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Alimentation et déterminisme environnemental de la reproduction des huîtres perlières *P. margaritifera* sur l'atoll d'Ahe (Archipel des Tuamotu, Polynésie Française)

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Étude du régime alimentaire et du déterminisme environnemental de la reproduction des huîtres perlières *Pinctada margaritifera* sur l'atoll d'Ahe (Archipel des Tuamotu-Gambier, Polynésie Française).

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INTRODUCTION GÉNÉRALE

Introduction générale

Bref historique de l'exploitation des huîtres perlières

L'huître perlière a toujours occupé une place importante dans la vie sociale des polynésiens (Le Pennec et al., 2010). Traditionnellement, ses valves étaient utilisées dans la confection d'ustensiles (pelles, grattoirs, hameçons, leurres de pêche) ou d'ornements (Chazine, 1985; Ono, 2010). Puis, au XIXème siècle, l'exploitation des stocks d'huîtres perlières est entrée dans l'ère industrielle afin de fournir la forte demande européenne en nacre, utilisée alors dans la confection d'objets de luxe et dans la fabrication des boutons. Entre 1890 et 1960, ces exportations varient de 20 tonnes à 1300 tonnes par an en fonction de la demande qui était fortement liée au contexte économique mondial. Puis, malgré la mise en place de mesures de protection des stocks (pêche limitée à certaines saisons), leur épuisement dans de nombreux atolls de Polynésie Française a entraîné une baisse progressive des exportations à partir des années 1960 (Intes, 1984).

Cette période correspond également aux premiers essais de greffes d'huîtres perlières réalisés sur la base d'une méthode développée au Japon et appliquée en Polynésie Française par le docteur Domard (Intes, 1984). Les résultats positifs de ces essais ont encouragé la création de fermes perlières privées et, en 1976, le service de la pêche lançait un programme de captage naturel de naissain dans l'objectif de diminuer progressivement l'exploitation des stocks naturels et de fournir les premières fermes perlières en naissain de qualité. La production, l'exportation et les recettes de l'industrie perlière se développent ensuite de façon exponentielle des années 1980 jusqu'en 2000 où les exportations culmineront à 11 tonnes de perles pour une valeur de 20 milliards de CFP (170 millions d'euros). A partir de l'an 2000 la surproduction, la mise sur le marché de perles de mauvaise qualité ainsi qu'un contexte économique mondial défavorable (suite notamment aux

attentats du 11 septembre 2001 et au krach boursier de 2001-2002) entraînent une baisse du prix de vente qui n'a cessé de diminuer jusqu'à aujourd'hui (de 1400 cfp g^{-1} en 2002 à 500 cfp g^{-1} en 2010, source ISPF), mettant en difficulté de nombreux producteurs (Le Pennec et al., 2010).

Malgré cette crise récente, l'exportation de perles noires constitue, après le tourisme, la seconde source de revenus pour la Polynésie Française (6 milliards de CFP soit 50 millions d'euros en 2010). Outre sa contribution aux recettes du Pays, cette activité génère des revenus conséquents pour les habitants de l'archipel des Tuamotu-Gambier, archipel éloigné du centre économique que représentent Tahiti et l'archipel de la société.

Contexte scientifique

La mise en place puis l'optimisation zootechnique de cette activité ont mobilisé les services du Territoire de la Polynésie Française dès 1963 (Intes, 1984) et cette mobilisation s'est maintenue jusqu'à aujourd'hui. Les projets de grande envergure (*e.g.* « ATOLL », « PGRN » : Programme Générale de Recherche sur la Nacre, groupement de recherche « ADEQUA » : Amélioration de la qualité des perles) fédérant les diverses institutions de recherche scientifique présentes en Polynésie Française ainsi que les différents projets de recherche indépendants réalisés par ces même instituts, ont permis de caractériser les spécificités physiologiques et écophysiologiques de l'huître perlière *Pinctada margaritifera* (Thielley, 1993; Loret,1999; Pouvreau,1999; Thomas,2009; Linard, 2010), le fonctionnement et la structure de l'écosystème lagonaire (Intes,1990, Charpy, 1994) et d'identifier les facteurs zootechniques et génétiques ayant une influence sur la qualité des perles (Joubert, 2011).

Les résultats du PGRN et du programme ATOLL ont notamment abouti à la construction d'un modèle de fonctionnement de l'écosystème des lagons d'atolls et d'un modèle bio-énergétique de croissance et de reproduction des huîtres perlières qui ont été utilisés pour définir des limites de densité d'élevage et d'exploitation des lagons (Niquil et al, 1998; Pouvreau et al., 2000).

Le programme de « pérennisation et de professionnalisation de la perliculture » mise en oeuvre par le Service de la Perliculture et financé par le Fond Européen de Développement (9 ème FED) entre 2008 et 2010 avait pour objectifs globaux l'augmentation du prix moyen de la perle et une meilleure rentabilité des fermes perlières afin d'assurer le maintien d'une activité économique dans les archipels éloignés. Ce programme avait également pour but d'aider les petits perliculteurs (78% de l'ensemble des producteurs de perles de culture de Tahiti et d'huîtres perlières) par l'amélioration durable des performances techniques et économiques de leurs exploitations. Pour cela, 3 actions ont été mises en place : une formation technique itinérante dans les atolls producteurs, une étude du marché international de la perle de culture de Tahiti et un programme de recherche scientifique visant à étudier les interactions entre les huîtres perlières et leur environnement.

La partie recherche scientifique de ce programme était divisée en trois thèmes : la description de la structure et du fonctionnement de l'écosystème planctonique, l'utilisation de ces ressources planctoniques par les huîtres perlières et le déterminisme environnemental de la reproduction des huîtres perlières.

Les travaux sur la description de la structure et du fonctionnement de l'écosystème planctonique ont été pilotés par l'IRD et réalisés en collaboration avec plusieurs instituts de recherche scientifique (IRD, Université de la Rochelle, Université de Caen, Université de la Méditerranée) spécialisés chacun dans un domaine d'étude de l'écosystème planctonique. Les travaux sur l'utilisation de ces ressources planctoniques par les huîtres perlières (qui constituent le chapitre 2 de cette thèse) ont été accomplis en collaboration avec cette équipe de spécialistes de l'écosystème planctonique. Enfin, le travail sur le déterminisme environnemental de la reproduction des huîtres perlières correspond aux chapitres 3 et 4 de cette thèse et a été réalisé grâce à l'étroite collaboration de l'Université de Polynésie Française avec l'Ifremer.

De plus, ce travail sur le déterminisme de la reproduction des huîtres perlières complète le travail sur la croissance et la dispersion des larves d'huîtres perlières (action Ecolarve financée par le service de la perliculture) afin de fournir les éléments essentiels à une meilleure compréhension de la variabilité spatio-temporelle du captage de naissain dans les atolls de Polynésie Française.

En effet, la production de perles noires est entièrement basée sur ce captage naturel dont l'irrégularité peut constituer un obstacle majeur à la rentabilité des entreprises. De plus, lorsque l'offre locale n'est pas suffisante, du naissain provenant d'autres atolls est importé. Ces transferts entre atolls sont potentiellement préjudiciables à long terme pour l'activité perlière et pour l'écosystème car ils sont susceptibles d'engendrer le transfert simultané d'épibiontes ou de pathologies; en outre ils représentent une menace pour la conservation de la diversité génétique des populations d'huîtres perlières (Arnaud-Haond et al., 2003).

Objectifs et plan de la thèse

Dans ce contexte, l'objectif de cette thèse est de décrire les principaux facteurs influençant la gamétogénèse et la synchronisation des pontes d'huîtres perlières et de fournir des indicateurs environnementaux permettant d'estimer le potentiel de croissance et de reproduction des huîtres perlières dans les lagons de Polynésie Française.

Ce travail est divisé en 4 chapitres qui abordent chacun un objectif spécifique permettant une meilleure compréhension des liens existants entre l'environnement et la reproduction des huîtres perlières. Les chapitres 2, 3 et 4 ont été rédigés en anglais sous forme d'articles (acceptés ou en préparation) et sont précédés d'un résumé détaillé en français.

Chapitre I Ce chapitre expose quelques généralités sur l'environnement dans lequel vivent les huîtres perlières, sur leur biologie ainsi que sur le fonctionnement et la construction des modèles bio-énergétiques.

Chapitre II Les variations de concentration du plancton sont susceptibles d'influencer le régime alimentaire des huîtres perlières et leurs capacités de croissance et de reproduction. Le premier objectif de cette thèse est donc de quantifier la contribution des différentes communautés planctoniques à la nutrition des huîtres perlières dans le lagon d'Ahe.

Chapitre III Le cycle de reproduction des bivalves est généralement déterminé par deux facteurs principaux : la température et la concentration en plancton. Cependant, les variations saisonnières de ces deux paramètres sont faibles dans les atolls de Polynésie Française. Les travaux présentés dans ce chapitre ont pour objectif de déterminer le rôle des variations à petite échelle de temps de ces deux facteurs sur la gamétogenèse et la synchronisation des pontes d'huîtres perlières.

Chapitre IV Les modèles bio-énergétiques de croissance et de reproduction constituent des outils d'intérêt pour l'étude de l'influence de la variabilité des paramètres environnementaux sur la reproduction des organismes. Le troisième objectif de ce travail est donc de mettre au point un modèle de croissance mécaniste répondant de la théorie du budget d'énergie dynamique (DEB) pour les adultes d'huîtres perlières et par ce moyen de vérifier les conclusions du chapitre précédent.

Enfin, une dernière partie permettra de synthétiser les principaux résultats et d'évoquer les perspectives de recherche permises par nos résultats.

CHAPITRE I

Généralités sur l'environnement de l'huître perlière *P. margaritifera*, sa biologie et les modèles bio-énergétiques de croissance et de reproduction

Généralités sur l'environnement de l'huître perlière *P. margaritifera*, sa biologie et les modèles bio-énergétiques de croissance et de reproduction

Environnement et Répartition des huîtres

L'huître perlière est présente dans toute la zone intertropicale de l'océan Indien et de l'océan Pacifique. L'abondance de cette espèce dans les atolls de l'archipel des Tuamotu-Gambier (78 atolls dont 30 sont exploités pour la production de perles, Figure 1) est particulièrement élevée et montre à quel point cette espèce est adaptée aux conditions hydrobiologiques de ces atolls.

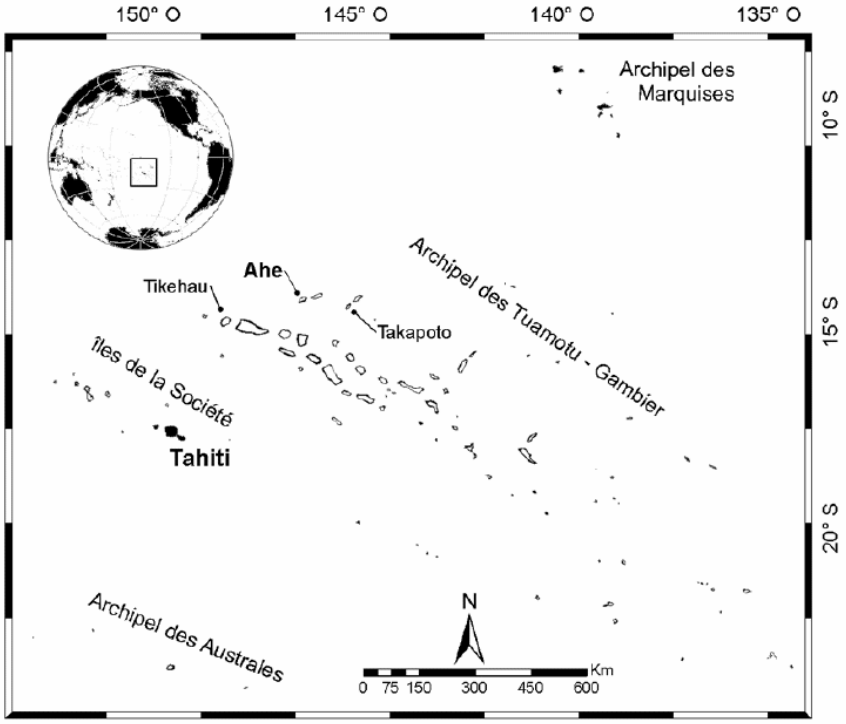

Figure 1 : Localisation des archipels constituant la Polynésie Française et des atolls d'Ahe, de Tikehau et de Takapoto dans l'archipel des Tuamotu.

Ces atolls sont le résultat de la lente submersion d'un volcan (pendant 1 à 50 millions

d'années) autour duquel s'est progressivement développée une ceinture récifale, érigée par diverses espèces de scléractiniaires et d'algues encroûtantes. Cette ceinture récifale délimite la surface d'un lagon intérieur et peut être marquée par la présence de bandes de terre émergées d'une largeur de quelques centaines de mètres (*motu*), de chenaux de faible profondeur < 50 cm (*hoa*) et de passes plus profondes (> 50cm et jusqu'à plusieurs dizaines de mètres de profondeur) qui permettent les échanges entre l'océan et le lagon (Figure 2).

La morphologie de la couronne récifale (principalement liée à la répartition et à la taille des *motu*, *hoa* et passes), son exposition au vent et à la houle ainsi que la bathymétrie et la superficie du lagon ont une influence majeure sur le fonctionnement hydrodynamique et sur l'hydrobiologie du lagon : (i) sur le temps de résidence des masses d'eau (Pages et al, 2001a) (ii) sur la circulation des masses d'eau (Lenarhdt, 1991), (iii) sur la concentration moyenne du phytoplancton (Pages et al, 2001) et (iv) sur la structure et la biomasse des populations benthiques et pélagiques (Adjeroud, 1997, Adjeroud, 2000).

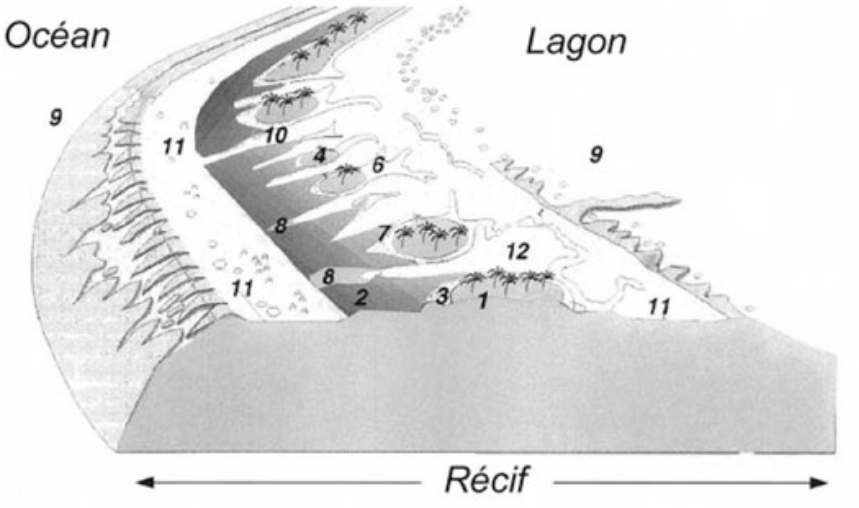

Figure 2 : Schéma de la morphologie du récif d'un atoll. 1 : végétation, 2 : conglomérat, 3 : débris coralliens, 4 : motu, 5 : platier intertidal, 6 : débris coralliens intertidaux, 7 : conglomérats intertidaux, 8 : chenal résiduel, 9 : eaux profondes, 10 : chenaux (hoa), 11 : platier, 12 : lagune fermée. D'après Battistini et al. (1975).

Par comparaison avec les écosystèmes côtiers des zones tempérées, ceux des lagons d'atolls

de Polynésie Française sont décrit comme stables et homogènes car la biomasse et la production des différentes communautés planctoniques ne présentent pas de variations saisonnières importantes (e.g. Torréton & Dufour, 1996; Charpy & Charpy-Roubaud, 1991).

Ils sont également qualifiés d'oligotrophes car la faible concentration en nutriments limite la biomasse des communautés phytoplanctoniques (Dufour et al, 1999). Les concentrations en phytoplancton et en matière organique particulaire sont donc relativement faibles et dépassent rarement 1 µg chl *a* l^{-1} et 0.7 mg l^{-1} respectivement (Pages et al., 2001; Pouvreau et al. 2000).

En terme d'abondance, les communautés planctoniques sont caractérisées par une nette dominance des organismes picoplanctoniques (bactéries hétérotrophes, cyanobactéries et picoeucaryotes) avec une concentration comprise entre 2.2 x 10^8 cell l^{-1} et 23.0 x 10^8 cell l^{-1} sur les organismes unicellulaires nano-microplanctoniques dont la concentration totale varie entre 2 x 10^4 cell l^{-1} et 2 x 10^6 cell l^{-1} (Gonzales et al., 1998; Loret, 1999; Sournia et Ricard, 1976).

Ces populations nano-microplanctoniques sont largement dominées par les petits nanoflagellés hétérotrophes et autotrophes (environ 2 x 10^6 cell l^{-1}) et par les dinoflagellés autotrophes, mixotrophes ou hétérotrophes (environ 2 x 10^4 cell l^{-1}) tandis que les concentrations en ciliés (environ 1000 cell l^{-1}) et en diatomées sont généralement faibles (Gonzales et al., 1998; Loret, 1999; Sournia et Ricard, 1976).

En termes de biomasse, les bactéries représentent environ 53% de la biomasse carbonée totale (0.7µm -2000µm) contre 14.2% pour le phytoplancton < 5µm, 14.2% pour le phytoplancton > 5µm, 6% pour le nanozooplancton (5-35µm), 4.7% pour le microzooplancton (35-200µm) et 7.9% pour le meso-macro-zooplancton (200-2000µm) (Charpy & Charpy-Roubaud,1990).

Sur Tikehau, le zooplancton d'une taille comprise entre 35-200µm est principalement composé de nauplii (39%), de copépodes (31%), de larves de bivalves (20%) et de larves de polychètes (10%) (Le borgne et al., 1989).

Bien que les variations saisonnières de concentration en chlorophylle *a* soient peu marquées, des variations spatiales et temporelles ont été observées à plusieurs reprises et ont été associées au régime des vents (Sournia & Ricard, 1976; Buestel et Pouvreau, 2000; Thomas, 2009).

Alimentation

L'huître perlière est un bivalve filtreur suspensivore. Le mouvement synchronisé des cils vibratiles situés sur les filaments branchiaux créent un courant d'eau dans la cavité palléale et les particules planctoniques capturées par les branchies sont alors enrobées de mucus avant d'être acheminées vers les palpes labiaux qui effectuent un tri pré-ingestif des particules. Les particules non-ingérées sont rejetées sous forme de pseudo-fécès tandis que les particules ingérées sont digérées, les substances assimilables sont absorbées et finalement, les particules ingérées mais non digérées sont rejetées sous forme de fécès (Figure 3) (Pouvreau et al., 2000).

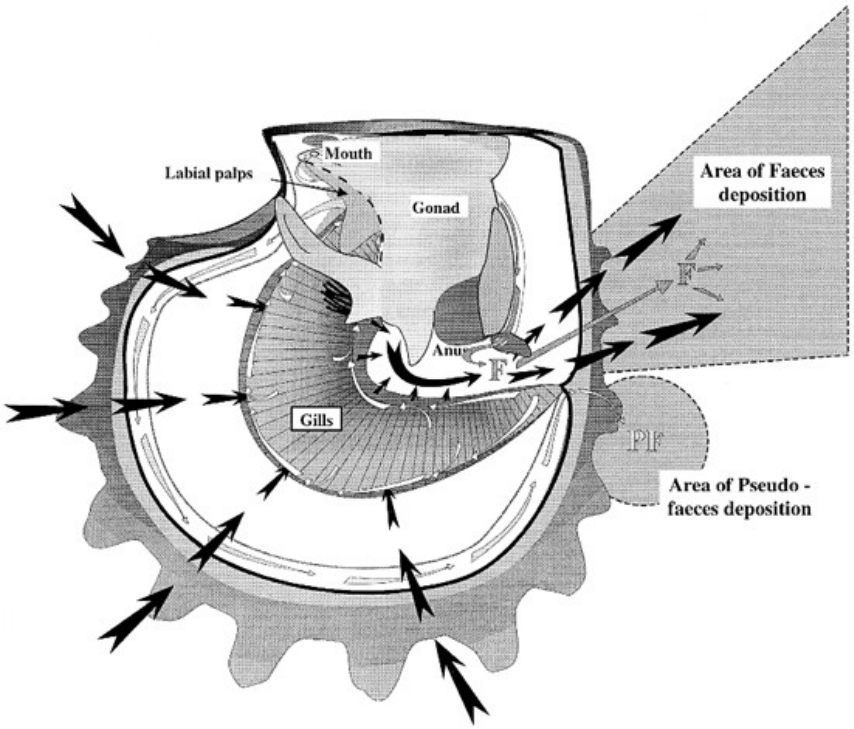

Figure 3 : Processus de filtration de l'eau et trajet des particules planctoniques retenues par l'huître perlière. D'après Pouvreau et al (2000)

Des mesures réalisées en laboratoire avec des micro-algues d'élevage ont montré que le taux

de rétention des huîtres perlières varie en fonction de la taille des micro-algues (Pouvreau et al., 2000). Le taux de rétention des particules d'1 µm est d'environ 10% et augmente de façon linéaire jusqu'à atteindre environ 100% pour les particules d'une taille supérieure à 5µm (Figure 4). Le faible taux de rétention des particules < 2 µm, qui constituent la biomasse planctonique dominante des lagons d'atolls polynésiens, est compensée par des taux de filtration particulièrement élevés compris entre 13 l h^{-1} g^{-1} et 25 l h^{-1} g^{-1} (Pouvreau et al., 2000; Yukihira et al.,2000). Les travaux menés sur l'atoll de Takapoto par Pouvreau et al (2000) ont également montré que les concentrations en matière organique particulaire et en matière minérale particulaire avaient une influence sur les taux de filtration.

L'étude des contenus stomacaux par Loret et al. (2000) a permis de mettre en évidence la diversité du régime alimentaire des huîtres perlières qui est constitué en majorité de petits flagellés autotrophes (cryptophycées, prymnésiophycées et chlorophycées) et de dinoflagellés.

Sur Takapoto, les *clearance rates* des dinoflagellés et des ciliés ont été estimés à 33 l h^{-1} g^{-1} et entre 12 l h^{-1} g^{-1} et 20 l h^{-1} g^{-1} respectivement, ce qui permettait aux huîtres perlières une rétention de carbone de 10.7 µgC h^{-1} g^{-1} et 12.7 µgC h^{-1} g^{-1} respectivement (Loret, 1999).

La sélection pré-ingestive opérée par les huîtres perlières semble être principalement liée à leur capacité de distinguer la nature organique ou minérale des particules (Pouvreau et al., 2000; Hawkins et al 1998). Cependant, bien que les pseudo-faeces soient constitués d'au minimum 80% de matière minérale, ils peuvent également contenir un certain nombre de cellules planctoniques appartenant à divers taxons (Loret, 1999). Sur Takapoto, la concentration des différents taxons de phytoplancton dans les pseudo-faeces présente des variations spatiales et temporelles importantes mais certaines espèces sont rejetées préférentiellement (diatomées) (Loret, 1999).

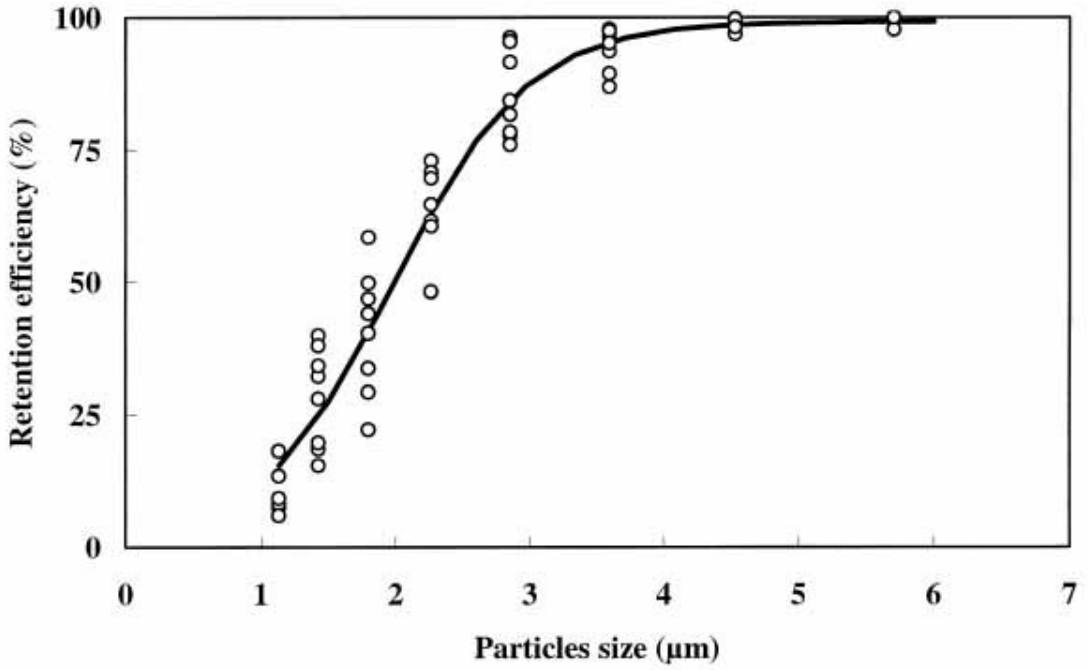

Figure 4 : Capacité de rétention des huîtres perlières en fonction de la taille des particules filtrées. (Pouvreau et al., 1999).

Reproduction

L'huître perlière *P. margaritifera* est hermaphrodite protandre. Le sex-ratio est équilibré à partir de 9 à 10 ans pour les populations naturelles mais déséquilibré en faveur des mâles pour les populations en élevage (Chavez-Vilalba et al., 2011, Theilley, 1993; Pouvreau et al., 2000; Le Moullac et al., 2011).

Les nombreux travaux sur le déterminisme environnemental de la reproduction des bivalves ont majoritairement été effectués dans des écosystèmes tempérés dans lesquels (i) la température a une influence majeure sur le déclenchement des différentes phases du cycle de reproduction : repos sexuel, maturation et ponte (Bayne & Newell, 1983; Gervis & Sims, 1992; Sastry, 1979; Fabioux et al., 2005) et (ii) la concentration en plancton a une influence sur l'effort de reproduction et sur la qualité des gamètes (Enriquez-diaz et al., 2008; Mac Donald & Thompson, 1985; Ruiz et al., 1992, Saucedo et al., 2002; Saxby, 2002; Bernard, 2011).

Le cycle reproductif des bivalves peut également être lié à une stratégie de gestion de l'énergie

caractérisée par une période de stockage d'énergie (principalement sous forme de glucides) qui sera utilisée ultérieurement pour la gamétogenèse (Berthelin et al., 2000). Les espèces qui suivent ce type de stratégie sont définies comme conservatrices par Bayne (1976). A l'inverse, celles qui ne font pas (ou peu) de réserves et dont la gamétogenèse est principalement dépendante des ressources immédiatement disponibles dans le milieu sont définies comme opportunistes.

Cependant ces différentes stratégies dans le phasage de la reproduction et du stockage de l'énergie ne semblent pas spécifiques aux espèces et une grande plasticité peut être observée parmi les différentes populations d'une même espèce. Les origines de cette variabilité populationnelle ont été attribuées soit à une adaptation aux conditions environnementales locales soit à des différences génétiques entre populations (Hilbish & Zimmerman, 1988; Loosanoff & Nomejko,1951; Paulet et al.,1988; Thompson,1984).

Dans les lagons polynésiens, les températures élevées (26°C – 32°C) font que ce paramètre n'a qu'une faible influence sur le cycle reproductif des huîtres perlières. En conséquence : un fort asynchronisme des stades de maturité est généralement observé entre les individus, la gamétogenèse est continue et les pontes peuvent avoir lieu toute l'année. Des épisodes de ponte intense ont cependant été observés dans le lagon de Takapoto, à des périodes variables selon les auteurs : en janvier - février puis septembre - octobre pour Thielley (1993) et Buestel et al (1995) lesquels ont également observé des émissions en mars - avril et juillet - aout; puis en août - octobre et février - mars pour Pouvreau (1999). Parmi ces travaux, les facteurs environnementaux étudiés n'ont pas permis de déterminer directement les causes de ces périodes de pontes intenses, qui impliquent une synchronisation des pontes individuelles sur des périodes plus ou moins longues.

En comparaison de certains bivalves tempérés les huîtres perlières n'accumulent pas des quantités importantes d'énergie. Elles ont donc été classées dans la catégorie des bivalves opportunistes et ont été caractérisées comme investissant tout surplus d'énergie dans la reproduction

(Pouvreau et al., 2000; Pouvreau, 1999). Le temps de maturation est estimé à environ 50 jours en milieu naturel et les huîtres perlières perdent environ 10% de leur poids sec lors de l'émission des gamètes ce qui représente approximativement 58 millions d'ovules ou 4 milliards de spermatozoïdes (Raissa, 2007).

Chez *P. margaritifera*, la fécondation est externe. L'embryogenèse aboutit à la formation d'une larve trocophore puis d'une larve D d'une taille moyenne de 80 µm environ 24H après la fécondation. Le développement pélagique se poursuit pendant une 20^{aine} de jours jusqu'à ce que la larve atteigne le stade pédivéligère et une taille de 230µm. Ce stade est caractérisé par le développement d'un pied qui permet à la larve de se déplacer sur un substrat solide avant sa fixation. Après la métamorphose, le naissain se fixera au substrat par la sécrétion de byssus. A ce stade, la croissance est relativement rapide et les huîtres perlières atteignent une hauteur de 10 cm au bout de deux ans (Pouvreau, 1999).

Modélisation

Les interactions entre les bivalves et leur environnement sont nombreuses et complexes : le métabolisme général des bivalves et leur besoin en énergie varie en fonction de la température; leur capacité de filtration augmente donc avec la température mais est également une fonction de leur taille et une fonction de la concentration en seston dans leur environnement. De l'eau qu'ils filtrent, ils ne retiennent que certaines particules planctoniques desquelles ils tirent l'énergie nécessaire à leur survie, leur croissance et leur reproduction. Enfin, dans leur environnement ils rejettent de grandes quantités de matière organique dissoute ou particulaire (excrétion azotée, mucus, faeces, pseudo-faeces) qui sont une source de nutriments et de matière organique pour les bactéries hétérotrophes et les populations phytoplanctoniques.

Face à la complexité de ces interactions, des modèles bio-énergétiques de croissance et de reproduction ont donc été développés pour les bivalves afin de mieux appréhender (i) leur

croissance et leur reproduction et (ii) l'impact des populations de bivalves sur leur environnement.

Pour *P. margaritifera*, 2 modèles bioénergétiques basés sur le concept du *Scope for Growth* ont été établis par Pouvreau et al. (2000) et Yukihira et al. (2000). Bien que ces modèles aient démontré leur capacité à simuler la croissance et la reproduction chez plusieurs espèces de bivalves, ils présentent quelques inconvénients majeurs : ils sont basés sur des relations allométriques empiriques et sur des budgets énergétiques ponctuels déterminés expérimentalement. Ces caractéristiques limitent (i) leur capacité à décrire les flux physiologiques alloués aux différentes fonctions de l'organisme en fonction des conditions environnementales, (ii) leur application directe à différents sites pour la même espèce et (iii) leur application à des espèces de bivalves proches de celles pour lesquelles le modèle a été construit.

Au cours des 20 dernières années, la théorie des budgets énergétiques dynamiques (Dynamic Energy Budgets, DEB), développée par Kooijman (2000) a permis la construction d'un nouveau type de modèles bio-énergétiques qui ne présente pas ces inconvénients. Cette théorie est basée sur une application rigoureuse (qui respecte les lois de la conservation de la matière et de l'énergie) des principes fondamentaux de la biologie des organismes (eg : réponse fonctionnelle de type Holling), de la biologie cellulaire (e.g nécessitée pour les cellules d'allouer de l'énergie à l'entretien de leur structure), de la biochimie (e.g température d'Arrhénius) ou de la biophysique (e.g relations entre un volume vivant et sa surface d'interaction avec l'environnement); et elle vise à schématiser et à quantifier de la façon la plus « simple » possible et la plus réaliste possible, les flux d'énergie liés à la survie, à la croissance et à la reproduction des organismes.

Cette approche a permis le développement de nouveaux modèles bio-énergétiques présentant 3 avantages majeurs (Van der Veer et al., 2009; Alunno-Bruscia et al., 2009) : (i) un modèle générique possédant un nombre restreint de paramètres suffit à décrire les flux d'énergie nécessaires à la survie, la croissance et la reproduction pour l'ensemble des organismes vivants, (ii) les

comparaisons entre organismes sont possibles en comparant les valeurs de ces paramètres, (iii) les valeurs de certains paramètres sont proches pour des espèces proches ce qui rend l'adaptation des modèles relativement simple entre ces espèces, (iv) les flux sont décrits de façon dynamique ce qui permet de suivre en permanence l'impact des variations de la température et de la concentration en nourriture sur la quantité d'énergie allouée aux différentes fonctions de l'organisme.

Son application dans les domaines de l'écophysiologie de la croissance et de la reproduction (Bourlès et al., 2009), de l'écotoxicologie (Bodiguel et al., 2009), de la pathologie bactérienne (Flye-Sainte-Marie et al, 2009), de la dynamique des populations (Bacher & Gangnery, 2006) ont permis de démontrer clairement l'efficacité du modèle DEB à prédire la croissance et la reproduction de nombreuses espèces et notamment de plusieurs espèces de bivalves (voir Van der Veer et Alunno-Bruscia, 2006; Alunno-Bruscia et al, 2009 pour une liste exhaustive de ces espèces).

La liste des paramètres et les équations du modèle DEB pour les adultes d'huîtres perlières sont détaillées dans le chapitre 3. Le fonctionnement global du modèle est schématisé ci dessous (Figure 5).

Le flux d'ingestion est une fonction de la concentration en plancton (fonction Holling de type II) et est modulé par la température. Une partie de cette énergie ingérée est assimilée et se déverse dans le compartiment des réserves. L'énergie nécessaire à la croissance de la structure et au développement de la gonade est puisée dans le compartiment des réserves et est répartie entre ces deux fonctions physiologiques selon un coefficient fixe. Pour assurer la survie des huîtres perlières, l'énergie destinée à la croissance de la structure et au développement de la gonade est allouée en priorité à la maintenance des cellules somatiques et des cellules reproductrices. Les flux d'énergie nécessaires pour assurer la maintenance sont également modulés par la température.

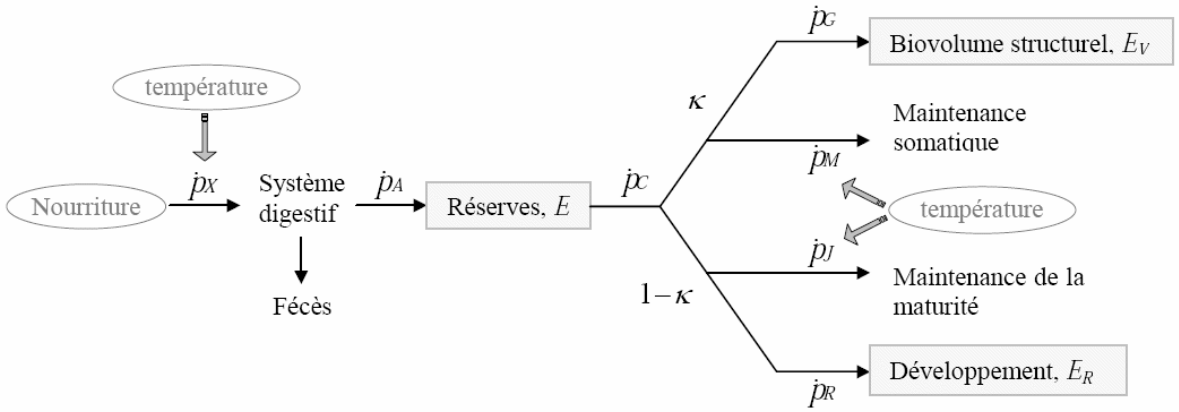

Figure 5 : Représentation schématique de l'allocation d'énergie selon la théorie DEB pour l'huître perlière *P. margaritifera*. Les variables d'état sont grisées et les variables de forçage sont représentées dans des cercles (Thomas et al., 2011)

CHAPITRE II

Régime alimentaire des huîtres perlières sur l'atoll d'Ahe (Tuamotu, Polynésie Française)

RÉSUMÉ DÉTAILLÉ

La survie, la croissance et la reproduction des bivalves reposent sur leur capacité à absorber suffisamment d'énergie en filtrant les particules planctoniques disponibles dans leur environnement.

Les lagons de Polynésie Française sont caractérisés par une faible concentration en plancton qui présente peu de variations saisonnières mais des variations importantes à l'échelle de quelques jours. Dans les lagons d'atolls polynésiens, les particules planctoniques < 5 µm (bactéries, cyanobactéries et petit nanoplancton) représentent 70% de la biomasse planctonique totale contre 23% pour les particules planctoniques comprises entre 5µm et 200µm (nanoplancton et microplancton). Le faible taux de rétention des particules < 2µm est compensé par un taux de filtration particulièrement élevé compris entre 13 l h^{-1} g^{-1} et 25 l h^{-1} g^{-1} et par un régime alimentaire diversifié, constitué aussi bien de phytoplancton (Chlorophycées, Primnesiophycés, Diatomophycées) que de protistes (Dinoflagellés et Cilliés).

Cependant, les résultats de ces travaux réalisés par divers auteurs qui ont employé des moyens expérimentaux différents, ne permettaient pas une description quantitative fine de la nutrition *in situ* des huîtres perlières. Notre objectif était donc de compléter ces connaissances par une description du régime alimentaire *in situ* des huîtres perlières en mesurant leur *Clearance Rates* (CR) pour 6 communautés planctoniques dans un contexte expérimental unique.

Les mesures de CR ont été réalisées sur l'atoll d'Ahe, dans des bacs alimentés en continu avec de l'eau du lagon, ce qui nous a permis de conserver en permanence les conditions hydrobiologiques du lagon et d'effectuer plus facilement des réplicas des mesures de CR. Les CR étaient calculés par comparaison entre les concentrations en plancton mesurées dans les bacs avec huîtres et dans des bacs témoins (sans huîtres). Dans ces bacs, la concentration de 6 catégories de plancton a été mesurée par des techniques d'études complémentaires : le picoplancton par cytométrie en flux; la concentration en chlorophylle *a* < 2µm (Chl *a* < 2µm), en chlorophylle *a* > 2µm (Chl *a* > 2 µm) et en chlorophylle *a* totale (Chl *a* Tot.) par extraction au méthanol; les ciliés, les dinoflagellés et les

nanoflagellés par comptage au microscope. Les mesures de CR ont été réalisées durant 3 campagnes d'une 20^{aine} de jours sur des huîtres perlières d'une taille comprise entre 25 et 115 mm. Au mois d'octobre 2008, les CR de l'ensemble des communautés planctoniques ont été mesurés tandis qu'aux mois de mai 2008 et de mai 2009, seuls les CR de Chl *a* > 2 µm et de Chl *a* < 2 µm ont été mesurés. Les CR ont ensuite été convertis en taux de filtration de carbone afin d'établir la proportion des différentes catégories de plancton dans le régime alimentaire des huîtres perlières.

La campagne d'octobre 2008 a été marquée par des vents plus forts (8.6 m s^{-1}) que pendant les campagnes de mai 2008 et d'octobre 2008 (3.0 m s^{-1} et 2.7 m s^{-1} respectivement), par une concentration plus forte en Chl *a* Tot (0.64 µg l^{-1}) que pendant les campagnes de mai 2008 et de mai 2009 (0.51 µg l^{-1} et 0.34 µg l^{-1} respectivement); et par une proportion plus importante de Chl *a* > 2 µm (40%) que pendant les campagnes de mai 2008 et d'octobre 2009 (13% et 30% respectivement). Pendant ces trois campagnes, les CR moyens sont restés stables avec des valeurs comprises entre 3.7 l h^{-1} g^{-1} et 4.5 l h^{-1} g^{-1} pour la Chl *a* < 2 µm et entre 13.7 l h^{-1} g^{-1} et 14.6 l h^{-1} g^{-1} pour la Chl *a* > 2 µm. Par le calcul des taux de filtration de carbone nous montrons une forte variation des proportions relatives de ces deux catégories de plancton dans le régime alimentaire des huîtres perlières en raison des variations de concentrations en Chl *a* > 2 µm et Chl *a* < 2 µm dans l'environnement.

En mai 2008, les concentrations moyennes en picoplancton, en nanoflagellés, en dinoflagellés et en ciliés étaient respectivement de 2.54 x 10^8 cell l^{-1} , 5.25 x 10^7 cell l^{-1}, 5.09 x 10^4 cell l^{-1} et 740 cell l^{-1}. Le CR des huîtres perlières, dont la valeur moyenne était comprise entre 0.5 l h^{-1} g^{-1} pour le picoplancton et 18.7 l h^{-1} g^{-1} pour les ciliés, était fortement lié au bio-volume du plancton (r^2 = 0.71 et p-value < 10^{-4}, n=16). Lors de cette campagne, les nanoflagellés ont représenté près de 90% du carbone filtré par les huîtres perlières en raison de leur concentration exceptionnellement élevée.

Ces résultats montrent que la structure des communautés planctoniques peut présenter des variations temporelles importantes qui semblent liées au régime des vents. En comparaison avec les

autres atolls de Polynésie, le lagon d'Ahe est relativement riche et présente une biomasse élevée de particule nano- et micro-planctonique. Nos résultats confirment que les huîtres perlières ont des capacités de filtration *in situ* élevées par rapport à de nombreux bivalves tempérés et montrent que le régime alimentaire des huîtres perlières varie fortement en fonction de l'abondance des différentes catégories de plancton. Bien que les particules > 2 µm et notamment les nanoflagellés constituent une part dominante du régime alimentaire des huîtres perlières, les particules < 2 µm peuvent également représenter un part non négligeable de leur alimentation. Ainsi, malgré les faibles concentrations de plancton des atolls de Polynésie Française, les huîtres perlières disposent en permanence d'une concentration en nourriture suffisante leur permettant d'assurer leur survie, leur croissance et leur reproduction tout au long de l'année.

Pearl Oysters *Pinctada margaritifera* grazing on natural plankton in Ahe atoll lagoon (Tuamotu archipelago, French Polynesia).

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ABSTRACT

In atoll lagoons of French Polynesia, growth and reproduction of pearl oysters are mainly driven by plankton concentration. However, the actual diet of black-lip pearl oysters *P. margaritifera* in these lagoons is poorly known. To fill this gap, we used the flow through chamber method to measure clearance rates of *P. margaritifera* in Ahe atoll lagoon (Tuamotu Archipelago, French Polynesia). We found : (i) that pearl oysters cleared plankton at a rate that was positively related to plankton biovolume, (ii) that nanoflagellates were the main source of carbon for the pearl oysters, (iii) that the quantity and origin of carbon filtrated by pearl oysters was highly dependent on the concentration and composition of plankton. These results provide essential elements for the comprehension of growth and reproduction variability of pearl oysters in atoll lagoons of French

Polynesia.

INTRODUCTION

For the last 40 years, farming of the black-lip pearl oyster *Pinctada margaritifera* has been the main aquaculture activity in French Polynesia atoll lagoons. In 2010, production and annual exportation of black pearls reached up to 12 metric tons, worth approximately 50 million Euros, making this industry the 2nd source of income for French Polynesia after tourism (Service de la Perliculture, pers. com.). However, this industry entirely relies on spat collection successes, which strongly depends on natural reproduction rates and on environmental conditions (Pouvreau et al., 2000a; Thomas et al., This issue).

French Polynesian atoll lagoons have been characterized in the past by stable and homogeneous temperature and salinity (*e. g*. Buestel and Pouvreau, 2000). The planktonic biological processes are controlled by the hydrodynamic regime and specifically by the water residence time (Charpy et al. 1997; Delesalle and Sournia, 1992; Torréton et al., 2002), which is closely linked to atoll geomorphology and water exchanges through the reef rims (Andréfouët et al., 2001; Charpy and Blanchot, 1998; Sournia and Ricard, 1976; Dumas et al. this issue).

The same lagoons were also characterized by concentrations of chlorophyll *a* and particulate organic carbon that rarely exceed 0.6 µg l^{-1} and 0.4 mg l^{-1}, respectively (Buestel and Pouvreau, 2000; Charpy et al., 1997); and by the dominance of planktonic particles inferior to 5 µm size which represented more than 70% of the total planktonic biomass (Buestel and Pouvreau, 2000; Charpy and Charpy-Roubaud, 1990; Niquil et al., 1998).

In the 1990s the feeding strategy of *P. margaritifera* was investigated with various methods including laboratory and *in situ* experiments : (1) batch and flow-through chamber methods were used by Pouvreau et al. (1999) and Yukihira et al. (1998b) to measure clearance rates of *P.*

margaritifera on various species of cultured algae, (2) batch method was used by Loret et al. (2000a) to study clearance rates of pearl oysters on natural assemblage of ciliates and dinoflagellates, (3) the biodeposit method was used by Pouvreau et al. (2000b) to measure *in situ* clearance rates of pearl oysters in Takapoto lagoon and, finally, (4) direct sampling of *P. margaritifera* gut content and HPLC analysis were used to determine which phytoplankton taxa were contributing to the pearl oysters' diet (Loret et al. 2000b).

These experiments demonstrated that (i) planktonic particles < 2µm were not efficiently retained, (ii) the diet of *P. margaritifera* included both autotrophic and heterotrophic plankton and (iii) *P. margaritifera* compensated the low concentration of efficiently retained planktonic particles (> 2µm) by relatively high pumping rates to meet its energy requirements. However, this knowledge remained too limited to fully characterize, quantitatively, the pearl oysters' diet.

In this context, this study aims to measure the clearance rates of pearl oysters for six types of autotrophic and/or heterotrophic plankton (picoplankton, nanoflagellates, dinoflagellates, ciliates, phytoplankton < 2µm and phytoplankton > 2µm), and to assess their relative contribution to the pearl oysters' diet in Ahe lagoon.

We selected the flow-through chamber method to measure clearance rates for two reasons: (i) it allows keeping the pearl oysters under the influence of natural fluctuations of environmental parameters and (ii) it facilitates repetitive sampling.

Complementary techniques such as flow cytometry, microscope counts and chlorophyll *a* extraction were used to measure the plankton concentration in the flow-through chambers.

MATERIAL AND METHODS

Study site

This study was conducted in Ahe atoll lagoon, located 500 km north of Tahiti Island in the north of the Tuamotu Archipelago (Figure 6). Ahe lagoon measures 142 km^2 with a mean depth close to 42 m. Ahe is defined as a semi-enclosed atoll. One active pass is located in the west part of the lagoon and several reef-flat spillways (less than 50 cm depth) are distributed along the reef rim, mainly in the south and west parts of the lagoon. The average water renewal time (ratio of lagoon volume to average water input rate) was estimated at 80 days (Dumas et al., This issue). With nearly 1350 spat collection stations and almost 11% of the lagoon dedicated to black-lip pearl oyster rearing, Ahe lagoon is a remarkable site for pearl culture and spat collection in French Polynesia.

Our study site and experimental set up were located in the northeast of the lagoon, 30 m off the coast, in a small pile building (Figure 6). Lagoon depth was approximately 2.5 m. Experimental devices were protected from direct sunlight and rain. Pearl oysters were subjected to natural light regimes and experiments were conducted after an acclimation period of four days in the flow-through grazing chambers.

The experiments took place in May 2008 (from 15th- 23rd), October 2008 (from 10th-23rd) and April/May 2009 (from 28th-10th). The rate at which pearl-oysters cleared phytoplankton from lagoon water (chlorophyll *a* used as a proxy) was measured during each of these three experimental periods. The rate at which pearl-oysters cleared picoplankton, nanoflagellates, dinoflagellates and ciliates from lagoon water were only measured during October 2008 experiments.

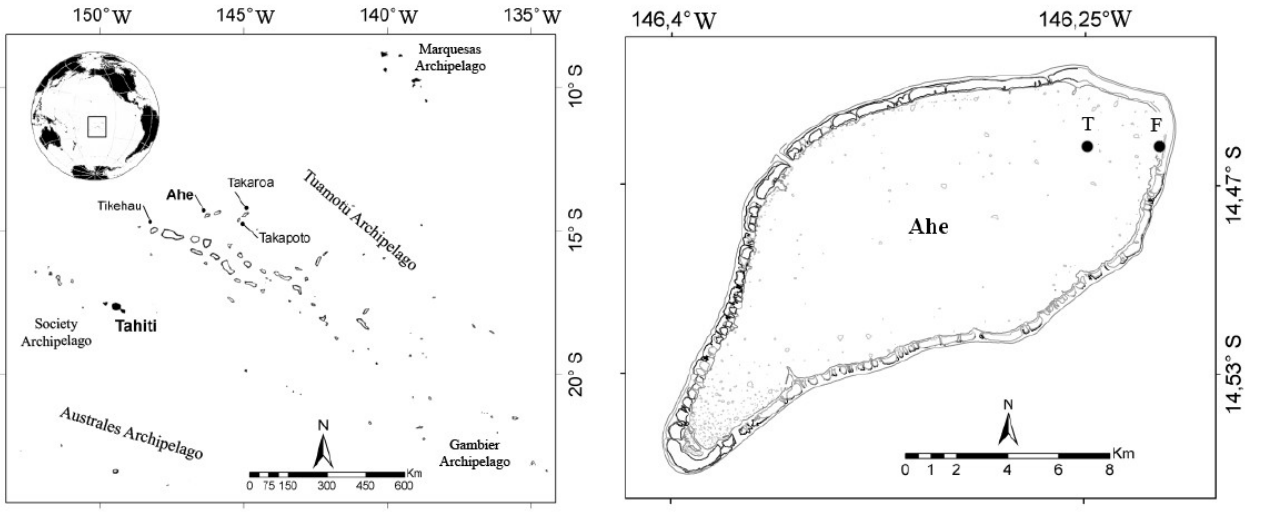

Figure 6 : Location of Ahe atoll. Location of the sites where filtration experiments were carried out (site F) and where we measured water temperature and salinity (site T) in Ahe lagoon (map by courtesy of Yoann Thomas).

Environmental parameters

Hourly wind direction and velocity were obtained from Takaroa atoll meteorological station (Météo France data) located about 120 km east of Ahe (145°3'4''W, 14°28'57''S). Lack of any orographic effects around atolls allows using this distant measurement, which was in good agreement with local value and numerical models output at Ahe atoll (Dumas et al., This issue).

Water temperature (°C) and salinity (PSU) were obtained from a Sea Bird probe (SBE V19 plus) immersed at a 10 meter depth, next to an experimental breeding station located approximately 3 km away from our study site (Figure 6).

Phytoplankton concentration

Water samples (200ml) were filtered firstly on Millipore filters (2 µm of pore size) and then on GF/F Whatman filters (ca. 0.7 µm pore size). Chlorophyll *a* (Chl *a*) retained on these filters was extracted from phytoplankton cells during 4h in the dark at 4°C in 6 ml of methanol 100%. Chl *a* concentration in these extracts was determined using a Turner design TD 700 fluorimeter calibrated with Chl *a* standard (Sigma) and equipped with the set of optical filters recommended by

Welshemeyer (1994) for direct measurement of Chl *a.*

We measured concentration of phytoplankton < 2 µm (Chl *a* < 2 µm, in µg l^{-1}) and > 2 µm (Chl *a* > 2 µm, in µg l^{-1}) respectively from the Chl *a* concentration measured in GF/F filters extracts and from the Chl *a* concentration in Millipore filters extracts. To convert Chl *a* > 2 µm and Chl *a* < 2 µm concentrations into carbon biomass, we used ratios equal to 50 µgC µgChla^{-1} and to 82 µgC µgChla^{-1}, respectively (Charpy and Charpy-Roubaud, 1990; Charpy, 1996).

Picoplankton concentration

In this study, picoplankton abundance (Pico. in cell l^{-1}) is defined as the sum of bacteria, cyanobacteria (*Synechococcus sp.* and *Prochlorococcus sp.*) and picoeukaryotes abundances.

Bacteria and picoautotrophic cells were fixed with 0.2 µm filtered formaldehyde (final concentration 2%) and frozen in liquid nitrogen (N_2). Bacterial cells were counted by flow cytometry using the method described by Marie et al. (1997). One mL formaldehyde-fixed subsamples were incubated with DAPI at a final concentration of 1/10,000 for 15 min at room temperature in the dark. Each subsample was counted using a MoFlo cytometer (Dako Colorado Inc., Fort Collins, CO, USA). Stained bacterial cells, excited at 488 nm, were enumerated according to their right-angle light scatter (RALS) and green fluorescence (FL1) measured using a 530/30 nm filter. These cell parameters were recorded on a 4 decade logarithmic scale mapped onto 1024 channels. Fluorescent beads (0.94 µm, Polysciences Inc., Warrington, PA, USA) were systematically added to each sample. Standardized RALS and FL1 values (cell RALS and FL1 divided by 0.94 µm beads RALS and FL1, respectively) were used as an estimation of the relative size and nucleic acid content of bacterial cells, respectively (Troussellier et al., 1995). Listmode files were analyzed using SUMMIT software (Dako Colorado Inc., Fort Collins, CO, USA).

Picophytoplankton (*Prochlorococcus sp.* and *Synechococcus sp.* cells) and autotrophic

picoeukaryotes counts were performed with the same flow cytometer set up as described above. Cells excited at 488 nm were detected and directly enumerated according to their FALS and RALS properties and their orange fluorescence (585/42 nm) and red fluorescence (>650 nm) due to phycoerythrin and chlorophyll pigments, respectively. Fluorescent beads (0.94 µm) were also systematically added to each sample. Listmode files were analyzed using SUMMIT software (Dako Colorado Inc., Fort Collins, CO, USA).

To calculate an average carbon conversion factor for picoplankton, we used conversion factors of 14 fgC $cell^{-1}$ (Gundersen et al., 2002), 60 fgC $cell^{-1}$, 178 fgC $cell^{-1}$ (Charpy and Blanchot, 1998) and 836 fgC $cell^{-1}$ (Verity et al.,1992) for bacteria, *Prochlorococcus sp.*, *Synechococcus. sp.* and for picoeukaryotes respectively. These values were averages and weighted by the mean abundance of heterotrophic bacteria, *Prochlorococcus sp., Synechococcus sp.* and picoeukaryotes measured during this study. This community-scale conversion factor was then used to convert the total picoplankton concentration (cell l^{-1}) into carbon biomass (µgC l^{-1}).

Similarly, to calculate an average biovolume (BV, μm^3) per picoplankton cell we used biovolumes of 0.035 μm^3, 0.11μm^3, 0.38μm^3 and 1.2 μm^3 per heterotrophic bacteria (Sakka et al., 2000), *Prochlorococcus sp.* cell, *Synechococcus sp.* cell (Charpy and Blanchot, 1998), and picoeukaryote cell, respectively. These values were weighted by the mean abundance measured for each plankton type.

Nanoplankton and microplankton concentration

The taxonomic determination of protists was carried out in accordance with systematics literature (Kahl, 1931; Lee, 1985; Nezan, 1996; Paulmier, 1997; Ricard, 1987; Sournia, 1986).

For microplankton counts (dinoflagellates and ciliates), water samples (1 liter) were fixed with alcalin lugol iodine (2% final concentration). A first period of sedimentation was conducted

during 24h after which the top 900 mL of sample was slowly siphoned off with small-bore tubing. The remaining 100 mL was then stored at 4 °C in the dark before enumeration. A second sedimentation of 24h was carried out in Utermöhl settling chamber (Hydro-Bios combined plate chamber) and cell enumeration was made at 400 magnification using a Leica DMI 3000B inverted microscope with interference contrast. Cells were counted in every microscope field (at least 60 fields per samples) for five transversal bands covering the settling chamber width and disposed at equal distance of each other.

For nanoplankton counts, water samples (25 ml) were fixed and preserved with paraformaldehyde (1% final concentration). Samples were concentrated to 10 ml with a filtration tower mounted with 0.8 µm pore size black polycarbonate filters (Nuclepore) and stained with DAPI (2.5 x 10^{-4} g l^{-1} final concentration). Enumeration of stained nanoplanktonic cells was made under UV light excitation on at least 15 randomly selected fields, at the magnification of x1000.

Nanoplankton and microplankton abundances (in cell l^{-1}) were computed using the following equation :

$$A = (N_C / (N_{MF} \times S_{MF})) \times S_{SC} \times 1000 / V_S$$

where A = abundance of nanoplankton or microplankton (cell l^{-1}), N_C = total number of cells (in cell), N_{MF} = number of counted microscopic fields, S_{MF} = area of one microscopic field (mm^2), S_{SC} = area of settling chamber or filter (mm^2), V_S = sample volume (l).

An average biovolume for dinoflagellates (200 cells) and ciliates (about 50 cells) was calculated using the mean length and width of cells, which were determined with a calibrated ocular micrometer.

Using these mean biovolumes and the biovolume to carbon content relationship from Menden-Deuer and Lessard (2000), we calculated the carbon conversion factors for both dinoflagellates and ciliates. These conversion factors were then used to convert dinoflagellates and

ciliates concentration (cell l^{-1}) into carbon biomass (µgC l^{-1}).

For nanoplankton cells, we assumed an average biovolume of 509 μm^3 which was calculated from the cell diameters of chlorophytes, prasinophytes and cryptophytes measured by Loret et al. (2000b) in Takapoto lagoon and we assumed an average conversion factor of 4.7 x 10^{-6} µgC per cell of nanoplankton as in Ferrier-Pagès and Furla (2001).

Flow-through chambers

After a critical analysis of the methodological shortcomings and possible misinterpretations related to the different methods of studying bivalve feeding processes (Bayne, 2004; Filgueira et al., 2006; Pascoe et al., 2009; Petersen, 2004 and Riisgård, 2004 for the most recent reviews) we selected the flow through chamber method for the measurement of *in situ* clearance rates of *Pinctada margaritifera*.

Water was pumped from the lagoon at 1 meter deep to a 80 liter reservoir tank at a flow rate of approximately 300 l h^{-1}. We used a peristaltic pump to avoid the destruction of fragile planktonic organisms. From this tank, lagoon water was distributed by gravity into flow through grazing chambers. Flow rates were adjusted (between 5 l h^{-1} and 68 l h^{-1}) in each flow through chamber to prevent the pearl oyster from removing more than 30% of chlorophyll *a* (Hawkins et al., 1999). A control flow through chamber without pearl oyster was maintained in the same configuration as our grazing chambers.

To avoid "recirculation issues" and to ensure "sufficient mixing of the exhalant flow with the flow bypassing the bivalve" (Riisgård, 2001), our grazing chambers were divided into three compartments (Figure 7): the inflow compartment where the water was entering, the grazing compartment where the pearl oyster was filtering and the outflow compartment where the water was siphoned off. Inflow and grazing compartments were separated out by 2 homogenization grids. In

the grazing compartment, one pearl oyster was maintained on a PVC support at mid-height of the water column and the exhalant flow was directed to the outflow compartment through a PVC reduction of 5cm of diameter. In the outflow compartment, water was siphoned off 5 cm under the surface.

The siphoned water was sampled with 500 ml graduated test tube simultaneously from the control chamber and from the grazing chambers.

Experiments with the smallest pearl oysters (25 to 30 mm in height) were conducted in 25 liters grazing chambers (20 cm in diameter and 50 cm in length) whereas experiments with pearl oysters measuring 41mm to 115 mm in height were conducted in 50 liters grazing chambers (20 cm in diameter and 100 cm in length).

All flow-through chambers were emptied and cleaned every single day to remove faeces and pseudofaeces produced by the pearl oysters.

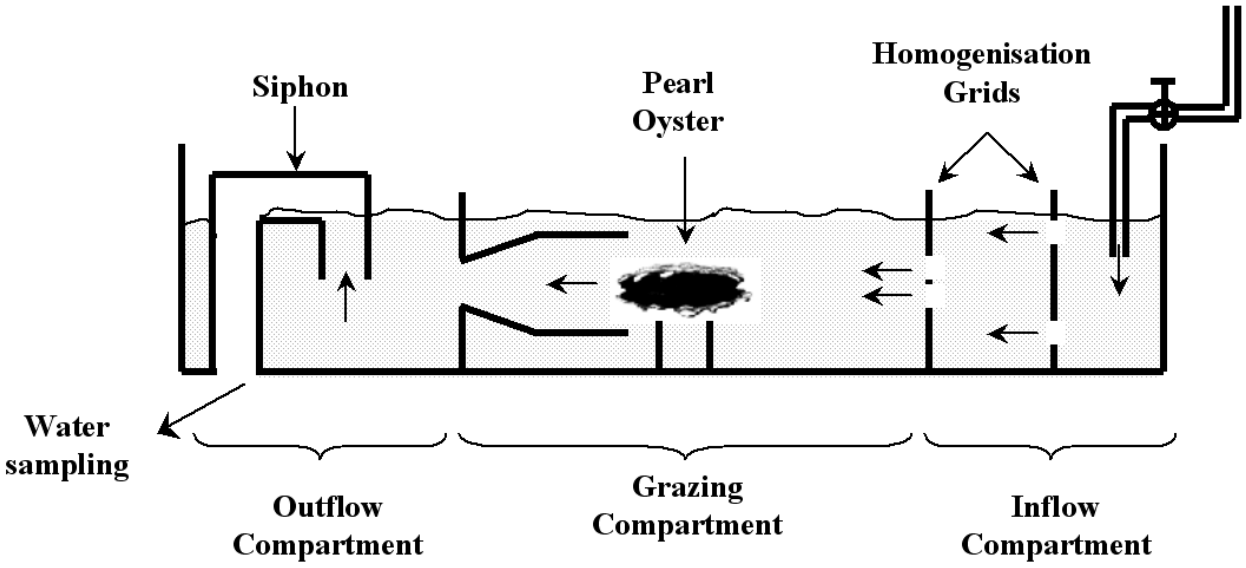

Figure 7 : Flow-through grazing chambers were divided into 3 compartments : inflow compartment, grazing compartment and outflow compartment.

Pearl Oysters

A total of 16 pearl oysters were used during these experiments. In May 2008, experiments were conducted with pearl oysters measuring 42±1 mm in height (mean ± sd) (n= 4) and with 113±4mm height pearl oysters (n=2). In October 2008, experiments were conducted with 28±2 mm height pearl oysters (n= 4), and in May 2009, with 75±6 mm height pearl oysters (n=6). For each size class of pearl oysters, the sampling strategy is indicated in Table 1.

The smallest pearl oysters (25 to 43 mm in height) were bred at the Ifremer center's hatchery of Vairao (Tahiti Island) and were stored in Ahe lagoon at one meter deep at least one week before starting the experiments. Pearl oysters from 74 mm to 115 mm came from the “Motu Tahiri” pearl farm in Ahe. All epibionts were cleaned off and pearl oysters were allowed to recover from any potential stress during three days before starting the experiments. Sampling was then conducted at least once a day during the experimental periods.

At the end of the experiments, each pearl oyster height was measured and the flesh was freeze-dried and weighed. Freeze dried flesh weight (DW in g) was used to normalize clearance rates per g of dry flesh.

Table 1 : Mean height ± standard deviation (in mm) and number of oysters (in parentheses) used during our experiments. Sampling strategy (*n*) for the measurement of clearance rates of pearl oysters (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm)

Survey	Oysters Height	Chl. *a* (*n*)	Pico. (*n*)	Nano. (*n*)	Dino. (*n*)	Cili. (*n*)
May 2008	42±1 (4)	8				
	113±4 (2)	9	-	-	-	-
October 2008	28±2 (4)	30	22	15	10	10
May 2009	75±6 (6)	50	-	-	-	-

Clearance rates

Clearance rate is defined as the volume of water entirely cleared of plankton by one pearl oyster per unit of time. It was calculated for each plankton type with the following equation,

modified from Hildreth and Crisp (1976) :

$$CRi = Fr \times (Cc - Cg)/ Cg$$

with CRi = clearance rates of pearl-oyster (in l h^{-1}, per individual), Cc and Cg = concentration of plankton at the exit of the control (Cc) and grazing (Cg) flow-through chambers (in µg Chl *a* l^{-1} or in cell l^{-1}), Fr = flow rate in the grazing flow-through chamber (l h^{-1}).

Clearance rates is known to follow an allometric relationship of the type $CR = aDW^b$ with DW = freeze dried flesh weight (in g), a and b = linear regression coefficients of log (CR_i) vs log (DW) (e.g. Pouvreau et al., 1999). Following this relationship, all CRi values were divided by DW^b and standardized clearance rates (CR) were expressed in l h^{-1} g^{-1}.

Carbon retention rates

Clearance rates can be defined as the capacity of pearl oysters to filter and retain particles from their environment. However, the amount of carbon retained by *P. margaritifera* also depends on the plankton biomass. To assess the contribution of each plankton type to the diet of *P. margaritifera*, we estimated carbon retention rates by the following equation :

$$RR = CB \times CR$$

where RR = Retention Rates of carbon in µgC g^{-1} h^{-1}, CB = Carbon Biomass in µgC l^{-1}, CR = Clearance Rates in (l h^{-1}).

Statistics

All analysis were conducted with the R freeware (http://www.r-project.org/). All data sets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). In most cases, data had to be log-transformed (natural log of *X*).

As wind velocity, salinity and water temperature data were not normal , highly heteroscedastic

and were also highly asymmetric between surveys, we only used their mean and associated 95% confidence interval (Efron and Tibshirani, 1986) for each survey.

Two-way analysis of variance (ANOVA) were used (i) to compare concentration of Chl *a* among surveys and within size class (> 2µm and < 2µm) and (ii) to compare CR of pearl-oysters among surveys and within size-class of Chl *a* (> 2µm and < 2µm). *A posteriori* multiple comparisons were carried out using Tukey HSD tests.

We used Pearson's correlation to examine relationships between Chl *a* concentration (> 2µm and < 2µm), flow rates in the flow-through grazing chambers and clearance rates of pearl oysters.

For each survey, exact binomial tests were used to compare the percentage of carbon retained by pearl oysters from Chl. *a* < 2µm and Chl. *a* > 2µm.

Non parametric Kruskal-Wallis tests were used to compare (1) abundance of ciliates, dinoflagellates, nanoplankton and picoplankton measured in October 2008, (2) clearance rates of ciliates, dinoflagellates, nanoplankton and picoplankton measured in October 2008. *A posteriori* multiple comparisons were carried out using the non parametric Steel-Dwass test (Critchlow and Fligner, 1991; Spurrier, 2006).

In all tests, significance was determined with an alpha level of 0.05.

RESULTS

Temperature, salinity, wind direction and speed

Mean water temperature ranged from 26.82±0.01 °C (in October 2008) to 29.1±0.05 °C (in May 2009). Mean salinity ranged from of 36.16±0.03 (in October 2008) to 36.87±0.09 in (May 2008) (Table 2).

East and southeast winds blew continuously in October 2008 with the highest velocity of the three surveys (8.63±0.41 m s^{-1}). In May 2008 and in May 2009, winds were predominantly blowing from the northwest and northeast (more than 75% of the time) with lower velocity (3.0±1.7 m s^{-1} and 2.7±1.4 m s^{-1} respectively) (Table 2).

Table 2 : Mean ± 95% confidence interval of wind velocity (m s^{-1}), water temperature (°C) and salinity (PSU) measured in May 2008, October 2008 and April/May 2009.

Survey	Wind Velocity	Water Temp.	Salinity
May 2008	2.83 ± 0.86	28.22 ± 0.03	36.87 ± 0.09
October 2008	8.63 ± 0.41	26.82 ± 0.01	36.16 ± 0.3
May 2009	2.65 ± 0.62	29.10 ± 0.05	36.23 ± 0.01

Chlorophyll *a* : concentration and clearance rates and carbon retention

Variations of Chl *a* < 2µm and Chl *a* > 2µm concentrations and of clearance rates are presented in Figure 8. Both Chl *a* > 2µm and Chl *a* < 2µm concentrations showed significant variations between surveys (Tables 3 and Table 4). Chl *a* > 2µm was lower than Chl *a* < 2µm in May 2008 and in May 2009, while concentrations were not significantly different in October 2008, a period when we observed the highest Chl *a* > 2 µm concentration (1.31 µg l^{-1}).

Conversion factors presented in Table 5 were used to convert mean Chl *a* > 2µm and Chl *a* < 2µm concentration into carbon biomass. Phytoplankton biomass ranged from 23 µgC l^{-1} (May 2009) to 42 µgC l^{-1} (October 2008).

The biomass temporal trends were similar to concentration trends. In May 2008 and May 2009, biomass of Chl *a* < 2 µm was higher than biomass Chl *a* > 2 µm while there was no significant difference between biomass Chl *a* < 2 µm and Chl *a* > 2 µm in October 2008.

The allometric relationship between Chl a > 2µm clearance rates and freeze dried dry flesh weight is presented in Figure 9. Linear regression of log (CRi) on log (DW) was significant (r^2 = 0.87 and $p<0.001$, n=16) and we established that $CRi = 13.3\ DW^{0.62}$. This relationship was further

used to standardize clearance rates for a 1g DW pearl oyster ($CR = CRi / DW^{0.62}$).

In all surveys, pearl oysters cleared Chl *a* > 2µm at a higher rate than Chl *a* < 2µm. Mean CR did not show any significant variations between surveys (Table 4). CR of pearl oysters was not influenced by variations of Chl *a* < 2µm and Chl *a* > 2µm concentration, neither by flow rates (Table 6).

In May 2008, pearl oysters retained significantly higher quantities of carbon from Chl *a* < 2µm than from Chl *a* > 2µm. In October 2008, it was the opposite : pearl oysters retained significantly higher quantities of carbon from Chl *a* > 2µm than from Chl *a* < 2µm. In May 2009, pearl oysters retained similar quantities of carbon from Chl *a* > 2µm and from Chl *a* < 2µm (Table 3).

Table 3 : Abundance (in Cell l^{-1} or in µgChl *a* l^{-1}), Carbon biomass (CB in µgC l^{-1} and B in %), Clearance Rates of pearl oysters (in l h^{-1} g^{-1}) and carbon retention rates (Carbon Retained in µgC h^{-1} g^{-1} and Carb. in %) measured in May 2008, October 2008 and April/May 2009 at our study site (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm).

Survey	Plankton Type	Abundance	CB	B (%)		Clearance Rates	Carbon Retained	Carb (%)	
May 2008	Chl *a* >2µm	0.10 ± 0.03	5	13		14.4 ± 6.0	72	32	
	Chl *a* <2µm	0.41 ± 0.08	34	87		4.5 ± 6.2	152	68	
October 2008	Chl *a* >2µm	0.34 ±0.32	17	40		13.7 ± 7.8	233	72	
	Chl *a* <2µm	0.30 ± 0.06	25	60		3.7 ± 2.5	92	28	
	Pico.	2.64 ± 0.71 x 10^8	28	10	100%	0.5 ± 5.4	15	0	100%
	Nano.	5.25 ± 0.80 x 10^7	247	85		11.8 ± 6.6	2918	93	
	Dino.	5.09 ± 4.32 x 10^4	11	4		15.9 ± 4.1	179	6	
	Cili.	740 ± 354	2	1		18.7 ± 10.0	29	1	
May 2009	Chl *a* >2µm	0.14 ± 0.06	7	30		14.6 ± 5.0	102	53	
	Chl *a* <2µm	0.20 ± 0.06	16	70		5.6 ± 2.5	92	47	

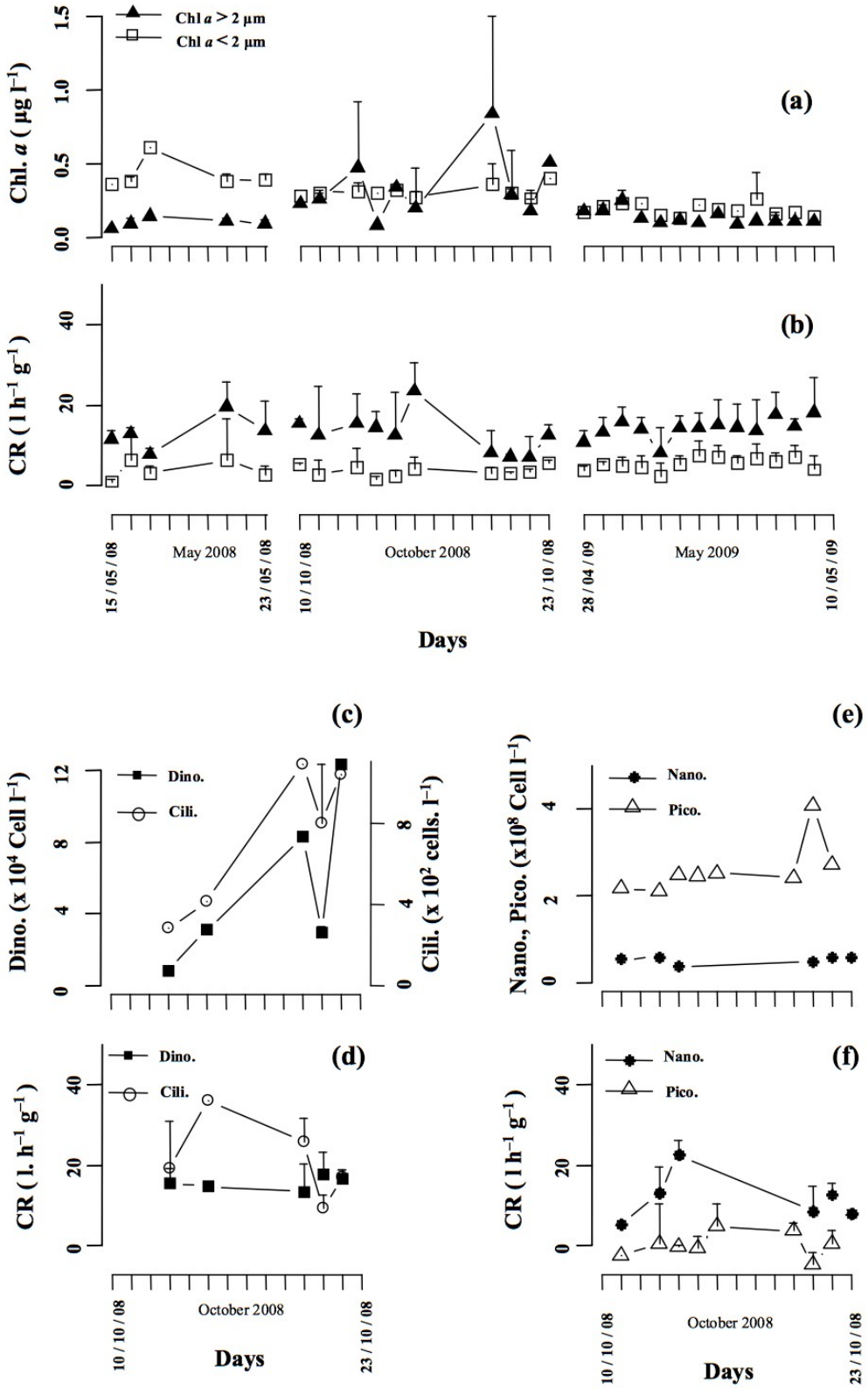

Figure 8 : Abundance of plankton (graphs a, c and e) and clearance of pearl oysters (graphs b, d and f) measured in May 2008, October 2008 and April/May 2009 in Ahe lagoon. (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm).

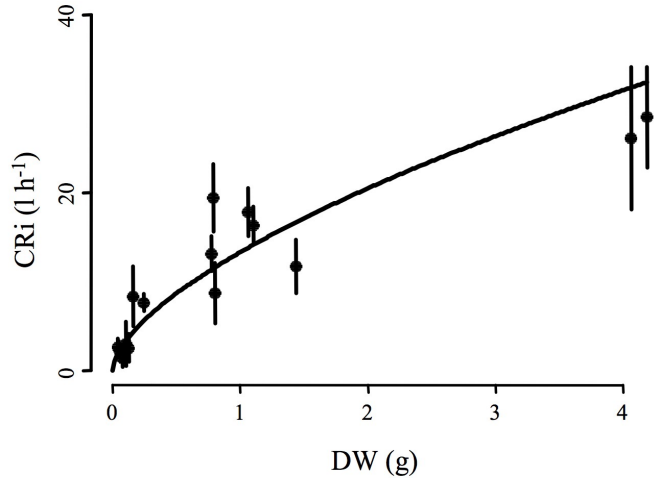

Figure 9 : Allometric relationship between clearance rates (CRi in l h^{-1}) and freeze dried flesh weight (DW in g) of pearl oysters. Each point represents the mean individual clearance rates of pearl-oysters (Chl *a* > 2µm) with bars corresponding to standard deviation. The curve corresponds to the equation $CRi = 13.3 \times DW^{0.62}$.

Table 4 : Analysis of variance table for statistical comparisons of concentration Chl. *a* and standardized clearance rates of pearl oysters within size class of Chl. *a* (> 2µm and < 2µm) and between survey.

Analysis	Source	*df*	*F*	*p*
Chl. *a* concentration among survey and size class of Chl *a*	Size class	1	25.3	0.000
	Survey	2	17.9	0.000
	Interaction	2	21.0	0.000
CR of pearl oysters among survey and size class of Chl *a*	Size class	1	154.6	0.000
	Survey	2	2.4	0.089
	Interaction	2	0.3	0.766

Planktonic microorganisms : concentration, clearance rates and carbon retention

This section presents results of the October 2008 survey, when the contribution of all plankton types to the pearl oysters diet was assessed. Variations of plankton concentrations and clearance rates are presented in Figure 8. Mean plankton concentration, mean clearance rates and mean carbon retention rates are presented in Table 3.

In October 2008, picoplankton and nanoflagellates were the two most abundant plankton types (Table 3). Picoplankton concentration ranged from 1.92×10^8 cell l^{-1} to 3.09×10^8 cell l^{-1} with a mean of $2.64 \pm 0.71 \times 10^8$ cell l^{-1}. We calculated an average carbon content per cell of picoplankton

of 1.1 x 10^{-7} µgC $Cell^{-1}$ (Table 5). Nanoflagellates concentration ranged from 3.77 x 10^7 cell l^{-1} to 6.04 x 10^7 cell l^{-1} with a mean concentration of 5.25±0.80 x 10^7 cell l^{-1}. Dinoflagellates concentration ranged from 0.86 x 10^4 cell l^{-1} to 12.3 x 10^4 cell l^{-1} with a mean concentration of 5.09±4.32 x 10^4 cell l^{-1}. From their mean length (14.1±5.0 µm) and width (10.9±3.7 µm), we calculated an average biovolume of 1,600 μm^3 and an average carbon content per cell of 2.2 x 10^{-4} µgC $Cell^{-1}$ (Table 5). Ciliates concentration ranged from 282 cell l^{-1} to 1093 cell l^{-1} with a mean of 740±354 cell l^{-1} (Table 3, Figure 8). From their mean length (30.3±13.4 µm) and width (23.4±9.4 µm) we calculated an average biovolume of 18,000 μm^3 and an average carbon content per cell of 2.1 x 10^{-3} µgC $Cell^{-1}$ (Table 5).

The mean concentration of picoplankton, nanoflagellates, dinoflagellates and ciliates were converted into carbon biomass using the conversion coefficients presented in Table 5. The total carbon biomass was 288 µgC l^{-1} and nanoflagellates constituted the bulk of total plankton biomass (85%).

Mean clearance rates of pearl oysters increased with the size of plankton (from 0.5 l h^{-1} g^{-1} for picoplankton to 18.7 l h^{-1} g^{-1} for ciliates) and there was a significant relationship (r^2 = 0.71, p = 0.000, n=16) between mean clearance rates of pearl oysters and biovolumes of plankton cells : CR = 0.42 ln (BV) + 0.35 (Figure 10).

Nanoflagellates were the dominant source of carbon retained by pearl oysters in October 2008 (93%). The second source of carbon for pearl oysters were dinoflagellates (6%).

Table 5 : Average biovolumes (BV in μm^3) and carbon content (C.C. in µgC $Cell^{-1}$ or in µgC µgChla^{-1}) computed from our data and from litterature data (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm).

Plankton type	B.V.	C.C.
Pico.	0.25	1.1×10^{-7}
Nano.	509	4.7×10^{-6}
Dino.	1606	2.2×10^{-4}
Cili.	18091	2.1×10^{-3}
Chl. *a* > 2 µm	-	50
Chl. *a* < 2 µm	-	82

Table 6 : Relationship between clearance rates of pearl oyster ($CR_{Chl\ a\ <\ 2\mu m}$ and $CR_{Chl\ a\ >\ 2\mu m}$), concentration of phytoplankton < 2 µm (Chl *a* < 2µm), of phytoplankton > 2 µm (Chl *a* > 2µm), and flow rates in the grazing chambers. Pearson's product moment correlation (*r*) and p-values (*p*) are indicated for each analysis.

	$CR_{Chl\ a\ <\ 2\mu m}$	$CR_{Chl\ a\ >\ 2\mu m}$
Chl *a* > 2µm	*r* = -0.08 *p* = 0.434	*r* = 0.09 *p* = 0.348
Chl *a* < 2µm	*r* = 0.01 *p* = 0.970	*r* = -0.04 *p* = 0.717
Flow rates	*r* = 0.20 *p* = 0.050	*r* = 0.08 *p* = 0.415

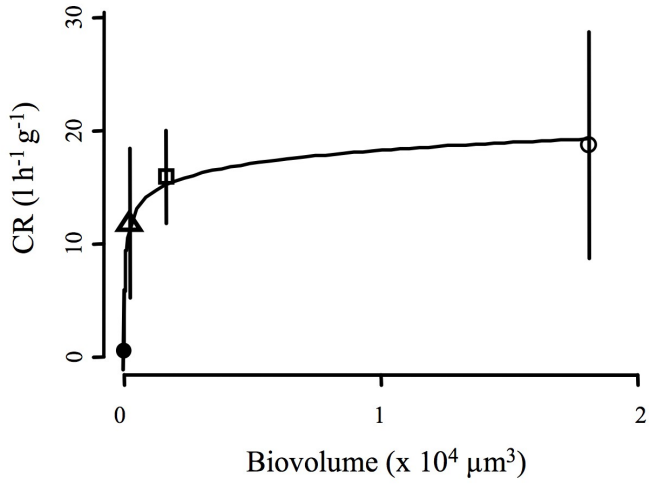

Figure 10 : Relationship between standardized clearance rates of pearl oysters (CR in l h^{-1} g^{-1}) and plankton biovolume (in μm^3). Each point represents the mean CR of pearl-oysters measured in October 2008 in Ahe lagoon. (picolankton = full circle, nanoplankton = empty triangle, dinoflagellates = empty square and ciliates = empty circle). Bars represent standard deviation. Curve represents the equation CR = 0.42 ln (BV) + 0.35.

DISCUSSION

Plankton concentration

Phytoplankton concentration measured during this study was in the upper range of phytoplankton concentration measured in Ahe lagoon and in several other Tuamotu atoll lagoons (Table 7).

In October 2008 phytoplankton concentration reached values > 1 µgChl *a* l^{-1} with a mean concentration above 0.65 µgChl *a* l^{-1}. In October 2008, we also observed (i) concentration of dinoflagellates being in the upper range of values measured in other lagoons (Takapoto and Tikehau) (Table 7), (ii) concentration of nanoflagellates that were approximately 10 times greater than those measured in Rangiroa, Tikehau and in Ahe lagoon at other sites/periods (Table 7), (iii) concentration of picoplankton in the lower range of values reported in other atolls (Table 7).

Thus, during the October 2008 experiments, the biomass of > 2µm planktonic particles (nanoplankton + dinoflagellates + ciliates) represented more than 90% of the total planktonic biomass. These observations are unusual in Tuamotu atoll lagoons where the biomass of > 2µm planktonic particles is approximately 36% of the total planktonic biomass, as reviewed by Pouvreau et al. (2000a).

Previous studies in French Polynesian atolls have shown that plankton concentration variations can be significant at small spatial and/or temporal scale, despite the average low concentration of plankton and despite weak seasonal trends (Buestel and Pouvreau, 2000; Charpy et al., This issue; Fournier et al, This issue; González et al., 1998; Pagano et al., This issue; Sournia and Ricard, 1976; Thomas et al., 2010). However, the exact mechanisms responsible for these changes remain unclear. Changes in hydrodynamic regimes are likely causal factors and warrant

further investigations. The availability of 3D circulation numerical models will allow in a near future a better understanding of these processes (Dumas et al. This issue).

Table 7 : Range of plankton concentration (in µgChl *a* l^{-1} or in Cell l^{-1}) measured during our experiments (This study), at other sites/periods in Ahe atoll lagoon (Ahe) and in other French Polynesian atoll lagoons (Other atolls).

	This study	Ahe	Other atolls
Chlorophyll *a*	0.25-1.76	0.08-0.85 [a]	0.02-1.24 [e]
Picoplankton. ($x10^8$)	1.9-3.1	1.0-5.1 [b]	2.2-23.2 [e]
Bacteria. ($x10^8$)	0.6-1.9	2.6-7.8 [b]	2.2-20.7 [e]
Synecococchus. ($x10^8$)	0.6-1.4	0.8-1.2 [b]	<0.1-2.8 [e]
Prochlorococcus. ($x10^8$)	0.1-0.8	0.6-1.4 [b]	<0.1-1.7 [e]
Picoeukaryotes. ($x10^6$)	1.4-5.3	2.8-4.6 [b]	<0.1-4.9 [e]
Nanoflagellates. ($x10^6$)	37.0-67.0	5.5-8.5[c]	0.7-2.0 [f]
Dinoflagellates. ($x10^5$)	0.09-1.2	<0.01-0.03[d]	<0.01-1.90 [g]
Ciliates. ($x10^3$)	0.3-1.1	<0.01-0.9[d]	<0.01-4.0 [g]

[(a)] Thomas et al. (2010), Fournier et al. (this issue), Charpy et al. (this issue); [(b)] Thomas et al. (2010); [(c)] Dupuy, (Pers. Com.); [(d)] Fournier et al. (this issue); [(e)] Charpy & Blanchot (1998), Torreton et al. (2002); [(f)] González et al. (1998); [(g)] González et al. (1998), Loret et al. (2000a).

Clearance rates

Mean CR of pearl oysters ranged between 11.8 l h^{-1} g^{-1} and 18.7 l h^{-1} g^{-1} for plankton > 2µm (Chl a > 2µm, nanoflagellates, dinoflagellates and ciliates). These values are in the range of CR measured by Yukihira et al. (1998b) (12.3 l h^{-1} g^{-1}) and Pouvreau et al. (1999) (25.9 l h^{-1} g^{-1}) during laboratory experiments with a monospecific solution of *Isochrysis galbana* retained at 98% by *P. margaritifera*.

Clearance rates of *P. margaritifera* are also close to clearance rates of the oyster *Crassostrea gigas* measured under low seston load conditions in Thau lagoon in France (16 l h^{-1} g^{-1} for > 5 µm flagellates) (Dupuy et al., 2000).

During our experiments, we did not measure any influence of plankton concentration variations on clearance rates (Table 6). However, bivalves filtration performances are known to decrease when seston load increases (e.g., Pouvreau et al., 2000b for *P. margaritifera*). Species

inhabiting high seston load environments display lower clearance rates than species in low seston load environments (Jørgensen, 1996; Yukihira et al., 1998a, Trottet et al., 2008). The low load of atoll lagoons compared to many temperate coastal environments explains the typically high, and stable, CR of *P. margaritifera* (and *C. gigas* when in a low seston load environment).

Clearance of picoplankton by pearl oysters was extremely low compared to clearance of nanoplankton and microplankton. Moreover, there was a clear positive relationship between clearance rates of *P. margaritifera* and biovolume of plankton cells (Figure 10). This relationship, obtained *in situ*, is in agreement with the relationship between retention efficiency and particle size obtained in laboratory by Pouvreau et al. (1999). Finally, numerous studies have shown that this relationship was explained by the gill structure, and especially by the disposition of cirri on gill filaments (e.g Pouvreau et al., 1999; Silverman et al., 1996; Wright et al., 1982).

For *P. margaritifera*, *in situ* clearance rates data are scarce in literature. However, comparisons between clearance rates values measured during our experiments and clearance rates values measured by Loret et al. (2000a) in Takapoto lagoon again highlight this obvious relationship between clearance rates and particle size / biovolume.

Indeed, mean CR of small (length : 14.1 µm; width : 10.9 µm) dinoflagellates (16 l h^{-1} g^{-1}) measured during this study was half lower than CR (33 l h^{-1} g^{-1}) of large (length : 83µm; width : 35 µm) dinoflagellates measured by Loret et al (2000a).

Conversely, mean CR of small (length : 30.3µm; width : 23.4 µm) ciliates (19 l h^{-1} g^{-1}) measured during this study was in the range of CR of 10 l h^{-1} g^{-1} for *Amphileptus sp* (length : 55 µm; width : 21 µm) and of 20 l h^{-1} g^{-1} for *Strombidium sp.* (length : 50 µm; width : 30 µm) measured by Loret et al. (2000a) in Takapoto lagoon.

Carbon retention rates

Obviously, plankton concentration measured in October 2008 was exceptionally high and did not represent the average plankton concentration in Ahe lagoon. Thus, to assess the average amount of carbon retained by pearl oysters in Ahe lagoon, we calculated the average concentration of Chl. *a* < 2 µm, Chl. *a* > 2 µm, picoplankton, nanoflagellates, dinoflagellates and ciliates from literature data (Table 8). Then, we converted these average plankton concentrations into their respective carbon biomass using the conversion factors in Table 5. Finally, we calculated the average carbon retention rates of pearl oysters for each plankton fraction using clearance rates measured in October 2008.

The average biomass of phytoplankton in Ahe was 26 µgC l^{-1} and Chl *a* > 2µm represented 27% of this biomass. However, pearl oysters retained similar amounts of carbon from Chl *a* < 2µm and from Chl *a* > 2µm (ca. 100 µgC h^{-1} g^{-1}) (Table 8).

The average total panktonic carbon biomass was 103 µgC l^{-1} (Table 8). Picoplankton represented 69% of this total carbon biomass and nanoflagellates represented 24%. Finally, dinoflagellates and ciliates represented only 7%. In contrast, carbon retained by pearls oysters originated mainly from nanoflagellates (64%), then from dinoflagellates and ciliates (27%), and finally from picoplankton (8%).

In October 2008, pearl oysters retained almost 8 times more planktonic carbon than average (*ca*. 3000 µgC h^{-1} g^{-1} and 400 µgC h^{-1} g^{-1}, respectively).

Table 8 : Average abundance (in Cell l^{-1} or in µgChl *a* l^{-1}), Carbon biomass (CB in µgC l^{-1} and B in %), and carbon retention rates of pearl oysters (Carbon Retained in µgC h^{-1} g^{-1} and Carb. in %) in Ahe lagoon. (Pico. = picoplankton, Nano. = nanoflagellates, Dino. = dinoflagellates, Cili. = Ciliates, Chl. *a* < 2 µm and Chl. *a* > 2 µm = phytoplankton < 2µm and > 2µm).

Plankton Type	Abundance	CB	B (%)		Carbon Retained	Carb (%)	
Chl *a* >2µm	0.14 [(a)]	7	27		102	49	
Chl *a* <2µm	0.23 [(a)]	19	73		105	51	
Pico.	6.5×10^8 [(b)]	71	69		38	8	
Nano.	5.3×10^6 [(c)]	25	24	100%	293	64	100%
Dino.	2.0×10^4 [(a)]	4	4		70	15	
Cil.	1.4×10^4 [(a)]	3	3		55	12	

[(a)] Fournier et al. (this issue); [(b)] Thomas et al. (2010); [(c)] Dupuy C., (Unp. Data.)

In Takapoto lagoon, pearl oysters retained similar quantities of carbon from dinoflagellates (64 µgC h^{-1} g^{-1}) compared to the average in Ahe (70 µgC h^{-1} g^{-1}). Dinoflagellates were larger in Takapoto lagoon but their concentration was lower than in Ahe lagoon (Loret et al., 2000a).

Pearl oysters retained higher quantities of carbon in Takapoto from ciliates (86 µgC h^{-1} g^{-1}) compared to the average in Ahe (55 µgC h^{-1} g^{-1}), where they were smaller and less abundant than in Takapoto (Loret et al., 2000a).

To our knowledge, there is no comparable *in situ* study that has measured the relative contribution of pico- nano- and micro- plankton to the diet of a tropical bivalve. In temperate environments, Trottet et al. (2008) and Dupuy et al. (2000) investigated the relative contribution of pico- nano- and micro- plankton in the blue mussel diet (*Mytilus edulis*) and in the cupped oyster diet (*Crassostrea gigas*), respectively. In Thau lagoon (France), *C. gigas* retained a total of 1634 µgC h^{-1} g^{-1}, and in Grand Entrée lagoon (Canada), total carbon retention of *M. edulis* ranged from 160 µgC h^{-1} g^{-1} to 1467 µgC h^{-1} g^{-1}.

In Thau lagoon, diatoms represented 87% of the total planktonic biomass and 80% of the carbon retained by *C. gigas* while in Grande Entrée lagoon, ciliates represented at least 50% of the

total planktonic biomass and at least 70% of the carbon retained by *M. edulis*.

Similarly to these two species, we report for *P. margaritifera* that (i) natural variations in the composition and abundance of plankton lead to important feeding variations (ii) particles of size > 2µm are the main source of carbon.

Conclusion and perspectives

The grazing experiments conducted in Ahe lagoon with the flow-through chamber method confirmed the *in situ* high clearance rates of *P. margaritifera* and highlighted the strong relationship between clearance rates and plankton size/biovolume. Our results also clearly demonstrated that, even if atoll lagoons of Tuamotu Archipelago are characterized by a low average biomass of plankton, the variations of this biomass and the variations in the structure of planktonic communities have a major influence on the feeding of pearl oysters. This will help, on the long run, to understand the inter-lagoon differences of pearl oysters' ecophysiology (growth, reproduction, see Fournier et al., This issue) and therefore the inter-lagoon differences in aquaculture and pearl farming potential.

However, food sources of *P. margaritifera* are highly diversified (Loret et al., 2000a; Nasr, 1984) and it is obvious that several plankton taxa/types were not considered in the present study due to their low concentration such as diatoms, small metazooplankton, coccolithophorids.

Despite their average low abundance, transitory peaks of diatoms, bivalve larvae and other metazoan larvae concentration have been observed in atoll lagoons (Fournier et al., this issue; Pagano et al, this issue; Sournia and Ricard, 1976). These plankton fractions may therefore represent significant food sources for pearl oysters.

For these reasons, further studies on pearl oysters nutrition should focus on the measurement of clearance rates and carbon retention rates of small metazooplankton, coccolithophorids and

diatoms.

ACKNOWLEDGEMENT

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CHAPITRE III

Les variations temporelles de concentration en plancton et leur influence sur la gamétogenèse et le synchronisme des pontes chez *P. margaritifera* dans le lagon de l'atoll d'Ahe.

RÉSUMÉ DÉTAILLÉ

En Polynésie Française, l'industrie perlière est entièrement dépendante d'un captage naturel de naissain fortement irrégulier. Afin de mieux appréhender la forte variabilité temporelle de ce captage il est nécessaire de mieux comprendre l'influence des facteurs environnementaux sur la maturation et la synchronisation des pontes chez les huîtres perlières.

La reproduction des bivalves a majoritairement été étudiée en milieu tempéré où le cycle de reproduction est principalement déterminé par la température, tandis que la concentration en plancton a une influence forte sur l'effort de reproduction. Dans les lagons polynésiens, les températures élevées (26°C – 32°C) font que ce paramètre n'a qu'une faible influence sur le cycle reproductif des huîtres perlières. En conséquence : un fort asynchronisme des stades de maturité est généralement observé entre les individus, la gamétogenèse est continue et les pontes peuvent avoir lieu toute l'année. Cependant, des épisodes de pontes synchronisées ont régulièrement été observés par différents auteurs. La faible capacité de mise en réserve des huîtres perlières en fait une espèce qualifiée d'opportuniste, investissant tout surplus d'énergie dans la reproduction lorsque les besoins énergétiques nécessaires à sa survie et à sa croissance sont assurés.

L'objectif de ce suivi de la reproduction de 4 mois sur l'atoll d'Ahe était donc de mieux comprendre l'influence des variations naturelles de la concentration du plancton sur la maturation et la ponte des huîtres perlières.

Lors de ce suivi, la concentration de deux indicateurs trophiques était mesurée tous les 3 jours près de la filière d'élevage : la concentration en phytoplancton était estimée par extraction et dosage au fluorimètre de la chlorophylle *a* pour 2 classes de tailles (< 2µm et > 2µm) et la concentration en microplancton était estimée par des comptages au microscope de diatomées, de dinoflagellés et de ciliés. Tous les 10 jours, 80 huîtres perlières étaient prélevées au hasard afin de mesurer 3 indicateurs de l'état de maturité des huîtres perlières : le poids sec des masses viscérales, un indice quantitatif de la taille de la gonade (rapport de surface entre la masse gonado-viscérale et la gonade

sur une coupe sagittale de la masse gonado-viscérale) et l'étude des stades de maturité histologiques. Des données météorologiques horaires ont également été obtenues à Météo France.

Au cours de ce suivi, la concentration en Chl *a* > 2µm, en Chl *a* < 2µm, en dinoflagellés, en diatomées et en ciliés étaient en moyenne de 0.14± 0.06 µg l^{-1}, de 0.23±0.07 µg l^{-1}, de 2.0±1.3 x 10^4 cell l^{-}, de 7.3±12.2 x 10^3 cell l^{-1} et de 1.4±1.0 x 10^3. cell l^{-1} respectivement. Pendant ce suivi de l'abondance des communautés planctoniques, nous avons pu observer : (i) une corrélation entre la concentration en Chl *a* > 2 µm et la concentration des dinoflagellés et des diatomées, (ii) une corrélation entre la force du vent et la concentration en Chl *a* > 2µm, en dinoflagellés et en diatomées, et (iii) une période de forte concentration en Chl *a* > 2 µm correspondant à la succession d'un pic de concentration en dinoflagellés et en diatomées.

Au début du suivi de la reproduction, une proportion importante d'huîtres perlières était mature. Puis nous avons observé une ponte majeure concernant 80% des individus et entraînant une perte moyenne de 20% de poids de chair sec dans la population sur une période de 3 semaines. La période de cette émission massive et synchronisée de gamètes (confirmée par histologie) correspondait à une période de forte abondance en Chl *a* > 2 µm et à la succession d'un pic de concentration en dinoflagellés et d'un pic de concentration en diatomées. De plus, sur l'ensemble du suivi, nous avons pu observer une corrélation significative entre la concentration en Chl *a* > 2 µm et l'intensité des variations de l'indice gonadique moyen de la population (maturation ou ponte).

Ces résultats montrent que le régime des vents a un impact majeur sur la concentration en plancton qui à son tour joue un rôle prépondérant dans la maturation des huîtres perlières et dans la synchronisation des pontes.

Lorsque le vent est suffisamment fort, il provoque (i) le déplacement des couches d'eau supérieures du lagon vers les côtes sous le vent et (ii) l'ascension vers la surface des couches d'eau inférieures du lagon le long des côtes situées au vent. Nous suggérons que la remontée de ces

couches d'eau inférieures, que la sédimentation a probablement enrichie en nutriments, en particules organiques en décomposition ou en particules phyto- ou zoo-planctoniques vivantes; serait responsable des pics de concentration en plancton observés durant ce suivi dans la zone du lagon situé au vent des vents dominants.

Ces résultats montrent également que les conditions de température et d'abondance en plancton permettent aux huîtres perlières de produire des gamètes toute l'année sur l'atoll d'Ahe. Au fur et à mesure de leur production, les gamètes s'accumulent dans les tissus gonadiques jusqu'à ce que la capacité maximale de stockage soit atteinte, ce qui provoque l'émission des gamètes. Lorsque la concentration en plancton augmente, la maturation est plus rapide, la capacité maximum de stockage des gonades est atteinte plus rapidement et la fréquence des émissions de gamètes augmente également. La concentration en plancton est donc un élément déterminant dans la synchronisation des pontes d'huîtres perlières dans les lagons de Polynésie française.

Influence of plankton concentration on gametogenesis and spawning of the Blacklip Pearl Oyster *P. margaritifera* in Ahe atoll lagoon (Tuamotu archipelago, French Polynesia).

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ABSTRACT

Pearl culture industry represents one of the dominant business sectors of French Polynesia. However, it still entirely relies on unpredictable spat collection success. Our aim was to assess the influence of natural plankton concentration fluctuations on maturation and spawning of the black lip pearl oyster *Pinctada margaritifera*, during a four months survey conducted in Ahe atoll lagoon. Plankton concentration was assessed by Chlorophyll *a* extraction and by microscope counts while gonadic index, gonado-visceral dry weights and histology were used to measure the oysters reproduction activity. We found that (i) plankton concentration fluctuations were mainly related to the wind regime, (ii) gametogenesis rate was mainly related to plankton concentration, (iii) spawning occurred when maximal gonad storage was reached, (iv) plankton concentration was the main spawning synchronizing factor. These results contribute explaining *P. margaritifera* spat collection variability in French Polynesian atoll lagoon.

INTRODUCTION

Pearl culture industry represents one of the dominant business sectors and sources of income in French Polynesia. However, it still entirely relies on unpredictable natural reproduction and spat collection success. Indeed, large spatio-temporal variability of recruitment rates of the pearl oyster *Pinctada margaritifera* has been reported (Andréfouët et al. 2006, Thomas et al., this issue, a) and a better knowledge of the factors that could determine reproduction is thus of particular interest.

Reproductive cycle of bivalve is generally driven by annual temperature cycles and by food availability (Bayne and Newell, 1983; Gervis and Sims, 1992; Sastry, 1979).

Most studies on bivalve reproduction have been conducted in temperate coastal ecosystems which are characterized by strong seasonal differences. Typically, temperature peaks in summer and concentration of phytoplankton is generally higher during spring, summer and early autumn than in late autumn and winter (Gómez and Gorsky, 2003; Valiela and Cebrián, 1999). In these environments, most bivalve species display an annual reproductive cycle characterized by a resting period during the coldest months of the year with spawning inhibited under a given temperature threshold (Dutertre et al., 2009). During spring and summer, the most favorable period for gametogenesis and spawning, food availability has a major impact on spawning frequency and reproduction effort (Enríquez-Díaz et al., 2008; Mac Donald and Thompson, 1985; Ruiz et al., 1992; Saucedo et al., 2002; Saxby, 2002). In addition to the seasonal determinism of gametes production and spawning, Bayne (1976) distinguished two types of energy management strategies used by bivalves to support gametogenesis. First, a “conservative strategy” where energy storage occurs prior to gametogenesis. Second, an “opportunistic strategy” where gametogenesis and storage can occur simultaneously. However, both reproductive cycles and storage strategies are not species specific and a great plasticity has been observed between different populations of the same species. These differences were explained either by an adaptative response to local environmental conditions or by a genetic difference between populations (Hilbish and Zimmerman,1988;

Loosanoff and Nomejko,1951; Paulet et al.,1988; Thompson, 1984).

In tropical ecosystems, characterized by low seasonal differences and high water temperature, gametogenesis is generally continuous and spawning can occur all year long (Arjarasirikoon et al., 2004; Báqueiro-Cárdenas and Aldana-Aranda, 2000; Fournier, 1992; García-Domínguez et al., 1996; Gervis and Sims, 1992; Lefort and Clavier, 1993; Luna-González et al., 2010).

In the atoll lagoons of the Tuamotu archipelago (French Polynesia), temperatures are high and stable (between 25°C and 31°C) and plankton and particulate organic carbon concentrations are low with little seasonal differences but with high day to day fluctuations (Buestel and Pouvreau, 2000; Charpy et al., 1997; Thomas et al., 2010). In these lagoons, *P. margaritifera* has a continuous and fast gametogenesis leading to frequent asynchronous spawning all year long (Pouvreau et al., 2000). Pouvreau et al. (2000) and Le Moullac et al. (2011) further demonstrated that *P. margaritifera* had low energy storage abilities and could therefore be defined as an "opportunistic" bivalve, investing all energy surplus into its reproduction. However, the precise influence of the natural fluctuations of plankton composition and concentration on *P. margaritifera* gametogenesis and spawning remain poorly known.

In the present study, we aim to measure the influence of natural plankton concentration fluctuations on maturation and spawning of the black lip pearl oyster *P. margaritifera* during a four months survey conducted in Ahe atoll lagoon. To reach this goal, we used digitized images of visceral-mass sections (including gonads) to calculate a quantitative descriptor of gonadal maturity. We measured chlorophyll *a* concentration as a proxy for phytoplankton concentration and we estimated microplankton concentration by microscope counts of dinoflagellates, diatoms and ciliates.

MATERIALS AND MEHTODS

For convenience sake, on all graphs, time is represented in days. Day 1 represents the 7th of February 2009 and Day 120 represents the 6th of June 2009.

Study site

This study was conducted in Ahe atoll lagoon, located 500 km northeast of Tahiti island in the north of the Tuamotu archipelago (Figure 11). Ahe lagoon has an area of 142 km^2 and a mean depth close to 42 m with several maxima close to 70 m. Ahe is defined as a semi-enclosed atoll. There is one active pass in the west part of the lagoon and several reef-flat spillways (less than 50 cm depth) are distributed along the reef rim, mainly in the south and west parts of the lagoon. The average water renewal time (ratio of lagoon volume to average water input rate) was estimated at 34 days (Pagès and Andréfouët, 2001). Dumas et al. (this issue) recently characterized with numerical models the spatial variation of residence and flushing time in different weather conditions, and the average renewal time was estimated to be around 80 days. In 2008, there were 86 farms, but after a dramatic decrease of black pearl value, only 65 farms were still active at the end of 2010 (Lo Yat A., pers. com.).

To study the reproduction of *P. margaritifera*, 2000 6-year old pearl oysters were hung at low density (<20 pearl oysters m^{-3}) in December 2008 on a breeding line located in the north east of Ahe lagoon at approximately 3 km off the coast, at 10m deep (Figure 11). Experiments started in February 2009.

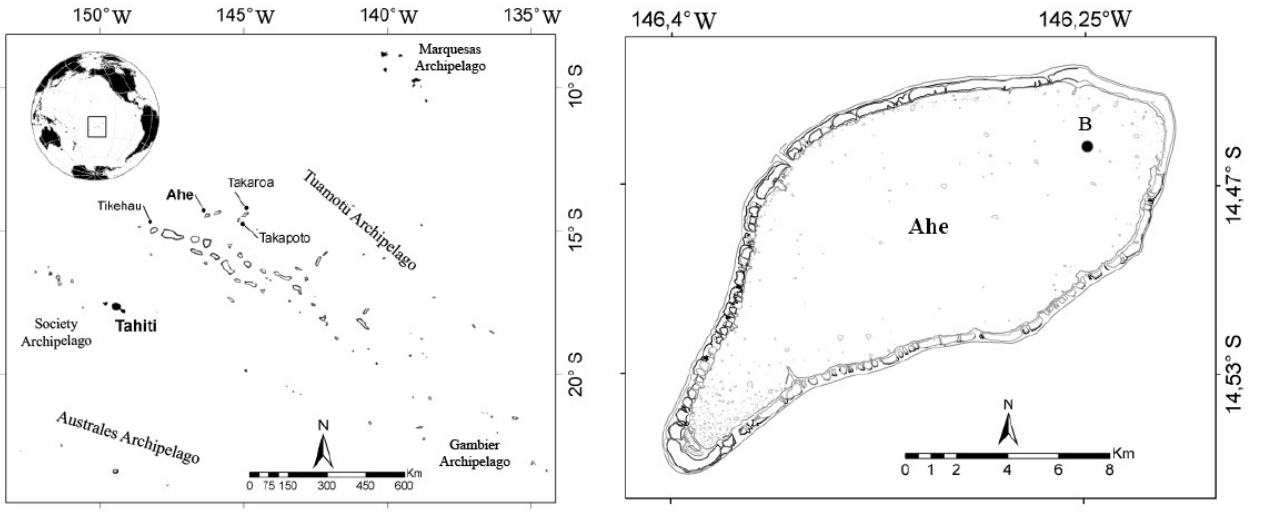

Figure 11 : Location of Ahe atoll and location of the experimental breeding station (B) in Ahe lagoon.

Meteorological and Hydrological parameters

Hourly wind direction and velocity were obtained from Takaroa atoll meteorological station (Météo France data) located about 120 km east of Ahe (145°3'4''W, 14°28'57''S). Daily mean of wind velocity was calculated from initial wind speed hourly values. Water temperature (°C) and salinity (PSU) were obtained from a Sea Bird (SBE V19 plus) probe immersed at a depth of 10 meters at the experimental breeding station (Figure 11).

Plankton concentrations

Plankton concentration was measured at the breeding site every 3 days, between the 11th of February (Day 5) and the 2nd of June (Day 116). All sampling and analysis were done in triplicate. Water was sampled at 10m deep with a 5 liters Niskin bottle and gently transferred into 5 liters containers which were kept in the dark in an isotherm container and immediately brought back to the laboratory for analysis. Phytoplankton concentration was measured on all samples while microplankton enumeration was carried out on samples collected between the 11th of March (Day 33) and the 19th of May (Day 102) only.

Methods used to measure phytoplankton and microplankton concentration are described in details by Fournier et al. (this issue). Briefly, phytoplankton concentrations were assessed by measuring chlorophyll *a* (Chl *a*) concentrations for two size class of particles : < 2 µm (Chl *a* < 2 µm) and > 2 µm (Chl *a* >2 µm). Water samples of 200 ml were filtrated sequentially on 2 µm Millipore filters and on GFF filters. Chl *a* concentration was measured with a Tuner Design TD 700 fluorometer equipped with the set of optical filters recommended by Welshemeyer (1994) for direct measurement of Chl *a*. Total phytoplankton (Chl *a* Tot.) concentration was defined as the sum of Chl *a* < 2 µm concentration and of Chl *a* >2 µm concentration.

To assess microplankton concentration, water samples (200ml) were fixed with alcalin lugol iodine. Enumeration of dinoflagellates, diatoms and dinoflagellates was carried out after sedimentation in Utermohl settling chambers (Hydro bios combined plate chamber), at 400 magnification with a Leica DMI 3000 inverted microscope and following the systematic litterature (Kahl, 1931; Lee, 1985; Nezan, 1996; Paulmier, 1997; Ricard, 1987 and Sournia, 1986).

Dissection and tissue dry weight, gonadic index and maturity stages

Every 10 days, 80 pearl oysters were randomly collected from the breeding line between 18th of February 2009 (Day 12) and 5th of June 2009 (Day 119). After collection, pearl oysters were cleaned from epibionths and immediately brought back to laboratory. Dorso-ventral height and antero-posterior length (Gervis and Sims, 1992) were measured to the nearest mm with a soft stainless ruler. Mantle+gills, muscle and gonado-visceral mass were dissected, drained during 1h on absorbent paper and weighed to the nearest 0.01g. Wet weight of drained gonado-visceral mass (GVM), mantle+gills (MAN) and abductor muscle (MUS) were then converted into dry weights using their respective moisture content : 86 % for GVM, 87 % for Ma. and 75 % for Mu. These values come from average moisture content of 215 freeze-dried pearl oysters sampled in Ahe lagoon, collected, dissected and drained as described above (unpublished personal data).

Between the 25th of March (Day 47) and the 9th of May (Day 92), from each set of 80 oysters, we measured the gonadic index of 40 randomly selected oysters and assessed their histological maturity stages. Specifically, gonado-visceral mass of pearl oysters were fixed during five days in a solution of 10% formalin prepared with seawater; transferred into 70% ethanol for preservation; cut in the sagittal plane and digitized with an Epson 2400 scanner. On the digitized images, gonad areas (GAR, in pixels) and total gonado-visceral mass areas (GVMA, in pixels) were measured with the help of Image J freeware (http://rsbweb.nih.gov/ij/). Gonadi index (GI, in %) was then computed for each individual using the following equation : GI = GAR / GVMA.

Once digitized, gonado-visceral mass was dehydrated through a graded ethanol series, embedded in paraffin, sectioned at 3–4 µm on a rotary microtome, stained with Giemsa dye and, finally, mounted on microscope slides. Sections were made in the gonad area, between the proximal end of the gut loop and the base of the foot. Slide preparations were examined under a light microscope at 200x magnification to assess maturity stages, which were based on the description made by Pouvreau et al. (2000a) :

- Stage 0 : indeterminate or inactive, no evidence of gonadal development

- Stage 1 : Early gametogenesis, follicles small, gonia numerous

- Stage 2 : Actively developing but matures gametes are not observed

- Stage 3 : Near ripe follicles with mature gametes

- Stage 4 : Spawning ripe, follicles distended, confluent and entirely filled

- Rp : partially spawned, partially empty lumen

- Rt : spent, completely empty lumen

Data analysis, statistics

Mean of GI , of GVM dry weight, of MAN dry weight and of MUS dry weight were calculated for each sampling date. Since data were not normal, we used the non parametric Kruskal-Wallis test for the comparison of these four variables among sampling dates. *A posteriori* multiple comparisons were carried out using the non parametric Steel-Dwass test (Critchlow and Fligner, 1991; Spurrier, 2006).

As data were not normal, confidence intervals of GI , GVM dry weight, MAN dry weight and MUS dry weight were calculated using a boot strap method (Efron and Tibshirani,1986).

Spearman correlation analyzes were used to examine the relationships between wind velocity and concentration of Chl *a* < 2 µm, Chl *a* > 2 µm, Chl *a* Tot., dinoflagellates, diatoms and ciliates.

Relationships between reproduction intensity and plankton concentration

To characterize the relationships between reproductive activity of pearl oysters and plankton concentration, it was critical to identify the time lag between food availability and GI. For this, we first calculated the absolute variation of GI. The absolute variation of GI between two sampling dates was calculated using the following equation : $GIV = |GI_D - GI_{D-10}|$, where GIV = absolute gonadic index variation (%), GI = mean gonadic index of pearl oysters at the sampling date D (GI_D) and at the previous sampling date (10 days before $GI_D = GI_{D-10}$). Then, we calculated the running mean of phytoplankton concentration for six periods of time (5, 10, 15, 20, 25 and 30 days). Finally for each day D corresponding to GI_D sampling date, we used Spearman correlation analysis to test the relationships between absolute variation of GI_D and time integrated phytoplankton averages.

We used the same procedure to test the relationships between phytoplankton concentration and variations of GVM, MAN and MUS dry weights.

In all tests, significance was determined with an alpha level of 0.05.

All analysis were conducted with the R freeware (http://www.r-project.org/).

RESULTS

Hydrobiological parameters

Oxygen concentration and salinity were stable during the period of our study (6.0±0.1 mg l^{-1} and 36.2±0.0 PSU, respectively). Water temperature ranged from 28.6°C to 29.2°C and maximum daily variation was 0.3°C.

Wind speed ranged from 0.7 m s^{-1} to 11 m s^{-1} (Figure 12a). When blowing from the east and south directions, wind velocity was higher than 6 m s^{-1} whereas it was lower than 6 m s^{-1} when blowing from west and north directions. Temperature, salinity, wind speed and wind direction corresponded to the usual climatic conditions expected during this period of the year (Buestel and Pouvreau, 1999; Thomas et al., 2010).

Chl *a* Tot. ranged from 0.22 µg l^{-1} to 0.60 µg l^{-1}. Mean concentration of Chl *a* < 2 µm (0.23 µg l^{-1}) was significantly higher than mean concentration of Chl *a* > 2 µm (0.14 µg l^{-1}) (Wilcoxon test, W = 157, p = 0.000). However, between Day 52 and Day 65, Chl *a* > 2 µm concentration was higher than Chl *a* < 2 µm's (Figure 12b).

The mean dinoflagellates concentration was 20.0±13.1 x 10^3 cell l^{-1}. The mean diatoms concentration was 7.3±12.2 x 10^3 cell l^{-1} and the mean concentration of ciliates was of 1.4±1 x 10^3 cell l^{-1}. Dinoflagellates constituted the dominant microplankton community (Figure 12c) except between Days 54 and 71 when diatoms concentration reached up to 6 x 10^5 cell l^{-1}.

All peaks of Chl *a* Tot. concentration occurred at the time of wind velocity peaks (Figure 12a, Days 15, 34, 57, 64,73, 99, 103 and 116). The lowest Chl *a* Tot concentrations were measured during low wind periods (Figure 12a, Days 23, 78 to 92 and 109). Chl *a* Tot. concentration and

wind velocity were significantly correlated (Table 9).

The four peaks of microplankton concentration were concurrent to Chl *a* > 2 µm concentration peaks (Figures 12b and 2c, Days 34, 57, 64 and 99). Similarly, the lowest microplankton concentration corresponded to the lowest Chl *a* > 2 µm concentration (Figures 12b and 2c, Days 73 to 92). Chl *a* > 2 µm and microplankton concentrationx were also significantly correlated (Table 9).

Table 9: Relationships between wind velocity (W.V.) and concentration of phytoplankton < 2µm (Chl *a* < 2 µm), phytoplankton > 2µm (Chl *a* > 2 µm), total phytoplankton (Chl *a* Tot.), dinoflagellates (Dino.), diatoms (Diato.), ciliates (Cili.) and total microplankton (MicPk); r = Spearman's rho, p = p-value. Significant correlations are indicated in bold type characters (α = 0.05).

	W.V.	Chl. *a* Tot.	Chl. *a* >2µm	Chl. *a* <2µm	Dino.	Diato.
Chl *a* Tot	**r = 0.56** **p = 0.000**					
Chl *a* >2µm	**r = 0.53** **p = 0.000**	-				
Chl *a* < 2µm	**r = 0.43** **p = 0.005**	-	**r = 0.45** **p = 0.004**			
Dino.	**r = 0.46** **p = 0.026**	**r = 0.54** **p = 0.007**	**r = 0.62** **p = 0.002**	r = 0.35 p = 0.103		
Diato.	**r = 0.49** **p = 0.018**	r = 0.21 p = 0.326	**r = 0.56** **p = 0.005**	r = -0.26 p = 0.224	r = 0.21 p = 0.335	
Cili.	r = 0.33 p = 0.129	r = 0.35 p = 0.106	r = 0.20 p = 0.371	r = 0.29 p = 0.185	r = 0.52 p = 0.118	**r = 0.11** **p = 0.000**
MicPk.	**r = 0.59** **p = 0.003**	**r = 0.56** **p = 0.006**	**r = 0.82** **p= 0.000**	r = 0.02 p = 0.910	-	-

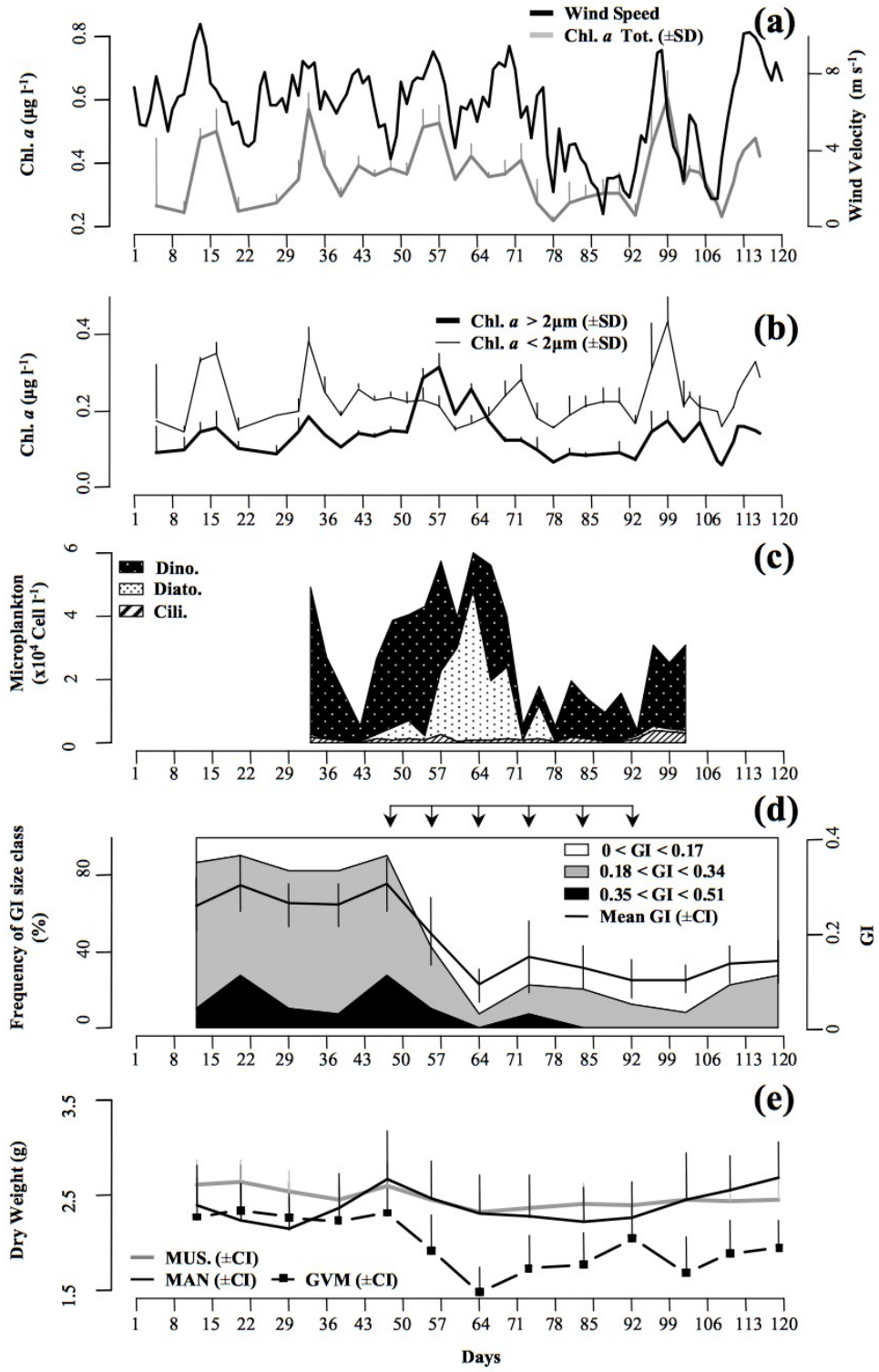

Figure 12 : (a) Wind velocity and total phytoplankton concentration (Chl *a* Tot.); (b) Chl *a* > 2µm and Chl *a* < 2µm concentration; (c) dinoflagellates (Din.), diatoms (Diat.) and ciliates (Cili.) concentrations; (d) mean gonad-index and frequency of 3 size class of gonadic index; (e) Dry weight of abductor muscle (MUS), of Mantle+Gills (MAN) and of gonado-visceral mass (GVM). All parameters were measured in Ahe atoll lagoon between the 7th of February 2009 (day 1) and the 6th of June 2009 (day 120). On figure 12d, arrows indicate the dates at which maturity stages of pearl oysters were assessed by histology.

GI and Maturity Stages

The fluctuation of the mean gonadic index (GI) and the GI size class frequency observed during our study are presented in Figure 12d. Mean GI displayed significant variations between sampling dates (Table 10).

From Day 12 to Day 47, the mean GI was at its highest and ranged from 0.24 to 0.29, with more than 70% of pearl oysters presenting a GI > 0.17. Between Day 47 and Day 64, a major spawning occurred. The mean GI decreased sharply from 0.29 down to 0.08 and the frequency of low GI (< 0.17) increased from 10% to 93%. Between Day 64 and Day 119, mean GI reached its lowest value (ca. 0.14) and low GI (< 0.17) frequency was high (70%).

The histological maturity stages also confirmed the main spawning event. Indeed, the frequency of ripe individuals decreased from 85% to 8% between Day 47 and Day 64 (Figure 13).

During the study period, the mean GI increased when Chl *a* >2µm concentration was > 0.1 µg l^{-1} (between Day 12 and 20, Day 38 and 47, Day 64 and 73) and decreased when Chl *a* >2µm concentration was > 0.1 µg l^{-1} (between Day 20 and 29, Day 47 and 55, Day 73 and 83).Conversely, when Chl *a* > 2 µm concentration was < 0.1 µg l^{-1}, we only observed slight variations of mean GI (between day 12 and 20, day 38 and 47, day 64 and 73). These graphical observations were confirmed by a significant correlation between GIV and Chl *a* > 2 µm concentration running mean (Table 11).

Table 10 : Results of Kruskal-Wallis tests used for the comparisons of gonadic index (GI), gonado-visceral mass dry weight (GVM DW), mantle+gills dry weight (Ma. DW), muscle dry weight (Mu. DW) among sampling dates.

Test	df	Khi²	*p*
GI among sampling dates	12	246	0.000
GVM DW among sampling dates	12	172.6	0.000
Ma. DW among sampling dates	12	52.7	0.000
Mu. DW among sampling dates	12	23.3	0.025

Table 11 : Relationships between GIV (gonadic index variations) and simple moving average of phytoplankton concentration (phytoplankton < 2µm = Chl *a* < 2 µm, phytoplankton > 2µm = Chl *a* > 2 µm, total phytoplankton = Chl *a* Tot.) calculated for 6 different periods (5 to 30 days). Relationships between gonado-visceral mass dry weight variation and the same moving averages of phytoplankton concentration; r = Spearman's rho, p = p-value. Significant correlations are indicated in bold type characters (α = 0.05).

	GIV			Gonado-Visceral mass Variation		
Period (Days)	< 2µm	> 2µm	Tot	< 2µm	> 2µm	Tot
5	r = -0.45 p = 0.14	r = 0.20 p = 0.53	r = -0.20 p = 0.53	r = -0.15 p = 0.65	r = 0.43 p = 0.17	r = 0.15 p = 0.63
10	r = -0.36 p = 0.26	r = 0.49 p = 0.11	r = 0.15 p = 0.63	r = -0.01 p = 0.97	r = 0.4 p = 0.2	r = 0.4 p = 0.2
15	r = -0.32 p = 0.31	**r = 0.64** **p = 0.03**	r = 0.44 p = 0.15	r = -0.11 p = 0.73	r = 0.42 p = 0.17	r = 0.49 p = 0.11
20	r = -0.22 p = 0.50	**r = 0.59** **p = 0.04**	r = 0.43 p = 0.16	r = 0.10 p = 0.76	r = 0.18 p = 0.57	r = 0.38 p = 0.23
25	r = -0.34 p = 0.28	r = 0.39 p = 0.21	r = 0.29 p = 0.35	r = -0.04 p = 0.90	r = 0.13 p = 0.68	r = 0.18 p = 0.57
30	r = -0.27 p = 0.39	r = 0.34 p = 0.28	r = 0.31 p = 0.32	r = 0.06 p = 0.85	r = 0.10 p = 0.75	r = 0.22 p = 0.48

Dry weights

Variation of mean gonado-visceral mass (GVM) dry weight, mean mantle+gills (MAN) dry weight and mean abductor muscle (MUS) dry weight are presented in Figure 12e. Mean GVM dry weight, MAN dry weight and MUS dry weight showed significant variations between sampling dates (Table 10).

Between Day 12 and Day 47 GVM dry weight was significantly higher than on Day 64. This confirmed the major spawning event observed during this period.

The MAN dry weight significantly increased when Chl *a* > 2 µm concentration was > 0.1µg l^{-1} (from Days 29 to 47 and Days 92 to 119) and significantly decreased during the main spawning event (between Day 47 and Day 74).

Compared to GVM and MAN dry weights, MUS dry weight was rather constant. We only

observed a slight increase preceding the major spawning and a slight decrease after.

No significant relationships were reported between GVM dry weight and phytoplankton concentration (Table 11) neither between MUS or MAN dry weight and phytoplankton concentration (data not shown).

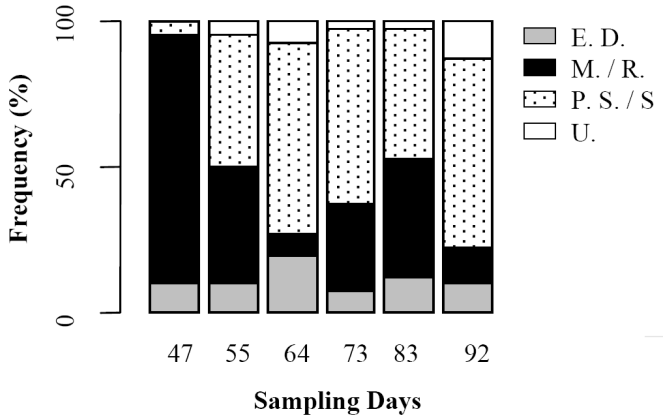

Figure 13 : Frequency of maturity stages observed by histology between the 25th of March (day 47) and the 9th of May (day 92). E.D. = Early development (=Stage 1 + 2); M. / R. = Maturing + Ripe (=Stage 3 + 4) ; P.S. /S. = Partially spawn + Spent (=Stage Rp + Rt), U = Lack of gonadal tissue.

DISCUSSION

Hydrobiological parameters

We report a Chl *a* Tot. mean concentration in the higher range of concentrations reported by Charpy and Blanchot (1998) and Pagès et al. (2001) in other French Polynesian atolls. The maximum concentration of Chl *a* Tot. observed during this study (0.6 µg l^{-1}) was close to values reported by Pagès et al. (2001) in Takaroa atoll lagoon during a phytoplankton bloom. Mean concentration of ciliates and dinoflagellates were higher in Ahe lagoon than those reported in Takapoto lagoon by Loret et al. (2000) but were in the range of Tikehau atoll lagoon (González et al., 1998).

Previous studies in French Polynesian atolls have shown that plankton concentration

variations can be significant at small spatial and/or temporal scale, despite the average low concentrations and the weak seasonal differences (Buestel and Pouvreau, 2000; Charpy et al., this issue; Fournier et al, this issue; González et al., 1998; Pagano et al., this issue; Sournia et Ricard, 1976; Thomas et al., 2010). However, the exact mechanisms responsible for these changes remain unclear. Here, we report that the main fluctuations of Chl *a* > 2 µm, Chl *a* < 2 µm, diatoms and dinoflagellates concentration were clearly related to the wind regime variations.

The link between wind and plankton concentrations can be explained by a process of nutrient enrichment in the water column. First, in semi enclosed atoll lagoons, high winds (10 m s^{-1}) induce an overturning circulation that brings deep bottom water layer to the windward coast of the atoll (Dumas et al., this issue; Lenhardt, 1991). This process brings nutrients accumulated in deep water layers to the surface layer. Indeed, nutrient release from the sediment added to remineralization of the settling organic particles tend to enrich bottom water layers of atoll lagoons (Charpy and Charpy-Roubaud, 1991; Charpy-Roubaud at al., 1996; Gerber and Marshall, 1982). Since experimental nitrogen and phosphorous enrichment of lagoonal water samples have increased growth and abundance of phytoplankton and heterotrophic flagellates (Ferrier-Pagès and Furla, 2001), this nutrient flow likely enhanced as well the plankton biomass and concentration in the eastern lagoon. Specifically here, south and east winds blowing at speed > 6 m s^{-1} probably brought enriched bottom water layer to the surface in the northeastern part of Ahe lagoon which promoted plankton growth and abundance where our breeding line was located. This process is indirectly confirmed by the low concentrations of Chl *a* Tot. observed during west and north winds < 6 m s^{-1}, and by results of Fournier et al. (this issue) who measured high Chl *a* Tot. concentration (> 1µg l^{-1}) in October 2008 in the northeastern part of the lagoon during steady east and south-east wind.

Reproduction of pearl oysters

Dispersion of individual GI and histological results show that, during the period of our study,

P. margaritifera exhibited a continuous reproductive activity with an extremely short resting period and a fast initiation of gametogenesis. These results are in agreement with previous studies which reported fast and continuous gametogenesis in tropical bivalves (Arjarasirikoon, 2004; Baqueiro-Cárdenas and Aldana-Aranda, 2000; García-Domínguez, 1996; Gervis and Sims, 1992).

Ahe lagoon displays fairly similar hydrobiological conditions to Takapoto lagoon where Pouvreau et al. (2000) showed that gametogenesis and spawning were occurring all year long. It is therefore obvious that *P. margaritifera* is characterized by continuous gametogenesis and spawning in Ahe atoll lagoon.

The effect of temperature, food availability and food quality on gametogenesis rate has mainly been studied for temperate species during experimental broodstock conditioning. These conditioning experiments demonstrated that an increase of temperature and food level were matched by an increase of maturation rate until an optimal combination of temperature and food was reached (Chávez-Villalba et al., 2002; Chávez-Villalba et al., 2003; Pronker et al., 2008; Martínez et al., 2003). From a bioenergetic point of view, these results are explained by the global increase of physiological rates when temperature increases and by the increase of energy inflow when food availability increases (Kooijman, 2000). Similar increase of gametogenesis rate with temperature and algae concentrations was observed in *P. margaritifera* conditioning experiments (personnal unpublished data).

Once individuals are mature, a thermal stress is generally used to artificially induce spawning in hatcheries of temperate (Helm, 2004) and tropical bivalves (Gervis and Sims, 1992). However, factors inducing spawning in natural conditions remain unclear for temperate bivalves. A combination of several environmental factors have explained spawning, including thermal amplitude, phytoplankton blooms, tidal cycles and lunar phases (Bernard, 2011; Bonardelli et al., 1996; Starr et al., 1990).

Pouvreau et al. (2000) and Le Moullac et al. (2011) have shown that gametogenesis and spawning of pearl oysters can occur all year long within a temperature range of 23°C to 31°C. Thus, in Tuamotu atoll lagoons, temperature is not a limiting factor for gametogenesis. The same authors have also concluded that sufficient plankton food is naturally available to sustain constant gametogenesis and spawning all year long. However, our results clearly demonstrate that gametogenesis rate and spawning of pearl oysters are directly related to plankton concentration.

In fact, conditions of food and temperature are met for *P. margaritifera* to produce gametes continuously all year long, but at a rate that varies with plankton concentration. Then, gametes accumulate in gonads until the maximum storage capacity is reached, which leads to spawning. Thus, when plankton concentration increases, the amount of energy allocated to gametogenesis increases, the maximum storage size of gonad is reached faster, and the number of individual spawning in the population increases as well.

Plankton concentration is therefore the main spawning synchronizing factor for pearl oysters in atoll lagoons. However, artificial spawning conducted at the Ifremer center of Vairao (Tahiti, French Polynesia) has revealed that female spawning was conditioned to the previous release of the male gametes (Le Moullac, pers com.). The impact of this gender synchronization is unknown *in situ* but is likely to play a role in the spawning synchronization of pearl oysters.

As discussed above, plankton concentration variations can be significant at small spatial and/or temporal scale. Thus, reproduction dynamics of pearl oysters is also likely to be highly variable from one site to another. A peak of plankton concentration at one site could induce a synchronized spawning of all individuals, while at other sites spawning may be reduced to a small percentage of individuals.

Seasonal variations of plankton concentration are commonly assumed to be low in Tuamotu atoll lagoons (e. g. Charpy, 1996). However, during the “warm” season (November to April),

Buestel and Pouvreau (1999) and Thomas et al. (2010) measured higher concentration of phytoplankton than during the “fresh” season in Takapoto and Ahe lagoons, respectively. Available data are too scarce to demonstrate the impact of these seasonal variations of plankton concentration on reproduction dynamics of pearl oysters. However, Pouvreau et al. (2000) reported more intense spawning during the warm season than during the cooler season and we also observed a major spawning at the end of the warm season.

To conclude, our results are in agreement with Pouvreau et al. 2000a who showed that *P. margaritifera* was an opportunistic species with very low energy storage abilities and which invests all surplus of energy into its reproduction. More specifically, our results clearly demonstrated that even if spawning can occur all year long, gametogenesis rate and spawning are tightly linked to the variation of food availability which itself is related to wind regimes. Thus, spatial and/or temporal variability of the plankton concentration obviously leads to spatial and temporal heterogeneity of spawning intensity in the lagoon.

In association with the results of Thomas et al. (this issue a, b) who described the patterns of bivalve larval dispersal and growth in Ahe lagoon, our findings provide a comprehensive description of the processes involved in the inherent variability of spat collection success, observed empirically in Tuamotu atolls after decades of black pearl farming.

In fact, wind regime determines lagoon hydrodynamics regime which drives larval dispersal and impacts both food availability and reproduction dynamics. The monitoring of wind and of > 2µm plankton biomass is therefore a priority to predict spawning and infer subsequent larval dispersal (Thomas et al. this issue b).

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CHAPITRE IV

Application d'un modèle bio-énergétique de type DEB à l'huître perlière *P. margaritifera.*

RÉSUMÉ DÉTAILLÉ

Depuis une vingtaine d'années, le développement des modèles bio-énergétiques de croissance et de reproduction des bivalves a permis une meilleure compréhension de l'influence des conditions environnementales sur la croissance et la reproduction des bivalves et une meilleure estimation de la capacité trophique des écosystèmes dans lesquels ils sont cultivés. Dans cette étude, notre objectif était donc de vérifier les conclusions du chapitre précédent sur le déterminisme trophique de la reproduction des huîtres perlières par l'application d'un modèle basé sur la théorie des budgets énergétiques dynamiques (DEB).

La structure et les équations du modèle DEB établi pour *C. gigas* ont servi de base à la mise en place du modèle DEB pour *P. margaritifera*. Les valeurs des paramètres spécifiques à l'huître perlière ont été calculées en suivant les principes théoriques de la modélisation DEB et en combinant nos propres jeux de données avec les données disponibles dans la littérature. Ce modèle a ensuite été utilisé pour simuler le poids sec des huîtres perlières sur l'atoll d'Ahe (1 cohorte d'huîtres perlières âgées de 6 ans) et sur l'atoll de Takapoto (3 cohortes d'huîtres perlières âgées de 1, 2 et 3 ans). Les variables forçantes des modèles de type DEB sont la température et la densité de nourriture. Dans notre cas, les indicateurs de la concentration en plancton étaient : la concentration en chlorophylle *a* > 2µm sur Ahe (Chl *a* > 2 µm) et la concentration en matière organique particulaire (POM) sur Takapoto.

Sur Ahe, une première simulation a été réalisée avec une valeur initiale de la variable d'état E_R (qui représente la quantité d'énergie stockée dans les gonades) calculée à partir de l'indice gonadique moyen de la population observé au début du suivi de la reproduction. Puis, 30 simulations ont été réalisées avec une valeur de E_R différente pour chaque simulation et calculée à partir des indices gonadiques individuels observés au début du suivi de la reproduction.

Sur Takapoto, une première série de simulations a été faite avec le modèle initial pour les 3 cohortes; puis une seconde série de simulations a été faite après avoir augmenté le paramètre κ

(qui représente le coefficient de répartition de l'énergie entre la reproduction et la croissance) afin de diminuer la quantité d'énergie allouée à la reproduction.

Les valeurs des paramètres du modèle DEB pour l'huître perlière sont récapitulées dans le tableau 14. La forte valeur du $\{\dot{P}_{Am}\}$ (taux d'assimilation maximum) des huîtres perlières confirme que ses capacités de filtrations élevées lui permettent d'acquérir une quantité d'énergie importante malgré la faible concentration en plancton qui caractérisent les lagon d'atolls.

Sur Ahe, les simulations du modèle sont en accord avec les valeurs de poids secs observés. La croissance et l'effort de reproduction sont simulés efficacement par le modèle. De plus, dans 90% des simulations, les pontes se déroulent lorsque la concentration en Chl *a* > 2 µm est > 0.1 µg l^{-1} et dans 63% des simulations, les pontes se déroulent pendant la période où la concentration en Chl *a* > 2 µm est la plus élevée. La durée minimale de la maturation simulée par le modèle est de 60 jours. Ces résultats confirment les conclusions du chapitre 2 concernant le déterminisme trophique de la gamétogenèse et de la synchronisation des pontes. Ils montrent également que la concentration en Chl *a* > 2 µm est un indicateur pertinent de la concentration en plancton « utile » pour les huîtres perlières.

Sur Takapoto, malgré la simulation efficace de la croissance des différentes cohortes, l'effort de reproduction est relativement élevé avec une durée moyenne de maturation de 23 jours. En modifiant la valeur du paramètre κ on obtient une durée moyenne de maturation de 46 jours. La POM ne semble donc pas être un indicateur suffisamment précis de la concentration en plancton « utile » pour les huîtres perlières.

Bien que nous ayons pu obtenir des résultats satisfaisants avec cette première version du modèle DEB pour l'huître perlière, les valeurs estimées pour les paramètres DEB sont à confirmer et à affiner afin d'optimiser les prédictions du modèle. Pour cela, il est nécessaire d'effectuer des expérimentations complémentaires en laboratoire (mesures de croissance, de reproduction et de

respiration lors de conditionnements à différents niveau de température et de densité en algues) et d'obtenir des jeux de données intégrant le suivi *in situ* des sources de nourriture et de la croissance des huîtres perlières sur des périodes longues (plusieurs années) afin d'optimiser la validation du modèle.

Étant donné le fort asynchronisme de la reproduction des huîtres perlières nous suggérons que ce modèle DEB soit intégré dans un modèle populationnel de type IBM (Individual Based Model) (e.g. Bacher & Gangnery, 2006) en y incluant une variabilité individuelle au niveau du paramètre X_K (coefficient de demi saturation). Ce type de modèle serait en effet beaucoup plus adapté pour simuler aussi bien l'asynchronisme globale de l'état de maturité des populations d'huîtres perlières que les périodes de synchronisation des pontes.

Environmental determinism of growth and reproduction of the Blacklip Pearl Oyster *P. margaritifera* in atoll lagoons of French Polynesia : investigations through DEB theory.

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ABSTRACT

In atoll lagoons of French Polynesia, *P. margaritifera* is known to have a continuous annual reproductive cycle. Spawning can occur all year long and is generally asynchronous but some periods of synchronized spawning can also be observed. In these lagoons characterized by high and relatively stable temperature, spawning synchronization factors are poorly known but are of primary importance since the pearl culture industry relies on natural spat collection. In that context, this study aimed to apply to *P. margaritifera* a generic bio-energetic model of bivalve growth and reproduction based on the Dynamic Energy Budget theory (DEB), in order to assess quantitatively the influence of food concentration on reproduction effort and on spawning synchronization. A first set of DEB parameters values for *P. margaritifera* were computed by combining our own experimental data sets with published previous studies. Then this model was used to simulate soft tissue dry weights variations of two pearl oysters populations from Takapoto atoll lagoon and Ahe atoll lagoon by using temperature, particulate organic matter concentration (POM) and Chlorophyll *a* > 2µm concentrations (Chl *a* > 2 µm) as forcing variables. These simulations demonstrate clearly the influence of variations of plankton concentration on maturation and spawning of pearl oysters. Advantages, drawbacks and possible improvements of this first pearl oyster DEB model are

discussed.

INTRODUCTION

During the last two decades, much effort has been devoted to modeling of bivalve growth and reproduction in order to assess quantitatively the influence of environmental parameters on growth and reproduction (e.g. Ross and Nisbet, 1990; Barillé et al., 1997; Pouvreau et al., 2000; Kooijman, 2000; Van de Veer and Alunno-Bruscia, 2006; Alunno-Bruscia et al., 2009) and the carrying capacity of ecosystems (eg : Bacher, 1989; Dame & Prins, 1998; Pouvreau et al., 2000; Bacher & Gangnery, 2006; Brigolin et al., 2009; Guyondet et al., 2010).

For *P. margaritifera*, 2 bioenergetic models based on the scope for growth concept were constructed by Pouvreau et al., (2000). Although this model has shown its ability to simulate effectively growth and reproduction of various bivalve species , they present several drawbacks : they are based on empirical allometric relationships and on static energy budget, which are determined experimentally (Bayne, 1998). These characteristics limit (i) their ability to describe the allocation of energy flow to the physiological functions of an organism in a dynamically varying environment and (ii) their application to simulation of growth and reproduction at various sites for one single species.

In order to overtake these limitations, Kooijman (2000) developed the Dynamic Energy Budgets theory, which offered a new framework for the development of bio-energetic modeling of growth and reproduction. This theory is based on the first principles of biology and biochemistry such as energy and mass conservation theory or general system theory and aims (i) to describe and quantify energy and mass acquisition by an organism from its environment by a Holling type functional response and (ii) to quantify energy and mass flows within an organism by a mechanistic description of the basic energy budgets from the cellular level to the individual level. This approach presents four main advantages : (i) one single generic model built on a limited number of primary

parameters is used to describe energy flow in organisms, (ii) comparison between species is possible by comparing the values of these parameters, (iii) values of some of these parameters are considered to be similar between related species (which are called “intensive” parameters) and (iv) energy flows are described dynamically. In the last decade, models based upon this approach have undergone constant development and have shown their efficiency to describe growth and reproduction over an increasing number of species (e.g. Van der Veer et al., 2009; Pecquerie et al., 2009; Bodiguel et al., 2009; Péry and Garric, 2006), and bivalve species in particular (Pouvreau et al, 2006, Bourlès et al.,2009 Bernard et al., 2011, Thomas et al. 2011; Van der Veer et al., 2006; Van Haren & Kooijman, 1993).

In atoll lagoons of French Polynesia, *P. margaritifera* is known to have a continuous annual reproductive cycle with asynchronous spawning occurring all year long and with periods of more synchronized spawning (Theilley, 1993; Pouvreau et al, 2000; Fournier et al., in press). However, in these lagoons characterized by high and relatively stable temperature, spawning synchronization factors are poorly known.

In that context, this study aimed to apply the generic DEB model to *P. margaritifera* in order to assess quantitatively the influence of food concentration on reproduction effort and on spawning synchronization. In the present study, we propose a first set of DEB parameters values for *P. margaritifera* estimated (i) from our own experimental data sets when available, (ii) from parameters of other species when relevant and (iii) by using the compound parameters properties when possible (Kooijman,2000). This model was then used to simulate soft tissue dry weights variations of two pearl oyster populations in Takapoto lagoon and Ahe lagoon, and using particulate organic matter concentration (POM) and chlorophyll *a* > 2µm concentration (Chl *a* > 2 µm) as food forcing variables, respectively. Eventually, simulated dry weights were compared to observed dry weights to assess the reliability of this first pearl oyster DEB model.

MATERIALS AND METHODS

Structure and equations of this first pearl oyster DEB model were based on the oyster DEB model of *Crassostrea gigas* which was fully described in Pouvreau et al. (2006) and Bourlès et al. (2009). Hence, only a brief summary of the main outlines is presented here.

General Model Design

The DEB model assumes that the energy which is assimilated through ingested food is first stored in a reserve compartment. A fixed fraction κ of the energy flow from reserves is then used for growth and somatic maintenance, with a priority for maintenance. The remaining energy fraction (1−κ) is spent on maturity maintenance and development in embryos and juveniles or on reproduction, i.e. gamete production and spawning, in adults.

Notation and symbols follow Kooijman (2000). Quantities are expressed per unit of structural volume with square brackets [ ], or per unit of surface area of the structural body volume with braces {}. All rates have dots, indicating the dimension per time.

The energy ingestion rate $\dot{p}_x$ is proportional to the surface area of the structural body volume $V^{2/3}$ and depends upon food density X in the environment by a Holling type II functional response:

$$\dot{p}_x = \{\dot{p}_{Xm}\} f V_{2/3} \text{ ; with } f = \frac{X}{X + X_K} \quad (1)$$

where $\{\dot{p}_{Xm}\}$ is the maximum ingestion rate per unit of surface area and f is the dimensionless functional response which can vary between 0 and 1. X_K is the saturation coefficient, or Michaelis–Menten constant which represents the food density at which the ingestion rate is half the maximum. The half-saturation coefficient is the only free-fitted parameter of the oyster-DEB model, as it is supposed to vary according to food quality (Kooijman, 2006), and therefore according to shellfish growing area.

The assimilation rate $\dot{p}_A$ is given as:

$$\dot{p}_A = \{\dot{p}_{Am}\} f V^{2/3} \quad (2)$$

where $\{\dot{p}_{Am}\}$ is the maximum surface area-specific assimilation rate. Its precise value depends on the oyster diet. The ratio $\{\dot{p}_{Am}\} / \{\dot{p}_{Xm}\}$ gives the conversion efficiency of ingested food into assimilated energy, known as the assimilation efficiency A.E.

Assimilation rate $\dot{p}_A$ contributes to the energy reserve dynamics :

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

where $\dot{p}_C$ is the utilisation rate of the reserve energy.

The kappa rule (κ) states that a fixed fraction of $\dot{p}_C$ is allocated to somatic maintenance and growth. Maintenance rate $\dot{p}_M$ is proportional to the structural volume V, so $\dot{p}_M = [\dot{p}_M] V$, with $[\dot{p}_M]$ the maintenance cost per unit of volume. Therefore, the structural body volume V changes as:

$$\frac{dV}{dt} = \frac{\kappa \dot{p}_C - \dot{p}_M}{[E_G]} \quad (4)$$

where $[E_G]$ denotes the volume-specific costs for structure. Kooijman (2000, chapter 3.4) showed that $\dot{p}_C$, the energy used (fixed and dissipated) by the body tissues for development, growth, reproduction and maintenance, can be written as:

$$\dot{p}_C = \frac{[E]}{[E_G] + \kappa [E]} \left(\frac{[E_G] \{\dot{p}_{Am}\} V^{2/3}}{E_m} + \dot{p}_M V \right) \quad (5)$$

where $[E]$ represents the energy density and equals E / V, and $[E_m]$ is the maximum energy density in the reserve compartment. Thus, $[E]$ can vary between 0 and $[E_m]$.

As κ is the fraction of the energy utilization rate $\dot{p}_C$ spent on somatic maintenance plus growth, the remaining $(1-\kappa)\, \dot{p}_C$ is allocated to maturity maintenance and maturity in embryos and juveniles, or reproduction (i.e. gamete production and spawning) in adults. Giving that the somatic and maturity maintenance rate coefficients are equal, the maturity maintenance $\dot{p}_J$ is proportional

to the structure V until juveniles reach sexual maturity at volume V_P. Maturity maintenance does not increase beyond this level. Thus, $\dot{p}_J$ is defined as:

$$\dot{p}_J = \left(\frac{1-\kappa}{\kappa}\right) Min(V, V_p)[\dot{p}_M] \quad (6)$$

The dynamics for energy allocated first to maturation in juveniles, and then to the reproduction buffer E_R in adults are:

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

Shell length L (cm) is proportional to the structural body volume V:

$$L = \frac{V^{1/3}}{\delta_m} \quad (8)$$

where δ_m is the dimensionless shape coefficient.

Physiological processes, e.g. assimilation, maintenance and structural growth in the DEB model, depend on the body temperature. Within a species-specific temperature tolerance range, theory says that physiological rates increase exponentially with temperature, as described by the Arrhenius relation:

$$\dot{k}(T) = \dot{k}_1 \exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\} \quad (9)$$

where $\dot{k}(T)$ is a physiological rate at ambient temperature T (in K), $\dot{k}_1$ is its value at a chosen reference temperature T1, and TA is the Arrhenius temperature (in K) similar for all physiological rates of an animal. The basic Arrhenius correction $\exp\left\{\frac{T_A}{T_1} - \frac{T_A}{T}\right\}$ is applied in the range of an optimal temperature to the maximum specific ingestion rate and to the volume specific maintenance rate and is equal to 1 when T=T1. No experimental data were available to determine the boundaries

of the optimal temperature range in which physiological processes are corrected by the Arrhenius temperature. However, the range of forcing temperatures used in the simulations conducted in the present study were usual temperatures for atoll lagoons and were in the range of experimental temperatures tested by Yukihira et al., (2000). We therefore assumed that these temperatures were in the range of optimal temperature for the pearl oysters.

Parameters estimation

No other work had already been done to estimate DEB parameters values for *P. margaritifera*. We therefore used the methods recommended by Van der Veer et al. (2006), Van der Meer (2006) and Bernard et al. (2011) to obtain a first set of DEB parameters for the pearl oyster *P. margaritifera*. Thus, we combined our own experimental data sets, the values of DEB parameters of other species when this procedure was relevant and the compound parameter properties to complete DEB parameters estimates for *P. margaritifera*.

Shape Coefficient (δ_m) The estimates of the shape parameter value was based on dorso-ventral shell height and fresh flesh mass data (n = 3900) from Takapoto atoll (Pouvreau et al., 2000) and Gambier archipelago (Le Moullac et al., 2011). The model (eq. n°8) was adjusted to the lowest values of fresh flesh weight in order to consider only immature individuals with low storage level and to establish the relationship between height and structural body mass.

Arrhenius Temperature The estimates of Arrhenius temperature was based on oxygen consumption rates and ammonia excretion rates measured between 19°C and 32°C by Yukihira et al. (2000), combined to the estimates of Arrhenius temperature for various temperate bivalve species (Van der Veer et al., 2006).

Surface specific Maximum Ingestion Rate $\{\dot{p}_{Xm}\}$ **.** Surface specific maximum ingestion rate was estimated from values of maximum retention rate and of assimilation efficiency measured during an experiment conducted at the Ifremer/COP center of Tahiti.

Pearl oysters of 108±7mm (n = 20) were reared in a 300 l raceway supplied with lagoon sea-water maintained at 30°C and at an inflow rate of 300 l h-1. A solution of cultured *Chaetoceros gracilis* and *Isochrysis galbana* (50% V/V) was added at defined flow rates to reach ambient concentrations ranging from 4000 cell ml^{-1} to 100000 cell ml^{-1}, with each concentration of algae maintained during 48h. A control raceway was set up with the same conditions of temperature, renewal rate and inflow rate of algal solution. The concentration of algae was monitored continuously with a calibrated fluorometer in the control raceway and in the raceway containing the pearl oysters.

We assumed that pseudo-feces production was negligible and we calculated the ingestion rate I_R (in cell h^{-1} $individual^{-1}$) at each concentration level of algae by the the following formula :

$$I_R = F \times \left(\frac{[A]_C - [A]_{PO}}{N} \right)$$

with I_R = Ingestion rate (cell h^{-1} $individual^{-1}$), $[A]_{PO}$ = concentration of algae in the raceway containing the pearl oysters (cell ml^{-1}), $[A]_C$ = concentration of algae in the control raceway (cell ml^{-1}) and F = flow rate (l h^{-1}), N = number of oysters in the batch.

Linear regression of 1/ I_R against $1/[A]_{PO}$ was used to model retention rate as a function of algae concentration. From this regression curve we estimated the I_{Rmax} which represents the maximum ingestion rate (in cell h^{-1} $individual^{-1}$). $\{\dot{p}_{Xm}\}$ (in J cm^{-2} d^{-1}) was computed from I_{Rmax} with the available conversion factors (Table 12) and by standardizing per surface volume of pearl oyster soft tissue. Surface volume of soft tissue was obtained by computing the mean length of pearl oysters with the shape relationship (eq. n°8)

Table 12 : Coefficients used to convert algal retention rate from cell h^{-1} into J h^{-1}.

Conversion coefficients	*I. galbana*	*C. gracilis*
Volume ratio of algal culture solution into distributed algal mixture	0.5	0.5
Cell ratio into distributed algal mixture	2/3	1/3
Algal dry weight (pg $cell^{-1}$)[a]	20	70
Energy Content of dried algae (J pg^{-1})[b]	2.03×10^{-8}	2.03×10^{-8}
Energy Content per cell of microalgae (J $cell^{-1}$)	4.1×10^{-7}	1.4×10^{-6}

[a] Gonzalèz-Araya et al., 2011 and Renaud et al, 2011; [b] Yukihira et al., 2000

Volume specific maintenance rate $[\dot{P}_M]$, Maximum storage density [E_m] and Partition coefficient κ. No experimental data were available to allow the estimates of these three parameters. However, the volume specific maintenance cost is usually considered to be similar for bivalve species (Kooijman, 2000; Van der Veer et al., 2006). Thus, we considered the latest estimation of $[\dot{p}_M]$ value for *Crassostrea gigas* by Bernard et al. (2011) as an appropriate estimates of $[\dot{p}_M]$ for *P. margaritifera*.

Similarly, we considered the ν value of *C. gigas* as a reference value for *P. margaritifera* and we calculated the value of [*Em*] from the following relationship (eg : Van der Meer, 2006; Bernard et al., 2011) :

$$\nu = \frac{[\dot{p}_{Am}]}{[E_M]} \quad (10)$$

Finally, we set the maximum length L_M = 35cm and we estimated κ from the following formula :

$$\kappa = [\dot{p}_m] \frac{V_M^{1/3}}{\{\dot{p}_{Am}\}} \quad (11)$$

Model simulations

The model was implemented in Stella 8.1 software. The forcing variables used to run the

model were temperature and food density relative to the experiment concerned. The model was tested on two data sets. The first data set consisted in a one year monitoring of pearl oysters dry weight and particulate organic matter concentration (POM) in Takapoto atoll lagoon (Pouvreau et al., 2000). The second data set consisted in a five months monitoring of pearl oysters dry weight, pearl oysters gonadic index and chlorophyll *a* > 2µm concentration (chl *a* > 2µm) (Fournier et al., in press).

Spearman's correlation coefficients and statistics were used to test the goodness of fit between simulated and observed dry weights.

Ahe and Takapoto data sets

A detailed description of sampling schemes and methods used in Ahe atoll lagoon and in Takapoto atoll lagoon are given in Fournier et al. (in press) and Pouvreau et al. (2000), respectively.

In both sites, pearl oysters were hung at low density (<20 pearl oysters m-3) on breeding lines located in the north east of Ahe atoll lagoon (one size class of 6 years old pearl oysters) or in the south east of Takapoto atoll lagoon (3 age-groups : age-group I = 1 year old pearl oysters, age-group II = 2 years old pearl oysters and age group III = 3 years old pearl oysters), at 10 m deep in Ahe lagoon and at 7m deep in Takapoto lagoon. Due to their small size, pearl oysters of age group I were stored in pearl nets in Takapoto atoll lagoon.

In Ahe atoll lagoon, 80 pearl oysters were sampled every ten days from the 18th of February (day 1) to the 5th of June (day 108). In Takapoto atoll lagoon, 30 pearl oysters were sampled every 15 days from March 1997 to April 1998.

Their shell height was measured and they were dissected, drained during 1h and their flesh mass weighed. In Ahe atoll lagoon, at each sampling time, gonado-visceral mass of 40 pearl oysters were fixed in salted formalin (10%) during 4 days and were cut in the sagittal plan which was digitized. These digitized images were used to calculate a quantitative descriptor of gonadal

maturity. At each sampling time, gonado-visceral mass, muscle and mantle+gills of 40 pearl oysters sampled in Ahe lagoon and of 30 pearl oysters sampled in Takapoto lagoon were freeze-dried and weighed.

In Takapoto and Ahe atoll lagoons, water sampling was conducted at breeding sites in order to measure chl *a* > 2µm concentration according to Welshemeyer (1994) (every three days in Ahe atoll lagoon) or particulate organic matter according to Amino & Chaussepied (1983) (every week in Takapoto atoll lagoon). Water temperature was obtained at the depth of the breeding line from a Sea Bird (SBE V19 plus) probe in Ahe atoll lagoon and from an Hydrolab probe in Takapoto atoll lagoon.

Simulations

These data sets were used to run a first set of simulations with the pearl oyster model. For these simulations, state variables (E, E_V and E_R) were set in order to respect (i) mean flesh dry weight at the beginning of the experiment, (ii) equilibrium between the functional response and the energy density at the beginning of the experiment and (iii) the mean gonadic index observed at the beginning of the experiment for the simulations conducted in Ahe atoll lagoon.

Then, these data sets were used to run a second set of simulations in order to investigate (i) the effect of food concentration on spawning synchronization in Ahe atoll lagoon and (ii) optimization of the simulation of reproduction effort of pearl oysters in Takapoto atoll lagoon. In order to investigate the effect of plankton concentration on spawning synchronization we used exactly the same values of DEB parameters (including X_K) and state variables, except the initial quantity of energy contained in gonadal tissue (called E_R in the model), which were calculated for each simulation (n=30) from the individual initial values of gonadic index observed in the first sampling conducted in Ahe atoll lagoon (Figure 14) and using the following formula :

$$E_R = \frac{E_{Rmax} * GI_{IND}}{GI_{MAX}}$$

with E_R = initial quantity of energy contained in gonadal tissue (J); E_{Rmax} = maximum quantity of energy contained in gonadal tissue (36000 J); GI_{IND} = Observed Individual Gonadic Index and GI_{MAX} = maximum Gonadic Index observed during this study (=0.5). Finally, in order to optimize the simulation of the reproductive effort with the Takapoto atoll lagoon data set, we increased κ to 0.7 to decrease the energy flow allocated into reproduction. For these simulations the X_K values had to be re-adjusted. Except these modifications, state variables and DEB parameters were kept identical as in the first simulation with the Takapoto atoll lagoon data-set (Table 13).

Table 13 : Initial values of state variables for simulations conducted in Ahe atoll lagoon and in Takapoto atoll lagoon for 3 age groups.

	Ahe (mean)	Takapoto (Age-group I)	Takapoto (Age-group II)	Takapoto (Age-group III)
E_V	55000	2000	16000	34000
E	28000	1000	10000	25000
E_R	22000	500	5000	8000

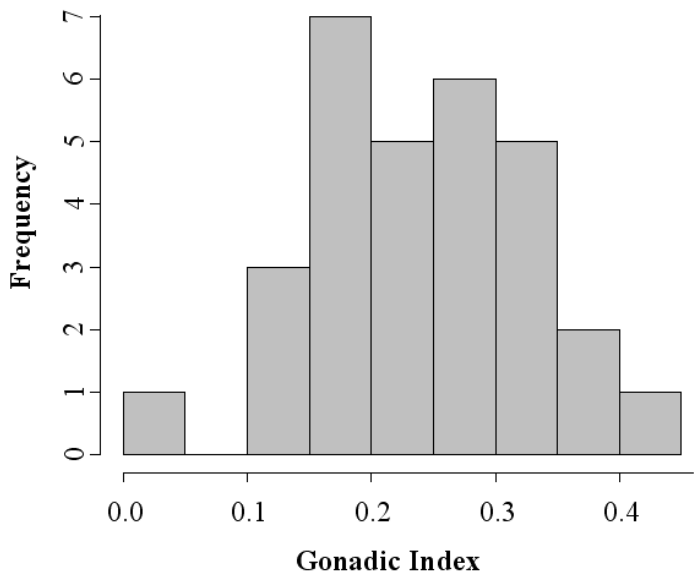

Figure 14 : Absolute frequency of Gonadic Index (n = 30) observed in Ahe atoll lagoon at the first sample date

RESULTS

Parameters estimates

From length and fresh flesh mass data of pearl oysters collected in the Gambier archipelago (Le Moullac et al., 2011) and Takapoto atoll lagoon (Pouvreau et al., 2000), shape coefficient was adjusted at 0.22 (Figure 15).

Linear regression of standardized excretion rates and oxygen consumption rates of *P. margaritifera* (Yukihira et al., 2000) on the reference Arrhenius temperature determined for various bivalve species by Van der Veer et al. (2006) resulted in an $R^2 = 0.74$ with a p-value =0.004 (Figure 16). We therefore considered the Arrhenius temperature estimates for bivalves established by Van der Veer et al. (2006) (T_A = 5800 K) as an appropriate estimate of Arrhenius temperature estimates for *P. margaritifera*.

Results of the ingestion experiments conducted at 30°C are presented in Figure 17 From the linear regression of $1/ I_R$ against $[A]_{PO}$ we obtained an I_{Rmax} = 6 x10^8 cell h^{-1} $individual^{-1}$. This value was computed with the shape relationship (eq n°8), the conversion coefficients presented in Table 12, and corrected with the Arrhenius relationship (eq n°9) to obtain a $\{\dot{p}_{Xm}\}$ = 1060 J cm^{-2} d^{-1} at the reference temperature of 23°C.

At the reference temperature of 23°C, $[\dot{p}_M]$ was set to 54 J cm^{-3} d^{-1} for *P. margaritifera*, on the basis of the volume-specific maintenance cost value estimated by Bernard et al. (2011) for *C. gigas* (44 J cm^{-3} d^{-1} at 21°C).

Then, using the max-surface specific assimilation rate ($\{\dot{p}_{Am}\}$ = 795 J cm^{-2} d^{-1} (*P. margaritifera*, this study) and the energy conductance v = 0.18 (*C. gigas*, e.g. Bernard et al., 2011); we can calculate a maximum energy density $[E_m]$ = 4400 J cm^{-3} according to eq n°10

Finally, we set the maximum length of *P. margaritifera* at L_m = 35 cm and we computed

$[\dot{p}_M]$, $\{\dot{p}_{Am}\}$ and $V_M^{1/3}$ according to eq. n°11 to obtain the partition coefficient $\kappa = 0.53$.

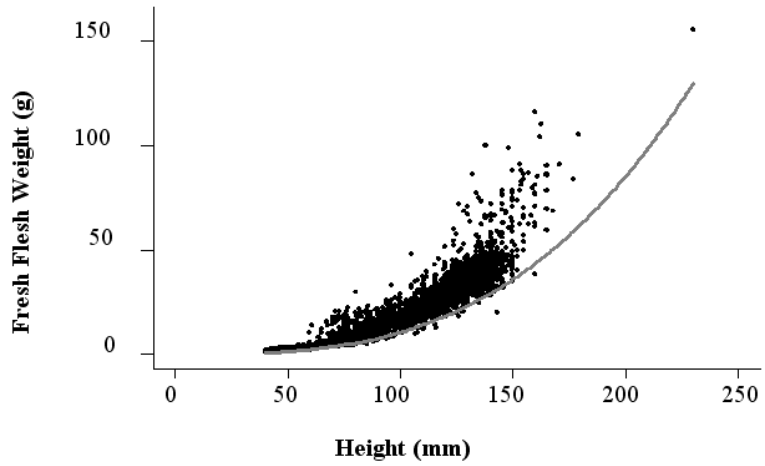

Figure 15 : Shell length (L, mm) and somatic wet mass (g) relationship for *P. margaritifera* (n=3893). Observed wet weights (dots) include structure and reserves; continuous curve (δ_m= 0.22) represents the "minimal envelope" between length (L), and structural body volume (V) (without reserves).

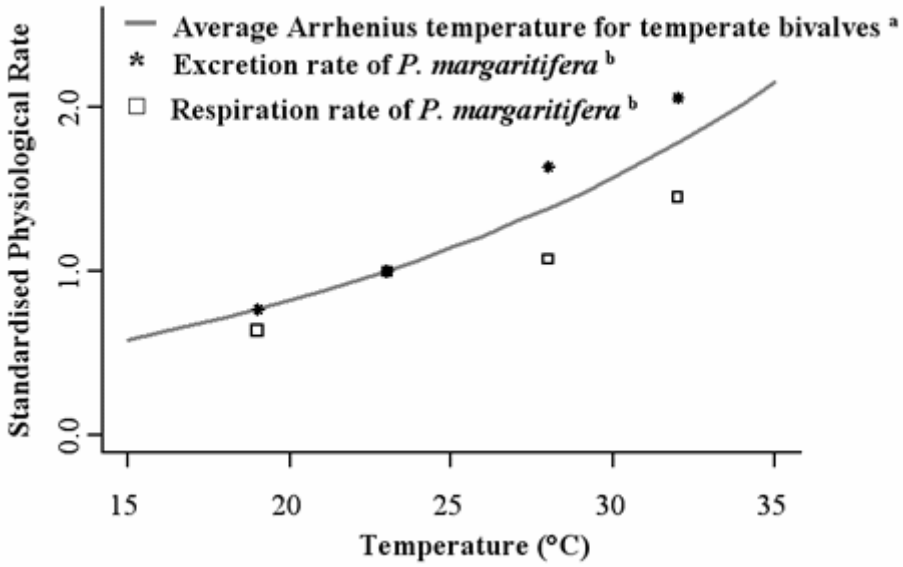

Figure 16 : Reference Arrhenius temperature for bivalve (curve)([a] Van der Veer et al., 2006) and standardized rate of oxygen consumption and urea excretion for *P. margaritifera* ([b]Yukihira et al.,2000)

Table 14 : Parameters used in the DEB model

Symbol	Meaning	Value	Units	Source
	General parameters			
δm	Shape coefficient	0.22	-	This study
$[\dot{p}_M]$	Vol. specific maintenance cost	54	J cm^{-3} d^{-1}	Bernard et al., 2011
$[E_G]$	Vol. specific cost for structure	1900	J cm^{-3}	Bourlès et al., 2009
$[E_m]$	Maximum storage density	4400	J cm^{-3}	This study
ν	Energy conductance	0.18	-	Bourlès et al., 2009
κ	Fraction of Pc spent on growth	0.526	-	This study
A.E	assimilation efficiency	0.75	-	Van der Veer et al., 2006
L_m	Taille maximale	35	cm	This study
	Feeding processes			
$\{\dot{p}_{Xm}\}$	Max surf area specific ingestion rate	1060	J cm^{-2} d^{-1}	This study
$\{\dot{p}_{Am}\}$	Max surf area specific assimilation rate	795	J cm^{-2} d^{-1}	This study
	Temperature effect			
T1	Reference Temperature for rates	296	°K	Arbitrary
TA	Arrhenius temperature	5800	°K	This study
Lp	Size at sexual maturity	4	cm	Pouvreau et al., 2000
Vp	structural volume at sexual maturity	0.73	cm^3	Pouvreau et al., 2000
	Additional parameters			
μ_E	Energy Content of reserves	17.5	J mg^{-1}	van der Veer et al., 2006
GI	Gonado-somatic index triggering spawning	25	%	Pouvreau et al., 2000
dv	Dry mass ratio for structure	0.15	-	Pers. Unpubl. data

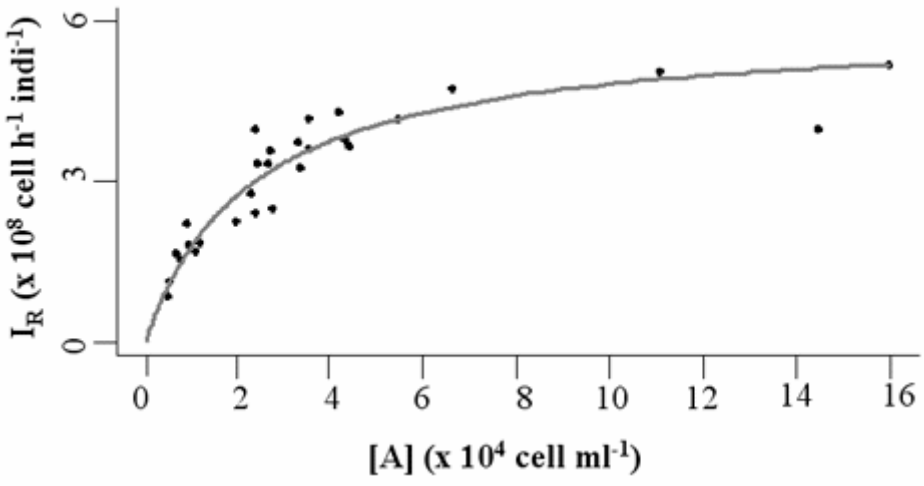

Figure 17 : Relationship between algal concentration ([*A*], cell ml^{-1}) and retention rate of *P. margaritifera* (I_R, cell h^{-1} $individual^{-1}$) obtained at 30°C . Dots = observed values, curve = Holling type II functional response model.

Simulations

Ahe

Variations of temperature, of chl *a* > 2 µm, of observed and simulated flesh dry weights are presented all together in Figure 18. Half saturation coefficient (X_K) value of chl *a* > 2 µm was adjusted at 0.42 µg chl *a* l^{-1}. Simulated dry weights were close to observed dry weights especially between day 1 and day 36, and between day 80 and day 108. The main spawning event which occurred between day 36 and day 53 was also well represented by the model as indicated by the loss of dry weight (Table 15).

Observed dry weights and the 30 simulated dry weights are presented in Figure 19. Despite the high dispersion of the initial quantity of energy contained in gonadal tissue (E_R), we can see in Figure 19b that spawning occurred for 63% of simulated individuals during the main Chl *a* > 2 µm peak (day 43 to day 60) and that 90% of the individuals spawned when Chl *a* > 2 µm was over 1 µg l^{-1}. Moreover, the minimum maturation period of these 30 simulations was of 60 days.

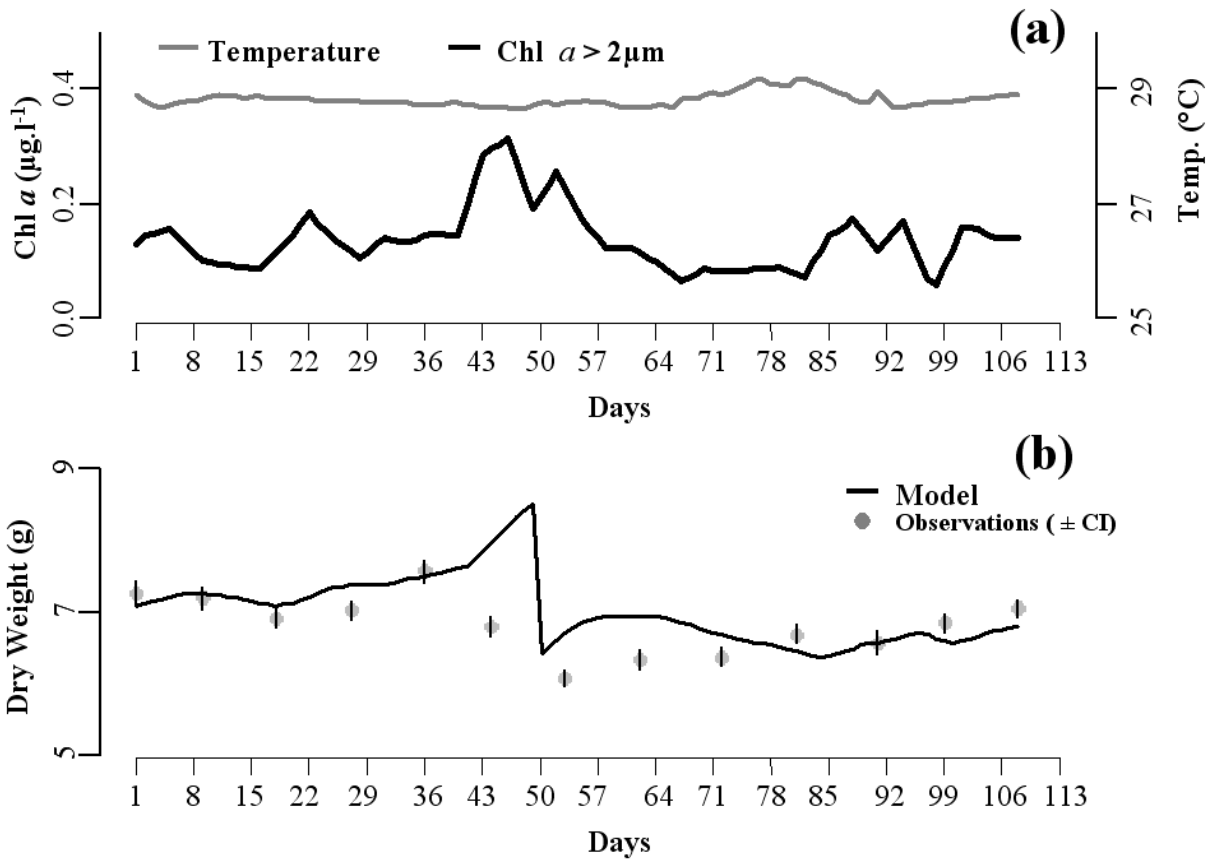

Figure 18 : Variations of (a) Temperature (°C) and Chl a > 2µm concentration (mg l^{-1}); and (b) dry weights of pearl oysters (in g) (observed mean ± CI = symbols; simulated = line) in Ahe atoll lagoon during 108 days.

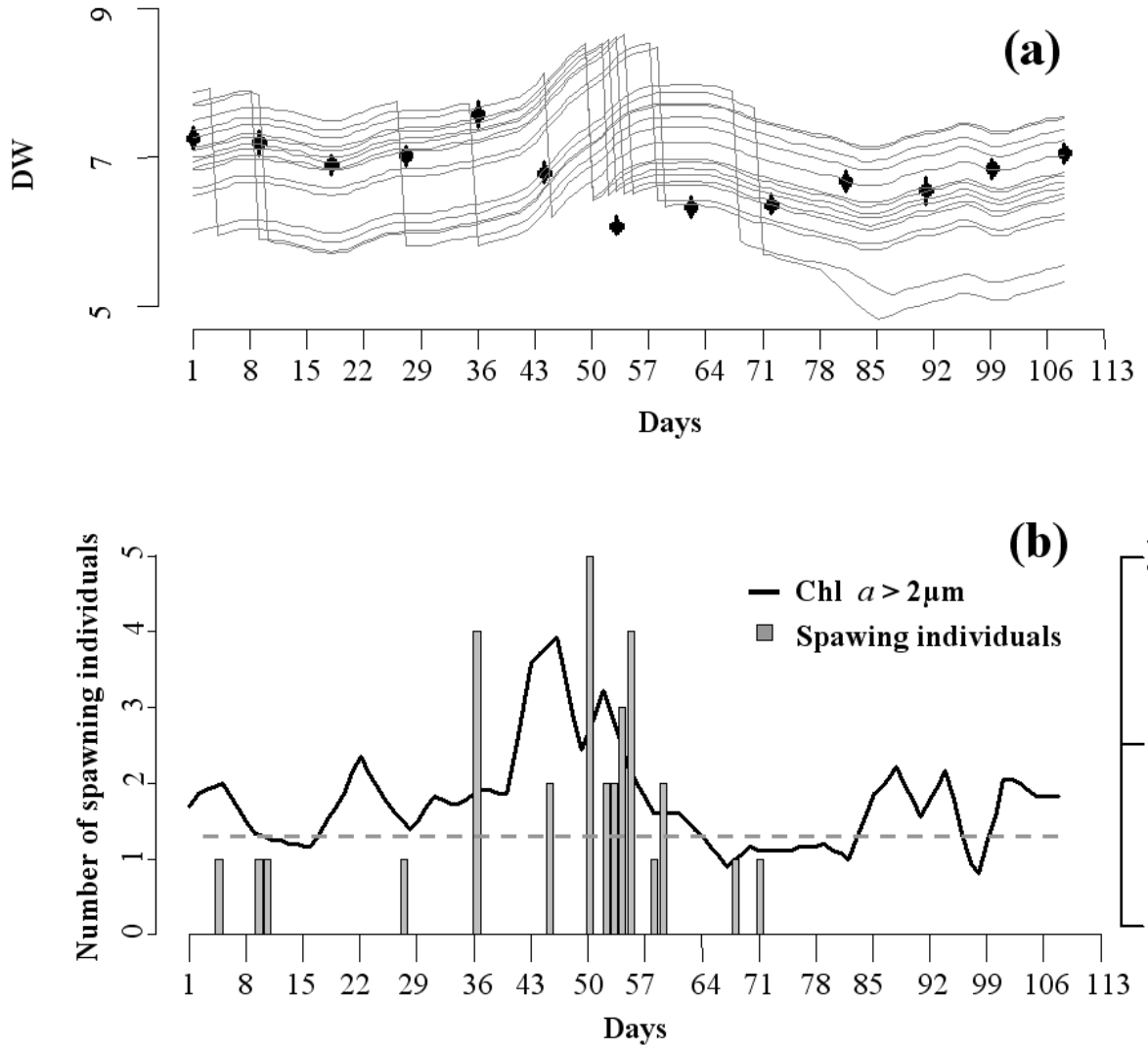

Figure 19 : (a) Observed (dots) and simulated (grey lines) pearl oysters dry weights (in g) in Ahe atoll lagoon during 108 days, (b) Absolute frequence of spwanings among the 30 simulated pearl oysters (grey bars) and Chl a > 2 µm (full line) in Ahe atoll lagoon.

Takapoto

Variation of temperature, POM, observed dry weights and simulated dry weights for the 3 age groups are presented in Figure 7. Half saturation coefficient (X_K) of POM was adjusted to 1.2 mg l^{-1} for age-group I, to 1.0 mg l^{-1} for age-group II and to 0.9 mg l^{-1} for age-group III.

Global growth of pearl oysters was relatively well represented by the model (Figure 20, Table 15). Nevertheless, the model predicts 16 spawning events over one year which corresponds to an average maturation period of 23 days and spawning periods observed in the data are not well

reproduced by the model.

Figure 21 presents the second simulation which was conducted with a κ set to 0.7 in order to improve simulation of reproductive effort of *P. margaritifera* in Takapoto atoll lagoon. Half saturation coefficient (X_K) of POM was therefore adjusted to 2.5 mg l^{-1} for age-group I, to 1.45 mg l^{-1} for age-group II and to 1.35 mg l^{-1} for age-group III. Global growth of pearl oysters was also well predicted by this modified model (Table 15). The model predicts 8 spawning events over one year which corresponds to an average maturation period of 46 days. In contrast with the previous version, spawning periods seem to be well reproduced for the 3 cohorts by this modified model.

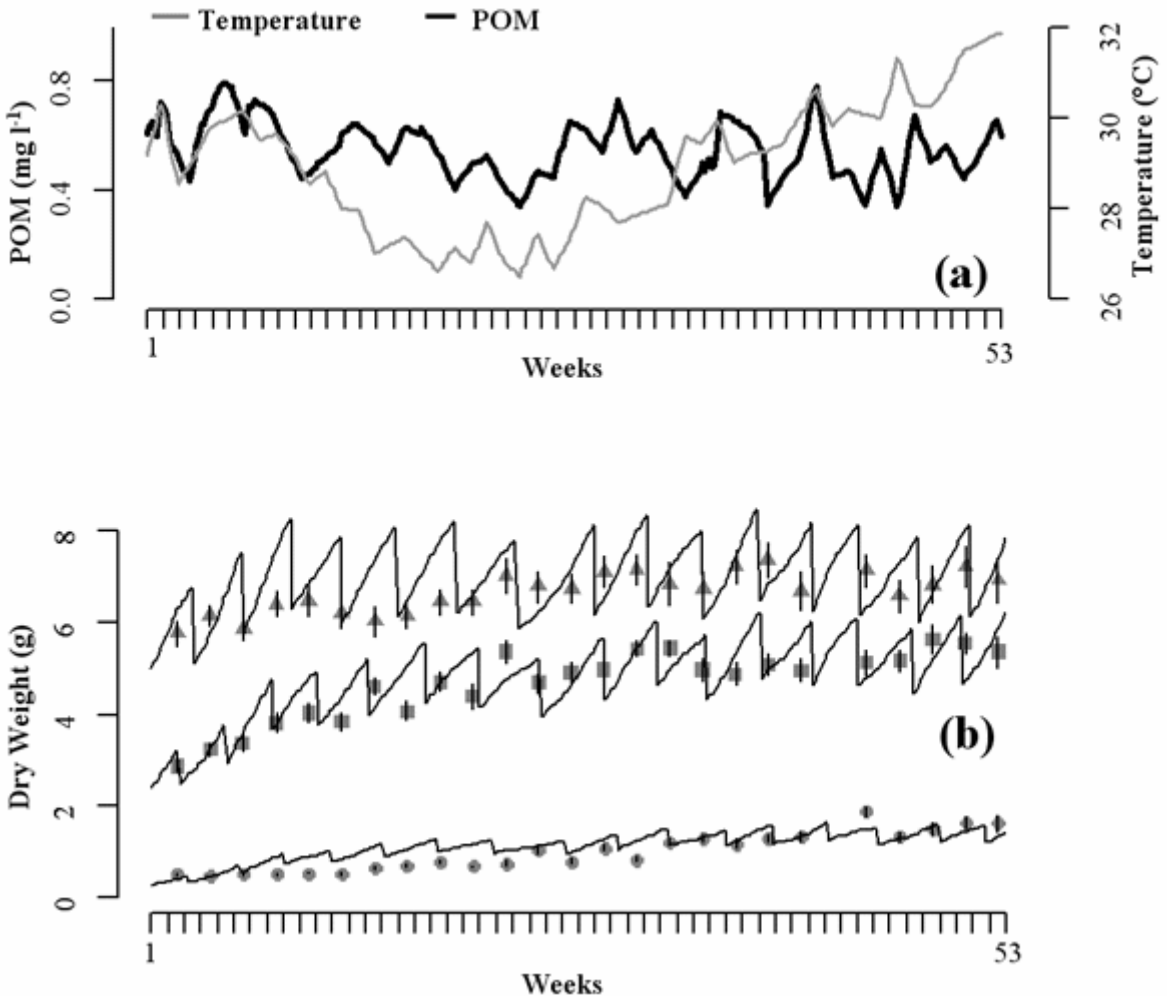

Figure 20 : Variation of (a) Temperature (°C) and POM concentration (mg l^{-1}); and (b) dry weights of 3 age groups of pearl oysters (in g) (observed mean ± CI = symbols; simulated = line) (observed = symbols; simulated = line) in Takpoto atoll lagoon during 365 days.

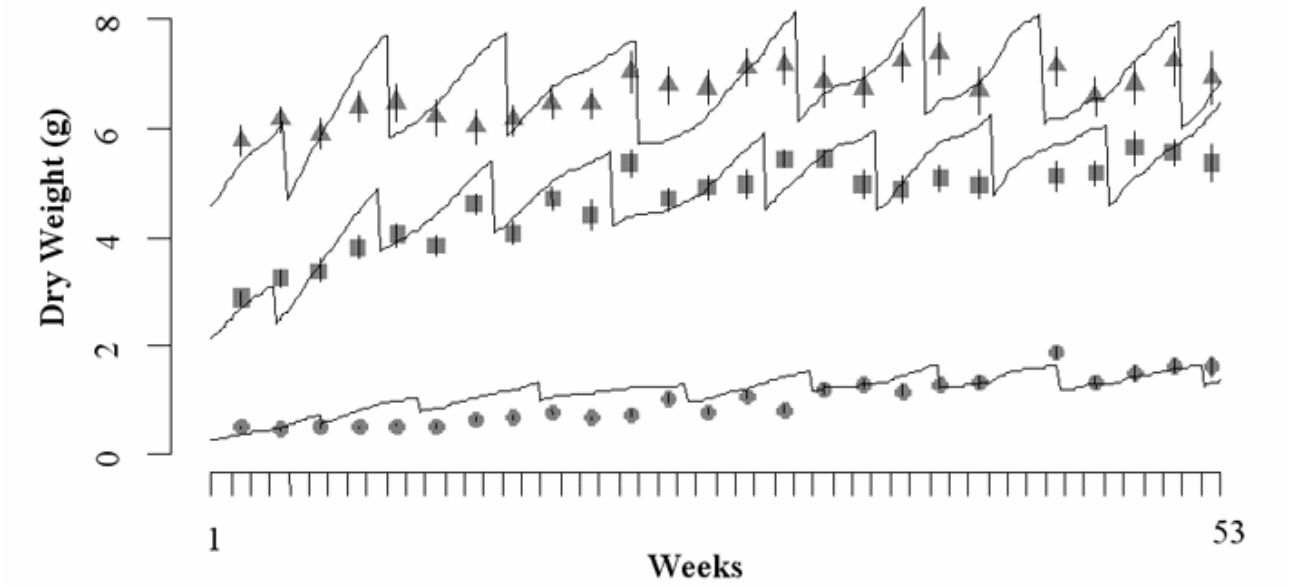

Figure 21 : Variation of dry weights of 3 age groups of pearl oysters (in g) (observed mean ± CI = symbols; simulated = line) (observed = symbols; simulated = line) in Takpoto atoll lagoon during 365 days, with a modified partition coefficient (kappa = 0.7).

Table 15 : Correlation coefficient and associated p-value between simulated and observed dry weights.

	Spearman's Rho	p-value
Ahe	0.56	0.049
Takapoto	0,96	$<10^{-15}$
Takapoto (κ modified)	0,96	$<10^{-15}$

DISCUSSION

Parameters estimates

Estimating DEB parameters is a continuous process and even for bivalve species that have been widely studied such as *C. gigas* or *M. edulis*, values of DEB parameters are still under question (Van der Veer et al., 2006, Bernard et al., 2011, Saraiva et al., 2011). Thus, the set of parameters established in the present study should be considered as a first estimates of DEB parameters value for *P. margaritifera* and should be re-investigated in future studies.

However, it is remarkable how the standardized oxygen consumption rates and ammonium

excretion rate of *P. margaritifera* measured by Yukihira et al. (2000) between 19°C and 32°C fit well with the average curve of Arrhenius temperature established by Van der Veer at al. (2006) for temperate bivalves.

As no data were available on the temperature tolerance range of *P. margaritifera*, we did not add any boundaries to the Arrhenius relationship. This was not a limiting factor in our simulations since the forcing temperature measured in Ahe and Takapoto atoll lagoons didn't exceed the range of temperature tested by Yukihira et al. (2000).

Previous studies have demonstrated that temperature had a specific effect on filtration rates of bivalves (Ren et al., 2000; Hawkins et al., 2002; Le Moullac, 2008). Accordingly, in the DEB model of *C. gigas,* a specific Arrhenius correction was applied to $\{\dot{p}_{Xm}\}$ (Bourlès et al., 2009; Bernard et al., 2011). In order to be in accordance with the DEB theory, which states that within the optimal temperature tolerance range all physiological processes are affected in the same way by temperature (Kooijman, 2000), we did not apply a specific Arrhenius correction to $\{\dot{p}_{Xm}\}$ in the present work.

However, experiments are currently taking place at the Ifremer center of Tahiti to measure the specific influence of temperature on ingestion rates. The results of these experiments should therefore be taken into account for further improvements of the pearl oyster DEB model.

Our estimates of the $\{\dot{P}_{Am}\}$ for *P. margaritifera* from our ingestion experiments (795 J cm^{-2} d^{-1} at 23°C) was close to the values reported for *C. gigas* by Ren & Scheil (2008) (550 J cm^{-2} d^{-1} at 18°C) and by Bernard et al. (2011) (770 at 20°C) but was higher than the values reported for other temperate species of bivalves by Van der Veer et al (2006) (from 33 J cm^{-2} d^{-1} to 420 J cm^{-2} d^{-1} at 20°C).

This high value of $\{\dot{P}_{Am}\}$ confirms the high clearance rates of pearl oysters which allow them to ingest sufficient quantities of energy, even under the low seston load conditions that characterizes Tuamotu atoll lagoons.

The shape parameter was estimated from more than 3000 values of shell height and wet weight of pearl oysters, which originated from the Tuamotu archipelago and from the Gambier archipelago. Thus, our estimates of the shape parameter should be considered as a reliable value for *P. margaritifera*.

The lack of physiological data sets didn't allow us to estimate directly $[\dot{P}_M]$ and $[E_m]$ for *P. margaritifera*. Nonetheless, DEB theory states that related species should display similar values of $[\dot{p}_M]$ and ν. We therefore relied on the latest estimations of $[\dot{p}_M]$ and ν for *C. gigas*, and we used these values to compute $[E_m]$ and κ from the appropriate relationships (eq. n°11 and n°12).

The DEB theory states that organisms reach their infinite length when environmental conditions allow maximal ingestion constantly, i.e. when functional response is constantly equal to one. Maximum size of *P. margaritifera* observed in atolls of French Polynesia and of Cook Islands were of 26 cm and 25 cm, respectively (Sims, 1992; Domard, 1962). From the estimates of Von Bertalanffy parameters the infinite length L_{inf} was estimated at 19 cm on the island of Tahiti by Pouvreau et al. (2001) and at 31 cm in the Cook island by Gervis & Sims (1992). We therefore assumed a L_m of 35 cm in order to calculate the value of κ for *P. margaritifera* (eq. n°12).

In simulations conducted in Takapoto atoll lagoon, the functional response never exceeded 0.55 and the maximum length observed in this atoll was of 18 cm (Zanini & Salvat, 2000). These results are additional evidence that support a L_m of more than 30 cm for the pearl oysters in an environment presenting optimal concentration and composition of plantkon.

Simulations

Ahe

The goodness of fit between simulated and observed dry weight in Ahe lagoon shows that this first pearl oyster model predicts correctly growth and reproduction of *P. margaritifera.*

In atoll lagoons of Tuamotu archipelago, reproduction of *P. margaritifera* is characterized by continuous and fast gametogenesis which lead to frequent asynchronous spawning all year long. Moreover, Pouvreau et al. (2000) indicated that the spawning intensity was not constant through the year and that major gametes releases were generally observed during the warmer season. The variation of temperature is rather low in atoll lagoons of French Polynesia and it is generally not considered as a limiting or a triggering factors for spawning. (Pouvreau et al, 2000; Le Moullac et al., 2011).

The high dispersion of gonadic indexes in pearl oysters populations and the frequent asynchronous spawning suggest that a model simulating growth and reproduction for a single "average" individual is not optimal to predict the variations of the average dry weight of the population. In order to overcome this problem, our approach was to run 30 individual simulations which differed only by their initial reproductive buffer (E_R) values, which were set according to the gonadic index distribution observed at the beginning of the experiment.

The results of these 30 simulations confirm the conclusions of Fournier et al (2012) : (i) conditions of food and temperature are met for *P. margaritifera* to produce gametes continuously all year long; (ii) these gametes accumulate until the gonad's maximum storage capacity is reached, which leads to spawning. Thus, when plankton concentration increases, the amount of energy allocated to gametogenesis increases and the gonad's maximum storage capacity is reached faster, which increases the number of individuals spawning.

These 30 simulations also suggest that a population model would be more appropriate to simulate the dispersion of gonadic indexes in the pearl oyster population. Bacher and Gangnery (2006) showed that a population model of the type "Individual Based Model" (IBM) can be coupled with a DEB model to simulate efficiently the individual growth variability of *Crassostrea gigas*. Similarly, we suggest that the coupling of and IBM population model with the pearl oyster DEB model would be an efficient tool to assess the great dispersion of gonadic indexes of pearl oysters.

Finally, the goodness of fit between simulated and observed dry weights on Ahe atoll lagoon shows that Chl *a* > 2µm is an appropriate food forcing variable for the pearl oyster DEB model and demonstrates that the variability of Chl *a* > 2µm is a relevant indicator of the variability of food availability for pearl oysters.

This is in total accordance with Fournier et al. (2012 a, b) (i) who demonstrated that Chl *a* > 2µm concentration was correlated with microplankton concentration (ii) who recorded simultaneously extremely high concentration of Chl *a* > 2µm and of nanoflagellates (iii) who showed that nanoflagellates and microplankton are the main carbon sources for pearl oysters and (iv) who measured a significant relationship between the reproductive intensity of pearl oysters and the concentration of Chl *a* > 2µm.

Takapoto

Growth was well captured by the pearl oyster DEB model in Takapoto lagoon. However, reproduction effort was not simulated accurately. Indeed, the maturation length predicted by the model was of 23 days which is much shorter than the maturation length predicted by our DEB model in Ahe lagoon (60 days) and by the SFG model in Takapoto lagoon (30 to 60 days) (Pouvreau, 1999).

POM contains a large diversity of planktonic particles which aren't digested equally by pearl oysters (Loret et al., 2000). Moreover, the proportion of the different types of particles are highly variable at short time scales (Sournia & Ricard, 1976; Buestel & Pouvreau, 1999, Thomas et al., 2009, Fournier et al, 2012). POM quality is therefore highly variable and the adjustment of the X_K is no more adapted to adjust the energy intake of the model. We suggest that the use of POM concentration as the food forcing variable in the pearl oyster DEB model leads to the poor simulation of reproduction effort in Takapoto lagoon.

Simulations conducted with an arbitrary κ set to 0.7 increased the maturation length to 46

days which better fits with the values obtained in Ahe lagoon with this DEB model or in Takapoto lagoon with the SFG model. These results clearly demonstrated the ability of the pearl oyster DEB model to simulate correctly both growth and reproduction effort. However this modification of κ was entirely empirical and does not respect the basic rules of the DEB theory.

Additional data sets integrating a simultaneous monitoring of growth, reproduction and food availability during several years (or sites) are needed to calibrate more precisely the DEB parameters (Saraiva et al., 2011; van der Meer et al., 2006).

Comparison of DEB and SFG for *P. margaritifera*

The data set obtained in Takapoto atoll lagoon was also used by Pouvreau et al. (2000) to validate their bio-energetic model of growth and reproduction, based on the scope for growth concept (SFG). These two models gave similar growth predictions for the 3 age groups in Takapoto atoll lagoon ($R^2 = 0.99$ for the SFG model and rho = 0.96 for the DEB model).

Nevertheless, the pearl oyster SFG model presents two main drawbacks : (i) it's a static model, which limits its ability to quantify the effects of a constantly varying environment on energy stocks and flows and (ii) it has 41 parameters (while the pearl oyster DEB model has only 17 parameters).

Our results show that, compared to the pearl oyster SFG model, this first pearl oyster DEB model is much more efficient in simulating growth and reproduction of pearl oysters and should therefore be used preferentially in further studies on pearl oyster bio-energetics.

CONCLUSION

Estimates of DEB parameters for *P. margaritifera* allowed us to simulate correctly its growth and reproduction and to demonstrate clearly the influence of plankton concentration on maturation and spawning synchronization of pearl oysters in atoll lagoons of French Polynesia. Nevertheless,

in order to confirm and optimize the values of DEB parameters, this first version of the pearl oyster model still needs to be validated with other data-sets. Finally, considering the DEB model of pearl oyster larval growth and the hydrodynamic model of larval dispersal established by Thomas et al. (2012); our DEB model of adults growth and reproduction constitute a promising complementary tool for the comprehensive description of spat collection variability in atoll lagoons of French Polynesia.

SYNTHÉSE ET PERSPECTIVES

SYNTHESE

Variation temporelle de l'abondance des populations planctoniques

Les lagons d'atolls sont généralement considérés comme des écosystèmes stables et homogènes du fait des faibles variations saisonnières et des faibles concentrations en chlorophylle *a* et en nutriments.

Cependant, de nombreux travaux font état de variations spatio-temporelles de l'abondance des communautés planctoniques à petite échelle de temps. L'origine de ces variations est mal connue et a parfois été attribuée à la remise en suspension de nutriments ou de matière particulaire d'origine benthique dans les atolls peu profonds.

Dans le lagon d'Ahe, nous avons mesuré la concentration en pico-, nano- et microplancton de façon ponctuelle entre 2008 et 2009 (lors des mesures de taux de filtration des huîtres perlières) puis en continu entre février et juin 2009 lors du suivi de la reproduction des huîtres perlières.

Le microplancton était largement dominé par les dinoflagellés (en moyenne 2 x 10^4 cell l^{-1}) mais nous avons aussi mesuré un pic de diatomées dont la concentration a atteint un maximum de 6 x 10^5 cell l^{-1} au mois d'Avril 2009.

Le picophytoplancton représentait entre 60% et 80 % du phytoplancton total mais nous avons également pu observer des pics de nano-microphytoplancton au cours desquels la concentration en picophytoplancton ne représentait plus que 30% du phytoplancton total.

Au cours du mois d'Octobre 2008, nous avons pu observer des concentrations maximales en chlorophylle *a* (>1.5 µg l^{-1}) et de nanoflagellés (>8 x 10^7 cell l^{-1}) largement supérieures aux concentrations maximales mesurées précédemment dans le lagon d'Ahe ou dans les lagons des autres atolls de Polynésie Française.

Ces résultats montrent que globalement (1) le lagon d'Ahe présente une concentration moyenne en plancton proche des valeurs hautes mesurées sur d'autres atolls de Polynésie Française et (2) qu'à petite échelle de temps, la structure de l'écosystème planctonique peut présenter des variations importantes.

Enfin, nous avons pu clairement établir une corrélation positive entre la force du vent et la concentration en plancton. Actuellement, les données dont nous disposons ne nous permettent pas de fournir une explication définitive à cette relation.

Cependant, des études récentes traitant de l'hydrodynamisme du lagon d'Ahe ont montré que lorsque le vent est suffisamment fort, il entraîne la formation (i) d'un courant de surface se déplaçant dans la direction du vent, (ii) d'un courant de fond compensatoire se déplaçant dans la direction opposée à celle du vent et (iii) d'un courant ascendant provoquant la remontée des couches d'eau inférieures vers la surface le long des côtes situées au vent (Figure 22).

Nous suggérons donc que les pics de concentration en plancton mesurés sur notre site d'étude (situé au nord est du lagon d'Ahe) étaient provoqués par la remontée des couches d'eau profondes elles même provoquée par des vents d'Est soutenus.

Lors de la mise en place de ces « *micro-upwellings »,* plusieurs hypothèses sont susceptibles d'expliquer les variations d'abondance et de composition du plancton que nous avons observé : (i) la sédimentation et la reminéralisation de la matière organique entraîneraient l'enrichissement en matière organique, en azote et en phosphore de la couche d'eau inférieure; et l'apport de ces nutriments à la couche d'eau supérieure soutiendrait une augmentation de la biomasse planctonique; (ii) la remontée des populations phytoplanctoniques (qui serait en concentration plus importante près du fond) et leur développement rapide dans les conditions lumineuses plus favorables à la surface.

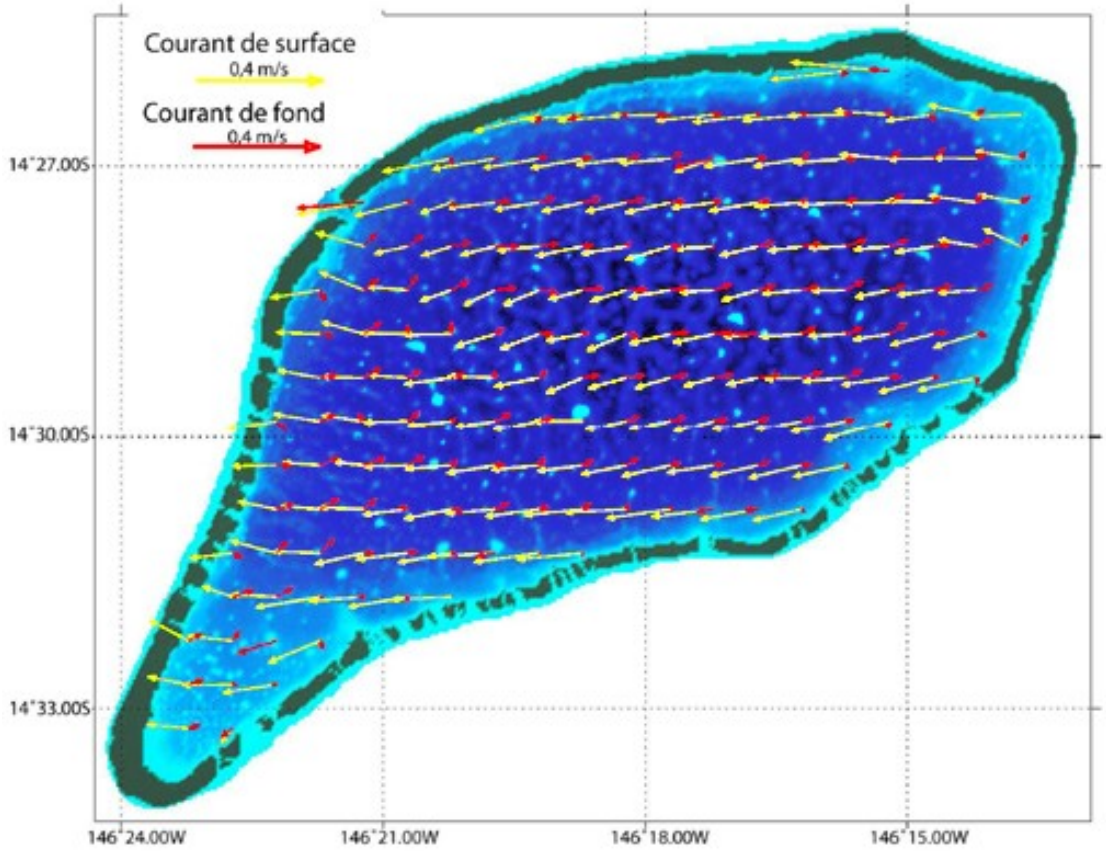

Figure 22 : Visualisation du courant de surface et de fond par vent d'est soutenu (10 m/s) sur l'atoll d'Ahe. (D'arpès, Legendre et al., com pers)

Nutrition des huîtres perlières

Comme en témoigne la littérature prolifique sur le sujet, mesurer le *clearance rates* des bivalves est une opération délicate qui requiert une grande attention quant au choix des méthodes expérimentales, à leur mise en oeuvre et à l'analyse des résultats obtenus. Les enceintes closes, couramment utilisées pour mesurer les *clearance rates*, présentent de nombreux désavantages et nous avons donc choisi d'utiliser des enceintes de filtration à flux ouvert qui nous ont permis : (i) de maintenir en permanence des conditions hydrobiologiques identiques à celles du lagon, (ii) d'éviter de manipuler et de stresser les animaux juste avant les expérimentations, (iii) de ne pas être limité par le volume d'eau lors des prélèvements et (iv) de réaliser de nombreux répliquas temporels.

Ces expérimentations nous ont permis de confirmer les fortes capacité de filtration des huîtres

perlières ainsi que la forte relation entre leur *clearance rates* et le biovolume du plancton. Cependant, nous n'avons pas mesuré d'influence de la concentration en plancton sur les *clearance rates* : malgré les variations importantes de concentration en phytoplancton > 2 µm et en phytoplancton < 2 µm, leur *clearance rates* respectifs sont restés stables entre les 3 campagnes de mesure.

Le calcul des taux de rétention de carbone nous a permis d'estimer la proportion que représente chaque communauté planctonique dans le régime alimentaire des huîtres perlières.

Au cours des trois campagnes, les concentrations en phytoplancton > 2µm et en phytoplancton < 2µm ont fortement variés. La proportion de ces deux communautés dans le régime alimentaire des huîtres perlières a donc varié en conséquence.

Parmi les sources potentielles de nourriture étudiées, les nanoflagellés constituaient la source de carbone majoritaire pour les huîtres perlières tandis que les dinoflagellés et les ciliés représentaient une source de carbone de moindre importance et que le picoplancton ne représentait pas une source de carbone significative.

Cependant, nos résultats montrent que le plancton > 2µm est la source de nourriture dominante pour les huîtres perlières, les variations de concentration et de composition du plancton entraînent clairement des fluctuations dans les quantités de carbone retenu ainsi que de la source de ce carbone retenu.

Reproduction

Les travaux réalisés au cours des années 1990 sur la croissance et la reproduction des huîtres perlières laissaient présager un fort déterminisme trophique de la reproduction des huîtres perlières. Afin de mesurer directement l'impact des variations rapides de la concentration et de la composition du plancton sur la reproduction des huîtres perlières, nous avons donc privilégié une stratégie d'échantillonnage caractérisée par un pas de temps resserré (10 jours pour les huîtres perlières et 3

jours pour le plancton), par un nombre important d'individus (80 huîtres perlières par échantillonnage) ainsi que par une description plus précise des ressources planctoniques (phytoplancton > 2µm et < 2µm, comptages de dinoflagellés, de diatomée, et de ciliés).

Ce suivi, réalisé entre les mois de Février et Juin 2009, nous a permis d'observer une période d'émission massive de gamètes correspondant à la ponte de 80% des individus de la population sur une période de trois semaines (en Mars/Avril 2009). Simultanément à cette ponte intense nous avons également pu observer un pic de concentration en chl *a* > 2 µm ainsi que la succession d'un pic de concentration en dinoflagellés et d'un pic de concentration en diatomées.

Sur la période de quatre mois qu'a duré le suivi de la reproduction, nous avons pu clairement établir que l'intensité de la reproduction (maturation ou pontes) était corrélée aux variations de concentration en chl *a* > 2µm. De plus, les périodes de faible concentration en chl *a* > 2µm ne se sont pas traduites par la disparition des cellules goniales chez les mâles contrairement à ce qui a pu être observé lors de conditionnements expérimentaux réalisés avec de faibles concentrations en algues.

L'ensemble de ces résultats nous a permis d'établir les spécificités suivantes sur la reproduction des huîtres perlières dans les lagons d'atolls polynésiens : (i) malgré les variations importantes de concentration en plancton, les huîtres perlières trouvent suffisamment d'énergie pour assurer en permanence la production des gamètes qui s'accumulent au fur et à mesure de leur production dans la gonade, (ii) lorsque la capacité de stockage maximale de la gonade est atteinte, cela provoque la ponte et (iii) lorsque la concentration en nano-microphytoplancton augmente, le taux de production des gamètes est plus élevé, la capacité de stockage maximale de la gonade est atteinte plus rapidement, ce qui provoque un nombre plus élevé de pontes dans la population des huîtres perlières.

Ce travail nous a donc permis d'établir clairement que la concentration en plancton est le

facteur principal responsable de la synchronisation des pontes des huîtres perlières *P. margaritifera* dans les atolls de Polynésie française.

Enfin, cette étude nous a également permis de comparer les différents indicateurs utilisés pour mesurer le statut des gonades. L'histologie nous a permis de confirmer que les pertes de poids sec ou que les baisses d'indices gonadiques correspondaient bien à des pontes et nous a également permis de mesurer l'intégrité des lignées goniales mâles. Cependant, même si le poids sec des masses gonado-viscérales est suffisant pour détecter les émissions massives de gamètes, nos résultats montrent qu'il s'agit un indicateur beaucoup moins précis que l'indice gonadique (Figure 23).

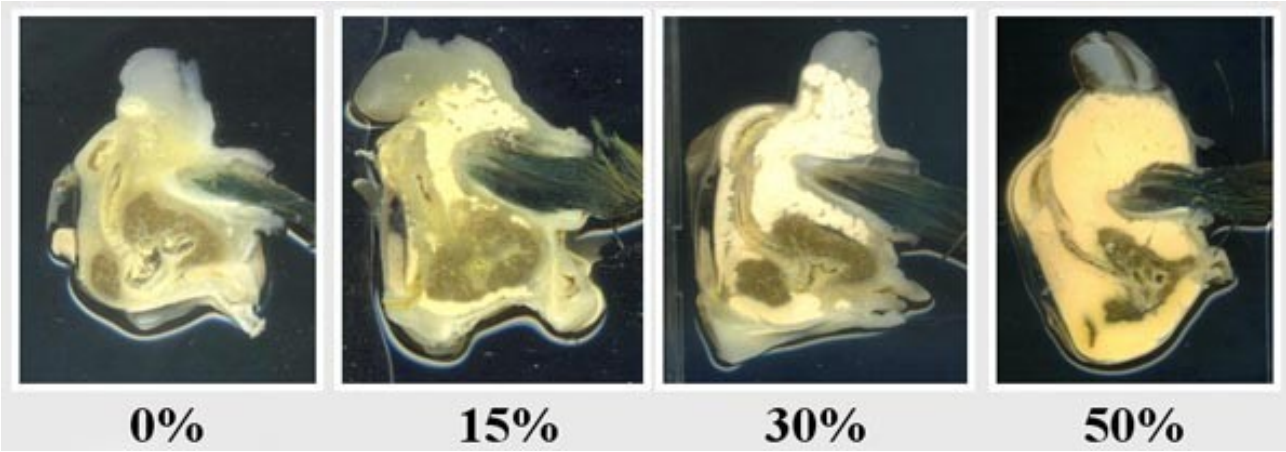

Figure 23 : Mesure de l'indice gonadique à partir de coupe sagittale de masse gonado-viscérales d'huîtres perlières.

Modélisation

Les valeurs des paramètres spécifiques à l'huître perlière ont été calculées en suivant les principes théoriques de la modélisation DEB et en combinant nos propres jeux de données avec les données disponibles dans la littérature.

Cette première estimation des paramètres DEB pour l'huître perlière nous a permis de simuler efficacement la reproduction des huîtres perlières sur Ahe et nous a permis également de confirmer

les conclusions du chapitre 2 sur le cycle de reproduction des huîtres perlières : lorsque la concentration en plancton augmente, la quantité d'énergie allouée à la reproduction augmente, la capacité maximum de stockage des gonades est atteinte plus rapidement et la proportion d'individus émettant leurs gamètes augmente également dans la population. De plus, ces résultats montrent que la concentration en chl $a > 2\mu m$ est un indicateur pertinent des concentrations en plancton « utile » pour les huîtres perlières.

Ces résultats montrent également qu'un modèle individuel moyen n'est pas optimal pour simuler la forte dispersion des indices gonadiques, l'asynchronisme global des pontes et les phénomènes ponctuels de synchronisation des pontes, qui caractérisent les populations d'huîtres perlières. Nous suggérons (i) que ce modèle DEB soit intégré à un modèle populationnel de type IBM (Individual Based Model) et (ii) que de la variabilité individuelle soit induite par un effet aléatoire affectant la valeur du paramètre X_K.

Sur Takapoto, la croissance globale des trois cohortes d'huîtres perlières est correctement simulée par le modèle mais l'effort de reproduction des huîtres perlières est largement surestimé. La MOP inclus une grande diversité de communautés planctoniques et de matière détritique, dont les proportions relatives varient en permanence. Nous avons donc supposé que la MOP était un indicateur trophique de mauvaise qualité qui ne permettait pas au modèle de simuler correctement la quantité d'énergie absorbée par les huîtres perlières.

Indicateur trophique de la concentration en nourriture pour les huîtres perlières.

Le régime alimentaire des huîtres perlières est principalement composé de nanoplancton et de microplancton. A l'heure actuelle, seuls les comptages au microscope permettent à la fois de décrire la composition taxonomique du nano-microplancton et d'estimer l'abondance et le biovolume de ces particules. Malheureusement, le temps nécessaire à l'étude des échantillons d'eau au microscope est beaucoup trop important pour que ce type de mesure soit utilisé en routine dans les atolls de

Polynésie Française. La définition d'un indicateur trophique qui permettrait d'estimer plus simplement et plus rapidement la quantité de nourriture disponible pour les huîtres perlières est donc indispensable pour les prochaines études sur l'écophysiologie de la croissance et de la reproduction des huîtres perlières.

D'après nos résultats, la concentration en chl *a* > 2µm semble être l'indicateur trophique le plus pertinent : (i) il est relativement simple à mesurer, (ii) sa concentration est corrélée à la concentration en particule microplanctonique (dinoflagellés + diatomées), (iii) la dynamique de la gamétogénèse et la synchronisation des pontes sont largement liées aux variations de sa concentration et (iv) son utilisation comme variable forçante du modèle DEB nous permet d'obtenir des simulations proches de la réalité.

Enfin, d'après les variations rapides de la concentration en chl *a* > 2µm et la durée des pics de concentration en plancton, de plancton observé sur Ahe, il nous semble nécessaire de faire ces mesures avec un pas de temps de 3 jours au maximum pour être sûr de ne pas passer à côté d'un pic de nourriture important pour les huîtres perlières.

PERSPECTIVES

Ces travaux ont permis de répondre aux questions posées concernant le régime alimentaire et le déterminisme environnemental de la reproduction des huîtres perlières. Toutefois, il reste encore de nombreuses inconnues aussi bien dans le domaine de la nutrition des huîtres perlières que dans l'écophysiologie de la reproduction ou dans l'estimation et la validation des paramètres DEB et du modèle.

Communautés planctoniques et indicateur trophique

Bien que l'étude des communautés planctonique ne soit pas le thème principal de ce travail, les variations de concentration en plancton ont une importance majeure dans toutes les problématiques que nous avons abordées : la nutrition, la reproduction et la modélisation.

Nous avons mesuré des concentrations en plancton relativement variables et parfois très élevées en comparaison des valeurs indiquées dans la littérature. Nous suggérons les variations du régimes des vents et leurs conséquences sur l'hydrodynamisme du lagon sont en grande partie responsables de ces variations de concentration en plancton. Cependant, des travaux supplémentaires sont nécessaires pour confirmer ces hypothèses. Le modèle hydrodynamique de circulation des masses d'eau dans le lagon d'Ahe établi par Legendre et al. (com pers) permettra certainement d'apporter un début de réponse à cette problématique.

La concentration en chl *a* > 2µm semble effectivement être un indicateur trophique pertinent de la concentration en nourriture disponible pour les huîtres perlières. Cependant, nous n'avons pas mesuré les concentrations en nanoflagellés durant le suivi de la reproduction *in situ* et nous n'avons donc pas d'informations concernant la corrélation éventuelle entre l'abondance en nanoflagellés et la concentration en chl *a* > 2µm. Des mesures simultanées de concentration en nanoflagellés et en chl

a > 2µm sont donc nécessaires pour valider définitivement cet indicateur trophique.

Nutrition

Lorsque les *clearance rates* des huîtres perlières ont été mesurés, les concentrations en diatomées étaient trop faibles pour pouvoir quantifier leur filtration par les huîtres perlières. Cependant, leur importance dans le régime alimentaire des huîtres perlières n'est pas à négliger. En effet, la taille des diatomées (comprises entre 25 et 30 µm pour les espèces mesurées par Loret, 1999 dans le lagon de Takapoto) et leur concentration parfois élevée suggèrent qu'elles sont susceptibles d'avoir un rôle significatif dans le régime alimentaire des huîtres perlières.

Les coccolithophoracées représentent également une source de nourriture potentielle non négligeable pour les huîtres perlière : elles font partie du petit microplankton et leur concentration est relativement proche de celle des dinoflagellés dans les lagons d'atolls (Sournia et Ricard, 1976; Lefèbvre, Com. Pers). Cependant, le lugol ne permet pas de les fixer efficacement et nous n'avons donc pas pu mesurer leur consommation par les huîtres perlières.

Nous estimons donc que le régime alimentaire moyen des huîtres perlières est constitué d'environ 40 à 50% de microplancton (coccolithophoracées +diatomées + dinoflagellés + cilliés), d'environ 40 à 50% de nanoflagellés et de 5 à 10% de picoplancton.

Des expériences complémentaires sont donc nécessaires afin de mesurer les *clearance rates* et les biovolumes moyens des populations de diatomées et de coccolithophoracées du lagon d'Ahe afin de quantifier leur importance dans le régime alimentaire des huîtres perlières.

Lors de nos expérimentations, nous avons mesuré les *clearance rates*, ce qui nous a permis de quantifier la quantité de particules filtrées par les huîtres perlières. Or ces particules filtrées sont ensuite triées par les palpes labiaux et celles qui sont ingérées ne sont pas toutes assimilées avec la

même efficacité. Des travaux complémentaires sont donc indispensables pour quantifier *in situ* les taux d'ingestion et d'assimilation des huîtres perlières et pour évaluer plus précisément la contribution réelle des particules filtrées au régime alimentaire des huîtres perlières.

La biomasse du microzooplancton > 35µm (larves de bivalves, larves de polychètes, nauplii, copépodes) représente une faible biomasse en comparaison du nanoplancton et du microplancton < 35µm (Charpy & Charpy-Roubaud, 1990). Cependant, lors du suivi de la reproduction, nous avons observé ponctuellement (1 échantillon de 40 huîtres) qu'une grande quantité de larves de bivalves (espèces non identifiées) avait été ingérée par les huîtres perlières.

Des travaux récents ont également montré que les larves de bivalves pouvaient effectivement être ingérées par les huîtres perlières (Marc Pagano, Com. Pers.). L'importance de ce compartiment planctonique dans le régime alimentaire doit absolument être précisé pour deux raisons : la première est que ce compartiment pourrait en effet constituer une source de nourriture ponctuelle mais significative pour les huîtres perlières, la seconde est que l'ingestion de grandes quantités de larves de bivalves par les huîtres perlières (et le cannibalisme éventuel que cela impliquerait) pourrait avoir des conséquences majeures sur les variations spatio-temporelles du collectage de naissain.

Reproduction

Lors du suivi de 4 mois sur l'atoll d'Ahe, nous avons pu montrer une forte influence des concentrations en microplancton et en chl *a* > 2µm sur la reproduction.

Cependant, les nanoflagellés constituent une fraction importante du régime alimentaire des huîtres perlières. Des travaux complémentaires sont donc nécessaires pour mesurer l'impact des variations de concentration de cette catégorie de plancton sur la croissance et la reproduction des huîtres perlières. *A minima* il est nécessaire de vérifier l'existence éventuelle d'une corrélation entre la concentration en nanoflagellés et la concentration en Chl *a* > 2µm.

Nous avons montré que la concentration en plancton est un élément déterminant dans la synchronisation des émissions de gamètes chez les huîtres perlières. Les pontes provoquées de manière artificielle au COP ont montré que les femelles pondaient uniquement lorsqu'un mâle avait émis ses gamètes. Les causes exactes de ce phénomène restent mal connues mais il est fortement probable qu'il joue un rôle dans la synchronisation des pontes entre mâles et femelles dans le milieu naturel.

Ainsi, lors de la ponte majeure observée au cours de notre suivi de la reproduction, nous avons constaté par histologie une proportion non négligeable de pontes partielles (20%) ainsi qu'une diminution significative de la taille moyenne des ovocytes. Il est probable que chez une certaine proportion de femelles n'ayant pas atteint leur stade de maturité maximale, la ponte ait pu être induite par l'émission des gamètes mâles dans le milieu.

L'asynchronisme des populations et la nécessité de disséquer les individus pour avoir une mesure fiable de l'indice gonadique sont des obstacles importants à la description précise de la dynamique de la reproduction au niveau de l'individu. En effet, nous utilisons des données décrivant l'évolution de la population pour estimer des caractéristiques individuelles (e.g temps nécessaires à la maturation, indice gonadique maximale, perte de poids sec lors de la ponte...). Une précision plus importante sur ces paramètres pourrait être obtenue soit (i) par le suivi d'une population d'huîtres perlières synchronisée artificiellement par la sélection préalable d'individus à un stade de maturité identique, soit (ii) par l'optimisation d'une méthode de mesure non destructrice de l'indice gonadique et par son utilisation au cours du suivi de la maturation des mêmes individus (IRM, photo *in vivo* de la gonade...).

Modélisation

Cette première estimation des paramètres DEB pour l'huître perlière nous a permis de simuler efficacement la reproduction des huîtres perlières et de confirmer la forte influence des

concentrations en phytoplancton > 2µm sur la gamétogenèse des huîtres perlières et sur la synchronisation des pontes.

L'estimation des paramètres DEB est un processus continu et même pour des espèces comme *C. gigas* ou *M. edulis* dont la physiologie et l'écophysiologie ont été le sujet de nombreux travaux, les valeurs des paramètres DEB ne sont pas encore fixées. Pour l'huître perlière,ces valeurs doivent donc être considérées comme une première estimation. Ces paramètres doivent être optimisées (i) par une estimation indirecte des flux physiologiques (mesures de respiration, de filtration ou de croissance d'huîtres perlières placées dans des conditions trophiques et à des température variables (e.g, van der Veer et al., 2006) et (ii) par la collecte *in situ* de séries temporelles de croissance, de reproduction, de concentration en nourriture et de température qui serviront à estimer directement les paramètres DEB par la méthode des « co-variations » récemment développée par Saraiva et al. (2011).

La Chl *a* > 2µm est un indicateur pertinent des concentrations en plancton utile pour les huîtres perlières. Cependant, comme l'a montré Bourlès et al.(2009) chez *C. gigas*, le modèle DEB est susceptible de donner de bien meilleurs résultats avec les concentrations totales en phytoplancton (exprimées en nombre de cellules ou en biovolume cellulaire) qu'avec des concentrations en chlorophylle *a* comme variable forçante. D'une façon générale, la qualité des indicateurs trophiques est primordiale pour réaliser des simulations qui s'approchent le plus possible des observations de croissance et de reproduction observées.

Nous suggérons que ce modèle DEB de l'huître perlière soit intégré dans un modèle populationnel de type IBM (Individual Based Model, *e.g.* Bacher et al. 2006) en y incluant une variabilité individuelle au niveau du paramètre X_K. (coefficient de demi-saturation). Ce type de modèle serait en effet beaucoup plus adapté pour simuler l'asynchronisme général de la maturité des huîtres perlières ainsi que les périodes de synchronisation des pontes.

Enfin, ce modèle de croissance et de reproduction des adultes est un outil prometteur pour la compréhension de la variabilité du captage des naissains d'huîtres perlières dans les lagons de Polynésie Française, particulièrement s'il est associé aux modèles de croissance et de dispersion des larves établis par Thomas (2009).

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TITRE : Alimentation et déterminisme environnemental de la reproduction des huîtres perlières *P. margaritifera* sur l'atoll d'Ahe (Archipel des Tuamotu, Polynésie Française)

Résumé

En Polynésie Française, la production des perles noires est entièrement basée sur la collecte naturelle de naissains de l'huître perlière *Pinctada margaritifera,* dont l'irrégularité peut constituer un obstacle majeur à la production ainsi qu'à la rentabilité des fermes perlières. Une meilleure compréhension des phénomènes responsables de la variabilité de la reproduction des huîtres perlières est donc déterminante pour mieux appréhender cette irrégularité du captage de naissains. L'objectif global de cette thèse était donc d'analyser les relations entre l'environnement et la reproduction des huîtres perlières en s'appuyant sur deux études réalisée *in situ* (une étude du régime alimentaire des huîtres perlières et un suivi de leur reproduction réalisés sur l'atoll d'Ahe) et sur la construction d'un modèle bio-énergétique de croissance et de reproduction de type DEB. Sur l'atoll d'Ahe, nous avons observé de fortes variations temporelles de la concentration et de la composition du plancton, principalement liées au régime des vents. Nos résultats montrent que ces variations de concentration en plancton ont une influence majeure sur le régime alimentaire des huîtres perlières, sur la gamétogénèse et sur la synchronisation des pontes dans la population. Lors des pics de concentration en plancton, nous observons une intensification la gamétogenèse ce qui réduit le temps nécessaire à la maturation des huîtres perlières et favorise la synchronisation des émissions de gamètes. La concordance entre les observations de terrain et les simulations du modèle DEB de l'huître perlière démontre le fort déterminisme trophique de la reproduction des huîtres perlières dans les atolls de Polynésie Française. Les résultats de ces expérimentations et les simulations du modèle de croissance et de reproduction nous permettent donc de mieux appréhender les facteurs environnementaux responsables de la reproduction des huîtres perlières, dont la variabilité est en partie responsable de l'irrégularité du captage de naissains.

Mots clés : Reproduction, Nutrition, Phytoplancton, Polynésie Française

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TITLE : Diet and environmental déterminism of reproduction of pearl oysters *P. margaritifera* on Ahe atoll lagoon (Tuamotu Archipelago, French Polynesia)

Abstract

While pearl culture industry represents one of the dominant business sector of French Polynesia, it still entirely relies on natural reproduction and spat collection. A better knowledge of reproduction determinism is thus of particular interest for the black lip pearl oyster *P. margaritifera*. This work is based (i) on *in situ* studies of the pearl oyster diet and of the environmental determinism of reproduction; and (ii) on the application to the black lip pearl oyster of a bio-energetic and deterministic model of growth and reproduction based on the Dynamic Energy Budget theory. In Ahe atoll lagoon, plankton concentration and composition fluctuations were strongly linked to wind regimes. These variations had a great impact on the diet of pearl oysters which was dominated by nano-microplankton (especially nano-flagellates and dinoflagellates). Peaks of plankton concentration were associated with an increased gametogenesis rate and were shown to enhance spawning synchronisation in the population. Eventually, estimates of DEB parameters for P. margaritifera allowed us to correctly simulate its growth and reproduction and to clearly demonstrate the influence of plankton concentration on maturation and spawning synchronization of pearl oysters in atoll lagoons of French Polynesia. These results provide relevant features for a better understanding of spat collection variability in French Polynesian atoll lagoons. Moreover, the pearl oyster DEB model of adults growth and reproduction constitute a promising complementary tool for the comprehensive description of spat collection variability in atoll lagoons of French Polynesia.

Keywords : Pearl oysters, Reproduction, Diet, Phytoplankton, French Polynesia