2.41)	0.22	(0.01)	5.03	(0.03)	3.78	(0.13)	3.25	(0.25)
20:5(n-3) EPA	9.51	(9.51)	20.43	(4.56)	9.25	(0.95)	10.97	(0.76	11.72	(0.78)
22:5(n-6)	0.2	(0.20)	0.01	(0.00)	0.84	(0.19)	0.73	(0.15)	0.64	(0.07)
22:6(n-3) DHA	8.18	(2.64)	3.6	(0.55)	17.72	(2.04)	16.85	(3.16)	15.01	(2.11)
Sum MUFA	8.35	(1.29)	22.47	(5.89)	11.3	(0.57)	15.04	(1.53)	16.96	(1.10)
Sum (n-9)	2.48	(0.67)	0.09	(0.01)	3.05	(0.31)	2.66	(0.37)	2.57	(0.14)
Sum (n-7)	3.13	(0.29)	21.58	(2.22)	5.98	(0.36)	10.33	(1.80)	12.84	(0.96)
Sum PUFA	68.74	(6.28)	52.86	(6.33)	50.33	(3.23)	48.38	(1.91)	45.28	(0.43)
Sum (n-4)	0.09	(0.40)	23.8	(1.57)	0.11	(0.09)	0.49	(0.14)	0.65	(0.22)
Sum (n-6)	24.55	(5.49)	1.05	(0.26)	10.66	(0.18)	8.35	(0.43	6.76	(0.67)
Sum (n-3)	44.02	(4.07)	26.35	(2.62)	33.23	(3.21)	33.01	(2.03)	30.84	(0.62)
(n-3)/(n-6)	1.79	(0.57)	24.97	(2.81)	3.12	(0.25)	3.98	(0.34)	4.6	(0.53)
DHA/EPA	0.86	(0.31)	0.18	(0.01)	1.92	(0.03)	1.56	(0.41)	1.29	(0.26)

The effects of broodstock diets (R_s, T_w, R_sT_w) on larvae combined with the effects of larval diet (C_g, TC_g) on larval growth (expressed as total daily growth rate from D1 to D11) highlighted a predominant influence of initial breeders nutrition (2, 9.3, $p = 0.004$) but no influence of larva nutrition (1, 0.15, $p = 0.70$) as well as interaction effects (broodstock diet ∗ larval diet: (2, 0.2, $p = 0.80$)). Regardless of the experimental conditions unfed larvae did not grow significantly; at the end of the experiment larval length only reached 175–180 µm (Fig. 4a, b, c). In contrast, larvae originating from broodstock fed R_S exhibited the highest growth of 7 µm d^{-1}

leading to a final length of 258 μm (Fig. 4a). Larvae fed *C. gracilis* alone or the bi-specific diet TC*g* showed similar growth ($p = 0.012$) (Fig. 4b, c) with the exception of larvae originating from broodstock fed R*s* during the first week ($p < 0.01$) (Fig. 4a). Under these latter conditions, the mixed larval diet improved larval growth significantly, but this difference disappeared thereafter (Fig. 4a). When the larvae originating from broodstock fed R*s*T*w*, were provided solely T-Iso, larval growth was depressed with 4.8 μm d^{-1}, leading to a final size of 226 μm (Fig. 4c).

Larval survival

Survival was assessed at the end of larval rearing, on day 11. Low survival was observed for unfed larvae (b1%), regardless of broodstock diet. Overall, larvae originating from broodstock fed R*s* or T*w* exhibited higher survival from 58 to 74% for larvae fed *C. gracilis* or TC*g* (Fig. 5a). The lowest survival, 50 to 55% was recorded for larvae released by broodstock fed R*s*T*w*, regardless of larval feeding (Fig. 5a). The combined effects of broodstock and larval diets (which excluded T-Iso) on larval survival revealed a predominant influence of nutrition during conditioning (2, 6.91, $p=0.002$) these differences being significant only between BR*s*LTC*g* and BR*s*LC*g* ($p = 0.003$) and BR*s*LTC*g* and BT*w*LC*g* ($p = 0.002$).

Larval competence and metamorphosis

When fed the mixed larval diet TC*g*, compared to single-species diet C*g*, larvae exhibited higher competence (60–70%) (Fig. 5b). The combined effects of diets on

broodstock and larvae upon larval competence (which excluded T-Iso) revealed a predominant influence of broodstock nutrition (2, 757.6, $p < 0.0001$), and a significant influence of larval nutrition (1, 126.6, $p < 0.0001$). In contrast, no interaction effects (broodstock diet × larval diet) were noted (2, 0.7, $p = 0.49$). When fed T-Iso, however, larval competence represented 75% of that achieved with *C. gracilis* in larvae released by parents fed R_sT_w (31% vs. 41%).

Whatever the broodstock diet, higher settlement was recorded when larvae were fed the mixed larval diet TC_g (25–60%: Fig. 5c), but metamorphosis performance was poor (1.3%) when broodstock were fed *R. salina* and larvae *C. gracilis* (Fig. 5c). Settlement ranged accordingly from 1.3 to 60% but the experimental design was incomplete to allow overall statistical data treatment. Thus, the effect of broodstock diet on larval settlement is difficult to confirm, although feeding broodstock *R. salina* led to similar larval metamorphosis (≈26%) as in broodstock fed C. gracilis (≈ 30%) which is only half the value recorded when broodstock received the mixed diet (≈ 60%). Similarly, when considering parents fed single-species microalgal diets, overall mean post-larval yield (from initial larval release to the end of settlement) was 7.5% for larvae fed single diets vs. 28% for those fed bi-specific diet.

Discussion

It is difficult to differentiate the role of adult reserves from that of additional food. For the cupped oyster *C. gigas*, which has external fertilization and larval development, additional food for broodstock appears to be of less importance than

Table 3 : Review of larval development performances and survival in closed-flow system related to temperature, larval density and nutritional conditions, P: *Pavlova lutheri*, I: *Isochrysis galbana*, Ph: *Phaeodactylum tricornutum*, Sk: *Skeletonema costatum* (*marinoi*), Ts: *Tetraselmis suecica*, Cc: *Chaetoceros calcitrans*, Rh: *Rhodomonas* sp., Ti: *Isochrysis affinis galbana* (clone T-Iso), Cg: *Chaetoceros gracilis*, Ps: *Platymonas* (*Tetraselmis*) *suecica*, Pp: *Pseudoisochrysis paradoxa*, Ir: *Imantonia rotunda*, nd: not determined.

T °C	Density (larva ml^{-1})	Nutritional condition	Growth rate (% or µm d^{-1})	Survival (%)	Competence rate (%)	Metamorphosis rate (%)	Final Size (µm)	Rearing period (days)	Source
22-24	0.625	I, Ts, Cc, I+Ts, I+Cc, Ts+Cc, I+Ts+Cc	nd	83, 90, 93, 91, 98, 98	9, 26, 36, 42, 31, 59, 64	nd	278, 286, 286, 296, 293, 296, 311	8	Helm, 1969
nd	2.67	I+Ts	nd	65.5	nd	nd	297	12	Holland and Spencer, 1973
nd	1.75	P, I, Ph, Sk	51, 52, 52.2%	93, 93, 96, 79	nd	nd	295, 295, 295, 200	11	Ferreiro et al., 1990
20	1	I, P, I+P, I +Cc, I+P+Cc	8.5, 2.8, 6.4, 9.7, 5.4 µm d^{-1}	Nd	nd	25, 0, 30, 0, 0	303, 279, 318, 234, 266	12	Jonsson et al., 1990
16	nd	I+Ts	nd	nd	nd	nd	304	17	Labarta et al., 1999
22-24	0.175	I, Ts, I+Ts	47, 50, 57%	88, 87, 92	nd	18, 22, 40	261, 267, 279	11	Helm, 1977
26	nd	I, Ph, P, Ps, Pp, Cc, Ir	40, 46, 28, 28, 32, 40, 35%	53, 58, 46, 75, 36, 57, 37	nd	nd	260, 270, 255, 255, 245, 260, 250	8	Wilson, 1978
nd	1.75	Cc, Ts, P, Rh	54, 62, 45, 44%	97, 99, 5, 90	58, 66, 10, 1	nd	287, 296, 265, 264	12	Ferreiro et al., 1990
22	4.5	I	55%	30	24-79	nd	285	10	Walne, 1966
20	2.75	Sk+Ti+P	95%	15	nd	nd	299	14	Glize, 1994
21	4.5	Ti+Cg	11 µm d^{-1}	84	nd	nd	302	13	Coatanea et al., 1994
21	2	Ts, I+Ts, Ts +Cc	4.45, 5.32, 1.67 µm d^{-1}	75, 52, 72	nd	nd	219, 221, 204	12	Bertsson et al., 1997

the initial content of glycogen reserves before conditioning (Cannuel and Beninger, 2007). In some cases, food availability favors growth and maintenance rather than reproduction (Donaldson, 1991); whereas, Muranaka and Lannan (1984) found that the fecundity of *C. gigas* broodstock was 60% greater when fed an algal food supplement rather than starved. Such apparent contrasting results can be explained by trials reported by Chávez-Villalba et al. (2003) who showed that a 6% (dry weight of algae per dry meat oyster weight) addition per day of equal quantities of *Chaetoceros calcitrans* and T-Iso had a positive effect on *C. gigas* fertility, but these effects were closely related to the season and the amount of initial reserves in oysters at the beginning of conditioning. Thus, in spring, the mean fertility (number of eggs per female) of fed broodstock originating from six different French oyster areas was 5.23 million (± 2.91) vs. 0.77 million (± 1.21) for unfed groups, while in

summer fertility reached 30.74 million (±12.05) vs. 8.11 million (±7.20), respectively, for fed and unfed oysters. Gamete viability, however, did not differ significantly between conditions, nor did larval growth when larvae were fed a mixed diet (Chávez-Villalba et al., 2003).

On the other hand, Devauchelle and Mingant (1991) reported that the fecundity of the hermaphroditic scallop *P. maximus*, at similar gametogenic stages, decreased with feeding levels. Gonad activity suffered first at lower feeding levels, but only in the female part of the gonad. No eggs were obtained after 28 and 45 days of conditioning when scallops were kept unfed; whereas, fecundity increased by 8 to 25% with a food ration of 3×10^9 cells animal^{-1}day^{-1} and by 30–60% with 14×10^9cells animal^{-1}day^{-1}. Similar results were reported for *Pecten fumatus*, showing that gonad condition and egg production improved as feeding rates increased from 12.5 to 100 satiation (equivalent to 0.75 to 6×10^9 cells scallop^{-1}day^{-1} respectively) at all test temperatures in the 12–21 °C range (Heasman et al., 1996).

The genus *Ostrea* is larviparous; accordingly broodstock must sustain egg development, embryogenesis and larval growth in the gill cavity for at least 1 week (Walne, 1974). Starving females of the Chilean oyster *Ostrea chilensis* induced low spat growth and survival (Wilson et al., 1996); whereas, *O. edulis* broodstock receiving *T. suecica* enriched seawater produced earlier broods with subsequent larval growth and settlement improvement (Helm et al., 1973). Moreover, an algal diet representing 3 to 6% of the initial meat weight of oysters (dry weight to dry weight) per day increased larval production in *O. edulis* as did the type of algae delivered, the poorest result being obtained with the single diet *Dunaliella tertiolecta* (Millican and

Helm, 1994).

In the present work, when fed three different diets – *R. salina*, *T. weissflogii* and mixed *R. salina* plus *T. weissflogii* at 6% dry weight – *O. edulis* broodstock did not show any specific diet preference during the first month of experiment, grazing rate being quite similar for all diets delivered. Beyond day 35, microalgae uptake decreased sharply and similarly for all diets until day 50. Such grazing depression could be explained by larval incubation in brachial chamber as reported in *O. chilensis* by Chaparro et al. (2001) who linked larval in activity and water circulation interference in the pallial cavity. Furthermore, *O. edulis* fecundity development (expressed as number and intensity of released larvae) presented an antagonistic relationship to food consumption, with obvious "pre-spawning" activity and large fecundity differences depending upon broodstock diet: 0.16 million larvae per oyster were released for BR_S, 0.28 million for BT_W and 0.39 million for BR_ST_W. When compared to similar age (2-yr-old oyster), 0.5 million larvae released per oyster was reported by Walne (1974), which is in agreement with values found in the present work for broodstock fed the bi-specific diet (≈0.4 million) as well as oysters fed single diets (≈0.2 million).

Larval survival ranged from 50 to 75% with a significant effect of broodstock feeding leading to differences between single and bi-specific diets but no clear trend emerged as already reported by Helm (1969, 1977) or Berntsson et al. (1997) (Table 3). In static culture, higher survival has been reported (95%: Ferreiro et al., 1990; Helm, 1969), but no information is given on the use of antibiotics. Antibiotics utilization is, however, suspected as this was previously recommended (Walne,

1966). In a more recent work, the use of erythromycin was shown to be essential to the survival of larvae in French experimental and commercial hatcheries employing stagnant-water methods (Bédier, 2004). Flow through larval rearing techniques were developed accordingly, and this practice was highly successful with *C. gigas*, leading to survival >90% in 150 L (Rico-Villa et al., 2008) or in similar 5-L containers (Petton et al., 2009a). For *O. edulis*, the lowest larval survival reported here probably is attributable to poor optimization of the techniques, particularly the direction of food-enriched seawater flow that has shown to be more efficient from bottom to top for *C. gigas* larvae (Petton et al., 2009b).

Generally, a multispecific microalgal diet is beneficial to mollusk larval development (e.g. Marshall et al., 2010; Rico-Villa et al., 2006; Robert and Gérard, 1999). A successful mixed-algal diet must be composed of two or three species with high nutritive values, often combining diatoms and flagellates (Robert and Gérard, 1999). In *O. edulis*, mixed-algal species diets support generally better growth and competence than single-species diets (Helm et al., 2004, Table 3), and the results reported here showed that this ascertainment is true for T-Iso (Fig. 4c). In contrast, the difference in larval growth between the bi-specific diet TC*g* and *C. gracilis* is slim, regardless of broodstock diet. Indeed the additive effect of T-Iso for flat-oyster larval growth is slight, but high for metamorphosis as already shown for the cupped oyster *C. gigas* (Rico-Villa et al., 2006). This generalization disagrees, however with the work of Jonsson et al. (1999) on *O. edulis* larvae for which a negative effect on growth of *C. calcitrans* was reported. High fluctuations in diatom quality may be suspected because batches of *C. calcitrans* varied at harvest from 3×10^6 cells ml^{-1} to 7×10^6 cells ml^{-1} (Jonsson et al., 1999).

In the present study, larval competence ranged from 30 to 70% with a clear effect of larval nutrition and a positive influence of the bi-specific diet vs. single-species diet, as already pointed out with *C. gigas* (Marshall et al., 2010; Rico-Villa et al., 2006). Such differences also occurred for settlement; single-species larval diets led to lower metamorphosis performances (8%) compared to larval bispecific diets (28%), as well as for broodstock diet, for which bi-specific diets led to 60% settlement (vs. 30%).

Initial larval proximate condition (proteins, lipids, carbohydrates), however, did not reveal any differences. On the other hand, it has been shown that neutral lipids in the form of triacylglycerol (TAG), is an important source of energy for bivalve larvae, especially in periods of low food availability or during starvation (Ben Kheder et al., 2010a, b; Gallager et al., 1986; Whyte et al., 1992). More precisely, essential fatty acids (EFAs), particularly omega-3 fatty acids eicosapentaenoic acid (20:5n−3, EPA) and docosahexaenoic acid (22:6n-3, DHA), are important to bivalve-larval growth and devel- opment (Langdon and Waldock, 1981) because they are major membrane components (Hendriks et al., 2003) and possible modulators of membrane function (Palacios et al., 2005). DHA is, indeed, involved in maintaining suitable structure of membranes; whereas, EPA has a role as energy source and as a precursor of eicosanoids. In addition, the omega-6 fatty acids docosapentaenoic acid (22:5n-6, DPA) and arachidonic acid (20:4n−6, AA) have been identified as fatty acids affecting growth and survival of larval and postlarval stages (Milke et al., 2006, 2008; Pernet et al., 2005). Yet, initial *O. edulis* fatty acid composition did not reveal any differences and, larval initial potential seemed to be similar.

The present work confirms that one critical factor in conditioning *O. spp.* is feeding in terms of the species of microalgae delivered and/ or ration (Chaparro et al., 2006; Dunphy et al., 2006; Utting and Millican, 1997; Wilson et al., 1996). "Natural" food has however proved to be better for broodstock conditioning than hatchery con- trolled feeding (Helm et al., 1991; Millican and Helm, 1994) and progress in this field is accordingly expected. To better understand how microalgae are mobilized to support reproduction, studies will have to be carried out to include ingestion, assimilation and biochemical allocation to gonads.

Conclusion

- When *O. edulis* broodstock are fed *R. salina* or *T. weissflogii*, lower fecundity is recorded than when broodstock are fed a mix of the two species.
- When fed the mixed diet (T-Iso plus *C. gracilis*), development of larvae is overall better than with single-species microalgal diets.
- *C. gracilis* plays an important role in *O. edulis* larvae growth but addition of T-Iso improves significantly percentage metamorphosis.
- Broodstock diet has a clear influence on *O. edulis* larval development, including metamorphosis.

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Chapitre II

RÔLE DE LA NUTRITION SUR LE CONDITIONNEMENT DE L'HUÎTRE PLATE

Comme nous venons de le voir (précédent chapitre), le conditionnement de géniteurs est une étape importante dans la reproduction contrôlée des mollusques et spécifiquement chez l'huître plate. Son succès se traduit généralement par la quantité et la qualité d'ovocytes ou larves émises.

Bien que la température soit considérée comme un facteur clé pour la régulation de la reproduction (Chávez-Villalba et al., 2001 ; Heasman et al., 1996, Wilson et al., 1996), la nutrition a un impact important confirmant certains travaux antérieurs (Millican et Helm, 1994). Néanmoins, ces travaux, comme les nôtres présentés dans le chapitre précédent, ne décrivent pas les mécanismes permettant d'en comprendre le déroulement. Seul le résultat final est relevé et les étapes intermédiaires méconnues à ce jour. Bien qu'un apport bispécifique soit plus performant, nous avons volontairement décidé de travailler en régime monospécifique afin d'identifier les algues les plus adaptées sur le plan physiologique (aptitude à être assimilée).

En effet, pour qu'une microalgue puisse être considérée comme une bonne candidate au conditionnement il faut qu'elle soit tout d'abord ingérée, puis assimilée et enfin allouée efficacement dans la gonade.

L'objectif principal de ce chapitre est donc de décrire ces différents processus ainsi que la quantification de l'ingestion et de l'absorption pour huit différentes espèces de microalgues. L'allocation biochimique de chacune de ces espèces, dans les principaux organes (gonade, glande digestive, muscle et branchies), a été déterminée

par la suite afin d'estimer leur incorporation dans l'huître et une éventuelle affectation spécifique dans la gonade. Cette étude a été complétée par une analyse fine de la gamétogenèse à travers un suivi histologique au cours du conditionnement.

Ces travaux sont présentés sous forme de trois publications, deux déjà acceptées et la troisième en cours de correction pour Aquaculture. La première a été conduite au printemps 2008 sur quatre espèces de microalgue, le seconde à l'automne 2008 sur quatre autres espèces de microalgues et la troisième (Short Note) concerne l'impact de ces huit espèces sur le développement gonadique, au cours de ces deux périodes de conditionnement.

Ces différentes microalgues font partie des espèces décrites dans la littérature comme pouvant avoir un rôle dans le développement des phases précoces de bivalves (Robert et al., 2004).

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A physiological and biochemical approach to selecting the ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part A)

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Abstract

To select the best microalgae for *Ostrea edulis* conditioning, four single species diets were fed to batches of broodstock, which were then, compared using physiological and biochemical approaches. Ingestion and absorption were first studied, showing that *Chaetoceros gracilis* and *Skeletonema marinoï* were efficiently ingested (4.9–5.3 mg g^{-1} h^{-1}) and absorbed (1.9–2.5 mg g^{-1} h^{-1}) while *Tetraselmis suecica* led to the lowest physiological responses (0.36 and 0.12 mg g^{-1} h^{-1} respectively). When *O. edulis* were fed any microalgae except T-ISO with only 79 mg g^{-1}, gonads accumulated carbohydrates from 116 to 141 mg g^{-1} and the extent of accumulation depended on the species supplied in the order *T. suecica*<*C. gracilis*<*S. marinoï*. When oysters were fed either of the diatoms (*C. gracilis* or *S. marinoï*), an efficient transfer of 20:5(n-3) to all tissues, including the gonads, was recorded while no enrichment in 22:6(n-3) occurred in all tissues (polar fraction) when oysters were fed T-ISO. In contrast (22:5(n-6)), a characteristic fatty acid of T-ISO accumulated in all tissues, confirming its allocation despite particularly low ingestion (1.66 mg g^{-1} h^{-1}) and absorption (0.32 mg g^{-1} h^{-1}). When oysters were fed *C. gracilis* or *S. marinoï* an efficient transfer of cholesterol and campesterol from diatoms to the gonads was observed, while no sterol accumulation occurred in the gonad when fed *T. suecica*. Because of low ingestion, absorption and poor biochemical compounds' transfer, *T. suecica* is not recommended for *O. edulis* conditioning. T-ISO also exhibited low physiological performances but due to a specific 22:6(n-3) enrichment in the gonad neutral fraction (16.1%), its potential role in reproduction should be considered. *Chaetoceros gracilis* is highly recommended for *O. edulis* broodstock while a source of DHA other than that provided by T-ISO should be found due to its poor absorption. Because a mixed diet has been shown to be more efficient for *O. edulis* broodstock conditioning, complementary trials dealing with the effects of other species rich in 22:6(n-3) such as *Rhodomonas salina* or *Pavlova lutheri* should be performed.

Keywords: *Ostrea edulis*, conditioning, algal diets, ingestion, absorption, biochemical composition.

Introduction

The flat oyster *Ostrea edulis* held a dominant position in European shellfisheries from 1950 to 1980, and was accordingly the main biological model studied by Walne in Conway (UK), one of the pioneers of the mollusk hatchery rearing techniques (Walne 1966, 1970). A tremendous amount of work was carried out during this period (see the papers in Walne 1974) but since the appearance of diseases due to *Marteilia refringens* (Comps 1970) and *Bonamia ostreae* (Comps, Tige & Grizel 1980) in France and the extension of the latter across Europe (e.g. Holland: van Banning 1990, Spain: Montes, Villalba, López, Carballal & Mourelle 1991, Ireland: Culloty & Mulcahy 1996), such specific hatchery knowledge has progressively declined. However, the production demand for hatchery-produced juveniles is predicted to increase significantly for many mollusks, due either to genetic improvement through selection (Ward, English, Mc golddrick, Maguire, Nell & Thompson 2000; King, Janke, Roberts & Kaspar 2004) or due to a decline in natural stocks (Laing, Walker & Areal 2005). Moreover, the French oyster industry relied on monoculture (130 000 tonnes year^{-1} of *Crassostrea gigas*), which is nowadays impeded by severe juvenile mortality (Samain & McCombie 2008; Pernet, Barret, Marty, Moal, Le Gall & Boudry 2010). There is accordingly a need to diversify shellfish production in France and flat oyster cultivation enjoys renewed interest.

The control of reproduction is the first step in mollusc hatchery management (Utting & Spencer 1991; Helm, Bourne & Lovatelli 2004), in which temperature and

food are known to play a major role. The effect of food on broodstock conditioning is species specific both in quantity and in quality (Utting & Millican 1997). Thus, algal diets representing 3–6% of the initial dry weight of *O. edulis* broodstock increase larval production (Millican & Helm 1994) and low fecundity is reported when only fed *Dunaliella tertiolecta*. In *O. edulis*, reproductive effort is therefore high because the quality of food during conditioning will affect the growth and survival of larvae after their release (Walne 1970). There is little published work related to optimal algal size and algal densities in *O. edulis* broodstock conditioning. In contrast, more information is available on the nutritional requirements at different stages of development: broodstock (Frolov & Pankov 1992), larvae (Ferreiro, Pérez-camacho, Labarta, Beiras, Planas & Fernandez-Reiriz 1990) and juvenile (Enright, Newkirk, Craigie & Castell 1986). Nevertheless, the relationship between broodstock feeding and larval development remains poorly known. On comparing broods of *O. edulis* larvae originated from a closely controlled hatchery conditioning regime (unfiltered seawater) and from mature oysters taken from a wild population, an increased level of 20:5(n-3) and 22:6(n-3) was observed from late June (Helm, Holland, Utting & East 1991). In the hatchery, supplementary algal feeding improved fertility and larval development (Helm, Holland & Stephenson 1973), but conflicting results were reported some years later (Millican& Helm 1994). The latest report concluded that hatchery-conditioned broodstock showed lower fecundity than wild stock and pointed out that there remained considerable scope to improve broodstock maintenance. To fill this gap, an efficient broodstock diet was searched for by Berntsson, Jonsson, Wängberg and Carlsson (1997). However, unfed *O. edulis*

breeders released larvae that performed as well as those originated from fed parents. Moreover, a high variability in larval development achieved for the same food treatment made interpretation difficult (Berntsson *et al.* 1997). Today, <10 algal species of the Bacillariophyceae (i.e. diatoms) and Haptophyceae are routinely cultured in commercial bivalve hatcheries (Coutteau & Sorgeloos 1992; Robert, Chrétiennot-Dinet, Kaas, Martin-Jézéquel, Moal, Le Coz, Nicolas, Bernard, Connan, Le Dean, Gourrierec, Leroy & Quéré 2004). Microalgae are commonly used in multi-species diets, generally composed of one diatom and one or more flagellates, to ensure a better balance of essential nutrients (Robert & Trintignac 1996; Brown, Jeffrey, Volkman & Dunstan 1997). Although this practice is generally successful, mollusc feeding requirements are still poorly understood (Robert & Trintignac 1997; Knauer & Southgate 1999; Volkman & Brown 2006) and there is a crucial need for studies in this field.

To be suitable for broodstock conditioning, a microalga must be well ingested, digested, assimilated and allocated efficiently to the reproductive compartment. The present study focuses on the ecophysiological aspects of feeding in *O. edulis* conditioned with four different microalgae, with a study of the biochemical allocation of these diets to different tissues including the gonads.

Materials and Methods

Broodstock conditioning

In February 2008, *O. edulis* aged 18 months (≈5 cm length and 0.5 g flesh dry weight), originating from the Bay of Cancale (North Brittany, France), were distributed homogeneously in translucent 50 L tanks (30 oysters per tank for an equivalent biomass: ≈1 kg total weight and 16 g dry flesh weight). Triplicate tanks were set up for each of the four single species diets tested here. Seawater was maintained at 19 °C in a flow-through system at a flow rate of 12 L h^{-1}, and the oysters were fed continuously at 900 μm^3 L^{-1} per feeding condition by means of a peristaltic pump. Seawater (salinity: 34 g L^{-1}) was filtered on 1 μm polypropylene filter media following UV treatment. Four different microalgae were tested as mono-specific diets: *Isochrysis affinis galbana* (volumetric size ≈45 μm^3, dry weight 20 pg $cell^{-1}$, T-ISO strain CCAP 927/14), *Chaetoceros gracilis* (80 μm^3, 70 pg $cell^{-1}$, strain UTEX LB2658), *Skeletonema marinoï* (85 μm^3, 50 pg $cell^{-1}$, strain CCAP 1077/3) and *Tetraselmis suecica* (280 μm^3, 225 pg $cell^{-1}$, strain CCAP 66/4). Ingestion and absorption of these different microalgae were studied according to Beiras, Camacho and Albentosa (1994) over 6 consecutive weeks. It was hypothesized that the microalgae species that was best absorbed by the oysters represented the best potential diet; hence, we tested this hypothesis by examining nutrient biochemical allocation to the gonads in the polar fraction compared with other tissues.

Culture of microalgae

Microalgae were grown using a standard batch culture method (Robert *et al.* 2004). Actively growing starter cultures (6 L) were inoculated into Perspex translucent columns containing 300 L of 1 μm filtered seawater previously UV treated (salinity: 34 g L^{-1}) enriched with Conwy media (Walne 1966). Cultures were grown under continuous illumination (160 μmol photon m^{-2} s^{-1}) at 21 °C and aerated with a 2% CO_2/air mixture to maintain pH between 7.6 and 8.1. The cell count of cultures was monitored daily using a Mallasez haemocytometer (Batailler Labo, Treillères, France) or a Multisizer 3 (Beckman Coulter, Paris, France). Cultures attained the late-logarithmic phase after 3–5 days, at which time samples were removed for feeding to flat oysters and for biochemical analyses. A minimum of three different cultures of individual algal species were used throughout each of the experiments.

Ecophysiological data acquisition

Consumption and ingestion

Algal concentration was determined twice a day using a Coulter particle counter (Multisizer 3) at the inlet and outlet of each tank. In a flow-through culture system, algal consumption corresponds to: $C=(C_i-C_o) \times$ flow, where C_i is the food concentration of the incoming seawater and C_o is food concentration of the outgoing seawater. C was expressed in mg (dry weight algae) per g (dry weight of oyster meat) per hour. In most molluscs, some of the filtered particles may be rejected as pseudofaeces. In this case, ingestion is defined as: $I=C*PF$, where PF is

the amount of pseudofaeces. Because pseudofaeces production corresponds to an energetic loss for the animal, it should be reduced as much as possible to optimize conditioning efficiency. Preliminary trials were accordingly run on two single diets T-ISO and *C. gracilis* and showed that food delivery should be reduced to 4.5% dry weight g^{-1} of oyster to induce only a low level of pseudofaeces production (PF <10%). For all diets, PF was considered as nil so that consumption ≈ ingestion.

Absorption

In most molluscs, digested food will be assimilated and/or totally or partially rejected as faeces. Under such conditions, absorption is defined as $A=I\times$ absorption efficiency (ae), defined as: $ae=100 \times (OM_A-OM_F)/[(1-OM_F) \times OM_A]$, where OM_A is the microalgae relative organic matter content and OM_F is the faeces relative organic matter content (Conover 1966).

Tanks were drained and oysters cleaned three times a week (Monday, Wednesday and Friday) and daily faeces production, established precisely on a 24-h period, was accordingly achieved the two other days: on Tuesday for two types of diet (*I. aff. galbana* and *T. suecica*) and on Thursday for the other two diets. Faeces collections were performed using a vacuum pump onto a GF/C filter, precombusted at 450 °C. Faecal samples were washed with an ammonium formate solution (34 g L^{-1}) to remove salts. The total dry weight was determined after drying at 75 °C for at least 24 h by the difference from the filter tare while the OM fraction (%) was obtained from a second weighing after combustion in a muffle furnace at 450 °C for 4 h. Although feeding was adjusted to limit pseudofaeces production, care was taken to

collect exclusively faeces during sampling so that the results presented here referred to actual assimilation. Thus, for each diet, five representative sets of data, for which the coefficient of variation was <10%, were pooled to express the mean faeces production throughout the entire experimental period.

At the beginning and end of the experiment, the dry weight of soft body tissue of each individual (10–30 per tank) was measured to the nearest mg after freeze-drying at 60 °C for 72 h and the flesh dry weight increment in oysters fed the four different microalgae was used to calculate ingestion and absorption. Considering that growth was linear in that 6-week experimental period, oyster dry flesh weight at t time (Dw_t) was estimated on a weekly basis as $Dw_t = \sum_{i=0}^{t}((Dw_f - Dw_0)/6) + Dw_0$, where Dw_0 is the mean outset oyster dry weight and Dw_f is the mean final oyster dry weight.

Biochemical procedures

Microalgae

For dry weight analysis, 25 mL samples of a microalgal suspension were filtered through precombusted (450 °C, 24 h), preweighed, 47-mm GF/F Whatman filters. The filters were washed with 30 mL of 0.5 M ammonium formate to remove residual salts, dried at 80 °C overnight and then reweighed to determine the dry weight.

For fatty acid and sterol analyses, similar harvest methods were used but filters were stored at 20 °C in chloroform–methanol (2/1) for a period of up to 6 months before analysis based on methods similar to those used for oysters.

Oysters

At the beginning and end of the broodstock conditioning period, 15 oysters per feeding condition were dissected to sample four different organs: gonad (Gn), digestive gland (Dg), adductor muscle (Am) and gills (G). For each diet, three samples were prepared of each of the four organs, each sample containing the pooled tissues of five oysters; these samples were frozen in liquid nitrogen at -196 °C until analysis.

The different organs were then crushed using a ball grinder, and divided into two equal parts: the first one for protein and carbohydrate analysis (≈200 mg for each tissue dispensed in 2 mL distilled water) and the second for fatty acid and sterol analysis (≈200 mg for each tissue distributed in 6 mL Folch solution, Folch, Lee & Sloane-Stanley 1957). Aliquots of the first fraction were analysed separately for tissue dry weight, protein (Lowry, Rosebrough, Farr & Randall 1957) and carbohydrate contents (Dubois, Gilles, Hamilton, Rebers& Smith 1956). After centrifugation, the lipid extract was transferred to a clean tube, sealed under nitrogen and stored at -20 °C. Neutral and polar lipid extracts were separated on a silica gel micro-column as described by Marty, Delaunay, Moal and Samain (1992), and fatty acids in each fraction were analysed as described in Delaporte, Soudant, Lambert, Moal, Pouvreau and Samain (2006) with 23:0 as an internal standard. For

each tissue, the fatty acid composition was expressed as the absolute and relative contents of the total fatty acids of each lipid fraction. Sterols were analysed using the method described by Soudant, Val Sanles, Quéré, Le Coz, Marty, Moal, Samain and Sorgeloos (2000) after transesterification with sodium methoxide (MeONa; Eder *et al.* 1992) in a Chrompack CP 9002 gas chromatograph (Chrompack, Amdelft, the Netherlands) equipped with a RestekRt × 65 fused silica capillary column (Restek, Bellefonte, PA, USA) (15 m × 0.25 mm, 0.25 μm film thickness) using hydrogen as a carrier gas and cholestane as an internal standard. Fatty acids and sterols were identified by comparison of their retention time with standards. In the present work, fatty acids of the polar lipid fraction were only reported because they correspond to real assimilation (Delaporte, Soudant, Moal, Giudicelli, Lambert, Séguineau&Samain 2006). The composition of the neutral lipid fraction was also determined but mainly used to explain transfer from one organ to another or from the gonad to eggs and larvae, which was not the objective of the present work.

Statistical analyses

Statistical analyses were performed using Statistica software (version 8.0). Significant differences were detected between the means at the 5% threshold using anova and an *apostiori* multiple comparison test between the means (Tuckeys' test), after transformation of percentage data by the function [arcsin (racine × *i*/100)].

Results

Effect of food on physiological parameters

Whatever the diet supplied, cumulative oyster mortality was low at the end of the experiment (3%), lending confidence to the results reported here.

The mean outset oyster dry weight was 0.48 ± 0.03 g while the mean final oyster dry weights ranged from 0.33 ± 0.03 g (*T. suecica*) to 0.62 ± 0.04 g (*S. marinoi*). Similar growth dry weight increment was recorded when oyster were fed *I. aff. galbana* (0.58 ± 0.05 g) or *C. gracilis* (0.56 ± 0.05 g).

Skeletonema marinoï and *C. gracilis* induced the highest consumption ($7.0 \times 10^9 \pm 0.5 \times 10^9$ and $6.5 \times 10^9 \pm 0.1 \times 10^9$ $\mu m^3\ g^{-1}\ h^{-1}$), with no significant differences between these diatoms, while consumption recorded with *I. aff. galbana* was approximately half ($3.7 \times 10^9 \pm 0.1 \times 10^9$ $\mu m^3\ g^{-1}\ h^{-1}$). The lowest consumption occurred with *T. suecica* ($3.0 \times 10^9 \pm 0.4 \times 10^9$ $\mu m^3\ g^{-1}\ h^{-1}$). Ingestion ranged from 0.36 ± 0.05 to 5.32 ± 0.37 mg $g^{-1}\ h^{-1}$ for *T. suecica* and *S. marinoï* respectively (Fig. 6). These two diatoms also led to the highest absorption (2.5 ± 0.0 and 1.9 ± 0.2 mg $g^{-1}h^{-1}$ for *S. marinoï* and *C. gracilis* respectively) and absorption efficiencies (ae=46% and 38% respectively), while similar low absorption and absorption efficiency were recorded with T-ISO (0.32 ± 0.07 mg $g^{-1}\ h^{-1}$, 19%) and *T. suecica* (0.12 ± 0.0 mg $g^{-1}\ h^{-1}$, 33%) (Fig. 7).

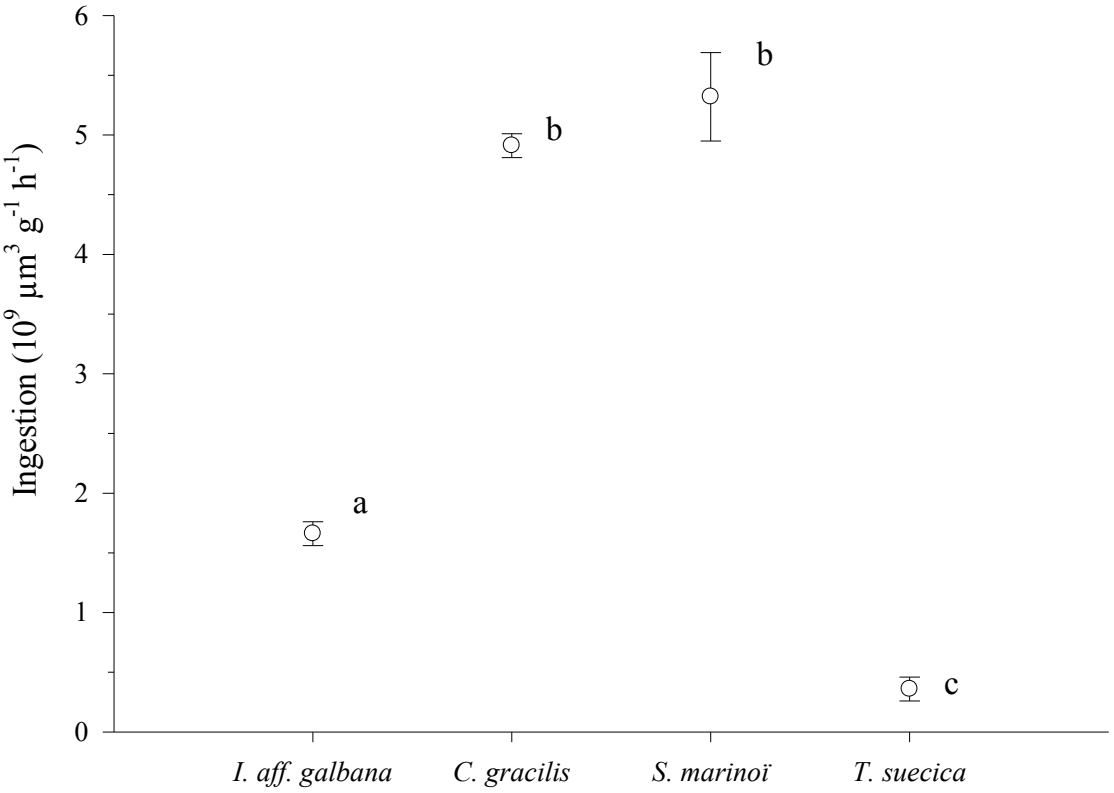

Fig. 6. Ingestion rate (mean ± SD) of *Ostrea edulis* broodstock fed different microalgae species for five consecutive weeks. Values with different letters are significantly different (P<0.05).

When *O. edulis* oysters were fed *I. aff. galbana*, *C. gracilis or S. marinoï* meat dry weight showed no significant increase over the 6-week experiment, ranging from 0.56 ± 0.06 to 0.62 ± 0.04 g at the end of the trial compared with 0.48 ± 0.03 g at the start. In contrast, those fed *T. suecica* exhibited a relative loss of weight in week 6 (0.33 ± 0.11 g), leading to significant differences between diets in week 6 (P<0.05)

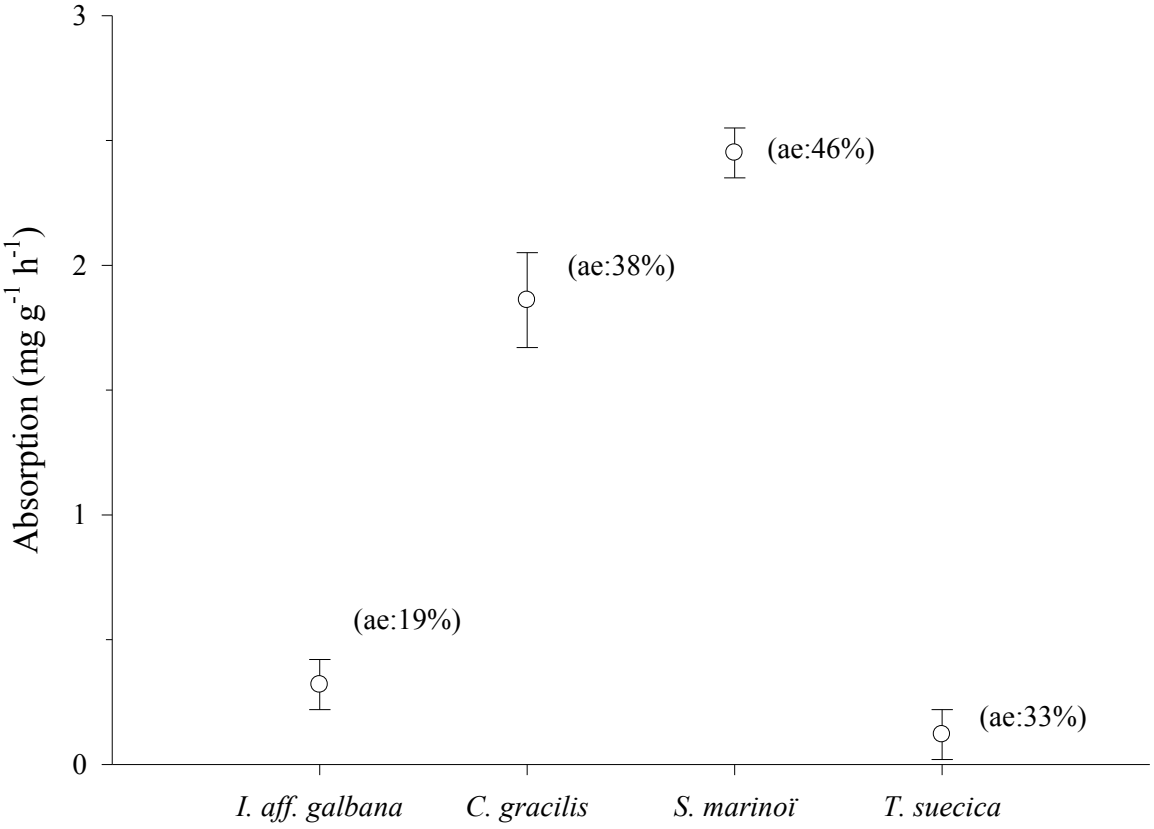

Fig. 7. Absorption (mean ± SD) and absorption efficiency (ae: %) of *Ostrea edulis* broodstock fed different microalgae species for 5 consecutive weeks.

Diet composition

The main fatty acids and sterols found in the four microalgae used as food for *O. edulis* are reported in Table 4. With ≈10% of total fatty acids T-ISO was rich in 22:6(n-3) but poor in 20:5(n-3) and exhibited accordingly a ratio 22:6(n-3)/20:5(n-3) that was particularly high (x = 31). With ≈12% (vs. ≈1–4% for the other species) T-ISO was also rich in 18:2(n-6) and 18:4(n-3). It was also characterized by 22:5(n-6) found at 1.7% (vs. 0–0.4 for both diatoms and *T. suecica* respectively) and by brassicasterol (99%) inexistent in the other microalgae (Table 4).

With 22–23% of total fatty acids *C. gracilis* and *S. marinoï* were rich in 20:5(n-3) but poor in 22:6(n-3) and exhibited accordingly a ratio 22:6(n-3)/20:5(n-3) that was particularly low (x<0.2). With 11.5–22.5% and 13.5–25.5%, both diatoms contained large proportions of 16:3(n-4) and 16:1(n-7) respectively (Table 4). *Chaetoceros gracilis* was also characterized by cholesterol (51%) and fucosterol (37%) while *S. marinoï* mainly contained campesterol (37%) and 24 mecholesterol (37%).

With 26% 18:1(n-9) was the prevailing mono-unsaturated fatty acid in *T. suecica* and with 10% 18:3(n-3) was the dominant poly-unsaturated fatty acid (Table 4). It contained at least five times less 22:6(n-3) and 20:5(n-3) with, however, a similarly low ratio 22:6(n-3)/20:5(n-3) as both diatoms. In contrast, it was characterized by 16:4(n-3), inexistent in both diatoms and weakly represented in T-ISO (0.5% vs. ≈6%) and by campesterol (89%: Table 4).

Effect of food on proximate biochemical composition

The mean gonad protein ranged from 408 to 529 mg g^{-1}(Table 5). There was no protein accumulation during the conditioning process when oysters were fed any microalga other than T-ISO (P<0.05). With the diatoms (*C. gracilis* and *S. marinoï*), a decrease in protein was observed in *O. edulis* digestive gland and adductor muscle, while *T. suecica* did not induce any significant change in any organ apart from a decrease in muscle (P<0.05). The mean gonad carbohydrates ranged from 79 to 141 mg g^{-1} (Table 5). Gonads accumulated carbohydrates when *O. edulis* were fed

	Oyster diets			
Fattyacid	*I. aff.galbana*	*C. gracilis*	*S. marinoï*	*T. suecica*
14:0	18.97 (8.83)	10.12 (1.13)	9.64 (0.46)	1.22 (0.77)
16:0	9.37 (0.19)	11.09 (0.71)	6.66 (0.53)	30.82 (1.23)
18:0	0.22 (0.47)	0.00 (0.00)	0.00 (0.00)	1.03 (0.15)
20:0	0.10 (0.01)	0.01 (0.01)	0.00 (0.00)	0.00 (0.00)
22:0	0.00 (0.00)	0.23 (0.01)	0.08 (0.08)	0.11 (0.11)
24:0	0.11 (0.05)	0.00 (0.00)	0.00 (0.00)	0.02 (0.04)
16:1(n-9)	0.63 (0.15)	0.00 (0.00)	0.00 (0.00)	2.79 (0.29)
16:1(n-7)	5.05 (0.39)	25.37 (0.57)	13.44 (1.63)	0.39 (0.25)
18:1(n-9)	12.09 (0.51)	0.77 (0.18)	0.00 (0.00)	26.39 (4.51)
18:1(n-7)	1.15 (0.10)	0.48 (0.08)	0.22 (0.06)	2.83 (0.90)
16:2(n-7)	0.22 (0.01)	2.64 (1.76)	5.76 (0.05)	0.00 (0.00)
16:2(n-6)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.55 (0.10)
16:2(n-4)	0.51 (0.12)	3.34 (0.38)	2.34 (0.04)	0.01 (0.01)
16:3(n-4)	0.29 (0.24)	11.61 (2.22)	22.55 (3.13)	0.00 (0.00)
16:4(n-3)	0.52 (0.01)	0.00 (0.00)	0.00 (0.00)	5.81 (3.91)
16:4(n-1)	0.00 (0.00)	0.00 (0.00)	1.78 (0.09)	0.00 (0.00)
18:2(n-6)	11.62 (3.47)	1.17 (0.43)	1.24 (0.05)	3.55 (0.46)
18:2(n-4)	0.01 (0.04)	0.05 (0.05)	0.12 (0.00)	0.00 (0.00)
18:3(n-6)	1.34 (0.26)	1.07 (0.04)	0.41 (0.13)	0.41 (0.14)
18:3(n-3)	6.12 (0.59)	0.21 (0.05)	0.55 (0.01)	9.69 (1.32)
18:4(n-3)	12.07 (3.19)	0.94 (0.06)	4.03 (0.72)	4.15 (1.02)
18:5(n-3)	2.15 (0.10)	0.00 (0.00)	0.00 (0.00)	0.09 (0.07)
20:4(n-6)	0.23 (0.19)	1.39 (0.09)	0.43 (0.04)	0.51 (0.08)
20:5(n-3)	0.33 (0.18)	22.66 (0.14)	21.88 (1.11)	4.44 (0.62)
22:5(n-6)	1.70 (1.03)	0.00 (0.00)	0.03 (0.00)	0.42 (0.29)
22:5(n-3)	0.09 (0.05)	0.03 (0.01)	0.03 (0.00)	0.01 (0.01)
22:6(n-3)	10.17 (2.54)	1.11 (0.51)	4.40 (0.21)	0.23 (0.08)
TO.SAT.	29.48 (9.40)	22.04 (0.50)	16.82 (1.08)	33.29 (1.31)
TO.MONO	20.45 (1.24)	28.18 (0.16)	15.48 (0.53)	34.19 (4.87)
TO.(n-9)	12.89 (0.34)	0.93 (0.34)	0.34 (0.00)	30.44 (4.71)
TO.(n-7)	7.28 (1.13)	26.44 (0.47)	14.24 (0.57)	3.31 (0.95)
TO.POLY	48.37 (8.04)	47.08 (1.40)	65.73 (1.61)	32.14 (5.05)
TO.(n-4)	0.81 (0.40)	15.00 (1.79)	25.01 (0.06)	0.01 (0.01)
TO.(n-6)	15.15 (7.23)	4.01 (0.63)	2.16 (0.26)	6.23 (0.75)
TO.(n-3)	32.07 (0.70)	25.33 (0.66)	30.96 (1.46)	25.52 (5.12)
(n-3)/(n-6)	2.12 (1.86)	6.32 (1.37)	14.35 (0.04)	4.14 (0.91)
22:6/20:5	31.07 (6.67)	0.05 (0.02)	0.20 (0.01)	0.05 (0.01)
fg cell^{-1}	1367.60 518.79	1848.86 87.29	1776.82 523.20	11565.40 3134.07)
Sterols				
Cholesterol	0.67 (0.09)	50.94 (1.99)	11.85 (2.43)	1.45 (0.79)
Brassicasterol	99.33 (0.09)	- -	- -	- -
Desmosterol	- -	- -	1.89 (0.12)	- -
Campesterol	- -	- -	36.68 (1.56)	88.92 (2.14)
24 MeCholesterol	- -	6.61 (1.94)	36.72 (4.06)	9.63 (1.35)
Fucosterol	- -	37.26 (0.64)	3.02 (1.8)	- -
Isofucosterol	- -	5.19 (0.7)	5.08 (1.66)	- -
fg cell^{-1}	83.71 7.68	103.39 13.22	58.46 4.71	330.31 108.67

Table 4. Fatty acid and sterol composition of total lipids of *Isochrysis affinis galbana*, *Chaetoceros gracilis*, *Skeletonema marinoï* and *Tetraselmis suecica* (weight % of total acids ± S.D.).

any microalgae, except T-ISO ($P<0.05$), and the extent of accumulation depended on the algal species supplied: *T. suecica*<*C. gracilis*<*S. marinoï* ($P<0.05$). In contrast, carbohydrates accumulated in the digestive gland irrespective of the diet, while no significant transfer occurred to the muscle or the gills (Table 5).

Initial values (spring)	Gonad	Digestive g.	Muscle	Gills
Protein (mg dwg^{-1})	408.0 (38)	523.9 (65)	843.9 (67)	705.5 (05)
Carbohydrate (mg dwg^{-1})	79.5 (13)	93.7 (7)	23.4 (1)	17.2 (1)
Values after 6 weeks of conditioning				
Isochrysisaffinisgalbana				
Protein (mg dwg^{-1})	528.7 (37)	581.2 (103)	724.6 (141)	540.6 (177)
Carbohydrate (mg dwg^{-1})	79.1 (12)	125.4 (16)	17.9 (5)	9.6 (3)
Chaetocerosgracilis				
Protein (mg dwg^{-1})	347.4 (23)	445.5 (12)	696.7 (39)	657.8 (61)
Carbohydrate (mg dwg^{-1})	122.4 (15)	109.9 (6)	26.6 (2)	14.9 (1)
Skeletonemamarinoï				
Protein (mg dwg^{-1})	393.5 (22)	454.1 (22)	697.3 (21)	722.8 (50)
Carbohydrate (mg dwg^{-1})	141.2 (15)	123.1 (5)	22.4 (4)	16.5 (1)
Tetraselmissuecica				
Protein (mg dwg^{-1})	447.6 (26)	614.33 (196)	659.6 (57)	643.3 (42)
Carbohydrate (mg dwg^{-1})	116.4 (4)	116.1 (14)	22.5 (5)	17.6 (2)

Table 5. Protein and carbohydrate contents in gonad, digestive gland, muscle and gills on European flat oyster *Ostrea edulis* broodstock, fed 4 microalgae species (values expressed in dwg tissue ± S.D; n=3).

The main fatty acids found over all organs, whatever the diets, were 16:0, 20:5(n-3) and 22:6(n-3), with values varying from 4.5% to 20.7% (gonad: Table 3; digestive gland: Table 7; adductor muscle: Table 8; gills: Table 9). When fed either diatom oysters showed a significant enrichment in EPA (20:5(n-3)) in the polar lipids of the gonad, although in the presence of *T. suecica* or T-ISO, EPA was either maintained or catabolized (Table 6). The three other organs showed similar

eicosapentaenoicacid content evolution in polar lipids (Tables 4–6). On the other hand, when oysters were fed T-ISO, there was a significant and exclusive enrichment in gonad docosahexaenoic acid (DHA, 22:6(n-3)) in the neutral fraction (data not shown here) but not in the polar fraction (Table 6). No DHA variation occurred in the other organs (Tables 7-9). When either diatom was supplied, DHA was catabolized in all organs but with *T. suecica* it remained constant (Tables 6-9). When oysters were fed T-ISO, enrichment in 22:5(n-6), a fatty acid characteristic of this haptophyte(Table 4), was recorded in all tissues (Tables 6-9). However, when oysters were fed *T. suecica* no 16:4(n-3) change was noted in any of the organs (Tables 6-9), even though this fatty acid is characteristic of this prasinophyte (Table 4).

No noticeable evolution occurred for the other main fatty acids including arachidonic acid, 20:4(n-6), which represented approximately 3–4% of the total fatty acids in all treatments (Tables 6-9).

When oysters were fed T-ISO, brassicasterol enrichment was measured in all the organs, while oysters fed both diatoms showed depletion in this sterol for the gonad, digestive gland and gills (Table 10). On the other hand, when flat oysters were fed *C. gracilis*, specific cholesterol enrichment occurred in all organs, except muscle. Similar trends were found for campesterol contents in oysters fed *S. marinoï* (Table 10). In contrast, when oysters were fed *T. suecica*, no differences were observed in the sterol composition (Table 10) despite campesterol's dominant position in that microalgae (Table 4).

Relative fecundity

When flat oysters were fed T-ISO, a total of 1.4 million larvae were harvested during the experimental period (6 weeks). After feeding with *C. gracilis* or *S. marinoï*, 1 million larvae were released, but only 0.3 million larvae were produced when the oysters were given *T. suecica*. Fecundity was therefore 0.04, 0.03 and 0.005 × 10^6 larvae per female in T-ISO, diatoms and *T. suecica* respectively (supposing an initial sex ratio of 1:1). At release, larvae varied in length from 165 to 179 μm, showing no significant differences (P<0.05) in size.

Discussion

Bivalve physiological parameters like consumption or ingestion depend on the reproductive cycle (Gabbott & Bayne 1973), the type and availability of food (Thompson & Bayne 1974; Defossez & Hawkins 1997; Albentosa, Fernández-Reiriz & Labarta 2007) and temperature (Ansell 1973; Newell & Branch 1980).

Impact of diet on physiological responses

Ingestion in *O. edulis* was clearly related to the diet. The high values reported for *C. gracilis* and *S. marinoï* (≈5 mg g^{-1} h^{-1}) contrast with those of T-ISO and *T. suecica* (<1.7 mg g^{-1} h^{-1}). Indeed, it is known that filter-feeding molluscs regulate their ingestion by a physical mechanism (Bayne, Hawkins & Navarro 1987; Navarro, Iglesias, Ortega & Larrexea 1994; Bacon, Macdonald & Ward 1998). Diatoms

showed better absorption compared with both T-ISO and *T. suecica*. When oysters were fed T-ISO, their absorption was six to eight fold less than with diatoms. This value represents 50% of that reported in *C. gigas* (Ropert & Goulletquer 2000) fed, however, a bi-specific diet of T-ISO and *T. suecica* (0.72 mg g^{-1} h^{-1}). These data also contrast with the values reported in *Glycymerisglycymeris* and *Phaphiaromboïdes*, where 80–90% ae was obtained with a multi-species diet supply (Savina & Pouvreau 2004).

Despite moderate T-ISO ingestion and absorption efficiency, no differences in *O. edulis* growth (expressed in meat dry weight) were observed compared with oysters receiving diatom diets. Although the absorption efficiency recorded in oysters fed *T. suecica* was close to the values obtained with diatoms (40% vs. 33%), the absorption value was 15–20-fold lower due to weak ingestion.

Impact of diet on the biochemical composition of oyster tissues

In the present work, gonads accumulated carbohydrates when *O. edulis* were fed three microalgae, except T-ISO, and the values can be ranked as follows: *T. suecica*<*C. gracilis*<*S. marinoi*. Carbohydrate storage and its utilization result in a balance between food supply and energetic demand for reproduction and growth. Carbohydrate exhaustion is consequently read as a catabolism of reserves for gametogenesis processes. Thus, carbohydrate depletion is reported during conditioning in *C. gigas* (Moal, Le Coz, Samain, Daniel & Bodoy 1991; Berthelin, Kellner et Mathieu 2000; Delaporte, Soudant, Lambert, Moal, Pouvreau and Samain, 2006).

	Initial				Oyster diets											
					I. aff. Galbana				*C. gracilis*				*S. marinoi*				*T. suecica*			
Fatty acid	Mean absolute contents [± SD (µg mg^{-1})]		Mean relative content [± SD (%)]		Mean absolute contents [± SD (µg mg^{-1})]		Mean relative content [± SD (%)]		Mean absolute contents [± SD (µg mg^{-1})]		Mean relative content [± SD (%)]		Mean absolute contents [± SD (µg mg^{-1})]		Mean relative content [± SD (%)]		Mean absolute contents [± SD (µg mg^{-1})]		Mean relative content [± SD (%)]	
14:0	0.48	± 0.48	2.16	± 1.25	0.60	± 0.04	2.83	± 0.33	0.35	± 0.03	2.40	± 0.31	0.49	± 0.05	3.23	± 0.13	0.22	± 0.18	1.24	± 0.39
16:0	3.26	± 2.17	16.18	± 3.25	3.24	± 0.23	15.20	± 0.86	2.29	± 0.03	15.81	± 0.78	2.18	± 0.23	14.32	± 0.73	3.40	± 3.04	16.87	± 1.65
18:0	0.85	± 0.27	4.67	± 0.56	0.79	± 0.07	3.71	± 0.21	0.64	± 0.03	4.40	± 0.05	0.64	± 0.03	4.24	± 0.34	1.15	± 1.02	5.67	± 0.36
16:1(n-9)	0.08	± 0.03	0.47	± 0.25	0.05	± 0.00	0.24	± 0.02	0.00	± 0.00	0.00	± 0.00	0.03	± 0.00	0.16	± 0.01	0.08	± 0.04	0.72	± 0.67
16:1(n-7)	0.56	± 0.58	2.50	± 1.55	0.27	± 0.01	1.28	± 0.10	0.82	± 0.08	5.64	± 0.74	0.44	± 0.06	2.90	± 0.12	0.28	± 0.23	1.41	± 0.16
18:1(n-9)	0.51	± 0.33	2.57	± 0.49	1.12	± 0.04	5.27	± 0.21	0.15	± 0.00	1.06	± 0.01	0.18	± 0.01	1.16	± 0.08	0.65	± 0.56	3.30	± 0.36
18:1(n-7)	0.42	± 0.40	1.91	± 1.00	0.32	± 0.01	1.51	± 0.06	0.50	± 0.01	3.43	± 0.06	0.50	± 0.08	3.29	± 0.15	0.26	± 0.22	1.34	± 0.16
20:1(n-9)	0.10	± 0.01	0.56	± 0.20	0.27	± 0.04	1.29	± 0.13	0.04	± 0.01	0.28	± 0.03	0.05	± 0.01	0.30	± 0.05	0.22	± 0.22	1.03	± 0.10
20:1(n-7)	0.92	± 0.35	4.94	± 0.47	0.81	± 0.10	3.80	± 0.30	0.81	± 0.05	5.55	± 0.13	1.17	± 0.15	7.69	± 0.34	1.18	± 1.10	5.48	± 0.16
16:3(n-6)	0.02	± 0.02	0.11	± 0.06	0.00	± 0.00	0.01	± 0.01	0.00	± 0.00	0.02	± 0.03	0.00	± 0.00	0.00	± 0.00	0.01	± 0.01	0.03	± 0.02
18:2(n-6)	0.19	± 0.17	0.90	± 0.41	1.11	± 0.04	5.21	± 0.30	0.07	± 0.01	0.50	± 0.07	0.30	± 0.03	2.00	± 0.15	0.15	± 0.13	0.75	± 0.10
18:3(n-3)	0.31	± 0.32	1.39	± 0.86	0.31	± 0.02	1.44	± 0.15	0.04	± 0.00	0.25	± 0.03	0.07	± 0.01	0.45	± 0.04	0.22	± 0.20	1.12	± 0.15
18:5(n-3)	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
20:4(n-6)	0.49	± 0.06	2.85	± 0.84	0.63	± 0.07	2.96	± 0.26	0.55	± 0.06	3.80	± 0.29	0.43	± 0.04	2.83	± 0.23	0.82	± 0.76	3.68	± 0.57
20:4(n-3)	0.09	± 0.09	0.41	± 0.23	0.05	± 0.01	0.23	± 0.03	0.03	± 0.00	0.21	± 0.02	0.02	± 0.01	0.14	± 0.02	0.06	± 0.06	0.30	± 0.02
20:5(n-3)	2.93	± 1.94	14.60	± 2.94	1.16	± 0.19	5.43	± 0.54	2.51	± 0.02	17.29	± 0.49	2.65	± 0.44	17.27	± 0.82	2.61	± 2.43	12.16	± 0.33
22:4(n-6)	0.08	± 0.02	0.51	± 0.25	0.15	± 0.02	0.70	± 0.05	0.08	± 0.00	0.57	± 0.03	0.07	± 0.01	0.43	± 0.05	0.16	± 0.14	0.78	± 0.05
22:5(n-6)	0.10	± 0.01	0.56	± 0.19	0.91	± 0.06	4.29	± 0.04	0.06	± 0.00	0.42	± 0.01	0.04	± 0.00	0.24	± 0.01	0.14	± 0.13	0.67	± 0.04
22:5(n-3)	0.22	± 0.03	1.29	± 0.35	0.17	± 0.02	0.82	± 0.06	0.21	± 0.01	1.44	± 0.14	0.19	± 0.05	1.23	± 0.23	0.34	± 0.31	1.65	± 0.05
22:6(n-3)	2.50	± 0.83	13.58	± 1.42	3.19	± 0.21	14.96	± 0.70	1.03	± 0.01	7.13	± 0.18	1.14	± 0.13	7.48	± 0.39	2.54	± 2.44	11.85	± 0.64
TO.MONO	3.13	± 1.89	15.84	± 2.21	3.59	± 0.17	16.84	± 0.30	2.85	± 0.04	19.63	± 0.49	2.87	± 0.35	18.78	± 0.06	3.38	± 3.03	16.47	± 0.77
TO.(n-9)	0.77	± 0.38	3.99	± 0.38	1.53	± 0.12	7.17	± 0.41	0.34	± 0.00	2.33	± 0.07	0.27	± 0.03	1.79	± 0.06	0.99	± 0.87	5.17	± 1.00
TO.(n-7)	1.91	± 1.33	9.37	± 2.21	1.41	± 0.11	6.61	± 0.31	2.13	± 0.01	14.64	± 0.65	2.13	± 0.28	13.90	± 0.16	1.72	± 1.55	8.27	± 0.27
TO.POLY	9.22	± 4.15	48.60	± 0.13	10.35	± 0.69	48.53	± 0.61	6.41	± 0.24	44.11	± 0.04	7.00	± 0.90	45.77	± 0.30	10.15	± 9.53	47.48	± 0.80
TO.(n-6)	1.00	± 0.34	5.46	± 0.60	3.08	± 0.16	14.46	± 0.18	0.86	± 0.05	5.94	± 0.09	0.91	± 0.08	5.96	± 0.24	1.37	± 1.25	6.38	± 0.39
TO.(n-3)	6.89	± 3.79	35.36	± 3.30	5.74	± 0.46	26.92	± 1.03	4.23	± 0.05	29.11	± 0.78	4.48	± 0.68	29.25	± 1.29	6.51	± 6.10	30.88	± 1.15
(n-3)/(n-6)	6.58	± 1.37	6.58	± 1.37	1.86	± 0.09	1.86	± 0.09	4.90	± 0.20	4.90	± 0.20	4.91	± 0.36	4.91	± 0.36	4.86	± 0.48	4.86	± 0.48
22:6/20:5	0.97	± 0.27	0.97	± 0.27	2.77	± 0.30	2.77	± 0.30	0.41	± 0.00	0.41	± 0.00	0.43	± 0.03	0.43	± 0.03	0.98	± 0.07	0.98	± 0.07
TOTAL	18.98	± 8.59	100.00	± 0.00	21.32	± 1.33	100.00	± 0.00	14.53	± 0.55	100.00	± 0.00	15.29	± 1.87	100.00	± 0.00	21.16	± 19.58	100.00	± 0.00

Table 6. Total polar fatty acid composition in gonad oysters fed mono-specific diets expressed in absolute contents (µg mg-1) and relative contents (weight % of total polar fatty acids. ± S.D.).

	Initial				Oyster diets															
					I. aff. galbana				*C. gracilis*				*S. marinoi*				*T. suecica*			
	Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content	
Fatty acid	[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]	
14:0	0.17	± 0.02	1.09	± 0.29	0.57	± 0.15	2.61	± 0.35	0.97	± 0.91	3.24	± 1.43	0.49	± 0.01	3.25	± 0.14	0.14	± 0.04	0.70	± 0.11
16:0	2.23	± 0.09	14.28	± 2.35	3.19	± 0.38	14.76	± 0.94	4.11	± 2.53	15.64	± 0.15	1.69	± 0.14	11.05	± 0.73	2.95	± 0.77	14.99	± 0.46
18 :0	0.93	± 0.07	5.90	± 0.48	1.03	± 0.21	4.71	± 0.22	0.93	± 0.22	4.06	± 1.70	0.80	± 0.05	5.25	± 0.24	1.16	± 0.33	5.85	± 0.28
16:1(n-9)	0.04	± 0.01	0.27	± 0.08	0.06	± 0.03	0.25	± 0.09	0.01	± 0.02	0.10	± 0.14	0.02	± 0.00	0.13	± 0.02	0.07	± 0.03	0.37	± 0.11
16:1(n-7)	0.21	± 0.01	1.36	± 0.27	0.22	± 0.06	1.02	± 0.11	2.40	± 2.52	7.59	± 4.81	0.41	± 0.02	2.67	± 0.22	0.22	± 0.06	1.14	± 0.02
18:1(n-9)	0.30	± 0.01	1.95	± 0.33	0.95	± 0.22	4.32	± 0.28	0.24	± 0.21	0.80	± 0.31	0.05	± 0.08	0.32	± 0.55	0.76	± 0.20	3.85	± 0.27
18:1(n-7)	0.18	± 0.02	1.14	± 0.08	0.31	± 0.07	1.43	± 0.07	1.53	± 1.48	5.04	± 2.48	0.49	± 0.03	3.19	± 0.21	0.26	± 0.06	1.32	± 0.04
20:1(n-9)	0.10	± 0.00	0.63	± 0.07	0.25	± 0.04	1.16	± 0.03	0.06	± 0.04	0.21	± 0.01	0.03	± 0.00	0.21	± 0.03	0.29	± 0.14	1.43	± 0.34
20:1(n-7)	0.80	± 0.08	5.04	± 0.23	0.81	± 0.13	3.70	± 0.08	1.07	± 0.71	4.00	± 0.21	0.89	± 0.07	5.82	± 0.35	1.01	± 0.30	5.11	± 0.39
16:3(n-6)	0.01	± 0.01	0.09	± 0.04	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.02	± 0.00	0.08	± 0.02
18:2(n-6)	0.08	± 0.01	0.52	± 0.05	0.99	± 0.20	4.54	± 0.29	0.15	± 0.14	0.50	± 0.22	0.22	± 0.00	1.42	± 0.02	0.15	± 0.03	0.78	± 0.03
18:3(n-3)	0.12	± 0.00	0.79	± 0.12	0.31	± 0.07	1.44	± 0.14	0.13	± 0.13	0.40	± 0.26	0.05	± 0.00	0.35	± 0.01	0.28	± 0.07	1.45	± 0.11
18:5(n-3)	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
20:4(n-6)	0.50	± 0.14	3.09	± 0.45	0.84	± 0.14	3.88	± 0.36	0.77	± 0.50	2.87	± 0.12	0.42	± 0.02	2.78	± 0.10	0.85	± 0.26	4.31	± 0.26
20:4(n-3)	0.05	± 0.01	0.34	± 0.01	0.05	± 0.00	0.25	± 0.03	0.09	± 0.09	0.27	± 0.17	0.02	± 0.00	0.12	± 0.01	0.05	± 0.01	0.26	± 0.01
20:5(n-3)	2.04	± 0.64	12.63	± 2.36	0.96	± 0.16	4.44	± 0.60	6.06	± 5.28	20.75	± 7.09	2.70	± 0.11	17.72	± 0.95	2.17	± 0.62	11.00	± 0.38
22:4(n-6)	0.09	± 0.02	0.54	± 0.05	0.12	± 0.02	0.57	± 0.03	0.04	± 0.01	0.23	± 0.19	0.04	± 0.00	0.25	± 0.02	0.14	± 0.04	0.72	± 0.02
22:5(n-6)	0.09	± 0.02	0.57	± 0.07	1.07	± 0.17	4.92	± 0.05	0.05	± 0.01	0.24	± 0.12	0.03	± 0.00	0.19	± 0.01	0.18	± 0.03	0.92	± 0.10
22:5(n-3)	0.25	± 0.07	1.56	± 0.20	0.15	± 0.02	0.68	± 0.07	0.18	± 0.03	0.78	± 0.35	0.13	± 0.01	0.88	± 0.03	0.30	± 0.09	1.53	± 0.06
22:6(n-3)	2.29	± 0.77	14.20	± 2.96	3.43	± 0.42	15.82	± 0.71	1.48	± 0.94	5.57	± 0.08	1.05	± 0.05	6.89	± 0.32	2.06	± 0.61	10.39	± 0.52
TO.MONO	2.06	± 0.14	13.11	± 1.14	3.24	± 0.68	14.83	± 0.60	6.08	± 4.93	21.38	± 5.40	2.38	± 0.05	15.59	± 0.13	3.21	± 0.69	16.41	± 0.75
TO.(n-9)	0.48	± 0.02	3.05	± 0.50	1.30	± 0.27	5.96	± 0.28	0.32	± 0.23	1.18	± 0.14	0.13	± 0.07	0.85	± 0.52	1.16	± 0.37	5.89	± 0.59
TO.(n-7)	1.19	± 0.09	7.55	± 0.57	1.36	± 0.26	6.21	± 0.16	5.04	± 4.75	16.74	± 7.58	1.79	± 0.04	11.75	± 0.10	1.49	± 0.41	7.57	± 0.39
TO.POLY	7.64	± 1.83	47.75	± 4.95	11.21	± 1.66	51.63	± 1.08	11.90	± 8.25	43.87	± 3.97	7.05	± 0.07	46.30	± 0.90	9.29	± 2.60	47.09	± 1.42
TO.(n-6)	0.83	± 0.20	5.19	± 0.55	3.31	± 0.56	15.20	± 0.02	1.25	± 0.86	4.63	± 0.37	0.78	± 0.03	5.10	± 0.11	1.42	± 0.38	7.20	± 0.27
TO.(n-3)	5.21	± 1.59	32.33	± 5.78	6.01	± 0.76	27.72	± 1.18	8.71	± 7.05	30.64	± 7.67	4.34	± 0.14	28.50	± 1.28	5.59	± 1.61	28.30	± 1.07
(n-3)/(n-6)	6.20	± 0.49	6.20	± 0.49	1.82	± 0.08	1.82	± 0.08	6.57	± 1.13	6.57	± 1.13	5.60	± 0.37	5.60	± 0.37	3.93	± 0.18	3.93	± 0.18
22:6/20:5	1.12	± 0.03	1.12	± 0.03	3.60	± 0.41	3.60	± 0.41	0.28	± 0.09	0.28	± 0.09	0.39	± 0.01	0.39	± 0.01	0.94	± 0.02	0.94	± 0.02
TOTAL	15.84	± 2.32	100.00	± 0.00	21.77	± 3.68	100.00	± 0.00	26.38	± 16.42	100.00	± 0.00	15.24	± 0.38	100.00	± 0.00	19.64	± 4.92	100.00	± 0.00

Table 7. Total polar fatty acid composition in gonad oysters fed mono-specific diets expressed in absolute contents (µg mg-1) and relative contents (weight % of total polar fatty acids. ± S.D.).

	Initial				Oyster diets															
					I. aff. galbana				*C. gracilis*				*S. marinoï*				*T. suecica*			
	Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content	
Fatty acid	[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]	
14:0	0.07	± 0.01	0.78	± 0.05	0.13	± 0.04	1.60	± 0.13	0.14	± 0.02	1.61	± 0.22	0.14	± 0.09	1.62	± 0.55	0.08	± 0.02	0.90	± 0.30
16:0	0.84	± 0.09	9.34	± 0.15	1.15	± 0.45	13.79	± 2.47	1.35	± 0.25	14.35	± 1.17	1.06	± 0.69	14.94	± 0.94	1.21	± 0.08	14.07	± 0.94
18:0	0.45	± 0.06	5.04	± 0.26	0.45	± 0.15	5.36	± 0.15	0.59	± 0.07	6.33	± 0.23	0.47	± 0.31	6.69	± 0.44	0.66	± 0.07	7.93	± 0.93
16:1(n-9)	0.02	± 0.01	0.20	± 0.10	0.00	± 0.00	0.00	± 0.00	0.01	± 0.01	0.07	± 0.13	0.00	± 0.01	0.06	± 0.10	0.00	± 0.00	0.00	± 0.00
16:1(n-7)	0.08	± 0.01	0.87	± 0.05	0.12	± 0.04	1.41	± 0.12	0.15	± 0.13	1.62	± 1.11	0.08	± 0.11	1.00	± 0.86	0.11	± 0.03	1.16	± 0.33
18:1(n-9)	0.17	± 0.01	1.87	± 0.14	0.23	± 0.07	2.85	± 0.44	0.11	± 0.01	1.29	± 0.21	0.11	± 0.08	1.60	± 0.43	0.18	± 0.02	2.12	± 0.29
18:1(n-7)	0.08	± 0.01	0.91	± 0.02	0.10	± 0.05	1.19	± 0.26	0.25	± 0.03	2.57	± 0.23	0.15	± 0.11	1.82	± 0.70	0.06	± 0.05	0.49	± 0.70
20:1(n-9)	0.07	± 0.01	0.81	± 0.04	0.16	± 0.04	1.89	± 0.22	0.09	± 0.02	1.01	± 0.23	0.09	± 0.06	1.42	± 0.27	0.13	± 0.02	1.48	± 0.21
20:1(n-7)	0.54	± 0.07	6.04	± 0.24	0.55	± 0.18	6.58	± 0.24	0.81	± 0.06	8.76	± 0.24	0.66	± 0.43	8.87	± 0.97	0.68	± 0.03	7.84	± 0.57
16:3(n-6)	0.00	± 0.00	0.02	± 0.02	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
18:2(n-6)	0.05	± 0.00	0.54	± 0.02	0.18	± 0.05	2.14	± 0.08	0.04	± 0.00	0.52	± 0.27	0.06	± 0.04	0.70	± 0.27	0.04	± 0.00	0.49	± 0.06
18:3(n-3)	0.07	± 0.00	0.78	± 0.06	0.08	± 0.03	0.91	± 0.15	0.04	± 0.00	0.45	± 0.07	0.04	± 0.03	0.61	± 0.09	0.06	± 0.01	0.73	± 0.12
18:5(n-3)	0.00	± 0.00	0.02	± 0.04	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
20:4(n-6)	0.26	± 0.04	2.85	± 0.14	0.27	± 0.09	3.21	± 0.43	0.36	± 0.04	3.72	± 0.30	0.24	± 0.16	3.42	± 0.31	0.33	± 0.02	3.78	± 0.21
20:4(n-3)	0.02	± 0.00	0.27	± 0.03	0.02	± 0.01	0.26	± 0.07	0.02	± 0.00	0.20	± 0.02	0.02	± 0.01	0.25	± 0.05	0.02	± 0.01	0.25	± 0.07
20:5(n-3)	1.32	± 0.17	14.74	± 0.23	0.95	± 0.31	11.46	± 0.71	1.67	± 0.14	17.62	± 0.62	1.21	± 0.80	16.62	± 1.21	1.36	± 0.05	15.98	± 1.04
22:4(n-6)	0.04	± 0.00	0.49	± 0.01	0.07	± 0.02	0.87	± 0.17	0.07	± 0.01	0.71	± 0.06	0.05	± 0.03	0.69	± 0.11	0.08	± 0.00	0.92	± 0.02
22:5(n-6)	0.06	± 0.01	0.69	± 0.02	0.18	± 0.05	2.23	± 0.27	0.06	± 0.01	0.65	± 0.04	0.04	± 0.03	0.65	± 0.10	0.07	± 0.00	0.78	± 0.00
22:5(n-3)	0.19	± 0.01	2.08	± 0.09	0.16	± 0.05	1.97	± 0.18	0.18	± 0.02	1.95	± 0.06	0.14	± 0.09	2.10	± 0.23	0.20	± 0.01	2.30	± 0.03
22:6(n-3)	1.55	± 0.19	17.27	± 0.14	1.53	± 0.49	18.44	± 1.04	1.37	± 0.13	14.77	± 0.37	1.10	± 0.75	15.86	± 1.17	1.53	± 0.02	17.94	± 0.59
TO.MONO	1.16	± 0.13	12.89	± 0.31	1.33	± 0.41	15.97	± 0.09	1.64	± 0.19	17.74	± 1.21	1.24	± 0.88	17.05	± 1.21	1.35	± 0.19	14.86	± 1.94
TO.(n-9)	0.27	± 0.02	3.06	± 0.20	0.41	± 0.12	5.01	± 0.71	0.24	± 0.04	2.73	± 0.44	0.23	± 0.16	3.37	± 0.62	0.33	± 0.04	3.66	± 0.45
TO.(n-7)	0.70	± 0.08	7.82	± 0.21	0.77	± 0.27	9.18	± 0.31	1.21	± 0.19	12.95	± 1.03	0.89	± 0.63	11.72	± 1.71	0.85	± 0.09	9.50	± 0.94
TO.POLY	4.49	± 0.53	49.93	± 0.27	5.02	± 1.54	60.44	± 3.05	5.45	± 0.42	58.03	± 2.64	4.06	± 2.81	56.38	± 2.90	5.16	± 0.11	60.07	± 0.73
TO.(n-6)	0.47	± 0.06	5.23	± 0.06	0.78	± 0.23	9.41	± 0.60	0.58	± 0.06	6.13	± 0.15	0.43	± 0.29	6.07	± 0.18	0.56	± 0.02	6.52	± 0.17
TO.(n-3)	3.32	± 0.39	36.92	± 0.18	3.54	± 1.07	42.67	± 2.25	4.04	± 0.30	42.69	± 2.54	2.91	± 2.03	40.73	± 3.53	3.88	± 0.08	45.43	± 1.82
(n-3)/(n-6)	7.06	± 0.10	7.06	± 0.10	4.54	± 0.06	4.54	± 0.06	7.03	± 0.36	6.96	± 0.33	6.63	± 0.36	6.71	± 0.42	6.98	± 0.32	6.98	± 0.46
22:6/20:5	1.17	± 0.02	1.17	± 0.02	1.61	± 0.02	1.61	± 0.02	0.82	± 0.02	0.84	± 0.05	0.89	± 0.06	0.96	± 0.14	1.12	± 0.03	1.12	± 0.04
TOTAL	8.98	± 1.02	100.00	± 0.00	8.31	± 2.59	100.00	± 0.00	9.35	± 0.85	100.00	± 0.00	7.15	± 4.84	100.00	± 0.00	8.66	± 0.31	100.00	± 0.00

Table 8. Total polar fatty acid composition in gonad oysters fed mono-specific diets expressed in absolute contents (µg mg-1) and relative contents (weight % of total polar fatty acids. ± S.D.).

	Initial				Oyster diets															
					I. aff. galbana				*C. gracilis*				*S. marinoï*				*T. suecica*			
	Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content		Mean absolute contents		Mean relative content	
Fatty acid	[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]		[± SD (µg mg^{-1})]		[± SD (%)]	
14:0	0.09	± 0.03	0.63	± 0.20	0.35	± 0.15	1.80	± 0.18	0.34	± 0.07	1.60	± 0.25	0.62	± 0.20	1.52	± 0.95	0.09	± 0.01	0.51	± 0.06
16:0	1.20	± 0.32	8.44	± 0.49	3.18	± 1.25	16.35	± 1.02	4.20	± 0.60	17.15	± 2.36	4.69	± 1.75	15.03	± 1.66	2.80	± 0.02	15.40	± 0.30
18:0	0.62	± 0.19	4.36	± 0.04	0.75	± 0.27	3.93	± 0.21	1.01	± 0.26	4.25	± 0.14	1.27	± 0.42	4.67	± 0.97	1.01	± 0.09	5.22	± 0.37
16:1(n-9)	0.02	± 0.02	0.14	± 0.13	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
16:1(n-7)	0.13	± 0.03	0.91	± 0.12	0.26	± 0.07	1.39	± 0.20	0.67	± 0.10	2.42	± 0.84	0.50	± 0.16	1.14	± 0.99	0.00	± 0.00	0.00	± 0.00
18:1(n-9)	0.18	± 0.04	1.30	± 0.14	0.70	± 0.29	3.61	± 0.44	0.14	± 0.02	0.58	± 0.05	0.12	± 0.10	0.95	± 1.02	0.46	± 0.11	2.97	± 0.66
18:1(n-7)	0.09	± 0.03	0.62	± 0.02	0.26	± 0.10	1.34	± 0.05	0.73	± 0.20	3.16	± 0.07	0.94	± 0.27	2.22	± 1.33	0.16	± 0.04	1.05	± 0.26
20:1(n-9)	0.10	± 0.03	0.73	± 0.01	0.30	± 0.11	1.57	± 0.06	0.31	± 0.22	1.12	± 1.12	0.27	± 0.22	1.40	± 0.97	0.28	± 0.03	1.66	± 0.19
20:1(n-7)	0.79	± 0.23	5.56	± 0.08	0.90	± 0.30	4.72	± 0.18	1.53	± 0.41	7.12	± 0.99	2.49	± 0.77	7.67	± 0.60	1.30	± 0.04	7.06	± 0.02
16:3(n-6)	0.01	± 0.01	0.07	± 0.02	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
18:2(n-6)	0.03	± 0.01	0.23	± 0.03	0.66	± 0.29	3.36	± 0.43	0.05	± 0.01	0.56	± 0.61	0.38	± 0.15	0.93	± 0.51	0.08	± 0.02	0.52	± 0.10
18:3(n-3)	0.04	± 0.01	0.28	± 0.03	0.11	± 0.04	0.56	± 0.04	0.02	± 0.00	0.10	± 0.05	0.05	± 0.01	0.23	± 0.13	0.09	± 0.03	0.64	± 0.19
18:5(n-3)	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00	0.00	± 0.00
20:4(n-6)	0.66	± 0.17	4.64	± 0.18	1.20	± 0.40	6.28	± 0.22	1.66	± 0.47	6.83	± 0.46	1.83	± 0.54	6.13	± 0.71	1.29	± 0.02	7.00	± 0.05
20:4(n-3)	0.01	± 0.00	0.06	± 0.00	0.01	± 0.00	0.05	± 0.01	0.01	± 0.00	0.03	± 0.02	0.01	± 0.00	0.05	± 0.05	0.02	± 0.00	0.12	± 0.00
20:5(n-3)	1.48	± 0.40	10.41	± 0.28	0.89	± 0.23	4.79	± 0.69	3.66	± 0.80	15.36	± 0.72	4.61	± 1.49	13.41	± 2.99	1.80	± 0.13	9.46	± 0.44
22:4(n-6)	0.05	± 0.09	0.29	± 0.50	0.21	± 0.07	1.07	± 0.01	0.18	± 0.04	0.75	± 0.07	0.07	± 0.12	0.40	± 0.69	0.21	± 0.02	1.12	± 0.06
22:5(n-6)	0.05	± 0.07	0.26	± 0.38	1.07	± 0.46	5.44	± 0.56	0.12	± 0.02	0.47	± 0.07	0.05	± 0.06	0.34	± 0.52	0.18	± 0.00	0.99	± 0.03
22:5(n-3)	0.23	± 0.05	1.67	± 0.16	0.17	± 0.05	0.91	± 0.10	0.31	± 0.05	1.26	± 0.18	0.33	± 0.09	1.33	± 0.46	0.32	± 0.04	1.62	± 0.17
22:6(n-3)	2.14	± 0.54	15.06	± 0.61	3.27	± 1.21	16.89	± 0.43	1.82	± 0.32	8.81	± 1.70	3.19	± 0.89	11.37	± 1.94	2.35	± 0.30	11.80	± 1.28
TO.MONO	1.73	± 0.49	12.11	± 0.36	2.88	± 1.04	14.96	± 0.54	4.02	± 1.07	16.91	± 0.55	5.34	± 0.92	16.88	± 3.69	2.65	± 0.18	15.34	± 1.37
TO.(n-9)	0.33	± 0.09	2.29	± 0.25	1.08	± 0.44	5.56	± 0.54	0.74	± 0.12	2.58	± 1.60	0.44	± 0.26	2.49	± 1.60	0.75	± 0.14	4.65	± 0.84
TO.(n-7)	1.01	± 0.28	7.10	± 0.11	1.43	± 0.46	7.49	± 0.37	2.93	± 0.71	12.71	± 0.20	3.93	± 1.18	11.04	± 2.87	1.47	± 0.01	8.14	± 0.27
TO.POLY	6.58	± 1.97	46.02	± 0.52	11.32	± 4.04	58.94	± 2.70	13.05	± 3.86	57.28	± 3.52	17.51	± 5.58	56.88	± 4.91	11.10	± 0.63	58.83	± 1.87
TO.(n-6)	0.88	± 0.36	6.06	± 0.70	3.32	± 1.31	17.03	± 0.91	2.08	± 0.56	8.95	± 0.17	2.42	± 0.78	8.08	± 1.48	1.83	± 0.00	10.11	± 0.25
TO.(n-3)	4.03	± 1.11	28.33	± 0.45	5.60	± 1.96	29.48	± 3.54	7.33	± 2.15	31.73	± 1.38	9.55	± 3.09	31.73	± 3.66	6.23	± 0.60	32.05	± 2.39
(n-3)/(n-6)	4.72	± 0.58	4.72	± 0.58	1.74	± 0.28	1.74	± 0.28	3.51	± 0.09	3.55	± 0.09	3.95	± 0.30	3.96	± 0.29	3.40	± 0.33	3.17	± 0.33
22:6/20:5	1.45	± 0.05	1.45	± 0.05	3.57	± 0.51	3.57	± 0.51	0.50	± 0.02	0.58	± 0.14	0.70	± 0.04	0.90	± 0.39	1.30	± 0.07	1.25	± 0.07
TOTAL	14.28	± 4.15	100.00	± 0.00	19.27	± 6.95	100.00	± 0.00	23.32	± 5.76	100.00	± 0.00	30.62	± 8.35	100.00	± 0.00	18.46	± 0.47	100.00	± 0.00

Table 9. Total polar fatty acid composition in gonad oysters fed mono-specific diets expressed in absolute contents (µg mg^{-1}) and relative contents (weight % of total polar fatty acids. ± S.D.).

Because oysters fed T-ISO exhibited lower carbohydrate and higher protein contents than the other diets in week 6, gametogenesis can be supposed to be more advanced or delayed. The former hypothesis is being tested with a histological study of gametogenesis in relation to diet (analysis in progress) but can already be proposed because the first *O. edulis* larval release occurred after feeding with this haptophyte (0.6 million in week 4). For marine bivalves, the reproductive cycle is initially linked to the glycogen storage cycle (Berthelin, Kelnner et Mathieu, 2000). After an initial period of storage, stocked glycogen is used concomitantly with food as an energetic support of gametogenesis. A similar relation between carbohydrate and protein has been already reported (Gabbott & Walker 1971) in the natural environment but the opposite pattern was described when broodstock was fed a mixed diet of *T. suecica*+*I. galbana* under controlled conditions. Such contrasting results within the same study (natural/hatchery: Gabbott & Walker 1971) and between these experiments and ours) could be explained by the low feeding concentration used in this previous study (2 cells μL^{-1}), which led to a decrease in the *O. edulis* condition index in the controlled environment. In the present work, flat oysters were fed *ad libitum* (1 billion cells T-ISO equivalent per day), representing 20–24 cells μL^{-1}, which is 10-fold higher than the level used by Gabbott & Walker (1971). In their study, the initial oyster reserves would certainly have played a major role in the allocation of biochemical components between the tissues. Lastly, the data in the Gabbott & Walker study were based on whole-body flesh whereas our results report the composition of specific tissues separately.

Using a similar approach (specific organ biochemical allocation), Delaporte, Soudant, Lambert, Moal, Pouvreau and Samain, (2006a) reported a similar trend in *C. gigas*, where the protein and glycogen contents were inversely correlated during conditioning, with the maximal protein value recorded during spawning.

Generally, lipids are issued from carbohydrate catabolism (lipogenesis). However, most of the lipids that accumulated in the gonads during gametogenesis are directly obtained from the diet or by transfer from other tissues (Utting & Millican 1997). This is particularly true for PUFAand sterols, which are weakly biosynthesized by bivalves (Chu & Greaves 1991) and thus cannot be obtained from carbohydrate lipogenesis or neosynthesized. Hence, tissue-specific fatty acid and sterol variations are well related to food composition, with a possible and variable metabolism buffering (Soudant, Marty, Moal, Robert, Quéré, Le Coz & Samain 1996; Palacios, Racotta, Kraffe, Marty, Moal & Samain 2005).

The initial fatty acid composition of polar lipids was similar in all tissues analysed, with a preponderance of PUFA (≈50%). The fatty acid contents of the n-3 family were always greater than those of n-6 family, leading to an n-3/n-6 ratio >1. These results are in accordance with data reported on a natural *O. edulis* population in Spain (Abad, Ruiz, Martinez, Mosquera & Sanchez 1995). In the present work, EPA and DHA were the major fatty acids detected in all the organs (gonad, digestive gland, muscle and gills). These results agree with fatty acid seasonal variations in *O. edulis* (Helm, Holland, Utting and East, 1991; Abad, Ruiz, Martinez, Mosquera and Sanchés, 1995) and *Crassostrea virginica* (Trider & Castell 1980).

	Control	*I. aff. galbana*	*C. gracilis*	*S. marinoï*	*T. suecica*
Gonad					
Brassicasterol	18.51 (0.65)	40.24 (2.78)	8.02 (5.05)	10.22 (0.84)	17.96 (0.50)
Cholesterol	31.67 (0.49)	27.88 (2.48)	47.43 (3.17)	29.39 (0.61)	33.20 (0.96)
Campesterol	3.13 (0.09)	2.40 (0.09)	5.52 (6.37)	17.53 (1.86)	3.76 (0.27)
24MeCholesterol	15.21 (0.62)	9.09 (0.88)	8.08 (5.39)	16.05 (1.79)	14.51 (0.66)
Fucosterol	1.05 (0.11)	0.50 (0.06)	8.37 (2.85)	0.82 (0.14)	0.86 (0.16)
Digestive gland					
Brassicasterol	18.39 (0.92)	44.97 (7.72)	8.58 (0.92)	11.35 (9.47)	16.08 (0.45)
Cholesterol	30.91 (0.34)	26.30 (3.42)	49.19 (1.68)	25.47 (3.17)	32.04 (1.10)
Campesterol	3.36 (0.08)	2.39 (0.21)	1.57 (0.08)	22.36 (2.16)	6.03 (0.52)
24MeCholesterol	14.66 (0.56)	9.47 (0.93)	10.37 (0.44)	16.69 (1.12)	16.80 (0.66)
Fucosterol	1.16 (0.13)	0.53 (0.09)	14.98 (1.05)	0.87 (0.54)	0.97 (0.12)
Muscle					
Brassicasterol	18.90 (0.17)	28.11 (0.43)	16.97 (0.95)	16.20 (0.31)	19.54 (0.49)
Cholesterol	33.88 (1.05)	34.75 (1.32)	39.54 (4.03)	38.14 (4.03)	34.83 (0.34)
Campesterol	3.26 (0.05)	2.83 (0.08)	5.46 (4.81)	6.35 (3.33)	3.72 (0.44)
24MeCholesterol	16.16 (0.26)	13.48 (0.84)	14.97 (2.54)	15.87 (1.96)	15.96 (0.12)
Fucosterol	0.93 (0.04)	0.61 (0.14)	2.90 (2.13)	2.37 (2.35)	0.74 (0.09)
Gills					
Brassicasterol	16.82 (0.10)	36.34 (5.16)	10.13 (0.42)	9.90 (1.49)	18.59 (0.41)
Cholesterol	32.98 (0.56)	24.06 (2.23)	46.40 (0.74)	27.11 (0.90)	32.26 (0.66)
Campesterol	3.15 (0.03)	2.11 (0.33)	1.67 (0.13)	18.40 (2.98)	3.92 (0.27)
24MeCholesterol	15.46 (0.16)	12.72 (0.58)	11.18 (0.27)	17.16 (0.10)	14.43 (0.70)
Fucosterol	1.12 (0.11)	0.70 (0.18)	11.26 (0.61)	1.04 (0.16)	0.99 (0.05)

Table 10. Sterol composition of *Ostrea edulis* broodstocks fed on mono-specific diets for different tissues expressed in mean relative content (wt % of total fatty acids ± SD, n=3).

When oysters were fed the diatoms *C. gracilis* and *S. marinoi*, there was a specific accumulation of 20:5(n-3) and when they were fed T-ISO such accretion occurred with 22:6(n-3). These results are partially in agreement with those of Frolov and Pankov (1992), who reported a high correlation (0.65) between the supply of these two particular fatty acids and their concentration in *O. edulis* gonads. Whatever the diet provided and its microalgal fatty acid composition, *O. edulis* gonads accumulated roughly the same amount of 20:5(n-3), varying from 8.5% to 10.7% (Frolov & Pankov 1992). Our study does not fully agree with this result because EPA varied

from 5.5% to 17.3% and a relation was shown between the microalgae EPA content and gonad enrichment, with the highest values corresponding to diatoms and the lowest to T-ISO. Moreover, 22:6(n-3) varied from 6.5% to 11.2% in Frolov and Pankov (1992) study, with a cumulative effect only in the case of a mixed diet while in the present work DHA exhibited a similar low-end value (7%) but a higher content (15%) with T-ISO supply, a microalga known to be particularly rich in this fatty acid. In *O. edulis* gonads, a specific enrichment in EPA and DHA occurred (Frolov & Pankov 1992), which seemed to indicate a specific role of these fatty acids in reproduction. This pattern must, however, be partially incorrect because the present work shows that such enrichment also occurs in the other organs for the neutral fraction (data not reported), with the exception of the gills for DHA. There is accordingly no specific allocation of these two fatty acids to *O. edulis* gonads.

Except the muscle, all tissues showed the same variations as those observed in the gonad. The good and similar relation between the FA supply in the food and its incorporation into the different oyster tissues emphasized the importance of the food and the active transfer from the digestive gland to other tissues during gametogenesis. The absence of a specific imprinting of the gonad compared with other tissues may signify a precocious stage of gametogenesis, with a gonad weakly developed. In contrast, the specific composition of muscle, more independent of the food composition, means a strict FA regulation of this tissue, and a specific need for DHA to maintain its function.

Sterols, on the other hand, are known to play an important role in living organisms as structural components of cell membranes, steroid hormones and vitamin D precursors (Soudant *et al.* 2000). After 6 weeks of experimentation, the relative sterol composition of the organs was significantly influenced by the diet composition. In oysters fed different mono-specific diets, the main sterols, characteristic of each microalgae species, were allocated to all tissues except muscle. Thus, for oysters fed *C. gracilis*, cholesterol, which represented 51% of total sterols in this microalga, was efficiently transferred to the gonad (from 32% on week 0 to 47% on week 6) and other organs. Similar enrichment was recorded for campesterol in oysters fed *S. marinoï* (37% of total sterols in this microalga). Diatoms have been shown to be well assimilated by *O. edulis* and biochemical studies confirmed that their main representative fatty acids (EPA) and sterols (cholesterol and campesterol) were efficiently transferred to the flat oyster gonad as well as to most of the other tissues.

In the present work, *T. suecica* is poorly absorbed when using a physiological assessment. This result was confirmed by biochemical analysis, where no significant enrichment in the main fatty acids was found, in any tissue, at the end of the 6-week feeding experiment. Thus, 16:4(n-3), which is characteristic of this prasinophyte (Volkman, Jeffrey, Nichols, Rogers & Garland 1989; Robert *et al.* 2004), did not show any variation in the gonad in the present work. Moreover, while campesterol represented 89% of sterols in *T. suecica*, oysters fed this prasinophyte did not show any differences in allocation of this specific sterol. Because such trends also occurred in the other organs, *T. suecica* did not offer any benefit for *O. edulis*

broodstock conditioning or maintenance under controlled conditions. The low food value of this alga for the flat oyster was also indicated by a relative decrease in the mean dry weight and weak fecundity in oysters fed this diet: larval release probably arose from the utilization of original oyster reserves.

From a physiological point of view, T-ISO ranked in an intermediate position. This result was confirmed with an additional trial (data not shown) using a refreshed strain of T-ISO (also obtained from the CCAP). Indeed, as absorption efficiency was only 20%, doubts can be raised about its effective value for broodstock conditioning. Biochemical analysis and specifically 22:5(n-6), characteristic of this haptophyte (Volkman,Jeffrey, Nichols, Rogers & Garland, 1989; Robert *et al.* 2004), clearly showed that this microalga was efficiently incorporated into the gonad and the other tissues, including the gills. Moreover, brassicasterol, which represented 99% of the total sterols in T-ISO, was efficiently transferred and accumulated in all *O. edulis* organs (from 17% in week 0 to 45% in week 6). Moreover, the highest fecundity was recorded when *O. edulis* was fed T-ISO, although such results should be analysed carefully because the entire flat oyster spawning season can last up to 3 months (Helm, Holland et Stephenson, 1973, González-Araya & Robert, unpubl. obs.), and a previous study under controlled conditions reported that early larval release and total fecundity were not related (González-Araya & Robert, unpubl. obs.). These two approaches therefore yielded conflicting results on the role of T-ISO in *O. edulis* conditioning. Such contradictory results have already been reported with this same microalga when considering *C. gigas* larval development (Rico-villa,

Le Coz, Mingant & Robert 2006). Indeed, when fed as a mono-specific diet, this microalga is poorly ingested and larval development is accordingly rather low. In contrast, associated with the diatom *Chaetoceros calcitrans* forma *pumilum*, in different proportions, microalgae uptake increases markedly, resulting in a higher and more reproducible larval performance (Rico-Villa, Le Coz, Mingant & Robert, 2006) than that obtained with the diatom alone. Such additive effects have often been attributed to a better balance in the dietary components (Helm, Bourne & Lovatelli, 2004), which is true when considering EPA and DHA but that should also be explained by a higher food uptake. Because T-ISO was not efficiently assimilated by *O. edulis*, it should be rejected from an ecophysiological point of view. However, its high DHA content (15%) and its efficient DHA transfer from microalgae to flat oyster tissues, including the gonad, make T-ISO difficult to replace. Another microalga, rich in DHA and exhibiting higher absorption efficiency, needs to be found. *Rhodomonas salina* is a potential species whose performances will be examined in a forthcoming paper.

Conclusions

1. *Chaetoceros gracilis* and *S. marinoi* are both efficient for *O. edulis* conditioning because of their physiological responses and efficient transfer of EPA and characteristic sterols.

2. In contrast, *T. suecica* holds no interest for *O. edulis* conditioning due to its low ingestion and absorption as well as the poor transfer of dietary components.
3. Conflicting results were found for T-ISO, showing relatively low ingestion and absorption values contrasting with efficient transfer and allocation of DHA and brassicasterol.
4. A mixed diet is therefore recommended for *O. edulis* conditioning to offer a dietary balance. *Chaetoceros gracilis* (or *S. marinoi*)+T-ISO is proposed but a substitute for T-ISO should also be sought.

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The selection of an ideal diet for *Ostrea edulis* (L.) broodstock conditioning (part B)

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Abstract

Four microalgae species (R*hodomonas salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana* and *Pavlova lutheri*) were evaluated to estimate their potential as food for *Ostrea edulis* (L.) reproductive conditioning. Best ingestion and absorption were observed with *R. salina* (3.44 and 1.59 mg g^{-1} h^{-1}, respectively), followed by *T. pseudonana* (2.75 and 0.98 mg g^{-1} h^{-1}) and *P. lutheri* (2.40 and 0.91 mg g^{-1} h^{-1}). Oysters fed *T. weissflogii* exhibited the lowest ingestion and absorption values (1.40 and 0.68 mg g^{-1} h^{-1}). Proximate composition (proteins and carbohydrates) and lipid content (fatty acids and sterols) analysed in four main tissues (gonad, digestive gland, muscle and gills) also differed significantly with diet. Protein ranged from 355 mg g^{-1} in the gonad of oysters fed *P. lutheri* to 837 mg g^{-1} in gills of oysters fed *T. weissflogii*; whereas carbohydrates ranged from 17.5 mg g^{-1} in gills of oysters fed *P. lutheri* to 271 mg g^{-1} in gonads of oysters fed *R. salina*. An overall poor enrichment in total PUFAs across all diets masked some of their potential impact on nutrition. In gonad, however, the major polyunsaturated fatty acids (polar lipid fraction) were EPA (≈19% for oysters fed *T. weissflogii* and 14% for those fed *P. lutheri*) and DHA (17% for oysters fed *P. lutheri* and 15% for those fed *R. salina*). Sterol contents showed a clear transfer from food to oyster tissues except with *P. lutheri*, from which neither methylpavlovol nor ethylpavlovol (characteristic of Pavlophyceae) were detected in oyster tissues. Histological analysis showed that gametogenesis was active in oysters fed *R. salina* and *T. weissflogii*, whereas only low gonadic development occurred in unfed oysters or those fed *P. lutheri*. *R. salina* is accordingly highly recommended for *O. edulis* broodstock conditioning whereas *P. lutheri* should be excluded.

Introduction

Broodstock conditioning is a key step in the process of rearing bivalves under standardized conditions. Its success has often been estimated in terms of the quality of bivalve eggs and larvae produced. Thus, initial egg lipid contents have been found to be positively correlated with either larval survival (e.g., *Mercenaria mercenaria* and *Crassostrea virginica*: Gallager and Mann, 1986; *Pecten maximus*: Le Pennec et al., 1991) or larval growth (*Ostrea edulis*: Helm et al., 1973). Although temperature has been considered to be the main environmental factor regulating bivalve reproduction (e.g., *C. gigas*: Fabioux et al., 2005; *Mytilus galloprovincialis*: Fearman and Moltschaniwsky, 2010); feeding (i.e., the amount of food supplied) also seems to be an important factor for in- creasing fecundity (e.g., *C. gigas*: Chavez-Villalba et al., 2003; *Argopecten purpuratus*: Martinez et al., 2000a, 2000b). In contrast, the influence of the relative food value of different phytoplankton species (nutritional quality) on mollusc gonadic development has been very little explored, especially in *O. edulis*. The pioneer works of Frolov and Pankov (1992) and Millican and Helm (1994) provided relevant information in this field but only a few studies (Berntsson et al., 1997) have been carried out since. Indeed the appearance in France in the 1970-1980s of epizooties of *Marteilia refringens* (Comps, 1970) and *Bonamia ostreae* (Comps et al., 1980) and their progressive extension throughout Europe (see review in Laing et al., 2005) led to the collapse of *O. edulis* culture and research then focused more effort on recently introduced species (e.g., *C. gigas*: Helm and Millican, 1977; Robert et al., 1982; and *R. philippinarum*: Helm, 1990; Utting and Spencer, 1991).

In Europe, particularly France and Spain, *O. edulis* remains an emblematic species and attempts to develop "resistant strains" have been made in both countries (Lallias et al., 2010; Montes et al., 2003; Naciri-Graven et al., 1988). Moreover, such interest in flat oyster cultivation is now increasing in France due to high *C. gigas* juvenile mortalities (Pernet et al., 2010; Samain and Mc Combie, 2008).

To allow *O. edulis* genetic improvement through selection, the reliability of hatchery methods for this species needs to be improved. As already pointed out, conditioning is an important step in hatchery production of molluscs and particular attention needs to be paid to flat oyster feeding during this stage because hatchery-conditioned broodstock has been found to have lower fecundity than wild stock (Helm et al., 1991). We had already made an initial study to look for an ideal diet for *O. edulis* (González-Araya et al., 2010). This work compared four monospecific microalgal diets based on ecophysiological and biochemical approaches, and assumed that the best microalgae should be those that were highly ingested, digested, assimilated and efficiently allocated to the reproductive compartment. The present study was designed to provide complementary information by testing the influence of four more microalgae on *O. edulis* consumption, ingestion, assimilation and reproduction.

Material and methods

The techniques used in this study were previously detailed in González-Araya et al. (2010), so only a brief outline will be given here.

Experimental design

In August 2008, *O. edulis* aged 18 months (≈5 cm length and 0.5 g flesh dry weight), originating from Bay of Quiberon (South Brittany, France) were submersed, at 5 m depth, for 1 month, in mesh bags tied to trestles in the Bay of Brest. They were then returned to the quarantine area of the Argenton hatchery, where they were maintained at 14 °C for an additional month, during which they were treated for a week with chloramphenicol at 8 mg l^{-1} to limit any development of vibrios. Thereafter, seawater temperature was increased by 1 °C weekly and, at beginning of October 2008, the flat oysters were transferred to translucent 50-l tanks where they were distributed homogeneously (30 oysters per tank, corresponding to an equivalent biomass of ≈1 kg total weight and 16 g dry flesh weight). During this pre-conditioning period oysters were fed a mixed diet of T. Iso and *Chaetoceros gracilis* used routinely in Argenton to feed most of mollusc at different stages of development (Ben Kheder et al., 2010). Triplicate tanks were set up for each of the four single diet species tested here. Oysters were maintained in a flow-through system at 19 °C and fed constantly at 900 $\mu m^3 \mu l^{-1}$ or unfed (only receiving continuously 1 µm-filtered-seawater). Four different microalgae were tested as mono-specific diets at the same biovolume (measured daily and accordingly including variation in cell volume): *Rhodomonas salina* (mean volumetric size 160 μm^3, mean dry weight 130 pg $cell^{-1}$ strain CCAP 978/24), *Thalassiosira weissflogii* (900 μm^3, 250 pg $cell^{-1}$, strain CCAP 1085/1), *Thalassiosira pseudonana* (40 µm3, 35 pg $cell^{-1}$, strain CCAP 1085/3) and *Pavlova lutheri* (40 μm^3, 20 pg $cell^{-1}$, strain CCAP 931/1).

The choice of these species was based on their frequency of utilization in different mollusc commercial hatcheries worldwide (Borowitzka, 1997; Robert and Trintignac, 1996). Ingestion and absorption of the different microalgae were studied according to Beiras et al. (1994) over six consecutive weeks. It was hypothesized that the microalgae species that was best absorbed by oysters represented the best potential diet; hence, we tested this hypothesis by examining nutrient biochemical allocation in the gonads in the polar fraction compared with other tissues to control potential reproductive specificity.

Microalgae were grown in standard batch culture, based on the step-by-step method (Robert et al., 2004). Cultures grown in 300-l cylinders were used as feed for oysters when they attained the late logarithmic phase after 3–5 days. Conwy medium (Walne, 1966) was used at 1 ml l^{-1} except for *R. salina*, which was cultivated on a double dose of nutrients to improve growth.

Ecophysiological measurements

Ingestion was estimated by measuring algal concentration twice a day using an electronic coulter counter (Multisizer 3) at the inlet (Ci) and outlet (Co) of each tank with consumption C=Ci−Co. For all diets, pseudofaeces production (PF) was b 10% and considered as nil so that consumption≈ingestion (C≈I).

Tanks were drained and oysters cleaned three times a week (Monday, Wednesday and Friday) and daily faeces production, established over precise 24-h periods, was accordingly measured on the two other days: Tuesday for *R. salina* and *T. weissflogii* and Thursday for the other two diets. Faeces samples were collected onto a 450 °C

pre-combusted GF/C (Glass Filter, type C : grade 1.2 µm) filter using a vacuum pump and washed with ammonium formate solution. Faeces total weight was measured after drying at 75 °C and the Organic Matter fraction (% OM) calculated by the difference after combustion at 450 °C for 4 h. The procedure was similar for microalgae, for which 25 ml of culture were filtered for each sample.

For each diet, the coefficient of variation was 10% and the data were accordingly pooled to express the mean faeces production over the entire experimental period. Under such conditions, absorption (A) was defined as A=I (Ingestion)×ae (absorption efficiency) and ae was defined as: ae=100×(OMA−OMF/ [(1−OMF)×OMA]), where OMA is the microalgae relative organic content and OMF the faeces relative organic content (Conover, 1966).

Biochemical analysis

At the beginning and end of the experimental period, 15 oysters per feeding condition were dissected to sample four different organs separately: gonads (Gn), digestive gland (Dg), adductor muscle (Am) and gills (G). For each diet, three pools were prepared of each of the four organs, each pool containing the tissues of five oysters; these were then stored at −80 °C for a period of up to 6 months prior to analysis.

Aliquots of the homogenate were analysed separately for protein (Lowry et al., 1957), carbohydrate (Dubois et al., 1956), fatty acids and sterols (Folch et al., 1957). Fatty acids were analysed after transesterification with BF3 according to Marty et al.

(1992) and Delaporte et al. (2006), whereas sterols were evaluated after transesterification with sodium methoxide (Eder et al., 1992; Soudant et al., 2000). Fatty acids and sterols were identified by comparing their retention time with standards (23:0 for FA and cholestane for sterols). In the present study, we initially only planned to report the fatty acids of the polar lipid fraction, as we did in our sister work (González-Araya, submitted for publication), because they correspond to real assimilation. However, we extended our analysis to some of the main fatty acids of the neutral lipid fraction because this additional data was useful to improve our understanding of the fate of these compounds, as mentioned in the discussion.

Gametogenesis survey

At the beginning and end of the experimental period, 15 oysters per diet were frozen and stored at −80 °C until gonad histological analysis. Gamete activity was assessed using Mann's maturity index (Mann, 1979) to classify oysters into five categories on a scale of 0–4 ranging from inactivity (0) to spent (4).

Statistical analyses

After logarithmic transformation (log10 [xi]) of ingestion and absorption data, and angular transformation of percentage data by the function [arcsin (square root xi/ 100)] for biochemical composition, statistical analyses were carried out using SIGMAPLOT software (version 11.0). Significant differences were detected

between the means at the 5% threshold using ANOVA and a posteriori multiple comparison test of the means (Pairwise Holm-Sidak Method).

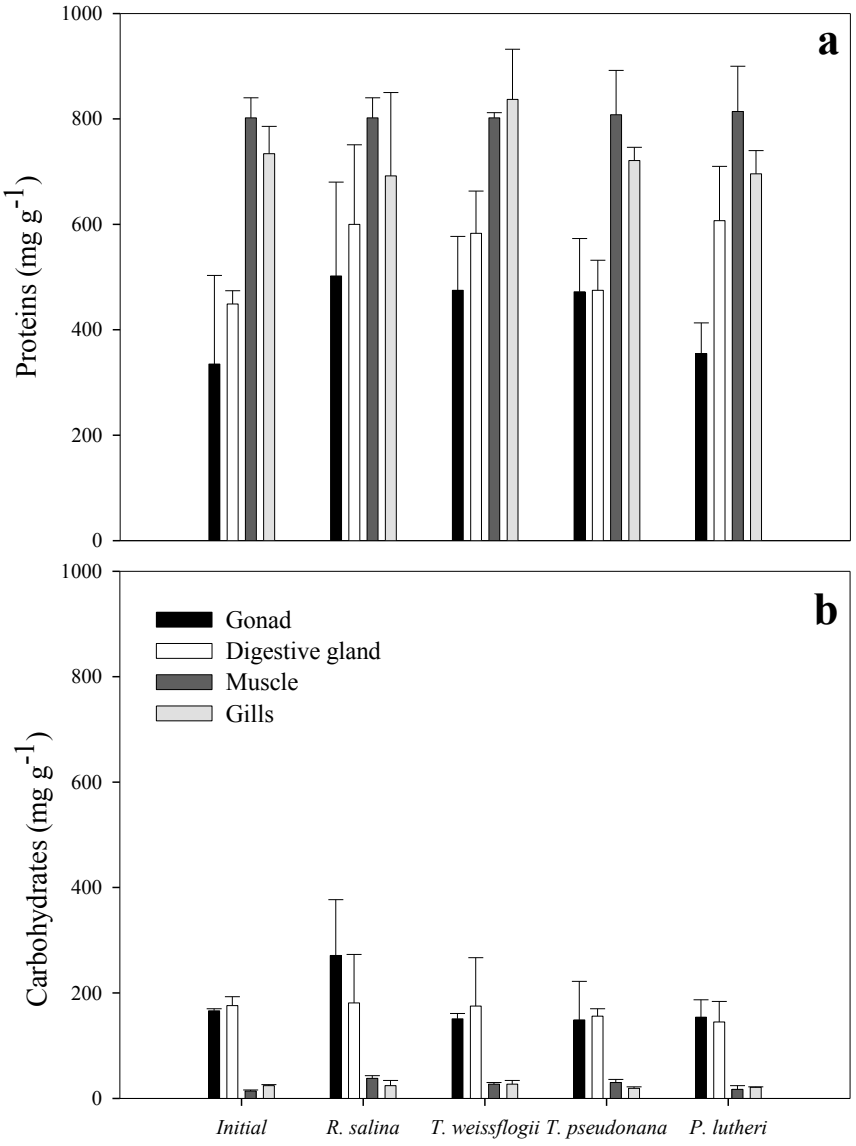

Fig. 8. Protein (a) and carbohydrate (b) contents of gonad, digestive gland, muscle and gills in European flat oyster *Ostrea edulis* (L.) broodstock, fed four microalgae species (values expressed in dw g^{-1} tissue ± S.D.; n = 3).

Results

Effects of food on physiological parameters

Cumulative mortality remained low in all diets (2%) meaning that they could be objectively compared.

The highest ingestion rate reached by *O. edulis* was 3.44± 0.22 mg g^{-1} h^{-1}, achieved when they were fed *R. salina*. In contrast, *T. weissflogii* led to low ingestion, with a mean value of 1.40 ± 0.17 mg g^{-1} h^{-1}. Oysters fed *T. pseudonana* or *P. lutheri* showed intermediate positions, with mean ingestion rates of 2.75 ± 0.22 and 2.41 ± 0.30 mg g^{-1} h^{-1}, respectively.

Similarly, flat oysters fed *R. salina* exhibited a significant higher absorption value (A = 1.62 ± 0.04 mg g^{-1} h^{-1}) than those fed the other three diets ($p< 0.001$). Despite *R. salina* and *T. weissflogii* showed similar absorption efficiencies (ae = 47% and 51% respectively), *R. salina* absorption was twice as high as that of *T. weissflogii* (A = 0.68 ± 0.02 mg g^{-1} h^{-1}). In contrast oysters fed *T. pseudonana* or *P. lutheri* showed similar absorption values and absorption efficiencies (A= 0.98 ± 0.10 and 0.91 ± 0.11 mg g^{-1} h^{-1}, ae = 33-34%, respectively). When flat oysters were supplied with feed composed of these two last microalgae, no significant differences were recorded in ingestion, absorption and assimilation values ($p>>0.05$).

Diet composition

Diatoms and flagellates differed in their fatty acid and sterol contents (Table 11). Whereas *R. salina* and *P. lutheri* were rich in 22:6(n-3), which represented 8 to 11% of total fatty acids, respectively (vs. 3.5 to 4.5% for the *Thalassiosira*). Diatoms were characterized by high EPA concentration (14.5 to 20.5%), but 20:5(n-3) content was surprisingly the highest in *P. lutheri* (23.5% vs. 9.5% in *R. salina*). *R. salina* was also particularly rich in 18:2(n-6) (18% vs. 0.5 to 2.5% for the three other microalgae; $p = 0.011$; Table 11). In contrast, *R. salina* was poor in 16:1(n-7), with only 0.7% vs. 16 to 20% for the other three diets and accordingly such overall differences were significant ($p = 0.036$). However, no mean row differences were found by use of a multiple posteriori test. Arachidonic acid (AA; 20:4 (n-6)) was highly variable between species without any overall trend. Thus, with only 0.2% AA *T. weissflogii* was the microalga with the poorest level, contrasting with the other *Thalassiosira* (*pseudonana*), which had the highest content (9.4%). AA represented 2.4% in *R. salina* but was a fifth of this amount in *P. lutheri* (≈0.5%). Lastly, with values ranging from 0.2 to 0.9, the DHA/EPA ratio did not fluctuate widely (Table 11).

With around 79% of total sterols, *T. weissflogii* and *T. pseudonana* were rich in 24-methylen-cholesterol compared with the two flagelates (vs. 0 to 2%). Despite lower contents (4.4 to 6%), cholesterol was also characteristic of the diatoms (vs. 0.4% for both flagellates) (Table 11). *R. salina* was characterized by a high brassicasterol content (97%); whereas, this sterol was inexistent in the other diets tested here. *P. lutheri* contained specific sterols, including methylpavlovol (36% of total sterols),

ethylpavlovol (16%), methylporifera (14%) and β-sitosterol (12%), that were not detected in the other diets (Table 11).

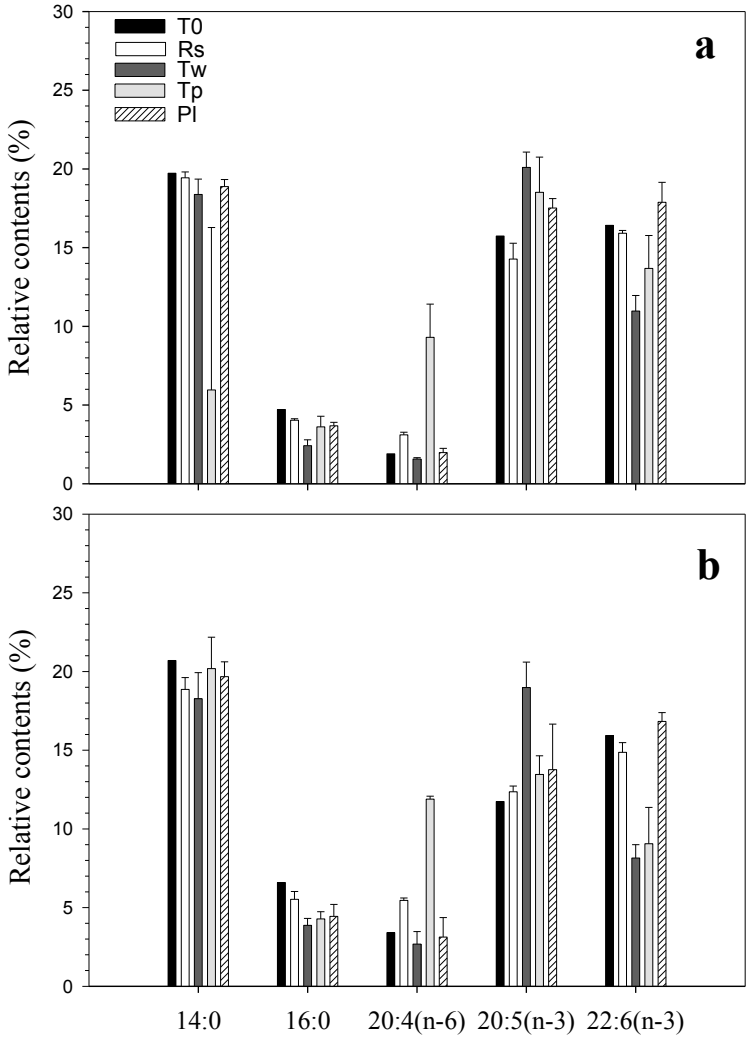

Fig. 9. Main fatty acid composition of neutral (a) and polar lipids (b) in gonad of *Ostrea edulis* broodstock according to diet: T0: Initial contents; Rs: *Rhodomonas salina*; Tw: *Thalassiosira weissflogii*; Tp: *Thalassiosira pseudonana* and Pl: *Pavlova lutheri*, expressed in relative contents (mean weight % of fatty acids ± S.D.)

Effect of food on oyster biochemical composition

Mean gonad protein content increased from 335 mg g^{-1} at the beginning of the experiment to 470 to 501 mg g^{-1} at the end in oysters fed all diets except *P. lutheri*, which led to no significant rise (355 mg g^{-1}). After 6 weeks, the highest gonad protein content was observed in oysters fed *R. salina* 501.5 ± 178 mg g^{-1}, but differences with the other diets were not significant ($p = 0.43$); (Fig. 1a). Protein increase was also independent of diet in flat oyster muscle (802–815 mg g^{-1} vs. 718 mg g^{-1} initially; $p = 0.63$), gills (692–837 mg g^{-1} vs. 734 mg g^{-1} initially; $p = 0.32$) and digestive gland (476–608 mg g^{-1} vs. 449 mg g^{-1} initially; $p = 0.18$) (Fig. 8a).

There was no enrichment in carbohydrate in oyster gonad, gills or digestive gland with any of the diets ($0.59 > p > 0.13$) (Fig. 8b). Oysters did, however, show carbohydrate accumulation in muscle ($p < 0.001$) with all the diets except *P. lutheri*: levels increased from an initial 13.9 ± 2 mg g^{-1} to 38.9 ± 5 mg g^{-1} with *R. salina*; where as, they only reached 17.5 ± 7 mg g^{-1} with *P. lutheri* (Fig. 8).

Whatever tissues were analyzed, the main polar fatty acids found over all diets were 16:0, 18:0, 20:4 (n-6), 20:5(n-3), 22:2j and 22:6(n-3) which represented ≈ 50-65% of total fatty acids (gonad = 62%: Table 12), (digestive gland = 64%: Table 13), (muscle = 55%: Table 14) gills = 52%: Table 15). These specific fatty acids represented initially 65-67% of total FA regardless oyster tissues.

	Oyster diets							
Fatty acid	*R. salina*		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
14:0	7.26	(2.88)	7.86	(1.23)	6.76	(0.89)	10.04	(0.63)
16:0	13.60	(2.23)	13.60	(0.88)	24.05	(0.22)	19.45	(2.20)
18:0	0.54	(0.23)	0.00	(0.00)	0.00	(0.03)	0.43	(0.10)
16:1(n-9)	[a] 1.12	(0.22)	[b] 0.00	(0.00)	[b] 0.00	(0.00)	[b] 0.00	(0.00)
16:1(n-7)	0.74	(0.36)	20.14	(3.25)	17.30	(0.10)	16.27	(4.21)
18:1(n-9)	1.26	(0.56)	0.00	(0.00)	0.99	(0.04)	1.31	(0.33)
18:1(n-7)	2.04	(0.32)	1.18	(0.08)	1.24	(0.08)	1.72	(0.03)
16:2(n-4)	[a] 0.09	(0.01)	[b] 5.85	(0.90)	[ab] 2.21	(0.02)	[a] 0.48	(0.06)
16:3(n-4)	[a] 0.00	(0.05)	[b] 17.95	(1.44)	[c] 5.39	(0.10)	[d] 0.09	(0.01)
18:2(n-6)	[a] 18.04	(4.03)	[b] 0.57	(0.01)	[b] 1.25	(0.02)	[b] 2.42	(0.30)
18:3(n-6)	3.76	(1.61)	0.27	(0.01)	2.56	(0.02)	1.72	(0.27)
18:3(n-3)	[a] 11.51	(2.46)	[b] 0.59	(0.09)	[b] 0.13	(0.01)	[ab] 1.57	(0.44)
18:4(n-3)	[a] 13.66	(1.58)	[b] 1.54	(0.01)	[ab] 5.23	(0.45)	[ab] 6.62	(0.55)
20:4(n-6)	[a] 2.41	(0.20)	[b] 0.22	(0.01)	[c] 9.41	(0.01)	0.46	(0.35)
20:5(n-3)	9.51	(0.85)	20.43	(4.56)	14.70	(0.89)	23.37	(2.08)
22:5(n-6)	0.20	(0.21)	0.00	(0.00)	0.00	(0.00)	1.01	(0.26)
22:6(n-3)	8.18	(2.64)	3.60	(0.55)	4.64	(0.03)	10.75	(0.39)
TO.MONO	8.35	(1.29)	22.47	(5.89)	19.67	(0.28)	19.86	(4.27)
TO.(n-9)	2.48	(0.67)	0.09	(0.01)	0.99	(0.16)	1.37	(0.11)
TO.(n-7)	3.13	(0.29)	21.58	(2.22)	18.55	(0.08)	18.14	(4.22)
TO.POLY	68.74	(6.28)	52.86	(6.33)	47.08	(0.75)	49.41	(2.87)
TO.(n-4)	[a] 0.09	(0.40)	[b] 23.80	(1.57)	[c] 7.60	(0.10)	[ad] 0.57	(0.07)
TO.(n-6)	[a] 24.55	(5.49)	[b] 1.05	(0.26)	[ab] 13.45	(0.04)	[ab] 5.94	(0.77)
TO.(n-3)	44.02	(4.07)	26.35	(2.62)	24.71	(1.15)	42.79	(2.26)
(n-3)/(n-6)	[a] 1.79	(0.57)	[b] 24.97	(2.81)	[a] 1.84	(1.36)	[a] 7.21	(0.49)
22:6/20:5	0.86	(0.31)	0.18	(0.01)	0.32	(0.01)	0.46	(0.03)
22:5/20:4	[a] 0.08	(0.14)	[a] 0.00	(0.00)	[a] 0.00	(0.00)	[b] 2.19	(0.55)
fg cell^{-1}	26081.65	(13746.85)	5874.23	(708.67)	7593.36	(562.47)	2672.66	(636.59)
Sterols								
Brassicasterol	[a] 97.26	(0.45)	-	-	-	-	-	-
Cholesterol	[a] 0.36	(0.32)	[b] 4.36	(0.77)	[b] 6.10	(0.08)	[a] 0.40	(0.06)
Campesterol	[a] 0.28	(0.18)	[ab] 3.20	(1.11)	[b] 6.96	(0.05)	[ab] 2.90	(0.07)
24-Me-cholesterol	[a] 2.10	(0.31)	[b] 78.60	(1.10)	[b] 79.46	(0.34)	-	-
Stigmasterol	-	-	-	-	-	-	[a] 16.43	(1.95)
Isofucosterol	-	-	[a] 11.20	(0.80)	[b] 3.97	(0.14)	-	-
Methylporifera	-	-	-	-	-	-	[a] 13.80	(0.89)
β-sitosterol	-	-	-	-	-	-	[a] 11.58	(1.41)
Fucosterol	-	-	[a] 2.96	(0. 14)	[a] 3.52	(0. 06)	-	-
Desmosterol	-	-	-	-	-	-	[a] 1.57	(0.11)
Methylpavlovol	-	-			-	-	[a] 36.08	(1.90)
Ethylpavlovol	-	-			-	-	[a] 16.43	(1.44)
fg cell^{-1}	434.26	(193.97)	1685.47	(449.12)	135.04	(36.74)	806.16	(57.64)

Table 11 : Fatty acid and sterol composition of total lipids of *Rhodomonas salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana* and *Pavlova lutheri* expressed in mean relative content (weight % of total polar fatty acids ± S.D., n=3). Values within the same line with a superscript letter are not significantly different at p=0.05.

Neither total fa nor specific fa enrichment of the gonad was found (Table 12). Thus, no significant differences between diets were found for 16:0 (p=0.99), with values ranging from 18.3 to 20.2% after six weeks of conditioning, compared with 20.7% at the beginning of the experiment (Table 12). Similarly no significant 18:0, 20:4 (n-6), 20:5(n-3), 22:2j, 22:6(n-3), values fluctuations were noted over time ($p = 0.83$; $p = 0.10$; $p = 0.88$; $p = 0.89$; $p = 0.98$ respectively: Table 12). In contrast, significant 20:2 (n-6) enrichment occurred when oysters were fed *R. salina* (1.3%) ($p = 0.03$) (Table 12). It was the only fa exhibiting significant differences over time in the gonad when values were expressed as relative content. When considering absolute content and beyond the main fatty acids only a significant 20:4 (n-6) increment was noted in oysters fed *T. pseudonana.*

There was no specific fatty acid transfer to the gonad because the trends found for the main fatty acids in the gonad were similar to those in the digestive gland except for 20:4(n-6), which accumulated when oysters were fed *T. pseudonana* (Table 13). When considering absolute content in minor fatty acids, an enrichment in 20:2(n-6) occurred when oysters were fed *R. salina* (Table 13); whereas, a decrease in 22:5(n-6) was noted for those fed *T. weissflogii* and *T. pseudonana* contrasting with the enrichment found with *P. lutheri* (Table 13). Most of the other minor fatty acids in the digestive gland showed differenceswhen considering absolute values but no clear trend can be seen (Table 13). A similar overall situation was also found for the main fatty acids in muscle except for 16.0, whose absolute values decreased ($p < 0.001$) with all diets (Table 14), 20:4(n-6) and 22:2j which increased in oysters fed *T. pseudonana* and *T. weissflogii* respectively (Table 14). As for digestive gland some specific trends can be seen in minor fatty acids when considering absolute values.

Thus an increase in 16:1(n-7), 18:1(n-7), 18:2(n-6) was noted in muscle when oysters were fed *T. weissflogii*, and *R. salina* contrasting with the decrease observed in 16:3(n-3) for all diets (Table 14). A similar overall situation was also found for the main fatty acids in gills except for 20:4(n-6) whose absolute value increased when oysters were fed *T. pseudonana* (Table 15). Similar trends as those reported in muscle can be seen in minor fatty acids when considering absolute values. Thus an increase in 16:1(n-7), 18:1(n-7), 18:2(n-6) was also noted in gills when oysters were fed *T. weissflogii*, and *R. salina*. (Table 15). In contrast a specific increase in 22:5(n-6) occurred with *P. lutheri*.

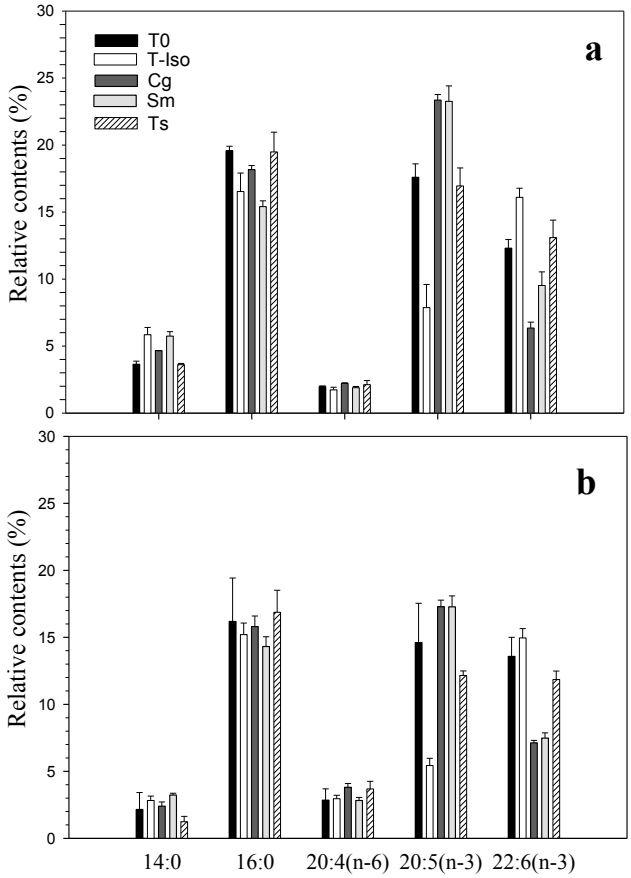

Fig. 10 Main fatty acid composition of neutral (a) and polar lipids (b) in gonad of *Ostrea edulis* broodstock according to diet: T0: Initial contents; T-Iso: *Isochrysis affinis galbana*; Cg: *Chaetoceros gracilis*; Sm: *Skeletonema marinoi*; Ts: *Tetraselmis suecica*, expressed in relative contents (mean weight % of fatty acids ± S.D.).

In the unfed oysters, no significant differences ($p>0.05$) were found in main polar fatty acid composition except for 16:0 in most of tissues at the end of the 6-week experiment (Table 16). Because no gonad development occurred in unfed oysters, it was impossible to separate this organ from the mantle and no data were therefore available on this tissue at the end of conditioning (Table 16).

When fed different microalgae, neutral fatty acid contents followed similar trends to polar fatty acids in all oyster tissues including gonad (Fig. 9). When oysters were fed *T. weissflogii* or *T. pseudonana*, an accumulation of 20:5(n-3) was noted but depletion of 22:6(n-3) was observed in oysters fed the same microalga. An accumulation of 20:4(n-6) was only observed in oysters fed *T. pseudonana* and, to a lesser extent, in oysters fed *R. salina*. No accumulation of 22:6(n-3) in gonad was observed in oysters fed *R. salina*, whatever fraction (neutral or polar) of lipids was analysed (Fig. 9).

Cholesterol allocation in all oyster tissues were not related to diet species because its content remained globally constant (Table 17) even with *T. weissflogii* and *T. pseudonana* supply, however rich in this sterol (Table 11). In contrast, in oysters fed *T. pseudonana*, 24-Methylen-cholesterol enrichment occurred in all tissues except digestive gland (Table 17). Such pattern was also found with *T. weissflogii*, however, to a lesser extend (Table 17). There was accordingly a clear food imprint for 24-Methylen-cholesterol that is the main sterol in both diatoms (80%: Table 11). When oysters were fed *R. salina*, brassicasterol enrichment seemed to occur in all tissues, doubling the concentrations, from ≈20% to 35-45% (Table 17) but such differences were not significant due to high variability in tissues samples of oysters fed *R. salina*

(CV=10-15%). On the other hand, campesterol allocation seemed to be more erratic, with a significant increase in gonad and gills of oysters fed *T. weissflogii* but an enhancement in the digestive gland of oysters fed *T. pseudonana* (Table 17). Lastly, when oysters were fed *P. lutheri*, no assimilation or incorporation of specific *P. lutheri* sterols (methylpavlovol and ethylpavlovol) was observed in any of the tissues which contrasted with the enrichment of β-sisterol in the gonad and gills (Table 17).

Gonad development

At the beginning of experiment, 33% of oysters had recently spawned (stage 4: 33%) or were sexually inactive (stage 0: 50%), while only 17% were at the initiation stage of gametogenesis (stage 1) (Table 18). At the end of experiment, a clear effect of the diets on gametogenesis evolution was visible. Gametogenesis had occurred more rapidly when oysters were fed *R. salina* and *T. weissflogii*, than when they were fed *T. pseudonana* and *P. lutheri*. When oysters were fed *R. salina* at least 50% of the population became ripe, while 40% spawned (Table 18). When fed *T. weissflogii*, 61% of the population became ripe during the experiment, while 20% were still in development (Table 18); thus, on week 6, a total of 1.5 million larvae were collected from oysters fed *R. salina* and 1 million from oysters fed *T. weissflogii*. Among oysters fed *T. pseudonana* 48% were ripe, but 25% of the population were still inactive (Table 8). In contrast, among oysters fed *P. lutheri*, only 7% of individuals were ripe, 43% were developing, and inactive individuals represented 40% of the population (Table 8). No larval release was recorded during the experimental period in either of these last two diets.

Discussion

Impact of diets on physiological responses

The four microalgae differed significantly in their value for *O. edulis* broodstock conditioning. First, they had a differential influence on response of flat oyster nutritional physiology. Ingestion of oysters fed *R. salina* (3.4 mg g^{-1} h^{-1}) was double that of oysters fed *T. weissflogii* (1.4 mg g^{-1} h^{-1}). This discrepancy could be explained by the difference in microalgae cell size between these species, which have a six-fold volume difference: 900 μm^3 vs. 160 μm^3. It may also explain the intermediate position occupied by *T. pseudonana* and *P. lutheri* (2.4 to 2.8 mg g^{-1} h^{-1}), both of which have a lower cell size (40 μm^3). Nevertheless, it has been reported that, among food particles of similar size, *Crassostrea gigas* larvae showed a target preference for *Chaetoceros calcitrans* forma *pumilum* (Rico-Villa et al., 2006). Moreover, it has been reported that external organic components of the diatom *Coscinodiscus perforatus* act as a major selection cue for *Pecten maximus* (Beninger and Decottignies, 2005). Lastly, epicellular molecules, such as lectins, have been showed to play a significant role in food selection of *Mytilus edulis* (Espinosa et al., 2010). Food selection seems to be an active process and, in the present case, *O. edulis* clearly shows a greater affinity preference for *R. salina.*

Fatty acid	Initial		Oyster diets							
			R. salina		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)
14:0	0.16 ± 0.00	1.16 ± 0.88	0.24 ± 0.06	1.63 ± 0.16	0.30 ± 0.11	2.23 ± 0.36	0.33 ± 0.03	3.21 ± 1.39	1.13 ± 1.56	3.28 ± 1.59
16:0	2.81 ± 0.00	20.70 ± 1.25	2.79 ± 0.65	18.86 ± 0.75	2.60 ± 1.20	18.27 ± 1.66	2.22 ± 0.54	20.18 ± 2.00	4.98 ± 5.33	19.68 ± 0.94
18:0	0.89 ± 0.00	6.59 ± 0.45	0.80 ± 0.09	5.52 ± 0.50	0.53 ± 0.16	3.87 ± 0.44	0.49 ± 0.19	4.28 ± 0.45	1.00 ± 0.93	4.44 ± 0.76
16:1(n-7)	0.32 ± 0.00	2.35 ± 1.13	0.14 ± 0.01	0.99 ± 0.11	0.98 ± 0.49	6.82 ± 0.69	0.56 ± 0.06	5.43 ± 2.35	1.16 ± 1.69	3.10 ± 2.02
18:1(n-9)	0.31 ± 0.00	2.27 ± 1.25	0.21 ± 0.03	1.41 ± 0.12	0.24 ± 0.15	1.62 ± 0.48	0.15 ± 0.04	1.35 ± 0.07	0.74 ± 0.94	2.43 ± 0.65
18:1(n-7)	0.23 ± 0.00	1.72 ± 1.69	0.15 ± 0.03	1.00 ± 0.02	0.58 ± 0.35	3.93 ± 0.77	0.38 ± 0.05	3.52 ± 0.82	0.95 ± 1.23	3.02 ± 0.99
16:3(n-3)	0.37 ± 0.05	2.72 ± 1.06	0.40 ± 0.08	2.86 ± 1.02	0.38 ± 0.25	2.65 ± 1.73	0.27 ± 0.19	2.20 ± 1.13	0.41 ± 0.05	2.96 ± 1.90
18:2(n-6)	0.17 ± 0.03	1.22 ± 0.29	0.95 ± 0.37	6.33 ± 1.27	0.13 ± 0.08	0.83 ± 0.25	0.15 ± 0.02	1.39 ± 0.40	0.46 ± 0.63	1.34 ± 0.63
18:4(n-3)	0.16 ± 0.14	1.19 ± 0.45	0.27 ± 0.09	1.80 ± 0.30	0.08 ± 0.03	0.59 ± 0.05	0.18 ± 0.02	1.71 ± 0.49	0.48 ± 0.69	1.33 ± 0.78
20 :2i	0.05 ± 0.03	0.33 ± 0.00	0.02 ± 0.00	0.12 ± 0.02	0.05 ± 0.02	0.37 ± 0.13	0.03 ± 0.01	0.26 ± 0.02	0.06 ± 0.03	0.33 ± 0.14
20 :2j	0.03 ± 0.00	0.22 ± 0.01	0.01 ± 0.00	0.07 ± 0.02	0.04 ± 0.02	0.29 ± 0.03	0.03 ± 0.02	0.26 ± 0.13	0.04 ± 0.02	0.21 ± 0.09
20:2(n-6)	$^{a'}$ 0.03 ± 0.02	^{ab} 0.22 ± 0.01	$^{b'}$ 0.19 ± 0.08	^{a} 1.26 ± 0.27	$^{a'}$ 0.02 ± 0.01	^{ab} 0.13 ± 0.06	$^{a'}$ 0.01 ± 0.00	^{b} 0.09 ± 0.00	$^{a'b'}$ 0.07 ± 0.08	^{ab} 0.25 ± 0.03
20:4(n-6)	$^{a'}$ 0.46 ± 0.09	3.41 ± 1.01	$^{a'b'}$ 0.80 ± 0.14	5.46 ± 0.15	$^{a'}$ 0.35 ± 0.08	2.68 ± 0.80	$^{b'}$ 1.33 ± 0.41	11.89 ± 0.19	$^{a'}$ 0.58 ± 0.37	3.13 ± 1.24
20:5(n-3)	1.59 ± 0.97	11.74 ± 2.33	1.82 ± 0.41	12.36 ± 0.36	2.71 ± 1.32	18.98 ± 1.62	1.48 ± 0.36	13.46 ± 1.19	4.05 ± 5.00	13.77 ± 2.89
22 :2i	$^{a'}$ 0.17 ± 0.02	1.22 ± 0.08	$^{b'}$ 0.10 ± 0.00	0.67 ± 0.12	$^{b'}$ 0.08 ± 0.02	0.56 ± 0.07	$^{b'}$ 0.06 ± 0.04	0.48 ± 0.26	$^{a'b'}$ 0.13 ± 0.02	0.92 ± 0.58
22 :2j	0.88 ± 0.31	6.52 ± 0.69	0.45 ± 0.02	3.15 ± 0.48	0.97 ± 0.38	6.94 ± 0.90	0.55 ± 0.34	4.52 ± 2.03	0.93 ± 0.34	5.86 ± 3.10
22:4(n-6)	0.09 ± 0.06	0.69 ± 0.09	0.08 ± 0.07	0.58 ± 0.50	0.05 ± 0.01	0.40 ± 0.17	0.11 ± 0.08	0.93 ± 0.44	0.07 ± 0.02	0.44 ± 0.24
22:5(n-6)	$^{a'b'}$ 0.15 ± 0.04	1.10 ± 0.09	$^{a'}$ 0.07 ± 0.05	0.54 ± 0.41	$^{a'}$ 0.05 ± 0.02	0.38 ± 0.05	$^{a'}$ 0.06 ± 0.04	0.47 ± 0.19	$^{b'}$ 0.45 ± 0.28	2.49 ± 1.06
22:5(n-3)	0.16 ± 0.10	1.15 ± 0.11	0.16 ± 0.01	1.11 ± 0.17	0.16 ± 0.03	1.22 ± 0.29	0.09 ± 0.05	0.73 ± 0.23	0.18 ± 0.15	0.85 ± 0.22
22:6(n-3)	2.16 ± 0.99	15.93 ± 2.89	2.18 ± 0.37	14.87 ± 0.62	1.15 ± 0.50	8.15 ± 0.85	1.07 ± 0.52	9.06 ± 2.30	4.27 ± 4.60	16.83 ± 0.56
TO.MONO	2.23 ± 0.57	16.45 ± 1.58	1.85 ± 0.34	12.58 ± 0.20	3.18 ± 1.51	22.25 ± 2.03	2.12 ± 0.55	19.19 ± 1.50	4.70 ± 5.61	16.62 ± 2.51
TO.(n-9)	0.46 ± 0.02	3.37 ± 1.36	0.35 ± 0.12	2.40 ± 0.71	0.33 ± 0.13	2.39 ± 0.00	0.24 ± 0.03	2.23 ± 0.56	0.96 ± 1.22	3.17 ± 0.83
TO.(n-7)	1.25 ± 0.09	9.23 ± 1.64	0.81 ± 0.12	5.54 ± 0.31	2.41 ± 1.16	16.85 ± 1.58	1.53 ± 0.32	14.00 ± 2.06	3.16 ± 3.87	10.85 ± 2.20
TO.POLY	6.87 ± 1.36	50.69 ± 3.58	8.49 ± 1.64	57.72 ± 0.71	6.67 ± 2.84	47.29 ± 2.87	5.74 ± 2.13	50.34 ± 4.06	13.11 ± 13.55	53.56 ± 3.35
TO.(n-4)	0.05 ± 0.00	0.37 ± 0.01	0.03 ± 0.01	0.18 ± 0.05	0.18 ± 0.12	1.23 ± 0.33	0.08 ± 0.00	0.72 ± 0.22	0.15 ± 0.21	0.45 ± 0.20
TO.(n-6)	0.93 ± 0.09	6.87 ± 0.99	2.26 ± 0.55	15.27 ± 0.74	0.65 ± 0.20	4.83 ± 0.91	1.77 ± 0.54	15.77 ± 0.38	1.82 ± 1.64	8.20 ± 1.50
TO.(n-3)	4.77 ± 1.25	35.14 ± 3.47	5.62 ± 1.06	38.25 ± 0.77	4.70 ± 2.11	33.04 ± 2.95	3.22 ± 1.17	28.29 ± 2.21	9.96 ± 11.24	37.54 ± 2.45
TO. NMI	1.13 ± 0.08	8.30 ± 1.87	0.58 ± 0.03	4.01 ± 0.63	1.14 ± 0.44	8.16 ± 1.07	0.68 ± 0.41	5.52 ± 2.42	1.15 ± 0.41	7.32 ± 3.89
(n-3)/(n-6)	$^{a'b'}$ 5.11 ± 2.33	5.11 ± 0.78	$^{a'}$ 2.51 ± 0.15	2.51 ± 0.15	$^{b'}$ 7.05 ± 1.76	7.05 ± 1.76	$^{a'}$ 1.80 ± 0.18	1.80 ± 0.18	$^{a'b'}$ 4.73 ± 1.26	4.73 ± 1.26
22:6/20:5	1.36 ± 0.78	1.36 ± 0.01	1.20 ± 0.08	1.20 ± 0.08	0.43 ± 0.05	0.43 ± 0.05	0.68 ± 0.21	0.68 ± 0.21	1.26 ± 0.24	1.26 ± 0.24
22:5/20:4	$^{a'}$ 0.32 ± 0.05	0.32 ± 0.08	$^{b'}$ 0.10 ± 0.07	0.10 ± 0.07	$^{a'b'}$ 0.15 ± 0.02	0.15 ± 0.02	$^{b'}$ 0.04 ± 0.02	0.04 ± 0.02	$^{c'}$ 0.80 ± 0.16	0.80 ± 0.16
TOTAL	13.56 ± 8.49	100.00 ± 0.00	14.71 ± 2.92	100.00 ± 0.00	13.99 ± 5.61	100.00 ± 0.00	11.23 ± 3.52	100.00 ± 0.00	25.53 ± 27.63	100.00 ± 0.00

Table 12 : Fatty acid composition of the polar fraction in gonad of flat oysters fed mono-specific diets (weight % of total acids±S.D.).Values within the same line with a common superscript letter, in the corresponding column (x′ for absolute value, x for relative value), are not significantly different at p=0.05.

A similar trend was observed for absorption, but there was also high absorption efficiency with *T. weissflogii*. (51%). Although the oysters fed *R. salina* showed the best ingestion and absorption of the study, the values remained lower than those previously reported with *C. gracilis* and *S. marinoi*(≈ 5 mg g^{-1} h^{-1}: González-Araya et al., 2011). Absorption efficiency (ae%) ranged from 33 to 51%, while those obtained with the four other microalgae tested in our sister study ranged from 19 to 46% (González-Araya et al., 2011). It is interesting to note that, whichever of the eight different species the microalgae was supplied, *O. edulis* absorption efficiency on

single diets was lower than that achieved with a mixed diet, as reported by Savina and Pouvreau (2004) who observed 80-90% ae with *Glycymeris glycymeris* and *Phaphia romboïdes*. It is also notable that high ae has been reported in tropical mollusc species fed single- species diets: 70–90% ae in *Pinctada maxima* fed T- Iso or *Dunaliella primolecta* and 80–90% ae in *Pinctada margaritifera* fed the same diets (Yukihira et al., 1998).

			Oyster diets							
Fatty acid	Initial		*R. salina*		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)	Contents (µg mg^{-1})	Rel. Contents (%)
14:0	0.20 ± 0.03	1.37 ± 0.16	0.22 ± 0.11	1.06 ± 0.16	0.23 ± 0.04	1.39 ± 0.15	0.20 ± 0.02	1.64 ± 0.05	0.25 ± 0.05	1.61 ± 0.24
16:0	2.70 ± 0.13	18.12 ± 0.61	3.53 ± 1.26	17.50 ± 0.24	2.84 ± 0.12	17.51 ± 0.36	2.27 ± 0.18	18.17 ± 0.35	3.07 ± 0.62	19.53 ± 1.65
18:0	1.01 ± 0.06	6.75 ± 0.23	1.49 ± 0.55	7.37 ± 0.25	0.85 ± 0.03	5.24 ± 0.14	0.73 ± 0.04	5.84 ± 0.20	0.87 ± 0.19	5.50 ± 0.27
16:1(n-7)	$^{a'c'}$ 0.24 ± 0.02	^{b} 1.64 ± 0.08	$^{a'}$ 0.11 ± 0.05	^{a} 0.54 ± 0.08	$^{b'}$ 0.67 ± 0.11	^{b} 4.12 ± 0.47	$^{c'}$ 0.33 ± 0.03	^{ab} 2.66 ± 0.12	$^{a'c'}$ 0.22 ± 0.04	^{ab} 1.41 ± 0.08
18:1(n-9)	$^{a'}$ 0.32 ± 0.01	2.16 ± 0.04	$^{a'b'}$ 0.22 ± 0.08	1.06 ± 0.06	$^{a'b'}$ 0.24 ± 0.02	1.50 ± 0.03	$^{b'}$ 0.15 ± 0.02	1.22 ± 0.04	$^{a'}$ 0.28 ± 0.05	1.79 ± 0.01
18:1(n-7)	$^{a'}$ 0.24 ± 0.01	^{ab} 1.63 ± 0.02	$^{b'}$ 0.15 ± 0.06	^{b} 0.72 ± 0.04	$^{c'}$ 0.53 ± 0.07	^{a} 3.28 ± 0.25	$^{a'd'}$ 0.33 ± 0.04	^{a} 2.62 ± 0.09	$^{e'}$ 0.00 ± 0.00	^{c} 0.00 ± 0.00
16:3(n-3)	0.37 ± 0.02	2.50 ± 0.07	0.77 ± 0.33	3.75 ± 0.33	0.53 ± 0.20	3.32 ± 1.50	0.35 ± 0.04	2.80 ± 0.19	0.51 ± 0.08	3.24 ± 0.19
18:2(n-6)	$^{a'}$ 0.15 ± 0.01	^{a} 1.03 ± 0.03	$^{b'}$ 0.91 ± 0.32	^{b} 4.49 ± 0.08	$^{a'}$ 0.09 ± 0.02	^{a} 0.58 ± 0.07	$^{a'}$ 0.10 ± 0.01	^{a} 0.82 ± 0.09	$^{a'}$ 0.14 ± 0.02	^{a} 0.91 ± 0.06
18:4(n-3)	$^{a'}$ 0.13 ± 0.02	0.85 ± 0.13	$^{b'}$ 0.27 ± 0.09	1.35 ± 0.05	$^{a'}$ 0.08 ± 0.01	0.50 ± 0.11	$^{a'}$ 0.14 ± 0.01	1.09 ± 0.04	$^{a'}$ 0.11 ± 0.02	0.68 ± 0.06
20 :2i	$^{a'}$ 0.08 ± 0.01	0.51 ± 0.03	$^{b'}$ 0.03 ± 0.01	0.16 ± 0.01	$^{a'c'}$ 0.08 ± 0.01	0.51 ± 0.08	$^{b'}$ 0.04 ± 0.00	0.32 ± 0.02	$^{a'}$ 0.08 ± 0.02	0.52 ± 0.06
20 :2j	$^{a'}$ 0.04 ± 0.00	0.26 ± 0.01	$^{b'}$ 0.01 ± 0.00	0.07 ± 0.01	$^{a'}$ 0.06 ± 0.01	0.35 ± 0.07	$^{a'}$ 0.05 ± 0.01	0.38 ± 0.03	$^{a'}$ 0.05 ± 0.01	0.30 ± 0.02
20:2(n-6)	$^{a'}$ 0.03 ± 0.00	^{a} 0.21 ± 0.00	$^{b'}$ 0.23 ± 0.06	^{b} 1.17 ± 0.11	$^{a'}$ 0.03 ± 0.01	^{a} 0.18 ± 0.05	$^{a'}$ 0.01 ± 0.00	^{a} 0.09 ± 0.01	$^{a'}$ 0.04 ± 0.01	^{a} 0.25 ± 0.00
20:4(n-6)	$^{a'b'}$ 0.72 ± 0.01	^{b} 4.86 ± 0.11	$^{a'c'}$ 1.33 ± 0.50	^{ab} 6.58 ± 0.26	$^{b'}$ 0.44 ± 0.03	^{b} 2.73 ± 0.10	$^{c'}$ 1.64 ± 0.20	^{a} 13.07 ± 0.54	$^{a'b'}$ 0.77 ± 0.17	^{b} 4.87 ± 0.20
20:5(n-3)	$^{a'b'}$ 1.71 ± 0.08	11.45 ± 0.36	$^{a'b'}$ 2.37 ± 0.82	11.76 ± 0.04	$^{a'}$ 2.86 ± 0.32	17.59 ± 0.92	$^{b'}$ 1.30 ± 0.16	10.36 ± 0.47	$^{a'b'}$ 1.74 ± 0.39	11.04 ± 0.51
22 :2i	$^{a'}$ 0.19 ± 0.00	^{a} 1.27 ± 0.00	$^{b'}$ 0.13 ± 0.04	^{ab} 0.64 ± 0.03	$^{c'}$ 0.08 ± 0.00	^{b} 0.47 ± 0.02	$^{c'}$ 0.07 ± 0.00	^{ab} 0.56 ± 0.02	$^{a'}$ 0.17 ± 0.04	^{ab} 1.09 ± 0.03
22 :2j	$^{a'b'}$ 1.18 ± 0.02	7.93 ± 0.18	$^{a'}$ 0.75 ± 0.27	3.69 ± 0.15	$^{b'}$ 1.63 ± 0.02	10.12 ± 0.73	$^{a'}$ 0.99 ± 0.11	7.94 ± 0.33	$^{b'}$ 1.63 ± 0.36	10.33 ± 0.21
22:4(n-6)	$^{a'b'}$ 0.10 ± 0.01	0.66 ± 0.05	$^{a'}$ 0.13 ± 0.05	0.64 ± 0.03	$^{b'}$ 0.04 ± 0.00	0.24 ± 0.03	$^{a'}$ 0.14 ± 0.02	1.14 ± 0.11	$^{a'b'}$ 0.08 ± 0.02	0.48 ± 0.01
22:5(n-6)	$^{a'}$ 0.21 ± 0.00	^{ab} 1.43 ± 0.06	$^{a'b'}$ 0.14 ± 0.06	^{a} 0.71 ± 0.02	$^{b'}$ 0.05 ± 0.00	^{a} 0.33 ± 0.01	$^{b'}$ 0.08 ± 0.01	^{a} 0.62 ± 0.05	$^{c'}$ 0.60 ± 0.07	^{b} 3.87 ± 0.29
22:5(n-3)	$^{a'b'}$ 0.17 ± 0.00	1.12 ± 0.04	$^{a'}$ 0.21 ± 0.08	1.01 ± 0.03	$^{a'b'}$ 0.16 ± 0.01	0.98 ± 0.02	$^{b'}$ 0.10 ± 0.01	0.81 ± 0.04	$^{a'b'}$ 0.11 ± 0.02	0.70 ± 0.06
22:6(n-3)	$^{a'b'}$ 2.40 ± 0.06	16.07 ± 0.46	$^{b'}$ 3.17 ± 1.07	15.75 ± 0.15	$^{a'}$ 1.48 ± 0.14	9.13 ± 0.35	$^{a'}$ 1.30 ± 0.09	10.40 ± 0.25	$^{a'b'}$ 2.45 ± 0.44	15.60 ± 0.74
TO.MONO	2.43 ± 0.08	16.32 ± 0.11	2.29 ± 0.83	11.31 ± 0.22	3.07 ± 0.26	18.93 ± 0.46	2.07 ± 0.17	16.58 ± 0.32	2.05 ± 0.40	13.04 ± 0.15
TO.(n-9)	$^{a'c'}$ 0.51 ± 0.03	3.41 ± 0.20	$^{a'c'}$ 0.55 ± 0.20	2.72 ± 0.06	$^{a'}$ 0.68 ± 0.05	4.23 ± 0.15	$^{b'}$ 0.21 ± 0.02	1.69 ± 0.03	$^{b'c'}$ 0.36 ± 0.06	2.30 ± 0.13
TO.(n-7)	$^{a'}$ 1.29 ± 0.04	8.64 ± 0.12	$^{a'}$ 0.94 ± 0.37	4.64 ± 0.22	$^{b'}$ 2.19 ± 0.21	13.47 ± 0.48	$^{a'}$ 1.42 ± 0.14	11.34 ± 0.15	$^{a'}$ 1.08 ± 0.21	6.88 ± 0.23
TO.POLY	7.90 ± 0.20	52.95 ± 1.02	11.74 ± 4.09	58.25 ± 0.75	8.03 ± 0.34	49.59 ± 1.00	6.58 ± 0.68	52.66 ± 1.15	9.04 ± 1.76	57.50 ± 1.59
TO.(n-4)	$^{a'}$ 0.06 ± 0.01	^{ab} 0.40 ± 0.03	$^{a'}$ 0.03 ± 0.01	^{a} 0.12 ± 0.02	$^{b'}$ 0.15 ± 0.02	^{b} 0.92 ± 0.05	$^{a'}$ 0.06 ± 0.01	^{ab} 0.46 ± 0.02	$^{a'}$ 0.04 ± 0.01	^{ab} 0.27 ± 0.02
TO.(n-6)	$^{a'}$ 1.27 ± 0.02	^{ab} 8.50 ± 0.13	$^{b'}$ 2.91 ± 1.02	^{a} 14.43 ± 0.24	$^{a'}$ 0.69 ± 0.06	^{b} 4.27 ± 0.09	$^{a'b'}$ 2.05 ± 0.24	^{a} 16.41 ± 0.65	$^{a'b'}$ 1.69 ± 0.30	^{ab} 10.75 ± 0.20
TO.(n-3)	$^{a'b'}$ 5.07 ± 0.16	34.01 ± 0.84	$^{a'}$ 7.88 ± 2.72	39.12 ± 0.32	$^{a'b'}$ 5.33 ± 0.27	32.90 ± 0.39	$^{b'}$ 3.32 ± 0.32	26.58 ± 0.59	$^{a'b'}$ 5.38 ± 1.04	34.23 ± 1.40
TO. NMI	$^{a'b'}$ 1.49 ± 0.03	9.97 ± 0.19	$^{a'}$ 0.92 ± 0.33	4.56 ± 0.18	$^{b'}$ 1.85 ± 0.02	11.45 ± 0.74	$^{a'}$ 1.15 ± 0.12	9.20 ± 0.33	$^{b'}$ 1.93 ± 0.42	12.24 ± 0.23
(n-3)/(n-6)	$^{a'}$ 4.00 ± 0.13	^{ab} 4.00 ± 0.13	$^{b'}$ 2.71 ± 0.02	^{a} 2.71 ± 0.02	$^{c'}$ 7.71 ± 0.25	^{b} 7.71 ± 0.25	$^{d'}$ 1.62 ± 0.04	^{aa} 1.62 ± 0.04	$^{e'}$ 3.18 ± 0.11	^{a} 3.18 ± 0.11
22:6/20:5	$^{a'}$ 1.40 ± 0.03	1.40 ± 0.03	$^{a'}$ 1.34 ± 0.02	1.34 ± 0.02	$^{b'}$ 0.52 ± 0.02	0.52 ± 0.02	$^{c'}$ 1.01 ± 0.07	1.01 ± 0.07	$^{a'}$ 1.41 ± 0.06	1.41 ± 0.06
22:5/20:4	$^{a'}$ 0.29 ± 0.01	^{ab} 0.29 ± 0.01	$^{b'}$ 0.11 ± 0.00	^{b} 0.11 ± 0.00	$^{b'}$ 0.12 ± 0.00	^{b} 0.12 ± 0.00	$^{b'}$ 0.05 ± 0.00	^{b} 0.05 ± 0.00	$^{c'}$ 0.80 ± 0.08	^{a} 0.80 ± 0.08
TOTAL	14.91 ± 0.40	100.00 ± 0.00	20.15 ± 6.95	100.00 ± 0.00	16.20 ± 1.00	100.00 ± 0.00	12.48 ± 1.07	100.00 ± 0.00	15.73 ± 3.10	100.00 ± 0.00

Table 13 : Fatty acid composition of the polar fraction in digestive gland of flat oysters fed mono-specific diets (weight % of total acids ± S.D.). Values within the same line with a common superscript letter, in the corresponding column (x′ for absolute value, x for relative value), are not significantly different at p=0.05.

Impact of diets on biochemical responses

In bivalves, mantle, digestive gland and adductor muscle tissues are considered to serve for storage and translocation of protein and glycogen, lipids and proteins, respectively (Barber and Blake, 1985; Saucedo et al., 2002). Thus, the reproductive cycle of a bivalve can be divided into two processes: storage accumulation (generally glycogen) and gametogenesis (Pouvreau et al., 2006), which is achieved by using previously accumulated reserves and/or available food. The seasonal changes in energy storage and depletion in relation to gametogenesis are well documented (Barber and Blake, 1985). Indeed, the adductor muscle of pectinids is known to be an important energy reserve site, and its utilization is associated with reproductive effort (Soudant et al., 1996). The utilization of carbohydrate from the *O. edulis* adductor muscle as source of energy was not clearly shown in the present study. Nevertheless, at the end of experiment, higher carbohydrate concentrations were recorded in gonads of oysters fed *R. salina*, which were also the oysters with the best gonadic development. For the other tissues analyzed, there were no statistical differences in either protein and carbohydrate contents during conditioning ($p>0.05$) regardless of diet. In the present study, conditioning was carried out from September 2008 to December 2008, and specific environmental effects prior to oyster collection could explain these results. In our experiment, despite a pre-conditioning period in which the oysters were exposed to low temperature and food conditions just after harvest, it is likely that reserves from summer storage remained in most tissues and that these contributed to the initial proximate values measured. This idea is in agreement with results already reported in *O. edulis* (Gabbott and

Walker, 1971) *C. gigas* (Delaporte et al., 2006a) and *Pinctada mazatlanica* (Saucedo et al., 2002), for which the apparent glycogen (80% of carbohydrate contents) and protein increase were observed from January to July, with a second minor peak from August to October.

			Oyster diets							
Fatty acid	Initial		*R. salina*		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)
14:0	$^{a'b'}$ 0.08 ± 0.01	1.02 ± 0.04	$^{a'}$ 0.07 ± 0.01	0.70 ± 0.07	$^{a'b'}$ 0.11 ± 0.02	1.14 ± 0.18	$^{b'}$ 0.12 ± 0.02	1.21 ± 0.11	$^{a'b'}$ 0.08 ± 0.02	0.94 ± 0.13
16:0	$^{a'}$ 1.36 ± 0.05	16.30 ± 0.11	$^{b'}$ 0.89 ± 0.01	9.45 ± 0.23	$^{b'}$ 0.96 ± 0.04	10.04 ± 0.25	$^{b'}$ 0.99 ± 0.14	10.32 ± 0.56	$^{b'}$ 0.84 ± 0.12	9.89 ± 0.60
18:0	0.52 ± 0.00	6.22 ± 0.24	0.56 ± 0.02	5.96 ± 0.19	0.52 ± 0.06	5.43 ± 0.54	0.58 ± 0.08	5.99 ± 0.45	0.52 ± 0.06	6.22 ± 1.31
16:1(n-7)	$^{a'}$ 0.07 ± 0.01	0.89 ± 0.10	$^{a'}$ 0.07 ± 0.01	0.71 ± 0.13	$^{b'}$ 0.28 ± 0.03	2.89 ± 0.28	$^{c'}$ 0.17 ± 0.01	1.76 ± 0.08	$^{a'}$ 0.08 ± 0.02	0.97 ± 0.13
18:1(n-9)	0.14 ± 0.01	1.64 ± 0.10	0.12 ± 0.01	1.29 ± 0.10	0.13 ± 0.02	1.35 ± 0.22	0.12 ± 0.00	1.24 ± 0.06	0.12 ± 0.01	1.39 ± 0.03
18:1(n-7)	$^{a'b'}$0.09 ± 0.01	1.10 ± 0.06	$^{b'}$0.06 ± 0.01	0.68 ± 0.12	$^{c'}$ 0.21 ± 0.01	^{c}2.16 ± 0.13	$^{c'}$0.17 ± 0.03	1.80 ± 0.13	$^{a'b'}$0.10 ± 0.02	1.22 ± 0.18
16:3(n-3)	$^{a'}$ 0.25 ± 0.13	3.03 ± 1.40	$^{b'}$ 0.05 ± 0.02	0.55 ± 0.25	$^{a'}$ 0.08 ± 0.02	0.87 ± 0.19	$^{a'}$ 0.08 ± 0.02	0.86 ± 0.19	$^{a'}$ 0.06 ± 0.01	0.75 ± 0.10
18:2(n-6)	$^{b'}$ 0.06 ± 0.01	^{ab} 0.72 ± 0.04	$^{a'}$ 0.24 ± 0.03	^{a} 2.55 ± 0.33	$^{c'}$ 0.00 ± 0.00	^{b} 0.00 ± 0.00	$^{b'}$ 0.06 ± 0.01	^{ab} 0.64 ± 0.06	$^{b'}$ 0.04 ± 0.01	^{ab} 0.51 ± 0.09
18:4(n-3)	$^{a'}$ 0.05 ± 0.01	0.55 ± 0.14	$^{a'}$ 0.05 ± 0.01	0.58 ± 0.09	$^{b'}$ 0.02 ± 0.00	0.21 ± 0.00	$^{a'c'}$ 0.06 ± 0.01	0.61 ± 0.04	$^{b'}$ 0.02 ± 0.01	0.22 ± 0.08
20 :2i	$^{a'}$ 0.02 ± 0.00	0.25 ± 0.01	$^{a'}$ 0.02 ± 0.00	0.18 ± 0.02	$^{b'}$ 0.04 ± 0.00	0.37 ± 0.04	$^{c'}$ 0.03 ± 0.00	0.31 ± 0.04	$^{c'}$ 0.03 ± 0.00	0.30 ± 0.01
20 :2j	$^{a'}$0.02 ± 0.00	0.28 ± 0.00	$^{b'}$0.03 ± 0.00	0.30 ± 0.03	$^{c'}$0.05 ± 0.00	0.48 ± 0.04	$^{c'}$0.05 ± 0.00	0.49 ± 0.01	$^{b'}$0.03 ± 0.00	0.40 ± 0.01
20:2(n-6)	$^{a'}$ 0.04 ± 0.00	^{ab} 0.46 ± 0.00	$^{b'}$ 0.13 ± 0.01	^{a} 1.35 ± 0.11	$^{a'}$ 0.03 ± 0.00	^{b} 0.34 ± 0.02	$^{a'}$ 0.03 ± 0.00	^{b} 0.35 ± 0.02	$^{a'}$ 0.03 ± 0.01	^{ab} 0.39 ± 0.04
20:4(n-6)	$^{a'}$ 0.29 ± 0.01	3.53 ± 0.04	$^{a'}$ 0.40 ± 0.03	4.27 ± 0.37	$^{a'}$ 0.29 ± 0.01	3.04 ± 0.05	$^{b'}$ 0.71 ± 0.11	7.36 ± 0.53	$^{a'}$ 0.30 ± 0.04	3.59 ± 0.15
20:5(n-3)	$^{a'c'}$ 1.21 ± 0.06	14.54 ± 0.08	$^{a'b'}$ 1.09 ± 0.04	11.47 ± 0.32	$^{c'}$ 1.39 ± 0.01	14.50 ± 0.30	$^{a'b'}$ 1.08 ± 0.08	11.30 ± 0.35	$^{b'}$ 0.96 ± 0.15	11.31 ± 0.75
22 :2i	$^{a'}$ 0.07 ± 0.00	0.89 ± 0.02	$^{a'}$ 0.07 ± 0.00	0.72 ± 0.03	$^{a'}$ 0.07 ± 0.00	0.68 ± 0.01	$^{b'}$ 0.06 ± 0.00	0.68 ± 0.05	$^{a'}$ 0.07 ± 0.00	0.88 ± 0.04
22 :2j	$^{a'b'}$ 0.49 ± 0.00	5.90 ± 0.20	$^{b'}$ 0.45 ± 0.02	4.79 ± 0.17	$^{c'd'}$ 0.6(± 0.02	6.89 ± 0.29	$^{d'e'}$ 0.60 ± 0.04	6.31 ± 0.35	$^{a'e'}$ 0.54 ± 0.04	6.42 ± 0.09
22:4(n-6)	$^{a'}$ 0.06 ± 0.00	0.76 ± 0.03	$^{b'}$ 0.08 ± 0.00	0.84 ± 0.03	$^{a'}$ 0.05 ± 0.00	0.57 ± 0.03	$^{b'}$ 0.09 ± 0.01	0.91 ± 0.15	$^{a'}$ 0.06 ± 0.01	0.73 ± 0.05
22:5(n-6)	$^{a'}$ 0.09 ± 0.00	1.09 ± 0.00	$^{a'}$ 0.09 ± 0.00	0.99 ± 0.03	$^{a'}$ 0.08 ± 0.00	0.81 ± 0.03	$^{a'}$ 0.08 ± 0.01	0.86 ± 0.05	$^{b'}$ 0.15 ± 0.02	1.77 ± 0.14
22:5(n-3)	$^{a'}$ 0.16 ± 0.01	1.94 ± 0.07	$^{a'b'}$ 0.15 ± 0.01	1.57 ± 0.12	$^{a'b'}$ 0.14 ± 0.00	1.45 ± 0.02	$^{a'b'}$ 0.14 ± 0.01	1.48 ± 0.06	$^{b'}$ 0.13 ± 0.01	1.49 ± 0.03
22:6(n-3)	1.73 ± 0.07	20.78 ± 0.08	1.64 ± 0.05	17.34 ± 0.40	1.40 ± 0.05	14.64 ± 0.61	1.42 ± 0.15	14.82 ± 0.37	1.55 ± 0.20	18.24 ± 0.68
TO.MONO	$^{a'}$ 1.36 ± 0.04	16.37 ± 0.17	$^{a'}$ 1.36 ± 0.09	14.31 ± 0.82	$^{b'}$ 1.85 ± 0.05	19.27 ± 0.25	$^{c'}$ 1.63 ± 0.08	17.05 ± 1.06	$^{a'}$ 1.30 ± 0.11	15.37 ± 0.34
TO.(n-9)	0.29 ± 0.01	3.50 ± 0.03	0.33 ± 0.02	3.53 ± 0.16	0.29 ± 0.06	3.02 ± 0.55	0.28 ± 0.01	2.89 ± 0.29	0.28 ± 0.01	3.33 ± 0.18
TO.(n-7)	$^{a'}$ 0.82 ± 0.05	9.82 ± 0.20	$^{a'}$ 0.70 ± 0.08	7.41 ± 0.80	$^{b'}$ 1.23 ± 0.01	12.84 ± 0.24	$^{c'}$ 1.06 ± 0.08	11.05 ± 0.67	$^{a'}$ 0.77 ± 0.08	9.14 ± 0.14
TO.POLY	4.75 ± 0.32	57.09 ± 1.34	4.85 ± 0.06	51.23 ± 0.52	4.52 ± 0.06	47.16 ± 1.13	4.70 ± 0.42	48.95 ± 0.27	4.18 ± 0.55	49.35 ± 1.91
TO.(n-4)	$^{a'}$ 0.02 ± 0.00	0.24 ± 0.04	$^{a'}$ 0.01 ± 0.00	0.14 ± 0.02	$^{b'}$ 0.05 ± 0.01	0.54 ± 0.09	$^{c'}$ 0.03 ± 0.00	0.28 ± 0.04	$^{a'}$ 0.01 ± 0.00	0.15 ± 0.03
TO.(n-6)	$^{a'}$ 0.56 ± 0.02	6.79 ± 0.05	$^{b'}$ 1.00 ± 0.06	10.57 ± 0.76	$^{a'}$ 0.49 ± 0.01	5.10 ± 0.08	$^{b'}$ 1.01 ± 0.13	10.53 ± 0.61	$^{a'}$ 0.61 ± 0.09	7.22 ± 0.45
TO.(n-3)	3.56 0.29	42.73 ± 1.65	3.27 ± 0.12	34.53 ± 1.03	3.17 ± 0.06	33.07 ± 0.96	2.91 ± 0.26	30.35 ± 0.30	2.88 ± 0.40	33.99 ± 1.60
TO. NMI	$^{a'b'}$ 0.61 ± 0.01	7.32 ± 0.21	$^{b'}$ 0.57 ± 0.02	5.99 ± 0.18	$^{c'd'}$ 0.81 ± 0.02	8.41 ± 0.30	$^{d'e'}$ 0.75 ± 0.05	7.79 ± 0.40	$^{a'e'}$ 0.68 ± 0.06	8.00 ± 0.12
(n-3)/(n-6)	$^{a'b'}$ 6.29 ± 0.29	6.29 ± 0.29	$^{c'}$ 3.28 ± 0.33	3.28 ± 0.33	$^{b'}$ 6.49 ± 0.28	6.49 ± 0.28	$^{c'}$ 2.89 ± 0.19	2.89 ± 0.19	$^{d'}$ 4.71 ± 0.15	4.71 ± 0.15
22:6/20:5	$^{a'}$ 1.43 ± 0.01	1.43 ± 0.01	$^{a'}$ 1.51 ± 0.02	1.51 ± 0.02	$^{b'}$ 1.01 ± 0.03	1.01 ± 0.03	$^{c'}$ 1.31 ± 0.07	1.31 ± 0.07	$^{d'}$ 1.61 ± 0.06	1.61 ± 0.06
22:5/20:4	$^{a'}$ 0.31 ± 0.00	0.31 ± 0.00	$^{b'}$ 0.23 ± 0.03	0.23 ± 0.03	$^{b'}$ 0.27 ± 0.01	0.27 ± 0.01	$^{c'}$ 0.12 ± 0.01	0.12 ± 0.01	$^{d'}$ 0.49 ± 0.03	0.49 ± 0.03
TOTAL	8.32 ± 0.36	100.00 ± 0.00	9.47 ± 0.11	100.00 ± 0.00	9.59 ± 0.14	100.00 ± 0.00	9.59 ± 0.82	100.00 ± 0.00	8.46 ± 0.80	100.00 ± 0.00

Table 14 : Fatty acid composition of the polar fraction in muscle of flat oysters fed mono-specific diets (weight % of total acids ± S.D.). Values within the same line with a common superscript letter, in the corresponding column (x′ for absolute value, x for relative value), are not significantly different at p=0.05.

Polyunsaturated fatty acids (PUFAs) EPA (20:5(n-3)) and DHA (22:6(n-3)) have been shown to be essential for a wide variety of molluscs, as well as prawns and fish larvae (Volkman et al., 1989). Thus, phytoplankton species deficient in EPA or DHA have been reported to be poor food value for *C. gigas* larvae and spat (Langdon and Waldock, 1981; Thompson and Harrison, 1992). Moreover, it is likely that DHA plays a major structural and functional role in the cell membranes involved in oogenesis and embryogenesis (Soudant et al., 1996) in *P. maximus* broodstock. The specific role of 20:5(n-3) has been found to be related to energetic functions during embryogenesis of *Crassodoma gigantea* (Whyte et al., 1992) and *P. maximus* larvae (Delaunay et al., 1992; Soudant et al, 1998).

For *O. edulis* broodstock fed diets rich in PUFAs, a transfer of these fatty acids to their larvae was reported by Frolov and Pankov (1992). Lastly, many studies have documented relations between the fatty acid profile of the diet and that of the gonad or larvae contents (Caers et al., 2000; Flores-Vergara et al., 2004; Knauer and Southgate, 1999). The results of the present study differ in that the fatty acid profiles of main tissues did not show a clear correlation with the diets as it has been shown in our previous work with four other microalgae (Fig. 10; González-Araya et al., 2011). These results were unexpected, because fatty acid composition in microalgae is generally specific. Here *R. salina* and *P. lutheri* exhibited high DHA contents, whereas the two *Thalassiosira* species showed high EPA values. Despite this specific composition, no accumulation of DHA above the initial con- tents was recorded in any organs of oysters fed *R. salina* or *P. lutheri*. Initial DHA contents seemed accordingly to be sufficient to cover the needs of the oysters, or had already

reached a maximum level of accumulation in the gonads. Broodstock for this experiment were collected at the end of summer, and it is possible that the second peak of phytoplankton in the natural environment contributed to the PUFAs already present in oyster gonads in their initial state in our experiment. This idea is supported by data from the phytoplankton survey network (REPHY, 2011), which

			Oyster diets							
Fatty acid	Initial		*R. salina*		*T. weissflogii*		*T. pseudonana*		*P. lutheri*	
	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)	Contents ($\mu g\ mg^{-1}$)	Rel. Contents (%)
14:0	0.19 ± 0.07	0.85 ± 0.04	0.13 ± 0.02	0.54 ± 0.05	0.24 ± 0.04	0.82 ± 0.04	0.22 ± 0.02	0.92 ± 0.06	0.20 ± 0.02	0.84 ± 0.06
16:0	3.79 ± 1.58	16.72 ± 0.15	1.84 ± 0.28	7.50 ± 0.51	2.34 ± 0.40	8.00 ± 0.19	2.02 ± 0.12	8.39 ± 0.24	1.90 ± 0.14	8.18 ± 0.49
18:0	1.41 ± 0.61	6.20 ± 0.04	1.28 ± 0.17	5.22 ± 0.33	1.22 ± 0.14	4.18 ± 0.10	1.07 ± 0.05	4.43 ± 0.08	0.97 ± 0.06	4.18 ± 0.32
16:1(n-7)	a' 0.19 ± 0.07	ab 0.87 ± 0.06	a' 0.12 ± 0.01	a 0.49 ± 0.02	b' 0.62 ± 0.09	b 2.13 ± 0.04	c' 0.37 ± 0.02	ab 1.54 ± 0.05	a'd' 0.23 ± 0.02	ab 0.98 ± 0.07
18:1(n-9)	0.39 ± 0.18	1.69 ± 0.05	0.27 ± 0.03	1.11 ± 0.01	0.33 ± 0.03	1.13 ± 0.06	0.25 ± 0.02	1.05 ± 0.11	0.31 ± 0.01	1.33 ± 0.05
18:1(n-7)	a'd' 0.2(± 0.10	1.13 ± 0.03	a' 0.12 ± 0.02	0.50 ± 0.05	b' 0.65 ± 0.14	2.23 ± 0.13	c'd' 0.40 ± 0.03	1.68 ± 0.09	d' 0.33 ± 0.03	1.43 ± 0.09
16:3(n-3)	a' 0.80 ± 0.02	3.90 ± 1.73	b' 0.12 ± 0.01	0.47 ± 0.05	b' 0.23 ± 0.11	0.82 ± 0.44	b' 0.23 ± 0.04	0.94 ± 0.13	b' 0.15 ± 0.01	0.67 ± 0.04
18:2(n-6)	a' 0.14 ± 0.06	a 0.63 ± 0.02	b' 0.59 ± 0.12	b 2.40 ± 0.28	a' 0.00 ± 0.00	c 0.00 ± 0.00	a' 0.09 ± 0.01	ac 0.37 ± 0.01	a' 0.08 ± 0.01	ac '0.36 ± 0.04
18:4(n-3)	a' 0.07 ± 0.02	0.35 ± 0.07	a' 0.07 ± 0.02	0.28 ± 0.06	b' 0.02 ± 0.00	0.08 ± 0.01	a'c' 0.07 ± 0.01	0.29 ± 0.03	b'd' 0.02 ± 0.00	0.10 ± 0.02
20 :2i	a' 0.12 ± 0.05	0.53 ± 0.01	a' 0.06 ± 0.00	0.24 ± 0.02	b' 0.23 ± 0.05	0.77 ± 0.09	a' 0.13 ± 0.01	0.56 ± 0.04	a' 0.14 ± 0.01	0.60 ± 0.07
20 :2j	a' 0.06 ± 0.02	abc 0.26 ± 0.00	a'b' 0.04 ± 0.01	a 0.16 ± 0.02	c' 0.17 ± 0.03	b0.58 ± 0.02	c'd' 0.15 ± 0.00	bc0.60 ± 0.03	c' 0.10 ± 0.00	abc 0.43 ± 0.01
20:2(n-6)	a' 0.05 ± 0.02	a 0.20 ± 0.00	b' 0.24 ± 0.04	b 0.97 ± 0.13	a' 0.06 ± 0.01	a 0.20 ± 0.01	a' 0.02 ± 0.00	a 0.10 ± 0.01	a' 0.04 ± 0.00	a 0.18 ± 0.02
20:4(n-6)	a' 1.47 ± 0.64	ab 6.49 ± 0.05	a' 1.98 ± 0.13	a 8.11 ± 0.24	a' 1.25 ± 0.18	b 4.29 ± 0.07	b' 3.34 ± 0.24	c 13.86 ± 0.53	a' 1.32 ± 0.07	a b 5.69 ± 0.18
20:5(n-3)	a' 2.22 ± 0.96	9.75 ± 0.07	a' 2.08 ± 0.26	8.50 ± 0.35	b' 4.21 ± 0.71	14.40 ± 0.55	a' 1.49 ± 0.10	6.18 ± 0.22	a' 1.71 ± 0.12	7.37 ± 0.36
22 :2i	a'b' 0.39 ± 0.17	1.72 ± 0.03	b'c' 0.22 ± 0.02	0.89 ± 0.08	b'c' 0.17 ± 0.02	0.58 ± 0.03	c' 0.16 ± 0.01	0.68 ± 0.03	b'c' 0.24 ± 0.01	1.04 ± 0.04
22 :2j	b'c' 2.28 ± 1.01	10.03 ± 0.16	a'b' 1.28 ± 0.13	5.25 ± 0.63	c' 3.37 ± 0.47	11.57 ± 0.55	b'c' 2.33 ± 0.02	9.66 ± 0.25	b'c' 2.35 ± 0.09	10.15 ± 0.40
22:4(n-6)	a' 0.20 ± 0.08	ab 0.89 ± 0.02	b' 0.27 ± 0.02	ab 1.09 ± 0.03	a' 0.14 ± 0.01	a 0.49 ± 0.03	c' 0.47 ± 0.03	b 1.95 ± 0.05	a'b' 0.17 ± 0.01	ac 0.73 ± 0.02
22:5(n-6)	a' 0.37 ± 0.15	ab 1.64 ± 0.03	a' 0.22 ± 0.02	a 0.91 ± 0.07	a'c' 0.17 ± 0.02	a 0.59 ± 0.03	a' 0.21 ± 0.00	a 0.86 ± 0.03	b' 0.85 ± 0.05	b 3.68 ± 0.29
22:5(n-3)	0.29 ± 0.12	1.28 ± 0.01	0.26 ± 0.02	1.07 ± 0.00	0.31 ± 0.05	1.06 ± 0.06	0.21 ± 0.01	0.85 ± 0.02	0.16 ± 0.01	0.68 ± 0.05
22:6(n-3)	3.70 ± 1.60	16.26 ± 0.13	3.79 ± 0.40	15.49 ± 0.36	3.24 ± 0.55	11.07 ± 0.61	2.61 ± 0.20	10.82 ± 0.48	3.23 ± 0.11	13.91 ± 0.18
TO.MONO	a'b' 3.25 ± 1.23	14.50 ± 0.77	a' 2.70 ± 0.31	11.04 ± 0.36	b' 4.67 ± 0.63	16.01 ± 0.24	a'b' 3.57 ± 0.07	14.81 ± 0.22	a'b' 3.02 ± 0.10	13.04 ± 0.25
TO.(n-9)	0.50 ± 0.14	2.30 ± 0.38	0.59 ± 0.07	2.42 ± 0.09	0.52 ± 0.04	1.79 ± 0.12	0.42 ± 0.03	1.75 ± 0.20	0.49 ± 0.01	2.10 ± 0.03
TO.(n-7)	a' 1.77 ± 0.75	ab 7.79 ± 0.03	a' 1.12 ± 0.08	a 4.58 ± 0.12	b' 2.99 ± 0.45	b10.23 ± 0.13	a'b' 2.17 ± 0.09	ab 9.02 ± 0.14	a' 1.65 ± 0.09	ab 7.10 ± 0.27
TO.POLY	12.70 ± 5.15	56.25 ± 1.28	12.48 ± 1.11	51.12 ± 0.27	14.05 ± 2.06	48.14 ± 0.14	11.87 ± 0.68	49.22 ± 1.16	11.57 ± 0.32	49.91 ± 0.34
TO.(n-4)	a' 0.08 ± 0.02	0.39 ± 0.08	b'd' 0.02 ± 0.00	0.09 ± 0.01	a'c' 0.08 ± 0.03	0.29 ± 0.09	a'b'c'd' 0.05 ± 0.01	0.21 ± 0.04	d' 0.03 ± 0.00	0.14 ± 0.01
TO.(n-6)	a'b' 2.28 ± 0.95	abc 10.05 ± 0.08	a'c' 3.38 ± 0.31	a 13.85 ± 0.15	b' 1.69 ± 0.24	b 5.80 ± 0.09	c' 4.20 ± 0.27	ac 17.41 ± 0.54	a'b' 2.51 ± 0.06	ab 10.82 ± 0.18
TO.(n-3)	7.49 ± 2.92	33.28 ± 1.31	7.48 ± 0.79	30.63 ± 0.77	8.31 ± 1.28	28.48 ± 0.75	4.83 ± 0.37	20.03 ± 0.86	6.20 ± 0.24	26.71 ± 0.45
TO. NMI	a'b' 2.85 ± 1.26	12.54 ± 0.19	a' 1.59 ± 0.16	6.54 ± 0.75	b' 3.94 ± 0.56	13.51 ± 0.64	a'b' 2.77 ± 0.02	11.50 ± 0.33	a'b' 2.84 ± 0.09	12.23 ± 0.37
(n-3)/(n-6)	a' 3.31 ± 0.10	ab 3.31 ± 0.10	b' 2.21 ± 0.04	ac 2.21 ± 0.04	c' 4.91 ± 0.06	b 4.91 ± 0.06	d' 1.15 ± 0.02	c 1.15 ± 0.02	e'2.47 ± 0.07	abc 2.47 ± 0.07
22:6/20:5	a' 1.67 ± 0.00	1.67 ± 0.00	e'b' 1.82 ± 0.03	1.82 ± 0.03	c'd' 0.77 ± 0.02	0.77 ± 0.02	a'b'd' 1.75 ± 0.07	1.75 ± 0.07	e' 1.89 ± 0.07	1.89 ± 0.07
22:5/20:4	a' 0.25 ± 0.01	ab 0.25 ± 0.01	b'c'd' 0.11 ± 0.01	a 0.11 ± 0.01	c' 0.14 ± 0.01	a 0.14 ± 0.01	d' 0.06 ± 0.00	a 0.06 ± 0.00	e' 0.65 ± 0.07	a 0.65 ± 0.07
TOTAL	22.69 ± 9.67	100.00 ± 0.00	24.41 ± 2.21	100.00 ± 0.00	29.17 ± 4.23	100.00 ± 0.00	24.10 ± 0.83	100.00 ± 0.00	23.19 ± 0.51	100.00 ± 0.00

Table 15 : Fatty acid composition of the polar fraction in gill of flat oysters fed mono-specific diets (weight % of total acids ± S.D.).Values within the same line with a common superscript letter, in the corresponding column (x′ for absolute value, x for relative value), are not significantly different at p=0.05.

show a second peak of phytoplankton at the end of summer 2008 in Bay of Quiberon (Brittany, France).

Indeed it is has been reported that proportions of 22:6(n-3) and 20:5(n-3) from neutral and polar lipids of artificially conditioned oysters are generally lower than those developing in the natural environment (Soudant et al., 1999). In our study, it is noteworthy that initial DHA contents at the beginning of autumn conditioning (16%: Fig. 9b) were similar to DHA values recorded at the end of spring conditioning in oysters fed T-Iso (16% vs 13.5% for spring initial value: Fig. 10b) known to be particularly rich in 22:6(n-3). Gonad needs apparently a determined DHA content which should be achieved during pre-conditioning period in the natural environment depending on this season at collection.

In contrast, an increase in EPA was only observed in gonads of oysters fed *T. weissflogii* when polar lipids were analyzed. When the neutral lipids were examined, however, EPA storage was found in all gonads except those of oysters fed *R. salina*. Such EPA accumulation during conditioning could be related to an insufficient initial 20:5(n-3) content (12%: Fig. 9b), which differs from the final values (17%: Fig. 3b) in all diets tested in this previous study (González-Araya et al., 2011).

Fatty acid 20:4(n-6) was incorporated in both neutral and polar lipids of the gonad, but this incorporation was only noted in oysters fed *T. pseudonana*. This fatty acid is a major precursor of prostaglandins, which may influence the reproduction process in molluscs (Osada et al., 1988). Maturation was however more active in oysters fed *R. salina* and *T. weissflogii*.

Sterols are known to play a variety of roles in living organisms, acting as structural components of cell membranes, steroid hormones and vitamin D precursors

(Soudant et al., 2000). In our study, the relative sterol composition of the organs was significantly influenced by diet. Thus, for oysters fed *T. weissflogii* and *T. pseudonana*, 24-Methylen-cholesterol, was efficiently transferred to the gonad. Similarly, brassicasterol was only accumulated in gonads of oysters fed *R. salina*.

Fatty acid	Oyster organs															
	Gonad				Digestive gland				Muscle				Gills			
	Initial Contents		Final Contents		Initial Contents		Final Contents		Initial Contents		Final Contents		Initial Contents		Final Contents	
	(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)	
14:0	1.16	± 0.88	n.d.	n.d.	1.37	± 0.16	1.01	± 0.22	1.02	± 0.04	0.81	± 0.06	0.85	± 0.04	0.56	± 0.07
16:0	20.70	± 1.25	n.d.	n.d.	18.12	± 0.61	16.37	± 0.90	16.30	± 0.11	* 9.89	± 0.22	16.72	± 0.15	* 7.42	± 0.25
18:0	6.59	± 0.45	n.d.	n.d.	6.75	± 0.23	6.43	± 0.06	6.22	± 0.24	6.20	± 0.23	6.20	± 0.04	5.27	± 0.42
20:4(n-6)	3.41	± 1.01	n.d.	n.d.	4.86	± 0.11	6.72	± 0.78	3.53	± 0.04	4.05	± 0.25	6.49	± 0.05	6.78	± 0.16
20:5(n-3)	11.74	± 2.33	n.d.	n.d.	11.45	± 0.36	11.98	± 0.60	14.54	± 0.08	12.04	± 0.60	9.75	± 0.07	8.77	± 0.42
22:6(n-3)	15.93	± 2.89	n.d.	n.d.	16.07	± 0.46	17.41	± 1.02	20.78	± 0.08	19.00	± 1.24	16.26	± 0.13	15.84	± 0.65
∑ PUFA	50.69	± 3.58	n.d.	n.d.	52.95	± 1.02	58.53	± 2.06	57.09	± 1.34	50.77	± 0.07	56.25	± 1.28	51.38	± 0.39

Table 16 : Main fatty acid composition of the polar fraction of the main tissues of unfed flat oysters (weight % of total acids±S.D.). * indicate significant differences at p=0.05.

In the present work, the sterols characteristic of *P. lutheri* (methylpavlovol and ethylpavlovol) did not show any accumulation in oyster gonads. Because the same trend was also observed in other tissues, we consider *P. lutheri* to be a poor microalgae species for *O. edulis* broodstock conditioning. This verdict is also based on the moderate physiological performances observed (moderate ingestion and absorption) and the poor gametogenesis development noted in oysters fed this species.

Despite the differing fatty acid and sterol composition of the diets, gametogenesis of *O. edulis* was globally successful, except when oysters were fed *P. lutheri* (as already mentioned) or not fed at all. This last point means that, although flat oyster tissue storage was clearly insufficient to support gametogenesis from an energetic point of

view, initial stored reserves could have been crucial for essential requirements poorly covered by some of the diets. Our present study found the highest *O. edulis*

	Oyster diets				
	Initial	*R. salina*	*T. weissflogii*	*T. pseudonana*	*P. lutheri*
Gonad					
Brassicasterol	[ab] 19.7± 1.2	[a] 44.2± 5.0	[b] 8.7± 1.0	[b] 8.8± 0.9	[ab] 12.7± 1.0
Cholesterol	36.3± 1.1	24.5± 3.6	26.1± 0.5	23.0± 0.3	29.3± 2.5
Campesterol	[a] 2.2± 0.2	[a] 1.7± 0.3	[b] 13.5± 0.8	[ab] 5.2± 0.3	[ab] 5.2± 0.7
24-Methylen-cholesterol	[a] 14.0± 0.8	[a] 11.1± 0.6	[ab] 29.9± 0.9	[b] 48.0± 2.4	[a] 8.9± 2.1
Stigmastérol	2.6± 0.0	5.9± 0.8	2.4± 0.5	0.3± 0.5	6.1± 1.0
Isofucosterol	2.5± 0.2	1.0± 0.2	2.5± 0.3	1.6± 1.4	2.1± 0.7
MethylPorifera	1.8± 0.2	1.1± 0.2	1.2± 0.1	1.4± 0.8	1.4± 0.1
β-sitosterol	[ab] 3.2± 0.4	[a] 1.8± 0.2	[ab] 3.5± 0.1	[a] 2.2± 0.7	[b] 22.7± 5.8
Fucosterol	3.6± 0.2	1.2± 0.2	3.8± 0.1	2.7± 0.3	2.2± 0.2
Desmosterol	3.7± 0.1	1.2± 0.1	2.3± 0.5	1.3± 0.4	1.1± 0.1
MethylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
EthylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
Digestive gland					
Brassicasterol	19.3± 0.2	45.0± 7.7	8.6± 0.9	11.3± 9.5	16.1± 0.5
Cholesterol	33.8± 0.3	26.3± 3.4	49.2± 1.7	25.5± 3.2	32.0± 1.1
Campesterol	[a] 2.4± 0.0	[a] 2.4± 0.2	[a] 1.6± 0.1	[b] 22.4± 2.2	[a] 6.0± 0.5
24-Methylen-cholesterol	14.1± 0.5	9.5± 0.9	10.4± 0.4	16.7± 1.1	16.8± 0.7
Stigmastérol	4.1± 0.5	4.0±1.0	2.1± 0.3	0.0± 0.0	2.9±1.0
Isofucosterol	2.5± 0.2	2.7± 0.6	4.1± 0.4	2.5± 0.6	6.8± 0.8
MethylPorifera	1.9± 0.1	0.4± 0.1	0.0± 0.0	5.2± 6.2	0.6± 0.3
β-sitosterol	[ab] 4.3± 0.1	[a] 1.8± 0.6	[ab] 2.0± 0.1	[b] 9.5± 0.8	[ab] 4.7± 0.2
Fucosterol	[a] 3.7± 0.1	[a] 0.5± 0.1	[b] 15.0± 1.0	[a] 0.9± 0.5	[a] 1.0± 0.1
Desmosterol	[a] 5.0± 0.2	[b] 0.9± 0.2	[b] 0.8± 0.1	[b] 1.0± 0.1	[b] 0.9± 0.2
MethylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
EthylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
Muscle					
Brassicasterol	20.3± 0.4	32.9± 2.9	15.0± 0.4	13.5± 1.0	18.5± 0.5
Cholesterol	35.3± 0.8	33.2± 1.4	31.8± 0.3	30.1± 0.8	35.2± 1.0
Campesterol	2.5± 0.0	1.9± 0.0	7.6± 0.3	3.5± 0.2	2.9± 0.9
24-Methylen-cholesterol	[ab] 16.6± 0.1	[b] 13.3± 1.0	[ac] 26.1± 0.4	[c] 35.9± 0.9	[b] 13.2± 0.8
Stigmastérol	3.1± 0.2	3.4± 0.9	3.8± 0.8	3.9± 0.7	4.6± 0.1
Isofucosterol	3.0± 0.3	2.7± 0.1	2.9± 0.2	2.5± 1.2	2.9± 0.9
MethylPorifera	1.5± 0.2	0.8± 0.7	0.9± 0.1	0.8± 0.1	1.1± 0.1
β-sitosterol	3.6± 0.3	2.8± 0.6	3.6± 0.3	2.2± 0.3	10.7± 1.7
Fucosterol	1.8± 0.1	1.0± 0.0	2.1± 0.2	1.3± 0.6	1.6± 0.1
Desmosterol	2.2± 0.2	0.9± 0.2	0.7± 0.2	0.7± 0.1	1.1± 0.2
MethylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
EthylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
Gills					
Brassicasterol	[ab] 19.2± 0.2	[b] 37.3± 3.2	[a] 9.3± 0.2	[a] 9.3± 0.4	[a] 12.6± 0.4
Cholesterol	33.4± 0.3	27.3± 3.8	27.6± 0.4	26.5± 0.6	27.4± 1.0
Campesterol	[a] 2.3± 0.2	[a] 1.7± 0.1	[b] 14.0± 0.5	[a] 5.3± 0.3	[a] 5.4± 0.2
24-Methylen-cholesterol	[bc]13.8± 0.2	[b] 11.8± 0.7	[ac] 31.8± 1.0	[a] 43.0± 0.9	[b] 9.2± 0.9
Stigmastérol	[a] 4.5± 0.1	[a] 7.7± 1.8	[b] 0.0± 0.0	[ab] 3.0± 0.3	[a] 7.5± 0.2
Isofucosterol	2.6± 0.0	1.1± 0.2	1.2± 0.5	1.5± 0.0	1.5± 0.2
MethylPorifera	2.0± 0.1	1.3± 0.2	1.5± 0.1	0.9± 0.2	1.3± 0.0
β-sitosterol	[a] 4.0± 0.2	[a] 1.9± 0.5	[a] 4.6± 0.2	[a] 2.5± 0.4	[b] 24.3± 2.5
Fucosterol	3.4± 0.0	1.1± 0.2	3.1± 0.1	2.4± 0.1	2.0± 0.1
Desmosterol	6.0± 0.1	4.7± 0.5	3.6± 0.2	2.2± 1.6	3.2± 0.1
MethylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0
EthylPavlovol	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0	0.0± 0.0

Table 17 : Sterol composition of different tissues of *Ostrea edulis* fed mono-specific diets (weight % ± S.D.). Values within the same line with a common superscript letter are not significantly different at p=0.05.

reproductive performances in oysters fed *R. salina*, while our sister work recommended *C. gracilis* or *Skeletonema marinoï* as the best from a comparison of four other species (González-Araya et al., 2010). T-Iso also showed some potential for *O. edulis* reproductive conditioning, although this was counterbalanced by low physiological performances with poor ingestion and absorption (González-Araya et al., 2011). *R. salina* offers similar biochemical characteristics as T-Iso but with higher physiological performances, which make it a better candidate. To balance diet and enhance flat oyster fecundity a mixed diet has been recommended (González Araya, submitted for publication). To improve reproductive performances a mixture based on *C. gracilis* (or *S. marinoi*) plus *R. salina* is highly recommended for *O. edulis* broodstock conditioning. The potential benefits of such microalgal assemblages for *O. edulis* broodstock fecundity and the quality of the larvae produced will be examined in a forthcoming paper.

	Stages of gonad maturation				
Broodstock diet	0	1	2	3	4
Initial	50.0	17.0	0.0	0.0	33.0
R. salina	0.0	3.5	3.5	51.7	41.3
T. weissflogii	6.5	9.7	16.1	61.3	6.4
T. pseudonana	24.2	18.2	6.1	48.5	3.0
P. lutheri	40.0	23.1	20.0	6.9	10.0

Table18 : Gonad development (%) in *Ostrea edulis* broodstock fed *R. salina*, *T. weissflogii*, *T. pseudonana* or *P. lutheri* for 6-weeks. Stages: 0, inactive; 1, early active; 2, late active; 3 ripe; 4, spent (see text for explanation: section Gonad development).

Conclusions

1. *R. salina* and *T. weissflogii* are both as efficient feeds for *O. edulis* conditioning.
2. Despite moderate physiological performances, *P. lutheri* has no value for *O. edulis* conditioning due to low or inexistent transfer of dietary components.
3. The analysis of physiological and biochemical performances of flat oysters fed eight different microalgae species tested in separate experiments led us to recommend a mixed diet for *O. edulis* conditioning based on the association of *C. gracilis* (or *S. marinoi*) plus *R. salina*.

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Article N° 4

Article en correction pour Aquaculture

The effect of eight single microalgal diets on sex-ratio and gonad development throughout European flat oyster (*Ostrea edulis* L.) conditioning

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Abstract

European flat oysters (*Ostrea edulis*) were conditioned at two different seasons (spring and autumn) during 40 days at 19°C, in 50 l transparent flow-through tanks (triplicate) and continuously fed an equivalent of 1 billion T-Iso cells $day^{-1}oyster^{-1}$ with eight different types of microalgae, four species per set of trial: *Isochrysis affinis galbana*, *Chaetoceros gracilis*, *Skeletonema marinoi*, *Tetraselmis suecica* (spring), *Rhodomonas salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana*, *Pavlova lutheri* (autumn).At the end of conditioning periods, oysters were sampled and processed for histology analysis for each diet condition. Each oyster was classified for its sex predominance and gonadic development stage. Oysters fed *S. marinoï* and *C. gracilis* exhibited the highest ratio of hermaphrodites with 96 and 77% respectively, where as those fed *T. suecica* showed the lowest hermaphrodite percentage (59%). When oysters were conditioned in autumn with four other species, oysters fed *R. salina* and *T. weissflogii* exhibited 83 and 87% of hermaphrodites. Regardless the diet a gonadic development occurred in spring with ≥ 60% ripe oysters (stage 3) and spawned oysters (stage 4). In autumn, the oysters fed *R. salina* and *T. weissflogii* were highly mature with 90% and 75% of stage 3 and 4 respectively where as those fed *P. lutheri* showed low evolution with only 17% of ripe oysters.

Keywords: *Ostrea edulis*, broodstock conditioning, gametogenesis, microalgal diets.

Introduction

In most of mollusks reproduction, temperature has been considered as the key factor controlling reproduction in the natural surrounding (Sastry, 1966; Barber and Blake, 1983) as well as in controlled condition (Wilson et al., 1996; Chávez-Villalba et al., 2001). Whereas the effect of food has been recognized more recently (Millican and Helm, 1994; Utting and Millican, 1997) the influence of diet quality on *Ostrea edulis* conditioning was however poorly known. We developed accordingly research in that field and it has been showed that mixed diets increased fertility and improved subsequent larval development (González-Araya et al., 2012). Moreover *Chaetoceros gracilis*, *Skeletonema marinoï* and *Rhodomonas salina* were highly ingested, assimilated, absorbed and well allocated in all flat oyster tissue including gonads (González-Araya et al., 2011, 2012). But the effects of different microalgae diets on fine reproduction process has not been analysed yet.

The aim of the present work is accordingly to study the influence of food on sex dominance and gametogenesis evolution during conditioning in *O. edulis*.

Material and methods

Broodstock conditioning

A total of 720 18-month-old flat oysters originated from Brittany were conditioned during 40 days at 19°C, in 50-L transparent flow-through tanks (triplicate) and continuously fed in equivalent of 1 billion T-Iso cells $day^{-1}oyster^{-1}$. Eight different

microalgae diets were delivered as single food to oysters at two different periods to study feeding physiological needs and its incidence on reproduction process. The first experimental period was assessed using four microalgae: *Isochrysis affinis galbana* (T: CCAP 927/14), *Chaetoceros gracilis* (C_g: UTEX LB2658), *Skeletonema marinoï* (S_m: CCAP 66/4), *Tetraselmis suecica* (T_s: CCAP 1077/3), whereas the second set of trials was run with *Rhodomonas salina* (R_s: CCAP 978/24), *Thalassiosira weissflogii* (T_w: CCAP 1085/1), *Thalassiosira pseudonana* (T_p: CCAP 1085/3) and *Pavlova lutheri* (P_l: CCAP 931/1). At the end of conditioning period, oysters were sampled from each tank and processed for histological analysis.

Histological analysis

Each oyster was carefully opened, and a 5-mm thick section of tissue was excised parallel to the anterior-posterior axis between the labial palps and the posterior adductor muscle. They were thereafter fixed in Davidson's solution and embedded in paraffin.Tissue sections achieved in this manner contain gametes representative of the whole gonad. Finally, 5-µm thick sections were stained with Harris's haematoxylin and eosin (Howard and Smith, 1983). Reproduction was studied on histological sections and each oyster was classified according to sex category and gonad development based on da Silva scale (2009). The sex was assigned as following: Indeterminate (I), when material had just collapsed or empty follicles are visible (Fig. 11a); male solely (M), when follicles contained only male gonadic material

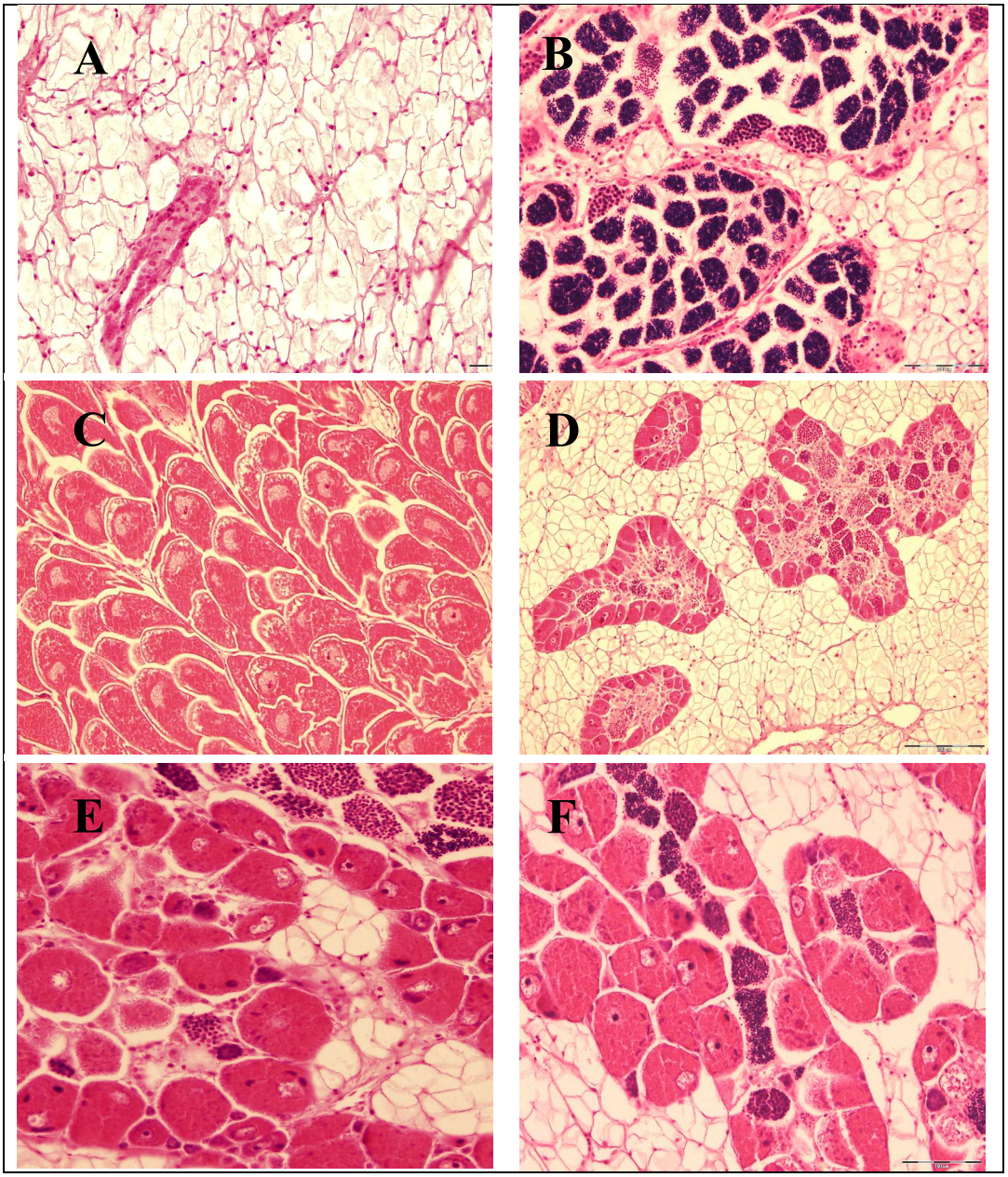

Fig. 11. Microphotographies of histological sections of *Ostrea edulis*, showing the gonad area of different sex categories. **a**: Indeterminate gonad. **b**: Male gonad solely, ripe gonad. **c**: Female gonad solely, ripe gonad. **d**: Hermaphrodite with both sex equally represented, ripe oocytes, initial and advanced spermatogenesis observed. **e**: Hermaphrodite predominantly male, ripe oocytes, partially spawning of spermatozoa balls observed. **f**: Hermaphrodite predominantly female, ripe gonad, ripe oocytes, spermatozoa balls observed.

(Fig. 11b); female solely (F), when follicles contained only female gonadic material (Fig. 11c); hermaphrodite (H), when female or male gonad material were observed, separately of prevalence sex (Fig. 11d-11e-11f).

Then a score of 0 to 4was assigned to these figures with 0= inactive gonad and4= empty due to spawning). Five stages per functional sex of gonad development were considered as following:

- Inactive or resting gonad (0)(Fig.11a): no evidence of ripe gametes development. The gonad is dilated and empty; follicles are located between mantle and digestive gland surrounded by abundant connective tissue.
- Early gametogenesis (1)(Fig. 11b): gonad follicles are more spread into the connective tissue with ovogonia and spermatogonia mostly attached to the follicle wall. In the male part, there are primary and secondary spermatocytes. In the female part, developing oocytes are attached to developing lines.
- Advanced gametogenesis (2)(Fig. 11c): gonad follicles are larger than in the previous phase, but connective tissue is still present. In the male section the development of few spermatogonia still occurred, but sparmatocytes and spermatid balls are dominant; in the female section, oocytes in vitellogenesis are dominant, while oocytes in post-vitellogenesis are sparse.
- Ripe gonad (3)(Figs. 11d, 11e): juxtaposed large follicles occupied the entire area between the mantle and digestive gland. Both male and functional female developing lines, follicles contained gametes, abundant spermatozoa balls and mature oocytes.

- Spawned (4) (Fig. 11f): gonad follicles are smaller than in the previous phase. Gametes have been released and residual mature oocytes and sperm balls could be observed in the follicle lumen. Phagocytes are often observed in the follicle lumen.

When hermaphrodites were detected, the subcategory of one sex predominance was not considered, because the aim of this experience was to study the overall effects of mono-specific diets on gonadic development without detailing hermaphrodite aspects.

Statistical analysis

Comparison of oyster distribution in gonad condition classes (sex and gonad development) between conditioning diet and sex category were analysed using a contingency test and a χ^2 test. Statistical analysis was performed using the software STATISTICA (version 8.0). Differences were considered statistically significant when $p \leq 0.05$.

Results

Oysters fed *S. marinoï* and *C. gracilis* during spring conditioning exhibited the highest percentage of hermaphrodites with 96 and 77% respectively where as those fed *T. suecica* showed the lowest hermaphrodites ratio (59%: Table 19). In autumn when oysters were fed *R. salina* and *T. weissflogii* 83 to 87% hermaphrodites were recorded whereas those fed *T. pseudonana* and *P. Lutheri* led to 58 and 33% hermaphrodites

respectively. However, the highest proportion of female was founded in oysters fed *P. lutheri* (33%) and the lowest in those fed *S. marinoï* (0%: Table 19). Except in oysters fed *R. salina*, male was observed in all samples (Table 1). High percentages of indeterminate were observed in the two initial conditions, spring (21%) and especially autumn (67%) as well as in oysters fed *T. suecica* (24%) and *P. lutheri* (23%). Regardless diet and season, gonadic development occurred. At the end of spring conditioning more than 60% of oysters were ripe (stage 3) or have recently spawned (stage 4) (Fig 12). The highest value of recently spawned individuals was recorded in oysters fed T-Iso, whereas those fed *S. marinoi* and *C. gracilis* showed the highest values of ripe gonads (70 and 55% respectively). Those receiving *T.suecica* and T-Iso exhibited 38 and 31% of ripe gonads respectively. Apart from initial condition, early gametogenesis was only observed in oysters fed *T. suecica* (3%) (Fig 12).

In autumn, the highest values of stages 3 and 4 were observed in oysters fed *R. salina* (>90%) and *T. weissflogii* (> 75%) whereas the lowest value was observed for those fed *P. lutheri* (17%). Initial and advanced gametogenesis (stages 1 and 2) were observed in oysters regardless diets, whereas stage 0 (indeterminate) was undetected in oysters fed *R. salina*. At the initiation of this fall conditioning period, 33% of recently spawned oysters and 67% of indeterminate were found.

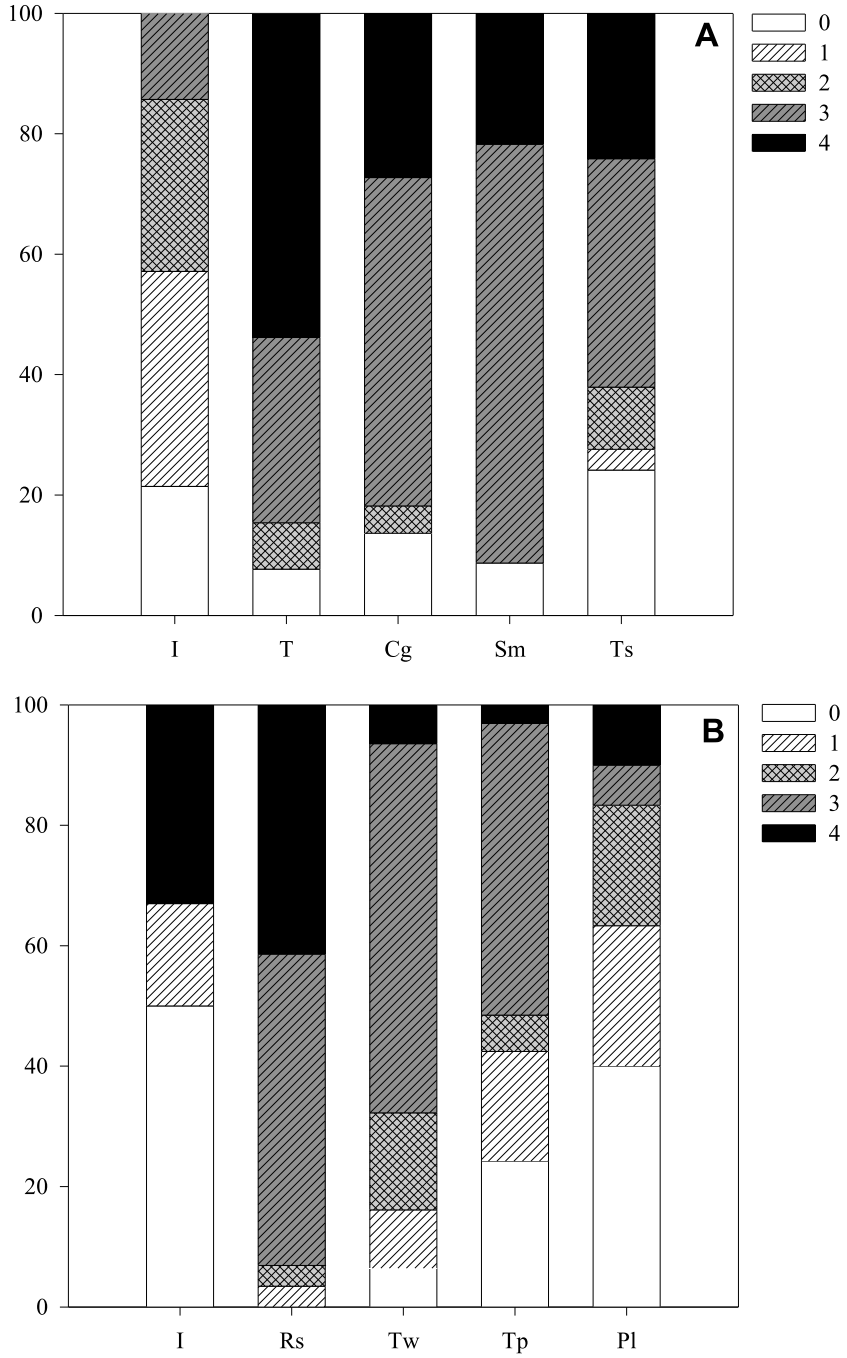

Fig. 12. Effects of microalgal diets fed *Ostrea edulis* on gametogenesis evolution: stage **0**: Inactive gonad. **1**: Early gametogenesis. **2**: Advanced gametogenesis. **3**: Ripe gonad. **4**: Spawned.
A: oysters fed T (*I. aff. galbana*), C_g (*C. gracilis*), S_m (*S. marinoi*), T_s(*T. suecica*).
B: oysters fed R_s(*R. salina*), T_w(*T. weissflogii*), T_p(*T. pseudonana*) and P_l(*P.lutheri*).
I: For both graphs means Initial condition, just before conditioning.

Discussion

At the end of conditioning periods, most of *O. edulis* examined in spring and autumn were hermaphrodites, excepted for initial condition in autumn with 67% of indeterminate. The presence of large number of hermaphrodites, as well as small percentage of males and females may indicate that, gametogenic phase followed the next one without complete resorption as already reported (Siddiqui and Ahmed, 2002).

Table 19. Effects of microalgal diets fed *Ostrea edulis* on sex repartition classes (%).

Sex category	Spring conditioning					Autumn conditioning				
	I	T	C_g	S_m	T_s	I	R_s	T_w	T_p	P_l
Indeterminate	21.4	0.0	4.5	0.0	24.1	66.7	0.0	6.5	12.1	23.3
Male	14.3	15.4	13.6	4.3	10.3	16.7	0.0	3.2	6.1	10.0
Female	0.0	15.4	4.5	0.0	6.9	16.7	17.2	3.2	24.2	33.3
Hermaphrodite	64.3	69.2	77.3	95.7	58.6	0.0	82.8	87.1	57.6	33.3

I: Initial condition; oyster broodstock fed: T: T-Iso; C_g: *C. gracilis*; S_m: *S. marinoi*; T_s: *T. suecica*; R*s*: *R. salina* T_w:*T. weissflogii*; T_p: *T. pseudonana*; P_l: *P. lutheri*.

Whereas temperature effects on gonad development and spawning are nowadays well known (e.g. Mann, 1979; Lossanof, 1962; Helm, 2004; da Silva et al., 2009), the influence of food on the reproductive pattern of bivalves which includes growth, maturation, spawning, resorption and resting period are less documented (Kang et al., 2000). The occurrence of greater proportion of ripe gonad (stage 3) and spawning (stage 4) in oysters fed *C. gracilis*, *S. marinoi*, *R. salina* and *T. weissfloggi* indicate that gametogenesis was more rapid than with other diets used. This result could be explained by specific biochemical allocations in gonads. Despite, the

absence of correlation between fatty acids concentration and gonadic development (González-Araya et al., 2011) it has been showed that cholesterol concentration in *C. gracilis* and *S. marinoi* were higher than in T-Iso and *T. suecica*. For autumn conditioning, similar concentrations of brassicasterol and cholesterol in gonads of oysters fed *R. salina* and *T. weissflogii* have been already reported (González-Araya et al., 2012). The role of cholesterol in mollusks gametogenesis is still unknown but bivalves have a limited capacity for cholesterol synthesis or bioconversion of phytosterols into cholesterol (Napolitano et al., 1993; Kanazawa, 2001). Phytosterols present in oysters reflect accordingly ingested microalgae. Different microalgae synthesize specific phytosterols (Palacios et al., 2007): for example, brassicasterol has been found in high concentrations (90%) in *Isochrysis sp.* (Volkman et al., 1981, Napolitano et al., 1993; Soudant et al., 1998), whereas 24-methylenecholesterol and also cholesterol could be recorded in diatomophyceae such as *Chaetoceros sp.*, *Skeletonema sp.*, *Thalassiosira* sp. (Napolitano et al., 1992; Soudant et al., 1998; González-Araya et al., 2011).

On eight microalgae commonly used in hatchery four of them (*C. gracilis*, *S. marinoï*, *R. salina* and *T. weissflogii*) promoted a better and faster *O. edulis* gonadic development. In contrast, oysters fed *T. suecica* and *P. lutheri* showed the lowest gametogenesis and are accordingly not recommended for broodstock conditioning. T-Iso occupied an intermediate position and combined with a diatom could represent an efficient diet for *O. edulis* broodstock conditioning but never as single diet.

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Chapitre III

RÔLE DE L'ALIMENTATION SUR LE DEVELOPPEMENT LARVAIRE D'*OSTREA EDULIS* L.

La valeur nutritionnelle des microalgues sur les développements larvaire et post larvaire a fait l'objet de nombreux travaux (Walne, 1970 ; Laing et Millican, 1986 ; Albentosa et al., 1996; Brown et al.,1997; Helm et al., 2004 ; Rivero-Rodríguez et al., 2007). Mais celle-ci est spécifique et son incidence peut également varier en fonction du stade étudié. Ainsi, *Isochrysis affinis galbana* a été considérée comme une bonne espèce en terme de croissance et survie larvaire chez *Mercenaria mercenaria* (Helm et Laing, 1987) mais celle-ci est médiocre pour les larves de *C. gigas* (Rico-Villa et al., 2006), *C. rhizophorae* (Helm et Laing, 1987) et *Donax trunculus* (Ruiz-Azcona et al., 1996). De même, *Pavlova lutheri* s'est avérée être de bonne qualité pour le développement larvaire de *Pecten maximus* (Delaunay et al., 1993) tandis que celle-ci ne permet pas la croissance ni la métamorphose des larves de *C. gigas* (Ponis et al., 2008).

D'une façon générale, les régimes monospécifiques sont moins performants que les régimes mixtes car ces derniers assurent un meilleur équilibre des composés biochimiques nécessaires aux stades précoces des mollusques. Même si l'apport de *Chaetoceros gracilis* s'avère cruciale chez *O. edulis*, nous avons précédemment montré que son association avec T-Iso est nécessaire pour assurer une bonne métamorphose. Mais ces performances sont dépendantes de la nutrition des géniteurs au cours du conditionnement et une carence a ainsi été suspectée. Pour

pousser plus en avant cette idée, nous avons nourri des larves d'*O. edulis* avec le même régime parental, de type monospécifique. Ce travail est présenté sous forme d'une publication (Short Note à soumettre).

Nous venons de voir également dans les précédents chapitres, que chez *O. edulis* le régime avait un impact très marqué sur la fertilité des géniteurs et sur le développement ultérieur des larves. Ce régime (*Thalassiosira weissflogii* et *Rhodomonas salina*), choisi arbitrairement à l'origine, s'est avéré être composé d'espèces bien ingérées, assimilées avec une bonne allocation de ces composants biochimiques dans les différents tissus de l'huître. D'autres diatomées telle que *C. gracilis* et *S. marinoï* ont également permis de bonnes performances physiologiques et nous avons de ce fait décliné deux régimes parentaux qui devaient autoriser l'émission de larves de qualité, en grand nombre.

Puis, nous avons, sur la base d'association deux à deux, recherché le meilleur régime pour le développement larvaire et la métamorphose d'*O. edulis* afin de proposer, comme nous l'avions fait chez les géniteurs, le régime larvaire le plus approprié. Ce travail est présenté sous forme d'une publication en cours de soumission et l'ensemble des analyses biochimiques (acides gras et stérols) acquis pour chaque régime larvaire est rapporté en Annexe III.

Article N° 5

Article soumis à **Aquaculture** (AQUA-S-12-00035)

Growth, survival and competence of larvae of *Ostrea edulis* (L.) fed four single diets from conditioning to pre-settlement

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Abstract

Growth, survival, and competence of *Ostrea edulis* larvae (L.) fed four single-species, microalgal diets, *Isochrysis affinis galbana* (T-Iso), *Chaetoceros gracilis*, *Skeletonema marinoi*, *Tetraselmis suecica*, from broodstock to pre-settlement, were studied. Lower larval growth (5.5 μm to 6.0 μm d^{-1}) was recorded in progeny continuously fed *S. marinoi* or *T. suecica* whereas, best growth was achieved with T-Iso (6.0 μm d^{-1}). Compared to a bi-specific diet of T-Iso plus *C. gracilis*, single diets led globally to lower larval growth or competence. Survival, however, was high (> 90%) regardless of microalgal diet, except for oysters and larvae fed exclusively T-Iso (53%) or *T. suecica* (2%). When used alone, the latter species showed, respectively, medium or poor food value for *O. edulis* larvae.

Keywords: *Ostrea edulis*, diet, phytoplankton, larval growth, survival, competence.

Introduction

Broodstock conditioning is an important step in the molluscan hatchery process. Indeed, conditioning allows the extension of the reproductive period (Helm et al., 2004) and leads generally to better gamete quality (Utting and Millican, 1997). The importance of temperature in molluscan reproduction has been thoroughly described (e.g. Mann 1979, Fabioux et al., 2005), but the role of nutrition in

quantitative aspects of reproduction is being recognized increasingly (e.g. Enriquez-Diaz et al., 2009). In *O. edulis,* a 6% daily ration has been shown to be highly effective for broodstock conditioning (Utting, 1993), and the importance of food quality based on different microalgal diets was pointed out by Millican and Helm (1994) and Gonzalez et al. (2012). These latter authors showed, moreover, that single-species, microalgal diets, specifically *Chaetoceros gracilis,* performed quite well compared to the mixed diet TCg (T-Iso + *C. gracilis*). In contrast, larvae fed T-Iso exhibited lower growth but this diet was only tested in a single experiment. Moreover larval performance was related clearly to initial broodstock diet (Gonzalez et al., 2012). Such interactions between broodstock diet and subsequent larval development were addressed in the present work by feeding *O. edulis* larvae the same single diet as broodstock. We hypothesised that maternal nutritional deficiencies could be revealed and compensated at larval development by feeding the larvae a mixed, balanced diet.

Materials and methods

A total of 360 18-month-old flat oysters originating from Brittany were conditioned for 40 days at 19 °C, in 50-L, transparent, flow-through tanks (triplicate) continuously fed an equivalent of 1 billion *Isochrysis affinis galbana* cells day^{-1} $oyster^{-1}$. Four diets were delivered during broodstock conditioning: *Isochrysis affinis galbana* (T-Iso: CCAP 927/14), *Chaetoceros gracilis* (UTEX LB2658), *Skeletonema marinoi* (CCAP 66/4), or *Tetraselmis suecica* (CCAP 1077/3). From each treatment, released larvae

were counted at each expulsion. When a sufficient number was obtained, larvae were distributed in 5-L, translucent, methacrylate cylinders and reared in flow-through conditions with UV treatment, in triplicate. Larvae at a density of 5 mL^{-1} were reared in seawater at 22 °C and 34 ppt salinity. Larvae were fed continuously the same single-species, microalgal diets as delivered to broodstock at 1,500 μm^3 μl^{-1} equivalent T-Iso volume (≈ 40 cells) or the mixed diet T-Iso plus *C. gracilis* (TC_g; v/v). Each broodstock diet was split into treatments with two larval diets, i.e., 8 different larval conditions were assessed: **B**T**L**T, **B**T**L**TC_g, **B**C_g**L**C_g, **B**C_g**L**TC_g, **B**S_m**L**S_m, **B**S_m**L**TC_g, **B**T_s**L**T_s, and **B**T_s**L**TC_g. Thus, the designation **B**T**L**T means that broodstock was fed T-Iso and larvae from them were fed T-Iso; whereas, **B**S_m**L**TC_g means that broodstock was fed *S. marinoi* and larvae from them were fed T-Iso plus *C. gracilis*.

Larval length and survival were quantified on days 3, 6 and 9 under the light microscope by use of an image analysis technique (WinImager 2.0 and Imaq Vision Builder 6.0 software for image capture and treatment, respectively) and direct counting of empty shells (dead larvae). The number of pediveligers ready to set (competent larvae possessing an eyespot) was estimated at the end of the experiment, on day 11.

Statistical analyses were performed using STATISTICA software (version 8.0). Significant differences were detected between the means at the 5% threshold using ANOVA and a posteriori multiple comparison test between the means (Tukeys' test), after transformation of percentage data by the function (arcsin[sqr(χ/100)]) to respect homogeneity of residues distribution (Sokal and Rohlf, 2001).

Results

Effect of diets on fecundity and initial larval size

The number of larvae released after six weeks of conditioning was high when broodstock were fed T-Iso (1.4 million) and low when oysters received *T. suecica* (0.3 million); whereas, similar fecundity was recorded when broodstock were fed either of the diatoms, *S. marinoi* or *C. gracilis* (1 million). The initial larval size ranged from 172 µm (± 2.1) to 179 µm (± 2.7) with no significant difference when oysters were fed either diatoms or T-Iso. In contrast, newly-released larvae were significantly smaller when broodstock were fed *T. suecica* (165 µm ± 3.5).

Effect of diets on larval growth and survival

The lowest larval growth was recorded for larvae fed *S. marinoi* and T-Iso plus *C. gracilis*, when the larvae originated from broodstock fed *S. marinoi* (5.5 and 6.0 µm d^{-1} respectively) and for larvae and broodstock fed *T. suecica* (single diet: 6.0 µm d^{-1}) (Fig. 13A). In contrast, larvae fed the mixed diet and originating from broodstock fed *T. suecica* grew significantly better (mixed diet: 8.0 µm d^{-1}). For larvae originated from broodstock fed T-Iso or *C. gracilis*, no differences ($p>0.05$) were found between treatments regardless of larval diet (single or mixed diet).

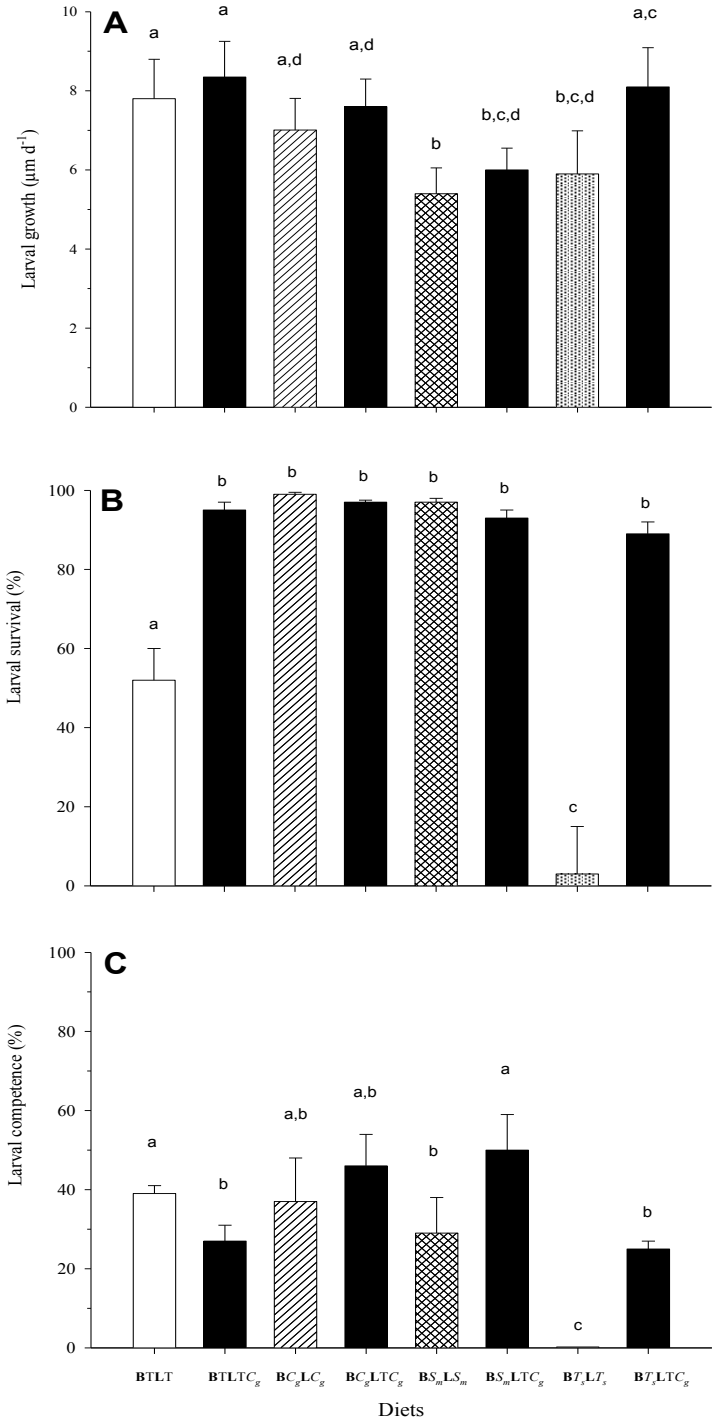

Fig. 13: Mean daily growth, from release to day 11 (A), survival (B) larval competence (C) (± S.D.) of *O. edulis* larvae (**L**) fed different single diets: T: T-Iso, *Cg*: *C. gracilis*, *Sm*: *S. marinoi* and *Ts*: *T. suecica* or bi-specific diet T*Cg*. Respective similar single diets were used during broodstock conditioning (**B**). Vertical bars represent the standard deviation and significant differences ($p<0.05$) among treatments are denoted by different letters.

Under most experimental conditions, larval survival was high, > 90% on day 11, except when larvae and broodstock both were fed single-species diets of T-Iso (53%) or *T. suecica* (2% : Fig. 13B).

Effect of diets on larval competence

Except for larvae fed *T. suecica*, which did not reach competence on day 11, no clear relationships between diet and competence to metamorphosis were found for the other treatments (Fig. 1C). At the end of the larval rearing period (day 11), 25% of pediveligers were eyed when fed the mixed diet and originating from broodstock fed T-Iso or *T. suecica*, but eyed larvae accounted for 46% to 50% of the total in larvae originating from broodstock fed *C. gracilis* or *S. marinoi*, respectively (Fig. 1C).

Discussion

Successful larval performance, especially metamorphosis in bivalves, relies on energy reserves stored during two developmental stages (Labarta et al., 1999). The first stage corresponds to embryonic development wherein endogenous reserves are provided by the parent (Bayne, 1973), and the second stage prior to metamorphosis, which depends on reserves stored during all of larval life. The latest depends on the

food value of algal diets supplied to growing larvae combined with endogenous reserves (Whyte et al., 1990).

In the present work, initial larval size was related to diet quality expressed as the type of microalgae delivered during broodstock conditioning. Thus, when larval growth relies on energetic reserves stored during embryogenesis, larvae released from broodstock fed single *T. suecica* were probably poorly enriched compared to other broodstock diets.

No differences in larval growth were found when larvae, fed single or mixed diets, originated from broodstock previously fed T-Iso or *C. gracilis*. In contrast, larvae originating from broodstock fed *S. marinoi* showed lower growth regardless of larval diet, single or mixed. This result contrasts with those of Enright et al. (1986) who classified *S. costatum* (named nowadays *S. marinoi*) as the second highest-ranked diatom for *O. edulis* growth (juvenile). Such apparent contradiction can be explained by the size of this microalga and the stage of bivalve development at which this diatom was delivered. Indeed, *S. marinoi* is a colonial diatom formed of spherical to cylindrical cells joined by long marginal processes. Colonies can be longer than 60 µm (Sauriau and Baud 1994), but in 300-L batch cultures in Argenton, colonies are usually composed of 2 to 4 cells, and the chains rarely exceed 10-15µm in length (Robert, unpublished data). Accordingly, this phytoplankton size is more appropriate for older bivalve stages than larvae (Robert and Gérard, 1999). When fed *S. marinoi*, *O. edulis* larval performance was, nevertheless, partially in agreement with those reported by Ferreiro et al (1990), who classified this diatom as the worst tested microalga, supporting *O. edulis* larval growth of 0.4 µm d^{-1} (12- fold lower than

in the present work). On the other hand, larvae fed the mixed diet and originating from broodstock fed *T. suecica* showed surprisingly similar growth as larvae released by broodstock previously fed T-Iso or *C. gracilis*. Larvae fed solely *T. suecica* exhibited lower growth, reaching however 6 μm d^{-1} (*vs* 8 μm d^{-1} for the mixed larval diet). This relatively good larval growth was, however, counterbalanced by low survival and can be accordingly considered to not represent the mean population response. These results contrast with those reported by Berntsson et al. (1998) for broodstock fed single *T. suecica* or this species mixed with *C. calcitrans*. Thus, larvae fed the bi-specific diet (*I. affinis galbana* plus *C. calcitrans:* TC_2) grew less when broodstock were fed single diets, with values ranging from 1.4 to 1.9 μm d^{-1} (4-5 fold less than in the present work).

Generally, larval survival was high (≥ 90%), except for larvae fed *T. suecica* and originating from broodstock also fed *T. suecica* (2%). When broodstock were fed *T. suecica* alone and released larvae were fed the mixed diet, larval survival was similar to that reported by Berntsson et al. (1998) with survival ≥ 90%. When fed continuously single *T. suecica* from broodstock through the entire larval life, some deficiency occurred leading to high mortality. By means of a physiological approach coupled with biochemical analysis, *T. suecica* has been shown to be poorly ingested, weakly absorbed and led to a poor transfer of its dietary components to flat oyster tissues, including gonads (González-Araya et al., 2011). We note that a same trend was observed for T-Iso with, however, higher survival (53%); this species was also shown to be poorly ingested and digested, but with better biochemical allocation (González-Araya et al., 2011). On the other hand, competence to metamorphose did

not reveal clearly the influence of diet. Larvae continuously fed *S. marinoi*, from broodstock to pre-settlement, exhibited a low level of competence, which is in agreement with corresponding data on growth. A similar trend occurred with larvae fed single *T. suecica*, which is in agreement with data on survival. Moreover, except for T-Iso, mixed diets (TC_g) led to higher larval competence, which is partially in agreement with our previous work (González-Araya et al., 2012).

When analysing the overall *O. edulis* development performances, larvae fed single T-Iso from broodstock to pre-settlement exhibited nutritional deficiencies which were expressed with 50% mortality. Those fed *S. marinoi* showed lower growth and competence clearly related to its size which did not fit with this early stage. Lastly, when larvae were fed *T. suecica*, more severe nutritional deficiencies occurred, leading to the death of the entire larval population. Regardless of broodstock feeding, however, these potential deficits seem to be mitigated when expelled larvae are fed TC_g, which has been shown to be an effective bi-specific diet for *Crassostrea gigas* larvae (Rico-Villa et al., 2006; Ben Kheder et al., 2010). Several studies (Helm et al., 1991, Jonsson et al., 1999) have suggested that some PUFAs are essential for survival and development of *O. edulis* larvae. This topic will be investigated in a forthcoming study designed to select balanced broodstock diets by applying different microalgae assemblages.

Acknowledgments

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Article N° 6

Article en cours de préparation pour soumission à

Comparative Biochemistry and Physiology Part B

Effects of microalgal diets on the growth, survival and settlement of *Ostrea edulis*(L.) larvae.

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Introduction

Broodstock conditioning is a major step in the operation of bivalve hatcheries. The maturation of broodstock is controlled by both endogenous and exogenous factors;the temperature and food have been described like the most important (Mann, 1979; Newel et al., 1982; Barber & Blake, 2006). It has been shown that the quality of food offered to molluscan broodstock as having a major influence on their reproductive performance and on the quality of eggs and larvae produced (Robinson, 1992; Farias & Uriarte, 2001; Uriarte et al., 2004). Microalgae have been used as food for various life stages in bivalves, and live microalgae have constituted the sole food for culture bivalve larvae and juveniles (Helm & Bourne, 2004; Liu et al., 2009). The most commonly used microalgae are species of genera *Isochrysis*, *Pavlova* (prymnesiophytes); *Chaetoceros*, *Skeletonema*, *Thalassiosira* (diatoms); *Dunaliela*, *Nanochloris*, *Tetraselmis* (chlorophytes); and *Rhodomonas* (cryptophyte)(Coutteau & Sorgeloos, 1992; Brown et al., 1997, Helm & Bourne, 2004; Liu et al., 2009).

The microalgae used in the culture of larvae and juveniles has been the subject of many studies for different bivalves groups, e.g. clams (Albentosa et al., 1994, 1996, oysters (Laing & Millican, 1986; Brown et al., 1997; Rivero-Rodríguez et al., 2007), mussels (Walcker et al., 2002) and scallops (Milke et al., 2004, 2006; Pernet et al., 2005). The food value of microalage can vary considerably with bivalve species examined, e.g. for larvae of *Mercenaria mercenaria* and *Tapes semidecussata*, the *I. aff. galbana* was found to be good monospecific diet, however, for larvae of *Crassostrea gigas* and *Crassostrea rhizophorae* and clam *Donaxtrunculus* (Helm & Laing, 1987; Ruiz-

Azcona et al., 1996). While *Pavlova lutheri* was successfully used for larvae of *Pecten maximus* (Delaunay et al., 1993), not satisfactory results were found by Rico-Villa et al (2006) in *Crassostrea gigas* larvae. In contrast, the single diatoms have been found to be of high nutritional value for *C. gigas* (Knuckey et al., 2002), *Ostrea edulis* (Enright et al., 1986). The mixed diets, generally, produce better growth of bivalve larvae and juveniles and this effect is normally attributed to mix of essential nutrients. Wilson et al. (1996) suggested that broodstock nutrition was important for theviability of the larvae of *Ostrea chilensis*, while Berntsson et al. (1997) have demonstrated the effect of broodstock diet on fatty acid composition, survival and growth rates in larvae of *O. edulis*. They found that the larval growth rate was significantly correlatedwith the content of the total polyunsaturated fatty acids PUFAs and especially the22:6n-3 representative. It has been suggested that lipid can be used as an index ofgrowth and viability in the larvae of three species of bivalve (Gallager et al., 1986)

In the present study, we aimed to investigate further the effect of dietary fatty acid and sterol composition on physiological and biochemical responses in *O. edulis* larvae. In the same way, the nutritional value of several selected microalgae species (single o mixed), shown in literature to be good food value to other bivalves, for larvae of *O. edulis* was assessed. The identification of optimum diets for *O. edulis* would be the key for the aquaculture of this species.

Material and methods

Conditioning and larval rearing

A total of 300 four-year-old flat oysters, *Bonamia* and *Marteilia* free, originated from Norway were conditioned from March to August 2009. Oyster broodstock were held in 700 L flow-through tanks at 19°C and continuously fed on equivalent of 2 billion cells per day per oyster. Two diets were used for conditioning broodstock; *Rhodomonas salina* + *Chaetoceros gracilis* (Rs:CCAP 978/24; Cg:UTEX LB2658, respectively), *R. salina* + *Thalassiosira weissflogii* (Rs:CCAP 978/24; Tw:CCAP 1085/3, respectively) or unfed. Oocytes from unfed oysters were therefore exclusively sourced from *O. edulis* reserves, built in the natural surrounding before transfer to the laboratory. Duplicate tanks were used for each condition. The broodstock were continuously fed by means of a peristaltic pump for each feeding condition. For each treatment, the released larvae were collected and counted at each "spawning" (expelled larvae) from the water surface by means of sieves places under the outflow. When a sufficient number of larvae was obtained to allow the settlement of 24 experimental condition, they were distributed in 5 L translucent methacrylate cylinders, reared in flow-through with UV treatment, in triplicate. Larvae were held at a density of 5 larvae mL^{-1}, 22°C of seawater temperature and an ambient salinity (34‰). Larvae were fed different microalgal assemblages, based on *Isochrysis affinis galbana* (T), *C. gracilis* (Cg) and *Pavlova lutheri*(P) at 1500 μm^3 μL^{-1} T-ISO equivalent.

Larval length and survival were estimated on days 3, 6 and 9 under microspcope by use of image analysis technique (WinImager 2.0 and Imaq Vision Builder 6.0 software for images capture and treatment, respectively). When 50% eyed larvae (competent) were recorded in at least two different experimental conditions, all fed larvae, originated from broodstock whatever their conditioning diet, were transferred in PVC containers with a 125µm nylon mesh based and kept in 150 L raceway tank. Calibrated oyster shellfish chips (400 µm) were distributed for each bottom tray as cultch. Seawater, filtered at 1 µm and treated with UV, was continuously provided at 22 °C and ambient salinity (34%) with downweller system at a flow rate of 9 L h^{-1}. larvae were stocked from 6000 to 13000 per unit corresponding to 0.6 to 1.3 larvae mL^{-1} or 4 to 8 post-larvae cm^{-2} and were continuously fed the sale diet (TCgP) for one week at the end of which metamorphosis was estimated by determining the number of remaining larvae (absence of dissoconch). Because unfed larvae did not show any competence after 8 days of rearing they were discarded from metamorphosis triggering.

Statistical analysis

The homogeneity of variances of means was tested by use of the univariate test (Levene's test). Significant differences were detected by using ANOVA, *P* being set at 0.05 and a posteriori multiple comparison test between the means (Tuckey's test), after transformation of percentage data by the function [arcsin(racine x i/100)]

Results

Effects on larval growth and survival

Larvae originated from unfed broodstock were able to grow normally (Fig. 14) but less than 1.5% competence was recorded on day 9. In contrast, independent of broodstock diet, the unfed larvae showed poor growth (0.1 to 0.4 µm d^{-1}; Fig. 14).

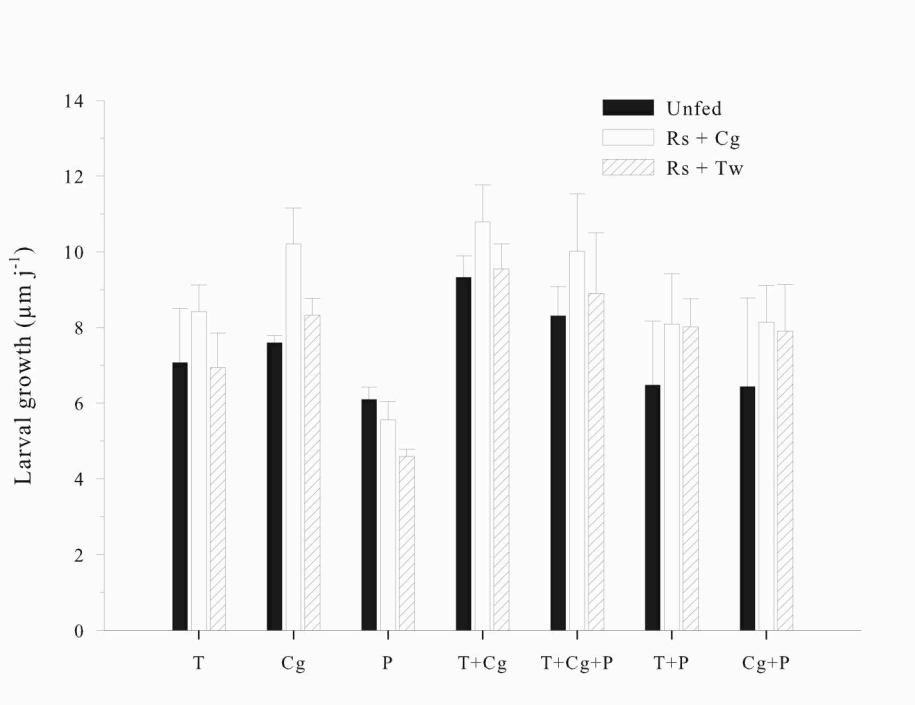

Figure 14: Larval growth (µm) for larvae fed *Isochrysis affinis galbana* (**I**), *Chaetoceros gracilis* (**Cg**), *Pavlova lutheri* (**P**), bi-specific diet **I**+**Cg**, **P**+**Cg**, **I**+**P** and multi-specific diet **I**+**Cg**+**P** originating from *Ostrea edulis* broodstock previously fed *Rhodomonas salina* + *Chaetoceros gracilis* (**Rs**+**Cg**) and *Rhodomonas salina* + *Thalassisosira weissflogii* (**Rs**+**Tw**).

Those fed the single diet of *P. lutheri* exhibited low growth (4.5 to 6.0 µm d^{-1}) and less than 4% competence on day 9. On the contrary, with growth from 9.5 to 11 µm d^{-1}, larvae fed the mixed TCg diet showed the best development with 80% competent to metamorphosis (Fig. 14).

Larvae originating from unfed broodstock exhibited generally lower survival, ranging from 38% to 93 (Fig. 15). In this specific condition (unfed parents) unfed larvae were more resistant than fed larvae. The fed larvae originating from parents receiving Rs+Cg showed better survival (>90%) than those released from parents fed Rs+Tw, where survival was more variable (67-91%: Fig. 15).

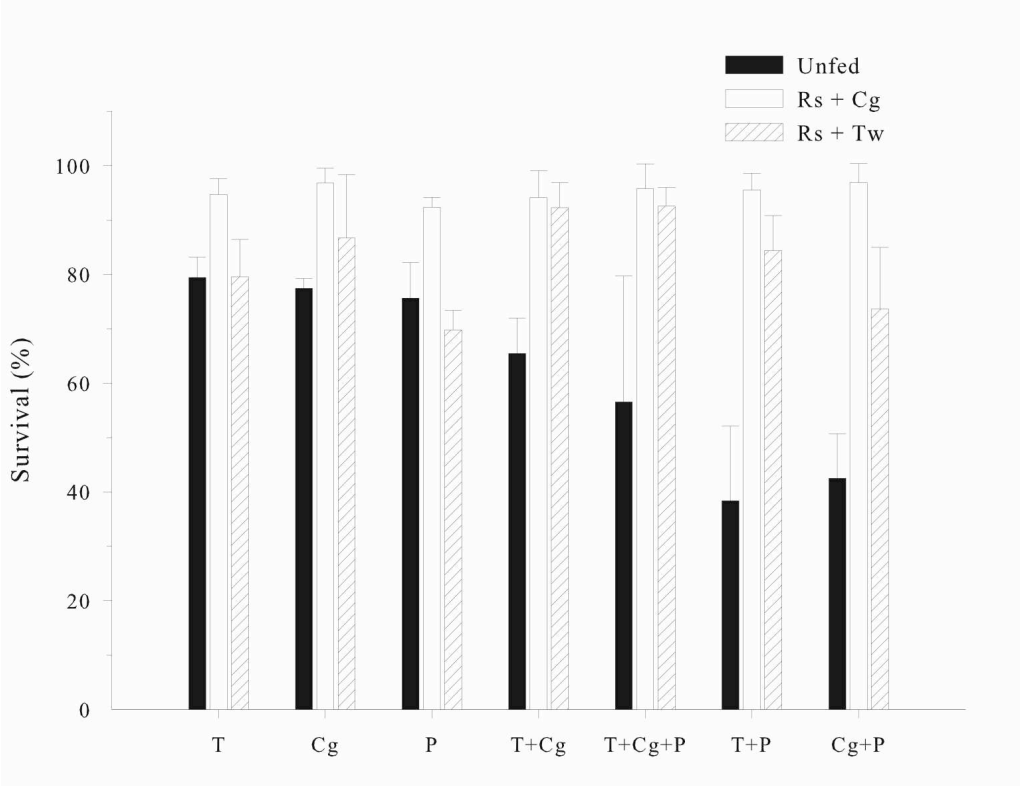

Figure 15: Survival (%) for larvae fed *Isochrysis affinis galbana* (**I**), *Chaetoceros gracilis* (**Cg**), *Pavlova lutheri* (**P**), bi-specific diet **I+Cg**, **P+Cg**, **I+P** and multi-specific diet **I+Cg+P** originating from *Ostrea edulis* broodstock previously fed *Rhodomonas salina* + *Chaetoceros gracilis* (**Rs+Cg**) and *Rhodomonas salina* + *Thalassisosira weissflogii* (**Rs+Tw**).

Effects on metamorphosis

Larvae originating from unfed broodstock, independent of larval diet, did not successfully metamorphosis and died. The larvae fed TCG showed the highest

metamorphosis rate varying from 30 to 60% depending on original broodstock diet (Fig. 16). When *P. lutheri* was added to this bispecific diet, metamorphosis was not improved. This demonstrated the poor food additive value of *P. lutheri* as confirmed by the lowest metamorphosis performances when used as single diet for larvae (1-6%: Fig. 16). Moreover, it depressed metamorphosis when combined with T or Cg in the diet during larval development. The success of metamorphosis was clearly linked to original broodstock feeding as already pointed out with better results recorded for oysters fed *R. salina* + *C. gracilis* (Fig. 16).

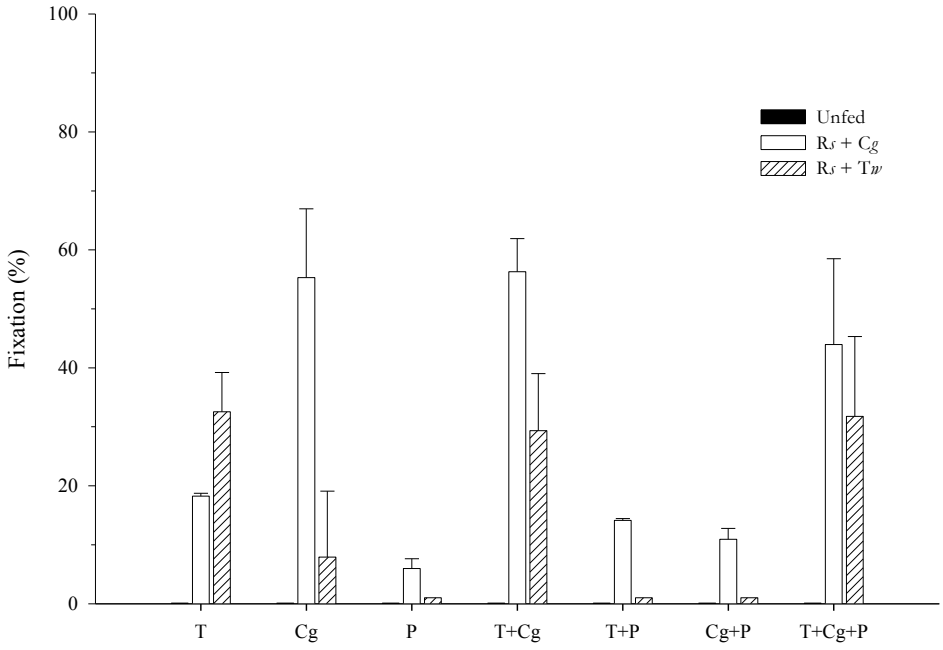

Figure 16: Fixation (%) for larvae fed *Isochrysis affinis galbana* (**I**), *Chaetoceros gracilis* (**Cg**), *Pavlova lutheri* (**P**), bi-specific diet **I**+**Cg**, **P**+**Cg**, **I**+**P** and multi-specific diet **I**+**Cg**+**P** originating from*Ostrea edulis*broodstock previously fed *Rhodomonas salina* + *Chaetoceros gracilis* (**Rs**+**Cg**) and *Rhodomonas salina* + *Thalassisosira weissflogii* (**Rs**+**Tw**).

Discussion

Successful larval performance, especially metamorphosis in bivalves, relies on energy reserves stored during two development stages (Labarta et al., 1999). The first stage corresponds to embryonic development wherein endogenous reserves are provided by the parent (Bayne 1973), and the second stage prior to metamorphosis, wich depends on reserves stored during all of larval life. The latest depends on the food value of algal diets supplied to growing larvae combined to endogenous reserves (Whyte et al., 1990).

In the present work, no differences in larval growth were found when larvae originating from broodstock previously fed *Rs* + *Cg* in larvae fed T, P, T+P, I+P or T+C+P. In contrast, larvae fed C or T+Cg showed higher growth compared to other diets tested. Indeed, *P. lutheri* led to the lowest *O. edulis* larval performances (growth an d metamorphosis) when used as single and had non-additive food value impact when associated to T-Iso or *C. gracilis*.

When used as single diet the diatom *C. gracilis* (C) led to generally better performances of larvae, but, in contrast with *C. gigas*, the use of T-Iso as single feed, allowed for satisfying *O. edulis* larval development.

Generally, larval survival was high (> 75%), except for larvae unfed, independently of broodstock diet.

When analysing the overall *O. edulis* larval performances, the different values obtained showed that competence to metamorphosis did not clearly linked to influence of diet. Larvae continuously fed, but originating from broodstock unfed,

exhibited a normally growth compared to other broodstock diets, however, the percentage of fixation was 0% (zero).

Rico-Villa et al. (2006) and Ben Kheder et al. (2010) showed that the potential deficits in broodstock diet for *C. gigas*, seem to be mitigated when larvae are fed TCg. Nevertheless, several studies (Helm et al., 1991; Jonsson et al., 1999) have suggested that some PUFAs are essential for survival and development of *O. edulis* larvae. This idea is been investigated, and a forthcoming study helps us to understand the different process in specific biochemical allocation of larvae of *O. edulis*.

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Conclusions et perspectives

DISCUSSION ET CONCLUSION GENERALES

Rappel du contexte :

Dans les années 60, la production de l'huître plate est proche de 20 000 tonnes par an. A la fin de cette décennie, *O. edulis* subit des mortalités dans les élevages estuariens liées au parasite (*Martelia refringens*) (Grizel et al., 1974). En 1970, alors que les élevages avaient été transférés en eau profonde, un nouveau protozoaire (*Bonamia ostreae*) apparaît en provoquant l'effondrement de la production d'huîtres plates à 1 000 – 1 500 tonnes. Ce phénomène a également affecté plusieurs pays européens, dont l'Espagne, l'Irlande et l'Italie.

Au début des années 90, Ifremer a mis en place un programme de sélection génétique de l'huître plate résistante ou tolérante à *B. ostreae* (Martin et al., 1993) et des lignées ont été obtenues par pression de sélection.

Si les efforts fournis au cours de ces 30 dernières années se sont concrétisés par l'obtention d'une souche de première génération d'*O. edulis* résistante/tolérante à *B. ostreae* et testée avec succès par les producteurs. La maîtrise des différentes étapes du cycle de production de cette espèce devrait permettre un approvisionnement régulier de la filière ostréicole.

Quelles que soient les espèces concernées, poissons, crustacés ou mollusques, la production de juvéniles par des écloseries commerciales passe nécessairement par trois étapes : conditionnement de géniteurs, développement larvaire et obtention de juvénile ou naissain.

Par rapport aux autres espèces de mollusques, l'huître plate possède certaines particularités. Elle présente en effet un cycle de vie dit larvipare. Lors de la ponte, les ovocytes ne sont pas émis dans le milieu extérieur. La fécondation se produit, elle aussi, à l'intérieur de l'huître, dans la chambre branchiale, par aspiration des spermatozoïdes, expulsés préalablement dans le milieu environnant. Les œufs sont conservés dans la chambre branchiale puis les larves sont libérées dans le milieu après 8 à 10 jours à une taille moyenne de 160-170 μm. Une deuxième phase de développement larvaire, cette fois externe ou pélagique, prendra place pendant 6-15 jours en fonction des conditions du milieu (température, salinité, nourriture) jusqu'à la métamorphose et l'obtention ultérieure des juvéniles.

Les expériences menées dans le cadre de cette thèse ont permis d'évaluer l'effet de l'alimentation à la fois sur les géniteurs et sur le développement larvaire de l'huître plate est estimer les interactions.

Mise au point du protocole de production de l'huître plate

Les connaissances concernant le conditionnement des géniteurs de l'huître plate ont été acquises 50 ans auparavant (Walne, 1970). Pendant les années 70-80, l'impact de deux épizooties (marteiliose et bonamiose) a limité l'acquisition de nouvelles connaissances sur cette espèce, à l'exception des aspects pathologiques. Toutefois, quelques travaux menés par Ferreiro et al. (1990), Frolov et Pankov (1992), Millican et Helm (1994), Bernsston et al. (1997) ont permis d'améliorer les connaissances sur les besoins nutritionnels des géniteurs et larves d'huitre plate, mais de façon

sporadique. Dans ce contexte, le travail réalisé dans le cadre de cette thèse a permis de déterminer les besoins nutritionnels des géniteurs et des larves jusqu'à l'obtention de juvénile. L'étape de la métamorphose a donc été inclue dans notre étude.

Dans un premier temps, l'évaluation d'un ou de plusieurs régimes nutritionnels sur les géniteurs et larves, ne paraissait pas affecter notre plan de travail ; cependant, Chaparro et al. (2001) ont démontré la capacité des larves d'*O. chilensis* à ingérer et absorber des microalgues et des particules en suspension pendant la période d'incubation. Ce comportement modifie l'effet du régime parental puisque les larves sont aptes à ingérer la nourriture apportée aux géniteurs pendant la phase incubatrice du développement. De ce fait, nous avons délibérément choisi deux microalgues différentes de celles communément décrites dans la littérature et considérées comme des espèces adaptées au conditionnement et à l'élevage larvaire de bivalves (*Isochrysis affinis galbana* : T-Iso, *Chaetoceros gracilis*). Il s'agit de: *Rhodomonas salina*, rarement utilisée en écloserie, à l'exception de la coquille Saint Jacques *Pecten maximus* (Robert, et al., 1994 ; Tremblay et al., 2007) et *Thalassiosira weissflogii*, toutes deux de grande taille (180 μm^3 et 950 μm^3, respectivement) et ne pourront pas être ingérées par des jeunes larves d'*Ostrea edulis*. Bien que n'ayant pas été utilisée fréquemment en écloserie de mollusques, *T. weissflogii* possède des caractéristiques morphologiques et biochimiques qui suggèrent son utilisation potentielle en remplacement d'autres diatomées considérées comme performantes.

Une fécondité plus élevée des géniteurs nourris sur régime bispécifique (3,8 10^7 larves), par rapport aux régimes mono spécifique (*R. salina* : 1,6 10^7 ; *T. weissflogii* : 2,8 10^7 larves émises) a été constatée à la fin du conditionnement.

Deux conditions nutritionnelles ont été testées par la suite sur les larves, *C. gracilis* et un régime bispécifique (T-Iso plus *C. gracilis*). La performance de ces deux régimes avait préalablement été démontrée sur des larves de *C. gigas* (Rico-Villa et al., 2006)., Afin de vérifier l'impact du régime parental sur la qualité des larves, une condition à jeun était également testée.

Après 11 jours d'élevage larvaire, les larves issues de géniteurs nourris sur *R. salina* ont présenté une meilleure croissance larvaire comparée aux autres régimes parentaux. Cependant, de faibles taux de métamorphose (1 %) sont observés lorsque les larves étaient alimentées sur *C. gracilis*.

A l'inverse, le plus fort taux de métamorphose (60 %) est observé chez les larves nourries sur régime bispécifique *C. gracilis* + T-Iso et issues de géniteurs alimentés par *R. salina* + *T. weissflogii*.

Les larves ne pouvant pas s'alimenter à partir des régimes parentaux, ces premiers résultats ont suggéré un lien entre le régime parental et les performances larvaires chez l'huître plate. Mais, ce lien n'était identifié qu'à la métamorphose, contrairement à *C. gigas* chez qui l'impact de la nourriture peut être évalué par la croissance (Rico-Villa et al., 2006) ou *P. maximus* où la mortalité est considérée comme un indicateur des performances larvaires (Robert et al., 1994 ; Soudant et al., 1996).

Définition du régime nutritionnel parental

Conditionnement des géniteurs

Le conditionnement des géniteurs d'huître plate pratiqué dans les écloseries de bivalves ne prend pas en compte la composition biochimique de l'alimentation. La nourriture des géniteurs ayant un effet sur le développement larvaire et la nourriture des larves ayant également une forte influence, il était donc nécessaire d'aborder la problématique en deux étapes successives. Nous avons, tout d'abord, focalisé nos efforts sur le régime parental.

Pour ce faire, des conditionnements d'huître plate ont été réalisés, l'un au printemps et l'autre à l'automne, car il était impossible de mener de front de tels travaux sur huit espèces d'algues et en triplicata. Le choix des micro algues s'est porté sur des espèces couramment utilisées en écloserie de mollusques.

Or, nous venions de démontrer que l'apport de régimes bispécifiques générait une plus forte fécondité et une qualité larvaire supérieure, décelable qu'à la métamorphose. Il aurait donc été logique d'aborder la recherche des meilleurs régimes nutritionnels par la mise en place de différentes combinaisons deux à deux de microalgues. En considérant l'association d'une diatomée avec un flagellé (permettant a priori une meilleure couverture des besoins essentiels sans que ceux-ci ne soient parfaitement identifiés) cela nous conduisait à 16 combinaisons et donc à quatre d'expérimentations successives, chaque régime étant mené en triplicata.

Nous avons donc opté pour une stratégie reposant sur des apports monospécifiques

dans un premier temps, avec des analyses basées principalement sur deux critères : une réponse physiologique (ingestion, assimilation et absorption) puis une réponse biochimique prenant en compte l'allocation des composants (acides gras et stérols) dans les quatre principaux organes de l'huître : gonade, glande digestive, muscle et branchie.

Ce protocole d'évaluation de l'effet de chaque espèce de microalgue reposait sur l'hypothèse qu'une algue fortement ingérée et absorbée produirait une empreinte biochimique marquée, que ce soit au niveau proximal ou en acides gras et stérols spécifiques des différentes espèces de microalgues. Si, cette théorie était correcte, l'analyse histologique du développement de la gonade devait confirmer nos données physiologiques et biochimiques. Un tel contrôle a donc été parallèlement réalisé.

Parmi les huit espèces de microalgues testées, les meilleures réponses physiologiques ont été obtenues en utilisant deux diatomées *C. gracilis* et *S. marinoï*, mais aussi la cryptophyceae *R. salina,* les valeurs d'ingestion et d'absorption observées étant supérieures à $3\ 10^9\ \mu m^3\ g^{-1}\ h^{-1}$ et 1,5 mg $g^{-1}\ h^{-1}$ respectivement. Quel que soit le régime distribué, les huîtres alimentées avec les diatomées *C. gracilis*, *S. marinoï*, et *T. weissflogii*, ont présenté une augmentation spécifique en EPA (20:5(n-3)), tandis qu'une accumulation en DHA (22:6(n-3)) a été mesurée chez les huîtres nourries avec T-Iso et *R. salina*. Un enrichissement en Brassicasterol a été constaté chez les huîtres recevant T-Iso et *R. salina*, tandis qu'une accumulation en Cholestérol et Campesterol était notée chez les huîtres nourries avec *C. gracilis*, *T. weissflogii* et *S. marinoï* respectivement.

Cependant, et malgré un marquage spécifique des microalgues, nous n'avons pas pu relier ces empreintes à la croissance ou la gamétogenèse. L'analyse physiologique couplée à une étude fine de l'évolution gonadique, a permis néanmoins de proposer un régime alimentaire adapté aux besoins des huîtres plates.

De ce fait, nous avons sélectionné le régime bispécifique *C. gracilis* + *R. salina*, cette dernière apportant les mêmes composants biochimiques que T-Iso, mais en étant bien mieux ingérée et assimilée.

La faible ingestion et assimilation mesurée chez les huîtres nourries avec T-Iso, suggèrent de faibles performances physiologiques liées à cette microalgue. Bien que la fécondité soit équivalente à celle d'huîtres recevant des diatomées, des expériences réalisées au cours de cette thèse, mais non intégrées dans le présent travail, ont démontré que l'ingestion et l'assimilation de T-Iso chez l'huître plate et creuse sont inférieures à celle de *C. gracilis*. De plus, en inversant au cours du conditionnement les espèces de microalgue, on observe une baisse de consommation des huitres plates lorsque T-Iso est remplacée par *C gracilis* mais aussi une augmentation de celle-ci lorsque l'on passait de T-Iso à *C. gracilis*. Malgré un volume cellulaire deux fois moindre que celle de *C. gracilis* (40 μm^3 *vs* 80 μm^3), cette microalgue devrait être ingérée de façon satisfaisante et au moins équivalente à celle de *P. lutheri* présentant la même taille. T-Iso, par rapport à *C. gracilis*, serait d'une moindre appétence. Cette notion, longtemps réfutée, a été démontrée chez *Pecten maximus* (Beninger et Decottignies, 2005) avec la diatomée *Coxinodiscus perforatus*. Ces mécanismes commencent à être explorés chez *Mytilus edulis* (Espinosa et al., 2010).

Élevage larvaire

Le régime nutritionnel parental ayant été défini (*R. salina* + *C. gracilis*), nous nous sommes attelés à la recherche du meilleur régime larvaire.

Tout d'abord, nous avons testé trois espèces de microalgues dont la taille est compatible avec celles des larves d'*O. edulis* : T-Iso, *C. gracilis* et *P. lutheri*. Le même régime parental bispécifique utilisé dans le protocole (Chapitre I ; *R. salina* plus *T. weissflogii*) a été appliqué comme « contrôle » afin d'évaluer la pertinence du choix d'un nouveau régime. Des géniteurs non nourris au cours du conditionnement ont été également suivis afin de mesurer l'impact d'un apport nutritionnel sur la fécondité et leur possible effet sur la composition biochimique des larves.

Les principaux résultats de ces conditionnements montrent une augmentation significative de la fécondité par rapport au régime contrôle (0,65 10^6 *vs* 0,23 10^6 larves par géniteur), soit un triplement des performances. D'autre part, seules les larves nourries avec *C. gracilis* ou T-Iso plus *C. gracilis* présentaient un taux de croissance supérieur à 10 µm j^{-1}. Cependant, nous avons observé que les larves issues de géniteurs maintenus à jeun, avaient des taux de croissance similaires à celles issues de géniteurs recevant le régime contrôle (*R. salina* plus *T. weissflogii*) : cette observation pourrait suggérer un faible effet du régime parental sur les performances larvaires, si ces travaux s'étaient limités au seul développement larvaire.

C'est au stade de la métamorphose que nous avons pu constater un effet significatif du régime nutritionnel qu'il soit larvaire ou parental sur l'huître plate, confirmant

ainsi nos premiers travaux (chapitre I). Ainsi, les larves issues de géniteurs non nourries ne se sont pas métamorphosées quel que soit le régime larvaire appliqué. À l'inverse, un taux de métamorphose supérieur à 50% a été enregistré chez des larves issues des géniteurs recevant le régime *R. salina* plus *C. gracilis* et nourries avec *C. gracilis* et T-Iso plus *C. gracilis*.

Ces résultats confirment que l'efficacité d'un régime alimentaire n'est observée, chez l'huître plate, que lors de la métamorphose. Une conclusion antérieure à cette étape, aurait été biaisée, car les différentes performances larvaires (survie, croissance et compétence) observées au cours des différentes expériences, n'ont jamais permis de dégager clairement une tendance, que ce soit en apport monospécifique ou en mélange.

PERSPECTIVES

L'effet significatif du régime nutritionnel sur la composition en acides gras des larves a permis d'en déduire la faible activité de bioconversion des acides gras alimentaires. Par ailleurs, aucune différence significative du régime nutritionnel sur la croissance et la survie chez les larves de l'huître plate n'a été constatée. Cependant, lorsque les larves atteignent la métamorphose, il semblerait que la composition du régime nutritionnel ait une influence sur cette étape décisive dans l'obtention d'un juvénile.

Les lipides jouent deux rôles fondamentaux dans le métabolisme cellulaire, en assurant, d'une part, le stockage de l'énergie sous forme de triglycérides et, d'autre part, le maintien et le fonctionnement des membranes cellulaires sous la forme de phospholipides et de cholestérol. Ces deux rôles sont sous la dépendance directe de l'alimentation qui doit fournir suffisamment d'énergie pour permettre à l'animal de stocker des triglycérides et les acides gras essentiels qui sont incorporés dans les phospholipides. Dans ce contexte, l'étude de la nutrition « lipidique » et la détermination des besoins en acides gras essentiels apparaît comme un besoin, permettant d'améliorer et de complémenter notre étude.

Les résultats obtenus montrent que l'effet de l'alimentation est presque intégralement retrouvé dans la composition en acides gras, que ce soit dans les différents organes des géniteurs ou les larves de l'huître plate. La composition des lipides polaires reflète à la fois celle de l'alimentation et l'action du métabolisme probablement parce que les mollusques bivalves ne convertissent pas les acides gras alimentaires et que le contrôle se fait principalement par mécanisme d'incorporation sélective des acides gras assimilés. L'incorporation préférentielle du 22 :6(n-3) suggère un rôle particulier de cet acide gras au niveau des lipides structuraux de même que le maintien du 20 :4(n-6) est probablement lié à son importance dans le métabolisme du phosphatidyl-inositol et à son rôle de précurseur de prostaglandines. Ces résultats montrent qu'une attention particulière doit être accordée au contrôle des conditions nutritionnelles des géniteurs dans les écloseries de bivalves.

Pour mieux comprendre l'impact d'une espèce de microalgue ou le rôle que celle-ci jouera dans les différentes étapes du cycle de développement d'*O. edulis*, quelques perspectives peuvent être également formulées. D'une part, une étude plus approfondie de l'empreinte biochimique d'une microalgue doit être envisagée, analysant l'accumulation des lipides dans les gonades, ovocytes et larves. Cette étude devra être basée sur l'évolution séparée, des différentes classes de lipides, triglycérides (plus de 90% de la composition des lipides neutres), phospholipides (principal composant des lipides polaires) et stérides (esters d'acides gras et stérols). Chez les mollusques bivalves, différentes solutions peuvent être envisagées afin d'étudier l'impact d'un ou plusieurs régimes nutritionnels sur l'accumulation ou assimilation des lipides dans différents organes, ovocytes et/ou larves. Or, l'huître plate présente un cycle de reproduction « atypique ». L'émission et fécondation d'ovocytes s'opèrent dans la chambre branchiale où aura lieu une première phase du développement larvaire pendant 8-10 jours, puis l'expulsion des larves dans le milieu naturel où aura lieu une deuxième phase du développement larvaire pendant 9-20 jours (Figure C-1). Cette difficulté, limitant la mise en route d'études plus approfondies sur la composition biochimique d'ovocytes et les jeunes larves (0-2 jours) nous a contraints à mettre au point des techniques non destructives, dites « non invasives » afin de suivre l'évolution de la gamétogenèse de façon macroscopique et microscopique. Dans ce contexte, deux techniques ont été évaluées. La première correspondant à des méthodes d'imagerie (IRM ; Annexe I), utilisées préalablement chez *C. gigas*, a permis la détermination des stades gamétogèniques (Pouvreau et al., 2006 ; Davenel et al., 2006). La seconde, basée sur

une observation directe des gonades après anesthésie et microponction, a été développée avec succès chez *P. fumatus* (Heasman et al., 1995), *C. gigas* (Namba et al., 1995) et *O. edulis* (Culloty et Mulcahy, 1992). Elle restait à être optimisée chez l'huître plate (Annexe II). Les résultats de ces expériences ont démontré que, contrairement aux espèces étudiées préalablement, ces techniques à leur niveau de développement actuel ne permettent pas, un suivi exhaustif de l'évolution de la gonade au cours du conditionnement. Quelle que soit la méthode utilisée, il existe encore des interrogations quant aux possibles effets liés à l'application de ces techniques : stress des animaux suite aux analyses d'IRM (champs magnétique) ou effet potentiellement néfaste d'un type d'anesthésiant sur les larves présentes dans la chambre branchiale.

Des travaux à court terme sur des microalgues supplémentées par des microcapsules lipidiques, pourraient permettre de préciser le rôle des certains composants (acides gras, triglycérides, phospholipides, stérols, etc) sur la gamétogenèse des géniteurs, la qualité des ovocytes, la croissance et la métamorphose des larves de *O. edulis*.

Les connaissances acquises au cours de cette thèse, ont permis l'amélioration des conditions nutritionnelles durant la phase de conditionnement des géniteurs et de l'élevage larvaire de l'huître plate. Cependant, ces améliorations ne sont observées qu'à la métamorphose, étape cruciale pour l'obtention de juvéniles. En effet, au cours de cette phase de nombreux remaniements structuraux nécessitent un apport

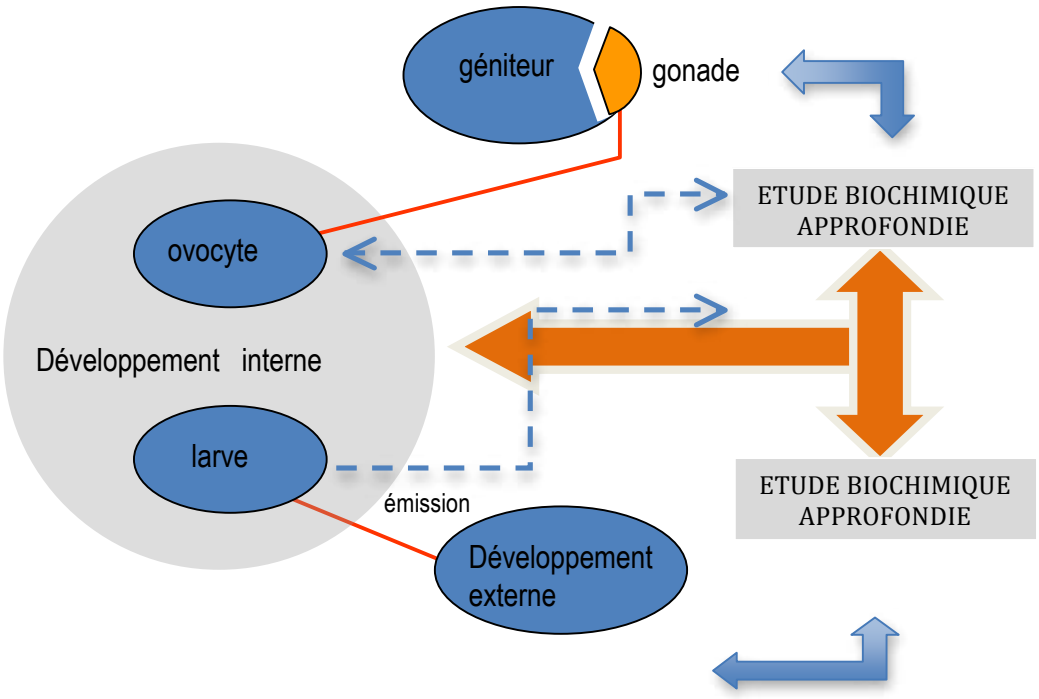

Figure C-1 : Schéma conceptuel de l'impact d'un régime nutritionnel chez l'huître plate.

important en acides gras polyinsaturés ((22 :6(n-3) notamment) pour la constitution des phospholipides membranaires. Enfin, il serait pertinent de réaliser de nouvelles expériences utilisant l'espèce T-Iso, afin de mieux comprendre son rôle dans le régime larvaire de l'huître plate.

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Annexes

Annexe I

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Anaesthesia and gonad sampling in the European flat oyster (*Ostrea edulis*)

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Abstract

Controlled reproduction of the European flat oyster requires the development of tools adapted to this species, including a practical anaesthesia and gonad sampling protocol to facilitate sex determination and the verification of gametogenesis. Three replicate groups of 10 oysters (mean weight ± SD: 29.9 ± 8.5 g) were anaesthetised in 5 L containers using magnesium chloride designed either for laboratory (Flucka®) or agricultural (Dead Sea Work®: DSW) use, at two concentrations (50 or 72 g L^{-1}). No significant differences were observed in the percentages of oysters anaesthetised or subsequent oyster mortality with the different anaesthetics or concentrations, but increasing water temperature from 14.9 to 18.8 °C significantly increased the number of oysters anaesthetised after 3 h. Increasing anaesthesia duration from 1 to 22 h significantly increased the percentage of oysters anaesthetised but did not affect subsequent oyster mortality. Gonad sampling of anaesthetised oysters did not increase oyster mortality either. A reliable anaesthesia protocol was, therefore, defined using 50 g L^{-1} DSW® magnesium chloride for a 2 to 3 h duration. This protocol was validated by monthly anaesthesia and gonad sampling on the same oysters over a three month period, during which a percentage of 95 ± 2% anaesthetised oysters was observed. Compared with controls (oysters that were neither anaesthetised nor sampled), oyster mortality of monthly anaesthetised batches showed no significant increase.

Keywords: Oyster; *Ostrea edulis*; Anaesthesia; Magnesium chloride; Gonad sampling

1. Introduction

Aquaculture production of the endemic European flat oyster *Ostrea edulis* decreased from 30 000 tonnes in 1961 to 6500 tonnes in 2007 (FAO, 2009) due to two parasites (*Marteilia refringens* and *Bonamia oestrea*) that caused a sharp decline in its survival, especially in France (Hegaret and Mazurié, 2005). The selection of resistant individuals has provided a means of improving oyster survival (Naciri-Graven et al., 1998), and thus offered a new opportunity for European flat oyster farming. Hatchery techniques, invaluable for the production of selected resistant lines, have been developed for the species, but adapted tools are still required to improve the control of its reproduction.

Anaesthesia is widely used in aquaculture to facilitate tissue biopsies and gametogenesis studies. In bivalves, tissue sampling often relies on destructive methods, since the shell has to be removed to obtain tissue samples. A reliable anaesthesia and tissue sampling protocol for European flat oyster would enable experiments to be conducted without sacrificing animals, and therefore also allow successive samplings of the same individual. This would facilitate descriptive studies such as those on gametogenesis, while avoiding inter-population variations of the results observed during successive samplings using a destructive method. Furthermore, avoiding oyster sacrifice in aquaculture is particularly valuable for selected animals.

Magnesium chloride has been used successfully to induce anaesthesia in the scallop *Pecten fumatus* (Heasman et al., 1995), Sydney rock oyster *Saccostrea glomerata* (Butt et al., 2008) and Pacific oyster *Crassostrea gigas* (Namba et al., 1995), with low subsequent mortality. In Pacific oyster, the use of agricultural magnesium chloride was found to be just as effective as a laboratory brand, but considerably less expensive (Suquet et al., 2009).

The examination of flat oyster reproductive development requires the development of tools adapted to this species, including a practical anaesthesia and tissue sampling

protocol that would enable both sex determination and gametogenesis description. Some elements of such a protocol have already been published (Culloty and Mulcahy, 1992), showing that magnesium chloride induces rapid anaesthesia and recovery of flat oysters. However, variations in results due to differences in anaesthesia conditions were not fully documented in this study. There remained a need to examine the effects of different types of magnesium chloride used, anaesthetic concentration, duration of anaesthetic treatment, oyster weight, water temperature and effects of gonad sampling on subsequent oyster survival. A detailed examination of these parameters under controlled conditions would help to establish a practical anaesthesia and sampling protocol for flat oyster.

Preliminary experiments conducted in our laboratory showed that adding microalgae with the magnesium chloride treatment, starving oysters before treatment or modifying light intensity, had no significant effect on anaesthesia success.

2. Materials and methods

20-month-old flat oysters (mean weight ± SD: 29.9 ± 8.5 g) were transferred from North Brittany (France) to the Argenton experimental hatchery (west Brittany). These oysters were maintained in flow-through seawater tanks at 19 °C and fed daily according to Chavez-Villalba et al. (2002).

The standard anaesthesia conditions used, unless otherwise stated, were three replicate groups of 10 oysters, each anaesthetised in 5 L containers using 50 g L^{-1} magnesium chloride designed for agricultural purposes (DSW: Dead Sea Work®, Israel: $MgCl_2$: 46 to 48%) dissolved in a mixture of fresh water (3 L) and seawater (2 L) to maintain salinity. Water temperature was maintained at 19 °C. After 3 h in the anaesthetic solution, the number of anaesthetised oysters was assessed. Oysters were considered as anaesthetised if shell closure was not observed after three successive pressures on their valves. Following the anaesthetic treatment, oysters were returned to clean seawater and their survival was monitored for one week. Six experiments were conducted during the present study; details and differences from

the standard conditions are listed below. Each experiment included at least three control groups (3 × 10 non anaesthetised oysters). When supplementary controls were added, these are detailed below for the appropriate experiments.

2.1. Magnesium chloride: source and concentration

Two types of magnesium chloride were used, the first designed for laboratory use (Flucka®, Czech Republic) and the second for agriculture use (DSW®). Each type was tested at two concentrations (50 and 72 g L^{-1}). To maintain salinity, the highest salt concentration was diluted in fresh water.

2.2. Water temperature and oyster weight

To determine the effect of water temperature, groups of oysters were anaesthetised at 14.9 ± 0.2 °C or 18.8 ± 0.2 °C. Water temperature was maintained using thermo-baths. The effect of oyster weight was studied by exposing groups of oysters with significantly ($F = 212.561$, d.f. = 1, $P < 0.001$) different mean weight (19.2 ± 4.2 g and 38.3 ± 5.9 g) to the anaesthetic.

2.3. Anaesthesia duration and gonad sampling

Groups of oysters were left in the anaesthetic solution for 1 h, 2 h, 3 h, 5 h or 22 h. Oyster valve opening was measured using a calliper rule and expressed as the opening rate: (valve opening/shell length) × 100. At the end of anaesthesia (the period the animals were left in the solution), the percentage of oysters that had recovered (those presenting closed valves) was assessed as a function of the time they had spent in clean seawater. Oyster gonads of anaesthetised animals were sampled using a 1 ml syringe and needle (0.6 × 30 mm, 23 gauge). Survival was then monitored compared with two control groups (control group 1: oysters were neither anaesthetised nor sampled, control group 2: oysters were anaesthetised but not sampled).

2.4. Method validation: oyster survival during a three month period with repeated anaesthesia and sampling

The protocol chosen in the present work was validated by studying the effect of repeated monthly anaesthesia and gonad sampling over a three month period (four samplings in all) on the overall survival of flat oysters.

2.5. Statistical analysis

Data are presented as mean ± SD. For each experiment, the percentage of anaesthetised oysters and percentage oyster survival one week following anaesthesia were recorded. The controls were excluded from these statistics since no oysters in these groups were anaesthetised and there was no mortality during the week of monitoring. On the other hand, controls were included in the analysis for the final validation experiment, since mortality was observed in these groups during the three-month experimental period. After angular transformation, percentages were compared using one way ANOVA. When the results were significant, a Tukey *a posteriori* test was used to compare treatments.

3. Results

No significant differences ($F = 3.388$, d.f. = 4) were found in the percentages of anaesthetised oysters or in the levels of oyster mortality ($F = 0.795$, d.f. = 4) between the treatments made with the different types of magnesium chloride (laboratory *vs.* agricultural) or at the different concentrations (Table 1). In contrast, the number of oysters anaesthetised was significantly lower ($F = 24.252$, d.f. = 1, $P < 0.01$) at 14.9 °C (50 ± 10%) compared to 18.8 °C (83 ± 6%), although there was no effect on oyster mortality ($F = 0.500$, d.f. = 1). Flat oyster weight had no significant effect either on the number of oysters anaesthetised (19.2 g oysters: 93 ± 12% and 38.3 g oysters: 77 ± 23%; $F = 2.167$, d.f. = 1) or on oyster survival (no mortality was observed).

Table 1. Effects of anaesthetics and their concentrations on the percentage of anaesthetised oysters and subsequent mortality (means ± SD).

Magnesium chloride	**Anaesthetised oysters (%)**	**Mortality (%)**
Laboratory (50 g L^{-1})	70 ± 27	3 ± 6
Laboratory (72 g L^{-1})	100 ± 0	7 ± 6
Agricultural (50 g L^{-1})	80 ± 20	3 ± 6
Agricultural (72 g L^{-1})	87 ± 15	0

Increasing treatment duration from 1 to 22 h significantly increased the percentage of anaesthetised oysters ($F = 5.085$, d.f. = 4, $P < 0.05$), 100% oysters opened in the longest treatment duration, but no effect ($F = 0.500$, d.f. = 4) was observed on subsequent oyster mortality (Fig. 1A). Furthermore, increasing treatment duration from 1 to 5 h significantly increased flat oyster valve opening rate ($F = 11.795$, d.f. = 4, $P < 0.001$, Fig. 1B), thus facilitating tissue biopsy. However, increasing flat oyster anaesthesia duration from 1 h to 22 h did not significantly change the percentage of oysters that recovered as a function of time in clean seawater ($F = 0.720$, d.f. = 20, Fig. 1C).

Gonad sampling of anaesthetised oysters did not significantly increase ($F = 0.074$, d.f. = 1) the mortality observed (3 ± 6%) compared with either of the control groups (no mortality in any group).

A mean percentage of 95 ± 2% anaesthetised oysters was observed in the monthly anaesthesia and gonad sampling test made over a three month period in the validation test. This percentage did not vary significantly as a function of anaesthesia rank ($F = 0.237$, d.f. = 3). Compared to the control oysters that were neither anaesthetised nor sampled (survival: 73.3 ± 15.3%), monthly anaesthesia and gonad sampling did not significantly decrease ($F = 3.904$, d.f. = 1) oyster survival (53.3 ± 5.8%).

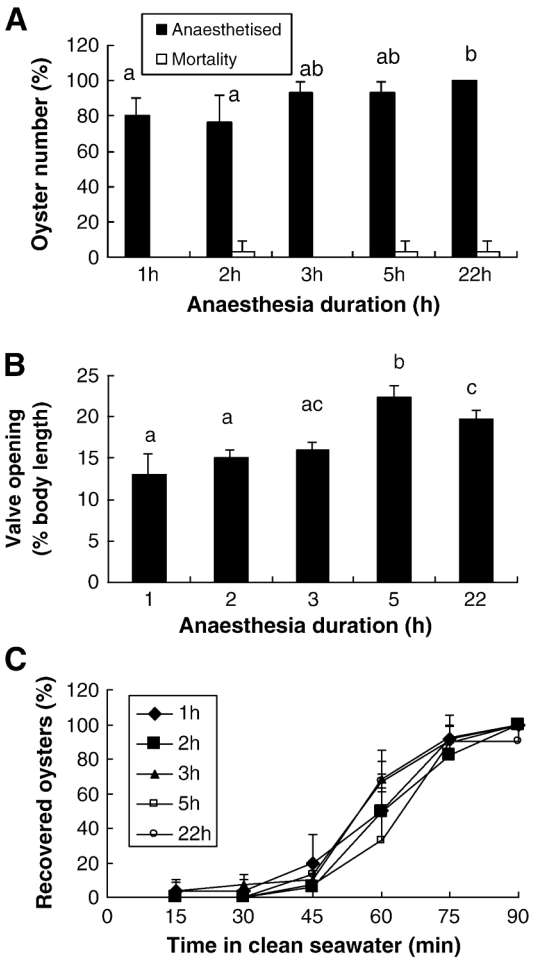

Fig. 1. Effect of anaesthesia duration on A: the percentage of anaesthetised oysters and subsequent mortality, B: valve opening, and C: recovery as a function of time (Different letters refer to significantly different results within the parameter).

4. Discussion

The present protocol complements information from the previous study published by Culloty and Mulcahy (1992) to provide a suitable anaesthesia protocol for European flat oysters: our study led to the selection of an agricultural magnesium chloride and examined the effects of water temperature, oyster weight and $MgCl_2$ exposure duration on flat oyster anaesthesia success.

Magnesium chloride has been found to be a suitable anaesthetic for many oyster species such as Pacific oyster (Namba et al., 1995) and European flat oyster (Culloty

and Mulcahy, 1992). According to results recorded in Pacific oyster (Suquet et al., 2009), the same magnesium chloride designed for agriculture (DSW®) is as effective as a laboratory brand but 14 times less expensive.

Raising the water temperature (from 14.9 to 18.8 °C) increased the percentage of flat oysters anaesthetised, in agreement with our previous results on Pacific oyster (Suquet et al*.,* 2009) comparing similar temperatures (15.3 °C and 19.5 °C) but in contrast to another study on Pacific oysters where the opposite trend was observed with a comparison of cooler temperatures (5 and 15 °C; Namba et al., 1995). Flat oyster weight had no significant effect either on the percentage of oysters anaesthetised or on oyster survival. The size of pearl oysters had also no significant effect on anaesthesia success (Norton et al., 1996).

The present study also provides a method that improves gonad sampling in European flat oysters, as increasing exposure duration to magnesium chloride increases the percentage of anaesthetised oysters and the extent of valve opening, thus facilitating tissue sampling. Furthermore, gonad biopsy did not modify flat oyster survival, as also observed in blacklip pearl oyster (Acosta-Salmon and Southgate, 2004). Monthly gonad sampling over a three month period did not increase flat oyster mortality. However, long term effects of anaesthesia on reproduction require further research. Furthermore, the representative aspect of sampling must be studied since Acosta-Salmon and Southgate (2004) demonstrated the effect of needle size on the interpretation of gonad stage.

In conclusion, a protocol for the European flat oyster anaesthesia and sampling was established from this work. Flat oysters can be anaesthetised by maintaining them for 2 to 3 h in a bath (2/3 freshwater and 1/3 seawater) containing 50 g L^{-1} DSW® magnesium chloride. Monthly anaesthesia and gonad sampling may be carried out, facilitating descriptive studies of European flat oyster cycles including gametogenesis.

Acknowledgments

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Annexe II

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Individual monitoring of gonad development in the European flat oyster *Ostrea edulis* by in vivo magnetic resonance imaging

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Abstract

Previous studies have shown that magnetic resonance imaging is a very appropriate non-invasive technique for quantifying the growth of somatic and gonadic tissues and sex determination in the Pacific oyster, *Crassostrea gigas*. Despite a thinner gonad, harder to distinguish than that of the Pacific oyster, we showed in this study that it was possible to distinguish and quantify the development of the gonad of the European flat oyster, *Ostrea edulis,* using a 3D MRI sequence with a suitable finer resolution. Compared to T2-weighted images, theoretically the most appropriate for good anatomical description, T1-weighted images were more suited to gonad observation. The gonad development was quantified by the number of voxels with higher grey level. Larvae were then depicted in the intervalvar cavity of spawning females. MRI imaging is a non-invasive method that is well suited to the description of gametogenesis in the European flat oyster.

Keywords: Oyster; *Ostrea edulis*; Gonad development; Magnetic resonance imaging

1. Introduction

The European flat oyster, *Ostrea edulis,* is a native European species and traditionally is an attractive product for consumption in a range of European countries. This species is highly valued and sought after on the European seafood market. However, supplies are limited because of declines in fisheries due to recruitment failure and the occurrence of diseases caused mainly by parasites and environmental stress.

Aquaculture may counteract decline in availability and, in terms of biodiversity, it is also important to sustain the production of healthy and genetically differentiated populations along European coasts. Spat production of flat oyster*s* has been a challenge over the years compared to the more easily cultured introduced species such as Pacific oyster, *Crassostrea gigas*. Research focused on the flat oyster is therefore essential to maintain large enough production to meet demands and retain the market share. However, the ecology and physiology of this bivalve are not fully understood, and consequently monitoring of growth and reproduction, both in the field and in hatcheries, is still based on empirical factors.

Investigation of soft tissues in bivalves classically relied on the removal of the hermetic shell that protects the animal (Chávez-Villalba et al., 2002, Dridi et al., 2007 and Fabioux et al., 2005). This has provided much valuable information, but has two major disadvantages: these methods are time consuming and necessarily destructive. Practical anaesthesia and sampling protocols have been developed in some bivalves such as Pacific oyster, enabling sex determination and maturation stage observation without sacrificing animals (Namba et al., 1995; Suquet et al., 2009). However, the assessment of biometric parameters (gonad wet weight and total body weight) facilitating the determination of condition index is prevented.

Non-invasive and quantitative procedures have therefore been developed and have proved promising after preliminary trials (Pouvreau et al., 2006). Non-invasive characterization of gonad maturation and sex determination of the Pacific oyster by

magnetic resonance imaging (MRI) has already been successfully applied using longitudinal relaxation T1-weighted MRI sequences (Davenel et al., 2006 and Hatt et al., 2009). MRI is the most appropriate technique for quantification of the growth of somatic and gonadic tissues because this method is non-invasive and successive sampling may be performed on the same individuals, thus facilitating descriptive studies such as gametogenesis.

We report here the ability of MRI to monitor gonad development in individual European flat oysters, *O. edulis,* until spawning. The European flat oyster is a larviparous species, the larvae of which are released after 7 to 10 days of development. Compared to the Pacific oyster, the fecundity of the European flat oyster is lower (1 to 2 million eggs; Walne, 1964), and smaller gonad size is reported. Any MRI protocol must therefore take into account these reproduction features.

2. Materials and methods

2.1. Origin of animals and preparation

Three hundred flat oysters (4 years of age, *Bonamia* and *Marteilia* free), originating from Norway, were transferred to the Argenton experimental hatchery (West Brittany). Oysters were maintained from April to August 2009, in 700 L flow-through tanks at 19 °C with permanent light, and continuously fed according to Chávez-Villalba et al. (2002).

Twelve non-anaesthetised oysters were brought 250 km to Cemagref in Rennes (Brittany, France) on five dates (April 7, April 29, May 13, June 2, and July 10, 2009) for MRI investigations without any specific anaesthetization procedure. Before MRI measurements, animals were soaked in sea water to expel any air bubbles that can cause artefacts on images. Sea water was maintained at room temperature and shell opening was facilitated by the addition of phytoplankton. After expelling air

bubbles, oyster shells were maintained closed with a rubber band during MRI scanning.

2.2. MRI measurements

In this new study, NMR measurements were performed on the European flat oyster at Cemagref (Rennes, Brittany, France) with a Siemens Avento imager operating at 1.5 T (60 MHz) equipped with a "knee" probe which allowed the simultaneous investigation of two oysters. The stronger magnetic field of this machine allowed us to obtain more precise images with higher spatial resolution and to monitor the development of the very thin gonad observed in individual flat oysters. Each oyster was scanned using two high resolution 3D MRI protocols (Table 1). A T2-weighted Turbo 3D Spin Echo sequence provided 48 contiguous images, highlighting water in the shell and chambers and pericardial cavities (Fig. 1a). A T1-weighted turbo gradient echo sequence provided 52 contiguous images, highlighting animal tissues and particularly gonadic tissues (Fig. 1b).

Table 1. Parameters of the two 3D MRI sequences used to describe and quantify flesh and gonad development.

MRI sequence	**T2-weighted 3D turbo spin echo**	**T1-weighted spoiled gradient echo**
Slice number	48	52
Slice thickness (mm)	1	1
FOV (mm × mm)	75 × 100	75 × 120
Matrix	192 × 256	160 × 256
Pixel size (mm × mm)	0.39 × 0.39	0.47 × 0.47
TR (ms)	2000	11
TE (ms)	150	4.76
Flip angle (°)	170	20
Bandwidth (Hz/pixel)	168	200
Excitation number	1	6
Acquisition time	13 min	8 min

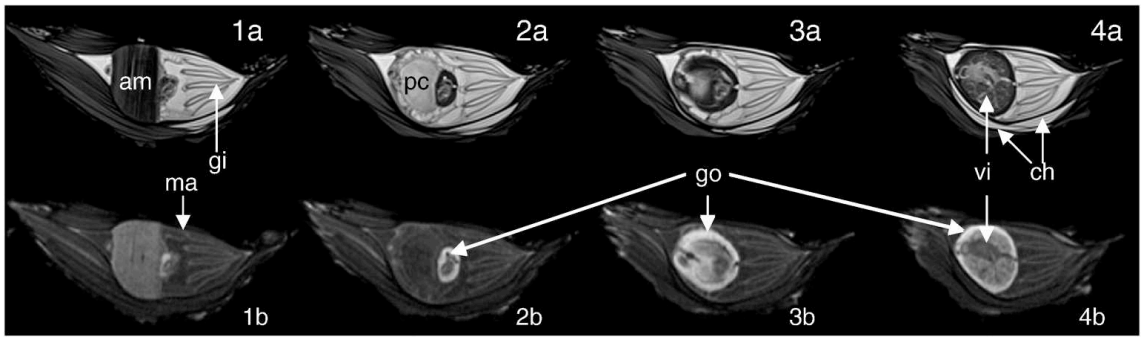

Fig. 1. The same four most representative transverse images through the body of flat oysters *Ostrea edulis* from the 48 images of the T2-weighted Turbo 3D Spin Echo sequence (a), and the 52 images of the T1-weighted gradient echo sequence (b). Main organs identified: adductor muscle (am), viscera (vi), gills (gi), gonad (go), pericardial cavity (pc), mantle (ma), and shell chamber (ch).

2.3. Image analysis

Examination of raw images obtained from an MRI scanner can lead to erroneous interpretations of variations in the grey level in the images: several sources of spatial inhomogeneities in the apparatus related to the inhomogeneity of the magnetic field and the geometry of the transmitting and receiving probes lead to undesirable variations in intensity in the images. Using homogeneous doped water phantoms as reference, we calculated that the grey levels of the oyster placed in the lower part of the MRI probe were 7% lower than the grey levels of the oyster placed in the upper part. The grey levels of the image, originating from the lower part of the raw images, were therefore multiplied by 1.07 after automated segmentation of the raw images in two under-images to separate each of the two oysters, to obtain comparable grey levels for all the oysters scanned.

Although it was possible to obtain a reliable assessment of the development of somatic and gonadic tissues by visualizing the images, we tried to reduce the influence of the human input by exploiting the overall histograms of voxels in all

the images and setting thresholds to separate tissues, while counting the somatic index reflecting the volume occupied by flesh and the gonad index reflecting the volume occupied by the gonad.

3. Results

3.1. Choice of MRI sequence to monitor gonad development and tissue evolution

Although the T2-weighted images (Fig. 1a) offered higher resolution and better signal to noise ratio and were theoretically the most appropriate for accurate anatomical description, the best contrasts for the characterization and segmentation of the gonad were obtained with T1-weighted images (Fig. 1b). In particular, the highlighting of the gonad before spawning was very significant, while the gonad gave a low signal that was difficult to differentiate from the signal of the background and the signal of the shell in T2-weighted images. Therefore, in addition to visual observation and the method successfully used previously to segment MRI T1-weighted images of the Pacific oyster, we sought to characterize the gonad development of each oyster with a gonad index (the number of voxels of grey level > 150) and the flesh occupation by a somatic index (the number of voxels of grey level > 90) to eliminate background, shell, and seawater in images (Fig. 2).

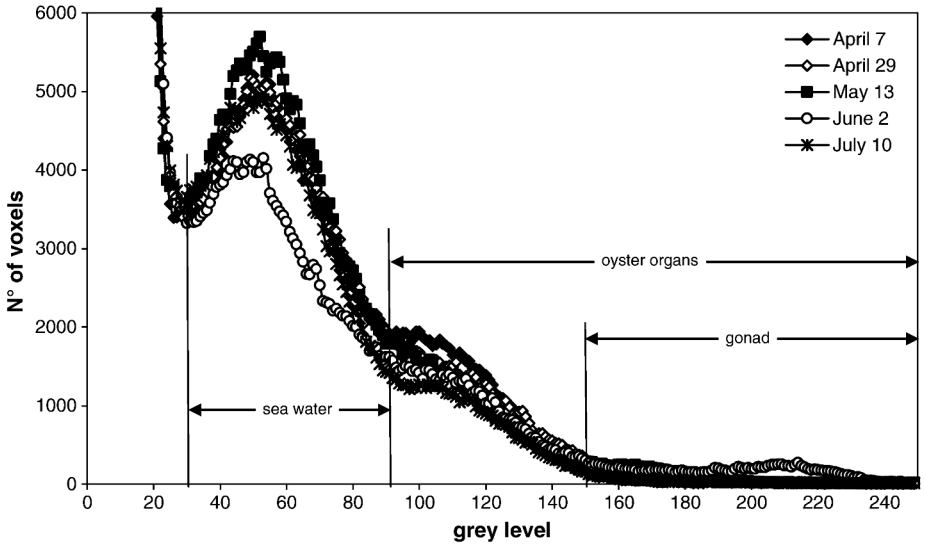

Fig. 2. Grey-level histograms from the 52 images from the T1-weighted spoiled gradient echo for oyster 12 at the five dates of MRI scanning.

3.2. Monitoring of gonad development and detection of spawning of gametes

Four of the twelve oysters died during the experiments: oyster 9 died after April 29, oyster 7 after May 13, and oysters 5 and 6 after June 2. The most representative T1-weighted images of the gonads of 10 of the 12 oysters scanned at the five dates is reported in Fig. 3, including oysters 5 and 6. Female gametes liberated in the intervalvar cavity, and probably fertilized by externally released sperm, were clearly visible for oysters 1, 2, 4, and 11 at different dates: larvae appeared as a grey volume in the gills. At the date preceding this stage when larvae were detected, the grey level of pixels corresponding to the gonad was very high and clearly visible in the images. We were therefore also able to determine the stage preceding spawning for oysters 5, 8, and 12, despite the fact that one corresponding animal died thereafter (oyster 5), or the larvae were not visible after their expulsion into the surroundings following an incubation period in the branchial chamber of 8–10 days, and probably because the time interval between the two successive samples was too long for these individual oysters. No gonad development was discernible for oysters 3, 6, and 10.

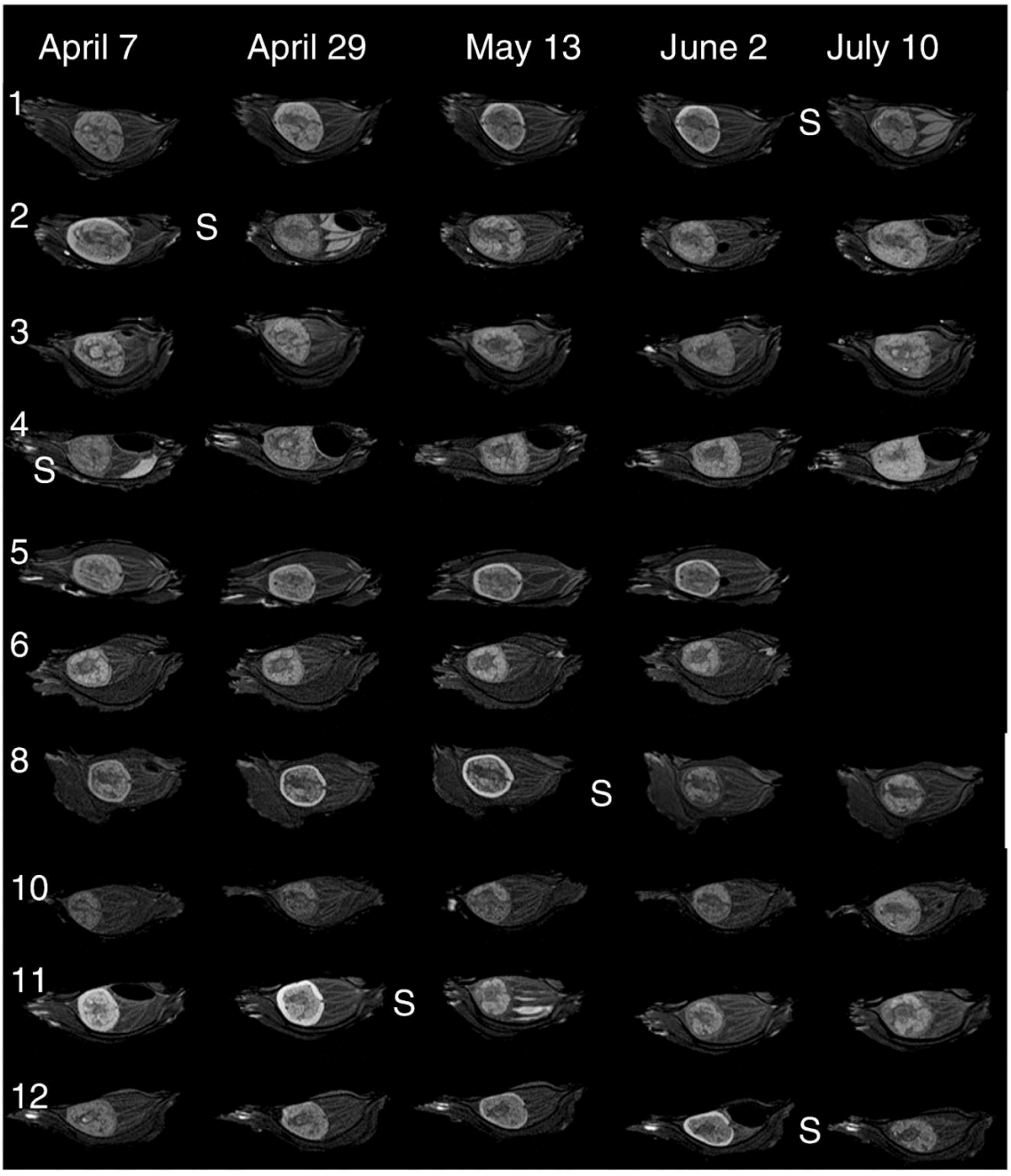

Fig. 3. The most representative MRI scanning images of gonad development of 10 laboratory conditioned flat oysters collected at five successive dates from April to July 2009 (S: spawning period).

It was possible to estimate the gonad development by counting the number of voxels at grey level > 150 (Fig. 4a and b). The number reached 5000 to 6200 voxels for oyster 1, 2, 8, 11, and 12 just before spawning, i.e., a gonad volume between 1.10 and 1.37 cm^3. Volume was only significantly lower (0.44 cm^3) for oyster 5 just before death.

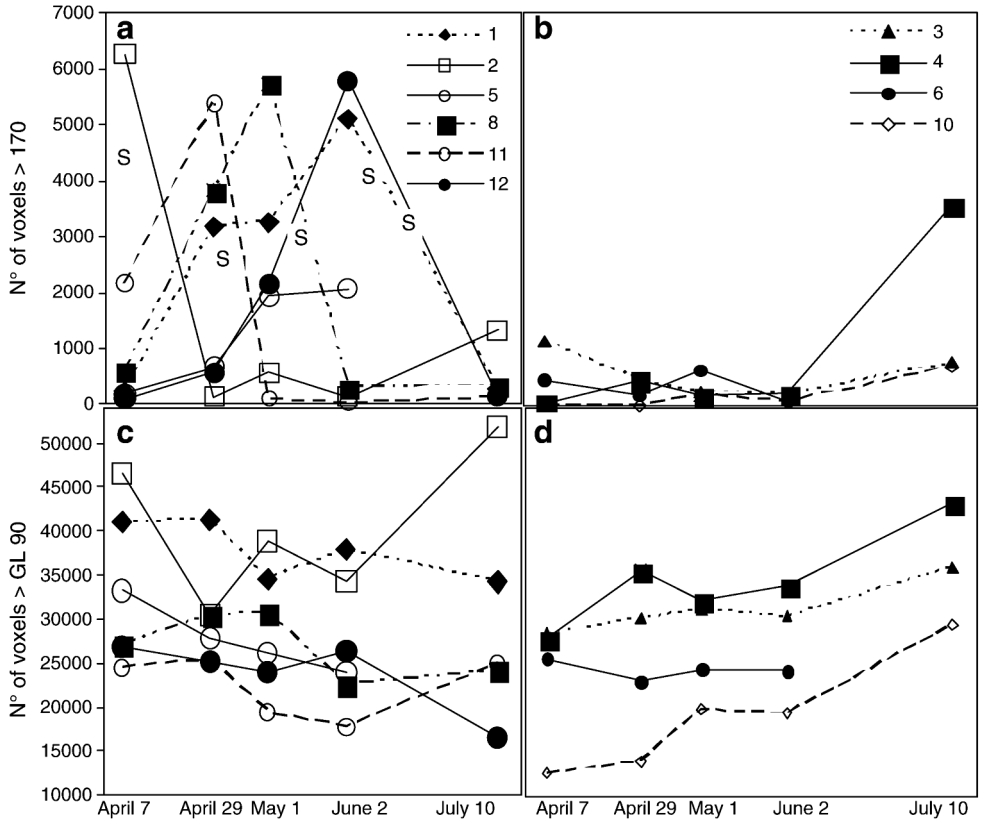

Fig. 4. Evolution of the gonad index (a, b) and the somatic index (c, d) of 6 oysters with apparent development of the gonad (a, c) and 4 oysters without apparent development of the gonad (b, d) at five dates of MRI scanning during April–July 2009.

By counting voxels at grey levels > 90 (Fig. 4c and d), it was possible to propose a raw somatic index. The index clearly increased from April to July for oysters 3 and 10 (Fig. 4d), but these oysters showed no apparent development of the gonad

during this period. It was the same for oysters 2 and 4 after spawning, the stage at which this index was the lowest. The index did not vary significantly during the period of gonad maturation before spawning.

4. Discussion

This new study confirms the potential of nuclear magnetic resonance in the non-invasive phenotype characterization of bivalves. Because of the development of a thick gonad, we have previously shown the possibility of monitoring and measuring the development of the gonad in the Pacific oyster with a low-field 0.2 T MRI imager and with medium spatial resolution ($0.94 \times 0.94 \times 4$ mm^3) (Davenel et al., 2006). In this study tracking individual European flat oysters, we individually monitored a dozen oysters between April and July 2009 during gonad maturation. Despite the development of a thinner gonad, using a higher field (1.5 T) with better spatial resolution ($0.47 \times 0.47 \times 1$ mm^3), we were able to monitor and quantify gonad development until spawning.

We confirmed that T1-weighted MRI sequences are particularly well suited to highlight the gonad of the European flat oyster. It was possible during monitoring to visualize the incubation of larvae in the intervalvar cavity, providing evidence that the gonad observed days earlier belonged to a female animal. Our previous studies on the Pacific oyster showed that the female gonad provided a clearer signal that the male gonad. The gonad index proposed to monitor the development of the gonads just before spawning and visual observation showed that seven of the ten oysters presented in Fig. 3 would clearly develop a female gonad during the observation period. The three others did not seem to have developed a gonad, unless it was a male gonad with a weaker signal than that of a female gonad and little different from that of the viscera. This hypothesis could not be confirmed during this study because we did not want to sacrifice any animal.

The somatic index adopted in this study, currently validated to reflect only the weight of flesh in the Pacific oyster (Davenel et al., 2006), was lowest when the

animal had just spawned. In the following period, it was more often growing progressively and then seemed to slow down during the gonad development phase. While the female gonad provided a clearer signal during this phase, the signal from the viscera tended to decrease. It is possible that there is accumulation of reserves before the gonad development phase and transfer of a proportion of the reserves accumulated in the organs to the gonad during gonad development. We have previously demonstrated that the somatic index proposed here in the case of the Pacific oyster (based on the number of voxels with grey levels higher than 90) is well correlated with flesh weight and thus mainly reflects the volume occupied by the flesh (Davenel et al, 2006). The grey level intensity in T1-weighted MRI images is highly correlated with dry matter content of tissues and particularly increased by the presence of lipids.

In conclusion, non-invasive imaging using MRI provides very valuable information for the individual monitoring of gonad development in the European flat oyster. Further studies, supplemented by biopsy and dissection, should be undertaken to characterize the development of the male gonad and to develop a new somatic index more suited to reflecting the accumulation of reserves including lipids. This would improve the existing relationship between the grey level in MRI image and dry matter content of the different tissues of the oyster.

Acknowledgement

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Annexe III

Table 1: Main fatty acids composition in the total lipids of *Isochrysis affinis galbana*, *Chaetoceros gracilis* and *Pavlova lutheri*, expressed in percent (% ± S.D.).

	Oyster diets		
Fatty acid	*I. aff. galbana*	*C. gracilis*	*P. lutheri*
14:0	18.97 (8.83)	10.12 (1.13)	10.04 (0.63)
16:0	9.37 (0.19)	11.09 (0.71)	19.45 (2.20)
18:0	0.22 (0.47)	0.00 (0.00)	0.43 (0.10)
20:0	0.10 (0.01)	0.01 (0.01)	0.00 (0.00)
22:0	0.00 (0.00)	0.23 (0.01)	0.06 (0.01)
24:0	0.11 (0.05)	0.00 (0.00)	0.02 (0.01)
16:1(n-9)	0.63 (0.15)	0.00 (0.00)	0.00 (0.00)
16:1(n-7)	5.05 (0.39)	25.37 (0.57)	16.27 (4.21)
18:1(n-9)	12.09 (0.51)	0.77 (0.18)	1.31 (0.39)
18:1(n-7)	1.15 (0.10)	0.48 (0.08)	1.72 (0.03)
16:2(n-7)	0.22 (0.01)	2.64 (1.76)	0.11 (0.09)
16:2(n-6)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
16:2(n-4)	0.51 (0.12)	3.34 (0.38)	0.48 (0.06)
16:3(n-4)	0.29 (0.24)	11.61 (2.22)	0.09 (0.01)
16:4(n-3)	0.52 (0.01)	0.00 (0.00)	0.00 (0.00)
16:4(n-1)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
18:2(n-6)	11.62 (3.47)	1.17 (0.43)	2.42 (0.30)
18:2(n-4)	0.01 (0.04)	0.05 (0.05)	0.33 (0.25)
18:3(n-6)	1.34 (0.26)	1.07 (0.04)	1.72 (0.27)
18:3(n-3)	6.12 (0.59)	0.21 (0.05)	1.57 (0.44)
18:4(n-3)	12.07 (3.19)	0.94 (0.06)	6.62 (0.55)
18:5(n-3)	2.15 (0.10)	0.00 (0.00)	0.00 (0.00)
20:4(n-6)	0.23 (0.19)	1.39 (0.09)	0.46 (0.35)
20:5(n-3)	0.33 (0.18)	22.66 (0.14)	23.37 (2.08)
22:5(n-6)	1.70 (1.03)	0.00 (0.00)	1.01 (0.26)
22:5(n-3)	0.09 (0.05)	0.03 (0.01)	0.66 (0.05)
22:6(n-3)	10.17 (2.54)	1.11 (0.51)	10.75 (0.34)
TO.SAT.	29.48 (9.40)	22.04 (0.50)	34.65 (1.96)
TO.MONO	20.45 (1.24)	28.18 (0.16)	19.86 (4.27)
TO.(n-9)	12.89 (0.34)	0.93 (0.34)	1.37 (0.11)
TO.(n-7)	7.28 (1.13)	26.44 (0.47)	18.74 (4.22)
TO.POLY	48.37 (8.04)	47.08 (1.40)	49.41 (2.87)
TO.(n-4)	0.81 (0.40)	15.00 (1.79)	0.57 (0.07)
TO.(n-6)	15.15 (7.23)	4.01 (0.63)	5.94 (0.77)
TO.(n-3)	32.07 (0.70)	25.33 (0.66)	42.79 (2.26)
(n-3)/(n-6)	2.12 (1.86)	6.32 (1.37)	7.21 (0.49)
22:6/20:5	31.07 (6.67)	0.05 (0.02)	0.46 (0.03)
fg cell^{-1}	1367.60 (518.79)	1848.86 (87.29)	2672.7 (636.6)
Sterols			
Cholesterol	0.67 (0.09)	50.94 (1.99)	0.40 (0.06)
Brassicasterol	99.33 (0.09)	0.00 (0.00)	0.00 (0.00)
Desmosterol	0.00 (0.00)	0.00 (0.00)	1.57 (0.11)
Campesterol	0.00 (0.00)	0.00 (0.00)	2.90 (0.07)
24 MeCholesterol	0.00 (0.00)	6.61 (1.94)	0.00 (0.00)
Fucosterol	0.00 (0.00)	37.26 (0.64)	0.00 (0.00)
Isofucosterol	0.00 (0.00)	5.19 (0.70)	0.00 (0.00)
Methylpavlovol	0.00 (0.00)	0.00 (0.00)	36.08 (1.90)
Ethylpavlovol	0.00 (0.00)	0.00 (0.00)	16.43 (1.44)
fg cell^{-1}	83.71 (7.68)	103.39 (13.22)	2672.7 (366.6)

Table 2: Total neutral fatty acids composition in *Ostrea edulis* larvae obtained from broodstock fed *Rhodomonas salina* + *Chaetoceros gracilis* and fed mono, bi and multi-specific diets expressed in relative contents (weight % of total neutral fatty acids. ± S.D.). **I**: T-Iso; **Cg**: *C. gracilis*; **P**: *Pavlova lutheri*; **I**+**Cg**: T-Iso + *C. gracilis*; **I**+**P**: T-Iso + *P. lutheri*; **P**+**Cg**: *P. lutheri* + *C. gracilis*; **I**+**Cg**+**P**: T-Iso + *C. gracilis* + *P. lutheri*.

Fatty acid	Initial	Oyster larval diets						
		I	**Cg**	**P**	**I + Cg**	**I + P**	**P + Cg**	**I + Cg + P**
	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]
14:0	3.9 ± 0.0	9.2 ± 0.3	5.7 ± 1.1	3.0 ± 0.5	8.6 ± 0.1	8.2 ± 0.1	5.8 ± 0.1	7.9 ± 0.0
16:0	18.9 ± 0.1	9.5 ± 0.4	11.6 ± 1.8	13.9 ± 2.1	10.7 ± 0.1	13.3 ± 0.0	13.9 ± 0.2	12.4 ± 0.3
18:0	3.7 ± 0.1	2.1 ± 0.2	2.8 ± 0.8	4.1 ± 0.6	2.3 ± 0.1	2.9 ± 0.1	3.2 ± 0.0	2.7 ± 0.2
16:1(n-7)	5.7 ± 0.1	3.3 ± 0.3	12.2 ± 1.6	8.0 ± 1.2	8.7 ± 0.1	7.0 ± 0.5	13.3 ± 0.0	9.9 ± 0.4
18:1(n-9)	1.1 ± 0.0	11.7 ± 0.1	1.0 ± 0.6	2.1 ± 0.3	5.7 ± 0.2	8.2 ± 0.1	1.2 ± 0.0	5.1 ± 0.0
18:1(n-7)	6.2 ± 0.0	3.6 ± 0.1	9.7 ± 1.2	6.4 ± 0.9	6.7 ± 0.0	6.7 ± 0.2	10.6 ± 0.1	8.3 ± 0.3
20:1(n-7)	4.6 ± 0.1	1.8 ± 0.0	3.2 ± 0.5	3.8 ± 0.5	2.4 ± 0.0	3.0 ± 0.1	3.9 ± 0.0	2.9 ± 0.1
16:2(n-7)	0.2 ± 0.3	0.0 ± 0.0	2.2 ± 1.0	0.8 ± 0.9	1.6 ± 0.0	0.0 ± 0.0	1.2 ± 1.7	1.3 ± 0.8
16:2(n-6)	0.3 ± 0.4	0.6 ± 0.1	0.4 ± 0.5	0.7 ± 1.1	0.0 ± 0.0	0.3 ± 0.2	1.1 ± 1.6	0.3 ± 0.4
16:2(n-4)	0.4 ± 0.0	0.2 ± 0.0	1.8 ± 0.9	0.1 ± 0.1	1.5 ± 0.0	0.2 ± 0.0	1.5 ± 0.2	1.2 ± 0.1
16:3(n-4)	0.3 ± 0.0	0.0 ± 0.0	4.6 ± 2.1	0.3 ± 0.1	3.6 ± 0.2	0.0 ± 0.0	2.7 ± 0.0	2.0 ± 0.0
16:4(n-1)	3.0 ± 0.3	0.8 ± 0.0	1.7 ± 0.9	3.5 ± 0.1	0.4 ± 0.6	1.3 ± 0.1	1.5 ± 0.0	1.3 ± 0.1
18:2(n-6)	4.2 ± 0.0	11.8 ± 0.1	0.8 ± 0.4	1.5 ± 0.2	6.3 ± 0.0	7.7 ± 0.2	0.3 ± 0.0	5.3 ± 0.0
18:3(n-3)	2.5 ± 0.0	6.1 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	2.8 ± 0.0	3.4 ± 0.0	0.3 ± 0.0	2.1 ± 0.1
18:4(n-3)	1.5 ± 0.0	10.5 ± 0.2	0.9 ± 0.1	1.0 ± 0.2	5.2 ± 0.1	5.6 ± 0.0	1.6 ± 0.0	4.2 ± 0.1
20:2i	0.9 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.1
20:2j	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.1
20:4(n-6)	2.3 ± 0.0	0.8 ± 0.0	1.2 ± 0.1	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.0	1.1 ± 0.0	0.8 ± 0.0
20:4(n-3)	0.3 ± 0.0	0.5 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:5(n-3)	14.3 ± 0.3	1.5 ± 0.1	22.8 ± 4.1	12.5 ± 2.3	14.5 ± 0.3	7.7 ± 0.1	20.2 ± 0.2	14.3 ± 0.3
22:2i	0.0 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
22:2j	5.6 ± 0.1	1.2 ± 0.0	2.7 ± 1.1	4.7 ± 0.5	1.1 ± 0.0	2.2 ± 0.1	3.0 ± 0.1	2.0 ± 0.1
22:5(n-6)	0.2 ± 0.0	2.9 ± 0.0	0.4 ± 0.5	1.3 ± 0.3	1.1 ± 0.0	2.2 ± 0.0	0.5 ± 0.0	1.2 ± 0.0
22:5(n-3)	0.8 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
22:6(n-3)	9.6 ± 0.2	14.4 ± 0.1	4.2 ± 3.3	9.7 ± 2.3	6.3 ± 0.0	12.9 ± 0.1	5.7 ± 0.0	7.5 ± 0.1
TO.MONO	20.3 ± 0.4	22.4 ± 0.3	27.8 ± 1.8	21.8 ± 3.0	25.5 ± 0.4	26.3 ± 0.1	30.2 ± 0.0	27.5 ± 0.1
TO.(n-9)	1.9 ± 0.7	12.9 ± 0.3	1.7 ± 0.6	2.6 ± 0.6	6.0 ± 0.3	8.7 ± 0.1	1.8 ± 0.1	5.5 ± 0.3
TO.(n-7)	16.5 ± 0.2	9.0 ± 0.0	25.2 ± 2.4	18.3 ± 2.7	18.3 ± 0.0	16.7 ± 0.2	27.8 ± 0.1	21.2 ± 0.2
TO.POLY	50.4 ± 1.4	55.7 ± 0.6	49.7 ± 1.0	43.4 ± 6.0	51.3 ± 0.9	48.8 ± 0.6	45.7 ± 0.6	48.6 ± 0.1
TO.(n-4)	1.4 ± 0.0	0.3 ± 0.0	8.2 ± 3.7	0.7 ± 0.3	7.4 ± 0.3	0.3 ± 0.0	5.5 ± 0.3	4.6 ± 0.1
TO.(n-6)	8.6 ± 0.4	18.3 ± 0.2	4.5 ± 1.8	7.2 ± 1.6	10.1 ± 0.1	13.1 ± 0.0	4.5 ± 1.5	9.4 ± 0.6
TO.(n-3)	30.3 ± 0.7	34.4 ± 0.4	29.7 ± 0.3	25.6 ± 4.9	30.1 ± 0.1	31.1 ± 0.3	29.2 ± 0.0	29.3 ± 0.3
TO. NMI	6.8 ± 0.2	2.0 ± 0.0	3.4 ± 1.3	5.7 ± 0.6	1.7 ± 0.0	3.1 ± 0.1	3.9 ± 0.1	2.6 ± 0.0
(n-3)/(n-6)	3.5 ± 0.1	1.9 ± 0.0	7.8 ± 2.0	3.6 ± 0.1	3.0 ± 0.0	2.4 ± 0.0	6.9 ± 2.3	3.1 ± 0.2
22:6/20:5	0.7 ± 0.0	9.3 ± 0.4	0.3 ± 0.3	0.8 ± 0.0	0.4 ± 0.0	1.7 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
22:5/20:4	0.1 ± 0.0	3.9 ± 0.0	0.3 ± 0.4	1.0 ± 0.0	1.3 ± 0.1	2.4 ± 0.0	0.5 ± 0.0	1.4 ± 0.0
TOTAL	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 3: Total neutral fatty acids composition in *Ostrea edulis* larvae obtained from broodstock fed *Rhodomonas salina* + *Thalassiosira weissflogii* and fed mono, bi and multi-specific diets expressed in relative contents (weight % of total neutral fatty acids. ± S.D.). **I**: T-Iso; **Cg**: *C. gracilis*; **P**: *Pavlova lutheri*; **I**+**Cg**: T-Iso + *C. gracilis*; **I**+**P**: T-Iso + *P. lutheri*; **P**+**Cg**: *P. lutheri* + *C. gracilis*; **I**+**Cg**+**P**: T-Iso + *C. gracilis* + *P. lutheri*.

Fatty acid	Initial	Oyster larval diets						
		I	**Cg**	**P**	**I + Cg**	**I + P**	**P + Cg**	**I + Cg + P**
	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]
14:0	5.0 ± 0.7	11.3 ± 0.3	6.7 ± 0.4	6.2 ± 0.4	9.5 ± 0.4	9.4 ± 0.1	5.6 ± 2.7	8.7 ± 0.2
16:0	29.4 ± 2.7	10.1 ± 0.0	12.8 ± 3.0	19.8 ± 1.0	11.5 ± 0.6	14.0 ± 0.3	11.6 ± 3.8	12.8 ± 0.3
18:0	6.8 ± 0.7	2.0 ± 0.0	3.0 ± 0.7	4.5 ± 0.1	2.6 ± 0.3	5.5 ± 1.1	2.5 ± 0.4	2.8 ± 0.0
16:1(n-7)	0.0 ± 0.0	3.8 ± 0.1	15.2 ± 0.2	7.4 ± 10.5	10.6 ± 0.0	9.1 ± 0.9	16.3 ± 0.2	11.0 ± 0.2
18:1(n-9)	2.3 ± 0.0	12.7 ± 0.2	1.0 ± 0.6	2.4 ± 0.1	5.7 ± 0.3	6.7 ± 0.9	0.8 ± 0.1	5.1 ± 0.2
18:1(n-7)	3.5 ± 4.9	4.1 ± 0.0	12.8 ± 0.1	13.0 ± 0.6	7.7 ± 0.4	7.1 ± 0.7	14.6 ± 2.4	8.2 ± 0.0
20:1(n-7)	7.3 ± 0.1	1.5 ± 0.1	3.2 ± 0.1	3.4 ± 0.0	2.3 ± 0.1	2.6 ± 0.7	2.8 ± 0.3	2.8 ± 0.0
16:2(n-7)	0.3 ± 0.5	0.5 ± 0.0	2.3 ± 1.1	0.0 ± 0.0	1.8 ± 0.0	0.6 ± 0.9	2.2 ± 0.2	1.1 ± 1.6
16:2(n-6)	0.8 ± 0.1	0.0 ± 0.0	0.1 ± 0.2	0.6 ± 0.2	0.0 ± 0.0	0.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
16:2(n-4)	0.2 ± 0.2	0.3 ± 0.0	1.9 ± 0.9	0.0 ± 0.0	0.8 ± 1.1	0.7 ± 0.2	1.9 ± 0.1	1.2 ± 0.2
16:3(n-4)	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 2.0	0.0 ± 0.0	3.3 ± 0.0	0.6 ± 0.0	3.1 ± 0.2	1.9 ± 0.1
16:4(n-1)	0.4 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.2
18:2(n-6)	0.0 ± 0.0	13.2 ± 0.0	1.1 ± 0.6	2.6 ± 0.1	6.4 ± 0.1	6.2 ± 1.2	1.2 ± 0.1	5.5 ± 0.2
18:3(n-3)	3.1 ± 0.1	6.0 ± 0.0	0.4 ± 0.2	0.8 ± 0.0	2.6 ± 0.0	2.5 ± 0.5	0.1 ± 0.2	1.9 ± 0.2
18:4(n-3)	2.0 ± 0.1	10.7 ± 0.1	1.1 ± 0.7	2.7 ± 0.1	5.0 ± 0.1	4.8 ± 0.8	1.4 ± 0.0	4.2 ± 0.2
20:2i	1.4 ± 0.1	0.2 ± 0.0	0.5 ± 0.0	0.2 ± 0.3	0.4 ± 0.0	0.6 ± 0.3	0.2 ± 0.3	0.4 ± 0.0
20:2j	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.0
20:4(n-6)	2.5 ± 0.0	0.6 ± 0.0	1.0 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.0
20:4(n-3)	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.2 ± 0.0
20:5(n-3)	11.6 ± 0.3	1.1 ± 0.0	22.4 ± 3.4	15.7 ± 0.6	14.3 ± 0.1	8.8 ± 1.5	25.8 ± 6.6	13.2 ± 0.2
22:2i	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
22:2j	6.0 ± 0.2	0.6 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	0.7 ± 0.1	1.1 ± 0.0	1.4 ± 0.1	1.9 ± 0.1
22:5(n-6)	0.0 ± 0.0	2.2 ± 0.0	0.2 ± 0.3	0.9 ± 0.0	0.9 ± 0.0	1.2 ± 0.2	0.1 ± 0.2	1.2 ± 0.0
22:5(n-3)	0.6 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0
22:6(n-3)	10.0 ± 0.1	11.5 ± 0.0	2.7 ± 2.2	7.6 ± 0.3	5.0 ± 0.0	7.0 ± 1.5	3.6 ± 0.1	7.9 ± 0.3
TO.MONO	14.9 ± 4.9	24.0 ± 0.3	33.4 ± 0.2	26.9 ± 9.8	28.1 ± 0.8	28.1 ± 0.1	34.8 ± 2.1	28.5 ± 0.2
TO.(n-9)	3.1 ± 0.0	13.6 ± 0.2	1.5 ± 0.4	2.4 ± 0.1	6.0 ± 0.5	7.1 ± 1.0	0.9 ± 0.2	5.3 ± 0.2
TO.(n-7)	10.8 ± 5.0	9.9 ± 0.0	31.2 ± 0.2	23.8 ± 9.9	21.0 ± 0.2	20.3 ± 1.2	33.8 ± 2.5	22.3 ± 0.1
TO.POLY	43.7 ± 0.9	51.3 ± 0.1	42.6 ± 3.8	35.2 ± 0.9	46.7 ± 1.1	39.9 ± 3.9	44.8 ± 4.7	46.6 ± 0.5
TO.(n-4)	0.9 ± 0.2	0.4 ± 0.0	7.1 ± 3.3	0.0 ± 0.0	5.9 ± 1.2	1.8 ± 0.2	6.5 ± 0.3	4.2 ± 0.0
TO.(n-6)	6.0 ± 0.5	18.4 ± 0.0	3.4 ± 1.2	6.1 ± 0.2	9.5 ± 0.2	10.0 ± 1.8	3.1 ± 0.8	9.0 ± 0.2
TO.(n-3)	28.6 ± 0.2	30.8 ± 0.1	27.5 ± 0.4	27.5 ± 1.0	27.9 ± 0.2	24.9 ± 3.5	31.4 ± 6.2	28.3 ± 1.1
TO. NMI	7.3 ± 0.2	0.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.3	1.3 ± 0.0	2.1 ± 0.4	1.6 ± 0.2	2.6 ± 0.0
(n-3)/(n-6)	4.8 ± 0.4	1.7 0.0	9.2 ± 2.2	4.5 ± 0.0	2.9 ± 0.1	2.5 ± 0.1	10.9 ± 4.8	3.1 ± 0.0
22:6/20:5	0.9 ± 0.0	10.5 0.0	0.2 ± 0.2	0.5 ± 0.0	0.3 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	0.6 ± 0.0
22:5/20:4	0.0 ± 0.0	3.8 0.3	0.3 ± 0.4	1.0 ± 0.0	1.1 ± 0.1	1.8 ± 0.1	0.2 ± 0.3	1.5 ± 0.1
TOTAL	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 4: Total polar fatty acids composition in *Ostrea edulis* larvae obtained from broodstock fed *Rhodomonas salina* + *Chaetoceros gracilis* and fed mono, bi and multi-specific diets expressed in relative contents (weight % of total neutral fatty acids. ± S.D.). **I**: T-Iso; **Cg**: *C. gracilis*; **P**: *Pavlova lutheri*; **I+Cg**: T-Iso + *C. gracilis*; **I+P**: T-Iso + *P. lutheri*; **P+Cg**: *P. lutheri* + *C. gracilis*; **I+Cg+P**: T-Iso + *C. gracilis* + *P. lutheri*.

Fatty acid	Initial	Oyster larval diets						
		I	**Cg**	**P**	**I + Cg**	**I + P**	**P + Cg**	**I + Cg + P**
	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]
14:0	1.2 ± 0.3	4.1 ± 0.6	2.8 ± 0.0	4.3 ± 3.4	2.9 ± 0.1	3.4 ± 0.1	2.6 ± 0.1	3.4 ± 0.2
16:0	14.1 ± 1.5	14.1 ± 0.3	14.8 ± 0.1	16.1 ± 2.8	13.7 ± 0.3	14.4 ± 0.3	16.1 ± 0.2	15.8 ± 0.7
18:0	2.6 ± 3.7	3.9 ± 0.4	4.7 ± 0.1	5.2 ± 1.0	4.2 ± 0.1	4.1 ± 0.4	5.0 ± 0.0	4.3 ± 0.0
16:1(n-7)	1.3 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 1.6	1.5 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:1(n-9)	0.6 ± 0.1	4.2 ± 1.7	0.7 ± 0.1	2.5 ± 2.0	2.6 ± 0.1	3.3 ± 0.0	0.9 ± 0.0	2.4 ± 0.0
18:1(n-7)	3.7 ± 0.4	3.5 ± 1.1	5.9 ± 0.1	2.2 ± 3.0	3.5 ± 0.1	3.7 ± 0.0	5.3 ± 0.1	4.3 ± 0.3
20:1(n-7)	5.4 ± 0.1	3.6 ± 1.1	5.9 ± 0.0	3.2 ± 1.7	4.8 ± 0.2	4.3 ± 0.1	5.8 ± 0.1	4.5 ± 0.5
16:2(n-7)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.3	0.4 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:2(n-6)	0.5 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.0 ± 0.0	0.2 ± 0.3	0.5 ± 0.2	0.0 ± 0.0	0.3 ± 0.4
16:2(n-4)	0.0 ± 0.0	0.1 ± 0.2	0.5 ± 0.0	0.1 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:3(n-4)	0.1 ± 0.1	0.1 ± 0.2	0.4 ± 0.0	0.0 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
16:4(n-1)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:2(n-6)	3.4 ± 0.2	5.2 ± 2.4	0.2 ± 0.0	0.0 ± 0.0	4.2 ± 0.0	4.3 ± 0.1	0.5 ± 0.8	3.5 ± 0.1
18:3(n-3)	1.3 ± 0.1	2.1 ± 0.8	0.4 ± 0.0	0.4 ± 0.0	1.5 ± 0.1	1.5 ± 0.1	0.4 ± 0.0	1.2 ± 0.1
18:4(n-3)	0.5 ± 0.1	2.3 ± 0.9	0.4 ± 0.0	0.5 ± 0.2	1.4 ± 0.0	1.6 ± 0.1	0.6 ± 0.1	1.2 ± 0.0
20:2i	0.9 ± 0.4	0.1 ± 0.2	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.1	0.2 ± 0.3
20:2j	0.4 ± 0.1	0.1 ± 0.2	0.5 ± 0.0	2.7 ± 3.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.2 ± 0.3
20:4(n-6)	5.2 ± 0.0	3.0 ± 0.1	2.7 ± 0.0	2.3 ± 1.1	2.4 ± 0.2	2.7 ± 0.1	2.8 ± 0.1	2.5 ± 0.0
20:4(n-3)	0.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
20:5(n-3)	22.9 ± 3.8	10.4 ± 8.3	28.2 ± 0.2	10.8 ± 4.8	16.2 ± 0.5	10.4 ± 0.4	23.2 ± 0.7	16.4 ± 0.6
22:2i	0.0 ± 0.0	0.9 ± 0.4	0.0 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.7 ± 0.1	0.0 ± 0.0	0.4 ± 0.0
22:2j	9.8 ± 1.8	5.5 ± 1.3	8.3 ± 0.1	6.9 ± 2.2	7.1 ± 0.3	6.7 ± 0.2	8.7 ± 0.1	7.0 ± 0.3
22:5(n-6)	0.3 ± 0.0	3.6 ± 1.6	0.1 ± 0.1	1.6 ± 0.7	2.8 ± 0.1	3.4 ± 0.2	1.0 ± 0.0	2.5 ± 0.0
22:5(n-3)	1.3 ± 0.0	0.7 ± 0.3	1.4 ± 0.0	1.0 ± 0.3	0.8 ± 0.0	0.6 ± 0.0	1.1 ± 0.0	0.8 ± 0.0
22:6(n-3)	4.5 ± 6.4	15.1 ± 5.2	3.9 ± 0.0	10.3 ± 3.8	12.1 ± 0.2	17.4 ± 0.1	9.1 ± 0.2	13.8 ± 0.4
TO.MONO	16.1 ± 1.1	14.0 ± 0.8	15.7 ± 0.0	15.0 ± 1.0	15.3 ± 1.6	15.1 ± 0.4	15.0 ± 0.0	14.3 ± 1.1
TO.(n-9)	2.2 ± 0.1	5.1 ± 1.8	1.6 ± 0.4	5.9 ± 5.3	3.2 ± 0.1	4.5 ± 0.3	1.9 ± 0.0	3.0 ± 0.1
TO.(n-7)	10.5 ± 1.4	7.1 ± 2.2	11.8 ± 0.1	7.1 ± 5.4	9.8 ± 1.8	8.0 ± 0.1	11.2 ± 0.2	8.9 ± 0.2
TO.POLY	53.7 ± 1.2	54.7 ± 1.0	52.5 ± 0.1	47.9 ± 2.7	55.5 ± 1.3	54.8 ± 1.3	52.4 ± 0.5	54.0 ± 0.4
TO.(n-4)	0.2 ± 0.3	0.4 ± 0.6	1.6 ± 0.0	0.2 ± 0.2	1.3 ± 0.0	0.0 ± 0.0	0.6 ± 0.2	0.6 ± 0.1
TO.(n-6)	10.6 ± 0.1	15.0 ± 4.5	5.5 ± 0.1	7.8 ± 0.6	11.4 ± 0.7	12.9 ± 0.7	6.3 ± 1.3	10.6 ± 0.6
TO.(n-3)	31.7 ± 3.2	32.6 ± 1.7	35.9 ± 0.2	28.4 ± 4.1	34.0 ± 0.6	33.7 ± 0.8	35.7 ± 0.9	35.0 ± 0.9
TO. NMI	11.1 ± 2.3	6.8 ± 1.3	9.5 ± 0.1	10.3 ± 0.8	8.3 ± 0.5	8.2 ± 0.2	9.8 ± 0.2	7.8 ± 0.9
(n-3)/(n-6)	3.0 ± 0.3	3.0 ± 1.7	6.5 ± 0.2	3.7 ± 0.8	3.0 ± 0.1	2.6 ± 0.1	5.8 ± 1.4	3.3 ± 0.1
22:6/20:5	0.2 ± 0.3	3.2 ± 1.4	0.1 ± 0.0	1.0 ± 0.1	0.7 ± 0.0	1.7 ± 0.1	0.4 ± 0.0	0.8 ± 0.0
22:5/20:4	0.1 ± 0.0	1.1 ± 0.5	0.0 ± 0.0	0.7 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	0.4 ± 0.0	1.0 ± 0.0
TOTAL	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 5: Total polar fatty acids composition in *Ostrea edulis* larvae obtained from broodstock fed *Rhodomonas salina* + *Thalassiosira weissflogii* and fed mono, bi and multi-specific diets expressed in relative contents (weight % of total neutral fatty acids. ± S.D.). **I**: T-Iso; **Cg**: *C. gracilis*; **P**: *Pavlova lutheri*; **I**+**Cg**: T-Iso + *C. gracilis*; **I**+**P**: T-Iso + *P. lutheri*; **P**+**Cg**: *P. lutheri* + *C. gracilis*; **I**+**Cg**+**P**: T-Iso + *C. gracilis* + *P. lutheri*.

Fatty acid	Initial	Oyster larval diets						
		I	**Cg**	**P**	**I + Cg**	**I + P**	**P + Cg**	**I + Cg + P**
	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]	Mean Relative Contents [± S.D. (%)]
14:0	1.2 ± 0.3	3.3 ± 0.1	3.5 ± 0.1	2.5 ± 0.1	3.4 ± 0.0	3.4 ± 0.1	3.2 ± 0.1	3.7 ± 0.1
16:0	14.1 ± 1.5	13.3 ± 1.5	15.9 ± 0.8	17.2 ± 1.3	13.9 ± 0.1	14.2 ± 0.5	16.7 ± 0.6	15.2 ± 0.6
18:0	2.6 ± 3.7	3.9 ± 0.3	4.6 ± 0.1	4.2 ± 0.4	4.3 ± 0.3	4.8 ± 0.4	5.0 ± 1.0	4.0 ± 0.1
16:1(n-7)	1.3 ± 1.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1.9
18:1(n-9)	0.6 ± 0.1	3.4 ± 1.2	0.8 ± 0.0	1.1 ± 0.0	2.6 ± 0.5	2.6 ± 0.5	1.3 ± 0.5	2.6 ± 0.1
18:1(n-7)	3.7 ± 0.4	3.1 ± 1.3	6.0 ± 0.1	5.0 ± 0.4	3.5 ± 0.1	3.5 ± 0.1	5.7 ± 0.5	4.4 ± 0.2
20:1(n-7)	5.4 ± 0.1	3.7 ± 0.3	4.7 ± 0.4	3.8 ± 0.3	4.1 ± 0.3	4.3 ± 0.3	4.8 ± 0.1	4.4 ± 0.2
16:2(n-7)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3
16:2(n-6)	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.0 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.2 ± 0.3
16:2(n-4)	0.0 ± 0.0	0.1 ± 0.2	0.5 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.1
16:3(n-4)	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0
16:4(n-1)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:2(n-6)	3.4 ± 0.2	4.4 ± 2.1	0.0 ± 0.0	1.4 ± 0.1	3.7 ± 0.2	3.8 ± 0.0	0.7 ± 0.9	3.9 ± 0.2
18:3(n-3)	1.3 ± 0.1	2.0 ± 0.6	0.7 ± 0.1	0.6 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	0.7 ± 0.2	1.3 ± 0.0
18:4(n-3)	0.5 ± 0.1	1.8 ± 0.7	0.3 ± 0.0	0.7 ± 0.0	1.4 ± 0.2	1.4 ± 0.2	0.6 ± 0.1	1.4 ± 0.0
20:2i	0.9 ± 0.4	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.3	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1
20:2j	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.1
20:4(n-6)	5.2 ± 0.0	2.7 ± 0.1	2.5 ± 0.2	2.2 ± 0.0	2.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.0	2.3 ± 0.0
20:4(n-3)	0.0 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
20:5(n-3)	22.9 ± 3.8	9.7 ± 8.3	27.7 ± 0.4	18.4 ± 0.9	13.1 ± 2.3	14.0 ± 2.7	21.9 ± 1.5	15.6 ± 0.3
22:2i	0.0 ± 0.0	1.2 ± 0.6	0.0 ± 0.0	0.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	0.0 ± 0.0	0.5 ± 0.0
22:2j	9.8 ± 1.8	6.6 ± 0.7	8.7 ± 0.9	8.6 ± 0.2	7.5 ± 0.2	7.3 ± 0.2	9.1 ± 0.2	7.2 ± 0.0
22:5(n-6)	0.3 ± 0.0	3.6 ± 1.7	0.0 ± 0.0	1.9 ± 0.0	2.8 ± 0.1	2.9 ± 0.2	1.0 ± 0.1	2.6 ± 0.0
22:5(n-3)	1.3 ± 0.0	0.7 ± 0.3	1.4 ± 0.0	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	1.0 ± 0.0	0.7 ± 0.0
22:6(n-3)	4.5 ± 6.4	16.4 ± 5.5	4.9 ± 0.3	14.7 ± 0.1	14.8 ± 1.7	15.5 ± 1.9	10.2 ± 0.5	13.8 ± 0.5
TO.MONO	16.1 ± 1.1	13.6 ± 0.3	14.6 ± 0.5	12.5 ± 0.1	16.1 ± 0.4	13.6 ± 0.6	14.6 ± 0.0	15.4 ± 1.9
TO.(n-9)	2.2 ± 0.1	4.9 ± 1.1	2.5 ± 0.1	1.3 ± 0.3	3.7 ± 0.3	3.2 ± 0.6	2.3 ± 0.5	3.1 ± 0.1
TO.(n-7)	10.5 ± 1.4	6.8 ± 1.7	10.7 ± 0.5	8.9 ± 0.1	10.5 ± 0.5	7.8 ± 0.3	10.5 ± 0.6	10.2 ± 1.9
TO.POLY	53.7 ± 1.2	55.0 ± 1.8	52.0 ± 1.2	54.4 ± 0.1	54.6 ± 0.1	56.2 ± 0.5	53.4 ± 1.7	56.0 ± 0.7
TO.(n-4)	0.2 ± 0.3	0.3 ± 0.4	1.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.7	0.4 ± 0.5	0.9 ± 0.0	0.5 ± 0.2
TO.(n-6)	10.6 ± 0.1	13.2 ± 4.0	4.8 ± 0.2	7.8 ± 0.1	10.2 ± 0.4	11.1 ± 0.1	5.6 ± 0.5	10.6 ± 0.6
TO.(n-3)	31.7 ± 3.2	33.0 ± 1.5	36.5 ± 0.4	37.2 ± 0.4	34.4 ± 0.5	35.9 ± 0.2	36.7 ± 1.9	36.1 ± 0.3
TO. NMI	11.1 ± 2.3	8.5 ± 0.3	9.6 ± 0.7	9.4 ± 0.6	8.8 ± 0.4	8.8 ± 0.3	10.2 ± 0.2	8.6 ± 0.2
(n-3)/(n-6)	3.0 ± 0.3	3.4 ± 2.0	7.6 ± 0.2	4.8 ± 0.0	3.4 ± 0.2	3.2 ± 0.0	6.6 ± 0.9	3.4 ± 0.2
22:6/20:5	0.2 ± 0.3	4.0 ± 1.8	0.2 ± 0.0	0.8 ± 0.0	1.2 ± 0.3	1.1 ± 0.4	0.5 ± 0.0	0.9 ± 0.0
22:5/20:4	0.1 ± 0.0	1.3 ± 0.6	0.0 ± 0.0	0.9 ± 0.0	1.3 ± 0.1	1.2 ± 0.2	0.4 ± 0.0	1.1 ± 0.0
TOTAL	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 6 : Main total sterol composition in released larvae (Initial) of *Ostrea edulis* broodstock fed *Rhodomonas salina* + *Chaetoceros gracilis*, and subsequent larval rearing fed mono, Bi and tri-specific diets, expressed in mean absolute contents (ng.larvae^{-1} ± S.D., n=3)

	Initial		*I. aff. Galbana* (I)		*C. gracilis* (C_g)		*Pavlova lutheri* (P_L)		I + C_g		I + P_L		C_g + P_L		I + C_g + P_L	
	Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})		Mean Absolute Contents ± S.D. (ng.larvae^{-1})	
Cholesterol	1.1	± 0.0	0.8	± 0.0	8.6	± 0.0	0.7	± 0.0	7.0	± 0.1	1.6	± 0.0	2.8	± 0.5	2.9	± 0.5
Brassicasterol	0.5	± 0.0	3.2	± 0.3	0.5	± 0.0	0.2	± 0.1	4.6	± 0.1	2.1	± 0.1	0.3	± 0.1	1.6	± 0.3
Desmosterol	0.1	± 0.0	0.2	± 0.0	0.8	± 0.0	0.2	± 0.1	1.1	± 0.1	0.4	± 0.0	0.4	± 0.1	0.5	± 0.1
Campesterol	0.0	± 0.0	0.1	± 0.1	0.3	± 0.3	0.3	± 0.1	0.1	± 0.1	0.8	± 0.0	0.5	± 0.1	0.7	± 0.1
24 Methylsterol	0.1	± 0.0	0.1	± 0.0	0.4	± 0.0	0.0	± 0.0	0.4	± 0.0	0.2	± 0.0	0.2	± 0.0	0.3	± 0.0
Stigmasterol	0.0	± 0.0	0.2	± 0.0	0.1	± 0.0	0.6	± 0.1	0.2	± 0.0	1.7	± 0.0	1.1	± 0.3	1.3	± 0.3
Fucosterol	0.1	± 0.0	0.0	± 0.0	1.1	± 0.0	0.0	± 0.0	0.6	± 0.1	0.2	± 0.0	0.2	± 0.0	0.3	± 0.1
Isofucosterol	0.0	± 0.0	0.0	± 0.0	0.1	± 0.0	0.0	± 0.0	0.1	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.1
MethylPavlovol	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
EthanolPavlovol	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0
Total	2.3	± 0.1	4.7	± 0.4	12.0	± 0.4	2.7	± 0.4	14.2	± 0.2	9.3	± 0.2	7.0	± 1.4	9.3	± 1.8

Table 7 :Main total sterol composition in released larvae (Initial) of *Ostrea edulis* broodstock fed *Rhodomonas salina* + *Chaetoceros gracilis*, and subsequent larval rearing fed mono, Bi and tri-specific diets, expressed in mean relative contents (% ± S.D., n=3)

	Initial	*I. aff. Galbana* (I)	*C. gracilis* (Cg)	*Pavlova lutheri* (PL)	I + C_g	I + P_L	C_g + P_L	I + C_g + P_L
	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)
Cholesterol	49.9 ± 0.7	16.4 ± 0.6	58.8 ± 15.5	24.4 ± 2.2	49.4 ± 0.0	16.9 ± 0.1	40.7 ± 0.6	31.7 ± 0.2
Brassicasterol	23.6 ± 0.0	69.1 ± 0.9	4.3 ± 0.1	5.4 ± 1.9	32.0 ± 0.1	22.6 ± 0.4	3.8 ± 0.2	16.9 ± 0.4
Desmosterol	5.7 ± 0.2	4.8 ± 0.5	5.8 ± 0.8	5.6 ± 1.9	7.6 ± 1.1	4.4 ± 0.2	5.5 ± 0.2	4.9 ± 0.1
Campesterol	2.1 ± 0.1	3.1 ± 1.0	5.3 ± 2.4	10.5 ± 0.3	0.5 ± 0.6	9.1 ± 0.2	7.6 ± 0.1	7.1 ± 0.1
24 Methylsterol	5.0 ± 0.4	2.1 ± 0.0	2.7 ± 1.3	0.0 ± 0.0	2.8 ± 0.1	2.4 ± 0.0	2.7 ± 0.0	2.8 ± 0.1
Stigmasterol	1.6 ± 0.5	4.5 ± 0.0	6.2 ± 7.6	21.7 ± 1.0	1.3 ± 0.0	18.7 ± 0.4	15.2 ± 0.8	14.2 ± 0.0
Fucosterol	5.0 ± 0.1	0.0 ± 0.0	7.1 ± 3.4	0.0 ± 0.0	4.0 ± 0.3	2.2 ± 0.1	3.2 ± 0.1	3.3 ± 0.2
Isofucosterol	1.0 ± 0.1	0.0 ± 0.0	0.7 ± 0.3	0.0 ± 0.0	0.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.6
MethylPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EthanolPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Table 8 : Main total sterol composition in released larvae (Initial) of *Ostrea edulis* broodstock fed *Rhodomonas salina* + *Thalassiosira weissflogii*, and subsequent larval rearing fed mono, Bi and tri-specific diets, expressed in mean absolute contents (ng.larvae^{-1} ± S.D., n=3)

	Initial	*I. aff. Galbana* (I)	*C. gracilis* (C_g)	*Pavlova lutheri* (P_L)	I + C_g	I + P_L	C_g + P_L	I + C_g + P_L
	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})	Mean Absolute Contents ± S.D. (ng.larvae^{-1})
Cholesterol	0.4 ± 0.1	0.9 ± 0.2	5.6 ± 0.5	0.7 ± 0.0	5.3 ± 0.5	1.1 ± 0.0	3.3 ± 0.2	3.3 ± 0.1
Brassicasterol	0.3 ± 0.3	5.8 ± 1.2	0.4 ± 0.1	0.3 ± 0.0	3.6 ± 0.4	1.4 ± 0.0	0.4 ± 0.0	2.3 ± 0.0
Desmosterol	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
Campesterol	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	0.5 ± 0.0	0.7 ± 0.0	0.9 ± 0.0
24 Methylsterol	0,2 ± 0.2	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Stigmasterol	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.9 ± 0.0	0.2 ± 0.0	0.9 ± 0.0	1.1 ± 0.2	1.6 ± 0.0
Fucosterol	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.1	0.1 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.3 ± 0.0
Isofucosterol	0,0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
MethylPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EthanolPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total	1.5 ± 0.6	7.4 ± 1.6	8.0 ± 1.0	5.0 ± 0.1	11.0 ± 1.1	5.7 ± 0.0	9.0 ± 0.8	11.8 ± 0.2

Table 9 : Main total sterol composition in released larvae (Initial) of *Ostrea edulis* broodstock fed *Rhodomonas salina* + *Thalassiosira weissflogii*, and subsequent larval rearing fed mono, Bi and tri-specific diets, expressed in mean relative contents (% ± S.D., n=3)

	Initial	*I. aff. Galbana* (I)	*C. gracilis* (Cg)	*Pavlova lutheri* (PL)	$I + C_g$	$I + P_L$	$C_g + P_L$	$I + C_g + P_L$
	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)	Mean Relative Contents ± S.D. (%)
Cholesterol	30.6 ± 7.1	11.8 ± 0.2	69.9 ± 2.2	13.3 ± 0.5	48.2 ± 0.2	19.3 ± 0.0	36.6 ± 0.4	27.6 ± 0.1
Brassicasterol	15.6 ± 15.1	78.3 ± 0.5	5.4 ± 0.6	5.1 ± 0.3	32.8 ± 0.1	23.7 ± 0.0	4.7 ± 0.1	19.8 ± 0.2
Desmosterol	10.3 ± 9.4	2.1 ± 0.4	6.0 ± 0.1	4.2 ± 0.3	5.5 ± 0.2	4.1 ± 0.0	5.7 ± 0.2	4.7 ± 0.0
Campesterol	19.2 ± 9.9	2.6 ± 0.4	1.8 ± 0.0	12.4 ± 0.1	2.1 ± 0.4	9.1 ± 0.0	7.5 ± 0.1	7.7 ± 0.1
24 Methylsterol	11,0 ± 11.8	2.4 ± 0.2	5.7 ± 1.0	3.4 ± 0.1	3.7 ± 0.1	2.9 ± 0.0	4.0 ± 0.0	3.2 ± 0.0
Stigmasterol	0.9 ± 1.3	2.7 ± 0.2	0.0 ± 0.0	18.8 ± 0.2	1.6 ± 0.1	15.5 ± 0.0	12.8 ± 0.7	13.9 ± 0.2
Fucosterol	1.0 ± 0.1	0.0 ± 0.0	10.6 ± 0.1	2.2 ± 0.6	4.7 ± 0.1	2.0 ± 0.0	5.3 ± 0.0	2.8 ± 0.0
Isofucosterol	0,4 ± 0.6	0.0 ± 0.0	0.5 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.1	0.0 ± 0.0
MethylPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
EthanolPavlovol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Incidence de la nutrition sur la reproduction et le développement larvaire d'*Ostrea edulis*

Résumé : Suite à l'émergence de deux maladies parasitaires, l'huître plate *Ostrea edulis*, autrefois abondamment cultivée en France, a subit un effondrement de sa production passant de 20 000 tonnes par an à environ 1 500 tonnes de nos jours. Depuis les années 80, des travaux de sélection ont ouvert des perspectives pour contrôler la production des juvéniles de cette espèce en écloserie. Cependant, les connaissances acquises chez l'huître plate n'avaient pas suffisamment évolué depuis les années 70 et ne permettaient pas en état la production fiable de naissain. Une réappropriation des connaissances sur cette espèce s'avérait donc nécessaire. Dans ce contexte, nous nous sommes intéressés à la gestion d'un paramètre clé en écloserie, la nutrition. Nous avons donc mis en place une série d'expérimentations sur le conditionnement des géniteurs, afin de préciser le rôle de l'alimentation sur la reproduction de l'huître plate. Pour chaque microalgue sélectionnée (T-Iso, *Chaetoceros gracilis*, *Skeletonema marinoï*, *Tetraselmis suecica*, *Rhodomona salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana* et *Pavlova lutheri*) l'ingestion, l'assimilation et l'absorption ont été successivement étudiées. Les meilleures réponses physiologiques étaient obtenues par les diatomées *C. gracilis* et *S. marinoï*, et par la cryptophyceae *R. salina* avec des valeurs d'ingestion et absorption supérieures à $3\ 10^9$ µm^3 g^{-1} h^{-1} et 1,5 mg g^{-1} h^{-1} respectivement. Au contraire, les géniteurs nourris avec *T. suecica* et *P. lutheri* ne dépassent pas $0{,}3\ 10^9$ µm^3 g^{-1} h^{-1} et 0,1 mg g^{-1} h^{-1} pour l'ingestion et l'absorption et de ce fait, ne sont pas adaptées à l'huître plate. Les principaux acides gras accumulés, quel que soit le régime, étaient 16 :0, 18 :0, 20 :4(n-3), 20 :5(n-3) et 22 :6(n-3) avec deux types de réponse en terme de transfert biochimique. Ainsi, le conditionnement opéré au printemps corrobore parfaitement les données écophysiologiques acquises parallèlement. A l'inverse, celui réalisé en automne ne met pas en évidence une incorporation marquée des acides gras. Ces différents approches nous ont permis de proposer un nouveau régime bispécifique basé sur l'apport de *C. gracilis* plus *R. salina* pour les géniteurs de l'huître plate. Celui-ci améliore très sensiblement la fécondité d'*O. edulis*. Les larves issues de ce nouveau régime parental, indépendamment du régime larvaire, se sont parfaitement développées jusqu'à la fin de l'élevage, avec une survie supérieure à 90%, mais avec des taux de croissance variant de 5 à 11 µm j^{-1}. Seules les larves recevant le mélange T-Iso plus *C. gracilis* étaient capables de se métamorphoser pour au moins 50% d'entre elles. Ces résultats démontrent que chez *O. edulis*, l'étape qui traduit l'efficacité d'un régime alimentaire est la métamorphose. Celle-ci dépend à la fois du régime parental et du régime larvaire.

The effects of nutrition on the reproduction and larval development of *Ostrea edulis*

Abstract : Since the appearance of two successive diseases the production of *Ostrea edulis* highly cultivated in France dropped from 20,000 tons in the 70's to 1,500 tons y^{-1} nowadays. Genetic improvement for resistant strain to *Bonamia* was successfully explored from the 80's and opened perspectives for the production of flat oyster spat in hatchery. The know-how previously achieved on *O. edulis* controlled reproduction was however insufficient to allow reliable production of juveniles. A re-appropriation of the knowledge of the biology of this species was necessary. A set of experiments was accordingly carried out, with a focus on a key parameter in hatchery, nutrition, which was studied in the present work from broodstock to larvae. To select the best microalgae for *O. edulis* conditioning, eight single species diets (T-Iso, *Chaetoceros gracilis*, *Skeletonema marinoi*, *Tetraselmis suecica*, *Rhodomona salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana* and *Pavlova lutheri*) were fed to batches of broodstock, which were then compared using ecophysiological and biochemical approaches. Both diatoms, *C. gracilis*, *S. marinoï* and the cryptophyte *R. salina* were highly ingested and absorbed with values ≥ $3\ 10^9$ µm^3 g^{-1} h^{-1} and 1.5 mg g^{-1} h^{-1} respectively; whereas *T. suecica* and *P. lutheri* led to the lowest physiological responses (≤ 0.3 µm^3 g^{-1} h^{-1} and 0.1 mg g^{-1} h^{-1}) and holds accordingly no interest for flat oyster conditioning. The main fatty acids accumulated whatever diet were 16 :0, 18 :0, 20 :4(n-3), 20 :5(n-3) et 22 :6(n-3) with two types of response concerning biochemical compounds' transfer. Thus, when broodstock were conditioned in spring, biochemical data corroborate physiological data. In contrast, when broodstock were conditioned in autumn, low biochemical compounds' transfer occurred, whereas ingestion and absorption were clearly related to the type of microalgae delivered. A new bi-specific diet composed of *C. gracilis* plus *R. salina* for *O. edulis* broodstock conditioning was therefore proposed which clearly increased flat oyster fecundity. Larvae, originated from parents fed *C. gracilis* plus *R. salina*, showed good development with survival ≥ 90%, whatever larval diets, with growth, however, varying from 5 to 11 µm d^{-1}. More than 50% of larvae fed T-Iso plus *C. gracilis* settled whereas poor metamorphosis was recorded when larvae were fed single diets. These results clearly showed that metamorphosis is an essential step for determining undoubtedly the effects of food on *O. edulis* larvae relying on broodstock and larval diets.