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DYNAMIQUE DES POPULATIONS DE PALOURDES JAPONAISES (*RUDITAPES PHILIPPINARUM*) DANS LE BASSIN D'ARCACHON CONSEQUENCES SUR LA GESTION DES POPULATIONS EXPLOITEES

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Résumé

La palourde japonaise *Ruditapes philippinarum* a été introduite au début des années 1980 dans le bassin d'Arcachon à des fins aquacoles. Elle s'est rapidement répandue dans toute la zone intertidale de la lagune, créant actuellement le premier gisement français en terme de stock exploitable. Cependant, de récentes évaluations du stock ont démontré une structure en taille déséquilibrée avec un déficit en juvéniles et en adultes de taille supérieure à 38 mm. Ce constat alarmant a posé des questions sur le devenir des populations de palourdes japonaises dans le bassin d'Arcachon et a motivé la présente thèse. Cette étude vise à mieux comprendre la dynamique de population de cette espèce et certains facteurs contrôlant cette dynamique. Le but *in fine* était d'améliorer la gestion du stock exploitable de cette espèce dans la lagune grâce à un modèle, incrémenté des différents résultats obtenus lors de cette étude. Les paramètres de croissance K et L_∞ du modèle de Von Bertalanffy ont été déterminés d'une part par un suivi régulier des populations naturelles, et d'autre part par une expérience de marquage-recapture d'une durée de deux ans. Les résultats montrent une croissance lente avec un K homogène dans tout le bassin et un L_∞ variable suivant le site. Cette étude met aussi en évidence une mortalité naturelle normale mais une reproduction déficiente (indices de condition bas, mauvais recrutement). Parmi les facteurs pouvant expliquer cette dynamique, différents pathogènes ont été suivis durant deux ans : les trématodes digènes, la maladie de l'anneau brun, la perkinsose. De plus, une pathologie émergente (maladie du muscle marron, BMD) a été découverte. Seules la perkinsose et la BMD ont révélé de fortes prévalences et intensités. La perkinsose entraîne des effets mitigés sur la croissance tandis que la BMD entraîne une remontée des palourdes en surface puis leur mort. Les ressources trophiques sont également importantes pour expliquer la croissance et ont été étudiées ici grâce aux isotopes stables de l'azote et du carbone. Cette étude a révélé une hétérogénéité des sources trophiques au sein de la lagune et une alimentation dominée par du phytoplancton. La proportion de phytoplancton ingérée a été corrélée à L_∞. Les paramètres de croissance et de mortalité ont été ensuite intégrés dans un modèle de gestion qui nous a permis de voir que si rien n'était fait en terme de gestion, le stock de palourdes continuerait de se dégrader. Ce modèle nous a permis de simuler différentes situations de gestion et suite aux résultats obtenus, un certain nombre de mesures a ensuite été adopté par les organismes gestionnaires.

Mots clés : *Ruditapes philippinarum*, dynamique de population, pathogènes, resources trophiques, gestion

Abstract

The Manila clam *Ruditapes philippinarum* was introduced into Arcachon Bay at the beginning of the 1980s for aquaculture purposes. It rapidly naturalized in all intertidal flats of the lagoon. Nowadays, Arcachon Bay ranks at the first French place in terms of exploitable stock. However, recent stock assessments have shown an unbalance size structure with a deficit in juvenile and adult clams (> 38 mm shell length). These alarming patterns asked many questions on the sustainability of Manila clam populations within Arcachon Bay and have motivated the present thesis. This study aimed to better understand the population dynamics of this species and also some factors controlling this dynamic. The final objective was to improve the Manila clam fishing management with a model raised with results of the study. Von Bertalanffy growth parameters (K and L_∞) were both determined by a field survey of populations and by a tagged-recapture experiment during two years. Growth appeared slow with a homogeneous K within the bay and different L_∞ according to the sites. This study also evidenced a normal natural mortality and an inefficient reproduction (low condition index, low recruitment). Among the factors that could explain these dynamics, different pathogens were monitored during two years: digenean trematodes, brown ring disease and perkinsosis. Moreover, an emergent pathology (brown muscle disease, BMD) has been discovered. Only perkinsosis and BMD revealed high prevalences and intensities. Perkinsosis induced mitigated effects on growth whereas BMD-infected clams rise to the surface of the sediment and died. Trophic sources were also important to explain growth and were studied with carbon and nitrogen stable isotopes. This study displayed a heterogeneous repartition of Manila clam trophic sources within the bay and a phytoplankton dominated diet. The proportion of ingested phytoplankton was correlated with L_∞. Growth and mortality parameters were integrated in a management model. If no new management measures were taken, the clam stock will continue to decrease. Different management situations have been simulated and new measures have been adopted by administrator organisms.

Keywords: *Ruditapes philippinarum*, population dynamics, pathogens, trophic sources, management

SOMMAIRE

Sommaire

Chapitre 1

Introduction générale

Ruditapes philippinarum

1. Contexte de l'étude et problématique

La conchyliculture est à l'échelle mondiale une activité dont les enjeux économiques sont majeurs. La filière conchylicole française est notamment l'une des plus importantes au monde avec une production de 191 650 tonnes et un chiffre d'affaires de 398 millions d'euros pour 3 700 entreprises et près de 20 000 emplois. La France se place ainsi au cinquième rang mondial derrière la Chine, les USA, le Japon et la Corée. La première espèce de bivalve produite est l'huître japonaise *Crassostrea gigas*, suivie par la palourde japonaise *Ruditapes philippinarum* (Gosling 2004).

La production mondiale de la palourde japonaise s'élève à 3 138 514 tonnes. Parmi les sites français bénéficiant d'une étude de stock, le bassin d'Arcachon se situait jusqu'en 2008 à la première place française en terme de stock total (4457 t) et de stock exploitable (1159 t). Le bassin d'Arcachon a vu sa production déclarée augmenter de 390 t à 1030 t entre 2005 et 2008 (Fig. 1.1).

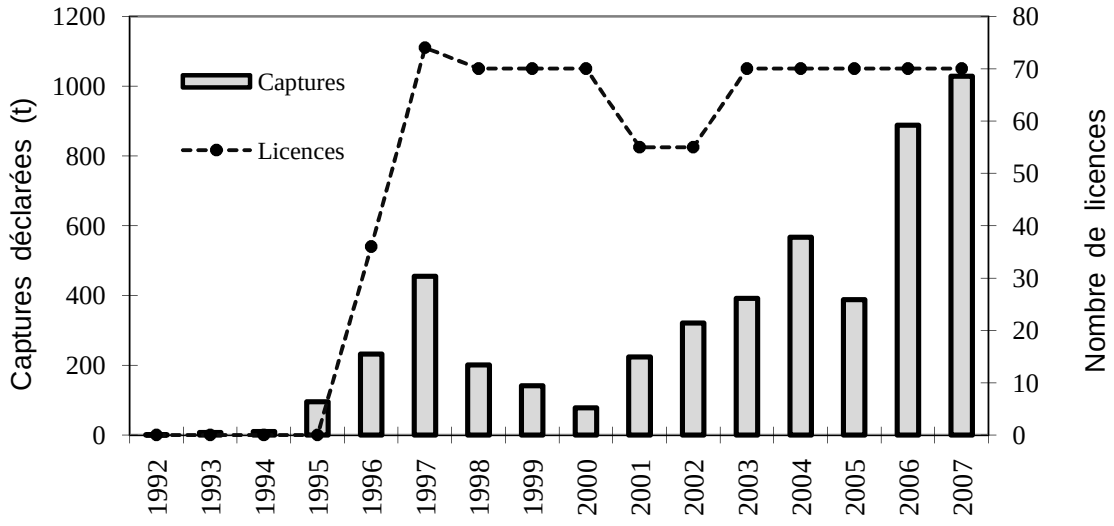

Figure 1.1 : Captures déclarées et nombre de licences attribuées aux professionnels entre 1992 et 2007 dans le bassin d'Arcachon (Caill-Milly et al. 2006).

La palourde japonaise *Ruditapes philippinarum* a été introduite en France en 1972 par la SATMAR (Société Atlantique de Mariculture) à des fins conchylicoles (Flassch et Leborgne 1992). La maîtrise de la production de naissain et des techniques de grossissement ainsi que la forte valeur marchande de cette espèce ont provoqué au début des années 80 un véritable engouement pour cette nouvelle culture. En effet, la forte capacité d'adaptation de la palourde japonaise à de nouveaux environnements ainsi que ses hautes performances de croissance ont entraîné une rapide installation et une prolifération de cette espèce sur la côte atlantique française. Elle fut introduite au début des années 1980 dans le bassin d'Arcachon, également à des fins aquacoles. De par le recrutement naturel, elle s'est rapidement propagée hors des zones d'élevage et établie dans toute la zone intertidale de la lagune. Une pêcherie s'est développée sur cette population néo-naturelle à partir du milieu des années 1990. Son activité est aujourd'hui importante en terme socio-économique puisqu'elle représente environ 1000 tonnes pour 70 licences de pêche attribuées (Fig. 1.1).

Dans l'objectif d'une meilleure gestion de l'activité, des campagnes d'estimation du stock de palourdes japonaises ont été réalisées en 2000, 2003, 2006 et 2008 (Bertignac et al. 2001, Caill-Milly et al. 2003, 2006, 2008). Ces campagnes ont mis en évidence que le bassin d'Arcachon possédait le plus grand stock total de palourdes japonaises parmi les sites bénéficiant d'une évaluation de stock, devant le golfe du Morbihan et l'étang de Thau. En revanche, la biomasse exploitable, estimée par exemple à 2730 t en 2003 était voisine de celle du golfe du Morbihan (Caill-Milly et al. 2003). Cependant, les distributions en taille sont caractérisées par des longueurs inférieures à celles des autres sites. Les structures en taille issues des campagnes d'évaluation de stock ainsi que d'autres études menées sur le bassin, démontrent une absence en juvéniles et un déficit d'adultes supérieurs à 38 mm (Blanchet 2004, Caill-Milly et al. 2006) (Fig. 1.2). Cela a engendré beaucoup d'inquiétudes de la part des professionnels étant donné que les palourdes ayant une taille réglementaire étaient quasi-absentes du bassin d'Arcachon (taille légale : 40 mm jusqu'à début 2008 puis 35 mm). De plus, la qualité selon les critères commerciaux des palourdes japonaises à Arcachon est inférieure (coquille érodée, faible poids de chair) à celle des autres sites français tels que le golfe du Morbihan. Tous ces problèmes ont stimulé des interrogations en termes de durabilité de l'exploitation des populations de palourdes dans le bassin d'Arcachon et ont souligné le manque d'étude de cette espèce dans son habitat naturel (herbiers intertidaux en majorité). La seule étude portant sur la croissance date de 1993 (Robert et al. 1993) et avait été réalisée dans des parcs d'élevage.

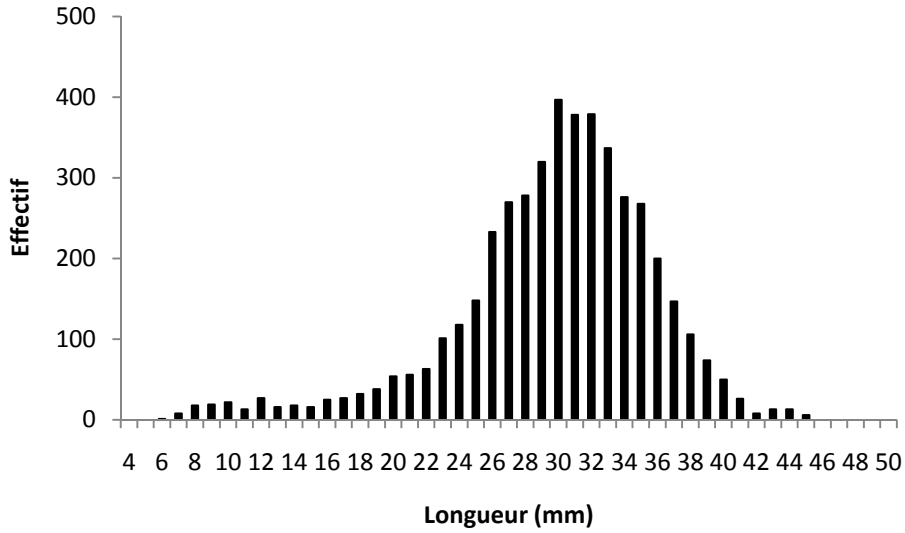

Figure 1.2 : Structure en taille de la population de palourdes dans le bassin d'Arcachon. Histogramme moyen issu de l'étude de stock de 2003 (Caill-Milly et al. 2003).

Devant le déséquilibre de la structure en taille de la population relativement inquiétant pour le devenir du stock, caractérisé par un faible recrutement de la palourde japonaise depuis 2001, la présente thèse a été mise en place. Les objectifs étaient d'étudier la dynamique de population des palourdes japonaises dans son ensemble ainsi que certains facteurs contrôlant cette dynamique. L'objectif final était de proposer un nouveau mode de gestion de la pêcherie pour assurer le maintien et la durabilité des populations de palourdes japonaises. Sur certains aspects liés aux pathologies, cette étude a été couplée, avec l'analyse d'une population d'une autre espèce de palourde, la palourde européenne *R. decussatus* sur un site espagnol, l'estuaire de Mundaka. Néanmoins, les enjeux étaient moins importants car ce site n'est pas exploité et le stock est de taille très réduite. Cette espèce apparaît uniquement dans les chapitres 3 et 5.

La thèse est divisée en trois grandes parties : I) l'analyse des paramètres de dynamique des populations qui correspond au chapitre 2 ; II) l'étude de certains facteurs contrôlant la dynamique des populations, les pathologies d'une part (chapitres 3 à 10) et les ressources trophiques d'autre part (chapitres 11 et 12) ; III) enfin, la gestion des populations exploitées fait l'objet du chapitre 13.

La partie I (chapitre 2) présente les paramètres de dynamique des populations de la palourde japonaise dans le bassin d'Arcachon, c'est-à-dire la modélisation de la croissance et de la mortalité, l'étude de la reproduction et le calcul de la production et de la productivité, paramètres intégrateurs de la dynamique de population. Cette étude est basée sur des campagnes terrain (suivi de populations) et des techniques de marquage recapture pour appréhender plus précisément les paramètres de croissance.

A la lumière des paramètres intégrateurs de la dynamique des palourdes, deux facteurs pouvant potentiellement influer sur la population ont été étudiés : les pathologies et les ressources trophiques (Partie II). Les chapitres 3 à 10 traitent donc des pathologies. De nos jours, il est reconnu que les maladies ne peuvent plus être séparées de la dynamique de population, de par les mortalités qu'elles peuvent entraîner mais également de par les retards de croissance, la castration des animaux, etc... qu'elles peuvent induire. L'émergence des maladies infectieuses représente un facteur limitant au bon développement de l'aquaculture et de la pêche et peut engendrer de lourdes pertes économiques (Carnegie 2005). En Amérique du nord, Europe, Asie et Pacifique, de nombreux mollusques d'importance commerciale ont été gravement touchés par des pathologies, entraînant l'effondrement des pêcheries et industries aquacoles (Carnegie 2005). Par exemple, le protozoaire *Haplosporidium nelsoni* contribue au déclin de l'huître *Crassostrea virginica* depuis 1957 sur la côte atlantique des Etats-Unis (Ford et Tripp 1996). Plus localement, la maladie de l'anneau brun a provoqué en 1987 des mortalités massives de palourdes japonaises sur toute la côte bretonne (Paillard 2004), entraînant un arrêt momentané de l'activité et de grosses pertes financières. De plus, le nombre restreint d'espèces, le transfert d'animaux entre bassins de production et pays favorisent la dissémination des maladies infectieuses (Goulletquer et Héral 1997, Paillard 2004, Carnegie 2005).

Les trématodes sont les parasites "privilégiés" des mollusques (Lauckner 1983). Une première approche a donc consisté à faire un bilan de leur présence dans les palourdes. Cela a été l'occasion d'aborder simultanément la théorie de l'"Enemies Release Hypothesis" stipulant

l'absence de pathogène chez les espèces introduites. Le chapitre 3 traite donc des parasites trématodes digènes qui infestent la palourde japonaise à Arcachon et la palourde européenne à Mundaka.

Beaucoup plus typique des palourdes, la perkinsose liée à la présence d'un protozoaire, pourrait expliquer l'affaiblissement de ces bivalves. Le chapitre 4 fait un état de la maladie dans le bassin d'Arcachon tandis que le chapitre 5 aborde les effets de ce parasite sur la croissance et la condition des deux espèces de palourdes.

Un accent tout particulier a été porté sur une nouvelle pathologie (la maladie du muscle marron, BMD) qui affecte le muscle adducteur postérieur de la palourde japonaise dans le bassin d'Arcachon. Cette pathologie a été découverte dans le cadre de la thèse. Le chapitre 6 dresse un bilan de cette maladie dans le bassin d'Arcachon et exclut la responsabilité étiologique de certains pathogènes comme les bactéries et les protozoaires. La recherche de l'agent causal s'est ensuite effectuée en microscopie électronique à transmission et l'étiologie virale est aujourd'hui privilégiée (chapitre 7). L'effet de la BMD sur la dynamique des populations de palourde a été examiné dans le chapitre 8. L'impact de cette maladie sur la santé des bivalves est reprise dans le chapitre 9 où un accent particulier est porté aux implications de cette maladie sur l'étude des réseaux trophiques par analyse des isotopes stables. Le chapitre 10 porte sur la maladie de l'anneau brun dans le bassin d'Arcachon.

De nombreuses études ont préalablement montré l'importance des ressources trophiques dans les performances de croissance des palourdes (Maître-Allain 1982, Laing et al. 1987, Goulletquer et Bacher 1988). Le chapitre 11 s'intéresse donc aux sources potentielles de nourriture de la palourde japonaise dans le bassin d'Arcachon tandis que le chapitre 12 recherche les différents facteurs influençant la croissance des palourdes. Dans les deux cas, l'approche fait appel aux isotopes stables du carbone et de l'azote.

Finalement, les différents paramètres de dynamique de population (croissance, mortalité) ont été insérés dans un modèle de gestion développé par l'AZTI (chapitre 13). Ce chapitre propose des solutions en termes de gestion pour une durabilité des populations de palourde japonaises dans le bassin d'Arcachon. Le dernier chapitre (14) est une synthèse des différents résultats obtenus au cours de cette étude et ouvre des perspectives de recherche.

2. Sites d'étude

2.1. Le bassin d'Arcachon

Certains aspects plus spécifiques aux thématiques abordées seront développés ultérieurement. Ici, sont regroupées les grandes lignes décrivant la lagune d'Arcachon. Le bassin d'Arcachon (44°40'N, 1°10'W) est une lagune semi-fermée située sur le littoral Aquitain du sud-ouest de la France (Fig. 1.3). La superficie totale de la lagune s'élève à 180 km^2. A basse mer, 115 km^2 d'estran se découvrent, dont 70 d'herbiers à *Zostera noltii* et 10 de parcs à huîtres (Auby 1991). Les chenaux principaux dont la profondeur est de 20 m sont prolongés par un réseau de chenaux secondaires peu profonds ("esteys"). Le bassin d'Arcachon est une zone de transition en termes de masses d'eaux car d'une part alimenté par l'océan Atlantique et d'autre part par des apports d'eaux douces (rivières, précipitations, nappe phréatique). Les apports d'eaux douces dans le bassin sont estimés à $1{,}25.10^9$ m^3 par an, soit $3{,}45.10^6$ m^3 par jour (Manaud et al. 1997). Ces apports sont essentiellement dus aux cours d'eau (83%) et dans une moindre mesure aux précipitations (11%) et au ruissellement de la nappe phréatique (< 6%). Concernant les apports issus des cours d'eau, ils proviennent principalement de la rivière Leyre au sud-est mais aussi d'un ensemble de petits cours d'eau situés tout autour du bassin et provenant de tout le bassin versant. Hormis la Leyre, les principaux tributaires alimentant le bassin et contribuant dans une moindre mesure aux apports d'eaux douces sont le canal des Etangs au nord et le canal des Landes au sud (Manaud et al. 1997).

Le bassin reçoit aussi des eaux marines de l'océan Atlantique par un système de passes de 2 à 3 km de large et de 20 km de long. Le volume d'eau oscillant à chaque marée est de l'ordre de 130 à 200.10^6 m^3 en période de mortes eaux, et de 370 à 400.10^6 m^3 en période de vives eaux. Au niveau de la zone de transition entre les eaux marines et les eaux lagunaires, se trouve une série de bancs de sable regroupés sous l'appellation de banc d'Arguin. Les courants de marées atteignent 1,75m/s au niveau des passes et dans la partie médiane du bassin. Ils sont beaucoup plus faibles vers le fond du bassin (< 1m/s). Le bassin d'Arcachon est donc une zone de transition où salinité et température varient au gré des apports marins et continentaux.

Le banc d'Arguin, situé à l'embouchure du bassin d'Arcachon est classé en zone de réserve nationale depuis 1973, notamment de par l'importance des populations estivales de sternes caujek qui viennent se reproduire (Campredon 1976). Les zones intertidales recouvertes d'herbier et situées à l'intérieur de la lagune accueillent chaque année durant

l'hiver un nombre croissant de bernaches cravant (*Branta bernicla*). Environ 40 000 bernaches hivernent chaque année dans le bassin d'Arcachon (http://www.lpo.fr/etudes/wetlands/index.shtml).

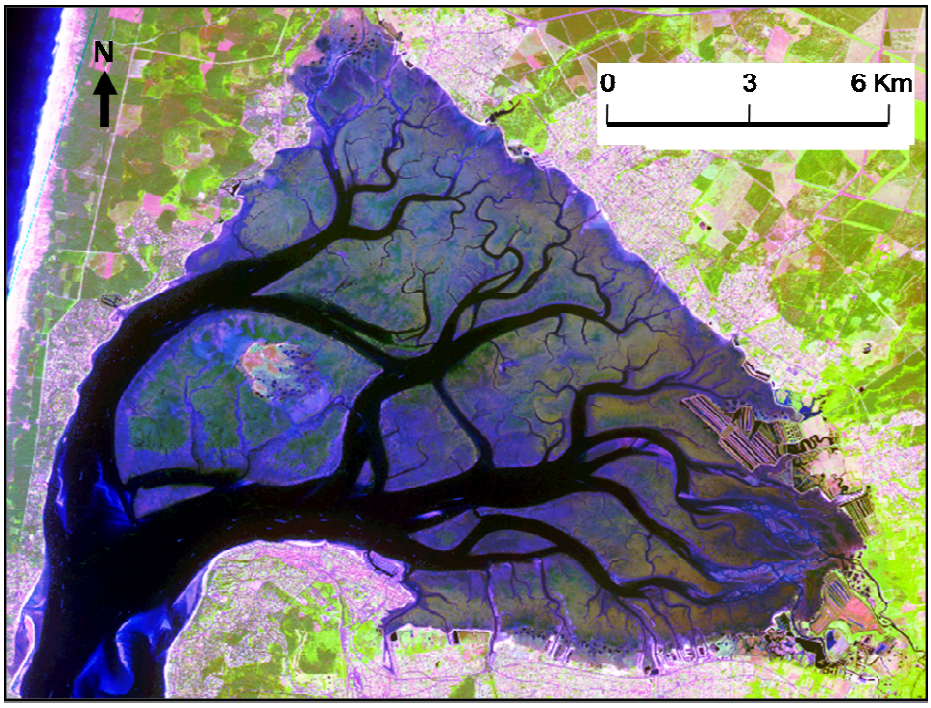

Figure 1.3 : Carte du bassin d'Arcachon (image, J.-M. Froidefond).

2.2. L'estuaire de Mundaka

L'estuaire de Mundaka (43°22'N, 2°43'W) est situé sur la côte nord de l'Espagne dans le Pays Basque, au sud-est du Golfe de Gascogne. C'est une zone mésotidale de transition où se mélangent les eaux douces du fleuve Oka et les eaux marines de l'océan Atlantique. Le système pélagique est constitué par la prédominance de masses d'eaux euryhalines à pleine mer et de masses d'eaux polyhalines à basse mer (Villate 1997). Cet estuaire s'étend sur 13 km de long et représente l'une des plus importantes zone humide du Pays Basque. Il est composé de larges zones intertidales sablo-vaseuses à gravier (marécages) qui deviennent progressivement sableuses en avançant vers l'océan. L'estuaire de Mundaka a été déclaré réserve de la Biosphère par l'UNESCO en 1984 (réserve d'Urdabai). Il est également le lieu d'hibernation de nombreuses espèces d'oiseaux migrateurs.

3. Présentation des deux espèces : la palourde japonaise (*Ruditapes philippinarum*) et la palourde européenne (*R. decussatus*)

3.1. Systématique et répartition géographique

La position systématique des deux espèces de palourdes ainsi que leur synonymie sont détaillées dans le tableau 1.1.

La palourde japonaise *R. philippinarum* est originaire de la province indopacifique : des mers des Philippines, de Chine et du Japon (Ponurovsky et Yakovlev 1992). Depuis le début du 20ème siècle, cette espèce a été introduite dans différentes parties du monde accidentellement avec l'importation du naissain de l'huître japonaise *Crassostrea gigas* ou volontairement pour l'aquaculture (Le Treut 1986). En 1920, elle fut introduite aux îles Hawaï puis dans les années 30 sur la côte pacifique de l'Amérique du nord où elle constitue des populations naturelles de l'Orégon à la Colombie Britannique. Elle a été importée en France en 1972 à partir des populations nord américaines puis en Angleterre, Espagne, Irlande, Italie et Allemagne (Ponurovsky et Yakokvlev 1992). En Europe, on observe des populations naturelles des côtes atlantiques bretonnes aux rias espagnoles, ainsi qu'en Méditerranée et en Adriatique. De nos jours, la palourde japonaise se distribue sur les côtes entre les latitudes 25°N et 45°N dans tout le globe.

La palourde européenne *R. decussatus* est présente dans le nord-est Atlantique dont elle est originaire, des côtes norvégiennes (61°N) à celles du Sénégal (12°N), jusqu'aux Açores (28°W), ainsi que dans les zones lagunaires et estuariennes de la majeure partie du bassin méditerranéen (Parache 1982, Lubet 1984).

Tableau1.1. Synonymie des palourdes japonaises et européennes (Le Treut 1986, Laruelle 1999).

	Palourde japonaise	**Palourde européenne**
Phylum	Mollusca	
Classe	Bivalvia Linné 1758	
Sous-classe	Heterodonta Neumayr 1884	
Ordre	Veneroidea Adams et Adams 1856	
Super-famille	Veneroidea Rafinesque 1815	
Famille	Veneridae Rafinesque 1815	
Sous-famille	Tapetinae Adams et Adams 1857	
Genre	*Ruditapes* Chiamenti 1900	
Espèce	*R. philippinarum* (Adams et Reeve 1850)	*R. decussatus* (Linné 1758)
Synonymie	*Tapes philippinarum* *Tapes semidecussatus* *Tapes denticulata* *Tapes indica* *Tapes grata* *Tapes quadriradiata* *Tapes violascens* *Tapes japonica* *Tapes bifurcata* *Tapes variegata*	*Tapes decussatus* *Tapes extensa* *Tapes decussata* *Tapes extensus*
	Venerupsis philippinarum *Venerupsis japonica* *Venerupsis semidecussata*	*Venerupis decussatus*
	Venus semidecussatus *Venus semidecussata* *Venus japonica* *Venus tesselata* *Venus philippinarum*	*Venus decussatus* *Venus truncata* *Venus vesta* *Venus florida* *Venus variegata* *Venus obscura* *Venus fusca* *Venus deflorata* *Venus litterata* *Venus sanguinolenta*
	Amygdala semidecussata *Amygdala philippinarum* *Amygdala ducalis* *Amygdala japonica* *Amygdala semidecussatus*	*Amygdala decussata*
	Paphia bifurcata *Paphia philippinarum*	*Cuneus reticulatus* *Cuneus decussata*
	Protothaca bifurcata *Protothaca philippinarum*	

3.2 Morphologie

La palourde japonaise *R. philippinarum* est difficile à différencier de l'espèce native, la palourde européenne, *R. decussatus* mais il existe des critères externes et internes pour les identifier (Tableau 1.2).

Tableau 1.2. : Critères de différenciation des palourdes européennes et japonaises (Le Treut 1986, Quero et Vayne 1998).

	Tapes philippinarum	*Tapes decussatus*
		
Critères externes	- coquille +/- ovale - stries concentriques et rayonnantes espacées et prononcées - ornementation souvent asymétrique	- coquille + large que haute et +/- rectangulaire - stries concentriques et rayonnantes fines et très serrées - ornementation symétrique
Critères internes	- sinus palléal régulièrement arrondi - siphons soudés aux 2/3	- sinus palléal anguleux dorsalement - siphons non soudés et plus longs

3.3. Biologie et écologie

L'habitat des deux espèces de palourdes est constitué par des baies abritées (lagunes, estuaires) où elles occupent la frange médiolittorale (Le Treut 1986). Ce sont des espèces fouisseuses. Les adultes vivent à des profondeurs variables dans le sédiment entre 7 et 12 cm de profondeur suivant la saison, les juvéniles étant situés plus en surface (Caill-Milly et al. 2003). La palourde japonaise supporte des substrats très variables : vaseux, sablo-vaseux et sableux. Tamura (1970 in Le Treut 1986) cite pourtant un milieu idéal composé de 20 à 60% de sable et de 20 à 30% de vase, avec des sables fins à moyens. La palourde européenne vit dans des sables variables : sablo-vaseux à sables à graviers avec une préférence pour le sable

moyen (Le Treut 1986). La palourde européenne peut vivre plus en profondeur dans le sédiment que la palourde japonaise, compte tenu d'une plus grande longueur de siphons.

La palourde est déposivore. Elle se nourrit principalement de phytoplancton et de matière organique particulaire qui sont amenés par un courant d'eau créé par son siphon inhalant à la surface du sédiment. La croissance de la palourde est régie par deux phénomènes : l'accumulation de matière organique et minérale dans le test qui fait varier les paramètres biométriques de la coquille et l'apport de matière organique dans l'organisme qui modifie le poids des chairs (Le Treut 1986).

Les facteurs les plus déterminants pour la croissance sont la température et les ressources trophiques, mais d'autres facteurs environnementaux ont aussi un rôle comme la turbidité, le temps d'immersion, les caractéristiques sédimentaires, la concentration en oxygène dissous, les parasites (Ohba 1959, Goulletquer et Bacher 1988, Soudant et al. 2004). La température de l'eau qui varie suivant la latitude et les saisons peut être en partie responsable des différences de croissance observées entre différentes zones géographiques et entre l'hiver et l'été. Par exemple la longueur n'excède pas 34,9 mm au Japon (Ohba 1959) alors qu'elle atteint 50 mm dans l'état de Washington (Nosho et Chew 1972). La température est un des facteurs écologiques le plus important dans la biologie de la palourde car elle détermine les périodes de croissance, les cycles sexuels et le niveau d'alimentation (Le Treut 1986). Les palourdes et en particulier les japonaises supportent des conditions très variables de température et de salinité selon les latitudes avec un preferendum thermique situé entre 19 et 21°C (Maître-Allain 1982). *R. decussatus* tolère une salinité oscillant entre 20 et 50 tandis que *R. philippinarum* supporte des salinités allant de 15 à 50 (Le Treut 1986). Les palourdes s'adaptent aux conditions difficiles en s'enfouissant plus profondément dans le sédiment et en obturant leurs valves (Le Treut 1986).

R. philippinarum et *R. decussatus* sont des espèces gonochoriques. Comme la plupart des bivalves, la gonade est un organe diffus dans la masse viscérale. Dans les populations naturelles, la palourde est sexuellement mature à partir de sa première année (Holland et Chew 1974). La gamétogénèse est initiée quand la température de l'eau dépasse 12°C (Mann 1979, Laruelle et al. 1994, Ngo et Choi 2004). Le cycle reproducteur est connu pour être variable et plusieurs pontes sont possibles dans une simple saison (Beninger et Lucas 1984, Ponurovsky et Yakovlev 1992). Des études concernant le cycle reproducteur de *R. philippinarum* ont souligné l'importance de la localisation géographique, c'est-à-dire de la latitude et de la température dans le contrôle de la gamétogenèse et de la ponte (Holland et

Chew 1974, Mann 1979, Bourne 1982, Beninger et lucas 1984, Rodriguez-Moscoto et al.1992, Ponurovsky 1992, Robert et al. 1993, Laruelle et al. 1994).

Les palourdes japonaises et européennes ont des larves méroplanctoniques. Les gamètes sont émis dans la colonne d'eau où ont lieu la fécondation et le développement de l'œuf. La phase pélagique larvaire dure trois à quatre semaines durant lesquelles la larve est la proie de nombreux prédateurs (Le Treut 1986). La mobilité des larves au gré des vents et des courants marins durant cette période assure une dissémination importante des populations de palourdes. Puis, une série de métamorphoses intervient et la larve va ensuite se fixer sur le substrat. Elle mesure alors 0,2 à 0,3 mm pour la palourde japonaise et 0,7 à 0,8 mm pour la palourde européenne (Tamura 1970 in Le Treut 1986).

Première partie :

Eléments de dynamique des populations à l'échelle du bassin d'Arcachon

Chapitre 2

Dynamique de population de la palourde japonaise dans le bassin d'Arcachon

The Manila clam population in Arcachon Bay (SW France): can it be kept sustainable?

Dang C., De Montaudouin X., Gam M., Paroissin C., Bru N., Caill-Milly. N.

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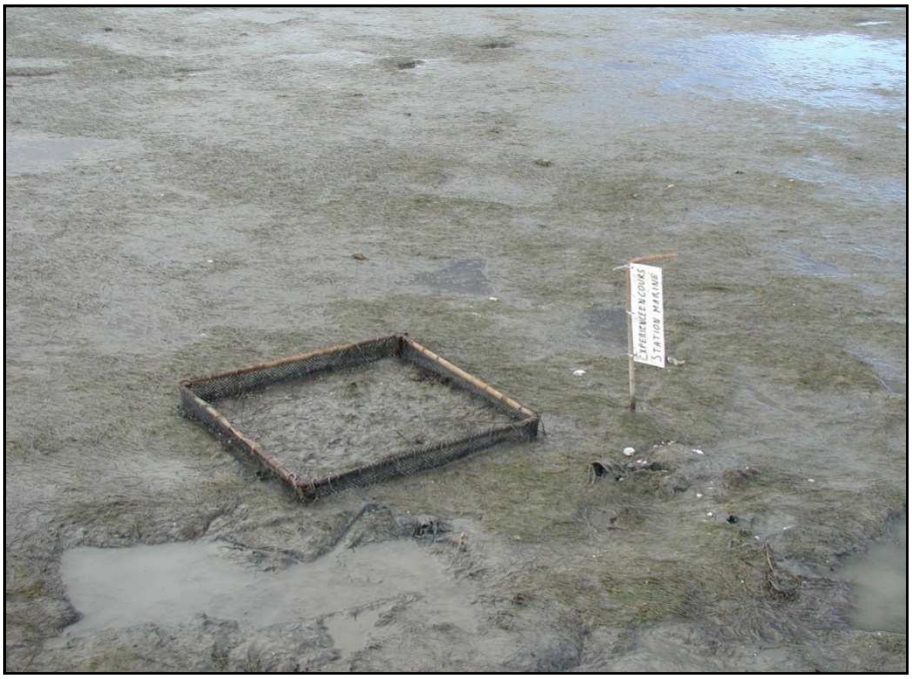

Cage (expérience de marquage-recapture)

Abstract

The venerid clam *Ruditapes philippinarum* is the most prominent suspension-feeding bivalve inhabiting muddy intertidal seagrass beds in Arcachon Bay (SW France). It is exploited by fishermen, and Arcachon Bay ranks number one in France in terms of production and total biomass of this species. Previous studies revealed a decrease in the standing stock of *R. philippinarum* since 2003 and unbalanced length-frequency distributions with a lack of juveniles and adults > 40 mm. Consequently, the population dynamics of this bivalve was studied at four intertidal sites and one oceanic site in Arcachon Bay. As clam size structure did not allow safely computing classical dynamics, field monitoring was coupled with field experiments (tagging-recapture) over two years. Condition index and maturation stages monitoring highlighted a high variability in spawning number and intensity between sites. Recruitment events in the exploited area spatially varied but with uniformly low values. Von Bertalanffy Growth Function (VBGF) parameters (K, L_∞) were determined using Appeldoorn and ELEFAN methods. In the exploited sites (inner lagoon), K was relatively high (mean = 0.72 yr^{-1}) but L_∞ was low (mean = 41.1 mm) resulting in a moderate growth performance index (Φ' = 2.99). Growth parameters were not correlated with immersion time and L_∞ was different between sites. Comparison of mortality coefficients (Z) between cage experiments and field monitoring suggested that fishing accounts for 65-75% of total adult mortality. Low recruitment, low growth rate and normal mortality rate led to low somatic production (4.1 and 8.7 g Shell-Free Dry Weight (SFDW). m^{-2}. yr^{-1}) and annual P/B ratio from 0.44 to 0.92 yr^{-1}. Under current conditions, the possibility of a sustainable population in Arcachon Bay will strongly depend on recruitment success and fishing management.

Keywords: Manila clam, *Ruditapes philippinarum*, growth, mortality, reproduction, population dynamic, production, productivity

1. Introduction

The Manila clam *Ruditapes philippinarum*, endemic from Indo-Pacific waters, is one of the most commercially exploited bivalve mollusks in the world. Its high adaptability to various rearing coastal systems make this clam a very suitable species for aquaculture. Introduced in different parts of the world along with the Pacific oyster *Crassostrea gigas* seeds, the Manila clam is now widely distributed along the Pacific coast of the United States, the European Atlantic coast, the Adriatic and Aegean seas and the Indo-Pacific region (Jensen et al. 2004). *R. philippinarum* was introduced in Europe at the beginning of the 1970s for culture purposes, initially to France (1972) and later to England, Spain and Italy (Flassch and Leborgne 1992). This species naturalized in all these countries and became a new commercially exploited resource.

In Arcachon Bay, the Manila clam was introduced in 1980 in designated growing areas as a commercially attractive species. *R. philippinarum* reproduced successfully in Arcachon Bay and rapidly naturalized in intertidal sea-grass beds (*Zostera noltii*) (Goulletquer et al. 1987). Today, Arcachon Bay ranks at the most important location in France in terms of national production and total biomass for a stock beneficiating of a survey. In 2007, fishermen exploited around 1000 metric tons (Caill-Milly et al. 2008). The minimum catch size of individuals permitted by the European Union was 40 mm. In 2008, the catch size was decreased to 35 mm.

To control the fishing effort and to create a sustainable fishery, three Manila clam standing stock studies that were carried out in Arcachon Bay highlighted an estimated total stock of 8095 mt in 2003, 7307 mt in 2006 and 4865 mt in 2008 (Caill-Milly et al. 2003, 2006, 2008). These studies evidenced that since 2003, total and exploitable (> 35 mm) standing stocks have been decreasing. Furthermore, in these three studies, the length-size frequency distributions were characterized by a lack of juveniles and an absence of clams larger than 40 mm. The lack of juveniles could be the consequence of a default of maturation and/or of spawning, a high larvae mortality or a recruitment deficiency. Two hypotheses could explain the lack of clams with > 40-mm shell length: 1) larger clams were removed by fishermen; 2) clams are not growing over 40 mm. In 1993, a study on reproduction and growth was carried out exclusively in clam growing areas (Robert et al. 1993). The lack of knowledge on *R. philippinarum* population dynamics in Arcachon Bay was the motivation for this study.

The present study focuses on the population dynamics of the Manila clam in its natural habitat (muddy intertidal flats) at Arcachon Bay. The aim of this study is to provide reliable population dynamics parameters in terms of growth, mortality and reproduction in order to implement a fishing strategy model (Bald et al. 2009). These data allowed us to calculate the production and the productivity (P/B = production/biomass ratio) of the clam population. One of the greatest difficulties was that clam population size-structure did not permit to compute classical dynamics analysis due to the difficulty in identifying cohorts, already mentioned by Ramón (2003). Consequently, field monitoring was coupled with field experiments involving individual tagged clams in enclosures (tagging-recapture method).

2. Materials and Methods

2.1. Study sites

Arcachon Bay is a 180-km^2 semi-sheltered lagoon on the southwest coast of France (Fig. 2.1). The inner lagoon is constituted of 110-km^2 of mudflats that are colonized by a vast *Zostera noltii* seagrass bed. The Manila clam *Ruditapes philippinarum* is, in terms of biomass, the dominant species of these intertidal flats (Blanchet et al. 2004). Arcachon Bay receives freshwater inputs mainly by the Leyre River and marine water via two channels located at the southwest end of the lagoon. These fresh and oceanic water inputs as well as the slow renewal of water by tides (Plus et al. 2006) induce salinity and temperature gradients within the bay (Bouchet 1968). Water salinity and sediment temperature of the Manila clam habitat vary from 4 to 35 psu and from -2 to 44°C, respectively (Dang et al., 2008). Sediment composition is mud to muddy sands. Four sites were investigated in the inner part of the bay: Ile aux Oiseaux, Andernos, Gujan and Lanton, and another site, Arguin, is located in the oceanic part of Arcachon Bay in the National Reserve of Banc d'Arguin (Fig. 2.1).

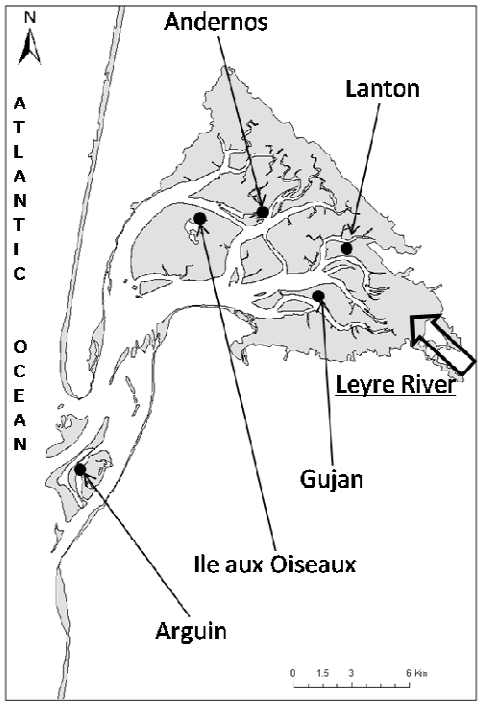

Figure 2.1. Position of the five sampling sites Arguin, Andernos, Ile aux Oiseaux, Gujan and Lanton in Arcachon Bay.

2.2. Environmental parameters

Sediment grain-size, sediment organic matter and elevation above chart datum (CD) were measured at each of the twenty cage locations at the end of 2006. Elevation above CD corresponds to the height (in meter) of the bottom surface. The ten first centimeters of the sediment were sampled by a 5 cm-diameter corer and frozen until analysis. Sediment granulometry was determined using a laser-diffractometer Malvern Master Sizer. This device uses the properties of diffraction and diffusion of particles with a laser beam. Total organic matter in the sediment was obtained by calcination (550°C, 2h). Water salinity was provided by Auby et al. (1999). Topographic surveys were conducted using a DGPS (Trimble 5700) with an accuracy of about 2.5 cm in the horizontal and 2 cm in the vertical. Immersion time was calculated from a tidal curve (tide height in metres *vs* time in hours) with a mean tidal coefficient of 63. Sediment temperature was measured with a Prosensor Thermic Tidbit probe (period = 1 hr, precision 0.4°C) at a single station per site (Arguin 2, Ile aux Oiseaux 2,

Andernos 4, Gujan 3 and Lanton 3) from December 2005 to October 2007. Data were collected regularly with an infrared optic shuttle and transferred to a PC with BoxCar Pro (version 3.51 for Windows) software.

2.3. Population size structure and reproductive patterns

Length-frequency data of the Manila clam population ambient the enclosures at Ile aux Oiseaux (November 2005 to September 2007) and at Andernos (October 2005 to September 2007) were assessed. These two sites were selected because their clam abundance is considered as 'representative' of the lagoon (Bertignac et al. 2001, Caill-Milly et al. 2003). Every other month, sampling consisted of collecting the top 15 cm of sediment (surface: 0.25-m^2) along a transect line (500 m) at each of the ten station established every 50 m, the first station being randomly selected. The sediment was sieved through a 1-mm mesh and *R. philippinarum* individuals were subsequently isolated in the laboratory and their shell length measured to divide into every size-classes from 6 to 44 mm.

In order to assess the reproductive patterns of *R. philippinarum* in Arcachon Bay, thirty clams from 30 to 40-mm shell length were sampled monthly over one year at Ile aux Oiseaux (December 2005 - February 2007) and Gujan (February 2006 - February 2007); and during two years at Andernos (December 2005 - October 2007) and Lanton (December 2005 - October 2007). Clams were dissected and their condition index was evaluated separately for males and females, to assess spawning synchronicity as

$$CI\ (‰) = dry\ flesh\ weight\ (mg)/dry\ shell\ weight\ (g)$$ (Walne and Mann 1975). During clam dissection, gonad development stage inspired from the macroscopic scale proposed by Lucas (1965) was estimated under a stereomicroscope. In stage A, the gonad is translucent and sex is not identifiable. In stage B, the sex is sometimes detectible and gonads are translucent to whitish and show the early stages of maturation. Gonads of clams in stage C are totally mature and strongly developed, with numerous spermatozoa and oocytes. Individuals may potentially spawn (in one or successive events) and remain in this stage C. The gonad is whitish and inflated. When the gonad is completely empty of gametes (stage D), it becomes brown, wrinkled and flaccid. This stage can last few months. The sex proportion at each of the four sites was determined.

2.4. Growth transplant experiment

Clams with shell-lengths comprised between 6 and 44 mm were collected at Ile aux Oiseaux in November 2005. Each individual was labeled with a numbered tag and measured to the nearest 0.1 mm shell length, before being released into different enclosures. Four enclosures were placed at each site (Arguin, Ile aux Oiseaux, Andernos, Gujan and Lanton), each one located at a different elevation from enclosure 1 (lowest level) to enclosure 4 (highest level). Each enclosure consisted in a 50 cm length x 50 cm width x 20 cm height topless metal frame meshed with a 2-mm plastic net, buried 15 cm into the sediment, projecting 5 cm into the overlying water column and anchored with iron bars. The sediment within the enclosure was flushed level with the surrounding sediment. We considered that enclosures just represented a barrier allowing us to retrieve clams and that they had a little effect on local environmental conditions (grain size grass-cover remained identical than outside the enclosure). Compared to the surrounding condition, enclosures were not considered as confounding treatments (Underwood 1997). All enclosures were emptied of native clams before the experiment. Clam density in each enclosure was 320 ind. m^{-2} and the biomass was 24.2 g Shell-Free Dry Weight (SFDW). m^{-2}. This represented higher values than the average in Arcachon Bay. However, such densities and biomass were locally observed (Blanchet et al. 2004, Caill-Milly et al. 2008). *R. philippinarum* were seasonally recaptured and their shell length was measured over a total period of two years. The obtained size increments were used for the estimation of growth parameters (see 2.5.1).

2.5. Data analysis

2.5.1. Growth

Von Bertalanffy growth function (VBGF) $L(t) = L_{\infty}[1 \quad \exp[\ K(t \quad t_0)]]$ predicts length as a function of age and is commonly used in bivalve growth analysis (Bourne 1982, Maronas et al. 2003, Fiori and Morsan 2004, Peharda et al. 2007). This particular function was used in our work in order to allow comparisons with previous studies. L_{∞} is the asymptotic shell length (mm), K is the growth coefficient (yr^{-1}) and t_0 is the theoretical age at zero length (yr).

In the present study, two methods were used to determine VBGF parameters *i.e.* K and L_{∞}, both using FISAT II software (version 1.2.2, FAO-ICLARM).

The first method uses the measurements taken during the growth transplant experiment, *i.e.* length increment data obtained from each of the four stations located at Arguin, Ile aux Oiseaux, Andernos, Lanton and Gujan (total of 20 stations). L_∞ and K were assessed using Appeldoorn's method (Gayanilo et al. 2005).

The second method uses the length-frequency data obtained in the 2-yr population size structure monitoring study at Andernos 4 and Ile aux Oiseaux 2. VBGF parameters were determined using the non-parametric Electronic Length-Frequency Analysis I (ELEFAN) method. This approach evaluates the best K/L_∞ pair by assessing Rn coefficient (Pauly and Moreau 1997). $R_n = (ESP/ASP) \times 1000$, ESP is the Explained Sum of Peaks and ASP is the Availables Sum of Peaks. A higher Rn value indicates a better fit between the components and growth curve. Limits of K and L_∞ values entered in FISAT were set at 0.4 to 1.3 yr^{-1} and 36 to 45 mm respectively.

The growth performance index (Φ') (Pauly and Munro 1984) was calculated using K and L_∞ using the following equation:

$$\Phi' = 2\mathrm{Log}(L_\infty) + \mathrm{Log}(K)$$

2.5.2. Mortality

In the same way as growth parameter determination, mortality rate Z was estimated using data from the growth transplant experiment and the population size structure monitoring. Mortality is the result of the decrease of the abundance, generally expressed as $N_t = N_0\, e^{-Zt}$ where N_0 is the number of individuals at relative age 0 and t is the relative age which is usually close to one year.

Firstly, in each cage, the number of clams at experiment completion was compared to the number of clams at start. Mortality was related to shell length at experiment start, using shell tagged figures. Because of low number of individuals, clams were pooled by 4-mm length shell size classes to calculate a percentage of mortality throughout the duration of the experiment. Knowing shell growth (VBGF), it was consequently possible to plot a mortality curve $Ln\, N_t = -Zt$ and to estimate the mortality Z coefficient. N_t is the abundance (ind) at relative age t, t is the time (yr) and Z is the mortality constant (yr^{-1}). At each station, Z was calculated for two intervals of clam shell lengths; [4-20 mm] and [20-40 mm], in order to compare results with the ELEFAN method.

Secondly, the instantaneous total mortality coefficient Z was estimated from the 2-yr population structure monitoring at Andernos and Ile aux Oiseaux. This approach consisted of using the length-catch curve converting method (Pauly 1983) of the FISAT II software (version 1.2.2, FAO-ICLARM) and the single negative exponential mortality model (Gayanilo et al. 2005). Length of pooled length-frequency samples were converted into ages with parameters of the VBGF. Mortality was calculated by linear regression analysis.
The curve was constructed by plotting the adjusted frequencies (N) of clams in each length-class (i.e. log (N/Δt)) against the length-converted mean relative age (t) (Pauly and Moreau 1997). The 2006 and 2007 years of the survey were considered together and VBGF parameters used were those calculated by Appeldoorn's method for Andernos 4 and Ile aux Oiseaux 2, which were the nearest enclosures.

2.5.3. Biomass, production and productivity

The annual mean biomass, the annual somatic production and the P/B ratio were evaluated from the population size structure monitoring for Manila clam populations at Andernos (immersion rate: 60-70%) and Ile aux Oiseaux (immersion rate: 45-50%) in 2006 and 2007. Utilized VBGF parameters (K and L_∞) were those obtained by the growth transplant experiment at Andernos 4 and Ile aux Oiseaux 2 (Appeldoorn's method) that were the nearest enclosures. In order to calculate production and biomass, the specific relationships between shell length, dry flesh weight and shell weight were estimated. Consequently, one hundred clams from 7 to 41 mm were collected at Andernos. Their dry flesh weight and shell length were evaluated to finally obtain the following equation:

$$log_{10}SFDW = -5.523 + 3.22\, log_{10}L \quad \text{(g)} \quad (R^2 = 0.93)$$

SFDW is the shell-free dry weight (g) and L corresponds to the shell length (mm).

The mean annual biomass was formulated by the following equation:

$$B = \sum N_i M_i \quad \text{(g SFDW. m}^{-2}\text{)}$$

N_i and M_i are the mean number of animals per m^2 and the mean individual somatic mass in size class i.
The total annual production was calculated according to the mass specific growth rate method (Brey 2001) from the size mass relation, the VBG equation and the pooled size-frequency distribution:

$$P = \sum N_i M_i G_i$$ (g SFDW. m^{-2}. yr^{-1})

G_i is the mass specific growth rate, which is represented by:

$$G_i = b\,K\,(L_\infty - L_i)/L_i$$ (yr^{-1})

b (= 3.22) is the specific constant of the size mass relation, K and L_∞, are the VBGF parameters and L_i is the mean size of class i.

The annual P/B ratio of *R. philippinarum* populations were evaluated from annual total production and annual mean biomass.

2.5.4. Statistical analysis

To compare condition index between each date for each site, Kruskall-Wallis non-parametric tests have been realized (Statistica software). Maximum type I error rates were set at $\alpha = 0.05$.

In order to test whether the elevation above chart datum and the sites have influence over clam growth parameters (K and L_∞ and Φ'), we use the non-parametric Kruskal-Wallis test with SAS software. When Kruskal-Wallis test indicated a significant heterogeneity between treatments, a non parametric version of the SNK test, based on the sums of ranks was used to compare treatments two by two.

3. Results

3.1. Environmental parameters

The elevation above chart datum CD was determined for each station of the 5 sites (Table 2.1). The lowest station was at 0.8 m (Lanton 1) and the highest station at 2.8 m (Arguin 4) that corresponded to an immersion time ranging from 96% to 38% respectively (Table 2.1). Grain-size analysis discriminated Arguin (medium sand, median = 360 µm) from the four other sites (muddy sediments, median = 50-160 µm) located in the inner part of Arcachon Bay (Table 2.1). Arguin displayed the lowest values of sediment organic matter (1%) and sediment silt (3.5%) whereas the Lanton site reached 8.5 ± 3% and 41 ± 12.9% respectively inside the lagoon. Daily mean sediment temperature for the five sites was monitored (Fig. 2.2). Arguin differed from other sites by reduced seasonal variability, *i.e.* lower temperatures in summer and higher temperatures in winter (Fig. 2.2). Sediment minimum temperatures varied between 0.2°C (Ile aux Oiseaux) and -1.7°C (Lanton), maximum temperature between 30°C (Arguin) and 43.7°C (Gujan) and mean temperature between 15.1°C (Arguin) and 16.1°C (Ile aux Oiseaux) (Fig. 2.2). Minimum water salinity ranged between 4.8 (Lanton, Gujan) and 32.2 (Arguin), maximum salinities between 26.7 (Lanton, Gujan) and 34.2 (Arguin). Thus, Arguin was the most stable site in terms of sediment temperature and water salinity; Lanton and Gujan displayed the highest variability.

Table 2.1. Growth (K, L_∞, Φ'), environmental parameters (tidal level, immersion time, sediment median grain-size, sediment silt, sediment organic matter) and natural mortality Z for clam shell length 4-20 mm (Z_1) and 20-40 mm (Z_2) with determination coefficient (R^2) for the 20 sampling sites in Arcachon Bay.

Sites	K (yr^{-1})	L_∞ (mm)	Φ'	Tidal level (m)	Immersion (% of time)	Sediment median (µm)	Sediment silt (%)	Sediment organic matter (%)	Time to reach 35 mm (yr)	Time to reach 40 mm (yr)	Z_1 (yr^{-1})-(R^2)	Z_2 (yr^{-1})-(R^2)
Arguin 1	0.88	45.89	3.27	1.57	75.00	360	3.5	1	1.7	2.4		
Arguin 2	0.70	46.70	3.18	1.84	70.83	360	3.5	1	2	2.8	0.27 (0.87)	0.42 (0.97)
Arguin 3	0.55	46.90	3.08	2.07	64.58	360	3.5	1	2.5	3.6	0.40 (0.90)	0.22 (0.94
Arguin 4	0.39	43.70	2.87	2.89	37.50	360	3.5	1	4.2	6.5	0.66 (0.88)	0.11 (0.97)
Ile aux Oiseaux 1	1.04	38.14	3.18	1.05	93.75	118	33.25	10.02	2.5		1.03 (0.99)	2.32 (1.00)
Ile aux Oiseaux 2	0.83	40.00	3.12	1.82	52.08	87	43.54	10.32	2.6	>10	0.83 (0.97)	0.51 (0.96)
Ile aux Oiseaux 3	0.92	38.90	3.14	2.03	64.58	76	47.72	10.95	2.6		0.61 (0.96)	2.66 (0.95)
Ile aux Oiseaux 4	0.68	38.20	3.00	2.63	45.14	97	42.45	13.05	3.7		0.61 (0.97)	1.36 (0.97)
Andernos 1	0.44	43.70	2.92	1.18	87.50	118	30.70	9.41	3.7	5.7	0.79 (0.69)	1.11 (0.99)
Andernos 2	1.21	41.30	3.31	1.72	72.92	70	47.88	9.15	1.6	3	1.10 (0.93)	0.45 (0.84)
Andernos 3	0.96	39.50	3.18	1.83	68.75	48	67.96	10.96	2.3		1.22 (0.92)	0.33 (0.77)
Andernos 4	0.97	39.45	3.18	2.11	60.42	163	14.54	3.33	2.3			
Lanton 1	0.45	43.60	2.93	0.83	95.83	95	40.73	10.17	3.7	5.7	0.35 (0.9)	0.75 (0.95)
Lanton 2	0.58	44.60	3.06	1.36	81.94	111	32.19	9.68	2.7	4	0.30 (0.86	0.22 (0.99)
Lanton 3	0.51	42.10	2.96	1.89	70.83	78	40.99	10.09	3.6	6	0.47 (1.00)	0.52 (1.00)
Lanton 4	0.49	43.90	2.98	1.91	65.97	94	36.38	8.49	3.3	5	0.23 (0.91)	0.35 (0.93)
Gujan 1	0.88	41.20	3.17	0.90	95.83	144	23.09	2.52	2.2	4.2	0.61 (0.98)	0.25 (1.00)
Gujan 2	0.52	42.40	2.97	1.46	75.00	69	47.17	5.63	3.4	5.7	0.68 (0.92)	0.27 (0.95)
Gujan 3	0.43	41.60	2.87	1.68	66.67	63	53.13	5.58	4.4	7.8	1.16 (0.95)	0.80 (0.94)
Gujan 4	0.57	40.10	2.96	2.21	56.25	58	54.48	6.13	3.7	> 10		

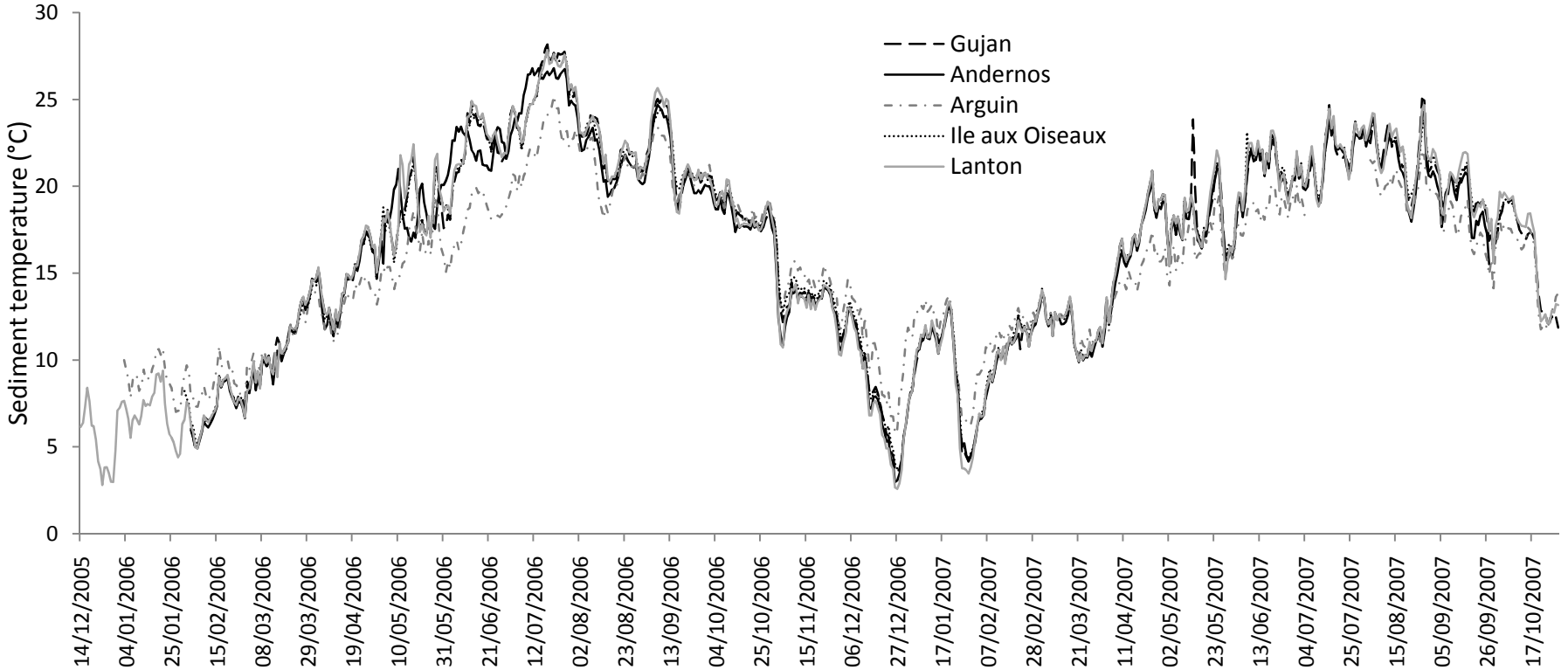

Figure 2.2. Mean daily temperatures for the five studied sites from December 2005 to October 2007.

3.2. Reproduction

Clam reproduction followed through Condition Index (CI) and maturation stages (Fig. 2.3, 4). In all sites, clams CI fluctuated with lowest values in winter and highest values in spring and summer (Fig. 2.3). In 2006, clams from Andernos and Gujan exhibited the highest CI with a peak at 65‰, when Lanton and Ile aux Oiseaux reached 50‰. Peaks occurred in June and September at Andernos (Kruskal-Wallis (KW) test, $P < 0.05$) (temperature (T°) = 22.2°C and 21.3°C, respectively), in May and July at Ile aux Oiseaux (KW test, $P < 0.05$) (T° = 18.4°C and 25.6°C, respectively), in June at Gujan ($P < 0.05$) (T° = 22°C) and in July at Lanton (KW test, $P < 0.05$) (T° = 25.6°C).

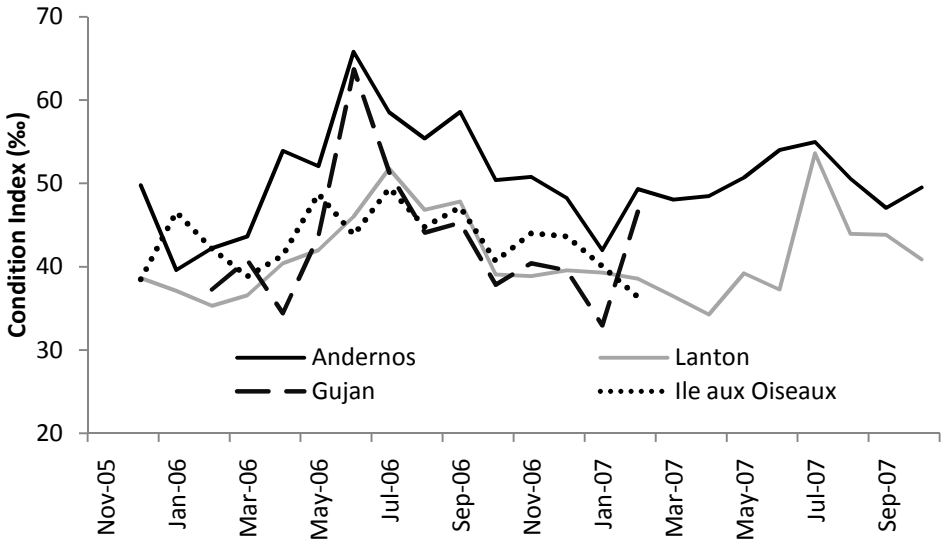

Figure 2.3. Condition index of *Ruditapes philippinarum* sampled in Andernos, Lanton, Gujan and Ile aux Oiseaux from December 2005 to October 2007.

The decrease of the CI was in relation with the spawning period after the gonad was ripe, *i.e.* in reproduction stage C (Fig. 2.4). Gametogenesis started in March (stage B), except in the inner station of Lanton where it occurred in April (Fig. 2.4). It coincided with a sediment temperature of 10°C. Spawning occurred from June to August and from September to October at Andernos, from May to June and from July to August at Ile aux Oiseaux, from June to August at Gujan and from July to September at Lanton. In 2007, only Andernos and Lanton were monitored. At Andernos, CI peak was much lower than in 2006 when it was similar to that encountered in Lanton.

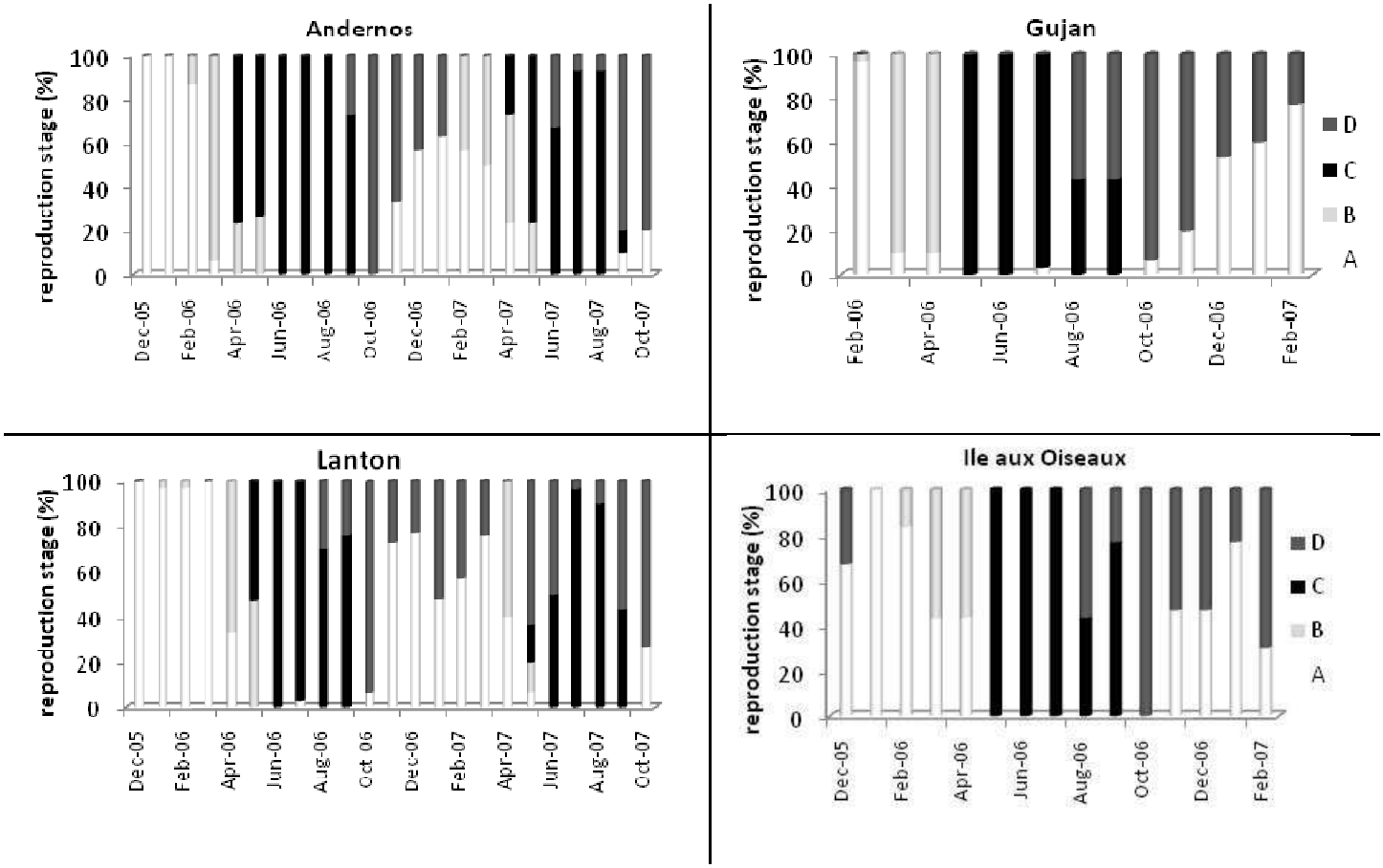

Figure 2.4. Reproduction stages (A, B, C and D) of *Ruditapes philippinarum* from December 2005 to October 2007 in Andernos, Lanton, Ile aux Oiseaux and Gujan.

Maximum CI and spawning periods were similar in both years with a later maturation in the inner station of Lanton. Conversely to 2006, in Andernos, only one spawning occurred from July to September 2007. Temperatures were lower in 2007 with 21.8°C at Lanton in July, and 21.6°C at Andernos in July than in 2006 with 25.6°C and 25.7°C, respectively.

The percentage of sex ratio between males and females was similar in the four sites. Males and females represented 22 and 19% of individuals at Andernos, 18 and 13 % at Gujan, 16 and 15% at Lanton and 19 and 22% at Ile aux Oiseaux, respectively. CI of males and females displayed the same evolution along time which showed a spawning synchronicity between sexes, except at Lanton in 2007 where CI of males evaluated inversely to female CI (Fig. 2.5).

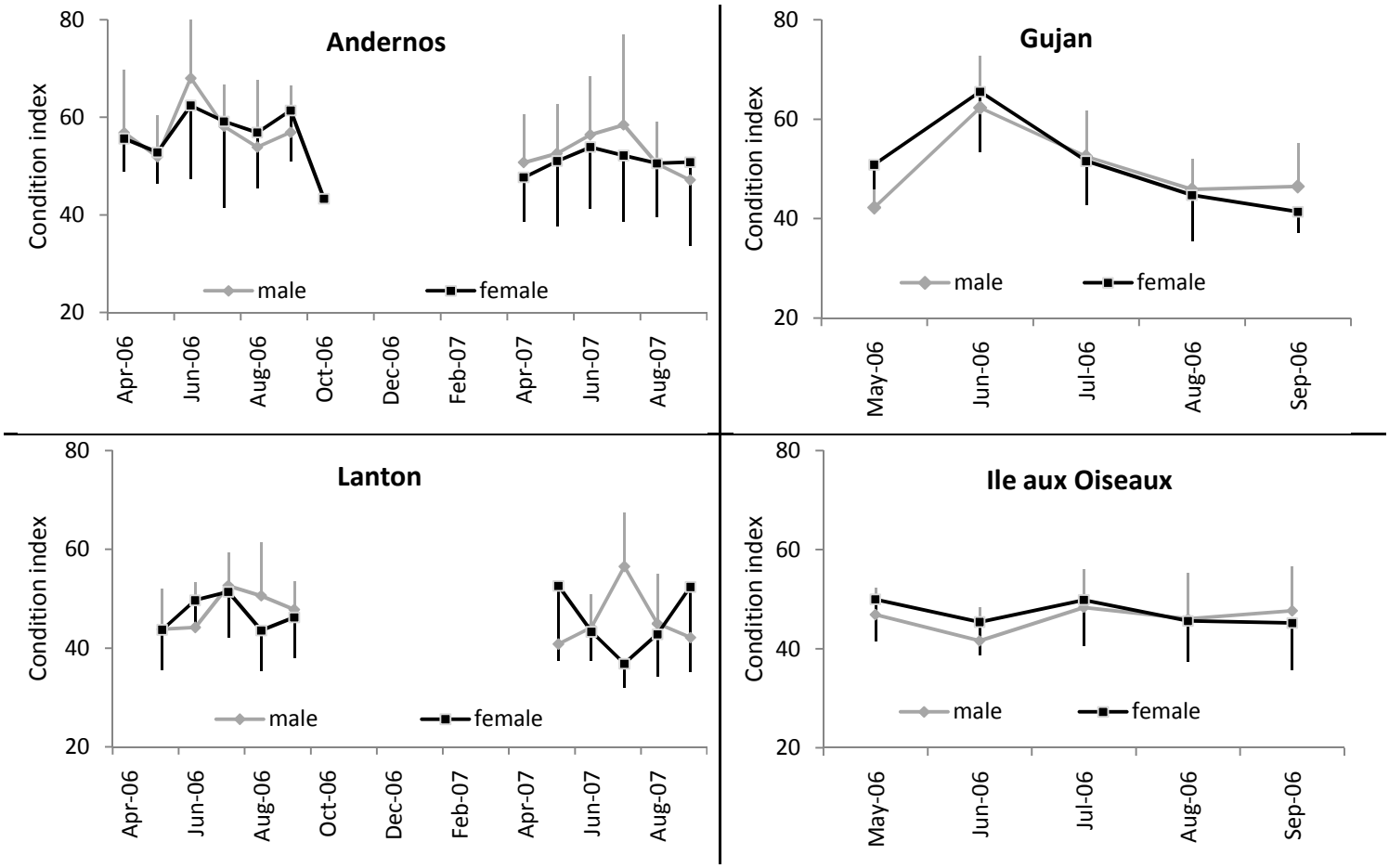

Figure 2.5. Condition index (± SD) of *Ruditapes philippinarum* male and female sampled in Andernos, Lanton, Gujan and Ile aux Oiseaux from December 2005 to October 2007.

3.3. Growth

The first step consisted in calculating VBGF parameters (K and L_∞) in the twenty cages through the growth transplant experiment, using Appeldoorn's method (FISAT II software) (Table 2.1). No significant difference was found between VBGF parameters and elevations above chart datum (CD) at each site (Kruskal-Wallis test, $P > 0.05$). K did not vary between the five sites (Kruskal-Wallis test, $P > 0.05$). L_∞ was significantly different between all studied sites, except between Andernos and Gujan. Growth performance index Φ' did not differ between sites and between elevations above CD at each site (Kruskal-Wallis test, $P > 0.05$).

The second step consisted in determining VBGF parameters K and L_∞ through the length distribution data analysis with the ELEFAN I's method (FISAT II software) at Ile aux Oiseaux and Andernos. Length size frequency distributions were represented in Figure 2.6.

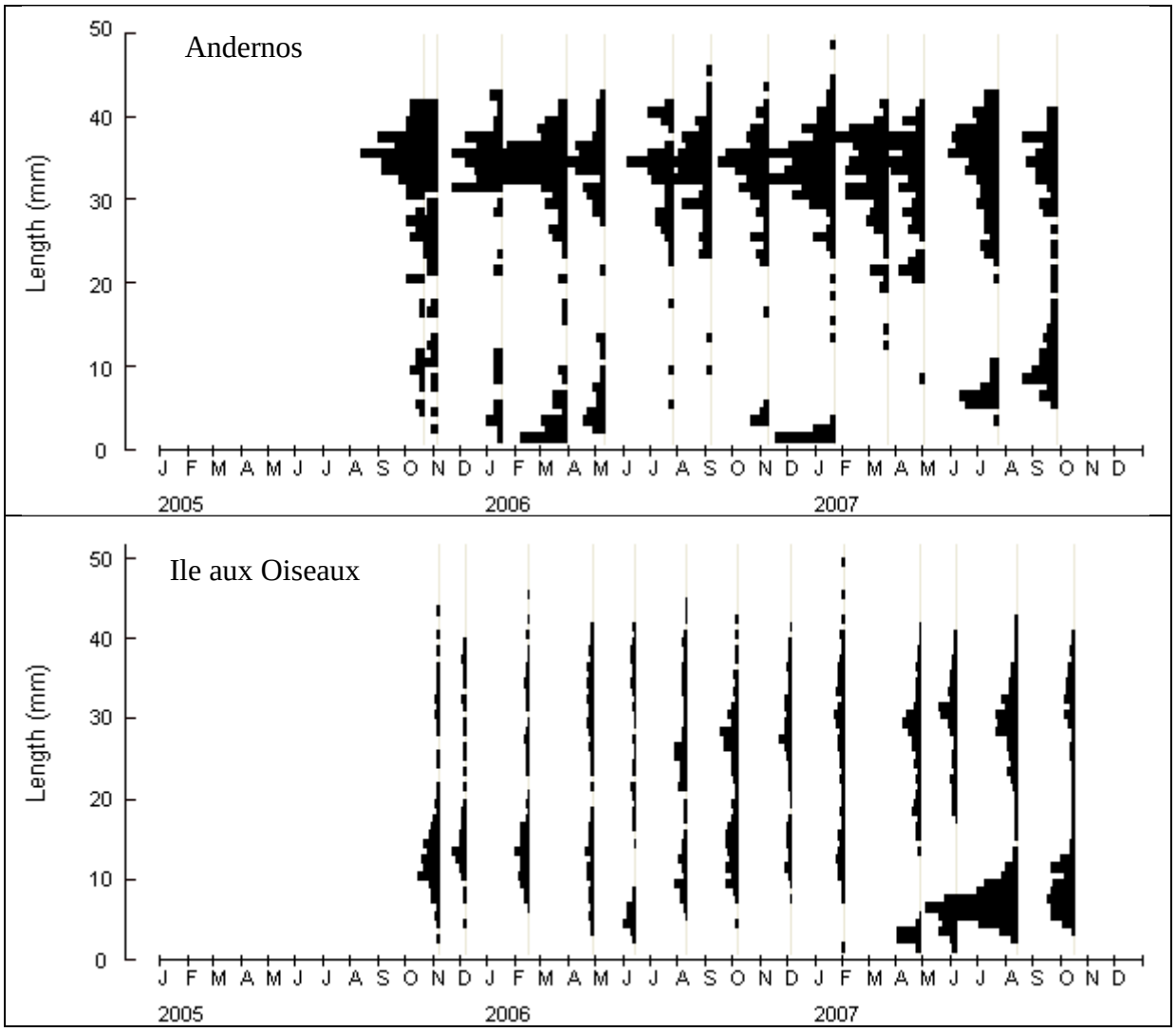

Figure 2.6. Every other month length size frequency distributions of Manila clam *Ruditapes philippinarum* from October 2005 to September 2007 at Andernos and from November 2005 to September 2007 at Ile aux Oiseaux.

These histograms showed the scarcity of juvenile *R. philippinarum* at Ile aux Oiseaux and especially at Andernos, as well as the scarcity of adults > 40 mm, comparing to the abundance of the clams of 28-40 mm in shell length. Few juveniles occurred in spring in March 2006 at Andernos and in April 2007 at Ile aux Oiseaux.

The best pairs K/L_∞ obtained by ELEFAN I were [1.03 yr^{-1}/37.8 mm] at Andernos and [0.81 yr^{-1}/37.8 mm] at Ile aux Oiseaux. The K value was similar to the one found by our growth experiment (nearest enclosures: Ile aux Oiseaux 2 and Andernos 4) on both sites whereas the asymptotic length was lower.

3.4. Mortality

The first method of Z assessment was based on individual mortality in experiment enclosures. Z mortality coefficients varied between stations and length-classes but were generally < 1.5 yr^{-1}, except on two occasions (larger length-class at Ile aux Oiseaux 1 and 3) (Table 2.1).

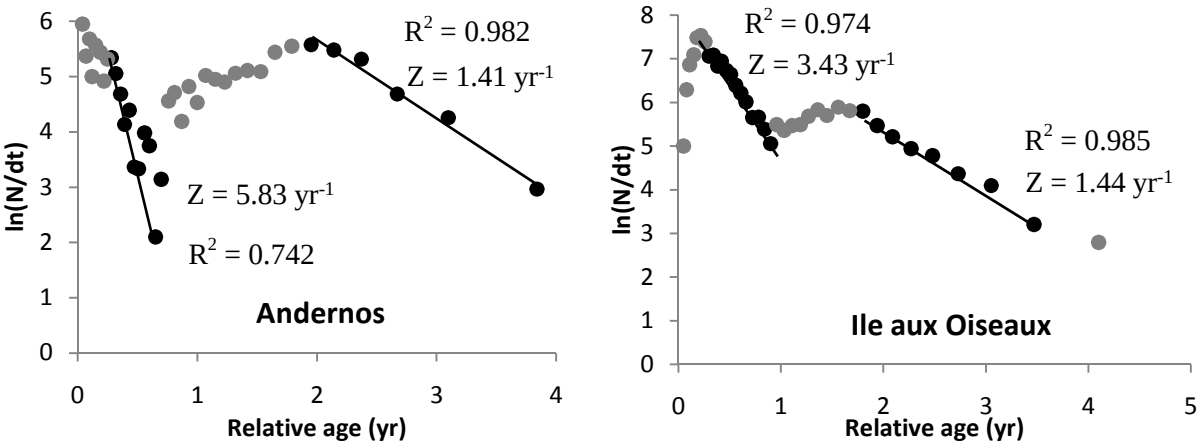

Figure 2.7. Size-converted catch curve of *Ruditapes philippinarum* calculated from the size-frequeny distribution and the Von Bertalanffy growth curve obtained by FISAT II software following the length catch curve converting method at Andernos and Ile aux Oiseaux.

The second estimation of Z was performed using the length-converted catch curve method (FISAT II) and the 2-yr population structure monitoring at Ile aux Oiseaux and Andernos. Size-classes were determined following the size-converted catch curve and determination coefficient (Fig. 2.7). For both sites, the mortality rate was higher for juveniles than adults. At Andernos, Z = 5.83 yr^{-1} (R^2 = 0.74) for size classes 9-19 mm and Z = 1.41 yr^{-1} (R^2 = 0.98) for 33-38 mm clams (Fig. 2.7). At Ile aux Oiseaux, Z = 3.43 yr^{-1} (R^2 = 0.97) for size classes 8-20 mm and Z = 1.44 yr^{-1} (R^2 = 0.98) for adult clams of 30-37 mm (Fig. 2.7).

3.5. Biomass, production and productivity (P/B)

Mean annual clam abundance was 41.8 and 44.5 ind. m^{-2} at Andernos in 2006 and 2007 respectively, and for Ile aux Oiseaux it was 63.6 and 120.9 ind. m^{-2} in 2006 and 2007 respectively (Table 2.2). Difference between both sites was due to the higher abundance of juveniles at Ile aux Oiseaux. It represented a mean annual biomass of 9.3 g SFDW. m^{-2} (2006), 10.1 g SFDW. m^{-2} (2007) at Andernos and 5.8 g SFDW. m^{-2} (2006), 10.5 g SFDW. m^{-2} (2007) at Ile aux Oiseaux (Table 2.2). The observed relationship between length and dry weight was used to estimate the production. The somatic annual production assessed was 4.1 g SFDW. m^{-2}. yr^{-1} (2006), 4.6 g SFDW. m^{-2}. yr^{-1} (2007) at Andernos and 5.3 g SFDW. m^{-2}. yr^{-1} (2006), 8.7 g SFDW. m^{-2}. yr^{-1} (2007) at Ile aux Oiseaux (Table 2.2).

Table 2.2. Annual mean biomass, annual mean density, mean body size, somatic annual production and P/B ratio calculated from the size frequency distribution, according to Brey's method, in Andernos and Ile aux Oiseaux for 2006 and 2007. B, biomass. DW, dry weight. P, production.

Site	B (g DW/m^2)	Mean density (clams/m^2)	Mean body size (g DW)	Somatic P ($g.m^{-2}.yr^{-1}$)	P/B (yr^{-1})
Andernos 2006	9.30	41.8	0.22	4.07	0.44
Andernos 2007	10.11	44.49	0.23	4.56	0.45
Ile aux Oiseaux 2006	5.76	63.64	0.09	5.28	0.92
Ile aux Oiseaux 2007	10.54	120.93	0.09	8.66	0.82

The distribution of total annual production P among the size classes is illustrated in Figure 2.8. The main part of production was supported by adult clams > 30 mm at Andernos 2006, 2007, and Ile aux Oiseaux 2007, whereas P was sustained by both juveniles and adults at Ile aux Oiseaux 2006. Production of adults was higher for Ile aux Oiseaux in 2007 compared to 2006 and with Andernos in 2006 and 2007 (Fig. 2.8). P/B ratios were similar for both years at Andernos, around 0.45 yr^{-1}, and was slightly higher in 2006 (0.92 yr^{-1}) than in 2007 (0.82 yr^{-1}) at Ile aux Oiseaux (Table 2.1).

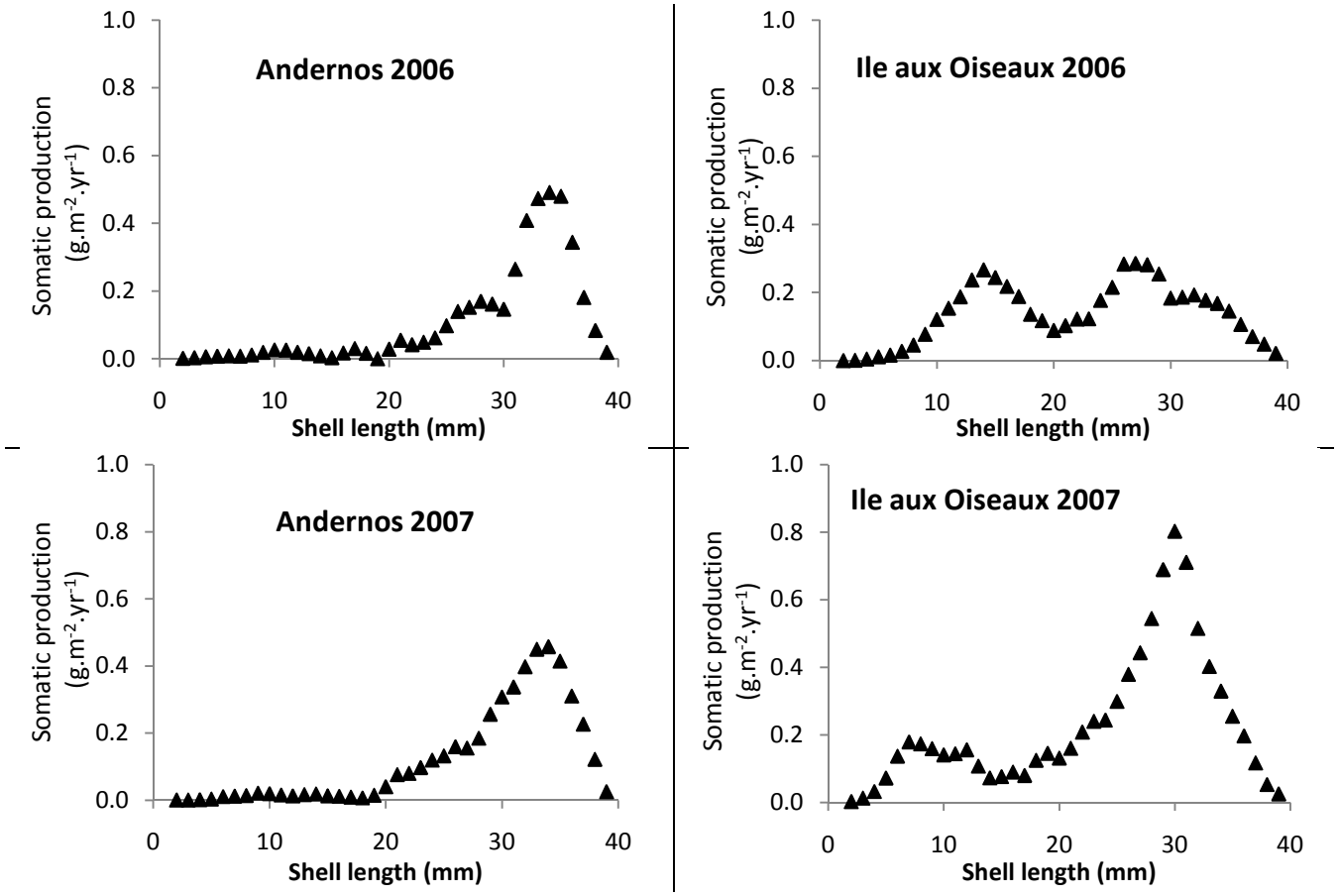

Figure 2.8. Somatic annual production (g SFDW. m^{-2}. yr^{-1}) of *Ruditapes philippinarum* per size classes in 2006 and 2007 at Andernos and Ile aux Oiseaux.

4. Discussion

This work was realized in a context of strong decrease of the Manila clam stock in Arcachon Bay. Recent studies showed a deficit of juveniles and a lack of large clams up to 40 mm. The present work highlighted bad performances of Manila clam population in terms of reproduction and growth. Indeed, gametogenesis displayed low values (analyzed through condition index evolution) and recruitment was poor (2006-07). Growth was characterized by a major difficulty to overpass 40 mm shell length. Surprisingly, K parameter was similar at all sites and levels above chart datum (CD) whereas L_∞ differed between sites (not elevation above CD) but remained in a rather low range. This situation makes the sustainability of the stock most hazardous, particularly in the actual exploited context.

This study confirmed the observations of previous authors, on the gametogenesis cycle of Manila clam, who found a high variability following geographical position (Drummond et al. 2006). Condition index is considered as a good indicator of the spawning period (Laruelle et al. 1994, Drummond et al. 2006, Toba et al. 2007). The maximum observed CI corresponds to a peak in ripeness following an accumulation of resources in preparation for spawning (Drummond et al. 2006). In Arcachon Bay, spawning periods varied according to the site and to the year with a delay of one month between the inner station of Lanton and the three others. Clams spawned twice in 2006 and once in 2007 at Andernos, once at Lanton in 2006 and 2007, twice at Ile aux Oiseaux and once at Gujan in 2006. Consequently, this study evidenced the spatial and temporal variability of spawning events at the km scale within Arcachon Bay. A previous study on three sites located in Arcachon Bay showed that in 1990, a single autumn spawning period was recorded (Robert et al. 1993). Different studies over the world showed that *R. philippinarum* displays different periods of spawning following sites (Table 2.3). In some cases, this period lasted for a few months as is the case in Venice lagoon (Meneghetti et al. 2004) or in the North-East Pacific (Holland and Chew 1974, Bourne 1982). No differences were evidenced between males and females about spawning chronology, as previously reported for *R. philippinarum* from north-western France (Laruelle 1999).

Table 2.3. Number and periods of spawning events following the geographic location.

Country	Location	Number of spawning events	periods	Reference
France	Arcachon Bay	1	autumn	(Robert et al. 1993)
	Ile Tudy (south Brittany)	2	April to August Late summer	(Beninger and Lucas 1984)
	Morbihan Gulf	3	June July September	(Laruelle et al. 1994)
	Bay of Brest	3	May June September	(Laruelle et al. 1994)
	Thau Lagoon	1	April to September	(Maitre-Allain 1985)
Spain	Ria de Vigo	2	June to November	(Rodriguez-Moscoto et al. 1992)
Italy	Lagoon of Venice	1	May to September	(Meneghetti et al. 2004)
Japan	Musaka shoal	2	Late spring Early to late fall	(Ohba 1959)
	Tokyo Bay Ariake Sound	2	Spring to early summer Fall to early winter	(Ishii et al. 2001)
	Tokyo Bay (Sanbanse and Kisarazu)	2	Summer Autumn	(Toba et al. 2007)
	Matsukawa-ura, (north Japan)	2	Late June to early August Late September to early October	(Kanazawa and Sato 2007)
Russia	Vostok Bay	1	summer	(Ponurovsky and Yakovlev 1992)
Canada	British Columbia	1	June to autumn	(Holland and Chew 1974, Bourne 1982)
USA	Hood Canal, Washington	1	Early June to September	(Nosho and Chew 1972)

Temperature is a forcing variable for reproduction. In the present study, it was measured in the sediment because clams live in tidal flats and experience successively aerial and water influence. Manila clam experimentally begin gamete maturation when the temperature reachs 12°C, even if gametogenesis may start at 8°C (Delgado and Pérez-Camacho 2007). This temperature threshold was confirmed by field observations (Ohba 1959, Holland and Chew 1974, Mann 1979, Xie and Burnell 1994). In the present study, maturation began in March when mean sediment temperature was 10.5°C in 2006 and 11.8°C in 2007. The lowest temperature limit for spawning is evaluated at 14°C (Beninger and Lucas 1984, Rodriguez-Moscoso et al. 1992). At Arcachon Bay, gonads started to be ripe in April-May, depending on the site, when sediment temperatures reached on average 14.5°C and 16.6°C in April 2006 and 2007 respectively, as well as 18.5°C and 18°C in May 2006 and 2007, respectively. Most individuals were ripe during three months from May to July at Gujan and Ile aux Oiseaux and from June to August at Andernos and Lanton. Spawning events began at various sediment temperatures, *i.e.* 18.5 °C at the minimum (May 2006, Ile aux Oiseaux) and 25.6 °C at the maximum (July 2006, Lanton and Ile aux Oiseaux). Our results were in accordance with literature with a beginning of maturation at 10°C and a spawning at a temperature up to 14°C. Even if it was an important factor in the determination of maturation and spawning periods, the variability of temperature during spawning in Arcachon Bay suggests that other environmental factors must be essential such as trophic sources (Laruelle et al. 1994) or salinity (proximity of the river) (Robert et al. 1993).

Clams size-structure was unbalanced in Arcachon Bay as showed by histograms at Andernos and Ile aux Oiseaux as well as by previous campaigns that evaluated the standing stock in 2003, 2006 and 2008 (Caill-Milly et al. 2003, 2006, 2008). Size structures of Manila clam were characterized by an absence of adults > 40 mm and a deficit of juveniles, which was particularly alarming for the survival of the population and for the exploitation by fishermen. However, clams develop sexual products at a shell length of 5-10 mm and reach maturity at 15-20 mm with all individuals able to spawn over 20 mm (Holland and Chew 1974).

The lack of juveniles could be explained by three hypotheses: (1) the reproduction was not efficient enough to produce larvae. The amplitude of variation of the CI from reproductive to resting periods is representative of the spawning intensity. In Laruelle et al. (1994) study, the CI was around 60‰ out of gametogenesis period, whereas maximum values reached 131‰ in the Bay of Brest and 142‰ in the Morbihan Gulf, against 66‰ in Arcachon Bay

(and around 40‰ for the resting period). Consequently, the amplitude of CI variation was considerably higher in the two Brittany sites than in Arcachon Bay. Values observed in Morbihan Gulf were close to those observed in clams from Arguin, *i.e.* located in oceanic conditions (Dang, unpublished data). The small average length of adult clams in Arcachon Bay can also exacerbate larvae depletion.

(2) Larvae were produced but endured high planktonic mortality, due to bad environmental conditions. Manila clam eggs require 1-2 days at a temperature of 13-16°C to hatch, followed by an optimal temperature of 25°C and salinity of 20-30 for larval survival (Robinson and Breese 1984). For instance, insufficient temperatures were the reason for the non-recruitment of Manila clams in Southern Ireland (Xie and Burnell 1994).

(3) Reproduction occurred, larvii survived but new recruits underwent mortality due to poor environmental conditions (low temperatures in winter) and/or predation. The density of 2-3 mm shell length clams in the present study was under 23 ind. m^{-2} in April 2007 at Ile aux Oiseaux 2 and 4 ind. m^{-2} in March 2006 at Andernos 4. These values are catastrophic for the population survival, when related to Ohba (1959) values that reported between 2000 and 2500 ind. m^{-2} for a length of 2-3 mm and to Humphreys et al. (2007) that found settlement densities were two orders of magnitude below those of Ohba (1959). Bad recruitment survival can be the consequence of many factors, including predation (Sanchez-Salazar et al. 1987, McArthur 1998, Hiddink et al. 2002). The density of crabs in these seagrass beds can reach 23 ind. m^{-2} (Blanchet et al. 2004). The role of grazing by Brent Geese remains unknown. Secondary settlement and juvenile migrations is unlikely (de Montaudouin 1997). Only two sites were sampled in this study but previous large-scale sampling campaigns collecting all size classes of clams > 5 mm, found low amounts of juveniles (Caill-Milly et al. 2003, 2006, 2008). In addition, a 2002 survey involving 49 stations sieved with 1-mm mesh revealed a lack of juvenile clams (Blanchet et al. 2004). Our study cannot privilege one of these hypotheses. However, the third hypothesis is unlikely since the juvenile mortality obtained in our study was low ($Z = 4.6\ yr^{-1}$).

Clam density (320 ind. m^{-2}) and biomass (24.2 g SFDW. m^{-2}) in our cage experiment were about twice as high as in the field, at Ile aux Oiseaux and Andernos. However, they probably have a little influence on Manila clam growth, as the cage densities were still lower than those found in many natural populations elsewhere. As well, a previous growth experiment at different clam densities failed to demonstrate an effect of clam abundance on growth between biomass ranging from 6.7 to 60.1 g SFDW. m^{-2} (Dang, unpublished data). In Tokyo Bay,

before the decline of clams at the end of the 90's, population density was constantly above 400 ind. m^{-2} (Toba 2004). Ponurovskii and Selin (1988) reported a population density from 490 to 896 ind. m^{-2} in Vostok Bay and Breber (2002) found 384 ind. m^{-2} of *R. philippinarum* in Goro lagoon. Unpublished data from the Morbihan Gulf showed adult (> 26 mm) densities between 40 and 69 ind. m^{-2} whereas it was between 28 ind. m^{-2} (Ile aux Oiseaux) and 33 ind. m^{-2} (Andernos) in Arcachon Bay. Consequently, abundance of juveniles and adults clam in Arcachon Bay was lower than that of Manila clam populations in many other investigated sites.

To explain the lack of adults > 40 mm, two hypotheses have been envisaged: large clams were collected by fishermen or clams was not able to exceed 40-mm shell-length. Given that asymptotic length was not larger than 44 mm inside the bay and also that fishermen exploit all large size classes, both hypotheses are acceptable. One solution to maintain clam population would have been that Manila clams in Arcachon Bay compensated low recruitment by fast growth and low mortality. The necessary time to reach 40-mm shell length depended on the site (Table 2.1). For instance, clams situated inside the bay necessitated between three and sometimes more than ten yrs to reach 40-mm shell length. At Ile aux Oiseaux (cages 1, 3, 4) and Andernos (cages 3, 4), 40-mm shell length cannot be attained. When considering the catch size tolerated in Arcachon Bay, *i.e.* 35 mm, clams from every sites can reach this length. The minimum time was 1.6 yrs at Andernos 2 and 1.7 yrs at Arguin 1 whereas it took 4.4 yrs at Gujan 3 (Table 2.1). The mean age to reach 35 mm was 2.9 yrs, with a minimum of 1.6 yrs at Andernos 2 and 4.4 yrs at Gujan 3. The growth performance index Φ' did vary neither between sites nor between elevations above CD. K values in Arcachon Bay (0.3 – 1.21 yr^{-1}) were relatively high compared to other sites such as British Columbia where they varied between 0.14 and 0.39 yr^{-1} (Bourne 1982). It can be partly interpreted by L_∞ that was dramatically low (< 45 mm in inner lagoon) compared to what was observed in Canada (47.6 – 84.5 mm) (Bourne 1982). However, low performances of L_∞ in Arcachon Bay were not unique and are found in native areas such as in Japanese waters (34.9 mm) (Ohba 1959).

The present study showed that growth efficiency (K and L_∞) was surprisingly independent from the elevation above CD and that L_∞ alone was different between sites. Influence of elevation above CD on growth has been demonstrated in many suspension-feeders like cockles *Cerastoderma edule* in Arcachon Bay (de Montaudouin 1996). A higher

immersion time corresponds to a higher suspension-feeding time (de Kergariou et al. 1981, Goulletquer et al. 1987) and consequently should induce high growth performances. However, Nakamura et al. (2002) and Masu et al. (2008) reported no difference in Manila clam shell length in Tokyo Bay between intertidal and subtidal stations. Bacher and Goulletquer (1989) reported that in intertidal areas, immersion time was an important factor in growth control, but even if this parameter interfered on growth performances, it was not the principal factor explaining the observed differences between sites. Similar observations have been made in a study by Masu et al. (2008) on Manila clam juveniles: shell growth was not exclusively determined by emersion and immersion but perhaps by metabolic rhythm as a result of collective effects of various environmental factors associated with tidal movements, as well as with shell valve movements. Clam growth is also influenced by food supply and water temperature (Thompson 1984). In Tokyo Bay, (Chew) 1989 postulated that the growth of Manila clam was affected by temperature, salinity, exposure regime and food availability whereas (Nakamura et al. 2002) showed that it could only be explained by food availability. Periods of high growth in *R. philippinarum* were associated with high temperatures, high chlorophyll, lipids and proteins concentrations in water column whereas periods of low growth were related to low temperatures, high turbidity and a high detritic food content in the water (Bacher and Goulletquer 1989). Differences in asymptotic length between sites may be explained by a various quality and quantity of trophic sources. The highest L_∞ of this study was found at Arguin. Oceanic sites are commonly characterized by better growth performances because of higher stability of environmental parameters (salinity, temperature) (Mann 1979, Bacher and Goulletquer 1989, Robert et al. 1993) and higher trophic supply (Bacher and Goulletquer 1989, Robert et al. 1993).

The mortality coefficient Z was assessed in cages and *in situ* for two length-classes, < 20 mm (juveniles) and > 20 mm (adults). In cages, averaged Z was 0.67 yr^{-1} (= 50% mortality in 12 mo), for juveniles. In both monitored sites (Andernos and Ile aux Oiseaux), juvenile mortality (Z) was 4 - 5 times higher than in cages located in the same site. This difference may result in a lower predation in cages and in the fact that juveniles in cages were larger (> 6 mm) at the experiment start than individuals sampled with a 1-mm mesh size (monitoring). Two cages displayed particularly high mortalities for adult clams, due to predation by the oyster drill *Ocenebra erinacea* (Z = 2.3 - 2.6 yr^{-1}) as observed with empty shells. In other cages, average Z was 0.51 yr^{-1} (= 50% mortality in 16 mo). In both monitored

sites, (Andernos 4 and Ile aux Oiseaux 2), adult mortality (Z) was 3 - 4 times higher *in situ* than in cages. This difference could result in fishing activity around the cages.

The stock of *R. philippinarum* in Arcachon Bay considerably decreased from 2003 to 2008 and this study flags up the alarming situation of Manila clam populations in Arcachon Bay, as a result of a poor recruitment, slow growth and high fishing effort. The annual production was < 9 g SFDW. m^{-2} .yr^{-1} compared to 17 g SFDW. m^{-2}. yr^{-1} in British waters (Humphreys et al. 2007). Furthermore, the productivity in Arcachon Bay was low with a population renewal of around 0.44 yr^{-1} and 0.87 yr^{-1} (average of P/B in 2006 and 2007) at Andernos and Ile aux Oiseaux, respectively. These values were comparable to those of cockles *Cerastoderma edule* from the Wadden Sea which were comprised between 0.46 yr^{-1} and 0.66 yr^{-1} (Ramón 2003). They were also in the range of the venerid bivalve *Callista chione* with 0.45 yr^{-1} (Metaxatos 2004). A low P/B indicates the small capacity to survive to overexploitation because P/B is considered as an indicator of the maximum limit of sustainable exploitation of stock (Metaxatos 2004).

In recent years in Japan, at Tokyo Bay and Ariake Sound, adult stock of Manila clam has drastically decreased and been coupled by a poor juvenile recruitment (Ishii et al. 2001, Toba 2004). *R. philippinarum* recruitment occurred twice a year in Tokyo Bay and the decline of the adult stock was associated with the unstable recruitment of summer cohorts, which was the major component of recruitment in the past (Toba et al. 2007).

The possibility of a sustainable population of the Manila clam within Arcachon Bay, with such low growth and recruitment is doubtful. Consequently, the survival of Manila clam populations with current levels of extensive fishing activity is unlikely unless high and successful recruitment events occur.

Deuxième partie :

Facteurs naturels contrôlant la dynamique des populations

Comme évoqué en introduction, certains facteurs qu'ils soient environnementaux, trophiques, ou infectieux exercent des pressions sur la dynamique de population des palourdes. La deuxième partie de la thèse s'intéresse principalement à deux de ces facteurs, les pathologies et les ressources trophiques mais aussi à d'autres variables comme le niveau tidal ou encore la couverture en herbier.

Un facteur de contrôle de la dynamique de population qui n'est pas toujours pris en compte est les pathologies. En effet, les pathogènes interagissent avec la croissance (Lauckner 1987-a), la mortalité (Paillard 2004), la condition (Villalba et al. 2000, Rodríguez-Moscoto et al. 2002) et la reproduction (Ngo et Choi 2004, Park et al. 2006) des bivalves. En conséquence, la présente thèse dresse un bilan des différentes pathologies rencontrées chez la palourde japonaise : helminthose (chapitre 3), perkinsose (chapitres 4 et 5), maladie du muscle marron (BMD, chapitres 6 à 9) et la maladie de l'anneau brun (chapitre 10). L'impact des deux principales maladies (perkinsose et BMD) sur la dynamique de population a été considéré dans les chapitres 5 (*Perkinsus vs* taux de croissance et indice de condition) et 8 (BMD *vs* mortalité et indice de condition).

La dynamique de population de bivalves est aussi sous le contrôle de différents facteurs environnementaux tels que la température (Mann 1979), la salinité, les caractéristiques sédimentaires (Goulletquer et Bacher 1988), la turbidité (Daou et Goulletquer 1988) la concentration en oxygène dissous (Uzaki et al. 2003), la vitesse du courant (Grizzle et Morin 1989), la présence ou non d'un herbier (Irlandi 1996), le niveau tidal (Glock et Chew 1979) et les ressources trophiques (Langton et al. 1977). Parmi ces facteurs, les plus déterminants sont la température et les ressources trophiques. En raison de son influence directe sur la croissance, la gamétogénèse et le taux d'excrétion de *Ruditapes philippinarum*, la température apparaît comme un important facteur de contrôle (Mann 1979, Laing et al. 1987). La quantité et la qualité des ressources trophiques disponibles pour la palourde se révèlent être un facteur de contrôle majeur (Langton et al. 1977, Maître-Allain, 1982, Laing et al. 1987, Maître-Allain 1992). Le niveau tidal influence également les paramètres de dynamique de population ; un bas niveau équivaut à un temps de nutrition plus important pour l'animal et donc à une meilleure croissance (Glock et Chew 1979, Griffiths 1981, Bodoy et Plante-Cuny 1984, Goulletquer et Bacher 1988, de Montaudouin 1996). Parmi ces facteurs, ont été prises en compte dans la présente thèse, les ressources trophiques au niveau qualitatif (chapitres 11 et 12) grâce à l'étude des isotopes stables du carbone et de l'azote mais aussi le niveau tidal, la couverture en herbier et la température (chapitre 12).

Les pathologies

L’helminthose

Chapitre 3

Les parasites trématodes dans le bassin d'Arcachon et l'estuaire de Mundaka

Trematode parasites in the non indigenous Manila clam *Ruditapes philippinarum*

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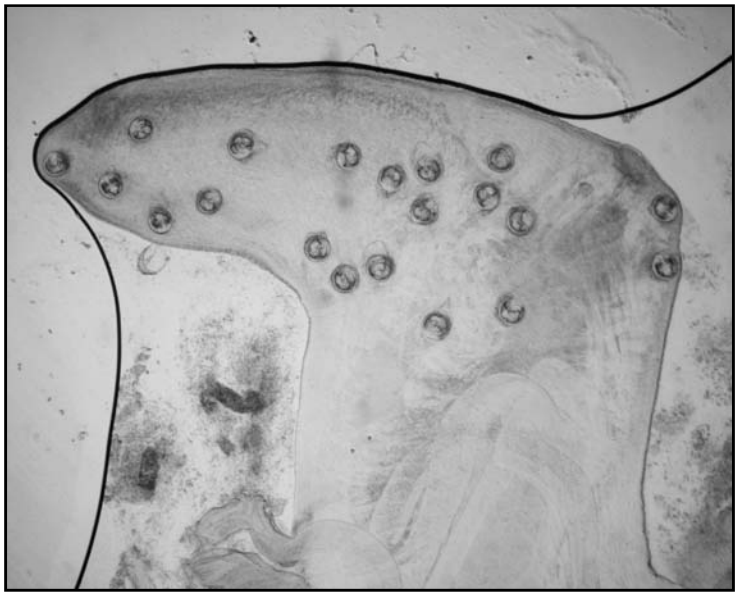


Curtuteria arguinae (Echinostomatidae) dans un pied de palourde

Abstract

Manila clam (*Ruditapes philippinarum*) was introduced in France in the 70's and has rapidly colonized coastal semi-sheltered ecosystems. The present study tested the 'Enemies Release Hypothesis' which states that the success of an introduced species is related to the scarcity of natural enemies in the introduced range compared with the native range. Digenean trematodes are dominant macroparasites of molluscs; therefore the interaction between Manila clam and these parasites was investigated. A two-year monitoring in Arcachon Bay (SW France) highlighted the very low digenean burden in Manila clams. Mean trematode abundance per host in Manila clams was compared with those of sympatric infaunal bivalves (*R. decussatus, Paphia aurea, Cerastoderma edule*). Manila clam trematode abundance was 29 to 624 times lower than for these bivalves, depending on species, sites and time. Similarly, mean trematode species richness per host individual was always lower in Manila clam than in sympatric bivalves (7 to 29 times). A comparison of metacercariaie abundance between *R. decussatus* and *C. edule* in Mundaka Estuary (Spain) showed that both species had similar trematode load but that *R. decussatus* was depleted in trematode species encysting in host tissues (non gymnophallid species).

Experimental infection by generalist trematode larvae (*Himasthla elongata* cercariae) confirmed that the *Ruditapes* genus was resistant to encysting trematodes, with an infection success 3 to 5 times inferior to that of *C. edule*.

According to the literature, the trematode infection in the native range of the Manila clam is also at a low level and consequently the ERH as an explanation for Manila clam success in Europe is not totally consistent in the case of trematode as enemies.

Keywords: *Ruditapes philippinarum*, Parasitism, Trematodes, Non-indigenous species, Arcachon Bay, Mundaka Estuary

1. Introduction

The Manila clam *Ruditapes philippinarum* originates from the Indo-Pacific region but progressively colonized coastal systems around the world (temperate and tropical climates) and became one of the most exploited bivalves. It was accidentally introduced into the United States presumably with Japanese oysters (*Crassostrea gigas*) seeds (1912-1961) and progressively spread from Vancouver to the Hawaiian islands (Bower et al. 1992). In Europe, *R. philippinarum* was primarily introduced to France (1972) then in England (1980), Spain and Italy (1985). In France, this bivalve underwent several infectious agents. Some of these parasites caused severe economical outbreaks like the bacteria *Vibrio tapetis* which induces the Brown Ring Disease (Paillard and Maes 1994). Others parasites remain for the moment as 'threats' either because their infection level is below risk like *Perkinsus* (Villalba et al. 2004, Lassalle et al. 2007) or because they are emerging pathogens with unknown host-population effects (Dang et al. 2008), at least at large spatial scale (Dang and de Montaudouin 2009).

Amongst macroparasites of bivalves, digenean trematodes are the most prevalent and abundant (Lauckner 1983). They have been described in many exploited species like mussels (de Montaudouin et al. 2000, Kim and Powell 2006, Krakau et al. 2006, Thieltges 2006a), cockles (de Montaudouin et al. 2000, Thieltges et al. 2006, Thieltges and Reise 2006, 2007, de Montaudouin et al. in press), oysters (Krakau et al. 2006, Thieltges et al. 2006), carpet-shell clams (Bartoli 1981, Navas et al. 1992, Gomez-León et al. 2007). In France, previous reports of Manila clam infection mentioned very low levels of trematode species richness, prevalence and abundance (de Montaudouin et al. 2000, Lassalle et al. 2007). The present study was performed in Arcachon Bay where the Manila clam standing stock and production is one of the highest in France (Caill-Milly et al. 2006). The most intuitive and appealing explanation of this non-indigenous species (NIS) success in its introduced range is that it is released from the effects of its natural enemies, trematodes in particular. The life cycle of trematodes usually requires three host species, molluscs being involved at two stages of this cycle (as first and second intermediate hosts). Indeed, the enemy release hypothesis (Calvo-Ugarteburu and McQuaid 1998b, Torchin et al. 2002, Colautti et al. 2004) states that the abundance or impact of some NIS is related to the scarcity of natural enemies in the introduced range compared with the native range (Blakeslee and Byers 2008, Genner et al. 2008, Kvach and Stepien 2008). This theory can be examined following two approaches (Colautti et al. 2004). First, 'communities studies' compare native species and NIS co-

occurring within the same community (Navas et al. 1992, Calvo-Ugarteburu and McQuaid 1998a, de Montaudouin et al. 2000, Bachelet et al. 2004, Krakau et al. 2006, Lassalle et al. 2007).

This is one of the aims of the present study. Manila clam infection by trematodes was monitored during two years and punctually compared to co-occuring bivalves (including close-related species like *R. decussatus* or *Paphia aurea*). To help interpret results, two concomitant studies were performed. 1) An experimental infection assessing the real potentiality of Manila clams to be infected by non-specific trematodes (compared to two native species) and 2) the parasite burden of the closely related carpet-shell clam *R. decussatus* was estimated to see if the infection pattern could be enlarged to the *Ruditapes* genus. Cockles *Cerastoderma edule* were also sampled because they give an idea of the maximal trematode infection range (de Montaudouin et al. 2000, in press). Second, 'biogeographical studies' examine native and introduced populations of a given host (Torchin et al. 2001). In the discussion, the trematode parasite burden observed in Arcachon bay will be compared with existing literature to what is observed elsewhere, including the Manila clam's native area.

2. Materials and methods

2.1. Sampling sites

Bivalves were sampled in different sites. Carpet-shell clams (*Ruditapes decussatus*) dominated in the Estuary of Mundaka (Spain, 43°22'N 2°43'W) with sympatric cockles (*Cerastoderma edule*), Manila clams (*Ruditapes philippinarum*) dominated in the inner area of Arcachon Bay (France, 44°40'N 1°10'W) with few sympatric *C. edule*, *R. decussatus* and golden carpet clams *Paphia aurea*, and *C. edule* dominated in the most oceanic part of Arcachon Bay with some sympatric *R. philippinarum* (Fig. 3.1).

The Estuary of Mundaka is a shallow meso-tidal 13-km system located in the south-east of the Bay of Biscay (Fig. 3.1). Clams were collected on a stony bank, in the outer part of the estuary that is a broad area open to the sea and filled by large intertidal flats. The pelagic system is characterised by the predominance of euryhaline waters at high tide and polyhaline waters at ebb tide (Villate 1997). This system maintains valuable estuarine habitats which support a rich wildlife (Cotano and Villate 2006).

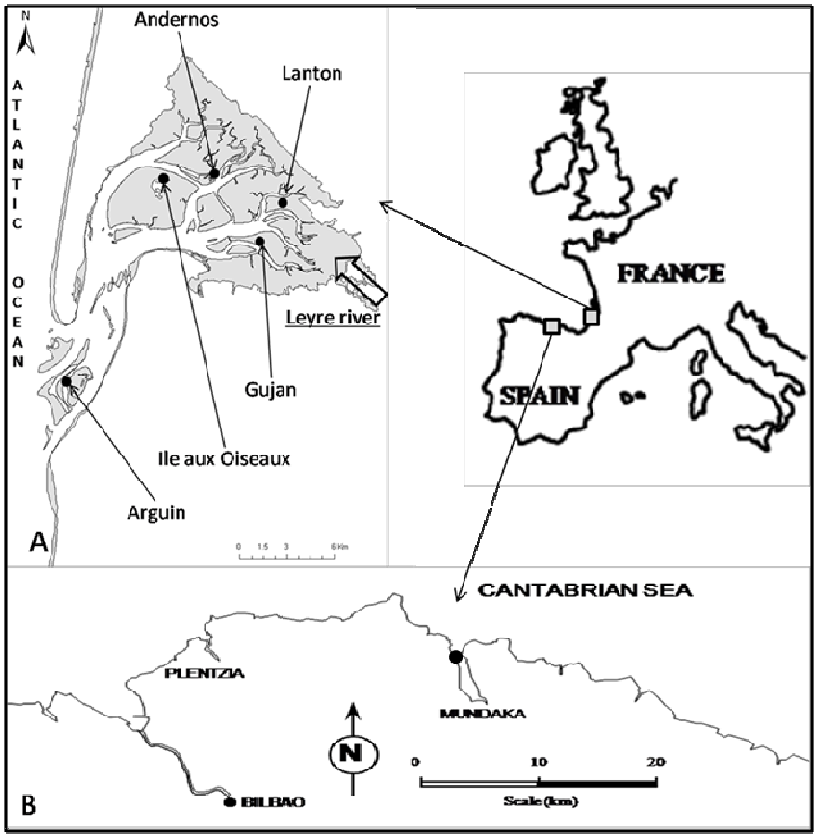


Figure 3.1. Sampling sites in Arcachon Bay and Mundaka Estuary.

Arcachon Bay is a 180-km² lagoon in the southwest of France (Fig. 3.1). The inner part is covered by 110 km² of muddy flats colonised by a vast seagrass bed. The biomass of these flats is dominated by Manila clams (Blanchet et al. 2004). The lagoon receives freshwater inputs mostly by the Leyre River in the south-eastern end of the bay (Fig. 3.1). The balance between marine and continental water inputs and the slow renewal of water by tides induces salinity and temperature gradients (Bouchet 1993). Four sites were sampled, Andernos, Gujan, Ile aux Oiseaux and Lanton (Fig. 3.1). The cockle population is situated in the oceanic part of Arcachon Bay, in the National Reserve of Banc d'Arguin. The habitat consists in moderately sheltered intertidal sand flats. The whole bay plays host to several marine bird populations (Campredon 1976) and the surrounding waters are inhabited by many fish species.

2.2. Sampling procedure and parasite analysis

Manila clams were sampled every other month from November 2005 to February 2007. At each occasion, ten individuals per site were collected by hand and measured at 1-mm precision with a calliper. Adults only were selected (between 30 and 45 mm for clams). Back at the laboratory, individuals were opened, the flesh separated and squeezed between two large glass slides. Trematodes were identified (de Montaudouin et al. 2000, Russell-Pinto et al. 2006, De Brabandere et al. 2007, de Montaudouin et al. in press) and counted under stereomicroscope. Trematode abundance was defined as the mean number of metacercariae per individual host, and prevalence as the percentage of infected hosts (Bush et al. 1997).

On a few occasions and when possible (species presence), ten individuals of sympatric *C. edule* were collected and treated as previously mentioned: at Andernos (October 2006, 18-23 mm shell length), Ile (October 2006, 16-25 mm). On a single occasion (February 2008) and in the only possible site (Andernos), ten individuals of four sympatric infaunal bivalves were dissected for trematodes: *R. philippinarum* (34-38 mm), *R. decussatus* (29-35 mm), *P. aurea* (14-22 mm) and *C. edule* (18-22 mm).

At Arguin, on one occasion (October 2006), Manila clam (28-31 mm shell length) and *C. edule* (25-35 mm) trematode burdens were compared. At two occasions, *C. edule* and *R. decussatus* trematode load were also compared at Mundaka (December 2005, 5-31 mm, and February 2007, 18-26 mm).

Each time, mean parasite abundance was compared between bivalve hosts, performing non-parametric tests: Kolmogorov Smirnov test with two independent populations, and Kruskal Wallis with four independent populations (February 2008, Andernos). Because gymnophallid trematodes display a peculiar infection pattern (non encysted metacercariae), statistic tests were also realised in excluding these species from the database.

2.3. Infection experiment

Due to bivalve availability constraints and the necessity to carry out experiments on bivalves of similar shell length, the experiment was performed during two different periods and at two temperatures: in October 2006 (*C. edule vs* Manila clam, T=16 and 22°C) and February 2007 (*C. edule vs R. decussatus*, T=16 and 22°C). Bivalves were collected and immediately sorted according to their shell length at Banc d'Arguin (*C. edule*: 14-21 mm in

October 2006 and 20-27 mm in February 2007), Ile aux Oiseaux (Manila clam: 14-26 mm) and Mundaka Estuary (*R. decussatus*: 17-29 mm). Fifteen individuals of each species were analysed for parasites prior to experiment (as a control). They were all free of the trematode parasite *Himasthla elongata* that was utilised for infection experiment. *H. elongata* cercariae were collected from infected periwinkles (*Littorina littorea*). Infected snails were kept at 15°C, and fed with macroalgae (*Ulva* sp.). To induce cercariae emission, each snail was isolated in a dish with seawater and exposed to light at about 25°C. The infection experiment was designed as a two-factor experiment in which species and temperature were fixed factors (two-ways ANOVA). The experimental units were 50 mL dishes. One individual was incubated in each dish with 40 cercariae for 48h at 16°C and 22°C (temperature range for infection). Cercariae were collected within less than 1h after emission from snails. There were 15 replicates. Upon completion the soft parts of bivalves were treated as previously described.

3. Results

3.1. Ruditapes philippinarum two-years monitoring in inner Arcachon Bay

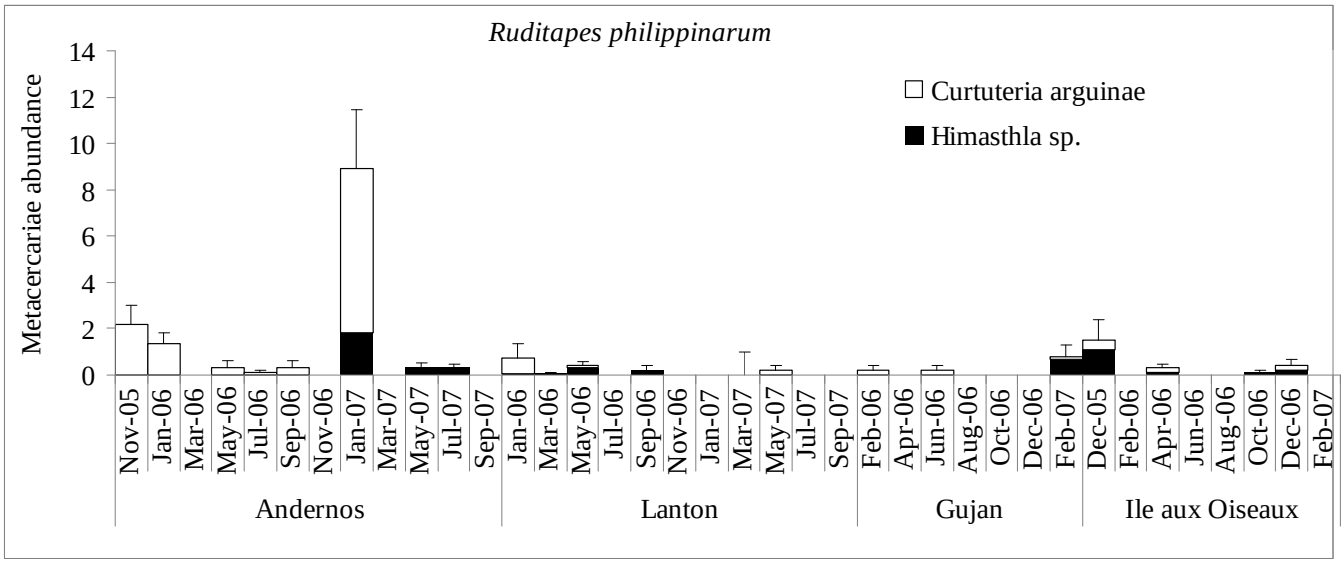


Figure 3.2. Mean total metacercariae abundance per host individual (+1 standard error) in *Ruditapes philippinarum* from the inner part of Arcachon Bay

In the four investigated sites of Arcachon Bay (Andernos, Lanton, Gujan, Ile aux Oiseaux), Manila clams were hardly infected by trematodes. A maximum of two Echinostomatid species (*Curtuteria arguinae* and *Himasthla continua*) was found (Table 3.1). The mean trematode abundance (calculated from all clams collected during the survey) was comprised between 0.1 and 1.1 metacercariae per Manila clam, following sites (Fig. 3.2).

Table 3.1: Mean trematode abundance (number of metacercariae per individual host) for each species: *Ca*: *Curtuteria arguinae*; *H*sp: *Himasthla* sp.; *Hi*: *Himasthla interrupta*; *Db*: *Diphterostomum brusinae*; *Pb*: *Psilostomum brevicole*; ?: Unknown metacercariae; *Mm*: *Meiogymnophallus minutus*; *Mf*: *M. fossarum*; *Bm*: *Bucephalus minutus* (for this species that uses cockles as its first intermediate host, the given value is a prevalence). Total parasite species richness (SR) and abundance (Ab) is listed.

Site	Date	Host species	*Ca*	*H*sp	*Hi*	*Db*	*Pb*	?	*Mm*	*Mf*	*Bm*	SR	Ab
Andernos	Oct-06	*C. edule*	0.2	8.4								2	8.6
	Oct-06	*R. philippinarum*	0.3									1	0.3
	Feb-08	*C. edule*	0.2	21.4		0.5					10%	4	22.1
	Feb-08	*R. decussatus*	1.2	2.0				3.5				3	6.7
	Feb-08	*P. aurea*	1.1	21.1		0.7		19.2				4	42.1
	Feb-08	*R. philippinarum*	0.1									1	0.1
Arguin	Oct-06	*C. edule*	8.1	2.4	28.1	0.3	3.0		707			6	749
	Oct-06	*R. philippinarum*	0.7	0.2			0.3					3	1.2
Ile aux Oiseaux	Oct-06	*C. edule*	0.1	34.5								2	34.6
	Oct-06	*R. philippinarum*		0.1								1	0.1
Mundaka	Dec-05	*C. edule*				2.7			88.8	21.5		3	113.6
	Dec-05	*R. decussatus*								139.9		1	139.9
	Feb-07	*C. edule*				2.7			41.3			2	44
	Feb-07	*R. decussatus*								83.5		1	83.5

3.2. Trematode infection: comparison between *Ruditapes philippinarum* and sympatric bivalves

At Andernos, in October 2006, Manila clam infection (0.3 metacercariae/Manila clam) was lower than those of *Cerastoderma edule* (Kolmogorov Smirnov test, $p < 0.001$) and was due to a single trematode species, *Curtuteria arguinae*. In *C. edule*, the mean infection reached 8.6 metacercariae per cockle belonging to two Echinostomatid species (Table 3.1, Figure 3.3). In February 2008, in the same site (Andernos), Manila clam infection (0.1 metacercariae/Manila clam, belonging to *Curtuteria arguinae* species) was compared with infection of three sympatric bivalves (Kruskal-Wallis test, $p < 0.01$): *R. decussatus* harboured 6.7 metacercariae per individual, belonging to three trematode species; golden carpet clams *Paphia aurea* hosted four trematode species and a mean of 42.1 metacercariae per individual; and *C. edule* were infected by four trematode species and 22.1 metacercariae per individual (Table 3.1, Figure 3.3).

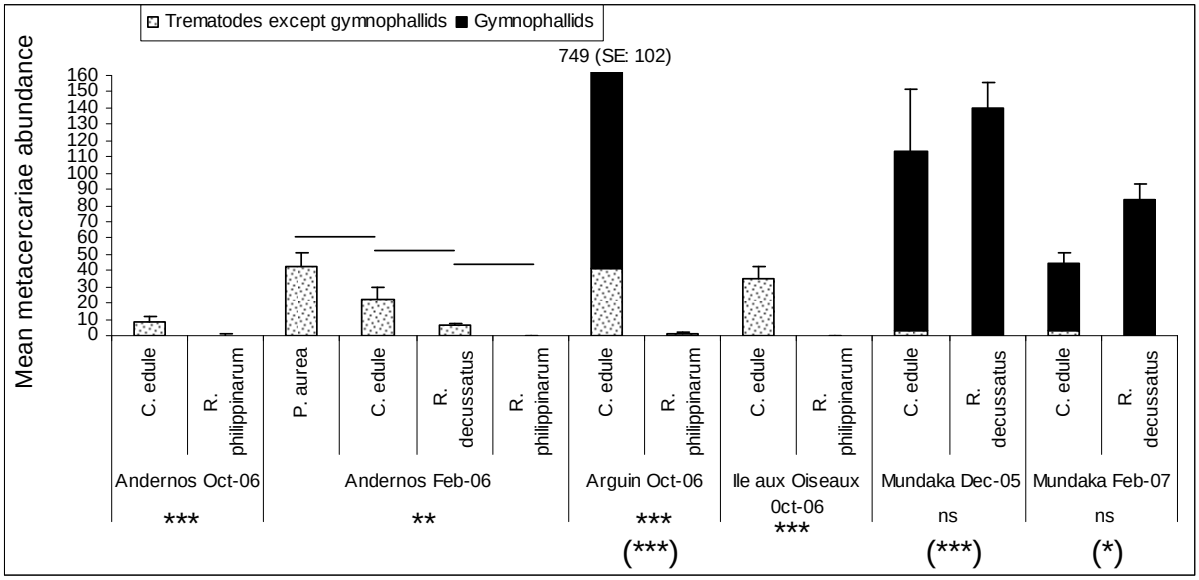


Figure 3.3. Comparison of total mean trematode abundance (+1 SE) in four infaunal bivalves (*Ruditapes philippinarum, R. decussatus, Paphia aurea* and *Cerastoderma edule*), living in sympatry in different sites (see Fig. 3.1). For each site, parasite abundances between species were compared: ns, $p > 0.05$; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. Statistics without gymnophallid species were also performed (results between brackets).

A posteriori multiple comparisons of mean ranks (two by two) revealed that Manila clams were not significantly less infected than *R. decussatus* ($p > 0.05$), but were less infected than *C. edule* and *P. aurea* ($p < 0.05$) (Figure 3.3). At both dates, the mean trematode species richness per host individual was low in Manila clam (0.1 species/host individual) compared with the other bivalves (1.2 in *C. edule* to 2.9 in *P. aurea*).

At Arguin in October 2006, Manila clam infection (1.2 metacercariae/Manila clam) was lower to those of cockle (Kolmogorov Smirnov test, $p < 0.001$). Indeed, in *C. edule*, the mean infection reached 748.6 metacercariae per cockle with a strong dominance of gymnophallid trematodes (707 metacercariae/host individual) (Table 3.1, Figure 3.3). When gymnophallid were not counted, the infection in *C. edule* remained higher than in Manila clam ($p < 0.001$) (Figure 3.2). The mean trematode species richness per host individual was 0.7 in Manila clam and 5.0 in *C. edule* (Table 3.1).

At Ile aux Oiseaux in October 2006, *C. edule* was infected by two digenean species with a mean of 34.6 metacercariae per individual, compared to sympatric Manila clams that were infected by 0.1 metacercariae per host individual (Kolmogorov Smirnov test, < 0.001) belonging to one digenean species (Table 3.1, Figure 3.3). The mean trematode species richness per host individual was 0.1 in Manila clam and 1.1 in *C. edule* (Table 3.1).

3.3. Ruditapes decussatus and *Cerastoderma edule* in Mundaka Estuary

This comparison was performed to obtain more data on the potential infection of *Ruditapes* genus by trematodes. At both dates (December 2005 and February 2007), *Ruditapes decussatus* was infected by a single species of the Gymnophallid family, *Meiogymnophallus fossarum* (Table 3.1). In December 2005, sympatric cockles were infected by three digenean species, *M. fossarum*, *M. minutus* and *Diphterostomum brusinae* (Table 3.1) but the metacercariae abundance per host was similar (Kolmogorv Smirnov test, $p > 0.05$) than in *R. philippinarum* (Table 3.1, Figure 3.3). When excluding gymnophallid species, the mean number of metacercariae per host individual was higher in *C. edule* (2.7) than in Manila clam (0), at both dates ($p < 0.001$ and $p < 0.05$, respectively) (Figure 3.3). In February 2007, both Manila clams and *C. edule* were less infected than in December 2005 (Table 3.1, Figure 3.3).

3.4. Infection experiment

In October 2006 (*C. edule vs* Manila clam), the success of infection of *C. edule* was five times higher than for Manila clam ($p < 0.001$) with no effect of temperature ($p = 0.35$) (Table 3.2). Indeed, the percentage of recovery of metacercariae in *C. edule* (infection success) was 63% of the 40 cercariae that were introduced at experiment start, against 14% in Manila clams (Figure 3.4). In term of *Himasthla elongata* prevalence (0% of bivalve individuals were infected in controls), all *C. edule* individuals were infected at experiment completion (100%), whereas 67-87% of Manila clams were parasitized.

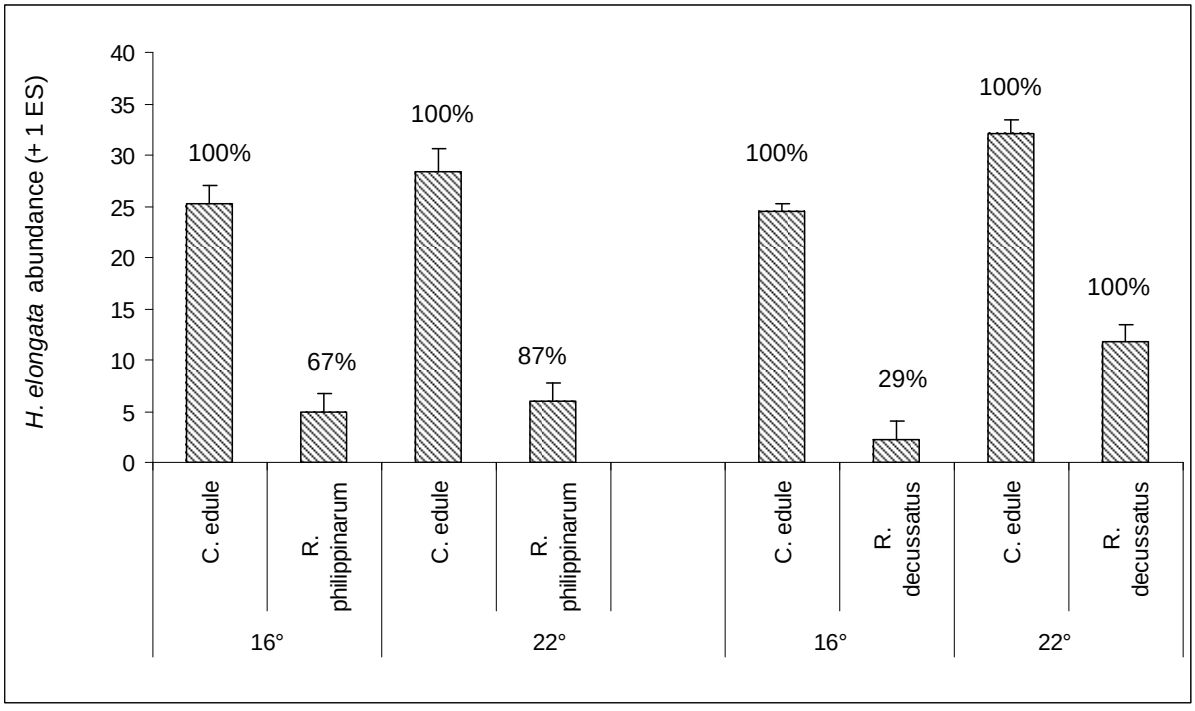


Figure 3.4. Mean number of metacercariae per individual host (+ 1 SE) of *Himasthla elongata* in cockles (*Cerastoderma edule*), carpet shell clams (*Ruditapes decussatus*) and Manila clams (*Ruditapes philippinarum*) after a challenge with 40 cercariae, at 16°C and 22°C. Percentage of infected bivalves (= prevalence) is mentioned. At experiment start, *H. elongata* abundance in all bivalve individuals was nil.

In the second experiment design (February 2007, *C. edule vs R. decussatus*), success of infection in *C. edule* was similar to what was observed previously. The success of infection of *C. edule* compared with *R. decussatus* was higher ($p<0.001$) but here the magnitude depended on temperature ($p<0.001$) (Table 3.2, Figure 3.4). At 16°C, infection of *R. decussatus* was eleven times lower than in *C. edule*. At 22°C, infection of *R. decussatus* was three folds lower than in *C. edule*.

4. Discussion

The level of infection of the Manila clam *Ruditapes philippinarum* by digenean trematodes in Arcachon Bay was very low, as well in term of prevalence than in term of abundance. This observation was consistent with a previous study in 1997-98 (de Montaudouin et al. 2000). As a first intermediate host, where clams would host the parasite in its asexual reproduction stage, there was no observation either in our study, or in any country of the introduced range (Bower 1992; Navas et al. 1992). It could be argued that Manila clam is resistant to trematodes as are many molluscs (Pechenik et al. 2001) but there are some report of such infection in the native area (Lee et al. 2001, Ngo and Choi 2004). The whole host-parasite system could have been transferred (Torchin et al. 2005) however it rarely happens due the generally low prevalence of trematode using molluscs as first intermediate hosts and consequently to the low probability to transfer such parasitized hosts. Moreover, this kind of infection generally induces severe pathologies (da Silva et al. 2002, de Montaudouin et al. 2003, Fredensborg et al. 2005, Thieltges 2006b) and therefore the probability in it being transferred alive is also low. These two arguments create an invasion bottleneck for those parasites. Considering the high specificity of the parasite-host system at this stage it is not surprising that Manila clams did not find new trematodes to harbour as first intermediate hosts in their introduced range. Bivalves can also serve as second intermediate host. In this case trematode larvae are in a latent stage (metacercariae) disseminated in more or less specific tissues of their host which is itself less specific. Again, our results demonstrated low trematode species richness, prevalence and abundance, in accordance with other sites in France (Lassalle et al. 2007). This lack of infection cannot be attributed to a lack of parasites. For instance, at Ile aux Oiseaux and Andernos the non specific trematode genus

Himasthla (Lauckner 1984, 1987b, de Montaudouin et al. 2000, Krakau et al. 2006) infected cockles with an abundance over 20 metacercariae per cockle *Cerastoderma edule* compared to less than 0.2 metacercariae per sympatric Manila clam. In South Spain, the prevalence was also low (0 to 8.3%) compared to those of carpet-shell clam *Ruditapes decussatus* (3.4-26.6%) (Navas et al. 1992). In Vancouver Island (British Columbia) where introduction was more ancient, the level of metacercariae species richness and prevalence was also very low, two species and 6.3% respectively (Bower 1992). In the native area, data remains scarce but the level of infection seemed to also be low (Endo and Hoshina 1974).

The hypothesis of a resistance of Manila clams against trematodes as observed in native and introduced range was consistent with results from infection experiment. Although *Himasthla* spp. can infect numerous bivalves (Lauckner 1983, 1984, Kesting et al. 1996, de Montaudouin et al. 2005, Krakau et al. 2006), it failed to properly infect *R. philippinarum*. Similar observations were made between Manila clam and *Himasthla quissetensis* with 3.2% of cercariae recovered as metacercariae (Cheng et al. 1966). To a lesser extent, the native *R. decussatus* also displayed rather low infection levels for encysting trematodes, both experimentally and during different surveys (this study) or in other sites in Europe (Navas et al. 1992, Gomez-León et al. 2007). Consequently, low levels of infection in Manila clams seems more closely related to specific characteristics that can be observed in other *Ruditapes* genus bivalves than to the introduced status of that species within the ‘enemies release hypothesis’ (Colautti et al. 2004). Amongst hypothesis explaining this resistance, personal observations during infections revealed the difficulty for cercariae to perforate the foot tissue of clams, which is tougher than those of cockles. In other words, the softness of tissues appears as a selective barrier. This could explain why gymnophallid rather succeeded in infecting *R. decussatus* at Mundaka. These trematode parasites do not encyst in tissues of their second intermediate host and remain either at the flesh surface (*Meiogymnophallus minutus*) or in the thinner tissues of the mantle (*M. fossarum*) (Bowers et al. 1990, Russell-Pinto et al. 1996).

5. Conclusion

The success of Manila clams in Arcachon Bay (and certainly in many sites along the European coastline) cannot be attributed to the ERH alone. Indeed the loss of enemies against which host seems well defended in native sites would be of little consequence for host populations in the introduced range (Colautti et al. 2004). It can even be stated that Manila clams undergo more pathogen agents than in the native range. Indeed, wild populations in Arcachon Bay can cumulate Perkinsosis and Brown Ring Disease (as elsewhere in the world (Paillard et al. 1997, Park et al. 2006-b; Villalba et al. 2004) but also Brown Muscle Disease that has been so far described in Arcachon Bay only (Dang et al. 2008, Dang and de Montaudouin 2009) and was ascribed to virus-like particles (Dang et al. 2009-a). Nevertheless, the lack of trematode infection in Manila clams was certainly a supplementary advantage for this species to successfully established, considering the well documented detrimental effects of trematodes on bivalves fitness, as well as first intermediate hosts (Jonsson and André 1992, Thieltges 2006b) than as second intermediate hosts (Wenne and Klusek 1985, Wegeberg and Jensen 1999, Desclaux et al. 2004, Thieltges 2006a).

La perkinsose

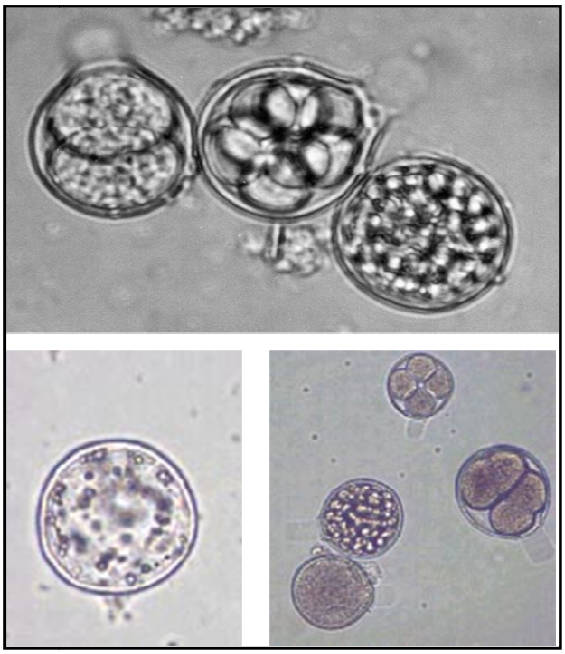
Chapitre 4

Variations spatio-temporelles de la perkinsose dans le bassin d'Arcachon

Spatio-temporal patterns of perkinsosis in the Manila clam *Ruditapes philippinarum* from Arcachon Bay (SW France)

Dang C., de Montaudouin X., Caill-Milly N., Trumbić Ž.

In revision, *Diseases of Aquatic Organisms.*



Perkinsus olseni (photo, K. S. Choi)

Abstract

Pathogens belonging to the genus *Perkinsus* infect many bivalve molluscan species around the world, including the Manila clam *Ruditapes philippinarum*. We investigated the spatial distribution of this parasite at fifty stations scattered within Arcachon Bay (SW France). Prevalence of perkinsosis was 93% and mean infection abundance was 96 x 10^3 cells g^{-1} wet gill tissues. Lowest mean abundances were found close to the Leyre River mouth and a significant negative correlation was observed between mean abundance and salinity. Perkinsosis was rare at the oceanic site where salinities and other environmental parameters were stable. A second aim of this study was to survey perkinsosis during annual cycles at four sites within Arcachon Bay. Prevalence and intensities (± SE) of the disease were high, between 70 and 100% and 130 x 10^3 ± 6.7 x 10^3 cells g^{-1} wet gill on average. No seasonal cycle was evident. Clams were infected at 9-mm shell length and infection increased with clam size. The third objective was to determine the purification and infection kinetics by means of a 21-month reciprocal transplant between a nearly *Perkinsus* sp.-free area and a highly affected site. Purification appeared to be a very slow process and was similar at the site with favorable conditions for *Perkinsus* sp. as at the site with unfavorable conditions. Conversely, infection acquisition appeared to be a punctual and spatially focused event. Consequently, the overall lack of a clear seasonal infection pattern is interpreted as the combination of punctual infection events and slow purification kinetics.

Keywords: *Ruditapes philippinarum*, *Perkinsus* sp., Arcachon Bay, clam, spatio-temporal variations, purification, infection

1. Introduction

Pathogens of the genus *Perkinsus* occur around the world and infect numerous species of oysters, clams and abalones. They have been associated with massive mortalities of these commercially important mollusks including *Crassostrea virginica* in the USA (Andrews and Hewatt 1957) and *Ruditapes decussatus* in Spain and Portugal (Ruano and Cachola 1986, Azevedo 1989). The *Perkinsus* genus includes several species of molluscan parasites, including *P. marinus* from *C. virginica* (Mackin et al. 1950, Burreson et al. 1994), *P. olseni* from the blacklip abalone *Haliotis ruber* (Lester and Davis 1981) and clams *R. decussatus* and *R. philippinarum* (Azevedo 1989, Navas et al. 1992), *P. qugwadi* from Japanese scallops *Patinopecten yessoensis* (Blackbourn et al. 1998), *P. chesapeaki* from the clams *Mya arenaria* and *Macoma balthica* (McLaughlin and Faisal 1999, Coss et al. 2001, Dungan et al. 2002), *P. mediterraneus* from the European flat oyster *Ostrea edulis* (Casas et al. 2004), *P. honshuensis* from *R. philippinarum* (Dungan and Reece 2006) and *P. beihaiensis* from *C. ariakensis* (Moss et al. 2008).

The Manila clam *Ruditapes philippinarum* is from the Indo-Pacific but is now widely distributed all over Europe. It was introduced in France in 1972, rapidly became naturalized, and became a common bivalve of tidal flats along the Atlantic coast. The Manila clam has important commercial value. French production was 3000 mt in 2005. Arcachon Bay (a southwestern French Atlantic lagoon) ranks first in terms of national production with around 1000 mt produced in 2008.

In Asia, *Perkinsus* sp. has been identified in the Manila clam *Ruditapes philippinarum* in Korea (Choi and Park 1997, Park et al. 1999, Lee et al. 2001, Park and Choi 2001), in Japan (Hamaguchi et al. 1998, Choi et al. 2002), and in China (Liang et al. 2001). *Perkinsus olseni* is considered a severe pathogen with highly destructive potential in clams belonging to the genus *Ruditapes* in Spain, Portugal and Italy (Azevedo 1989, Figueras et al. 1992, Sagristá et al. 1995, Elandalloussi et al. 2008). In Europe, *Perkinsus* sp. was found in *R. philippinarum* in Spain (Navas et al. 1992), in Italy (Da Ros and Canzonier 1985, Da Ros et al. 1998) and in France (Lassalle et al. 2007).

Perkinsus spp. pose major threats to molluscan aquaculture around the world. Thus, it was important to determine the infection level and the dynamics of the *Perkinsus* sp. parasite in *R. philippinarum* from Arcachon Bay during an annual cycle. Previous studies gave an instantaneous picture of *Perkinsus* sp. infection in Arcachon Bay (Fouche et al. 1997, Lassalle

et al. 2007). The purposes of this study were (1) to map the infection distribution through thirty-four stations scattered within Arcachon Bay; (2) to survey *Perkinsus* sp. infection during annual cycles on four sites of Arcachon Bay; (3) to examine perkinsosis purification and infection kinetics through a transplant experiment.

2. Materials and methods

2.1. Study area

Arcachon Bay is a 180-km^2 semi-sheltered lagoon on the southwest coast of France (Fig. 4.1). Intertidal muddy flats occupy 110 km^2 and are mostly covered by *Zostera noltii* seagrass beds. The Manila clam *R. philippinarum* is generally located in the mid intertidal zone but can be found from 2.75 m above chart datum to shallow channels (Blanchet et al. 2005, Cottet et al. 2007). In terms of biomass, it is the dominant species of these mudflats. Arcachon Bay receives marine water inputs from the Atlantic Ocean and freshwater inputs mostly from the Leyre River but also from several small streams located around the bay. The mixing of these fresh and oceanic waters as well as the slow renewal by tide induces temperature and salinity gradients within the bay (Plus et al. 2006). Water salinity and sediment temperature of Manila clam habitat vary from 4 to 35 and from -2 to 44°C, respectively (Dang et al. 2008). Five sites scattered within the bay were investigated in the present study: Andernos, Ile aux Oiseaux, Gujan, Lanton, and the oceanic site of Arguin (Fig. 4.1).

Environmental characteristics of each site like sediment granulometry, sediment temperature, tidal level and immersion time were determined in a previous study (Dang et al. in revision-a) and are reported in Table 4.1. Water salinity for each site was provided by Auby et al. (1999). These environmental factors allowed determination of three groups among sites. First is the oceanic site of Arguin (1), which is characterized by higher sediment grain size and relative stability of environmental variables such as sediment temperature and water salinity (Table 4.1). The four sites situated in the inner bay display a lower sediment grain size and higher percentage of silt because they are located on muddy intertidal flats. Among these inner sites,

Andernos and Ile aux Oiseaux (2) present more oceanic features with higher salinities than Gujan and Lanton (3) (Table 4.1). Ile aux Oiseaux presents a higher tidal level (2.63 m) than Gujan (1.46 m) (Table 4.1).

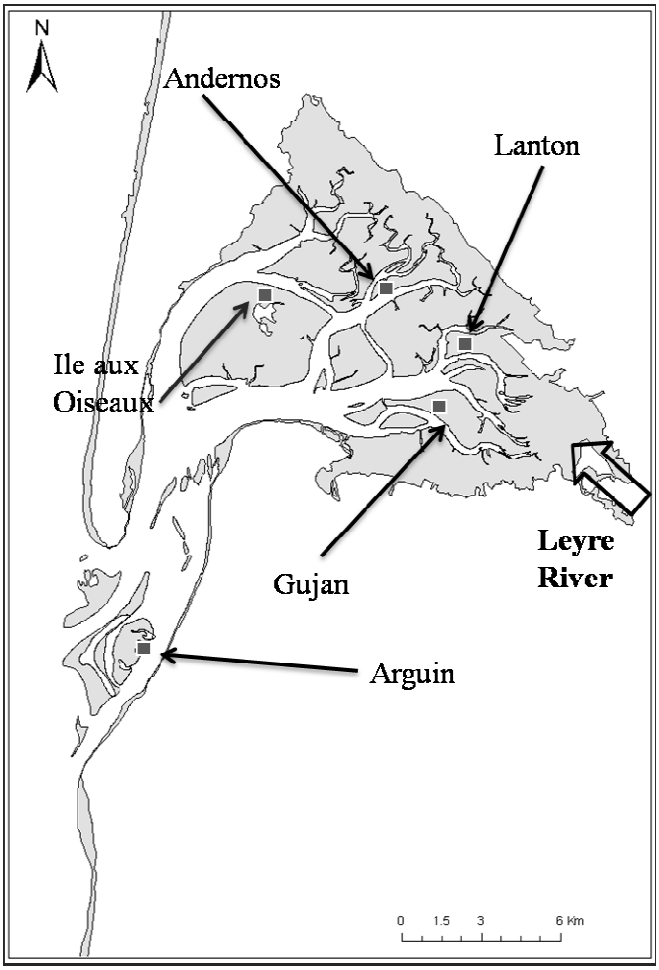


Figure 4.1. View of Arcachon Bay showing the four sampling stations selected for the temporal survey (Ile aux Oiseaux, Andernos, Lanton and Gujan) and the station involved in the cross-transplant experiment (Arguin).

Table 4.1. Environmental variables characterizing sampling sites: sediment grain size (median in µm), organic matter of sediment dry weight (%), sediment silt (%), sediment temperature (°C), water salinity, tidal level (m) and immersion time (%).

Site	Sediment grain size	Sediment organic matter	Sediment silt	Sediment temperature			Water salinity			Tidal level	Immersion time
				Min	Max	Mean	Min	Max	Mean		
Andernos	163	3.3	14.5	-1.0	35.4	15.8	18.5	34.5	30.0	2.11	60.42
Lanton	78	10.1	41.0	-1.7	37.8	16.0	4.8	34.4	26.7	1.89	70.83
Ile aux Oiseaux	97	13.0	42.4	0.2	37.9	16.1	12.1	34.8	29.6	2.63	45.14
Gujan	69	5.6	47.2	-0.2	43.7	16.1	4.8	34.4	26.7	1.46	75.00
Arguin	360	1.0	3.5	-0.2	30.0	15.1	31.2	35.4	34.2	2.07	64.58

2.2. Spatial distribution

Manila clams were sampled at 34 geographically referenced stations within Arcachon Bay between mid-May and mid-June 2006 (Fig. 4.2). Stations were randomly allocated within the ~ 70 km^2 distribution area of the species, as described by stock assessment studies (Caill-Milly et al. 2006). The clam density for each sampled station was given by Caill-Milly et al. (2006). Tidal elevation (m) of each station was provided by both SHOM (Service Hydrographique de la Marine, Brest) and DDE (Direction Départementale de l'Equipement de la Gironde, Bordeaux). Distances between each station and the ocean were calculated with the Arcview 3.2 Geographic Information System software. Temperature was provided by Auby et al. (1999). The number of days per year where the salinity was lower than 30 was estimated for each station by M. Plus (Ifremer, unpublished data).

Ten adult Manila clams (30-40 mm) were randomly collected by hand at each of the 34 stations and *Perkinsus* sp. was subsequently detected using the following protocol. Clams were opened, and gills were excised and processed as described by the quantitative method of Ray (1952) as modified by Choi et al. (1989). Gills were incubated in 10 mL of fluid thioglycolate medium (FTM) supplemented with 100µL of antibiotics (streptomycin and penicillin G) and 500µL of antifungal (Nystatin). Gills were incubated for at least five days in the dark at room temperature. After incubation, the FTM was discarded following centrifugation and remaining gill tissues were digested twice with 2N NaOH at 60°C for 1 hour. The resulting solution was centrifuged and the supernatant discarded. Finally the pellet was washed twice with a sterile solution of Phosphate Buffered Saline (PBS 1X) and resuspended in 1 mL of the same solution. The number of *Perkinsus* sp. hypnospores was estimated using a Malassez chamber under light microscopy. *Perkinsus* sp. density was expressed as the number of cells per gram wet weight of gill tissue. The prevalence of perkinsosis was defined as the percentage of individuals having their gills infected by *Perkinsus* sp. The parasitic abundance in gills was defined as the number of protozoans per analyzed clam. The intensity of the disease in gills was defined as the number of parasites per infected clam.

2.3. Clam shell length and infection abundance

To assess the size-specific infection abundance, clams from 7-42 mm were randomly collected by hand, but only at a single station (Lanton). Four individuals in each 2-mm shell length class were considered for perkinsosis analysis.

2.4. Temporal survey of four sites

To follow infection prevalence and intensity in adult clams in relation to time and space, four populations were surveyed during one year (February 2006-February 2007). However, these surveys were extended to a larger time scale where sampling was performed for other purposes. Every month, thirty adult clams (30-40 mm) were randomly collected by hand at each of the four sites: Ile aux Oiseaux (from December 2005 to February 2007), Andernos (from November 2005 to September 2007), Gujan (from February 2006 to February 2007) and Lanton (from January 2006 to November 2007).

2.5. Purification kinetics

In Arcachon Bay, clams from Arguin were almost free of perkinsosis whereas clams located inside the bay were highly infected by *Perkinsus* sp. At Arguin, the mean *Perkinsus* sp. abundance (± SE) was $2.1 \times 10^2 \pm 2.3 \times 10^2$ cells/g of wet gill whereas it was $1.2 \times 10^5 \pm 1.9 \times 10^5$ cells/g of wet gill at Lanton. Moreover, the mean prevalence was 7% at Arguin against 88% at Lanton.

In order to assess the perkinsosis purification kinetics, infected clams from Lanton were transplanted into enclosures at a site of much lower *Perkinsus* sp. abundance (Arguin) in November 2006. Four hundred specimens (average shell length ± SD = 31.5 ± 1.1 mm) were placed in four topless 0.25 m^2 enclosures. Every month, five clams per enclosure were randomly sampled (total = 20 clams) for perkinsosis diagnosis, and compared with 20 clams from the native site (Lanton).

2.6. Infection kinetics

In order to assess infection kinetics, reciprocal transplantation of four hundred adult clams (average shell length ±SD: 35.3 ± 3.2 mm) from Arguin (low *Perkinsus* sp. abundance) to Lanton (high *Perkinsus* sp.) was realized between November 2006 and May 2007 following the same protocol than previously described at Arguin. Clams native to Arguin were also monitored as a control for this experiment.

Due to the low incidence of infection at Lanton during our experiment, other stations within inner Arcachon Bay were also investigated. Indeed, clams from different origins were implanted at different locations inside the bay for a growth experiment (Dang et al. in revision-a). In April 2006, clams from Arguin (20-44 mm) were introduced to Ile aux Oiseaux (n = 6), in June 2006 to Andernos (n = 10), in April 2007 to Lanton (n = 19) and in May 2007 to Gujan (n = 9). These clams (lightly infected) were maintained very close to (centimeters away from) clams from the inner bay stations (highly infected) for two years. Transplanted clams were held inside an enclosure at a density of 320 ind/m^2. They were collected by hand in October 2007 and their *Perkinsus* sp. infection quantified in order to investigate whether infection at Lanton was or was not representative of other parts of the bay (and whether clams from Arguin were resistant to perkinsosis).

2.7. Statistical analyses

In the spatial analysis, regressions were performed between *Perkinsus* sp. mean infection abundance and following five variables: tidal level, distance from the ocean, maximum temperature, period of salinity < 30, and clam density.

To determine correlation between clam shell length and perkinsosis mean abundance, a regression was performed.

In temporal surveys, differences in prevalence between sites were tested by analysis of variance (ANOVA) after arcsin$\sqrt{p}$ data transformation (p = prevalence/100) (Zar 1984). Differences in infection intensities between sites over time were tested with a two-way ANOVA (factors: site and time) after logarithmic data transformation. Maximum type I error rates were set at $\alpha = 0.05$. Prior to ANOVA, homogeneity of variance was confirmed using Cochran test.

Regressions were performed between time and *Perkinsus* sp. mean log abundance in purification and infection experiments. To evaluate whether purification was significant during the course of the experiment and to determine the influence of site, a two-way ANOVA was performed (factors: sites, time). Statistical analysis was performed using Statistica software 7.1.

3. Results

3.1. Spatial distribution

The prevalence of *Perkinsus* sp. in clams was relatively high, with values between 60 and 100%. Mean perkinsosis prevalence within the bay was 93%. The disease was present in all parts of the bay except at Arguin where this protozoan was rare. The mean prevalence at Arguin was 5% and the mean abundance of infection was very low (213 cells g^{-1}). Unlike at Arguin, mean abundances of infection in the inner bay stations were high and ranged between 1.1 x 10^3 and 230 x 10^3 cells per gram of wet gill with an average of 96 x 10^3 ± 9.9 x 10^3 (± SE) cells per gram of wet gill tissues (Fig. 4.2).

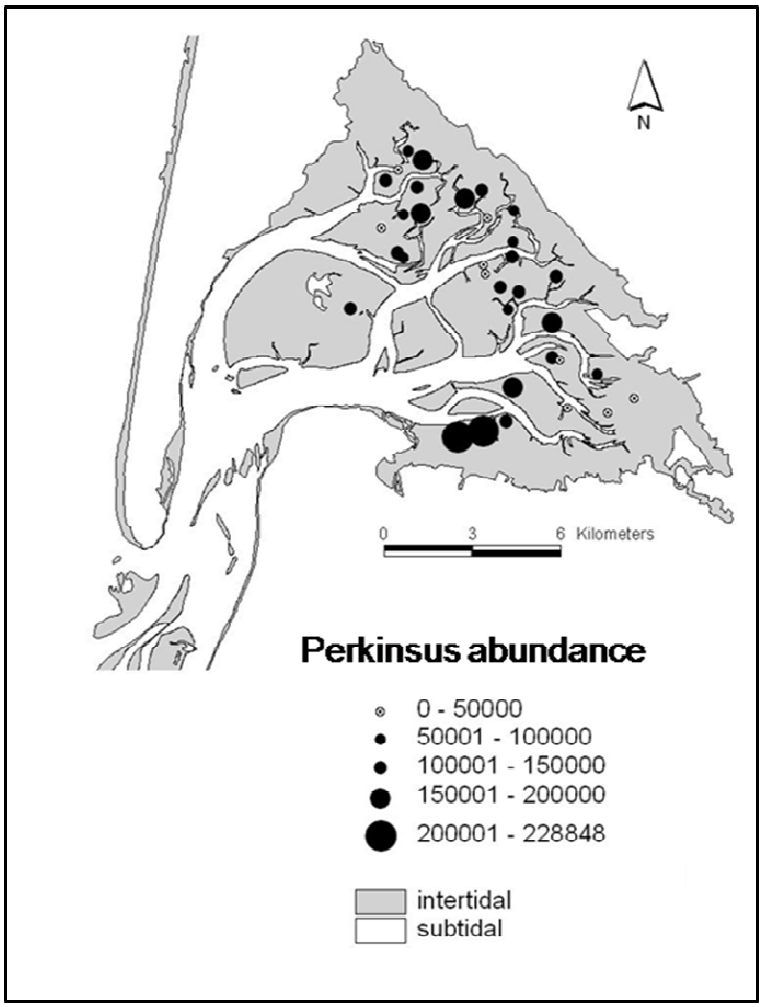


Figure 4.2. Mean *Perkinsus* sp. abundance (cells. g^{-1} wet gill) at 34 stations within Arcachon Bay that were sampled in May-June 2006.

Perkinsus sp. distribution within the bay seemed heterogeneous. However, with the exception of Arguin, the lowest values of mean infection abundance were found at the Leyre River mouth. Significant negative correlations were observed only between *Perkinsus* sp. mean abundance and distance to the ocean ($r = -0.39$, $n = 34$, $p = 0.022$,) and between *Perkinsus* sp. mean abundance and number of days where salinities are lower than 30 ($r = -0.37$, $n = 34$, $p = 0.033$,). No significant relation was observed between *Perkinsus* sp. infection and clam density for densities ranging from 10 to 160 ind/m^2.

3.2. Clam shell length and infection abundance

The minimum shell length range of infected clams at Lanton was 9-10 mm (Fig. 4.3). *Perkinsus* sp. infection abundance significantly increased with shell length (regression, $r = 0.23$, $n = 97$, $p = 0.024$) (Fig. 4.3).

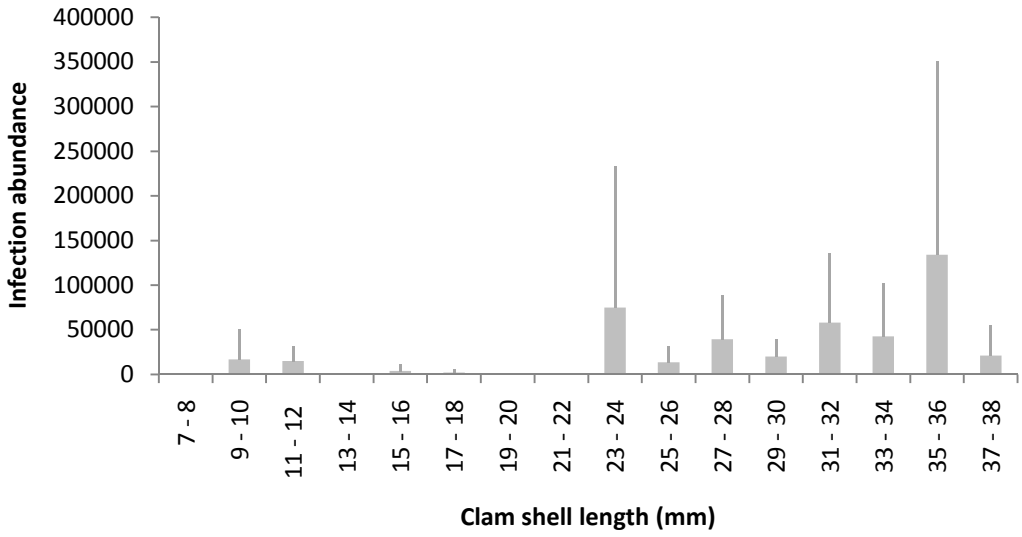
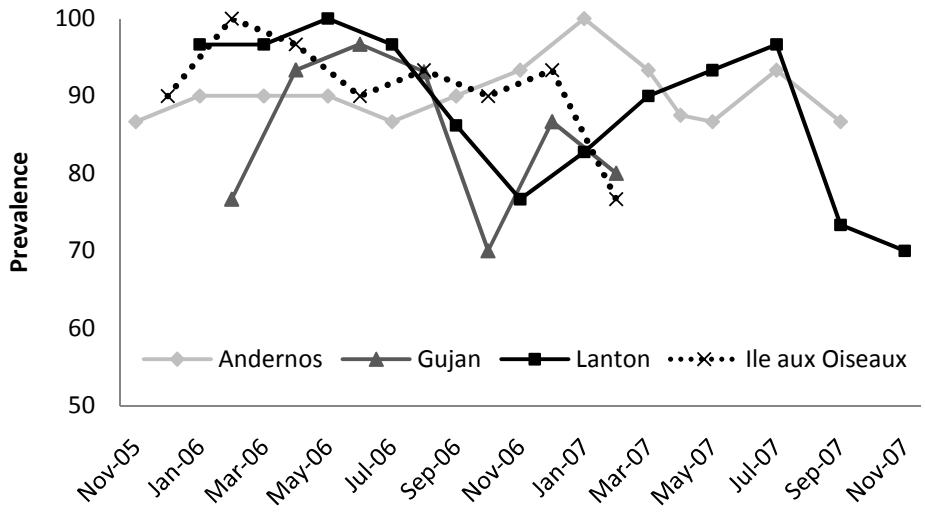


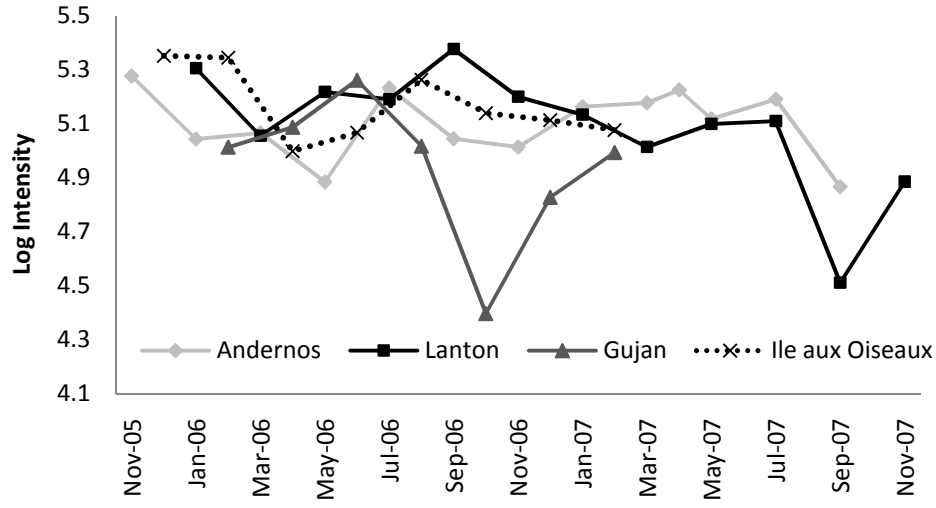
Figure 4.3. Size-specific *Perkinsus* sp. abundance (cells. g^{-1}. wet gill) ± SE (2-mm clam shell length categories) at Lanton (n = 4).

3.3. Temporal survey of four sites

Prevalence at the four inner sites was high, between 70 and 100% (Fig. 4.4a). Average yearly prevalences were not different between sites (one-way ANOVA, p = 0.56).



a



b

Figure 4.4. Prevalence (a) and mean intensity in logarithmic scale (b) of perkinsosis in the four inner sites of Ile aux Oiseaux, Andernos, Lanton and Gujan, from November 2005 to November 2007.

Mean intensities of infection were high, between 4 and 5 in logarithmic scale. No significant difference was found between sites ($F = 2.2$, $df = 3$, $p = 0.08$) (Fig. 4.4b) and with time ($F = 0.74$, $df = 6$, $p = 0.61$), with no significant interaction ($F = 1.2$, $df = 18$, $p = 0.26$). The average intensity (time and site) was $130 \times 10^3 \pm 6.7 \times 10^3$ (± SE) cells per gram of wet gill tissues.

3.4. Purification kinetics

In order to obtain information on the purification dynamic, clams originating from Lanton were held at Arguin for one year and a half. Mean infection abundance in a log scale (Fig 5) showed a significant decrease (regression, $r = -0.56$, $df = 15$, $p = 0.024$) (Fig. 4.5). Nevertheless, mean infection abundance remained high, with $120 \times 10^3 \pm 32 \times 10^3$ (± SE) cells g^{-1} wet gill at the start and $33 \times 10^3 \pm 10 \times 10^3$ (± SE) cells g^{-1} wet gill at completion. A significant decrease was also observed in the survey of control clams at Lanton (regression, $r = -0.79$, $df = 7$, $p = 0.019$) (Fig. 4.5). Purification did not depend on site (two-way ANOVA, $F = 0.04$, $df = 1$, $p = 0.84$) but was dependent on time (two-ways ANOVA, $F = 2.68$, $df = 7$, $p = 0.01$). These two factors did not interact (two-ways ANOVA, $F = 0.66$, $df = 7$, $p = 0.7$).

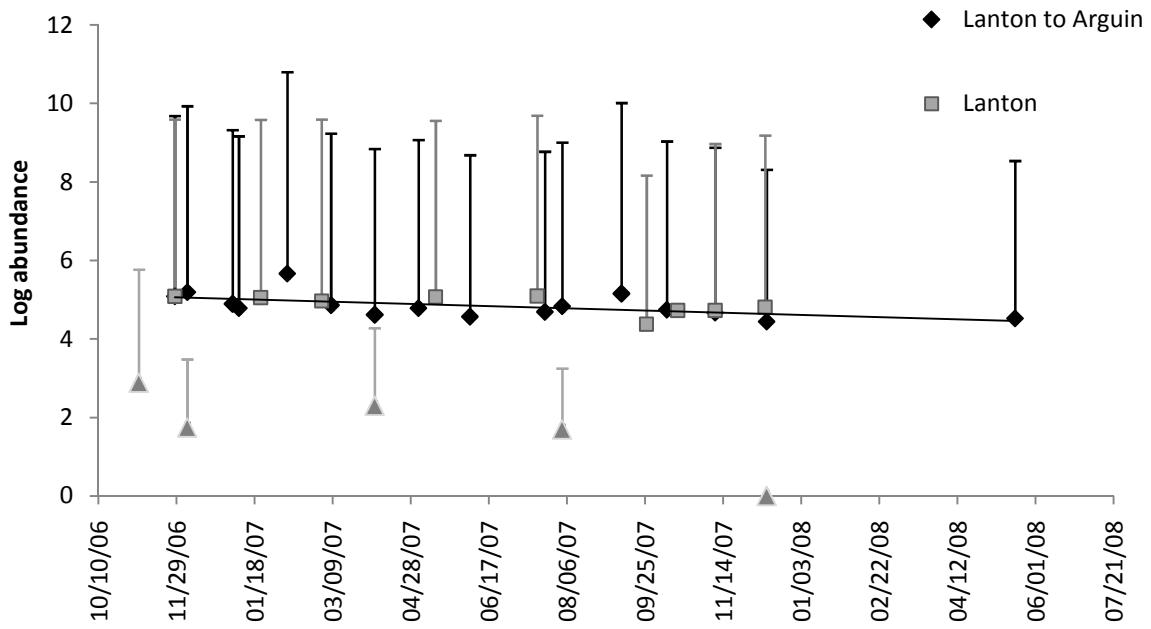


Figure 4.5. Mean infection abundance (cells. g^{-1}. wet gill) ± SE in logarithmic scale of perkinsosis in clams transplanted from Lanton to Arguin.

3.5. Infection kinetics

In order to understand the *Perkinsus* sp. infection dynamic, a reciprocal transplant experiment from Arguin to Lanton was realized. It revealed a stable mean infection abundance (± SE) with 0.76 x 10^3 ± 0.76 x 10^3 cells g^{-1} wet gill at the start and 1.3 x 10^3 ± 0.4 x 10^3 cells g^{-1} wet gill at completion (regression, r = 0.29, df = 16, p = 0.262) (Fig. 4.6).
In contrast, clams implanted from Arguin to Andernos in June 2006 (t-test, $p < 0.001$), to Gujan in May 2007 (t-test, $p < 0.05$) and to Ile aux Oiseaux in April 2006 (t-test, $p < 0.001$) developed a significantly increased *Perkinsus* sp. burden. The highest increase was at Andernos, where the mean abundance burden reached 160 x 10^3 ± 43 x 10^3 (± SE) cells g^{-1} wet gill at experiment completion. It was 0.76 x 10^3 ± 0.76 x 10^3 (± SE) cells g^{-1} wet gill at the start. At Ile aux Oiseaux, *Perkinsus* sp. mean abundance reached 35 x 10^3 ± 17 x 10^3 (± SE) cells g^{-1} wet gill in October 2007 after having been 0.76 x 10^3 ± 0.76 x 10^3 (± SE) cells g^{-1} wet gill in April 2006.

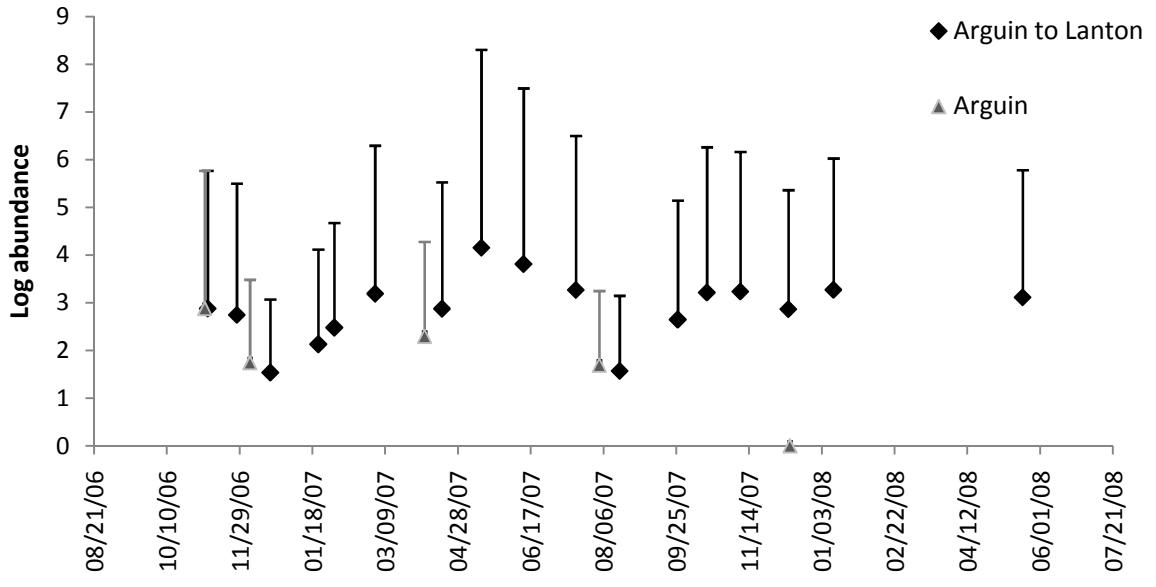


Figure 4.6. Mean infection abundance (cells. g^{-1}. wet gill) ± SE in logarithmic scale of perkinsosis in clams transplanted from Arguin to Lanton.

4. Discussion

No seasonality of perkinsosis was apparent in Arcachon Bay during this two-year survey. The stability of *Perkinsus* sp. infection could be explained by two hypotheses: 1) infection and purification did not occur or were negligible during the period of experiment; or 2) the stability of infection resulted from the same quantity of parasites acquired by and purged from the host.

The trend of decrease after peaks of infection intensity generally coincides with the death of the most heavily infected clams or with the regression of infection (Villalba et al. 2005). Considering that purification is a very slow process, decrease in infection intensities thus corresponded either to mortality or to appearance of a low-infected cohort. According to the low growth rate in Arcachon Bay (Dang et al. in revision-a), the latter hypothesis is doubtful. No changes in the physical environment (a low salinity event, for instance) could be related to the decrease of infection at Gujan from August 2006 to October 2006.

Salinity is an important abiotic factor structuring the density and prevalence of *Perkinsus* sp. The decrease in salinity induced by punctual events such as heavy rains could affect the local development of the disease (Leite et al. 2004). When *P. marinus*-infected oysters are transferred to a low salinity site, disease progression and mortality are delayed, suggesting a physiological effect of salinity on the parasite (Ray and Mackin 1954, Andrews and Hewatt 1957). For *P. olseni*, the optimal range of salinity is 25-35 (Auzoux-Bordenave et al. 1995). High infection intensity and prevalence of *P. olseni* generally correspond to high salinities (Ray and Mackin 1954, Andrews and Hewatt 1957, Burreson and Ragone Calvo 1996, Cigarría et al. 1997, Park and Choi 2001). In the present study, salinity could partly explain *Perkinsus* sp. distribution throughout the bay, with a negative correlation obtained between the number of days where salinities are below 30 and perkinsosis mean abundance. Moreover, the lowest mean abundances were found at the Leyre River mouth. However, Park and Choi (2001) showed that perkinsosis was absent from sites where salinity remained constant all year long. This could explain why perkinsosis was almost absent at Arguin (stable salinity year-round), even though this site presented the highest salinity of the bay. The stable salinity recorded all year at Arguin may explain the lack of perkinsosis. As well, Choi et al. (2002) reported that *Perkinsus* infection level relates significantly to sediment type: clams living on muddy flats tend to have higher levels of infection in comparison to those on sandy tidal flats.

In contrast to the present study, a seasonal cycle of infection intensity of *Perkinsus* sp. was observed during a five year survey of *Ruditapes decussatus* in Galicia, Spain (Villalba et al. 2005). Highest values of *Perkinsus* sp. infection were found from spring to early autumn, associated with increasing temperatures (above 15°C). Infection regression occurred in winter due to lower temperatures (annual minimum 9-10°C) (Villalba et al. 2005). Similarly, La Peyre et al. (2002) showed that *P. olseni* presented its highest metabolic activity at 15°C. The increasing north-south latitudinal gradient of *Perkinsus* sp. reported in Lassalle et al. (2007) confirmed that this protozoan prefers higher temperatures. Chu et al. (1996) and Park and Choi (2001) reported that high water temperatures enhance infection intensity and prevalence of *P. marinus*. However, no relation between spatio-temporal patterns and temperature could be deduced from this study.

The mean infection abundance at the end of the purification experiment was 3.8 times lower than at the beginning (14 mo after). However, even if environmental conditions at Arguin are not favorable for *Perkinsus* sp. development, purification was similar to that in the inner bay, where conditions are favorable for *Perkinsus* sp. development. Purification appeared to be a slow phenomenon, independent of the site as well as local conditions. Moreover, it seems that low salinity events have little impact on the *Perkinsus* sp. purification rate within the bay. Indeed, the Leyre River discharge was strongly higher in March 2006, March 2007 and June 2007 relative to the same months of the twenty past years (Météo France data). Precipitation was substantially higher relative to the twenty past years in March 2006, February 2007, March 2007 and May 2007 (Météo France data). These outputs of fresh water did not induce a decrease of *Perkinsus* sp. infection at studied sites, showing that the *Perkinsus* sp. at Arcachon is little affected by low salinity events.

In the reciprocal transplant experiment, *Perkinsus* sp. mean abundance evolution depended on the destination site. No infection occurred at Lanton between November 2006 and May 2008, although it is reputed to be a high infected site. However, infection occurred at Andernos between June 2006 and October 2007, at Ile aux Oiseaux between April 2006 and October 2007, and to a lesser extent at Gujan between May 2007 and October 2007. The greatest infection acquisition occurred at Andernos. These four inner sites are separated by a few kilometers. In the case of Lanton, no infection was observed for two years, suggesting that the *Perkinsus* sp. load in clams was acquired at least two years ago and hardly decreased. This dynamic (punctual high infection and high infection stability) explains that any seasonal

pattern was observed. Consequently, the hypothesis that the *Perkinsus* sp. load remained stable because of an equilibrium between infection and purification is not supported.

In conclusion, the infection phenomenon at Arcachon Bay appeared to be a punctual event in space and in time. The high infection load observed in Arcachon Bay was the result of few multiple infection events. This could suggest that large clams that have endured several infection events during their life spans, which explains why they were more infected than small clams. The present study showed that clams of 9-10 mm shell length are infected by *Perkinsus* sp. and that infection significantly increased with clam shell length. A similar association between infection intensity and clam size was observed in *R. decussatus* from Galicia (Villalba et al. 2005) and in *R. philippinarum* from Korea (Park et al. 1999, Park and Choi 2001). Infection was never found in carpet-shell clams < 20-mm shell length in Spain (Villalba et al. 2005), and the threshold was 15 mm shell length in Manila clam from Korea (Choi and Park 1997).

In the transplant experiment, clams from Arguin were placed into enclosures close to clams coming from the inner bay, i.e. infected by perkinsosis (Andernos, Lanton, Gujan and Ile aux Oiseaux). As no infection occurred at Lanton, the contamination between proximate live individuals did not happen. This indicated that the *Perkinsus* sp. present at Arcachon did not have a significant direct transmission from live host to live host. Transmission of *Perkinsus* sp. between clams apparently differed from that of *P. marinus* in oysters which occurs with host-spawning, excretory activities, alternate host or vector activities, heterotrophic parasite proliferation, or periodic resuspension of parasite cells present in the sediment (Bushek et al. 2002, Ragone Calvo et al. 2003). The principal mode of *P. marinus* transmission, however, is via the direct dissemination of parasite cells from dead oysters (Bushek et al. 2002, Ragone Calvo et al. 2003). Significant clam mortality may be required for substantial transmission of *Perkinsus* sp. at Arcachon as well.

Chapitre 5

Impact de la perkinsose sur la croissance et l'indice de condition des palourdes *Ruditapes* spp.

Correlation between *Perkinsus* sp. abundance, growth and condition in clams *Ruditapes* spp.

Dang C., de Montaudouin X., Bald J., Soudant P.

In preparation.

Perkinsus olseni dans des branchies de palourdes (coloré au lugol)

Abstract

Perkinsosis is one of the most widespread diseases affecting commercial important species of mollusks throughout the world. The present study aimed to evaluate the impact of *Perkinsus* sp. on the growth and condition index of the carpet-shell clam *Ruditapes decussatus* from the Mundaka Estuary and the Manila clam *R. philippinarum* from Arcachon Bay. Infected clams from Arcachon and Mundaka presented white nodules at the surface of gill, mantle, siphon and foot. A two-year field growth experiment of tagged-recapture clams was performed to determine the clam growth rate as well as condition index (CI) and *Perkinsus* infection intensity. During the time of the experiment, the *Perkinsus* sp. load did not vary. No correlation was observed between perkinsosis presence, growth rate and CI. In *R. decussatus* from Mundaka Estuary, the infection decreased the growth rate whereas in *R. philippinarum* from Arcachon Bay, interactions between perkinsosis and clam growth and condition depended on the studied station. Even if clam growth and condition could be modified by *Perkinsus* infection, the impact of this disease was considered as very low. Nevertheless, perkinsosis weakened clams by taken energy and inducing an immune response. This resulted in great consumption of energy at the expense of physiological activity of the clam. The impact of perkinsosis on mollusks depends especially on infection intensity level but also to environmental conditions.

Keywords: clams, *Perkinsus* sp., *Ruditapes philippinarum*, *Ruditapes decussatus*, growth, condition index

1. Introduction

Among molluscan diseases, perkinsosis has led to the most severe economic losses prompting considerable study of *Perkinsus* parasites. Protozoans of the genus *Perkinsus* have been associated with extensive mortalities in various commercially important species of mollusks including the eastern oyster *Crassostrea virginica* in the USA (Andrews and Hewatt 1957), the tridacnid clam *Tridacna gigas* in Australia (Goggin and Lester 1987), the carpet-shell clam *Ruditapes decussatus* in Spain and Portugal (Ruano and Cachola 1986, Azevedo 1989) and the Manila clam *Ruditapes philippinarum* in Korea (Choi and Park 1997). *Perkinsus* sp. infections were also associated with a major decline in clam *R. philippinarum* production in Japan (Hamaguchi et al. 1998), and with massive mortalities in cultured and wild Manila clams in South Korea (Choi and Park 1997, Park and Choi 2001). In Europe, *Perkinsus* sp. was observed in *R. philippinarum* and *R. decussatus* from Spain (Figueras et al. 1992, Navas et al. 1992, Elandaloussi et al. in press), Italy (Da Ros and Canzonier 1985, Da Ros et al. 1998) and France (Goggin 1992, Lassalle et al. 2007, Flye-Sainte-Marie 2008). This parasite also induced mortalities that could compromise clam production along the Atlantic and Mediterranean coasts of Spain (Rodríguez and Navas 1995, Sagristà et al. 1996).

Progression of *Perkinsus* sp. infection through host tissues causes lesions that may result in host death. The inflammatory reaction in venerid clams (Navas et al. 1992, Sagristá et al. 1995, Montes et al. 1996) primarily involves encapsulation of the parasite cells by haemocytes. Heavy infections produce lesions that are visible with naked eye. Normal structure of host tissues is lost and white milky nodules or pustules appear (Lee et al. 2001, Choi et al. 2002). At a sublethal level, hosts expend significant energy to counter the progression of the disease. Therefore, *Perkinsus* sp., at a sublethal stage of infection, is expected to alter reproduction, growth and condition index of infected hosts. However, there is a lack of information concerning these sublethal effects.

Mundaka Estuary (north-east coast of Spain) harbors a carpet-shell clam (*R. decussatus*) population which is not exploited. Preliminary monitoring showed high *Perkinsus* sp. intensities that may effect clam growth and condition. Arcachon Bay, a 180-km^2 lagoon on the southwestern Atlantic coast of France is well known for its oyster farming. It is also an important site for the French Manila clam *R. philippinarum* fishery but recent surveys revealed a dramatic decrease of the standing stock (Caill-Milly et al. 2008). This situation prompted several analyses concerning population dynamics (Dang et al. in revision-a) and

pathogens loads (Dang et al. 2008, Dang and de Montaudouin 2009, Dang et al. 2009), including *Perkinsus* sp.
Manila clams from Arcachon Bay and carpet-shell clams from Mundaka Estuary are infected by an unidentified *Perkinsus* species.

The aim of our study was to correlate *Perkinsus* intensity in clams with clam growth rates and condition index, through an *in situ* caging experiment with tagged bivalves. Such study necessitated to verify that *Perkinsus* load did not fluctuate during the experiment in order to know whether the parasite abundance at experiment completion was representative of the abundance during elapsed time.

2. Materials and Methods

2.1 Study areas

Carpet-shell clams (*Ruditapes decussatus*) were collected from the Mundaka Estuary (Spain, 43°22'N 2°43'W) and Manila clams (*R. philippinarum*) from Arcachon Bay (France, 44°40'N 1°10'W) (Fig. 5.1).

The Mundaka Estuary is a shallow meso-tidal 13-km estuary in the south-east of the Bay of Biscay (Fig. 5.1). This system is dominated by euryhaline waters at high tide and by polyhaline waters at ebb tide (Villate 1997). Carpet-shell clams were collected from the outer part of the estuary on a stony bank. This part of the estuary was composed of large intertidal flats opened to the sea.

Arcachon Bay is a 180-km² semi-sheltered lagoon on the southwest coast of France (Fig. 5.1). Fresh waters are released into the bay mainly by the Leyre River but also by many streams situated around the lagoon (Fig. 5.1). The lagoon receives marine waters from the Atlantic Ocean. The mixture of marine and continental water inputs as well as the slow renewal by tide result in temperature and salinity gradients throughout the bay (Plus et al. 2006). Muddy intertidal flats colonized by a vast *Zostera noltii* sea grass bed dominated the inner part of the lagoon (110 km^2) whereas intertidal sand flats (Arguin) are located at the mouth of the lagoon where it meets the Atlantic Ocean. Manila clams inhabit both of these

two contrasting environments. Experiments of the present study took place at the oceanic site of Arguin and at the inner site of Ile aux Oiseaux (Fig. 5.1). A preliminary survey showed that perkinsosis was rare at Arguin but that clams from Ile aux Oiseaux were heavily infected by *Perkinsus* (Dang et al. in revision-b).

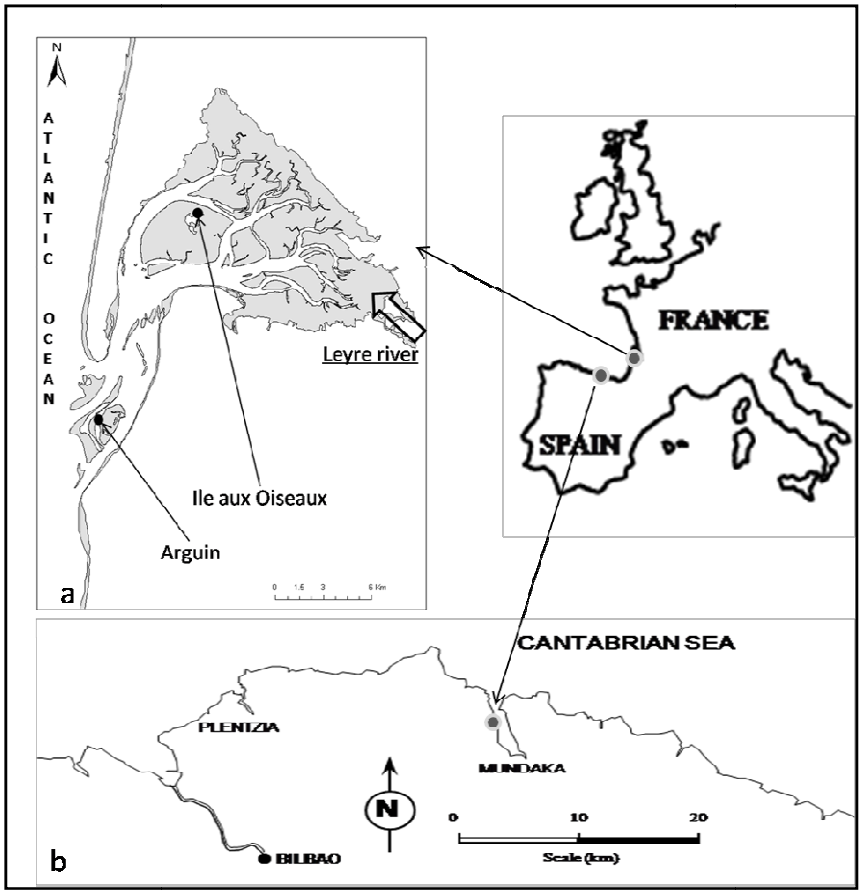


Figure 5.1. Map showing sampling sites of (a) Arguin and Ile aux Oiseaux in Arcachon Bay and (b) Mundaka Estuary.

Environmental parameters at both Arcachon Bay sites have already been characterized (Dang et al. in revision-a). Sediments at Ile aux Oiseaux and Arguin differ in median grain size (97 and 360 µm), organic matter content (13% and 1%, respectively) and silt and clay proportion (42.4% and 3.5%, respectively). Sediment temperature fluctuations are high at both sites (Arguin: minimum: -0.2, maximum: 30 and mean: 15.1°C; Ile aux Oiseaux: minimum: 0.2, maximum: 37.9 and mean: 16.1°C). The salinity is higher and more stable at Arguin (minimum: 1.2, maximum: 35.4, mean: 34.2) than at Ile aux Oiseaux (minimum: 12.1, maximum: 34.8, mean: 29.6).

2.2. Enclosure experiments

2.2.1. Mundaka Estuary

In order to observe the impact of perkinsosis intensity on clam growth and condition, carpet shell clams between 5 and 48 mm shell length were collected in December 2005 at Mundaka Estuary. Each clam was labelled with a numbered tag and measured to the closest 0.1 mm shell length with a calliper. Clams were subsequently planted in three enclosures at a density of 320 ind m^{-2}. Enclosures were emptied of native bivalves before the experiment. Each enclosure occupied a 0.25-m^2 surface and sides were covered with a 2-mm mesh plastic net to avoid clam migration. We assumed that enclosures represented a barrier for clams but had little effect on local environmental conditions. The three enclosures were situated at the same elevation above low tide level, i.e. 1.7 m, as the area covered was small and the bottom gradient was minimal. To compensate for natural mortality (Dang et al. in revision-a), new tagged clams of similar shell-length distributions were introduced three times into the enclosures. Clams were recaptured at the completion of the trial in October 2007. In order to verify, the stability of infection, thirty clams were collected in December 2005 and their *Perkinsus* abundance compared with that in enclosures at completion of the experiment.

2.2.2. Arcachon Bay

The same procedure was performed at Arcachon Bay. To observe the impact of perkinsosis on clams, transplantion of infected clams to two sites of contrasted environmental features was performed: Arguin (oceanic site) and Ile aux Oiseaux (higher continental influence) (Dang et al. in revision-a). Manila clams with shell-length between 6 and 44 mm were collected at Ile aux Oiseaux (infected site) in Arcachon Bay in December 2005. Each clam was tagged, measured and released into enclosures at a density of 320 ind m^2 that corresponded to a biomass of 24.2 g Shell-Free Dry Weight (SFDW) m^{-2}. Three enclosures were placed at each sites (Ile aux Oiseaux, Arguin) at various tidal levels. At Ile aux Oiseaux, enclosure LTL (low tidal leel) was situated at 1.8 m, enclosure MTL (medium tidal level) at 2.03 m and enclosure HTL (high tidal level) at 2.6 m. At Arguin, enclosures LTL, MTL and HTL were positioned at 1.8, 2.1 and 2.9 m respectively. In October 2007, clams were recaptured and analyzed. Between December 2005 and October 2007, new tagged clams of similar size distribution and origin were introduced three times into enclosures to compensate natural mortality (Dang et al. in revision-a). In order to evaluate the stability (or not) of

Perkinsus load in clams, thirty clams were also collected in December 2005 at the beginning of the trial. Perkinsosis intensity was compared to intensity obtained at the end of the experiment (October 2007) at the Ile aux Oiseaux and Arguin enclosures.

2.3. Clams analyses

At the end of the experiment, each clam shell was measured to the nearest 0.1 mm. Growth rate was calculated by the following equation: $G = \left[\left(L_f - L_i\right) \times 1000\right]/\left(t_f - t_i\right)$. G was the growth rate in µm day^{-1}, L_f was the final shell length (mm), L_i was the initial shell length (mm), t_f was the final time, i.e. the number of days accumulated at completion of the experiment, and t_i represented the initial time.

Clams were dissected and their gills were excised to quantify *Perkinsus* sp. infection by the Fluid thioglycolate medium (FTM) method of (Ray 1952) as modified by (Choi et al. 1989). Gills were incubated in 10 mL of FTM supplemented by 100µL of antibiotics (streptomycin and penicillin G) and 500 µL of antifungal (Nystatin). The incubation lasted an average of five days in the dark at room temperature. After this time, the FTM was discarded following centrifugations and gill tissues were digested twice with 2N NaOH at 60°C for 1 hour. Then, the resulting solution was centrifuged and the supernatant discarded. The pellet was rinsed two times in a Phosphate Buffered Saline (PBS 1X) solution and finally resuspended in 1 mL of PBS. Next, *Perkinsus* sp. hypnospores were enumerated with a Malassez chamber by light microscopy. Finally, *Perkinsus* sp. was reported to a number of cells per gram of wet gill tissues. The intensity of the disease was defined as the mean number of parasite cells per diseased clam.

Remaining tissues were collected in order to calculate the condition index (CI) (Walne and Mann 1975) following the relation:

$CI\ (‰) = \text{dry flesh weight (mg)}/\text{dry shell weight (g)}$.

The total dry flesh weight was calculated by adding dry flesh weight and dry gill weight. Dry gill weight was obtained by multiplying the wet gill weight by 0.153 (Flye-Sainte-Marie 2008).

2.4. Statistical data treatments

2.4.1. Growth rate and condition index versus Perkinsus *sp. presence*

Prior to analysis, infected individuals were selected in order to match shell length distribution of non-infected clams and to avoid a size dependent effect (de Montaudouin et al. 2000). For Ile aux Oiseaux and Arguin, condition index (CI) and growth rate of clams infected by *Perkinsus* were compared with those of clams free of this parasite by a two-way Anova (Sokal and Rohlf 1981) with tidal level and infection as fixed factors. A one-way Anova was used for Mundaka (infection only). Where Anova results, were significant Tukey post-hoc tests were used to identify different groups of stations.

2.4.2. Growth rate and condition index versus Perkinsus *sp. intensity*

In order to show the influence of *Perkinsus* sp. on clam growth, regressions were performed for each site between shell length and growth rate, and between shell length and *Perkinsus* sp. intensity. Residuals were extracted from each of these regressions. Then, to consider only interactions between *Perkinsus* sp. and growth rates independent of shell length, a regression was performed between residuals of growth rate and residuals of *Perkinsus* sp. intensities from both previous regressions (Leung and Poulin 2007). The same procedure was used to observe interactions between CI and *Perkinsus* sp. intensity.

2.4.3. Infection and purification

To verify the stability of *Perkinsus* intensity during our experiment, a t-test was carried out between values at start and at completion of the experiment for Arcachon and Mundaka.

3. Results

3.1. Effect of perkinsosis presence on clam condition and growth

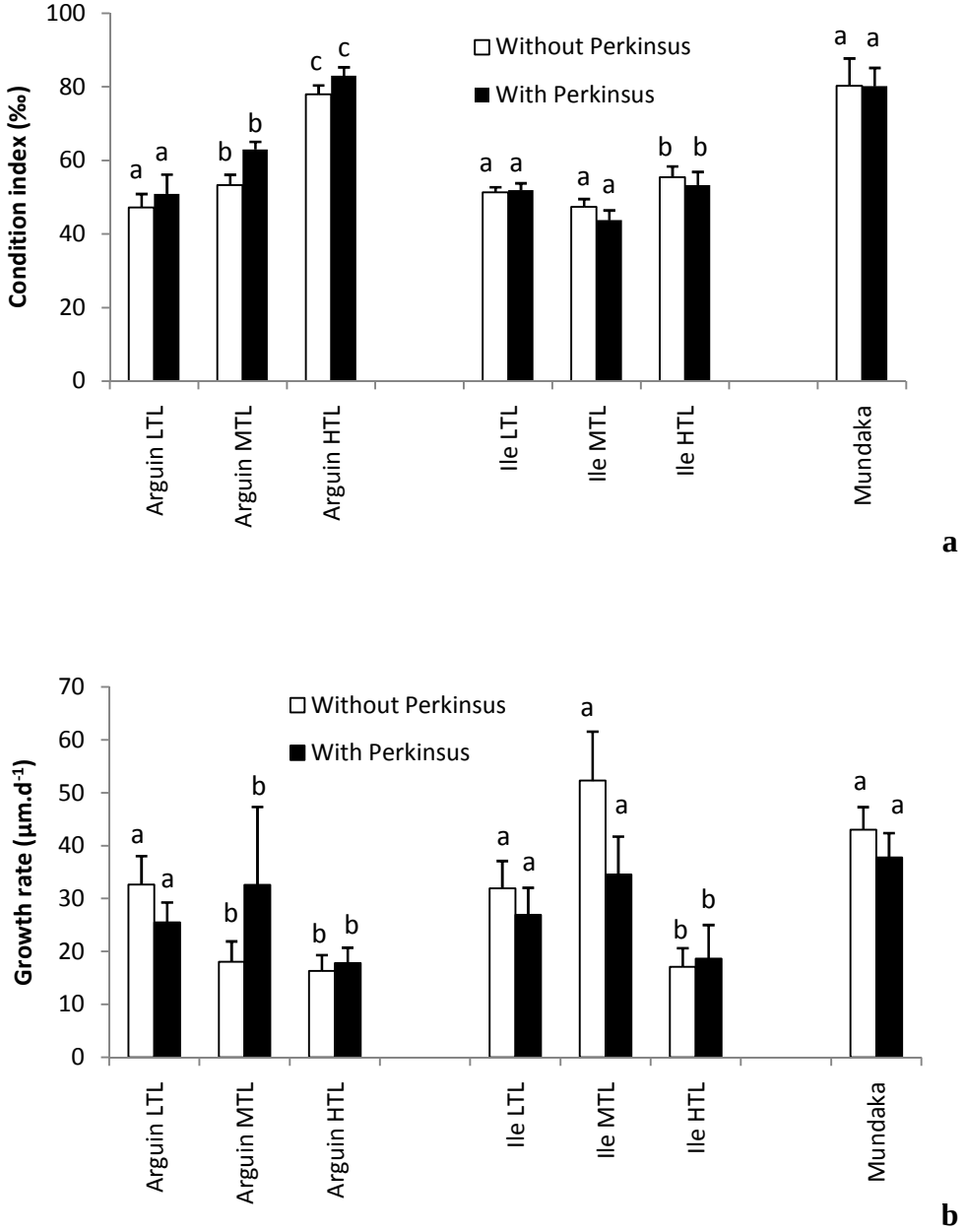


Figure 5.2. (a) Condition index (± SE) and (b) growth rate (± SE) of healthy and infected *Perkinsus* sp. infected clams at each site. The letters above the histograms illustrate the significant difference between the mean values, as a result of Tukey test.

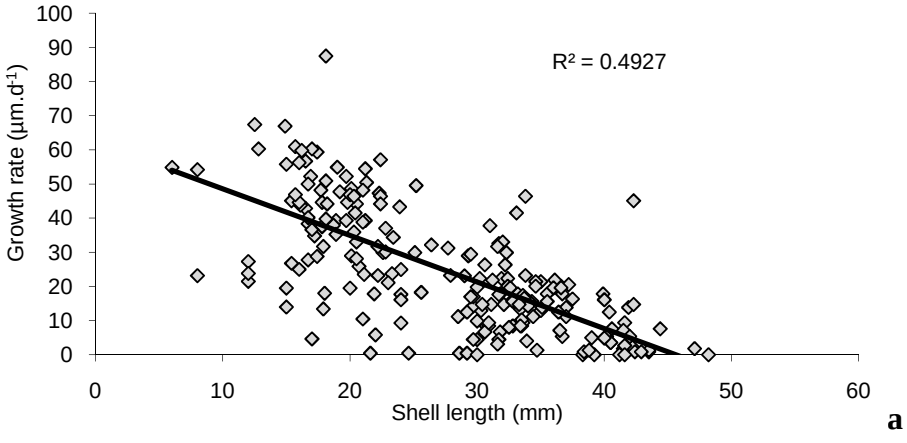
In Mundaka Estuary, *Perkinsus* sp. infection affected neither condition index (p = 0.99) (Fig. 5.2a) nor growth rate (p = 0.41) (Fig. 5.2b).

Condition indices (CI) at Arcachon were greater at the high tidal levels (HTL) (Ile aux Oiseaux: p = 0.007; Arguin: p < 0.0001) but were not affected by *Perkinsus* sp. infection (Ile aux Oiseaux: p = 0.43; Arguin: p = 0.19). Interactions between tidal level and *Perkinsus* sp. infection was not significant (Ile aux Oiseaux: p = 0.7; Arguin: p = 0.3) (Fig. 5.2a). Growth rates at Ile aux Oiseaux and Arguin decreased with higher tidal level (p < 0.001). Growth rate did not differ between infected and uninfected clams at either site (p = 0.18 and p = 0.49 respectively) (Fig. 5.2b). Interactions between tidal level and *Perkinsus* sp. infection was not significant (p = 0.35 and p = 0.6 respectively).

3.2. Growth rate and condition index in relation with *Perkinsus* sp. infection

*3.2.1. Mundaka (*Ruditapes decussatus*)*

At the end of the experiment, mean logarithmic infection intensity was 5.31 cells g^{-1} and the mean growth rate was 24.4 µm d^{-1} (Table 5.1). Growth rate and CI were negatively correlated with clam shell length (Table 5.1, Fig. 5.3a). Conversely no significant relationship was found between *Perkinsus* sp. infection intensity and clam shell length (Table 5.1, Fig. 5.3b). The analysis of residual regressions revealed negative correlations between *Perkinsus* sp. residuals and growth rate residuals (Table 5.1, Fig. 5.3c), and between *Perkinsus* sp. residuals and CI residuals (Table 5.1). However, determination coefficients were extremely low ($R^2 < 0.08$), highlighting a poor contribution of *Perkinsus* sp. to explain CI or growth rate variation.



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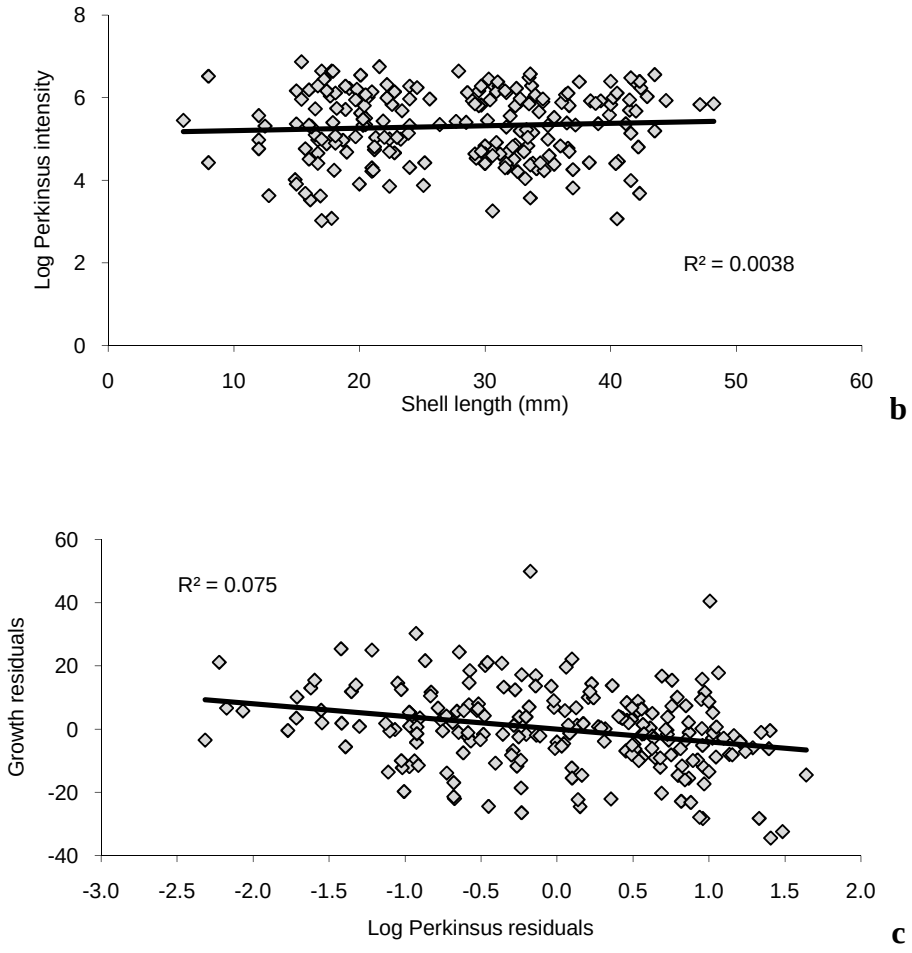

Figure 5.3. *Ruditapes decussatus* from Mundaka Estuary. Relationships between (a) clam growth rate (µm j^{-1}) and clam shell length (mm); (b) *Perkinsus* sp. infection intensity in logarithmic scale and clam shell length (mm); (c) regression residuals of clam growth rate obtained in (a) and regression residuals of *Perkinsus* sp. infection intensity obtained in (b).

*3.2.2. Arguin (*R. philippinarum*)*

Growth rate was the highest at the intermediate tidal level (Table 5.1). Clam growth was positively correlated with clam shell length at all tidal levels whereas *Perkinsus* sp. load was correlated with clam shell length for the two lower tidal levels only (Table 5.1). Growth residuals and *Perkinsus* sp. residuals were correlated only at the highest tidal level whereas correlations between CI residuals and *Perkinsus* sp. residuals were significant at the two lower levels (Table 5.1). However, as already found in Mundaka with *R. decussatus*, determination coefficients R^2 were very low.

Table 5.1. Means of *Perkinsus* sp. infection intensity in logarithmic scale (P), growth rate in µm d^{-1} (G) and condition index (CI) in ‰ in clams from each of the following enclosures: Mundaka, Arguin (Arg) LTL (low tidal level), Arguin MTL (medium tidal level), Arguin HTL (high tidal level), Ile aux Oiseaux (Ile) LTL, Ile aux Oiseaux MTL and Ile aux Oiseaux HTL. Presented *r* results is from different regressions between G, L (length), P, CI, R_G (residuals of growth rate), R_P (residuals of *Perkinsus* sp. infection intensity) and R_{CI} (residuals of condition index). Boldface *r* represents significant interaction ($P < 0.05$).

	Mundaka n=211	Arg LTL n=45	Arg MTL n=91	Arg HTL N=98	Ile LTL N=62	Ile MTL N=20	Ile HTL N=51
P	5.31	4.41	4.50	4.46	4.40	4.13	4.65
G	24.4	15.8	20.9	13.0	23.3	23.0	15.5
CI	77.95	49.86	62.73	78.62	46.72	38.60	48.11
G=f(L)	**-0.7**	**-0.69**	**-0.31**	**-0.69**	**-0.56**	**-0.51**	**-0.45**
P=f(L)	0.06	**0.44**	**0.26**	-0.06	-0.22	-0.26	-0.03
CI=f(L)	**-0.16**	-0.10	**0.31**	**-0.51**	**-0.63**	**-0.78**	**-0.62**
R_G=f(R_P)	**-0.27**	-0.28	-0.15	**-0.26**	-0.11	-0.11	-0.09
R_{CI}=f(R_P)	**-0.18**	**0.37**	**0.31**	-0.02	0.22	-0.40	0.04

*3.2.3. Ile aux Oiseaux (*R. philippinarum*)*

Clams from Ile aux Oiseaux presented lower CI than clams from Arguin. Conversely, mean growth rate was higher than at Arguin (Table 5.1). Negative correlations were observed between shell length and growth rate as well as between shell length and CI (Table 5.1). No relation was observed between *Perkinsus* sp. intensity and shell length. Residuals analysis indicated that *Perkinsus* sp. infection intensity, growth rate and CI were not correlated with each others (Table 5.1).

3.3. Infection and purification experiment

The infection load did not vary at Mundaka ($p = 0.07$) between the 675 days of the experiment whereas it decreased at Arguin ($p = 0.003$) and at Ile aux Oiseaux ($p = 0.008$). During the experiment, *Perkinsus* abundance increased by 6.1% at Mundaka whereas it decreased by 7.2% at Arguin and 10% at Ile aux Oiseaux.

4. Discussion

The present study showed that: 1) condition index of clams increases with the tidal level without any effect of presence/absence of *Perkinsus* sp.; 2) shell growth rate decreases with the tidal level without any effect of presence/absence of *Perkinsus* sp.; 3) within the infected clam population, the number of *Perkinsus* sp. has a minute effect on clam growth rate and CI.

To develop and proliferate, a parasite absorbs nutrients at the expense of its host. Defense responses against *P. marinus* and *P. olseni* have been found to be energetically costly to their hosts (Park et al. 2006). A defense reaction as well as the energy absorbed by *Perkinsus* sp. directly from its clam could consume much energy at the expense of physiological activities like reproduction, growth and consequently condition index (CI). (Villalba et al. 2004) reported that infection induced a negative effect on growth and condition, as a consequence of a decrease of the available energy in the infected host.

White creamy nodules have been observed in Manila clams from Arcachon Bay and carpet-shell clams from Mundaka Estuary. These nodules correspond to a high accumulation of *Perkinsus* sp. as well as tissue destruction. White nodules are very common in heavily infected clams. They were observed in *R. philippinarum* from Korea (Lee et al. 2001, Park and Choi 2001) and Japan (Choi et al. 2002) and have been described as the result of hemocytic encapsulations of trophozoïtes and massive hemocytic infiltrations producing tissue inflammation.

The accuracy of our experiment in Mundaka Estuary was underscored by the unchanged concentration of parasites cells between beginning and end of experiment. Growth rate significantly decreased with high *Perkinsus* loads, but the residuals analysis showed that

perkinsosis explained only 7% of clam growth variation, which is negligible. This suggested that at a concentration of 10^6 parasites per gram of wet tissue, perkinsosis could affect physiological function of clams. (Choi et al. 1989) calculated that 10^6 cells of *P. marinus* per gram of wet tissue in *Crassostrea virginica* may exceed the net production of the host oyster. Conversely, the influence of perkinsosis on CI can be considered as negligible at Mundaka. At the opposite, a significant decline in CI was observed in clams *R. decussatus* infected by *Perkinsus* sp. in Spain and Portugal (Rodríguez Moscoso et al. 2002, Villalba et al. 2005) (Leite et al. 2004) (Villalba et al. 2000), and in oysters *Crassostrea virginica* in US (Andrews 1961, Ford and Smolowitz 2007). A reduction of oyster growth due to *P. marinus* has also been recorded (Andrews 1961).

In infected *R. philippinarum* from Arcachon Bay, the effect of perkinsosis depended on the sampled sites. At Arguin, the overall impact of *Perkinsus* sp. on condition index and growth rates was small and at lower tidal level only. At Ile aux Oiseaux, with a similar range of infection, this impact was null. Consequently, the effect of perkinsosis in clams is moderate and depends on the parasite intensity but also on environmental conditions. Conflicting results have been reported on the effect of perkinsosis in different species of mollusks. (Goggin 1996) reported that *P. olseni* did not affect the wet tissue weight of the infected tridacnid clam *Tridacna gigas*. (Elston et al. 2004) reported a negative and significant correlation between the intensity of *Perkinsus* sp. infections and the CI of Manila clams from the Pacific coast of North and Central America. (Choi et al. 2002) did not observe an obvious correlation between infection intensity and the CI. Even if (Andrews 1961) found an effect of *P. marinus* on the growth of *C. virginica*, (Ford and Smolowitz 2007) did not document a consistent negative effect on shell growth. In Spain, no influence of *Perkinsus* sp. on *R. philippinarum* growth was noticed (Cigarría et al. 1997), but in that study, clams presented a very low prevalence.

Consequently, the impact of perkinsosis on mollusks could vary according to the infection intensity level and with the environmental condition of the studied site. Unfavorable environmental conditions could worsen the impact of *Perkinsus* sp. on clams. For instance, under certain conditions like high water temperature and low food abundance, the parasite's energy consumption in adult clams with strong infections would exceed the energy available for growth (Casas 2002 in (Villalba, 2004 #78). This would result in a lower CI and a lower growth rate. Furthermore, to explain the influence of perkinsosis on clam health, besides the fact that *Perkinsus* sp. consumes energy at the expense of clam, a high concentration of

Perkinsus in gills may decrease filtration efficiency. This could lead to a decrease of oxygen and food availability for clams and have direct repercussions on clam metabolism.

La maladie du muscle marron (BMD)

Chapitre 6

La maladie du muscle marron (BMD), pathologie émergente des palourdes japonaises dans le bassin d'Arcachon

Brown muscle disease (BMD), an emergent pathology affecting Manila clam *Ruditapes philippinarum* in Arcachon Bay (SW France**)**

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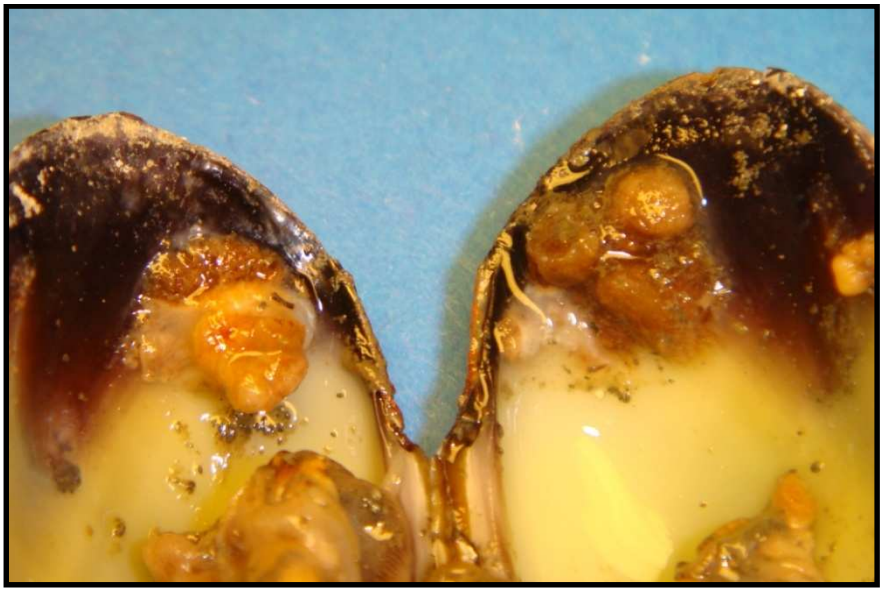


Muscle postérieur de palourde japonaise affecté par la BMD

Abstract

We describe an emerging pathology, brown muscle disease (BMD), which specifically affects the Manila clam *Ruditapes philippinarum* in Arcachon Bay (France). BMD induces a transformation of the posterior adductor muscle which becomes infused by conchiolin and calcified, reducing the ability of clams to bury. The disease affects both types of muscular tissue, with striated muscle becoming affected to a higher degree than smooth muscle. Two indices were created to quantify the symptoms: the Muscle Print Index, used for empty and live shells, and the Final Disease Index utilized for live clams only. Histological sections were made and observed under light microscopy to examine the muscular damage and to investigate a causal agent. Sections revealed an important inflammatory response with a large invasion of hemocytes into tissues and a heavy necrosis of muscular fibers. Additionally, molecular biology analyses were carried out to search for bacteria and protozoan agents using generic primers. In both histological and molecular assays, bacteria and protozoans were discounted. We monitored 4 sites scattered around the bay over 2 yr. The mean prevalence was 12% without seasonal variation in 3 sites against 30% and a winter peak in 1 site. The latter site was accurately surveyed and revealed that clams at the sediment surface (abnormal position) were affected 3 times more frequently than buried clams (normal position).

Key words: Brown muscle disease, Clam, *Ruditapes philippinarum*, Adductor muscle, Pathology, Light microscopy

1. Introduction

The Manila clam *Ruditapes philippinarum* is one of the most fished and farmed bivalve mollusks in the world. This species originates from the Indo-Pacific region (Ponurovsky and Yakovlev 1992). Since the beginning of the 20th century, it has been introduced in different parts of the world with the Pacific oyster *Crassostrea gigas* seeds (Flassch and Leborgne 1992). In Europe, *R. philippinarum* was first introduced for culture purposes to France in 1972 and later to England, Spain and Italy (Flassch and Leborgne 1992). Within a few years this species had established natural populations in these European countries, particularly along the French Atlantic coast. It was introduced to Arcachon Bay (SW France) in 1980 where it rapidly escaped from parks, colonized seagrass *Zostera noltii* beds and underwent intensive exploitation by fishermen. In 2006, Arcachon Bay harbored the most important stock of Manila clams in France (7600 metric tonnes, mt) and ranked first in terms of national production (450 mt yr^{-1}) (Caill-Milly et al. 2006).

Many infectious agents can infect the Manila clam, but the main pathologies affecting this bivalve are Brown Ring Disease (BRD) (Paillard 1992, 2004) and perkinsosis (Fouche et al. 1997, Ngo and Choi 2004, Villalba et al. 2004). BRD is induced by the bacterium *Vibrio tapetis* which disrupts the production of the periostracal lamina and causes an obvious abnormal conchiolin deposit on the inner side of the shell, forming a characteristic brown ring (Paillard 1992, Paillard and Maes 1995a, b). This disease led to mass mortalities in Brittany (NW France) in 1987 and decimated stocks of cultured clams (Paillard 2004). Subsequently, BRD was observed along the French Atlantic coast and in other countries including Spain, Ireland, England, Italy and Korea (Paillard 2004, Park et al. 2006). Perkinsosis, caused by the protozoan *Perkinsus* sp. affects numerous molluscan species all over the world and can lead to mass mortalities (Azevedo 1989, Burreson and Ragone Calvo 1996, Goggin 1996, Da Ros et al. 1998, Park and Choi 2001, Leite et al. 2004, Cremonte et al. 2005, Villalba et al. 2005). In Korea, this parasite has been the cause of a severe decrease in clam populations since 1993 (Park and Choi 2001). Previous studies in Arcachon bay revealed that clams could be infected by brown ring disease, perkinsosis (Lassalle et al. 2007) and also by digenean trematodes (de Montaudouin et al. 2000). However, prevalence and infection intensity were relatively low.

Mortalities have been evident in Arcachon Bay in recent years, particularly during winter. A preliminary study revealed a previously undescribed necrosis of the posterior adductor muscle. Macroscopic diagnosis under stereomicroscope eliminated the possibility

that macroparasites such as *Polydora* spp. (annelid), *Cliona* spp. (sponges) and trematodes were involved. As the posterior muscle was visually affected by the disease, we only considered this tissue in our study. Histopathological analysis of clam adductor posterior muscles has been undertaken in epizootiotopes since November 2005. The purpose of the present study was to describe the pathology of the disease designated herein as 'Brown Muscle Disease' (BMD) and to monitor temporal variations over a 2-yr survey program. Preliminary investigations of etiological agents are also included.

2. Materials and methods

2.1. Study area

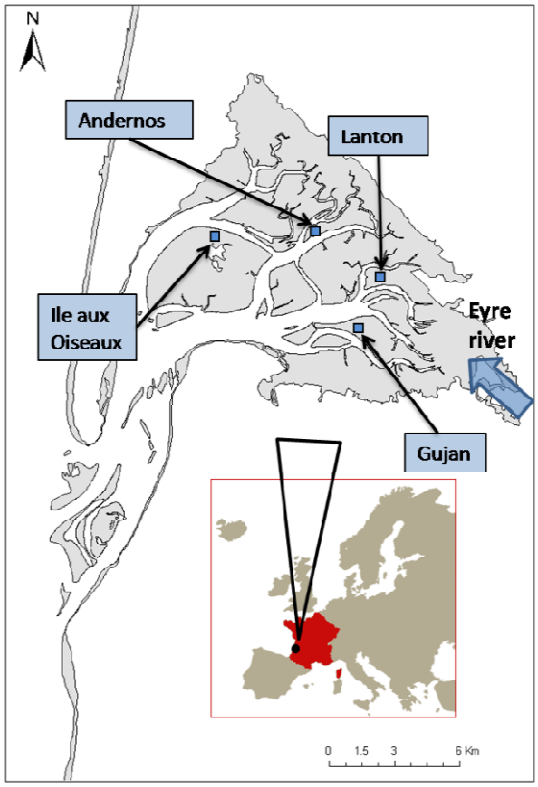


Figure 6.1. Location of sampling stations in Arcachon Bay.

Arcachon Bay (44°40' N, 1°10' W) is a 156 km^2 semi-sheltered lagoon in the southwest of France (Fig. 6.1). Tidal flats represent 110 km^2, with 70% covered by *Zostera noltii* seagrass beds and colonized by the Manila clam (Caill-Milly et al. 2003). Arcachon Bay is subject to both oceanic and continental influences, with a semi-diurnal macrotidal rhythm. External neritic waters enter through 2 channels situated at the southwest end of the lagoon and meet with freshwater inputs, principally from the Eyre River (Fig. 6.1). Clams are usually situated in the mid intertidal zone but can be found from 2.75 m above the 0 of low tide (Cottet et al. 2007) to the tidal channel. The 2 yr temporal survey was carried out at 4 different sites (Fig. 6.1) where the seagrass bed generally flourished on muddy sediments.

2.2. Environmental parameters

From November 2005 until November 2007 the sediment temperature was measured every hour using *in situ* electronic devices. Sediment granulometry was determined using a laser-diffractometer Malvern Master Sizer. This device uses a laser beam to determine the properties of diffraction and diffusion of particles. Water salinity data were provided by IFREMER Arcachon.

2.3. Histological assay

Ten clams were collected for each of the 3 disease stages, healthy, intermediate and advanced. Posterior adductor muscles were dissected, fixed in Bouin's fixative, dehydrated in ethanol followed by xylene, embedded in a paraffin-wax block and cut in a microtome. Sections (5 µm thick) were first deparaffinized, then rehydrated and finally stained with May-Grunwald-Giemsa. Histological sections of *Ruditapes philippinarum* muscle were observed by light microscopy for diagnosis of pathological alterations.

2.4. Molecular biological assays: DNA extraction and PCR analysis

In order to attempt the identification of a pathogen agent, DNA was extracted from the muscles of 5 infected clams and 5 healthy clams. Muscles were incubated in an extraction buffer (0.1 M NaCl, 20 mM EDTA, 0.3 M Tris) with proteinase K (0.2 mg ml^{-1}) and sodium dodecyl sulfate (0.6%) until complete digestion.Deproteinisation was then carried out using phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform, followed by a precipitation of nucleic acids (AcNa, 3M, 0.1 × vol; absolute ethanol, 2 × vol). The resulting pellet was washed in 70% ethanol and resuspended in H_2O with RNase (20 µg ml^{-1}). Extraction efficiency was verified by migration of DNA isolated in a 1% agarose Tris Acetic acid EDTA (TAE) buffer gel and stained with ethidium bromide (0.2 µg ml^{-1}). The universal primer pair for eukaryotic 18S rDNA CAS1S (GGAATTGACGGAAGGGCACC)/CAS2 (ACGGGCGGTGTGTACAAAGG) was used to control the quantity of DNA as well as the presence of inhibitory factors (Le Roux et al. 1999). Negative control was H_2O. After one hundredth dilution of our samples, the expected PCR product was obtained for reference genes.

Occurrence of bacteria and protozoans was investigated using universal primers described for these organisms. The amplification of bacterial DNA was carried out with the primer pair Eubu 1492 R (TACGGTTACCTTGTTACGACTT)/Eubu 27 F (AGAGTTTGATCATGGCTCACA) (Lane 1991) and the protozoan's DNA amplification with the primers 18 S Euk 581 F (GTGCCAGCAGCCGCG) / 18 S Euk 1134 R (TTTAAGTTTCAGCCTTGCG) (Carnegie et al. 2003).

PCR reactions were performed in 50 µl containing 2 µl of isolated DNA, 0.5 µl of each universal primer (100 µM), 3 µl of $MgCl_2$ 25 mM; 10 µl of 5× Go Taq flexi buffer; 1 µl of dNTP (10 mM), 0.2 µl of Taq polymerase (5 u μl^{-1}). PCR was conducted on an Eppendorf thermocycler and had the following profile for bacteria: 95°C for 1 min; 35 cycles of 95°C for 1 min, 48°C for 1 min, 72°C for 1 min and 72°C for 5 min, and for protozoans: 95°C for 1 min; 35 cycles of 95°C for 1 min, 62°C for 1 min, 72°C for 1 min and 72°C for 5 min. Positive controls were *Escherichia coli* and *Perkinsus* sp. for bacteria and protozoan primers respectively, and negative controls were H_2O. PCR products were analyzed on a 1% agarose TAE buffer gel stained with ethidium bromide.

2.5. Temporal survey

One hundred adult clams (30 to 40 mm) were collected monthly from December 2005 to February 2007 at the Ile aux Oiseaux and Gujan sites, and from December 2005 to November 2007 at Andernos and Lanton (Fig. 6.1). Clams were collected from their normal, i.e. buried, position. Individuals were opened, and muscle prints were observed on the inner side of the shells under a stereomicroscope to quantify the pathology. The term 'muscle print' defines the muscle scar with pieces of affected calcified muscle still attached to the shell (see Fig. 6.2a). The surface of the muscle print was divided into 4 equal sectors. The Muscle Print Index (MPI) was used to designate the surface colonized by the brown muscle print on a scale of 0 to 4 as follows: 0 (healthy), 1 (0–25%), 2 (25–50%), 3 (50–75%) and 4 (75–100%). When both valves displayed different pathology indices, the highest category was selected to characterize the stage of BMD. The advantage of MPI was that it enabled us to measure the pathology intensity on empty shells that were kept dry. Prevalence and MPI were determined monthly at each site.

2.6. Lanton survey

Ruditapes philippinarum, like many bivalves, possesses 1 anterior and 1 larger posterior adductor muscle. Each muscle consists of a smooth and a striated tissue (Fig. 6.2). At the most affected of the 4 sites (Lanton), a specific survey was carried out to distinguish the pathology intensity in both muscular tissues and both positions of clams, buried (normal) or at the surface (abnormal). Buried (N = 50) and unburied (N = 50) clams were collected monthly from November 2006 to April 2007.

The volume of each affected muscular tissue was evaluated. Each tissue was evaluated twice on a scale of 0 to 4 to describe (1) the affected depth and (2) the affected surface. For each tissue, the depth value was multiplied by the surface value to obtain a volume value from 0 (healthy) to 16 (totally diseased). As for the MPI, the highest value for each striated and each smooth tissue was selected and, thus, 2 Final Disease Indices (FDI) were obtained for each clam. These FDIs give a better diagnosis of the pathology but can be applied only when muscle tissues are still present. Four FDI stages were defined, based on the FDI values: Stage a (1–4), b (5–8), c (9–12) and d (13–16). Prevalence was calculated for buried and unburied clams.

Finally, in order to assess pathology specificity, other bivalves were collected and dissected between October 2006 and May 2007. One hundred cockles *Cerastoderma edule* (23–36 mm) and 100 carpet shell clams *Ruditapes decussatus* (14–23 mm) were processed.

3. Results

3.1. Environmental parameters

The environmental parameters displayed heterogeneity of the Manila clam habitat. Salinity and sediment temperature varied between sites and fluctuated yearly between 4 and 35 psu and between –2 and 44°C, respectively (Table 6.1). The sediment grain-size is also variable between sites. The median was 163 µm at Andernos, 78.5 µm at Lanton, 97 µm at Ile aux Oiseaux and 68.7 µm at Gujan (Table 6.1).

Table 6.1. Environmental variables characterizing the four sites: grain size (median in µm), sediment temperature (°C) and salinity (psu).

Site	Sediment grain size	Sediment temperature			Salinity		
		Min	Max	Mean	Min	Max	Mean
Andernos	163.0	-1.0	35.4	15.8	18.5	34.5	30.0
Lanton	78.5	-1.7	37.8	16.0	4.8	34.4	26.7
Ile aux Oiseaux	97.0	0.2	37.9	16.1	12.1	34.8	29.6
Gujan	68.7	-0.2	43.7	16.1	4.8	34.4	26.7

3.2. Clinical signs of BMD

All disease signs were visible to the naked eye and BMD was only observed in the posterior muscle. Diseased clams exhibited pathological signs, such as muscle atrophy and degraded tissues. The muscle becomes progressively brown and hard (Fig. 6.2), the brown color being linked to the diffusion of conchiolin within the muscle. Calcification was assumed because of the positive reaction with HCl. Three different macroscopic states of infection were noted: healthy, intermediate and advanced stages. In the intermediate stage, the striated muscle was often the first and the most severely affected. The first gross sign of the disease

was the appearance of one or several large yellowish nodules within the striated muscle and also of minute brown spots on the adductor muscle attachment. The nodules increased in size and the tissues became increasingly brown (conchiolin) and hard (calcified). First, the soft tissues of the muscle became brown (conchiolin) and in the next stage the muscle became hard, i.e. it calcified. BMD reduced the effective area of attachment of the adductor muscle to the shell. In the advanced stage, the smooth muscle was also modified and the striated muscle was completely brown and calcified as was the majority of the smooth muscle (Fig. 6.2). At the end, both muscular tissues were completely infused with conchiolin and calcified.

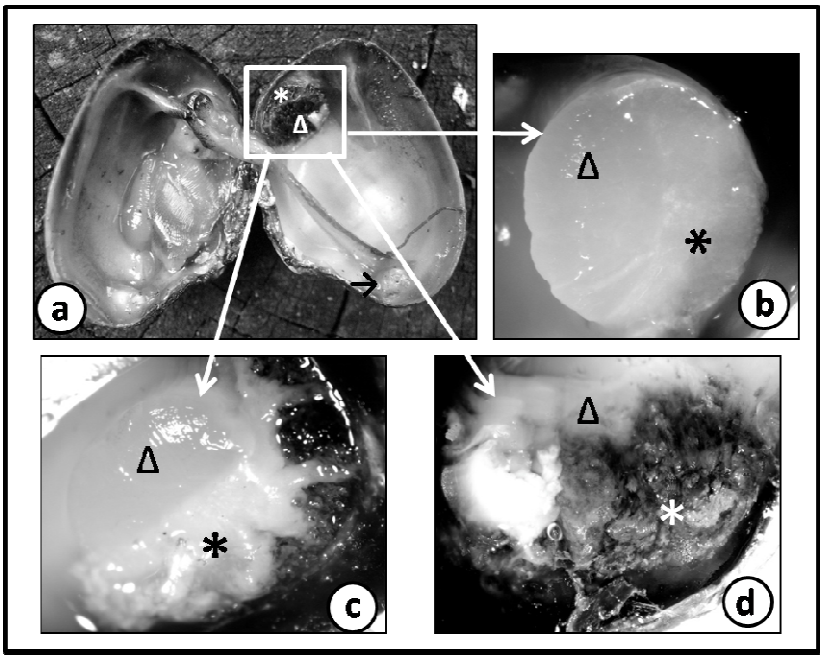


Figure 6.2 (a to d). External appearance of Manila clam posterior muscle (three macroscopic stages) with the distinction between striated (*) and smooth (Δ) muscles. (a) Whole clam with focus on "muscle print" (muscle scar and attached remnant muscle tissues) showing a brown posterior muscle (MPI = 4) (→**:** anterior muscle always normal); (b) Healthy muscle (MPI = 0); (c) Intermediate stage with the beginning of the brown conchiolin infiltration and calcification. The striated muscle is the first to become affected (MPI = 2); (d) Ultimate stage with the striated muscle completely brown and calcified as well as a major part of the smooth muscle (MPI = 4).

The survey of the Lanton site confirmed that the striated muscle was the first and the most infected of the 2 muscular tissues. Out of 600 analyzed clams, prevalence of BMD was 41.5% for smooth muscle and 48.7% for striated muscle. A percentage comparison test showed a significant difference between both muscles ($p < 0.05$). Stage a dominated in smooth muscle with 43% and Stage d prevailed in striated muscle with 38% (Fig. 6.3). The disease reached maximum intensity (Stage d) in 38% of clams for striated muscle compared with 20% in smooth muscle (Fig. 6.3). In the infected clams, 98% of striated muscles were infected, while disease did not spread to the smooth muscle in around 16% of the clams.

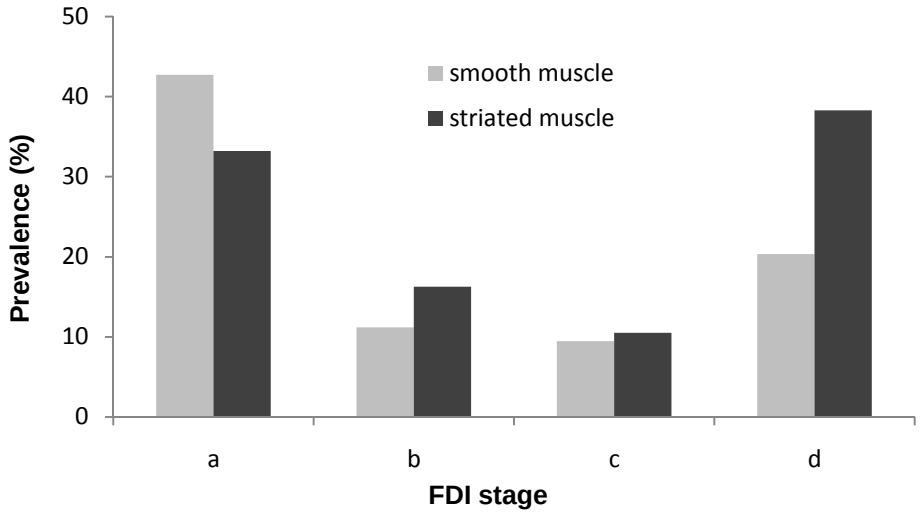
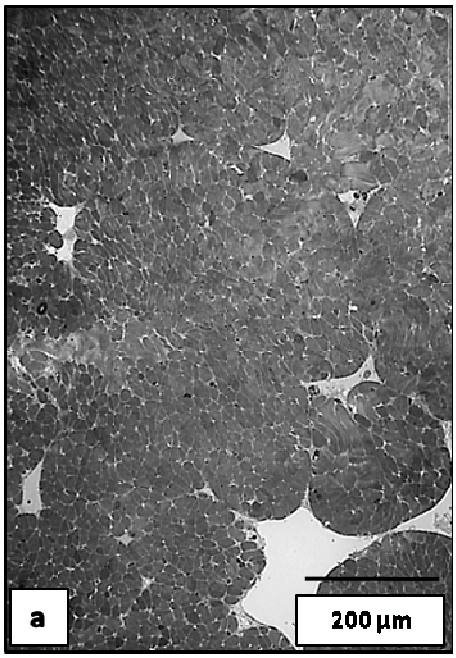


Figure 6.3. Final disease index (FDI) for smooth and striated muscle in Lanton (N = 600).

3.3. Histopathology

The 3 macroscopic stages —healthy, intermediate and advanced—were used for histological investigations (Fig. 6.4). The severity of the muscular damage corresponded to the intensity of coloration (from yellow to brown) and to the hardness of the tissue. In the healthy stage, the muscular fibers of the striated muscle were organized normally and composed of several myofibrils (Fig. 6.4). In the intermediate stage, the striated muscle displayed disorganized necrotic muscular fibers with a loss of surface adherence between muscular cells, a loss of muscle bundle orientation and substantial atrophy. Muscular fibers disappeared and were progressively replaced by intense hemocytic infiltration (Fig. 6.4b, d).


a
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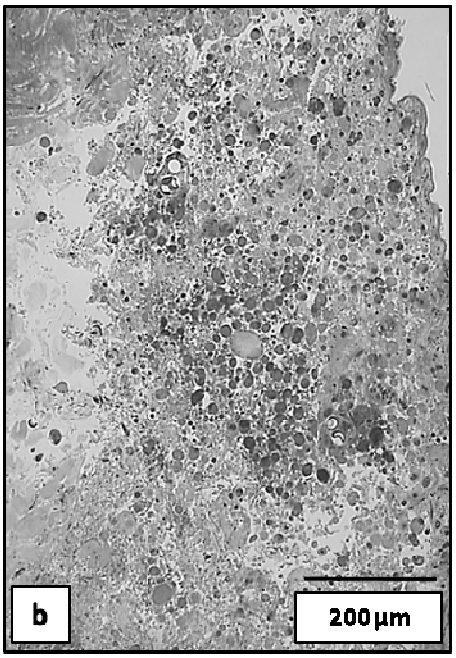
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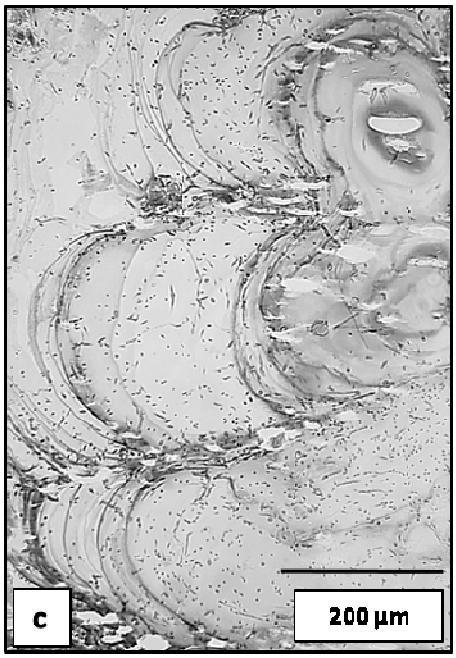
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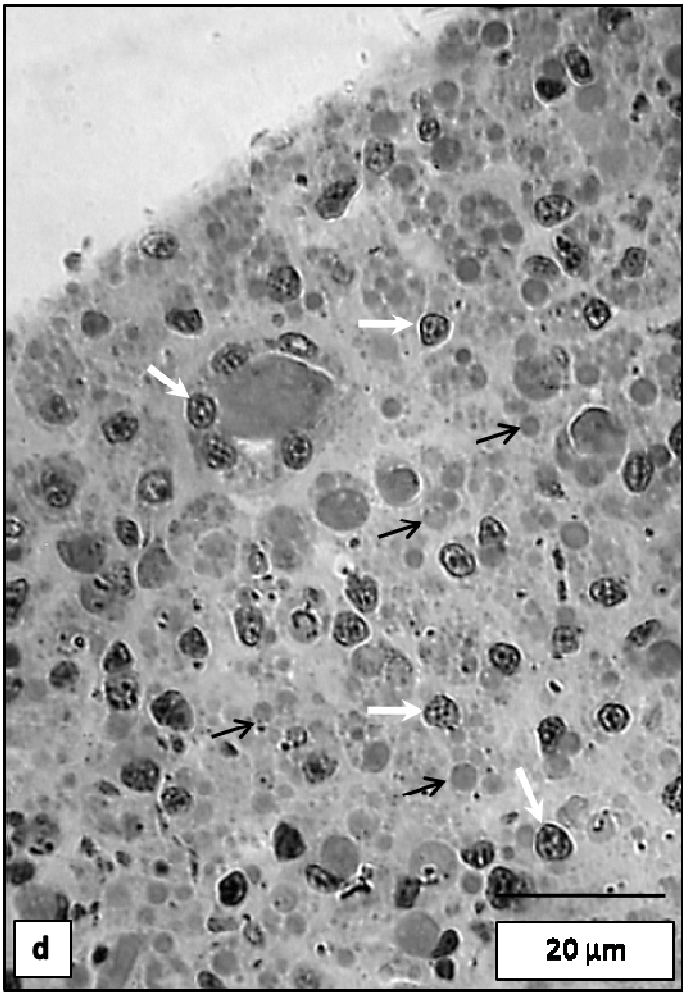

d
20 µm

Figure 6.4. (a) Histological sections of healthy clam and (b to d) diseased clam stained by May-Grunwald-Giemsa; (a) Muscular striated fibers of healthy clam (MPI = 0); (b) Section of diseased clam showing necrosis of muscular fibers and an intense hemocytic infiltration (MPI = 2); (c) Ultimate stage of the disease corresponding to a completely brown calcified muscle; no structure is recognizable (MPI = 4). (d) View of granulocytes with pycnotic nuclei (white arrows) and granules (black arrows).

These hemocytes were composed of numerous basophilic granulations and consequently were assumed to be granulocytes (Fig. 6.4d). These cells possessed pathological peripheral pycnotic nuclei. Several granulocytes can amalgamate to improve the defense mechanism of clams. The intensity of granulocytic concentration differed within different degrees of tissue destruction, thus indicating the progression of the pathology. Between these defense cells, remnants of muscular tissues were present (Fig. 6.4d). The advanced stage of the pathology (Fig. 6.4e, f) is characterized by the total destruction of the muscle, which was completely necrotized. Neither muscular fibers nor cells such as hemocytes were observed; all functional structures had disappeared due to calcified necrosis (Cabanne and Bonenfant 1986). Thus, no bacterial, protozoan, metazoan or fungal organisms were observed in these sections.

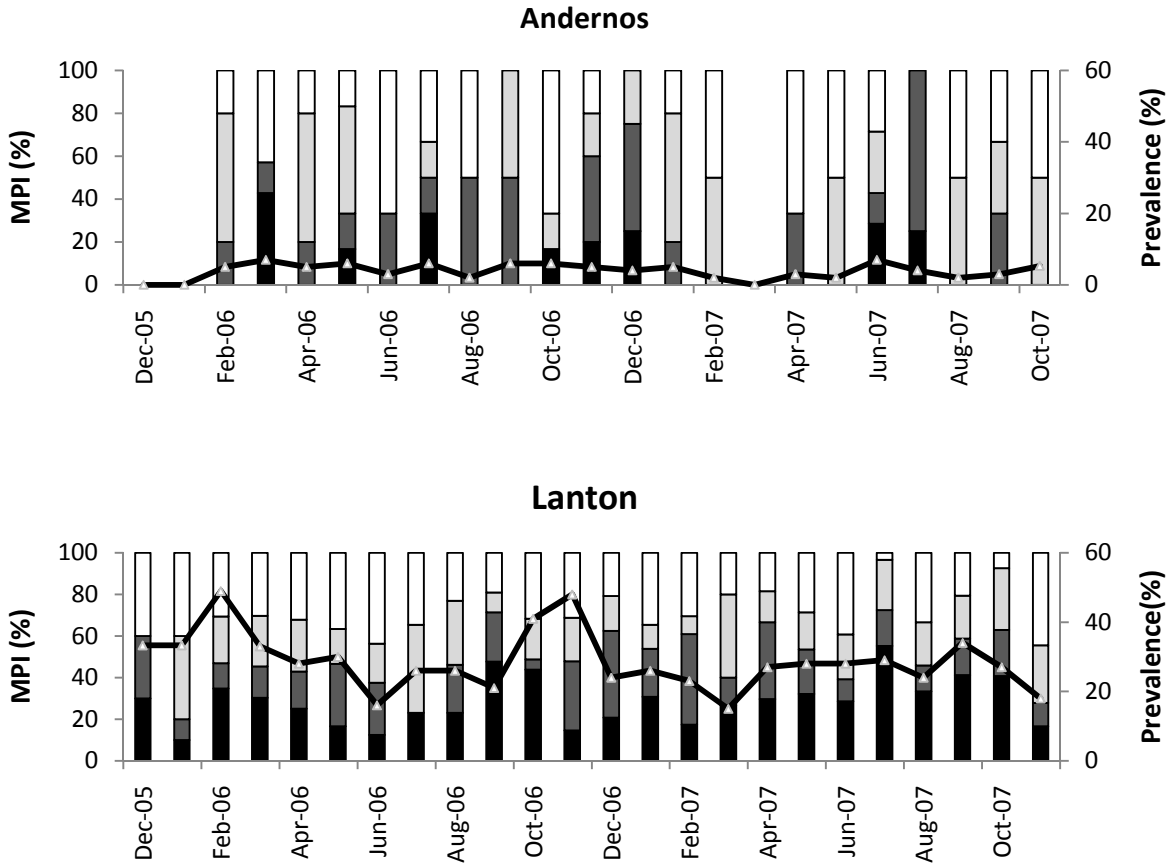
3.4. Molecular biological assays

With respect to the occurrence of bacteria, no PCR products were obtained in either healthy or infected muscles, while a band was observed with the positive control. Similar results were achieved for protozoans and some fungus species analyses. Consequently, PCR did not provide evidence of bacteria, protozoans or some fungi.

3.5. Temporal survey

Between December 2005 and November 2007, prevalence of BMD was between 0 and 48%, and was significantly different between sites (1-way ANOVA, $\arcsin\sqrt{p}$ transformed data, $p < 0.05$) (Fig. 6.5). A Tukey test distinguished 3 groups ($p < 0.05$): (1) Andernos, (2)

Gujan and Ile aux Oiseaux, (3) Lanton. Andernos appeared to be the least infected site with a mean prevalence of 4.5%. Ile aux Oiseaux and Gujan formed a second group with a mean BMD prevalence of 12%. No seasonal variations were observed in these 3 sites, in contrast to Lanton where the mean prevalence was 30.4%, with 2 significant peaks ($p < 0.05$) during cold periods in February 2006 and in November 2006 (prevalence of 48%).



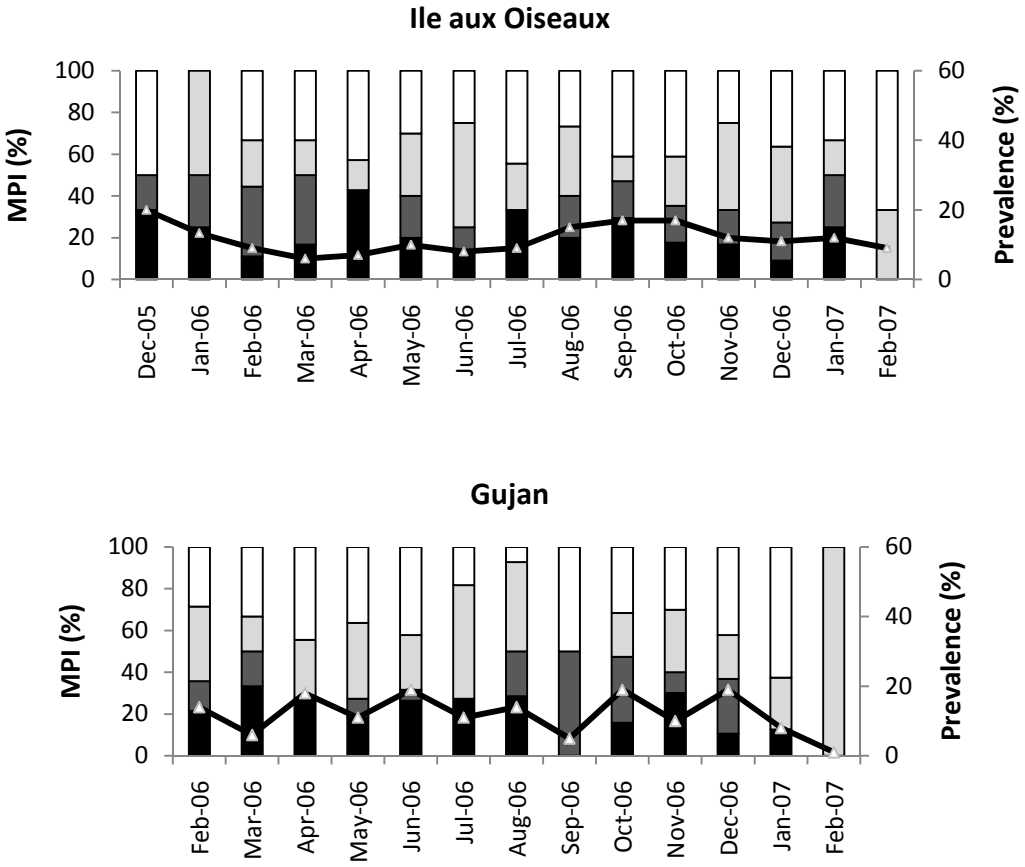


Figure 6.5. Evolution of the prevalence (—) and the muscle print index (MPI) with time in the four sites. (□ stage 1; ▫ stage 2; ▪ stage 3; ■ stage 4).

The intensity of the pathology was assessed by means of the MPI (Fig. 6.5). In contrast to prevalence, intensity was not highest during cold months. In Andernos, where the lowest prevalence was observed, MPI Stages 1 and 2 dominated with a respective mean of 31 and 34.3%. No seasonality was observed for prevalence, but the highest values of Stage 4 were found in March 2006 (42.8%), in July 2006 (33.3%) and in June 2007 (28.5%). Stages 1 and 2 prevailed in Gujan with 32.8 and 33.6% respectively. No important variations were shown, but Stage 4 was present each month during sampling, with the exception of September 2006 and February 2007. The same dominance was observed at Ile aux Oiseaux: Stage 1 (35.3%), 2 (26.8%), 3 (17.1%) and 4 (20.8%). Lanton differed from the other sites with the highest percentage of Stage 4 (28.9%). The high prevalence observed in Lanton during winter was not correlated with an increase of the MPI stages.

3.6. Lanton survey

The prevalence of infected clams was significantly higher ($p < 0.05$) in surface clams (78.3%) than in buried clams (26.8%). Furthermore, the MPI stages were higher in unburied than buried clams (Fig. 6.6). BMD reached maximum intensity in Stage 1 for buried clams (33.3%) and in Stage 4 for surface clams (58.9%).

BMD affected the 2 muscles differently, depending on the clams living position. Our observations of BMD prevalence in both muscle tissues reinforced the findings of the histological and macroscopic analyses, with the striated being the most heavily infected muscle. For smooth muscle, disease prevalence was 19% in buried clams compared with 64% in surface clams. For striated muscle, prevalences were 27% in buried clams compared with 71% in surface clams. In both cases (surface and buried), BMD prevalence was significantly different ($p < 0.05$) between smooth and striated muscles and also between surface and buried clams for each muscle.

Neither the 100 sympatric cockles *Cerastoderma edule* nor the 100 *Ruditapes decussatus* clams were infected by BMD.

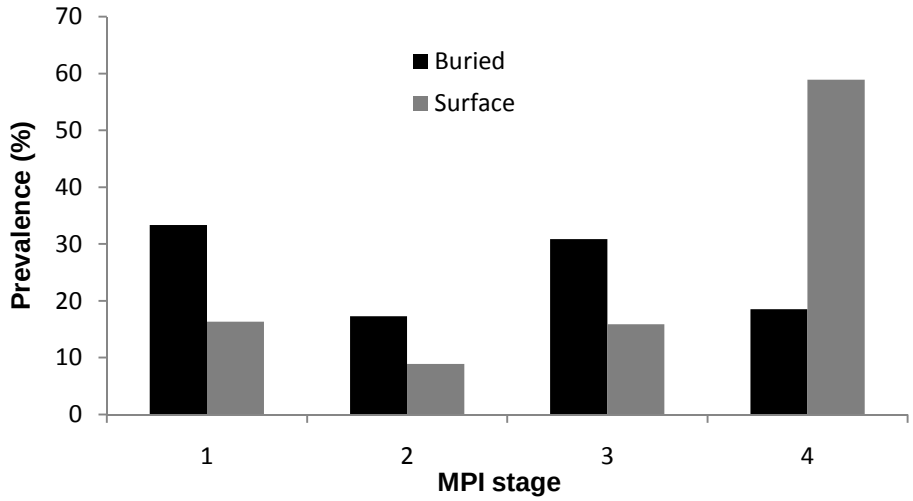


Figure 6.6. Muscle print index for buried and surface clams from Lanton (N = 600).

(☐ stage 1; ▒ stage 2; ▓ stage 3; ■ stage 4)

4. Discussion

Manila clam mortalities in Arcachon Bay were associated with high prevalence of a characteristic and visible (to the naked eye) sign: an important atrophy and a hardening of the adductor muscle which becomes progressively infused by brown conchiolin and calcified. BMD leads to the total destruction of the muscle in advanced stages of the disease. BMD has never been described in any clam in any part of the world, but similar signs were described in the Japanese pearl oyster *Pinctada fucata martensii* (Miyazaki et al. 1999). The disease affected the mantle lobe, the foot, cardiac and adductor muscles which became dark, although no hardening or calcification were reported. This disease was responsible for mass mortalities and drastic economic losses (Miyazaki et al. 1999). Another disease called Syndrome 85 was described in the black-lip pearl oyster *Pinctada margaritifera* and caused mass mortalities in 1985 in French Polynesia (Comps et al. 1999, 2001). In addition to producing the same symptoms previously described in the Japanese pearl oyster for the adductor muscle, Syndrome 85 also affected the shell by causing a brown deposit of organic matter on the inner side of valves. Organic deposits in the shell are usually the sign of a reaction stimulated by a wound, a parasite, debris or hemocytes (Perkins 1996). Currently it seems that BMD only affects the posterior muscle of Manila clams in Arcachon Bay, and even if some similarities were found with oyster diseases, this pathology is new because of the total muscle transformation, its brown conchiolin infusion and its progressive calcification. BMD does not resemble any other known disease in *Ruditapes philippinarum*. Muscle fibers were, however, extensively necrotized and degenerated, as in the Japanese pearl oyster disease (Miyazaki et al. 1999).

Because there appeared to be disturbances in the activity of the adductor muscle, this muscular damage disrupted the life cycle of the clams by inhibiting the valve opening and closing processes. These functional perturbations could disturb feeding and respiratory activities. Clams were unable to remain buried and migrated to the surface of the sediment; this phenomenon led to their death. BMD is the most important pathology in Arcachon Bay since the introduction of the Manila clam.

Clams from the Lanton site revealed a seasonality in the occurrence but not in the intensity stages (MPI) of BMD. Prevalence was higher during cold periods, and 2 winter peaks were observed. The decrease in prevalence from February 2006 to June 2006 and from November 2006 to March 2007 indicated either tissue restoration (unlikely) or mortality

events which were confirmed by our field observations (considerable increase in surface clams) in the 4 sampled sites, including areas of high mortality. This would mean that the disease did not necessarily have to be at an advanced MPI stage for death to result. BMD may induce a weakening of the whole clam and, consequently, affected animals are more sensitive to environmental conditions such as cold temperatures in winter or pathogenic agents. At Lanton, prevalence of BMD in buried clams was relatively high at 27%, but still lower than prevalence in clams collected at the surface (79%). The ascent of bivalves toward the surface is often considered as the prelude to death (Desclaux et al. 2002). It seemed that BMD was the first cause of the vertical migration of clams toward the sediment surface, although this may be accelerated by a variety of factors, including physical factors such as cold temperatures in winter and/or temperature variations due to tidal rhythms (Lauckner 1987a), or high clam densities (Richardson et al. 1993).

The principal sign of BMD was that the posterior adductor muscle was visibly affected; the anterior always remained macroscopically healthy. The posterior muscle was located nearest the sediment surface and therefore was more vulnerable to certain pathogenic agents or environmental variations. Although a few other species of bivalves lived in sympatry with *Ruditapes philippinarum* (e.g. *Cerastoderma edule* and *R. decussatus*), none of them were affected by BMD, which consequently appears to be a species-specific disease. Symptoms varied between the two types of muscle tissue, with the striated muscle being the first and most severely infected. Striated tissue was likewise situated nearest the surface in the clams' living position, and this was certainly important with respect to contamination by a causal agent and/or for the development of the pathology. The disease was subsequently propagated to the smooth muscle. The 2 tissues each have a distinct function: the striated muscle is responsible for quick pulses of the valves, whereas the smooth muscle permits the slow movement of valves and holds the valves closed. Consequently, BMD affected valve activity and clam mobility. The presence of sediment inside clams showed that this disease also altered the hermeticism of the valves, which could be conducive to penetration by opportunist pathogens such as fungal, bacterial, viral or protozoan organisms.

BRD and perkinsosis were analyzed, but no significant differences were found between buried and unburied clams (authors' unpubl. data). Even though these 2 pathologies are known to severely affect clams in other geographical areas, they do not appear to affect clam populations in Arcachon Bay. Microscopy and molecular biology analyses did not

identify any etiological agents. PCR analysis excluded the presence of bacteria, protozoans and some fungi, as confirmed by the histological observations.

There are few pathogens which affect the muscles of mollusks; of these, the association between a protozoan *Haplosporidium* sp. and a Rickettsia-like prokaryote in Withering Syndrome affects the foot of abalone causing the reduction of muscular fibers, the loss of bundle orientation and finally, atrophy and necrosis (Balseiro et al. 2006). Another pathogen, a shell disease called 'maladie du pied', and caused by a fungus similar to *Ostracobable implexa*, has affected the adductor muscles of *Ostrea edulis* and *Crassostrea angulata* in Europe since 1887 (Alderman and Jones 1967, 1971). This disease begins with small rounded black lumps in the adductor muscle attachment area, which progressively enlarge and become coalescent. Eventually, the muscle attachment may become a raised boss, often with incomplete layers of more calcified shell overlying it (Alderman and Jones 1971, 1997). The signs of BMD may resemble shell disease and Japanese pearl oyster diseases. Some viruses can alter muscles, for example in the cephalopod *Octopus vulgaris*, by inducing tumors which disturb the functioning of muscle tentacles (Rungger et al. 1971).

The causative agent of BMD might be a fungus or a virus. The signs of disease were closer to those of Japanese pearl oyster diseases for which Miyazaki et al. (1999) demonstrated that the causal agent was a non-enveloped virus. This author assumed that this virus was the cause of all muscular damage: hemocytes infiltration, tissue necrosis, degeneration of muscle fibers and, eventually, oyster mortalities. Furthermore, paraspherical virus-like particles were observed in histological sections of oysters affected by Syndrome 85 and were considered highly likely to be the causal agent of the disease (Comps et al. 1999, 2001). In this context, further investigations should focus on the observation of affected muscle tissues and use transmission electron microscopy to detect virus-like particles, if present. Furthermore, to better understand the whole pathological process, other organs should be processed by light microscopy. Questions related to transmission of the disease within the wild bivalve population should also be addressed in future research on BMD.

Chapitre 7

Des particules virales associées à la maladie du muscle marron (BMD)

Virus-like particles associated with brown muscle disease in Manila clam, *Ruditapes philippinarum*, in Arcachon Bay (France)

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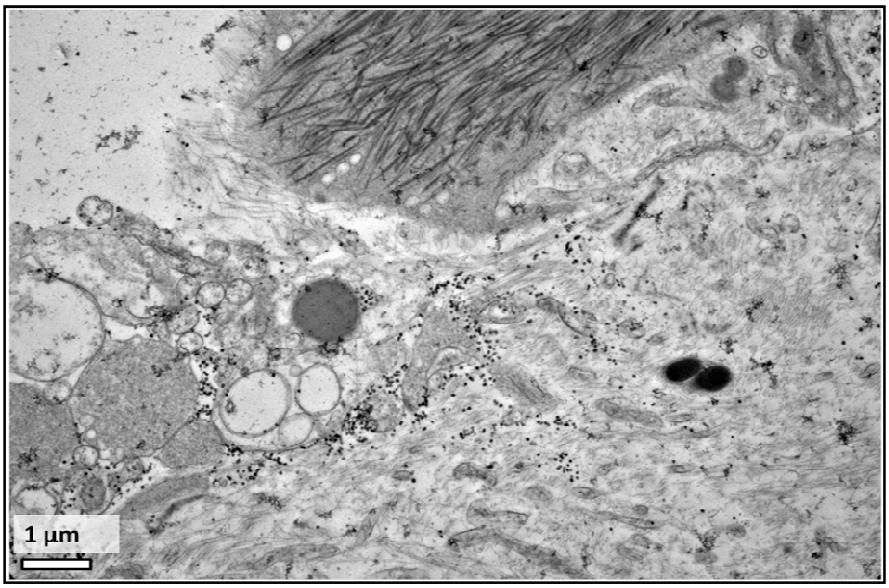


Particules virales dans une fibre musculaire dégradée

(muscle adducteur postérieur, *R. philippinarum*)

Abstract

Recently, Manila clam, *Ruditapes philippinarum*, populations have suffered mortalities in Arcachon Bay (SW France). Mortality was associated with extensive lesions of the posterior adductor muscle which become progressively brown and calcified. Ultrastructural observations by transmission electron microscopy revealed tissue degradation with necrotized muscle fibres and granulocytomas. Unenveloped virus-like particles (VLPs) were detected in muscle, granulocytic, epithelial and rectal cells. VLPs were abundant in the extracellular space, in the cytoplasm (free or enclosed in vesicles) and in the nucleoplasm of granulocytes. Nuclei and mitochondria of granulocytes displayed changes which suggested reactive oxygen species (ROS) production and apoptosis induction. VLPs exhibited an icosahedral structure with a diameter of 25 to 35 nm. These observations suggest that the VLPs could belong to the family Picornaviridae or the Parvoviridae.

Keywords: *Ruditapes philippinarum*, brown muscle disease, adductor muscle, transmission electron microscopy, necrosis, apoptosis.

1. Introduction

The Manila clam, *Ruditapes philippinarum,* is one of the most exploited bivalve molluscs in the world. This species has a relatively high commercial value in France. In 2006, Arcachon Bay (SW France) ranked first among commercial harvest areas in France in terms of standing stock (7600 mt) and production (450 mt). Normally, the Manila clam is an infaunal bivalve living a few centimetres under the sediment surface. Recently, in large areas within the bay, a significant numbers of molluscs were retrieved from the sediment surface. They were often gaping or were recently dead. Mortality was associated with lesions of the posterior adductor muscle that became progressively brown and calcified. This pathology has been named brown muscle disease (BMD) and aetiologic agents have been sought (Dang et al. 2008). Light microscopy has not revealed any causal agent. Bacterial and protozoan agents being excluded, a viral aetiology has been hypothesized.

The first report of viruses in bivalve molluscs was a herpes-like virus in oyster tissues (Farley et al. 1972). Subsequently, many reports of different virus families have been made in mollusc species worldwide (Comps et al. 1976, Comps and Duthoit 1976, Johnson 1984). Mortalities associated with viruses have been observed in many bivalve species in different parts of the world. For example, mortalities occurred in *Crassostrea angulata* from 1970 to 1973 along the French Atlantic coast as a consequence of an iridovirus (Comps et al. 1976; Comps and Duthoit 1976) and in *Crassostrea gigas* in 1977 due to a similar iridovirus (Comps and Bonami 1977). Mass mortality of the pearl oyster, *Pinctada margaritifira,* was recorded in 1985 with associated necrosis of the adductor muscle. Virus-like particles of 40 nm in diameter were detected (Comps et al. 1999, 2001). Other severe mortality events were reported in bivalve populations associated with the presence of virus-like particles (Johnson 1984, Jones et al. 1996, Hine and Wesney 1997, Miyazaki et al. 1999, Novoa and Figueras 2000).

This study reports the first morphological and ultrastructural description of virus-like particles associated with the brown muscle disease of Manila clam as well as the histological changes observed in the bivalve infected tissues.

2. Materials and methods

Specimens of *Ruditapes philippinarum* were collected from an intertidal site in Arcachon Bay. Manila clams selected for the study were from the three pathological stages previously described (Dang et al. 2008): healthy (n = 5), intermediate (n = 10) and advanced stages (n = 10). Clams were opened by sectioning the adductor muscle with a scalpel blade and striated muscles were dissected and fixed for histological examination. The muscle was fixed in 2.5 % glutaraldehyde buffered with 0.1 mmol. L^{-1} sodium cacodylate solution and synthetic sea water for 12 h at 4 °C, rinsed in a cacodylate buffer (0.1 mmol L^{-1}, NaCl 2 %), post-fixed in 1 % OsO_4 for 2 h, dehydrated through a graded series of ethanol (50 to 100 %), and embedded in araldite. Ultrathin sections (500—700 Å) were cut and examined without staining on a Philips Tecnai 12 transmission electron microscope (TEM).

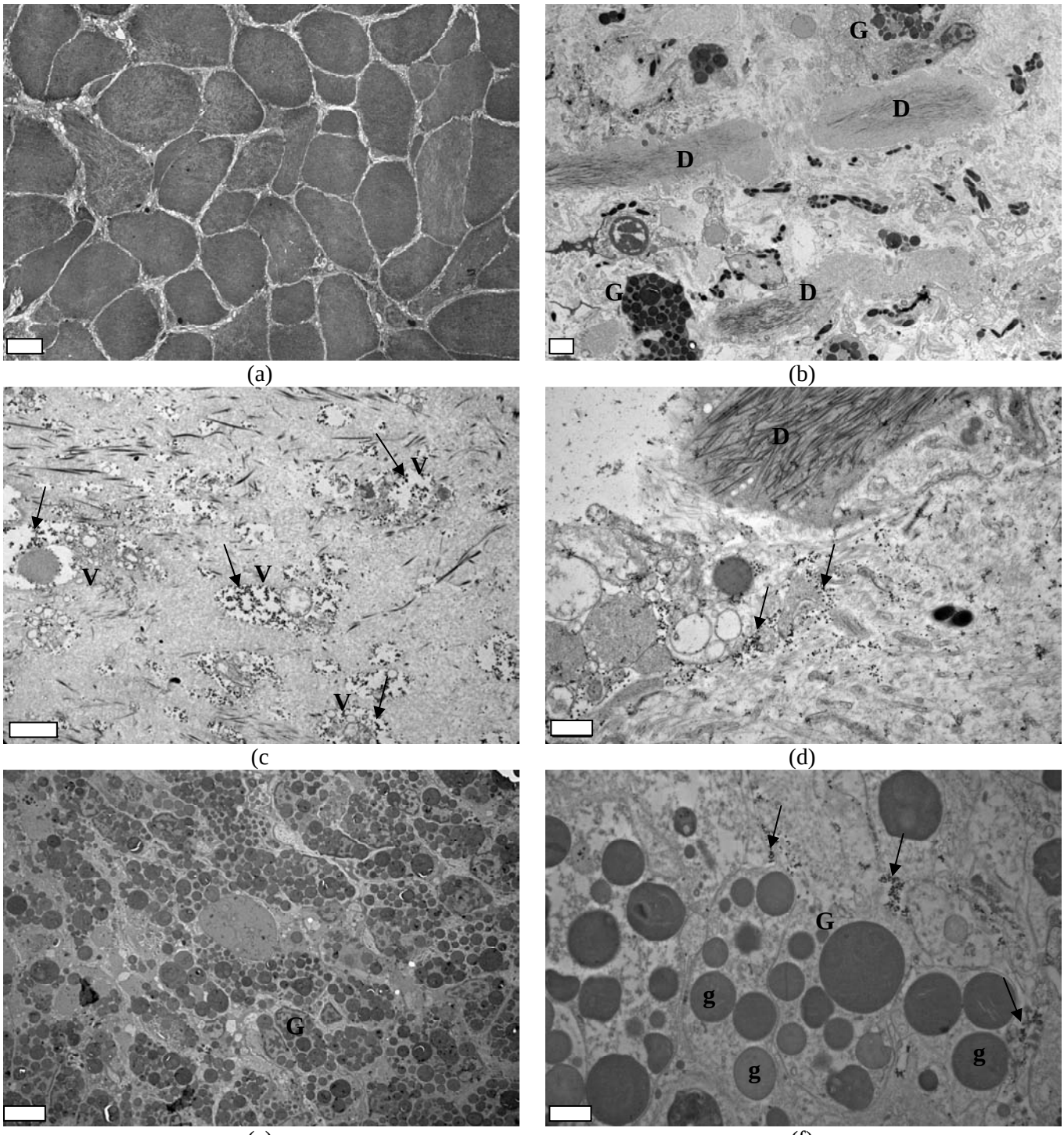
To observe the attachment zone of the muscle and pallial epithelium cells, muscles and shells from two uninfected and six diseased clams were decalcified. The shell and the muscle were decalcified in 7 % EDTA for 18 days. EDTA solution was changed every day until complete decalcification. Then, treatment of samples was as previously described.

3. Results

All infected clams in intermediate and advanced stages of the disease had electron-dense particles of 25 to 35 nm widespread in the tissues, in contrast to healthy clams in which no virus-like particles (VLPs) were detected.

Sections of clams observed by TEM showed a classical muscle arrangement in healthy specimens, with muscular fibres composed of several myofibrils (Fig. 7.1a). However, diseased clams exhibited an extensive necrosis and a complete muscular disorganization (Fig. 7.1b - f).

In clams showing an intermediate stage of brown muscle disease, two different situations were observed under TEM: 1), with remnants of muscular fibres and few granulocytes (Fig. 7.1b—d); 2), with extensive granulocytomas as previously described by light microscopy (Dang et al. 2008) (Fig. 7.1e—f). In both situations, the tissue was severely disrupted.



(a) (b) (c (d) (e) (f)

Figure 7.1. Ultrathin sections of *Ruditapes philippinarum.* **(a)** Transverse section of adductor muscle of healthy Manila clam (bar = 5 µm). **(b - f)** Adductor muscle of clam with intermediate of stage brown muscle disease. **(b)** Degraded fibres (DF) interspersed by granulocytes (G) (bar = 2 µm). **(c)** Virus-like particles (VLPs) (arrows) within vesicles (V) in degraded muscle fibre (bar = 2 µm). **(d)** Degraded fibre (DF) with VLPs (arrows) in sarcoplasm (bar = 1 µm). **(e)** Intense granulocyte (G) infiltration (granulocytomas) within the muscle (bar = 5 µm). **(f)** Extracellular VLPs (arrows) in a granulocyte infiltration. Granulocytes (G) contain many dark granules (g)(bar = 1 µm).

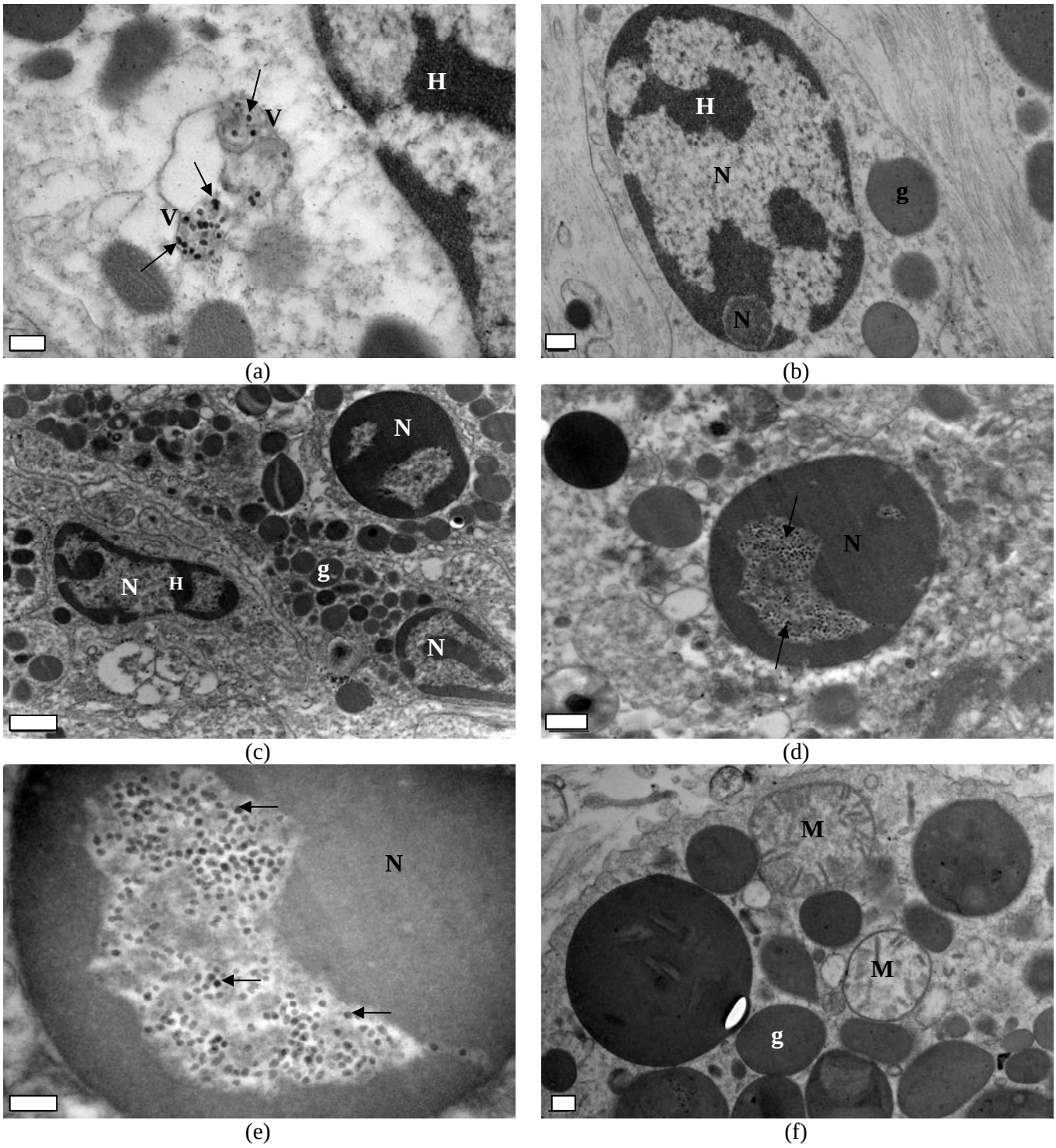


Figure. 7.2. Ultrathin sections of granulocytes in muscle of *Ruditapes philippinarum* with intermediate stage of brown muscle disease. **(a)** VLPs (arrows) within vesicles (V) in the cytoplasm; HC, heterochromatin (bar = 200 nm). **(b)** Nucleus (N) of a granulocyte with laterally displaced nucleolus (Nu) and prominent heterochromatin clumps (HC); g, granule (bar = 200 nm). **(c)** Nuclei (N) of granulocytes with condensed heterochromatin (HC); g, granule (bar = 1 µm). **(d)** Virus-like particles (arrows) in nucleoplasm of infected granulocyte; N, nucleus (bar = 500 nm). **(e)** Higher power of VLPs (arrows) in nucleoplasm (N) (bar = 200 nm). **(f)** Mitochondria (Mi) of infected granulocyte with loss of cristae architecture; g, granule (bar = 200 nm).

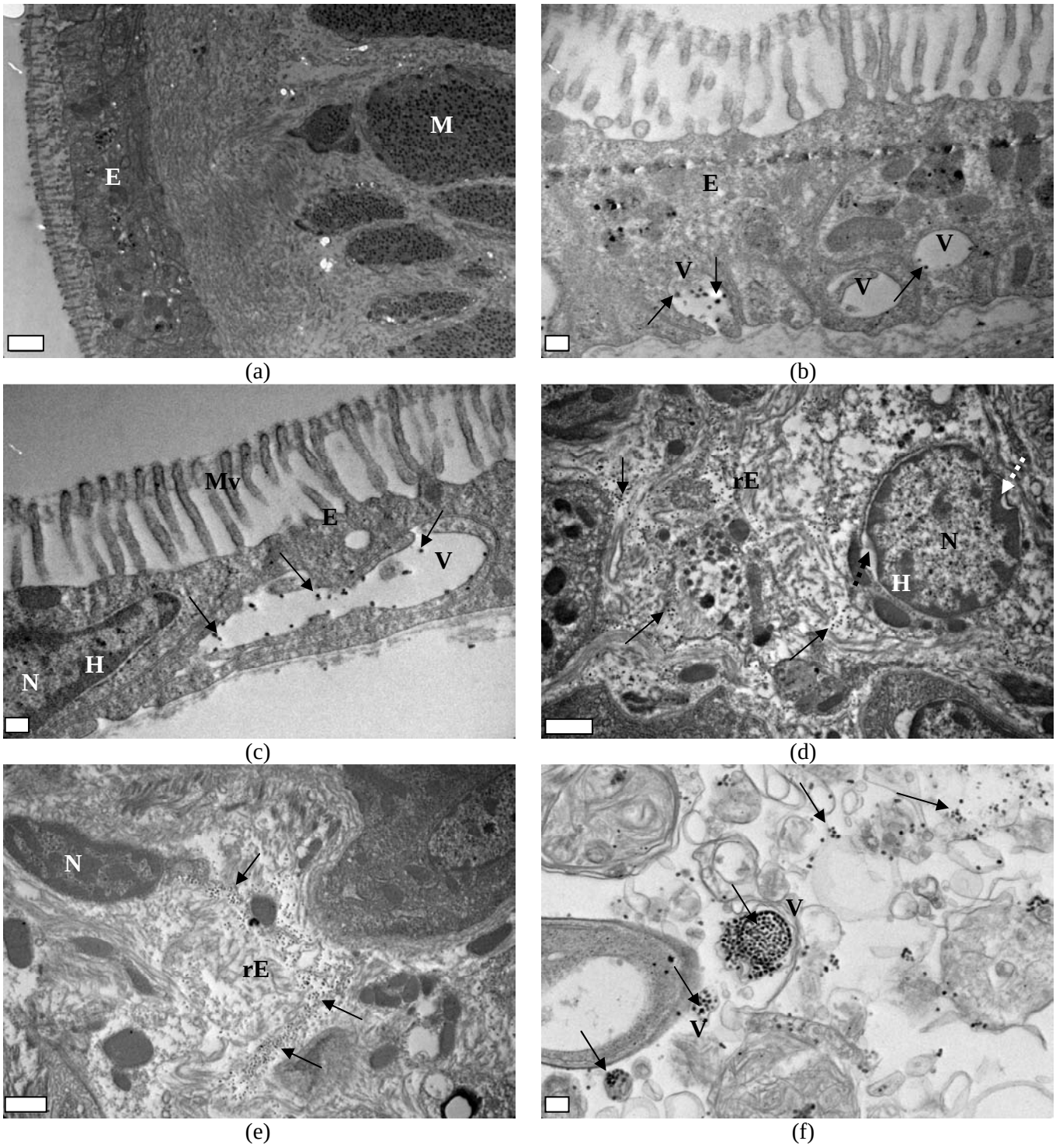


Figure 7.3. Intermediate stage of brown muscle disease in *Ruditapes philippinarum* tissues observed under TEM. **(a)** Attachment zone of muscle cells with muscle fibre (MF) and epithelial cells (EC) (bar = 1 µm). **(b – c)** VLPs (arrows) within vesicles (V) in the cytoplasm of epithelial cells (EC). Mv, microvillosity; N, nucleus; HC, heterochromatin (bar = 200 nm). **(d)** VLPs (arrows) in cytoplasm of rectal cell. Internal and external nuclear membranes are dissociated and form blisters (dotted arrows); N, nucleus; HC, heterochromatin; rER, rough endoplasmic reticulum (bar = 1 µm). **(e)** VLPs (arrows) in cytoplasm of rectal cells; N, nucleus; rER, rough endoplasmic reticulum (bar = 1 µm). **(f)** Advanced stage of brown muscle disease. VLPs (arrows) within vesicles (V) or free among cellular debris (200 nm).

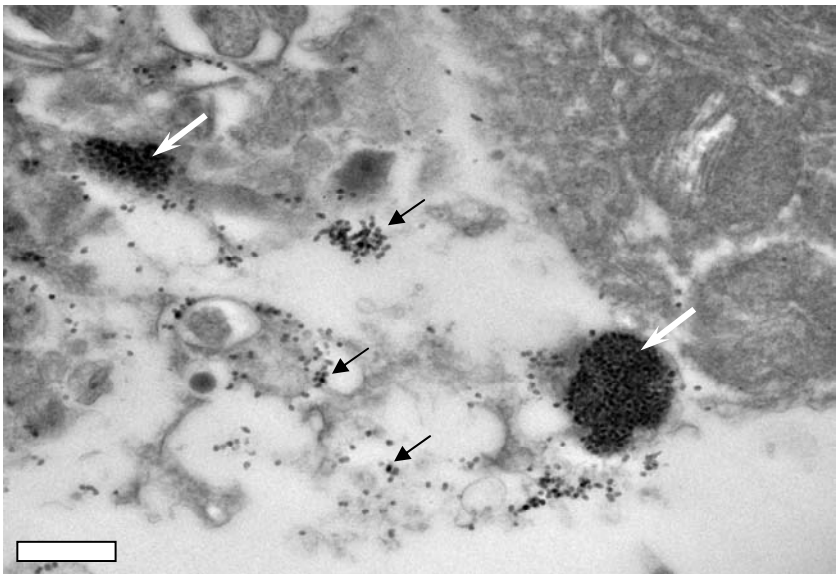
In clams showing the first intermediate stage of brown muscle disease, muscular fibres were completely necrotized, atrophied, and without a normal muscle bundle organization (Fig. 7.1b). At this stage, muscle cells were not discernible. Only remnants of fibres without any organisation were observed. These degraded muscle fibres were interspersed with haemocytes and particularly granulocytes (Fig. 7.1b). This is the first line of cellular defence in bivalves. Within the sarcoplasm, vesicles containing electron dense VLPs were seen (Fig. 7.1c).

VLPs could be found either in vacuoles inside fibres (Fig. 7.1c) or near damaged fibres (Fig. 7.1d), perhaps in sarcoplasm. The tissue was so degraded that it was not possible to determine the intra- or extracellular position of VLPs, but observations suggested that they were present in both locations.

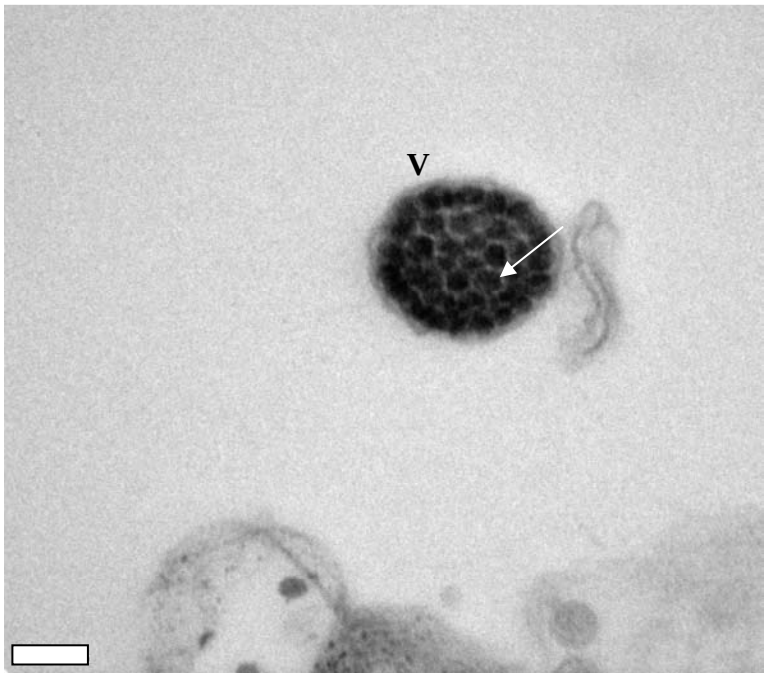
In a more advanced stage, significant haemocyte infiltration and granulocytoma formation were observed (Fig. 7.1e). These haemocytes contained numerous dark granules in their cytoplasm and were identified as granulocytes. They may have digested muscle cell debris.

VLP-associated granulocytomas were observed (Fig. 7.1f). As in muscle cells, VLPs could be located either extracellularly (Fig. 7.1f) or intracellularly (Fig. 7.2a, d, e). Indeed, VLPs could be found in vesicles inside the cytoplasm of the granulocyte (Fig. 7.2a).

The nuclei of granulocytes appeared abnormal with a prominent lateral displacement of the nucleoli (Fig. 7.2b), which may be an indication of replication, and a slightly higher degree of chromatin condensation. Inclusion bodies were absent. At a higher level of infection, nuclei appeared pyknotic and condensed (Fig. 7.2c, d). Within the nucleoplasm, VLPs were observed with the same structure and size as other VLPs (Fig. 7.2d, e). In these pyknotic nuclei the heterochromatin occupied almost the whole nucleus. However, VLPs were less electron-dense compared to the extracellular or cytoplasmic virions and their appearance resembled virions in the process of assembly. Furthermore, granulocytes possessed abnormal mitochondria with loss or disorganization of cristae (Fig. 7.2f). These organelles appeared empty with only short or fragmented cristae.



(a)



(b)

Figure 7.4. Advanced stage of brown muscle disease in *Ruditapes philippinarum* muscle observed under TEM. **(a)** Virus-like particles in paracrystalline arrays (white arrows) or free (arrows) among cellular debris (bar = 500 nm). **(b)** VLPs (arrow) within a vesicle (V). Note their icosahedral structure (bar = 100 nm).

Decalcification of samples permitted the observation of epithelial cells, responsible for shell secretion, and the attachment zone of muscular cells (Fig. 7.3a). VLPs were detected in the cytoplasm of these cells within endocytotic vesicles (Fig. 7.3b, c). VLPs were also detected in the cytoplasm of rectal cells, which are adjacent to muscle cells (Fig. 3d, e). They had invaded the rough endoplasmic reticulum (Fig. 7.3d, e). In Fig. 7.3d, the internal and external membranes of the nucleus appears irregular and dissociated. Blisters appeared between both nuclear membranes.

In the advanced stage, tissue was so disrupted that no structure was recognizable. Remnants of lysed cells as membranes and degradation bodies were present. Large amounts of VLPs were observed in this stage. They could be densely enclosed in vesicles, or free throughout the cellular debris (Fig. 7.3f). Paracrystalline arrays occurred frequently in this ultimate stage of brown muscle disease (Fig. 7.4a). VLPs were non-enveloped and measured from 25 to 35 nm in diameter (Fig. 7.4b). They appeared icosahedral in shape with an homogenous electron dense centre (Fig. 7.4b).

4. Discussion

Only one virus has been reported to infect and replicate in *R. philippinarum* tissues, a herpes-like virus which infects larvae. It induced sporadic mortalities in French hatcheries in 1997 (Renault et al. 2001-a). No virus has been described in adult Manila clams. However, virus-like particles (VLPs) have been found in *R. decussatus* in Spain (Novoa and Figueras 2000).

Similar clinical signs and associated virus particles were reported in the scallop *Patinopecten yessoensis* (Mori 1975), in the Japanese pearl oyster, *Pinctada fucata martensii* (Miyazaki et al. 1999), and in the Polynesian pearl oyster, *Pinctada margaritifira* (Comps et al. 1999, 2001). Like brown muscle disease, both oyster diseases featured an extensive necrosis and atrophy of the muscle fibres with a prominent inflammatory response and mass mortality. In the diseased muscle of the Japanese pearl oyster, inclusion bodies contained virions from 25 to 33 nm in size, presumably containing a RNA genome (Miyazaki et al. 1999). On other hand, 40 nm VLPs were reported from the granulocytoma tissue of the Polynesian pearl oyster (Comps et al. 1999).

In the Japanese pearl oyster, the pathology is combined with a yellowish to brown colouration of the adductor muscle but the disease also affects pallial, foot, gill and cardiac musculature. Based on gross observations, tissues other than the adductor muscle do not seem to be infected.

Clams affected by brown muscle disease showed highly degenerated muscle fibres and the disease could be diagnosed through gross observation. The gradation of colouration (from yellowish to brown) corresponds with infection intensity. In contrast with the report of Miyazaki et al. (1999) adductor muscle of infected Manila clam is calcified. At each stage of infection, VLPs were observed in different cellular types: muscular, epithelial, granulocytic and rectal as well as in different locations, i.e. intranuclear, cytoplasmic and extracellular.
The observation of VLPs in cells was dependent on cell type. VLPs were exclusively observed within endocytotic vesicles in epithelial cells and free in the cytoplasm of rectal cells, which might suggest a direct penetration. In rectal cells they were found in association with the rough endoreticulum. There was a close resemblance to the virus-like particles found in mussel, *Perna canaliculus* (Jones et al. 1996), and in scallop, *Pecten novaezelandiae* (Hine and Wesney 1997). In muscle cells, VLPs were observed inside vesicles and free in the sarcoplasm at higher infection levels when there is significant muscle necrosis. Miyazaki et al. (1999) reported the formation of membranous inclusion bodies in the sarcoplasm of necrotized muscle fibres which contained viral particles in the Japanese pearl oyster.
The presence of granulocytes forming granulocytomas was very marked in muscle, as in *Pinctada fucata martensii* (Miyazaki et al. 1999), *Pinctada margaritifira* (Comps et al. 1999) and *Mytilus edulis* (Rasmussen 1986). The lesions found in *Ruditapes philippinarum* displayed some similarities to granulocytomas which developed in *M. edulis* infected by a picornavirus (Rasmussen 1986). This latter virus replicated in cytoplasmic vesicles within granulocytes which aggregated to form the granulocytomas. The first cell type colonized by VLPs in brown muscle disease is unknown, although logically infection should begin in muscle cells. Granulocytes might subsequently phagocytose infected muscle cells and themselves become infected. It is also possible that VLPs pass from muscle cells to adjacent rectal cells.

Nuclei of granulocytes and rectal cells presented alterations like heterochromatin margination, lateral displacement of the nucleus and dilatation of the nuclear cisternae (blisters), features indicating the imminent pyknosis and consequent death of the cells (Cabanne and Bonenfant 1986). Some of these alterations suggest apoptosis-like aggregation of condensed chromatin

along the inner nuclear membrane and overall signs of nucleus collapse (Després et al. 1996, Wattre 1999). Viral protein may be responsible for apoptosis either during particle penetration or during the release of structural protein into the cell (Després et al. 1996, Hanon et al. 1996). In some pyknotic nuclei, a high concentration of VLPs was observed, which argues for virus multiplication in the nucleus and consequently a possible DNA-based viral genome. In this case, VLPs were less electron-dense, which suggests an early stage of assembly.

In granulocytes mitochondrial ultrastructure showed cristae loss and disorganization. The same phenomenon was reported in *Pinctada fucata martensii* muscle fibres (Miyazaki et al. 1999). It could be hypothesized that damaged mitochondria with disorganization of cristae could reflect apoptosis in this organelle. In this context, recent reports suggest that some viruses are able to induce reactive oxygen species (ROS)-mediated cell apoptosis (Wang and Weinman 2006, Schmitt and Reiter 2008). Activated granulocytes certainly play an important role in virus infections and their destruction can delay viral clearance, facilitating the spread of virus in the host. The induction of apoptosis provides a means for the virus to establish a balanced long-term relationship with the host immune system, sacrificing other cells to undergo infection and thereby promoting viral survival (Renault et al. 2001-b, Després et al. 1996, Miller and White 1998, Wattre 1999).

Studies on viral agents in molluscs are limited by the lack of molluscan cell lines to culture the putative viruses. Consequently, determination of virus family is mostly carried out by TEM. Using morphological criteria, VLPs of brown muscle disease could belong either to the Picornaviridae (Rueckert 1971, Godman 1973, Moore and Eley 1991) or Parvoviridae (Mayor 1973, Tijssen and Arella 1991). The VLPs were non-enveloped and the capsid was apparently icosahedral and measured approximately 25 to 35 nm in diameter. Picornaviridae have single-stranded RNA and Parvoviridae single-stranded DNA genomes. *Ruditapes philippinarum* VLPs are very similar in size, structure and cellular position to the Bay of Piran shrimp virus (BPSV) (Vogt 1996). BPS virions are spherical to angular, non-enveloped and measure around 22 to 27 nm in sections. Like the present VLPs, BPSV could be found extracellularly, accumulated in the cytoplasm but also abundant within membrane whorls, in association with the rough endoplasmic reticulum, or within the nucleoplasm, depending on the cell type. BPSV has been described as probably belonging to the Picornaviridae or the Parvoviridae.

At first glance, the Manila clam VLP seems to be a picornavirus, as it is located in the cytoplasm like the virus found by Miyazaki et al. (1999) in the Japanese pearl oyster. Viruses

related to the Picornaviridae are host species specific. The extensive cellular damage and incomplete maturation of the VLP as found by electron microscopy are in agreement with observations related to picornavirus infection in other animal species (Godman 1973).
However, the putative assemblage of the VLP within the nucleus would argue for inclusion in the Parvoviridae. Furthermore, a similar cytoplasmic virus, the IHHNV (infectious hypodermal and haematopoietic virus) of penaeid shrimp previously assumed to be a picornavirus, has been assigned to the Parvoviridae by molecular analysis (Mari et al. 1993).

On the basis of our investigations, the high infection rate, behavioural changes and gross pathological changes in infected clams, and the occurrence of immune defence reactions suggest that this is severe pathogenic infection. However, further attempts to isolate the virus are required to confirm the role of these virus-like particles as the cause of brown muscle disease and to conduct transmission experiments on healthy animals. Molecular analysis will be necessary to unequivocally relate this virus to a family.

Chapitre 8

BMD et dynamique de population dans le bassin d'Arcachon

Brown Muscle Disease and Manila clam *Ruditapes philippinarum* dynamics in Arcachon Bay, France

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Mortalités massives de palourde japonaise à Lanton

Abstract

Brown Muscle Disease (BMD) affects Manila clam *Ruditapes philippinarum*. It was described for the first time in 2005 in Arcachon Bay, France. The pathology consists in a progressive necrosis of the posterior adductor muscle, valve gaping, clam migration to the sediment surface and death. The present study aimed to quantify the prevalence of BMD in the bay and to evaluate the effect of BMD on Manila clam dynamics. The prevalence was assessed on 50 stations spread within Arcachon Bay. About 62 % of Manila clam habitat surface was infected by BMD. A survey of buried and surface clams was conducted from November 2006 to March 2008 in Lanton, a site infected by BMD. Modal progression analysis separated confidently cohorts from 2003 to 2005 recruitments. This pathology only affected adult clams (>25 mm, > 2yr). For both buried and surface individuals, shell length was significantly correlated with BMD infection. Surface clams had prevalence (67%) higher than buried clams (23%) and showed greater mortality rate after 15 d in running water: 82% against 12% for buried individuals. The final disease index (FDI) and the condition index (CI) were monthly evaluated on 50 clams located at each position in the sediment. CI displayed a significant decrease following BMD's infection from light to severe disease stages. Length data analysis through Bhattacharya's method (FISAT II software) allowed identifying four cohorts. The 2003's cohort enabled to calculate mortality rate that was 39% for 5 months and to estimate that BMD was responsible of 95 % of that mortality. The temperature was certainly an important factor in BMD transmission, as cohort dynamics results argued that BMD developed during spring and summer.

Key words: clam, disease, dynamic, brown muscle disease, mortality, *Ruditapes philippinarum*.

1. Introduction

Brown Muscle Disease (BMD) was described for the first time in 2005 in Arcachon Bay (Dang et al. 2008). This pathology affects the Manila clam *Ruditapes philippinarum* (Adams and Reeve 1850), one of the most exploited bivalve mollusks in the world. This species has a high commercial value in France. Arcachon Bay (SW France) harbors the most important national stock and production (Caill-Milly et al. 2006). BMD induces a transformation of the posterior adductor muscle, which becomes infused by conchiolin and calcified (Dang et al. 2008). The disease affects both types of muscular tissue, with striated muscle becoming impacted to a higher degree than smooth muscle. Histological observations revealed an important inflammatory response with a large invasion of hemocytes into tissues and a heavy necrosis of muscular fibers. The causal agent of the disease is not identified. Both histological and molecular assays discarded bacteria and protozoans, and macroscopic survey under binocular excluded digenean trematodes (Dang et al. 2008). More recently, ultrastructural observations by transmission electron microscopy revealed the presence of virus-like particles (Dang et al. 2009-a).

Preliminary results from a one-year monthly survey conducted on adult clams in four sites of Arcachon Bay showed that mean prevalence could reach 30%. In some places, peaks of prevalence occurred during the cold season (Dang et al. 2008). At one occasion, adult clams were also collected at the surface of the sediment (*i.e.* an abnormal position for this endogenous bivalve) and exhibited a three times higher prevalence than buried clams (78% *vs* 27%). These studies revealed the deleterious effect of the disease at the individual scale but did not allow to assess neither its impact at the population scale nor a clear phenology of the disease/clam system. Previous diseases have already seriously affected clam populations like Brown Ring Disease (BRD) (Allam et al. 2002) or perkinsosis (Ngo and Choi 2004, Villalba et al. 2004, 2005), but also due to virus-like particles (Novoa and Figueras 2000).

The present paper proposes to give an insight on the effect of BMD on clam populations and to clarify some aspects of the disease transmission. The first step consisted in verifying that BMD was not a local pathology through a large scale sampling campaign performed within Arcachon Bay. Then, an attempt was made to describe clam dynamics and link it to BMD occurrence, prevalence and intensity. Finally, the impact of BMD on clam condition and mortality was assessed.

2. Materials and Methods

2.1. Study area

Arcachon Bay (44°40'N, 1°10'W) is a 156-km² semi-sheltered lagoon in the southwest of France (Fig. 8.1). Tidal flats represent 110 km², partly covered by *Zostera noltii* seagrass beds and colonized by Manila clams (Blanchet et al. 2004, Caill-Milly et al. 2006). Tidal range varies between 0.9 and 4.9 m depending on site and tide coefficient. Clams are generally situated in the mid intertidal zone but can be found from 2.75 m above the 0 of low tide (Cottet et al. 2007) to the tidal channel (Blanchet et al. 2005). Most of the present study was conducted at Lanton, a site where the Brown Muscle Disease was most prevalent (Dang et al. 2008). Lanton is in the inner part of the Bay with an influence of the Leyre River (Fig. 8.1). The sampling site was situated at 1.9 m above the level of low tide. Sediments were fine muddy sands (median grain size = 78.5 µm; 41% silt and clay), sediment temperature fluctuated between -1.7 and 37.8°C and salinity between 4.8 and 26.7 psu (Dang et al. 2008).

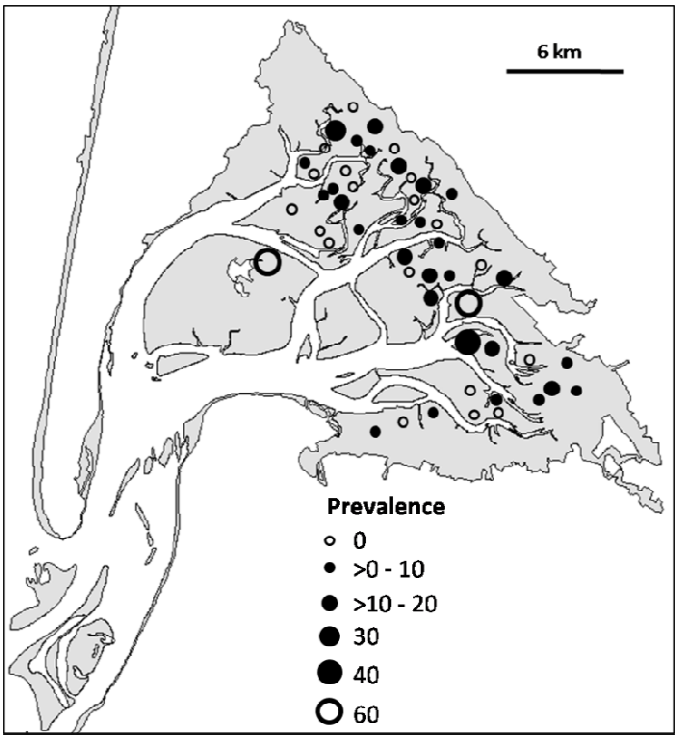

Figure 8.1. Prevalence of brown muscle disease in buried Manila clams *Ruditapes philippinarum* sampled in 50 stations within Arcachon Bay.

2.2. Spatial survey

In May and June 2006, 480 stations were sampled in Arcachon Bay following a stratified strategy in order to evaluate the Manila clam standing stock (Caill-Milly et al. 2006). To obtain a sufficient number of clams for BMD analysis, these stations were pooled in fifty stations (Fig. 8.1). Ten adult clams (from 30 to 40 mm) were collected from each pooled station. Shells were observed with the naked eye and under a binocular microscope to determine the BMD prevalence.

2.3. Manila clam dynamics

From November 2006 to March 2008, clams were monthly collected at Lanton. During that period, sediment temperature was recorded every hour with an automatic probe (BoxCar Pro, Version 3.51). The sampling strategy had to integrate the fact that clam density was rather low, the quantity of juveniles negligible (Caill-Milly et al. 2006), that it was impossible to sieve samples *in situ* due to the absence of proximal water at low tide, and it was difficult to walk in these very fine sediments. It should also give estimation of the ratio of surface clams (sometimes very scarce). Consequently, it was decided to collect clams by hand following three radials of 50-cm width. Buried clams were sampled along five meters (total monthly sample area: three 2.5-m² radials) and live surface clams were collected along twenty meters (total monthly sample area: three 10-m² radials). All clams were measured at the nearest 0.1 mm. Modal progression analysis (Bhattacharya 1967) was performed using FISAT software (FISAT II, version 1.2.2, FAO-ICLARM) to separate the different cohorts (Gayanilo et al. 2005). This analysis was performed with respect of Manila clam individual growth and recruitment parameters previously determined in this site (Dang et al. in revision-b): it was assumed that recruitment occurred in October and that growth followed Von Bertalanffy growth function:

$L_t = 42.10\ (1\text{-}e^{-0.51t})$

with L_t = shell length (mm) and t = time (yr).

At each occasion, fifty buried clams and fifty surface clams were dissected to measure condition index (CI) and Brown Muscle Disease. CI was defined as the ratio of dry flesh weight (mg) to shell weight (g). Finally, thirty clams monthly sampled in depth and the same

quantity collected in surface were maintained separately in the laboratory, in running seawater for 15 days at ambient temperature. This experiment ran for one year, from August 2007 to July 2008. During this period, all dead clams were counted and dissected. At the end, the surviving individuals were all dissected.

2.4. Brown Muscle Disease and pathology-dependent mortality

At each occasion, fifty clams from each position (buried and surface) were opened and an index of the pathology estimated. On the posterior muscle (the only affected one), two Final Disease Indices (FDI) were estimated, one for striated muscle and one for smooth muscle. The value increases from 1 to 16 with pathology progression (Dang et al. 2008). The mean of both values (mFDI) was calculated for each clam and four stages were defined, based on mFDI values: Stage a (1-4), b (5-8), c (9-12) and d (13-16). In a group (or a cohort) of clams, the mean intensity of BMD was defined as the mean of mFDI of each clam, including zero values corresponding to healthy individuals.

Pathology dependent mortality was estimated for 2003's clam cohort. The assumption was that clams do not recover from BMD and was based on our observation (see discussion). Therefore, when following the fate of a cohort, the decrease of the mean BMD intensity can be interpreted as the death of the most affected clams. The number of clams that should have disappeared was calculated between a peak of BMD and a minimum. In November 2006, when BMD intensity was the highest, a matrix was constructed with the mFDI of each clam considered in the mean BMD intensity. Then, clam with the highest mFDI was taken off the matrix, simulating the death of this clam and thus obtaining a new, lower mean intensity. This was sequentially reiterated until the BMD intensity reached the lowest value corresponding to the following March.

3. Results

3.1. Spread of BMD in Arcachon Bay

A total of 31 stations out of 50 harbored BMD-affected clams (Fig. 8.1). Considering the sampling strategy (Caill-Milly et al. 2006), it can be considered that 62% of the clam habitat was affected by BMD. However, prevalence was generally <20% (27 stations) and was over 30% at only three occasions.

3.2. Manila clam abundance

Between November 2006 and March 2008, the mean clam abundance collected by hand was 40 ind/m² with 89% being buried (Fig. 8.2). Clams' shell length was comprised between 9 and 42 mm, but hand sampling was considered as fully efficient over 20 mm. The highest proportions of surface clams (>15% of total abundance) were found when the averaged temperature of the last 24 hours was under 12°C. Conversely, the minimum proportion of surface clam was generally found when temperature was over 18°C (Fig. 8.2). In November 2007, however, the proportion of surface clam was very low (1%) despite a temperature 11°C.

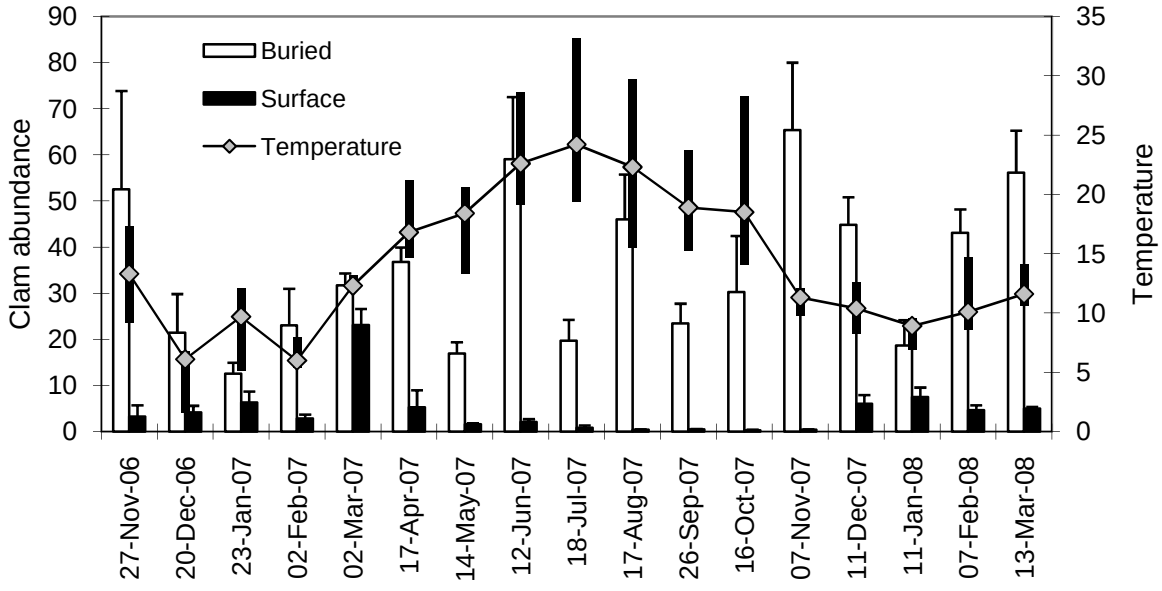

Figure 8.2. Abundance of Manila clams *Ruditapes philippinarum* (ind/m², +1 standard error) found in buried position or at the sediment surface and mean temperature during the 24 previous hours (°C, bars for minimum and maximum).

In pooling all clams (3125 individuals), a correlation was demonstrated between shell length and BMD infection. Infection in buried individuals was null between 9 and 26 mm, and started at 27 mm (Fig. 8.3a). Assuming October recruitment and the above-cited Von Bertalanffy growth (also assumed independent of pathology) function, 27-mm shell length corresponded to a 2-yr individual. At that date BMD prevalence reached 24%. Then, prevalence slowly and regularly increased (linear correlation, $R^2 = 0.72$, $p < 0.05$) to reach 33% at a length of 39 mm (5-yr individuals). Infection in surface individuals started at 25 mm (Fig. 8.3b) that also corresponded to the smallest clams found at the sediment surface. This length corresponded to a 1.8-yr individual infected in July. At that date BMD prevalence reached 25%. Then, prevalence slowly and regularly increased (linear correlation, $R^2 = 0.35$, $p < 0.05$) to reach 80% at a length of 38 mm (4.6-yr individuals). The average percentages within diseased clams were 36, 20, 19 and 24% for stages a, b, c and d, respectively. Each stage was however considered as equally represented (Chi2, $p > 0.05$).

3.3. Health of surface clams

During the 15 d period following sampling, surface clams died throughout the year in greater proportion (Kolmogorov-Smirnov test, $p < 0.01$) than buried clams (Fig. 8.4). Mean mortality was 12.4% for surface clam with a maximum of 82% (July). Between May and October, the mortality was always over 20%. Conversely, mean mortality was only 0.5% for buried clams with a summer maximum at 7%.

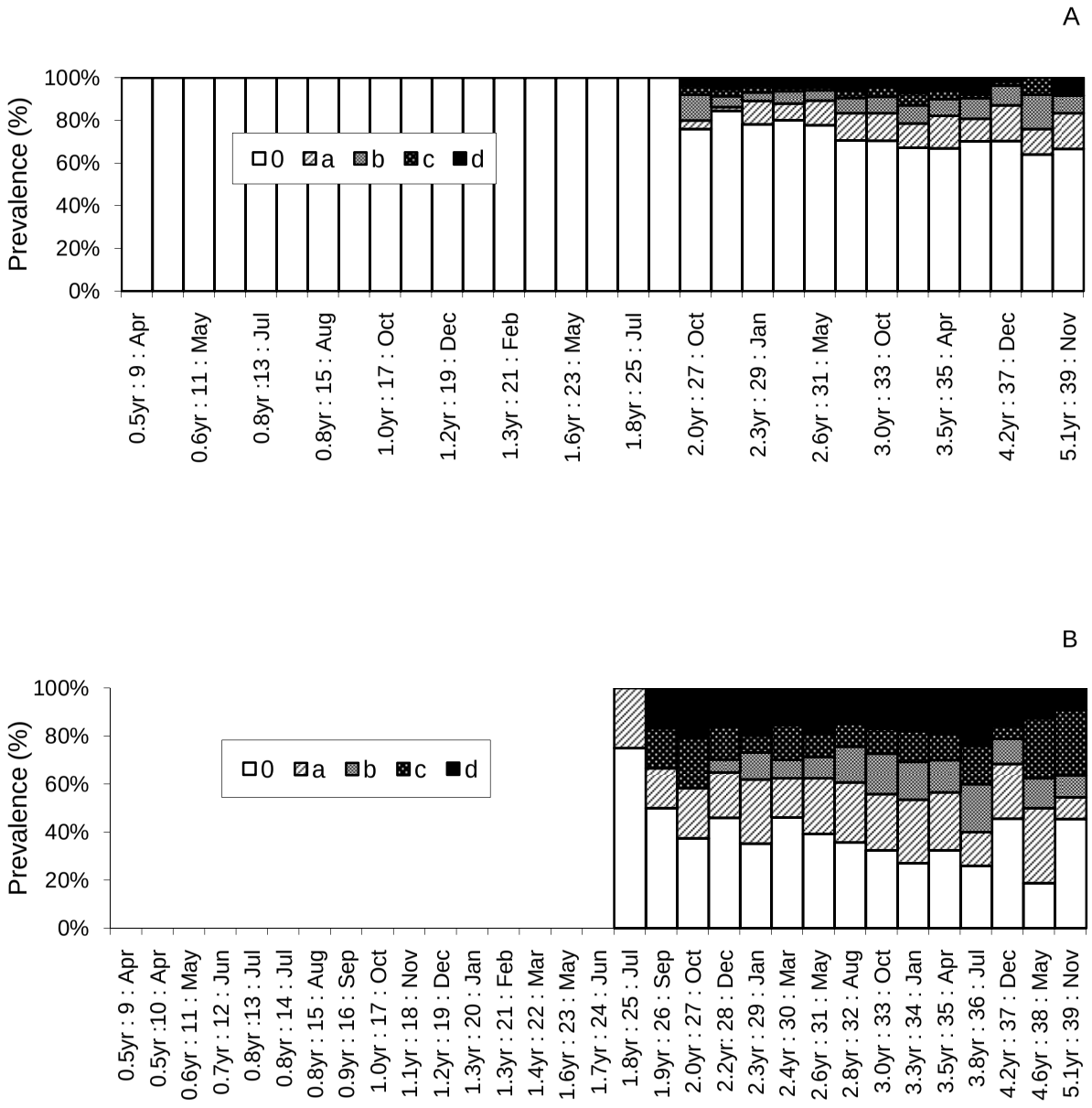

Figure 8.3. Prevalence of brown muscle disease in Manila clams *Ruditapes philippinarum*, including the different stages of the pathology (see text). X-axis: for example "0.5yr: 9: Apr" means that the predicted age of the clam is 0.5 yr, corresponding to a shell length of 9 mm, expected to occur in the field in April. A: buried clams ; B: surface clams

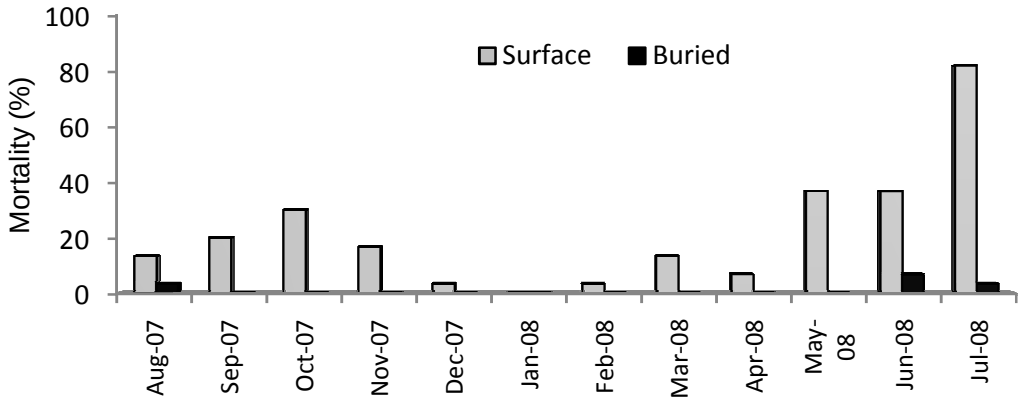

Figure 8.4. Mortality rates of Manila clams *Ruditapes philippinarum* collected in the field at the sediment surface and in buried position after 15 days in running water in the laboratory.

The monthly survey showed that surface clams were more heavily affected by BMD (Fig. 8.5). Indeed, the mean prevalence was 67% (minimum: 44%, maximum: 96%) against 26% for buried clams (minimum: 14%, maximum: 36%). There was no clear seasonal pattern. Clam condition index (CI) was affected by BMD (ANOVA, $p < 0.05$) (Fig. 8.6). In healthy clams, CI fluctuated between 37.8 and 69.8‰, following the reproductive cycle, with an annual mean of 43.8‰. CI index decreased with the severity of the disease, with annual means of 40.7, 38.8, 36.9 and 35.2‰ for stages a, b, c and d, respectively. The mean decrease of the CI in stage-d clams was 20% of healthy clam's CI but could occasionally reach 30% (January).

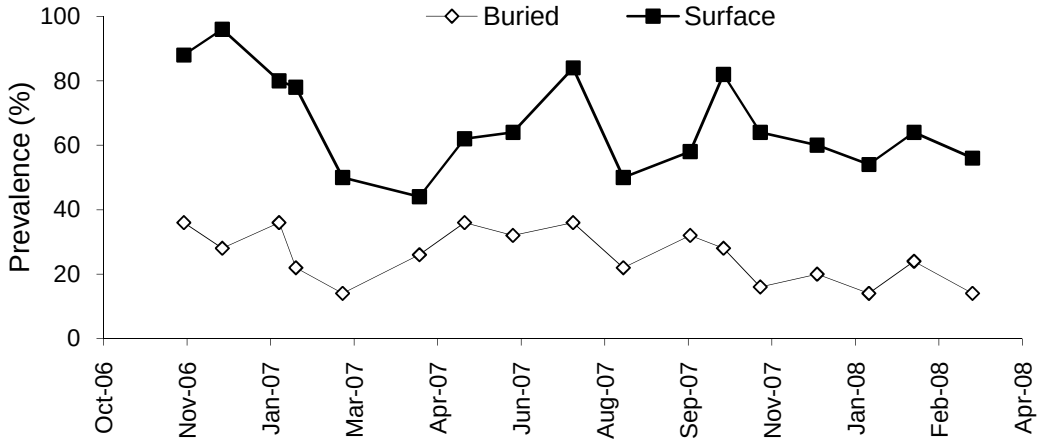

Figure 8.5. Prevalence of brown muscle disease in Manila clams *Ruditapes philippinarum* collected in the field at the sediment surface and in buried position.

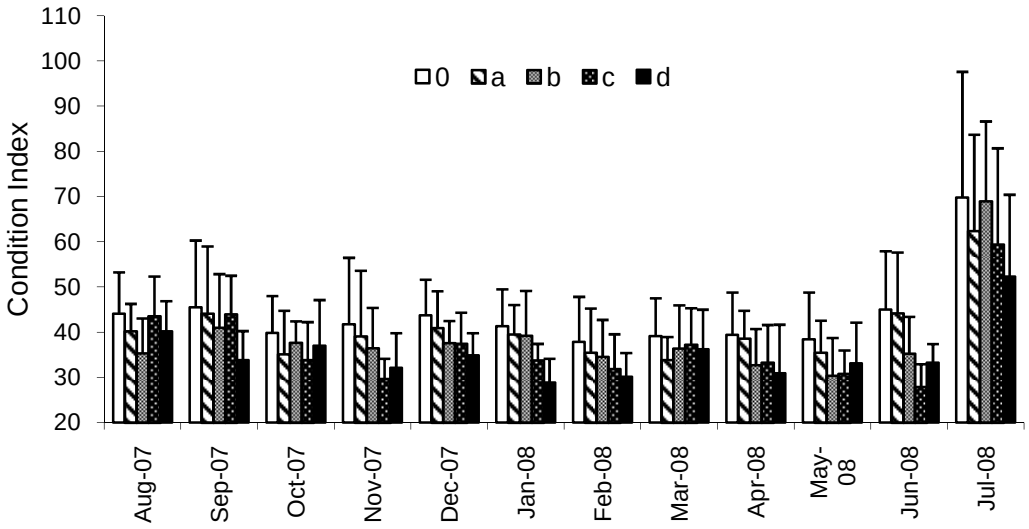

Figure 8.6. Condition index (+1 standard deviation) of Manila clams *Ruditapes philippinarum* in healthy clams (0) and brown muscle diseased clams (stages a to d, see text).

3.4. BMD-dependent mortality and BMD transmission period

Pooled data did authorize neither to detect any seasonality in BMD transmission nor to estimate the part of mortality due to BMD. To reach this aim, it was necessary to work at cohort scale. Modal progression analysis (Bhattacharya's method) separated confidently (separation index > 2) four cohorts, from 2003 to 2005. However, due to hand sampling, 2003 was the only cohort that was exhaustively collected, with mean shell length = 30.9 mm at the beginning of the study (November 2006) and 35.9 mm at the cohort disappearance (July 2007) (Fig. 8.7). At start, the clam density was 43 ind/m², *i.e.* 75% of the total population abundance (Fig. 8.2), and mFDI of the cohort was 6.1 (Fig. 8.8). Most individuals were buried (more than 80% except in January 2007 with only 64%). After five months (in March 2007), the density dropped to 16.7 ind/m², corresponding to a mortality rate of 39%. At that time, mFDI had decreased (ANOVA, $p < 0.05$) and reached the lowest value with 1.7. To obtain this mFDI, it was necessary to "kill" 32% of the individuals (the most affected by BMD, see Materials and Methods). Consequently, it was assumed that 95% of the total mortality was due to BMD. mFDI remained even until April 2007 and progressively increased afterwards with sediment temperature , especially in July 2007.

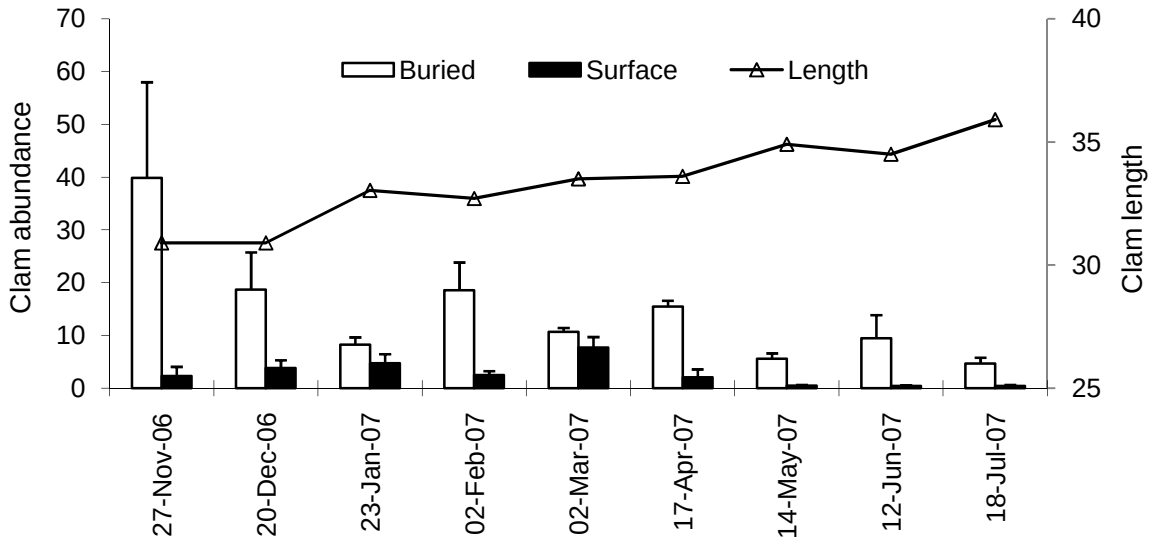

Figure 8.7. Abundance (ind/m², +1 standard error) of Manila clams *Ruditapes philippinarum* from 2003's cohort, found in buried position or at the sediment surface and mean shell length (mm).

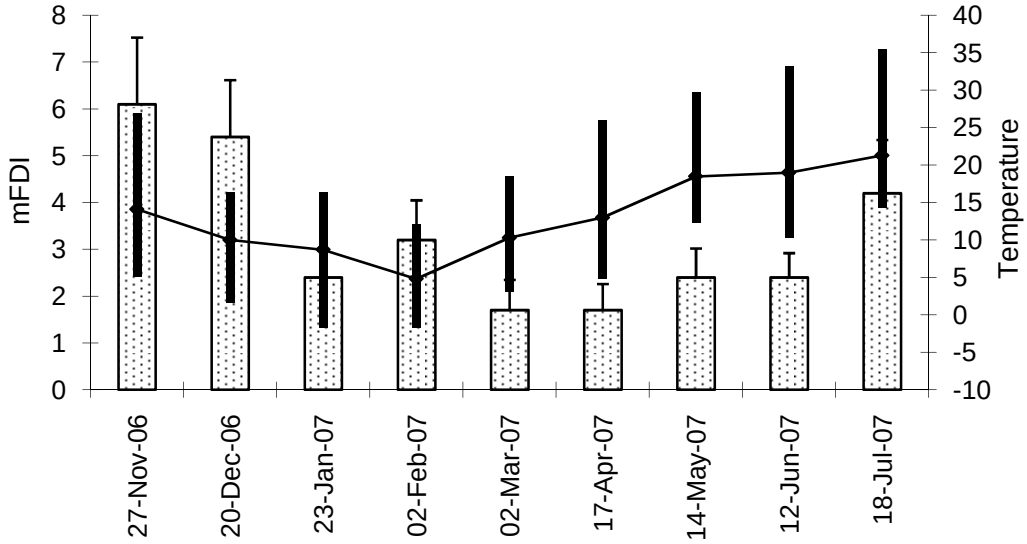

Figure 8.8. Mean FDI of Manila clams *Ruditapes philippinarum* from 2003's cohort and mean sediment temperature of the 24 previous hours (°C, bars for minimum and maximum).

4. Discussion

Brown Muscle Disease (BMD) appeared as a prevalent pathology for Manila clams in Arcachon Bay where it can be considered as the second disease in term of prevalence, behind perkinsosis (Lassalle et al. 2007), but before helminthosis (Dang et al. 2009-b) or brown ring disease (Lassalle et al. 2007). BMD affected adult Manila clams only (>25 mm or >2 yr). Three non mutually exclusive hypotheses could be evoked to explain the lack of young BMD-affected clams. 1) BMD has not affected clams for two years, and consequently did not occur in the youngest cohorts. However, the regular increase of prevalence with shell length (and clam age) made this option doubtful.

2) Young clams rapidly died when contracting BMD and were never sampled at intermediate stages. The fact that diseased clams were retrieved at the surface of the sediment before those in buried situation (25 mm vs 27 mm, corresponding to 3 mo elapsed time) partly argued for this hypothesis. Indeed, it could be assumed that at these shell lengths diseased clams could survive but were still not able to maintain themselves in buried position. However, out of the 1084 clams collected at the surface of sediment, none had a shell length < 25 mm.

3) The pathology necessities adult hosts. Size-dependent pathology was well-described for trematodes parasites which utilize host's gonad to develop, in their mollusk first intermediate hosts (Kube et al. 2006, Lajtner et al. 2008) or for protozoans like *Perkinsus* genus which could infest all tissues of mollusks. This parasite can infect clams and the infection intensity has been correlated to the clam size (Choi and Park 1997, Park et al. 1999, Park and Choi 2001, Villalba et al. 2005). In *Ruditapes decussatus* from Spain, no infection was observed in clams < 20 mm (Villalba et al. 2005) whereas no infection was found in clams <15 mm in *Ruditapes philippinarum* from Korea (Choi and Park 1997). Other disease like brown ring disease affecting *R. philippinarum* was not size dependent and could attain both adults and juveniles (Paillard 2004, Paillard et al. 2006).

Results from 2003's clam cohort as well as from correlation between shell length and BMD infection suggested that BMD developed during spring and summer months when mean sediment temperature was >13°C, with a peak in July (T = 21°C). This was also the period when the most important proportion of clams laid at the sediment surface with the lowest expectation of life. Dang et al. (2008) had rather pointed out winter peaks of BMD infection but from a mixture of clam cohorts that did not allow correct estimations. Temperature is considered as a major key factor in disease transmission but with contrasting correlations following species. Brown ring disease occurs within the lower values of temperature range

(Paillard 2004) when perkinsosis is a rather ‘warm disease’ (Villalba et al. 2005). In the same way, temperature could also be an important factor in some viral diseases. For instance, the ayoka-virus affecting the Japanese pearl oyster *Pinctada fucata martensii* induces high mortalities and symptoms tends to occur during summer when the temperature is up to 25°C (Miyazaki et al. 1999). Furthermore, the lymphocystis disease virus which infects the Japanese flounders *Paralichthys olivaceus* only replicated in its host when water temperature was around 20°C (Kitamura et al. 2007).

The similar proportion of individuals at each BMD stage suggested that the disease progresses regularly. The muscle was not the only affected tissue because the condition index (CI) concomitantly decreased with a mean loss of 20%. The mean CI of clams at stage d (35‰) was lower than the lowest CI of healthy clams along their sexual cycle (38‰). The decrease of Manila clams CI was certainly due to a loss of energy and particularly glycogen reserves to struggle against BMD progression through immune responses. This was supported by the important hemocytic infiltration observed within the muscle (Dang et al. 2008, 2009). However, CI of diseased clams also followed seasonal rhythm with a peak in July, suggesting that they were still able to reproduce. Some clam diseases like perkinsosis or brown ring disease (BRD) could reduce the clam CI (Casas et al. 2002, Leite et al. 2004, Flye-Sainte-Marie et al. 2007b). *Perkinsus olseni* led to a decrease of CI in infected clams *R. decussatus* in Portugal (Leite et al. 2004) and *R. philippinarum* in Korea (Park et al. 1999). Some authors observed a significant reduction of the CI in infected clams only during gametogenesis and not during the post-spawning period (Casas 2002). Clams with high infection of BRD exhibited a reduction of 27 to 35% of their CI, indicating a significant disease-associated weight loss (Flye-Sainte-Marie et al. 2007-b).

Assessment of mortality due to BMD was a complicated task because BMD occurred in adults whose age was difficult to assess. However, we could separate 2003’s cohort during part of its lifespan. This was a good cohort to analyze because we were sure to collect all individuals (shell length at start > 30 mm) and the length was still under exploited threshold (36 mm against 40 mm for legal catch size in 2007). Mortality could consequently be considered as natural. Mortality rate due to BMD was assessed using a theory developed for digenean trematodes. The main hypothesis was that, in a given cohort, the decrease of the pathology intensity was due to the death of the most affected individuals (Anderson and Gordon 1982, Kennedy 1984, Lester 1984, de Montaudouin et al. 2003, Desclaux et al. 2004). However, the decrease of a given pathogenicity index (as mFDI, parasite intensity,…) may

result from different phenomenon: 1) emigration of parasitized animal, or recruitment, or immigration of unparasitized animals. With such a sedentary species and working at the cohort level, this hypothesis could be excluded; 2) recovery processes of diseased clams as described for the brown ring disease (Paillard 2004). None of our observations suggested the least sign of recovery. However this possibility cannot be completely eliminated: 3) death of the most affected clams. This is for the moment the most probable issue. The mortality of 2003's cohort clams during the five studied months was 39%. For such a class-size (>30 mm), mortality rates ranged between 3.1 and 3.7% per month (*i.e*. less than 19% in five months) in this precise site for BMD-free clams (Dang et al. in revision-b). This rate was considered as high but was recorded in enclosures that may attract predators as crabs or rough tingles (*Ocenebra*). The herein clam mortality values (39% in five months) could consequently be considered as much higher than expected. The calculated part of BMD responsibility in the clam mortality was 32% which gives to this factor a major importance (95%) in explaining natural adult mortality.

The present study evidenced the important impact (high prevalence and high mortality rates) of an emergent pathology on Manila clam population in Arcachon Bay. Even if the causal agent is not actually identified, BMD transmission and/or development period has been assessed. Further studies will focus on BMD etiology. As a viral etiology is highly suspected, the purification of viral particles will be attempted and an experimental infection will be realized. Thus, disease transmission parameters *i.e.* environmental factors (temperature, salinity) or minimum size of infection will be confirmed.

Chapitre 9

Impact de la BMD sur la composition isotopique du muscle adducteur des palourdes

Stable isotopes changes in the adductor muscle of diseased bivalve *Ruditapes philippinarum*

Dang C., de Montaudouin X., Sauriau P. G., Savoye N., Caill-Milly N., Martinez P.

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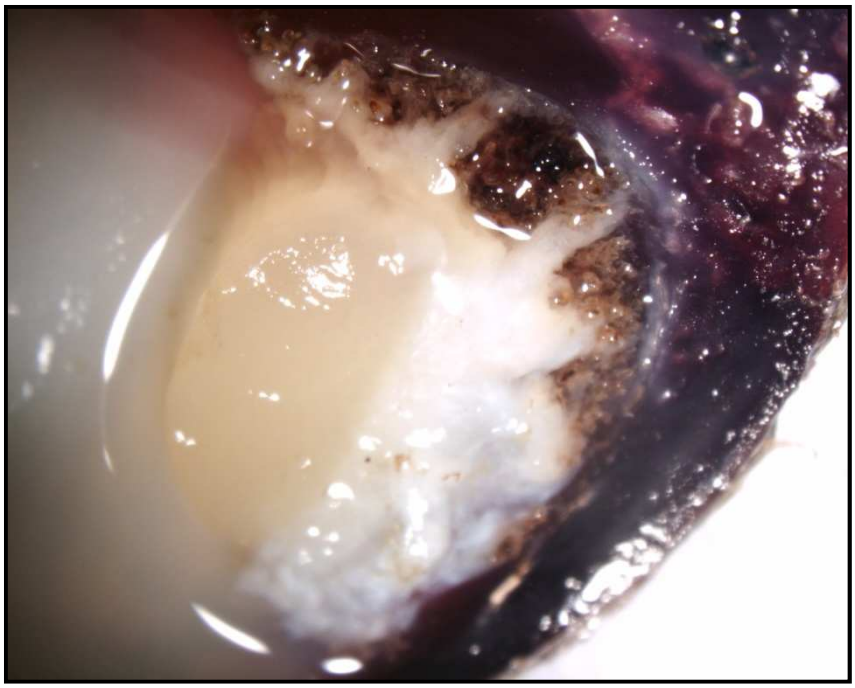


Muscle adducteur postérieur de palourde en stade intermédiaire de BMD

Abstract

In this article, we show how a disease could bias stable isotope analyzes of trophic networks and propose a strategy in the choice of tissues to be analyzed. In the past few years, a new pathology (brown muscle disease or BMD) affecting the posterior adductor muscle of *Ruditapes philippinarum* has emerged in Arcachon Bay. BMD induces a necrosis of muscle tissues which become infused by conchiolin and hence calcified. As muscle of mollusks are often used for trophic food webs studies through stable isotopic analyzes, this work investigated the effect of BMD on carbon and nitrogen isotopic ratios of anterior and posterior adductor muscles of clams collected in February and August 2007. Infected clams displayed a lower condition index and a posterior adductor muscle $\delta^{13}C$ enrichment of 1.2‰ in February and 0.7‰ in August. $\delta^{15}N$ of posterior muscles was however not affected by the disease. Anterior muscle of diseased clams remained healthy and displayed the same isotopic signature as both posterior and anterior muscular tissues of healthy clam. Acidification significantly depleted $\delta^{13}C$ in posterior muscles of infected clams, suggesting calcification, contrary to anterior muscles of infected clam and to both muscles of healthy clams, where no effect was observed. An X-ray diffractometry analysis confirmed the presence of $CaCO_3$ (aragonite). Trophic food web studies relying on stable isotope ratios should utilize only healthy animals or anterior adductor muscles when expertise in mollusk pathology is lacking.

Keywords: Clam, *Ruditapes philippinarum*, Stable isotopes, Adductor muscle, Disease, Calcification

1. Introduction

The Manila clam *Ruditapes philippinarum* (Adams and Reeve 1850) originates from Indo-Pacific waters and nowadays contributes to more than half of global yields of clams. Since 1930, Manila clam has been introduced with Pacific oyster (*Crassostrea gigas*) seed into different parts of the world, e.g. from the United States to Canada and to the Hawaiian islands (Flassch and Leborgne 1992). In Europe, *R. philippinarum* was primarily introduced into France for culture purposes in 1972 and later to England, Spain and Italy (Flassch and Leborgne 1992). Within a few years, the species established natural populations in most southern European countries and particularly along the French Atlantic coast. In Arcachon Bay (SW France), *R. philippinarum* was first introduced in 1980 as a commercially attractive species. It rapidly escaped from clam-growing areas and colonized intertidal sea-grass beds (*Zostera noltii*). Today, *R. philippinarum* undergoes intensive exploitation by local fishermen and has a relatively high economic value.

Since its introduction to France, *R. philippinarum* has endured several infectious pathologies such as the brown ring disease (BRD) caused by the bacterium *Vibrio tapetis* (Paillard 2004), perkinsosis induced by the protozoan *Perkinsus* sp. (Lassalle et al. 2007), and to a lesser extent, trematode infections (Lassalle et al. 2007). Recently, a presumptive viral pathology called the brown muscle disease (BMD) has occurred in Manila clam populations from localities of Arcachon Bay (Dang et al. 2008, 2009). The anterior adductor muscle never exhibits macroscopic signs of the disease (Dang et al. 2008).

Stable isotope analyzes (SIA) are widely used to investigate dietary pattern and trophic relationships in the marine environment (Peterson and Fry 1987). $\delta^{13}C$ values help in identifying primary food sources assimilated by consumers (Fry and Sherr 1984) whereas $\delta^{15}N$ values allow determination of their trophic levels (DeNiro and Epstein 1981). SIA have also recently been used to characterize host-parasite systems and provide a means of exploring relationships between a host and its parasites (Deudero et al. 2002).

The objectives of this study were to investigate the effects of BMD on the stable isotopic carbon and nitrogen ratios of adductor muscles, of Manila clams. Condition index and SIA are expected to provide complementary information on possible metabolic disturbances within muscles, as anterior and posterior adductor muscles of healthy and BMD-infected clams were comparatively analyzed by SIA. The idea was to compare stable isotopic signatures of infected muscle (posterior) with anterior muscle which never develops

macroscopic signs of BMD in order to assess in what extent $\delta^{13}C$ and $\delta^{15}N$ values are affected by the disease and could lead to shortcomings in term of trophic relationships interpretations. The possibility that muscle was calcified by the disease was also investigated in order to test the possibility to acidify tissues prior to SIA.

2. Materials and methods

2.1. Sampling strategy and clams treatment

Specimens of *R. philippinarum* were collected at Lanton, an intertidal area of Arcachon Bay, which is located on the southwestern Atlantic coast of France. Manila clams were then selected at different macroscopic stages of the BMD: healthy (Fig. 9.1a), intermediate and advanced (Fig. 9.1b) (Dang et al. 2008). At the intermediate stage, the striated part of the posterior adductor muscle was more severely infected than the smooth part. The adductor muscle attachment was reduced by the presence of brown spots which enlarged (initial calcification). The advanced stage of BMD displays a quasi-complete brown calcification of the striated muscle like the major part of the smooth muscle.

Ten healthy and ten diseased clams (30-36 mm shell length) were sampled in February 2007. Diseased clams were only collected in the intermediate stage of BMD. Posterior adductor muscles were dissected and freeze-dried for 48 h. According to the preliminary results obtained in February, ten healthy and ten diseased clams of the same shell length were sampled at the same location in August 2007 and both anterior and posterior adductor muscles were analyzed. Infection was quantified in clams in advanced stages of BMD in August by the final disease index (FDI) proposed by Dang et al. (2008) from 0 (no infection observed) to 4 (fully infected). Clams were opened, their FDI evaluated and their posterior and anterior muscles dissected, freeze-dried and weighed. Dry weights (mg) of the remaining tissues together with dry weights of their shells (g) were utilized for the calculation of the condition index (CI). The condition index is reported as CI = dry flesh weight (mg)/ dry shell weight(g).

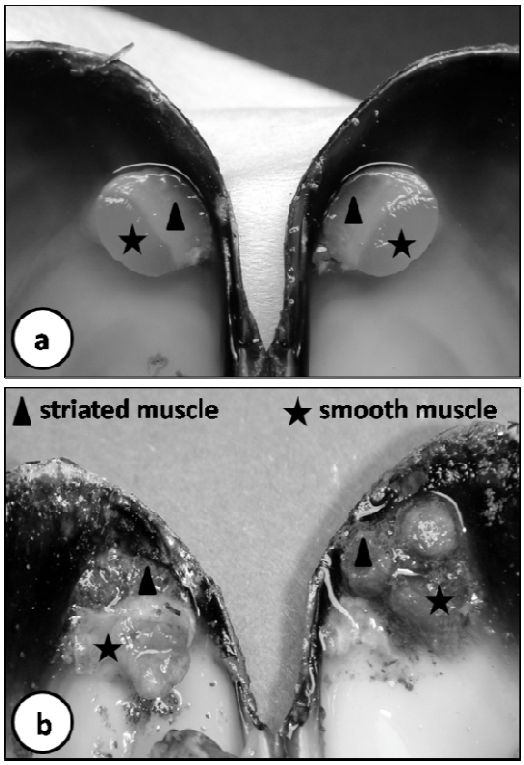


Figure 9.1. Naked-eye observations of healthy (FDI=0) (a) and diseased (FDI=d) (b) posterior adductor muscle (striated and smooth muscles) of *Ruditapes philippinarum*.

2.2. Isotopic and elemental analysis

Samples of clam muscle tissues were ground to a homogeneous fine powder and 1 mg was folded into tin cups (9-mm height, 5-mm diameter). Stable-isotope ratios measurements were performed on a Carlo Erba 2500 elemental analyzer in line with a VG Isoprime. The analytical precision was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$, estimated from several calibrated laboratory standards analyzed along with the samples. Replicates of February samples were performed to confirm the analytical reproducibility that was within 0.2‰. Stable isotopic ratios are reported as

$$\delta^{A}X = [(R_{sample}/R_{ref}) - 1] \times 1000$$

$$w\delta X = (R_{sample}/1) \times 1000$$

where A is the atomic mass of the heavy stable isotope of the element X, and R_{sample} and R_{ref} are the ratios of heavy to light isotope of sample and reference, respectively. References are

atmospheric nitrogen for $\delta^{15}N$ and Vienna Pee Dee Belemnita (PDB) for $\delta^{13}C$. Organic carbon and nitrogen contents were measured simultaneously with the stable isotopic ratios by integrating the voltage of the main ion beam (IRMS).

According to the extreme hardness of diseased posterior adductor muscles and following preliminary positive tests with HCl, the presence of calcification and therefore inorganic carbon was expected to occur. In many ecological studies, it has been common practice to acidify samples to remove inorganic carbonates which are less negative in $\delta^{13}C$ than other fractions (DeNiro and Epstein 1978).

To confirm this hypothesis and to exclusively evaluate the $\delta^{13}C$ of organic carbon in muscle tissue, all February and August samples were decarbonated by acidification for a second carbon isotopic analysis: 1.3 mg of powder was deposited into silver cups and acidified by adding 1M HCl drop by drop until the cessation of bubbling. At last, 140 µL was added and samples were left for 4 h in acid. Then, they were again dehydrated and freeze-dried for 48 h. Both carbon isotope ratios and carbon contents were measured, as previously described.

2.3. Powder X-ray diffractometry (XRD)

Powder XRD is a convenient, non-destructive tool used to differentiate between multiple phases of materials, owing to the unique diffraction patterns produced from the crystallographic structures of each polymorph. Since the diffraction pattern of each crystalline form of a compound is unique, XRD is particularly suited for the analyzes of solid mixtures. Moreover, the intensities of the peaks are unique to each phase and enable qualitative analyzes. The analysis is then expected to confirm calcification of the diseased muscle and to determine which crystal is present. Powder remaining from the isotopic analyzes of August posterior muscles was used. The ten healthy and the ten diseased samples were pooled in order to get enough healthy and diseased powder for the XRD analysis. Then, powder were placed in aluminium holders, pressed with a glass blade and exposed to CuKα radiation (Kα = 1.5418 Å) in a wide-angle X-ray powder diffractometer (Model, PANalytical X'pert MPD, Bragg-Brentano geometry). The angular range was 8.020°-79.980° and the angular step size was 0.02°. Eventually, diffraction patterns of samples were elaborated and compared to diffraction patterns of known crystals using DIFFRACplus software in order to determinate which crystals, if any, were present in the samples.

2.4. Statistical analyzes

Differences between diseased and healthy muscles were tested with analyzes of variance and/or non-parametric test. Maximum type I error rates were set at $\alpha = 0.05$. Homogeneity of variance was checked using Cochran test. Significant ANOVA results were followed by multiple comparisons using the conservative Tukey's HSD post-hoc test (Sokal and Rohlf 1981). When variances were not homogeneous, the non-parametric Mann-Whitney U test and Kruskal-Wallis test were used to assess differences between two and several groups of samples, respectively. Correlation between variables non-normally distributed was tested by the Spearman's rank correlation method. Statistical analysis was performed using Statistica software 7.1.

3. Results

3.1. Condition index and final disease index

Healthy and diseased adductor muscles can be easily distinguished (Fig. 9.1). Healthy clams displayed a significant higher condition index than infected clams ($p = 0.007$) with average values of 54.4 ± 4.8 ($\pm$ SE) and 34.4 ± 4.5, respectively. Final Disease Index (FDI) was calculated for each clam processed in August and FDI ranged between 8 and 16 for diseased clams. A significant negative correlation was found between FDI and the condition index ($r = -0.54$, $p = 0.013$, $n = 20$).

3.2. Pre-acidification stable isotopes analyzes

In February, $\delta^{13}C$ and $\delta^{15}N$ values of the posterior adductor muscle of *R. philippinarum* ranged from -17.5 to -16.6‰, and 8.8 to 9.7‰ respectively for healthy clams whereas these ratios varied from -16.2 to -14.1‰, and 8.5 to 9.5‰ respectively for diseased clams (Fig. 2a). $\delta^{13}C$ values of infected clams exhibited a higher range than those of healthy clams (2.1 *vs* 0.9‰). Muscles of diseased clams were significantly enriched in ^{13}C ($p = 0.0002$) by 1.2‰ on average (-15.6 *vs* -16.8‰) compared to healthy clams (Fig. 9.2a, Table 9.1). No significant difference was noted in the $\delta^{15}N$ values between healthy and diseased clams (8.9 *vs* 9.2‰) ($p = 0.111$). C/N ratios in healthy animals ranged from 3.8 to 4.1 with a mean value of 3.9 ± 0.02 (Fig. 9..2b, Table 9.1). Conversely, diseased clams exhibited scattered values between 3.9 and 5.5 (mean = 4.4 ± 0.16) (Fig. 9.2b). Posterior muscles of BMD infected clams had significantly higher C/N ratios than healthy bivalves ($p = 0.001$).

Table 9.1. Means (± SE, n = 10) of carbon and nitrogen stable isotope ratios (‰) and C/N content (mol.mol^{-1}) of *Ruditapes philippinarum* adductor muscles before and after acidification. H: healthy, D: diseased, P: posterior, A: anterior.

			Before acidification			**After acidification**
Date	**Status**	**Adductor muscle**	$\delta^{15}N$	$\delta^{13}C$	C/N	$\delta^{13}C$
February	H	P	9.2 (±0.11)	-16.82 (±0.13)	3.94 (±0.02)	-16.85 (± 0.13)
	D	P	8.9 (±0.13)	-15.62 (±0.23)	4.44 (±0.16)	-16.43 (± 0.12)
August	H	A	8.9 (±0.15)	-17.49 (±0.07)	3.68 (±0.02)	-17.60 (± 0.08)
		P	8.55 (±0.06)	-17.57 (±0.06)	3.70 (±0.02)	-17.53 (± 0.07)
	D	A	9.10 (±0.14)	-17.60 (±0.09)	3.85 (±0.02)	-17.40 (± 0.09)
		P	8.13 (±0.07)	-16.80 (±0.25)	4.59 (±0.07)	-17.89 (± 0.11)

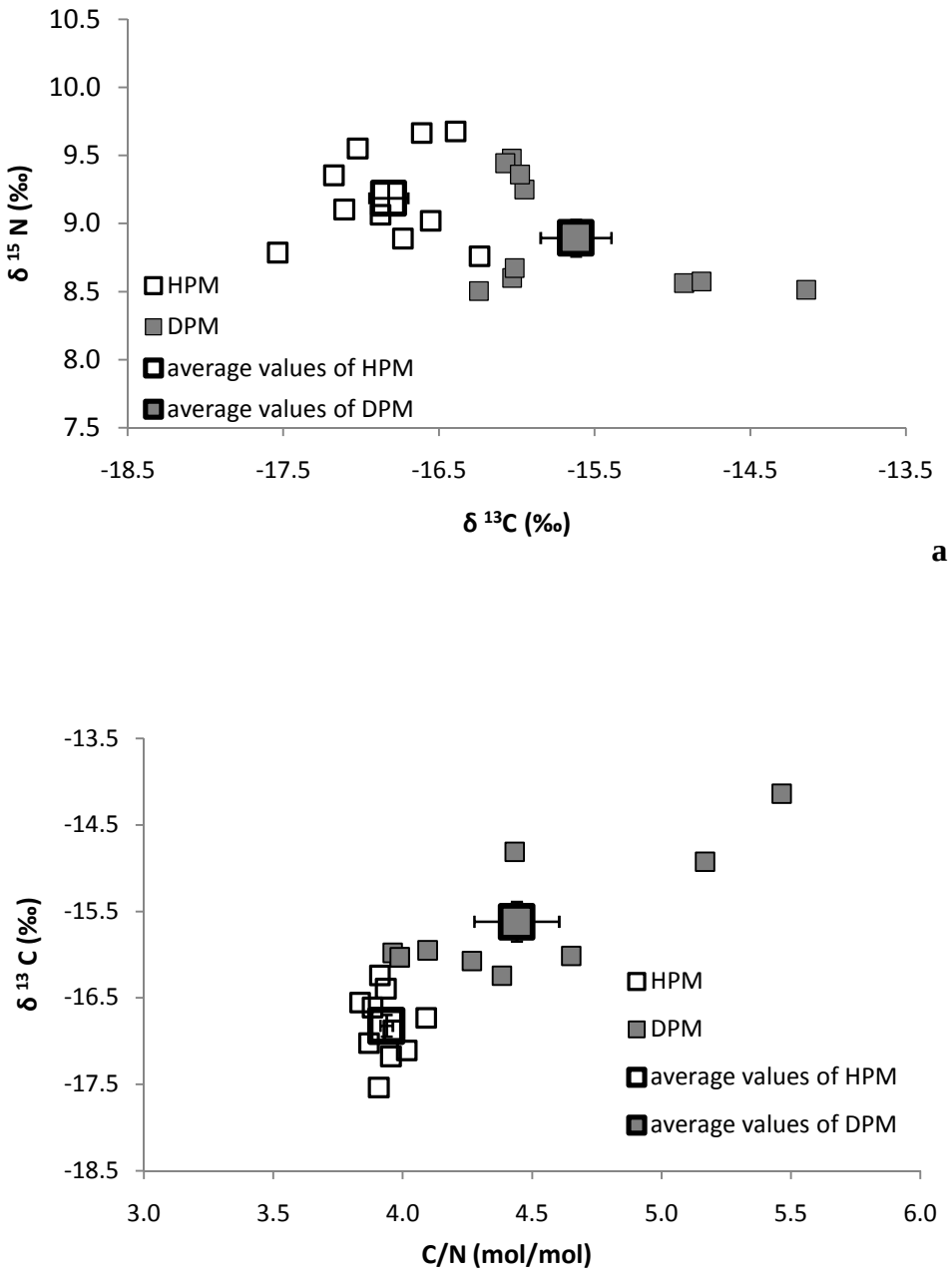


Figure 9.2. Dual plots of nitrogen *vs* carbon stable isotope ratios (a) and carbon stable isotope ratio *vs* C/N ratio (b) in posterior adductor muscles of *Ruditapes philippinarum* in February 2007. HPM, healthy posterior muscle. DPM, diseased posterior muscle. Averages are represented ± standard error.

In August, both anterior and posterior muscles were analyzed. Due to seasonal variations, posterior adductor muscles of healthy clams in August were depleted in ^{13}C compared to February (-17.6 *vs* -16.8‰) (Figs. 9.2a, 9.3a, Table 9.1). The dual plot of $\delta^{13}C$ and $\delta^{15}N$ in August exhibited the same trends as in February with a higher range in $\delta^{13}C$ for posterior muscles of diseased clams compared to those of healthy clams (2.4 *vs* 0.6‰) combined with a ^{13}C enrichment (0.7‰) in diseased clams. $\delta^{13}C$ and $\delta^{15}N$ values varied from -17.9 to -17.2‰, and 7.9 to 9‰, respectively, for healthy clams and from -17.9 to -15.6‰, and 7.8 to 8.4‰ respectively for infected clams (Fig. 9.3a). Mean values of carbon and nitrogen stable isotope signatures were -17.6 ± 0.05‰ and 8.6 ± 0.05‰ respectively for healthy bivalves, -16.8 ± 0.25‰ and 8.1 ± 0.07‰, respectively for diseased clams.

For anterior muscles, $\delta^{13}C$ and $\delta^{15}N$ values ranged from -17.8 to -17‰, and 8 to 9.7‰ with mean values averaging -17.5 ± 0.05 ‰ and 8.9 ± 0.11 ‰ respectively for healthy clam, and ranged from -17.8 to -17.1‰, and 8.7 to 9.9‰ with mean values of -17.6 ± 0.09‰ and 9.1 ± 0.13‰ respectively for diseased clams. There was no significant difference in both $\delta^{13}C$ and $\delta^{15}N$ values of anterior muscles between healthy and diseased clams ($p = 0.22$ and $p = 0.39$, respectively). $\delta^{13}C$ range was low and averaged 0.7‰ for both infected and no infected animals (Fig. 9.3a).

Posterior muscles of infected clams were significantly enriched in ^{13}C compared to anterior muscles of both healthy and infected clams and to posterior muscles of healthy bivalves ($p = 0.001$, Tukey Test, Fig. 9.3a, Fig. 9.4). $\delta^{15}N$ values of both infected and healthy clams anterior muscles were significantly different from those of both posterior muscles ($p < 0.001$, Tukey Test, Fig. 9.4). $\delta^{15}N$ values of posterior muscles were not significantly different from those of anterior muscles in healthy clams in contrast to diseased clams ($p < 0.001$, Tukey Test, Fig. 9.4).

C/N ratios ranged from 4.3 to 4.9 and averaged 4.6 ± 0.07 for posterior infected muscles and ranged from 3.6 to 3.9 for anterior diseased muscles and both healthy muscles (Fig. 9.3b). C/N ratios were significantly higher for posterior muscles in infected clams compared to anterior muscles in infected clams and both muscles in healthy clams ($p < 0.001$).

FDI calculated for each clam processed in August varied between 8 and 16 with a high proportion of clams in class 16. Significant positive rank correlations were found between FDI and both $\delta^{15}N$ and C/N ratios ($r = -0.5$, $p = 0.01$ and $r = 0.9$, $p < 0.001$, respectively, $n = 20$). No significant correlation was observed between FDI and $\delta^{13}C$ ($r = 0.3$, $p = 0.19$, $n = 20$).

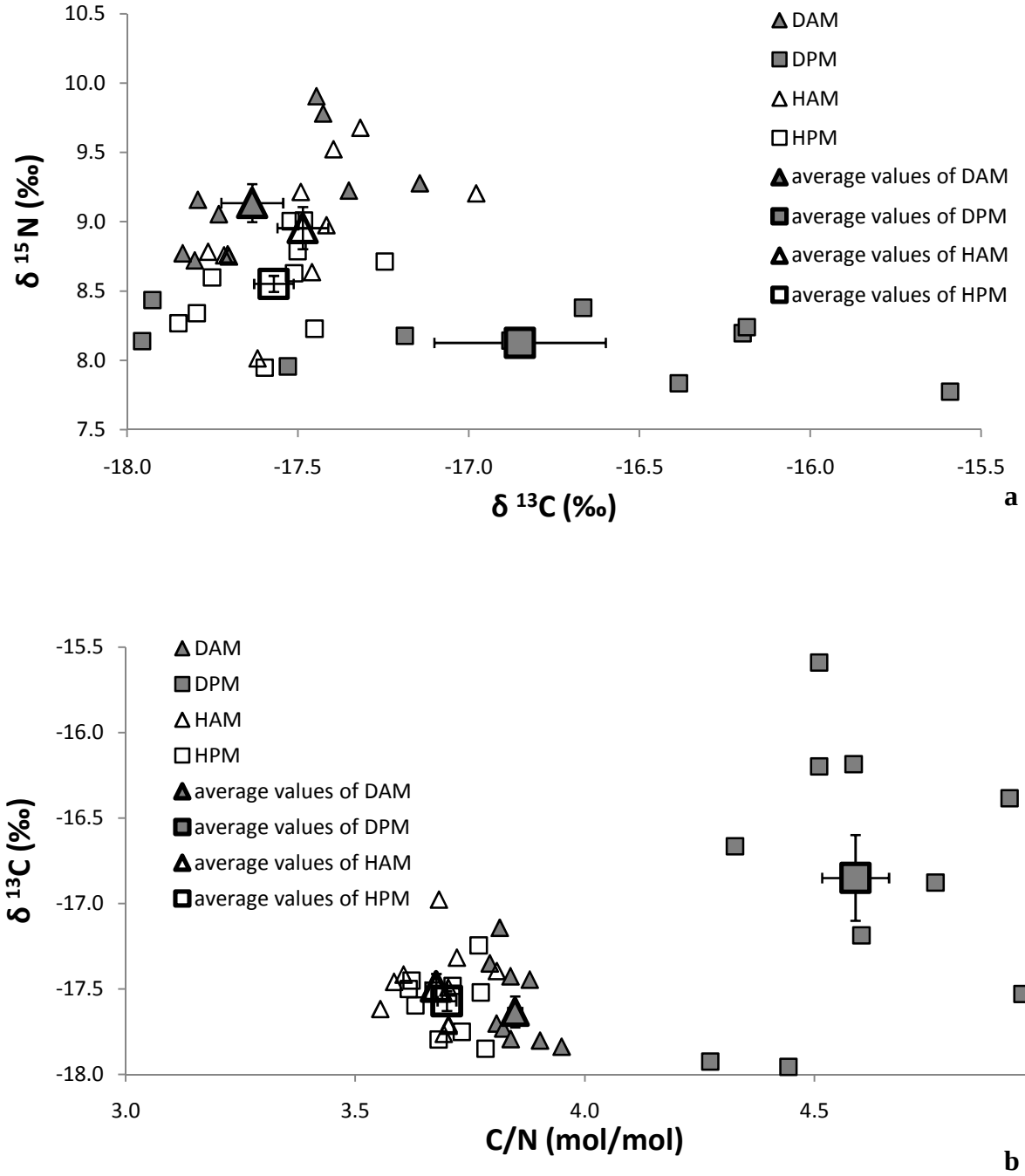


Figure 9.3. Dual plots of nitrogen *vs* carbon stable isotope ratios (a) and carbon stable isotope ratio *vs* C/N ratio (b) in anterior and posterior adductor muscles of *Ruditapes philippinarum* gathered in August 2007. HPM, healthy posterior muscle. DPM, diseased posterior muscle. HAM, healthy anterior muscle. DAM, diseased anterior muscle. Averages are represented ± standard error.

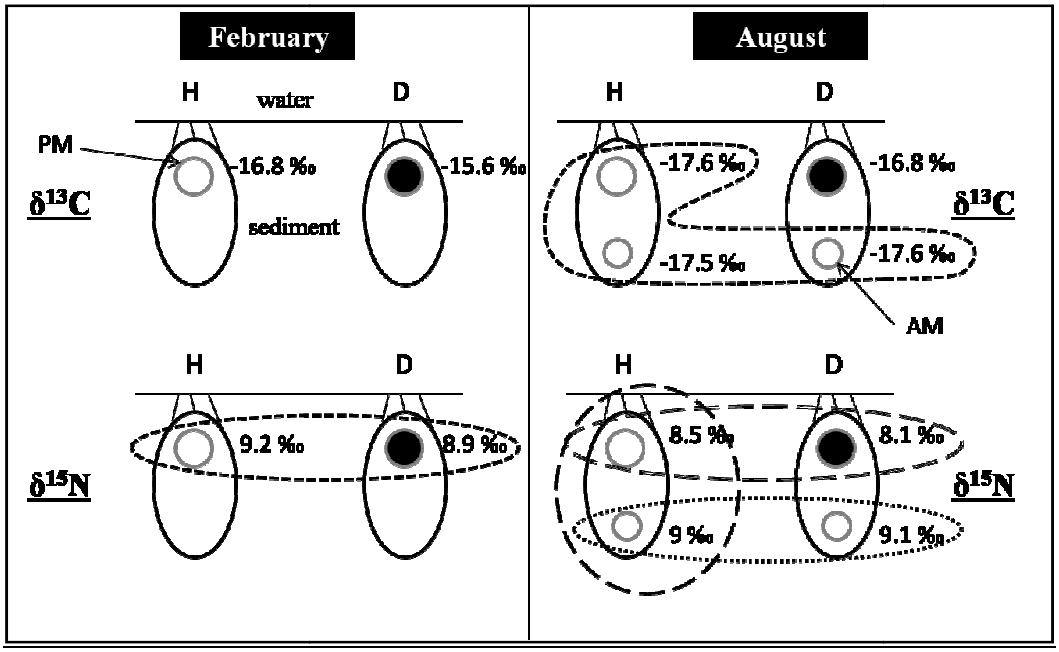


Figure 9.4. $\delta^{13}C$ and $\delta^{15}N$ averages of healthy (H) and diseased (D) clams in February and August 2007. Dotted lines gather values that were not significantly different (Anova, Tukey test $p > 0.05$). AM: anterior muscle; PM: posterior muscle.

3.3. Post- acidification stable isotopes analyzes

All clam samples from both February and August samples were analyzed for stable isotopes after acidification to remove inorganic carbon. No significant effect of the acidification process was noted for both muscles of healthy clams and for the anterior muscle of diseased clams ($p > 0.05$). In contrast, significant ^{13}C depletions of 0.8‰ in February and 1.1‰ in August were observed after acidification of the posterior muscle of diseased clams ($p = 0.005$) (Table 9.1). $\delta^{13}C$ values of infected posterior muscles were -15.6 and -16.8‰ before acidification and became -16.4 and -17.9‰ in February and August, respectively after acidification (Table 9.1). Acidification reduced the range in $\delta^{13}C$ values of the posterior infected muscle, which became similar to the low variability observed in $\delta^{13}C$ values of healthy clams (Table 9.1). However, the posterior adductor muscle after acidification remained significantly different ($p < 0.05$) from both healthy muscles and anterior muscle of diseased clams.

3.4. X-ray diffractometry

Apart from the occurrence of four peaks corresponding to aluminum of the holder, the XRD pattern of posterior adductor muscle of diseased and healthy clams greatly differed (Fig. 9.5). No mineral was detected within the healthy muscle sample. However, a bulge was noticed around 20° angle (2θ) and was assumed as the sign of amorphous organic material. This bulge was not found in the XRD pattern of diseased clams, which contained numerous peaks of crystalline compounds such as halite (NaCl) and aragonite ($CaCo_3$). Aragonite has an orthorhombic crystalline structure and was found in high concentration within the infected posterior muscles.

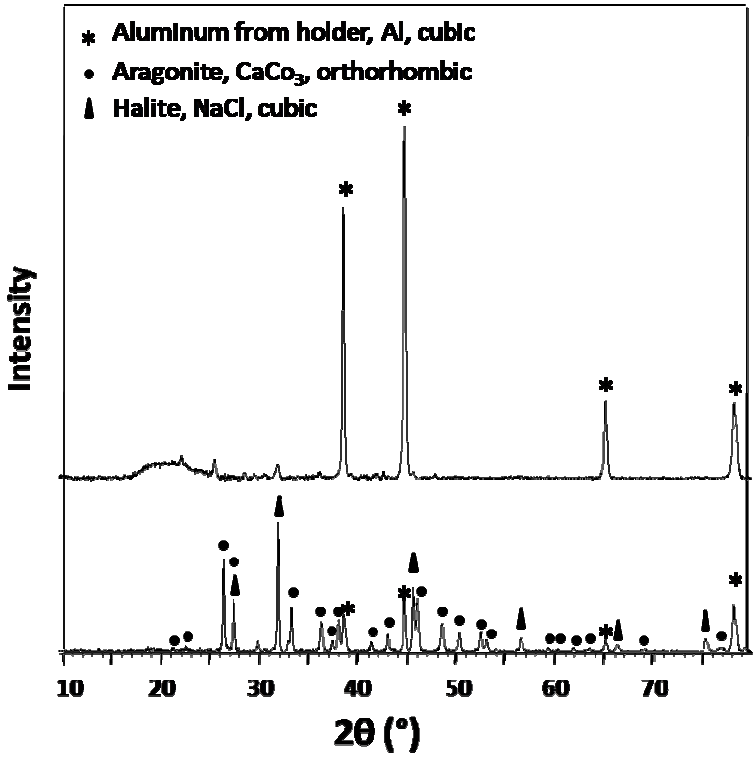


Figure 9.5. X-ray diffraction patterns of healthy (above) and diseased (below) muscles.

4. Discussion

BMD significantly modifies the $\delta^{13}C$ values of the posterior adductor muscle. Compared to healthy clams, the observed ^{13}C enrichment, *i.e.* 1.2‰ in February and 0.7‰ in August, is mainly but not exclusively due to the calcification by aragonite ($CaCo_3$). Indeed, after acidification, $\delta^{13}C$ of diseased posterior muscles is closer, but remains slightly different from $\delta^{13}C$ values of healthy muscles. Conversely, neither $\delta^{13}C$ of anterior muscles, nor $\delta^{15}N$ of both anterior and posterior muscles are modified by BMD.

Standards errors (SE) of carbon isotopic ratios of posterior adductor muscle of *R. philippinarum* were 0.13 and 0.06‰ in February and August respectively (Table 9.1). This variability was similar to that observed in other bivalve species. In general, SE were lower than 0.15 with $\delta^{13}C \pm$ SE of -22.6 ± 0.06‰ in *Crassostrea gigas* (Malet et al. 2007), -15.2 ± 0.03‰ in *Pecten maximus* (Lorrain et al. 2002), -16.4 ± 0.06‰ in *Mytilus galloprovinciallis* (Machás et al. 2003), -16.6 ± 0.08‰ in *Ruditapes decussatus* (Machás et al. 2003) and -14.3 ± 0.13 in *Cerastoderma edule* (Page and Lastra 2003). Conversely, diseased posterior muscle of Manila clam exhibited higher variability with $\delta^{13}C \pm$ SE values of -15.62 ± 0.23‰ and -16.80 ± 0.25‰ in February and August respectively (Table 9.1), according to various level of calcification within muscles.

The inter-individual variability in $\delta^{13}C$ in the present study was lower than 1‰ for posterior and anterior muscles of healthy clams and for anterior muscles of diseased clams. In contrast, this variability rose above 2‰ for diseased posterior muscles. This variability is very large compared to the usual trophic enrichment of 0.5-1‰ that occurs with trophic level increase in food webs (DeNiro and Epstein 1978, McCutchan et al. 2003). Consequently, BMD could bias the interpretation of isotopic data in the context of trophic studies if BMD were not recognized prior tissue analyzes. Indeed, the adductor muscle is commonly used in trophic studies of clams because of its long turnover rates compared to other tissues with fast turnover such as digestive glands and gonads (Kasai et al. 2004, Kanaya et al. 2005).

Even if BMD did not affect the isotopic signature of the anterior adductor muscle, the lower condition index of diseased clams compared to healthy clams suggests a general weakening of the animal. At this period of the year, the condition index of affected clams should be one and a half higher, like unaffected clams (Laruelle et al. 1994). The decrease of the condition index was in minor part due to the dry weight of muscles and in major part due to a weight loss of the whole animal.

BMD induces a calcification of the adductor posterior muscle as revealed by X-ray diffractometry. The presence of aragonite and not calcite within the muscle in clams infected by BMD appears normal because of its inner position within the clam. It may indicate that the calcification is controlled by biogenic processes, i.e. protein matrix (Goulletquer et al. 1989a), and immune response through enzyme activity (Jing et al. 2007).

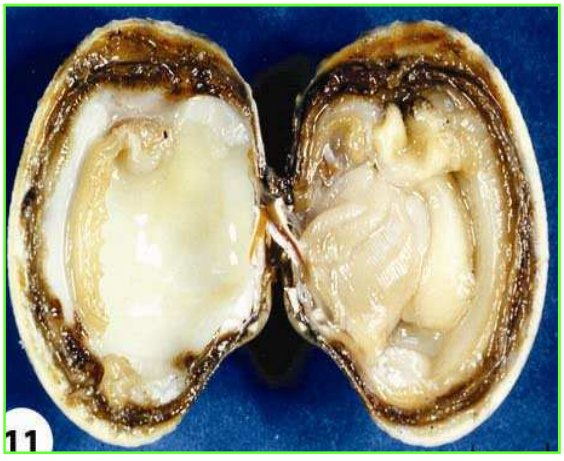
The isotopic signature measured in infected clams corresponded with the cellular response of clam in respect to an infectious agent and not to the infectious agent, like in many other studies where the parasite could be analyzed separately from the tissue (Deudero et al. 2002). Thus, the observed shift in $\delta^{13}C$ values actually reflects the metabolic consequences of the disease on the clam tissues and is thus a pathologic shift. To our knowledge, such pathologic shift in mollusk bivalve tissues has never been reported before.

Our results on SIA evidenced that precaution must be taken if Manila clams are planned to be included in trophic food web studies. Only healthy individuals should be considered after a closer examination of their posterior muscles or, as a conservative alternative, only anterior adductor muscles should be sampled, when expertise in mollusk pathology is lacking. Finally, this work displayed the importance to acidify Manila clam tissues to remove inorganic carbonates in trophic web studies.

La maladie de l’anneau brun

Chapitre 10

La maladie de l’anneau brun dans le bassin d’Arcachon



Palourde japonaise très infectée par la maladie de l’anneau brun (photo, C. Paillard)

Abstract

Brown ring disease (BRD) is a severe pathology that induced heavy mortalities in the Manila clam (*Ruditapes philippinarum*) stock from northwestern France in 1987. We investigated first the spatial distribution of BRD in Arcachon Bay and second the temporal dynamic (prevalence and infection level) of this disease. Spatial analysis revealed a mean low prevalence of 10.8% and a low level of infection, with 95% of clams at disease stage 1. The temporal survey also displayed low prevalence (16%) and a dominance of the lowest stage of infection. No seasonality of the disease was observed. Finally, BRD at Arcachon Bay was in a early stage of infection, similar to Korea.

Keywords: Manila clam, disease, brown ring disease

1. Introduction

The Manila clam *Ruditapes philippinarum* is endemic to the Yellow Sea (Ponurovsky and Yakovlev 1992) and is now one of the most cultivated species of shellfish around the world. Owing to its fast growth and productivity, this species was imported to France in 1972 for aquaculture purposes. This new clam culture became widely distributed along the French Atlantic coast by 1986. In spring and summer 1987, massive mortalities of cultured Manila clams occurred, mostly in North Finistère (France) (Paillard et al. 1989). Manila clam mortalities were associated with an obvious gross sign of pathology, a brown conchiolin deposit on the inner surface of the valves. This new pathology termed "brown ring disease" (BRD) (Paillard et al. 1989) decimated the cultured clam stock at this period. Afterwards, because of clam transfers, BRD spread to other European countries including England, Ireland, Italy, Spain and Portugal. Besides *R. philippinarum*, BRD has been detected in different natural populations of closely related clams like *R. decussatus*, *Paphia aurea* and *Tapes rhomboides*.

Paillard and Maes (1990) isolated from diseased Manila clams a bacteria first called *Vibrio* P1, which is now identified as *Vibrio tapetis*. They demonstrated the transmissibility of the disease by injecting these bacteria into healthy clams. Colonization of the shell matrix by *V. tapetis* disrupts the production of periostracal lamina and induces the characteristic sign of a brown deposit of melanised shell matrix on the inner surface of the valves (Paillard et al. 1994, Paillard and Maes 1995). Disease progression is estimated by the extent of the symptomatic deposit (Paillard and Maes 1994).

Manila clam standing stock and production in Arcachon Bay ranks highest among all French sites in which stocks are assessed (Caill-Milly et al. 2008). Arcachon Bay produced 1028 mt of clams in 2008 (Caill-Milly et al. 2008). A pathological survey of Manila clam populations was begun in 2006. Main pathologies, i.e. perkinsosis (Dang et al. 2009-b, in preparation), helminthosis (Dang et al. 2009-b), brown muscle disease (Dang et al. 2008) and BRD, were concomitantly monitored. The present study reports the occurrence of BRD in Arcachon Bay through a spatio-temporal survey.

2. Materials and methods

2.1. Sampling procedure

Ten Manila clams were collected at each of the fifty geographically referenced stations within Arcachon Bay between mid-May and mid-June 2006 during the stock assessment study (Caill-Milly et al. 2006) (Fig. 10.1). Stations were scattered throughout the local distribution area of this species.

Additionally, one hundred clams (30-40 mm) were collected monthly at each of the four studied sites of Andernos (November 2005-October 2007), Ile aux Oiseaux (December 2005-February 2007), Lanton (December 2005- October 2007), and Gujan (February 2006-February 2007) (Fig. 10.1).

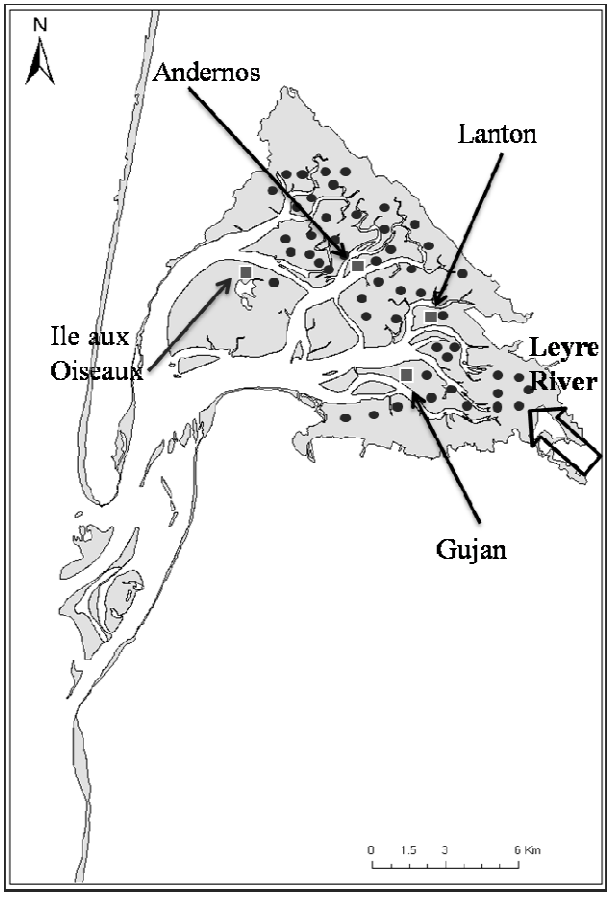


Figure 10.1. Map of Arcachon Bay representing the fifty sampled stations and the four sampling sites of Andernos, Ile aux Oiseaux, Lanton and Gujan.

2.2. Clam treatment

Clams were opened with a scalpel blade, the flesh was removed, and both valves were observed, first by eye and next using a stereomicroscope. Seven stages of BRD infection have been established according to the classification system of Paillard et al. (1994). In this classification, the degree of signs ranges from microscopic conchiolin brown spots on the inner face of the shell in the early stage of the disease (stage 1) to a thick and complete brown ring in the most advanced stage (stage 7).

3. Results

3.1. Spatial survey

The prevalence of BRD fluctuated greatly among the sampled sites but never exceeded 50% (Fig. 10.2). Sixteen of the fifty sampled sites did not exhibit infection. Mean prevalence within the bay was 10.8%.

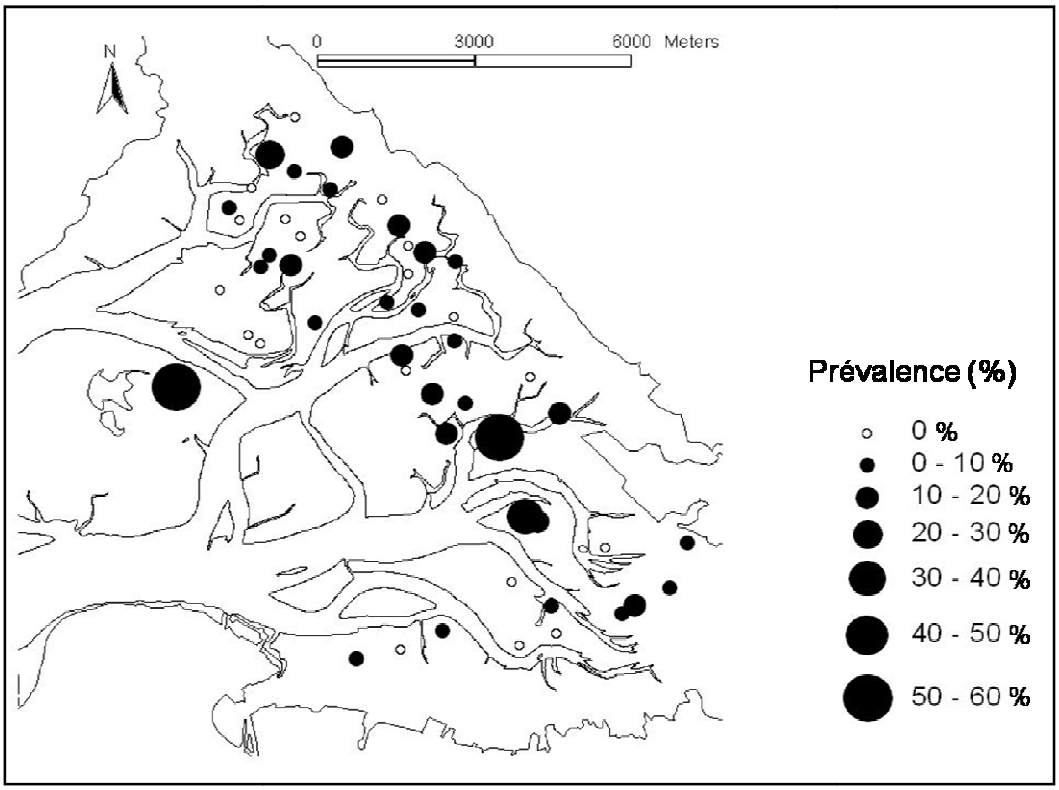


Figure 10.2. Prevalence of brown ring disease at the fifty sampled stations.

In affected clams, signs primarily consisted of microscopic brown spots along the pallial line at the inner edge of the shell, corresponding to stage 1 of the disease (Fig. 10.3). However, even if 68% of the sampled stations were infected by the disease, the infection level was very low with 95% of clams in stage 1 (Fig. 10.3). Only four stations had clams with disease greater than stage 1.

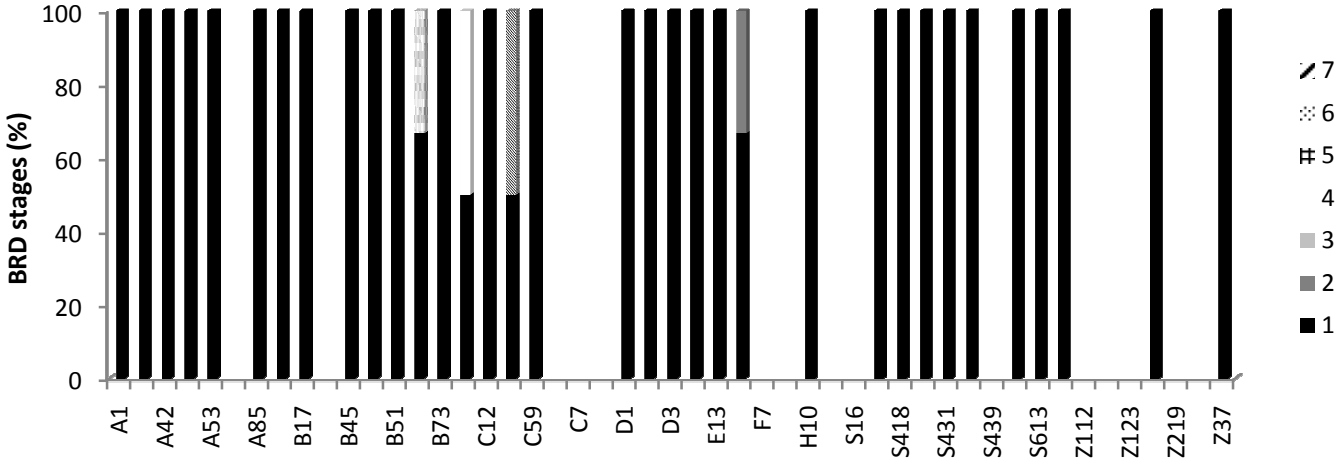


Figure 10.3. BRD infection stages in Manila clams from the fifty stations.

3.2. Temporal survey

Brown ring disease (BRD) was detected in *Ruditapes philippinarum* in the four sampled sites of Andernos, Lanton, Ile aux Oiseaux and Gujan. The prevalence varied by site and averaged 11% at Andernos, 14% at Gujan, 15% at Lanton, and 25% at Ile aux Oiseaux. Significant variation was observed over time at the four sites but without clear seasonal patterns (t-test, $P < 0.05$) (Fig. 10.4).

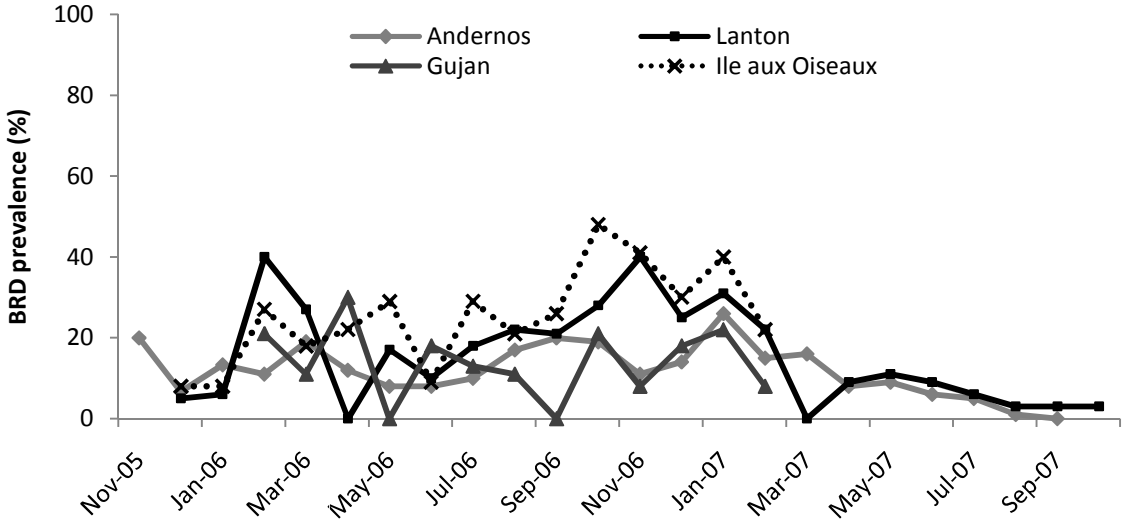
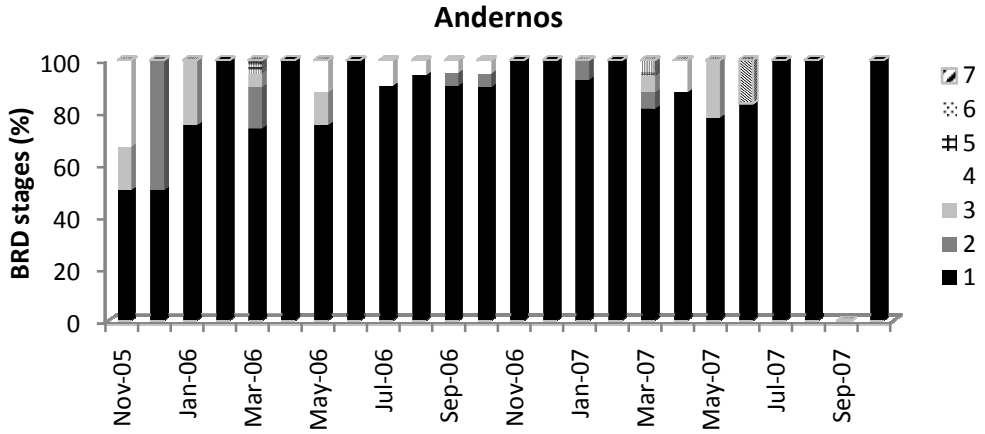


Figure 10.4. Prevalence of brown ring disease in *Ruditapes philippinarum* at Andernos, Lanton, Gujan and Ile aux Oiseaux.

At the four sampled sites, stage 1 dominated, with 80% of clams at stage 1 at Andernos, 88% at Lanton, 96% at Ile aux Oiseaux, and 82% at Gujan (Fig. 10.5). No seasonal pattern was detected (Fig. 10.5).



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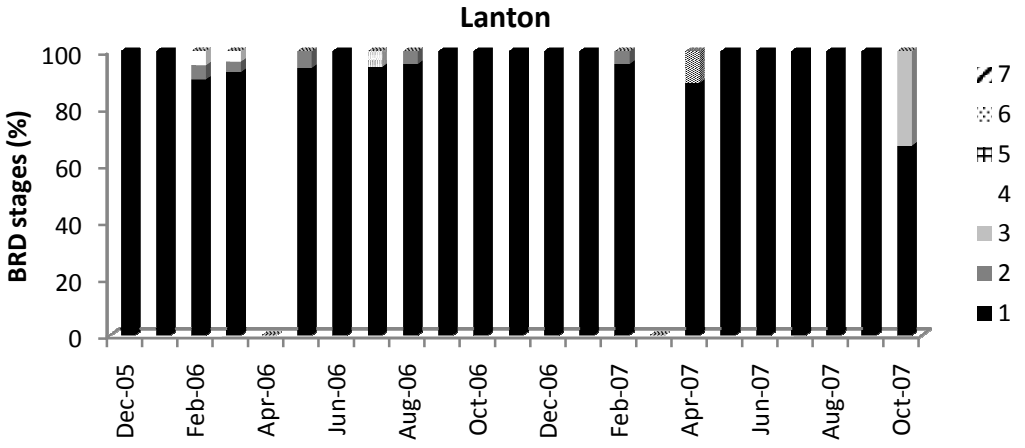

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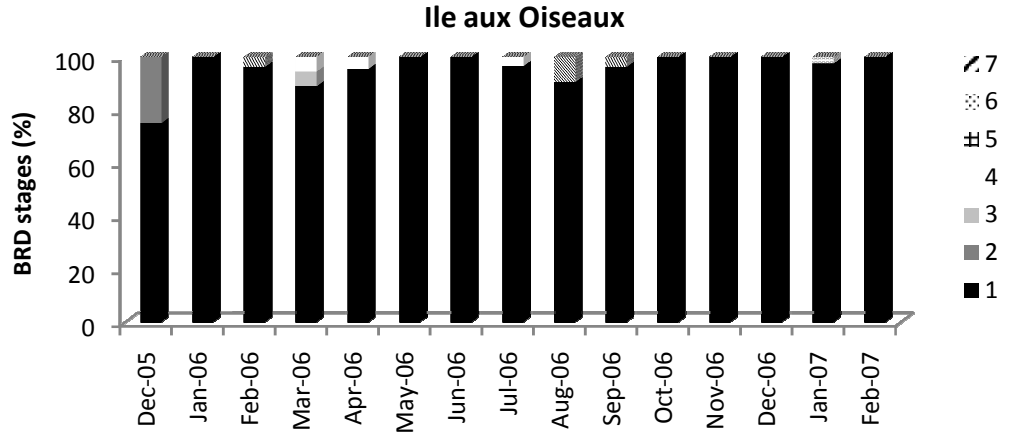

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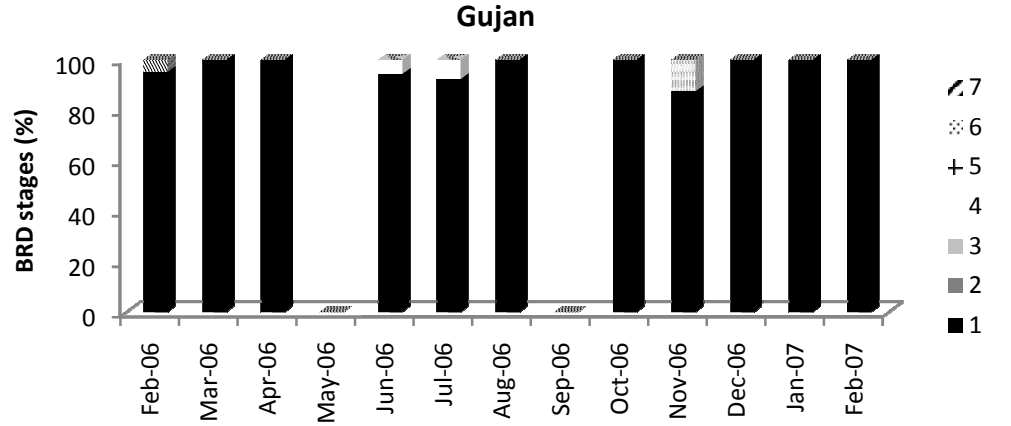


d

Figure 10.5. Brown ring disease infection stages in Manila clams from Andernos (a), Lanton (b), Ile aux Oiseaux (c) and Gujan (d).

4. Discussion

The aim of this study was to evaluate brown ring disease (BRD) in the Manila clam population from Arcachon Bay. BRD was present in the bay at low prevalence and at an early stage of infection (stage 1). Clams infected by the disease at Arcachon Bay presented only microscopic signs, i.e. minute irregularities within the shell along the pallial line.

BRD was reported for the first time at Andernos in Arcachon Bay by Lassalle et al. (2007). These authors examined the disease stage according to Paillard and Maes (1994) but they also searched for the bacteria by PCR amplification with specific primers. They found a high prevalence in shells, reaching 63%, which was associated with malformation and brown deposits on inner valve faces. Mean BRD prevalence was around 20%, whereas mean *Vibrio tapetis* prevalence was around 50% (Lassalle et al. 2007). Spatial monitoring in the present study displayed a mean BRD prevalence of 10.8% within Arcachon Bay and the temporal survey showed a mean of 16%. This low prevalence, associated with the light intensity of the disease (stage 1) indicated that the infection is in the early phase. BRD is present along the entire Atlantic coast (Paillard 2004). Prevalence of BRD in a natural population of Manila clam from the Morbihan Gulf (northwest France) seemed to be higher than at Arcachon, with 30% prevalence in January and April 2001 and 16% in August 2001 (Soudant et al. 2004). However, a monitoring in 2004-2005 at Morbihan revealed a low mean prevalence of 9.7% as well as low infection level (90% of infected clams had a disease stage below? Equal to or lower than? 4) (Flye-Sainte-Marie 2008). Infection level meanwhile was higher in northwestern France compared to Arcachon Bay. This could be explained by temperature patterns, as BRD is classified as a cold-water disease (Paillard 2004).

Infection in Arcachon Bay was comparable to infection in Manila clam populations from Korea, where Park et al. (2006-b) observed a prevalence of 36% with most infections stage 1. BRD infection was lower than in southern England, where BRD infection ranged between stages 1 and 5 (Allam et al. 2000). These authors demonstrated an effect of the disease on clam condition index. However, Flye-Sainte-Marie (2008) showed that clams were significantly affected only when infection was at stage 4 or more. Finally, BRD infection in Arcachon Bay was too low to cause mortalities.

Les ressources trophiques

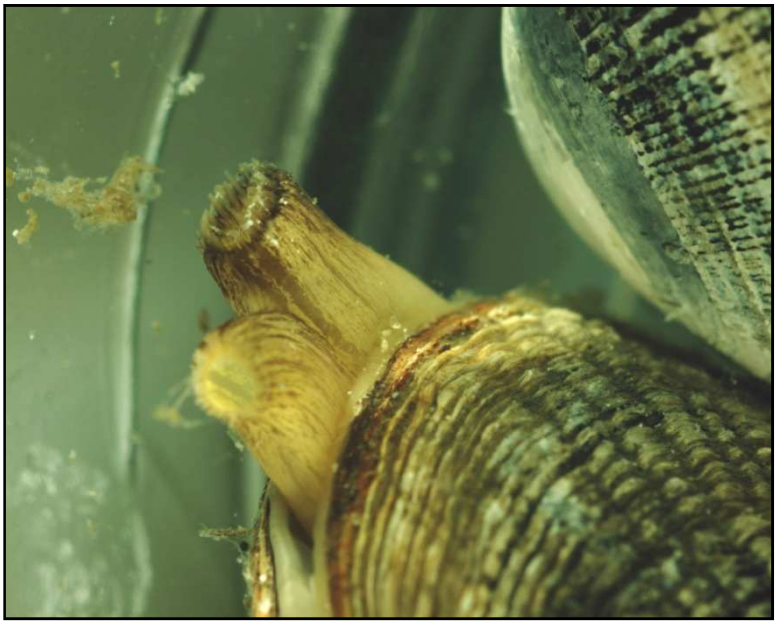
Chapitre 11

Détermination des sources trophiques de la palourde japonaise dans le bassin d'Arcachon par les isotopes stables ($\delta^{13}C$, $\delta^{15}N$)

Determination of diet in Manila clams by spatial analysis of stable isotopes

Dang C., Sauriau P.-G., Savoye N., Caill-Milly N., Martinez P., de Montaudouin X.

Published in *Marine Ecology Progress Series*, 387: 167-177.



Siphons de *Ruditapes philippinarum*

Abstract

Spatial changes in the dietary regime of *Ruditapes philippinarum* from intertidal sediments of Arcachon Bay were depicted by analyzing stable isotope ratios in both its adductor muscles and potential trophic sources. Manila clams were collected from 50 sites in May to June 2006. Dietary reconstruction was based on the IsoSource mixing model, considering trophic enrichments of 3.5‰ for carbon and 3.0‰ for nitrogen, which were determined experimentally. $\delta^{13}C$ and $\delta^{15}N$ values, respectively, averaged –28.2 and 5.2‰ for riverine particulate organic matter (POM), –20.6 and 4.7‰ for inner bay phytoplankton, –21.0 and 5.6‰ for outer bay phytoplankton, –16.8 and 4.8‰ for microphytobenthos, –18.4 and 3.9‰ for sedimentary organic matter (SOM) and 11.8 and 4.0‰ for *Zostera noltii*. Clam muscle $\delta^{13}C$ and $\delta^{15}N$ values ranged from –20.5 to –16.4‰, and from 7.6 to 9.3‰, respectively. $\delta^{15}N$ significantly decreased from southern to northern parts of the bay, while $\delta^{13}C$ values increased with tidal elevation. Outer bay phytoplankton was the major diet component of clams. Clams from the southeast mainly incorporated outer bay phytoplankton despite the proximity of the Leyre River, whereas clams from the northwest incorporated approximately equal parts of inner and outer bay phytoplankton, riverine POM, microphytobenthos and SOM. These patterns were consistent with spatial gradients driven by the dominant role of tidal hydrodynamics within the bay and land-use characteristics of the catchment.

KEY WORDS: *Ruditapes philippinarum*, muscle, C and N stable isotopes, trophic sources, mixing model, spatial variability, Arcachon Bay (SW France)

1. Introduction

The Manila clam *Ruditapes philippinarum* is native to the Indo-Pacific region and is one of the most fished and farmed bivalves in the world (Gosling 2004). The Manila clam was deliberately introduced into European waters, first in France for culture in 1972 and later in England, Spain and Italy (Flassch and Leborgne 1992). Within a few years, the species established wild populations in most of these European countries. In Arcachon Bay (SW France), the Manila clam was introduced in 1980 primarily for aquaculture. Due to successful reproduction, it rapidly colonized intertidal areas including *Zostera noltii* seagrass beds (Blanchet et al. 2004). Today, the species supports intensive commercial fisheries (Caill-Milly et al. 2006). Successive stock assessments of the Manila clam population revealed large heterogeneity in shell length frequency distributions over Arcachon Bay (Caill-Milly et al. 2006). Since the growth of Manila clam depends mainly on temperature and pelagic food sources (Langton et al. 1977, Mann 1979), spatial heterogeneity in both phytoplankton primary production (Glé et al. 2008) and benthic habitats (Blanchet et al. 2004) could lead to spatial heterogeneity in both its demography and growth performances. Furthermore, as the wild *R. philippinarum* population represents the second most prevalent suspension-feeder stock in Arcachon Bay, it is expected to play a key role in both the benthic food web and benthic–pelagic coupling within the bay.

Stable isotope analysis has been used widely to investigate time-integrated dietary patterns of organisms and transfers of organic matter within food webs (Peterson and Fry 1987, Post 2002). $\delta^{13}C$ helps to identify primary food sources assimilated by consumers, whereas δ15N is used to determine their trophic position (DeNiro and Epstein 1978, 1981, Post 2002). $\delta^{13}C$ and $\delta^{15}N$ in an organism respectively reflect the $\delta^{13}C$ and $\delta^{15}N$ of its diet with a trophic enrichment (δconsumer – δdiet) estimated to average 0.5 to 1‰ for $\delta^{13}C$ (DeNiro and Epstein 1978, Peterson and Fry 1987) and 3 to 4‰ for $\delta^{15}N$ (DeNiro and Epstein 1981, Post 2002). However, these average values hide large variations among species (Peterson and Fry 1987), feeding modes (McCutchan et al. 2003) and, as organisms are often analyzed whole, highly significant differences between their different tissues (Vanderklift and Ponsard 2003). For instance, Stephenson and Lyon (1982), Lorrain et al. (2002) and Malet et al. (2007) reported $\delta^{13}C$ differences greater than 2 to 3‰ between adductor muscles and digestive glands in suspension-feeding bivalves. An increasing practice in stable isotope studies on marine bivalves has been to analyze the adductor muscle instead of the whole body to depict both temporal (Lorrain et al. 2002, Page and Lastra 2003, Kasai et al. 2004, Malet et

al. 2007) and spatial trophodynamics (Machás et al. 2003, Page and Lastra 2003). Adductor muscles have slower turnover and much lower lipid contents than other tissues such as gonads and digestive glands (Paulet et al. 2006, Malet et al. 2007), and they are a good indicator of long-term diet (Peterson and Fry 1987). However, no species- and/or tissue-specific trophic enrichment values were available for bivalves in published reviews (Peterson and Fry 1987, Vander Zanden and Rasmussen 2001, Post 2002, McCutchan et al. 2003). Thus, it would be particularly useful to experimentally determine these values when using muscle instead of the whole body approach in tracing organic matter flows through suspension-feeding molluscs (Lorrain et al. 2002, Page and Lastra 2003, Malet et al. 2007).

The present study focused on large-scale spatial variations in the dietary pattern of the suspension-feeding bivalve *Ruditapes philippinarum* living in intertidal areas of Arcachon Bay. C and N isotopic ratios of its adductor muscles were determined together with those of its potential trophic sources. Trophic enrichment values for adductor muscle were estimated from a long-term diet-switch experiment with the diatom *Skeletonema costatum* and dietary inferences were deduced from the IsoSource mixing model. In light of known spatial variations in hydrological features within the bay, variations in dietary patterns of Manila clams are then discussed.

2. Materials and Methods

2.1. Study site

Arcachon Bay (44°40' N, 1°10' W) is a 156 km^2 semi-sheltered lagoon located on the south-west coast of France (Fig. 11.1). Tidal flats extend over 110 km^2, of which 70% are covered by dense *Zostera noltii* seagrass beds (Auby and Labourg 1996). Although *Z. noltii* meadows appeared homogeneous, macrozoobenthic community analysis revealed the existence of 4 distinct macrobenthic communities (Blanchet et al. 2004). The main forcing variables such as water mass characteristics and sediment grain-size are under the control of tidal hydrodynamics (Auby and Labourg 1996, Blanchet et al. 2004). Arcachon Bay is influenced by both oceanic and continental sources following a semi-diurnal macrotidal rhythm. Neritic waters enter the inner lagoon through 2 channels, the Piquey channel which extends to the north and the larger Teychan channel at the south (Fig. 11.1). The seawater exchange ranges from 160×10^6 to 400×10^6 m^3 per tide following the spring to neap tide

cycle, and meets freshwater inputs coming principally from the Leyre River on the east. According to Direction Régionale de l'Environement (DIREN) data, Leyre River discharge during 2006 was low compared to its average 20 yr discharge, and the maximum runoff was 25 m^3 s^{-1} in March 2006. The remaining freshwater inputs are provided by 17 secondary streams and diffuse sources located on the eastern side of the lagoon (De Wit et al. 2005). Sediment grain-size in *Z. noltii* beds varies from mud to sandy mud (Blanchet et al. 2004). Clams were usually located near the mid-tidal level within seagrass meadows (Caill-Milly et al. 2006). The Manila clam habitat in Arcachon Bay appears to be a mosaic of sedimentary biofacies where salinity and sediment temperature fluctuate yearly between 4 and 35 and between –2 and 44°C, respectively (Dang et al. 2008).

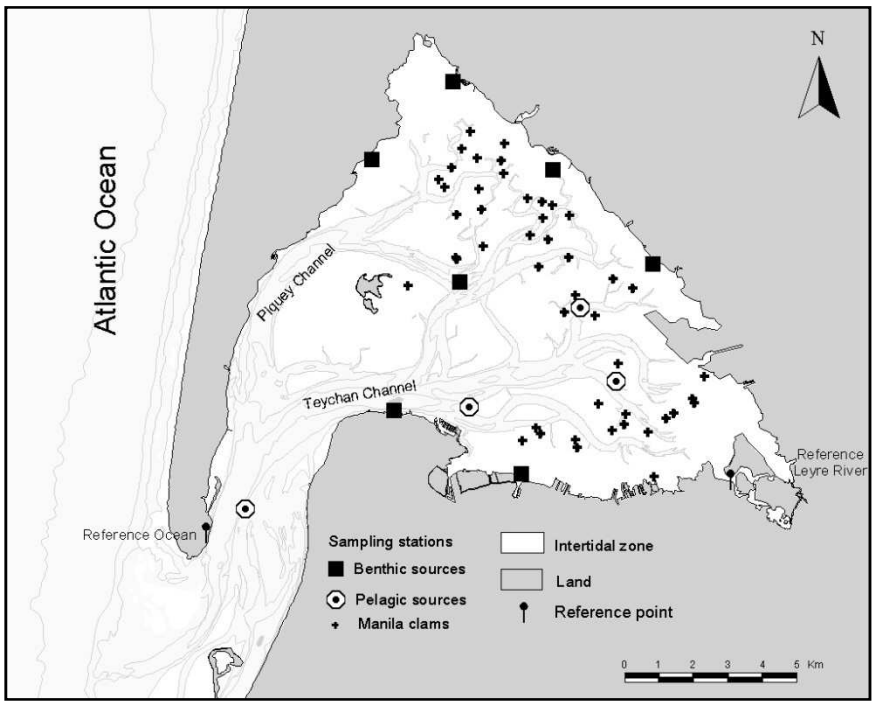


Figure 11.1. Map of Arcachon Bay showing location of sampling stations for benthic sources i.e. sediment, *Zostera noltii*, microphytobenthos (■), pelagic sources (⊙) and Manila clams *Ruditapes philippinarum* (✚). Intertidal areas (Miller and White), main channels (grey shaded) and reference points (↑).

2.2 Sampling strategy

Manila clams were sampled at 50 geographically referenced stations within Arcachon Bay between mid-May and mid-June 2006 (Fig. 11.1). Stations were evenly dispersed within the ca. 70 km2 distribution area of the species, as previously described by stock assessment studies (Caill-Milly et al. 2006). Tidal elevation (m) of each station was provided by both Service Hydrographique de la Marine (SHOM, Brest) and Direction Départementale de l'Equipement de la Gironde (DDE, Bordeaux). A total of 3 adult clams ranging from 30 to 36 mm in shell length were collected at each station.

Prior to the clam sampling (24 April to 15 May 2006), marine and continental water, sediment and benthic primary producers were sampled. Water was collected at 4 stations within Arcachon Bay and at 2 stations in the Leyre River (Fig. 11.1). Particulate organic carbon (POC) and nitrogen (PON), chlorophyll *a* (chl *a*) and $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter (POM) were then determined. The dominance of phytoplankton within the bulk POM was checked using a POC:chl *a* ratio of 200 (Cifuentes et al. 1988). When the water column POM exhibited POC:chl *a* ratios of 80 to 180, samples were used to characterize living phytoplankton. Surface sediment, microphytobenthos and living *Zostera noltii* leaves were sampled at 7 intertidal stations (Fig. 11.1). Water was sampled using a Niskin bottle and immediately poured into pre-cleaned plastic bottles before filtration. Surface sediment was sampled directly using acid-cleaned and pre-combusted glass vials. Living *Z. noltii* leaves were hand-sampled and placed within clean plastic bags.

2.3. Long-term diet-switch experiment

Batch cultures of the diatom *Skeletonema costatum* were kept in outdoor concrete tanks (80 m^3) under natural light conditions and continuous aeration (Baud and Bacher 1990, Sauriau et al. 1997). Salty groundwater pumped up from the subsoil was used as the culture medium since its nutrient composition was recognized to favour *S. costatum* growth (Baud and Bacher 1990). Diatom cultures in exponential growth phase (i.e. after 3 to 4 d and with cell concentrations of ca. 1.5×10^6 cells ml^{-1}) were used as monospecific algal food for rearing clams (Sauriau et al. 1997). Temperature and salinity in the tanks ranged from 15.1 to 19.2°C and 32.5 to 32.7, respectively. The food ration was ca. 2×10^9 algal cells d^{-1} $clam^{-1}$. Algal isotopic composition was monitored weekly by filtering four 0.5 l samples through pre-

combusted Whatman GF/F fiberglass filters (25 mm diameter). Filters were then immediately stored at –20°C until freeze-drying.

A total of 100 adult Manila clams were collected from the intertidal mudflats of Ile aux Oiseaux (Arcachon Bay) at the end of May 2006. They averaged 41.1 ± 1.1 mm shell length and 0.43 ± 0.08 g tissue dry weight. Clams were reared in 500 l tanks for 160 d. They were placed on trays filled with medium to coarse sand allowing them to bury and adopt a natural suspension-feeding behaviour. The flow of underground seawater was controlled in each tank at a rate of 10 l min^{-1} and was continuously aerated; 5 clams were randomly sampled on 1 June 2006 and then 5, 11, 20, 40, 76, 103, 126 and 160 d later. At each sampling date, individual clams were cleaned and placed in an aerated tank with filtered underground seawater for 48 h to allow gut content evacuation. All sampled clams were subsequently frozen at –20°C until dissection.

2.4. Sample processing

Clam shell length was measured by a digital caliper to the nearest mm. Clams were then opened, their posterior adductor muscles dissected, frozen at –20°C for storage and then frozen at –80°C prior to freeze-drying. Water samples were filtered onto 47 mm GF/F filters for chl *a* and onto pre-combusted 25 mm GF/F filters for POC, PON, $\delta^{13}C$ and $\delta^{15}N$. Filters for chl *a* were stored frozen (–80°C), whereas filters for C and N elemental and isotopic analysis were freeze-dried and then stored at room temperature. Sediment samples were frozen (–20°C). *Zostera noltii* leaves were cleaned of their epiphytes, decarbonated in a bath of 2% HCl and then rinsed with Q-water (C elemental and isotopic analysis), or only rinsed with Q-water (N elemental and isotopic analysis). Leaves were then dried overnight in an oven (60°C) and stored in aluminum foil at room temperature. Sediment for carbon and nitrogen elemental and isotopic analysis was decarbonated using 2N HCl (Kennedy et al. 2005). Filters for carbon and nitrogen elemental and isotopic analysis were decarbonated using HCl fumes (Lorrain et al. 2003). Prior to analysis, clam muscle and sediment samples were freeze-dried and ground using mortar and pestle to get a homogeneous fine powder. *Z. noltii* leaves were similarly ground.

2.5. Sample analysis

Chlorophyll *a* was analyzed by fluorescence (Yentsch and Menzel 1963). C and N elemental and isotopic analysis were performed using an elemental analyzer (Carlo Erba 2500) in line with an isotope ratio mass spectrometer (VG Isoprim). Data were corrected and calibrated against home made working standards (casein and glycin) and against certified standards (acetanilide, IAEA-N2, USGS-24). These standards allowed the analytical precision to be set at 0.2‰. Isotopic values are reported in the usual per mil unit (‰) following:

$$\delta^{A}X = \left[\left(R_{sample}/R_{ref}\right) - 1\right] \times 1000$$

where A is the atomic mass of the heavy stable isotope of the element X, and R_{sample} and R_{ref} are the ratios of heavy to light isotope for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Ref is Vienna Pee Dee Belemnite (PDB) for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$.

2.6. Trophic-enrichment estimates

Temporal changes in stable isotope ratios exhibited by clam muscles were fitted to the equation proposed by Tieszen et al. (1983): $Y_t = a + b \times e^{c \times t}$. In this time-based equation, Y_t (‰) is the δ ratio of clam tissues at time t (days since the first sampling date), *a* (‰) is related to the δ ratio of tissues in equilibrium with the new diet, *b* (‰) refers to the difference between initial and asymptotic δ ratio of tissues, and *c* (day^{-1}) is the turnover rate of tissues. All equations parameters were estimated by non-linear regression using SigmatPlot 1.02 (Jandel Scientific). Trophic-enrichment values for both $\delta^{13}C$ and $\delta^{15}N$ were then calculated as the difference between the δ ratios of tissues in equilibrium with the new diet and the average δ ratios of the diet. The half-life of $\delta^{13}C$ and $\delta^{15}N$ was defined as ln(0.5/*c*) in days, and was the time in which 50% of the isotope ratios were exchanged in the tissue.

2.7. Mixing model: IsoSource

Phytoplankton from both the inner and outer bay, Leyre River POM, microphytobenthos, sedimentary organic matter (SOM) and *Zostera noltii* leaves were considered as potential trophic resources for the Manila clam. Macroalgae were not considered as a potential food source for *Ruditapes philippinarum* due to their very low

prevalence in clam habitat within Arcachon Bay. The IsoSource software (Phillips and Gregg 2003, Phillips et al. 2005) was used to determine the relative contribution of each source to the mixed signature of clam adductor muscles at the 50 stations sampled. Experimentally determined muscle-specific trophic enrichments for both $\delta^{13}C$ and $\delta^{15}N$ were subtracted from adductor muscle values before IsoSource analysis.

2.8. Statistical analyses

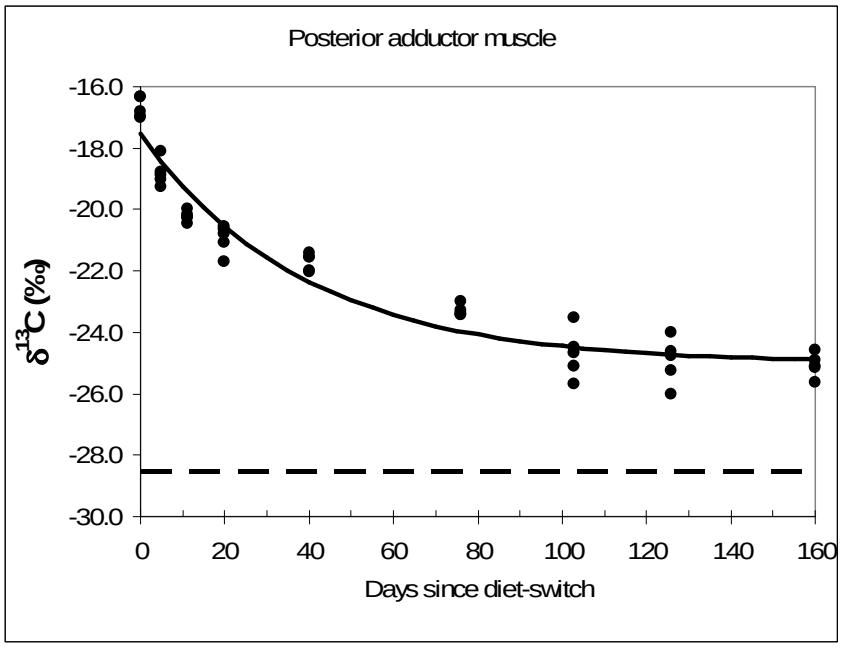
Statistical analyses were performed using Statistica 7.1 software (StatSoft). The maximum type I error rate was set at $\alpha = 0.05$. Results are expressed as means ± SD with n equal to the number of samples analyzed. Homogeneity of variance was checked using Cochran's test. First, a nested ANOVA was conducted to compare C and N isotopic ratios of the Manila clam muscle between the 50 stations (Sokal and Rohlf 1981). Second, single linear regressions were computed between $\delta^{13}C$, $\delta^{15}N$ and the distance (km) of each station from the Leyre River mouth, tidal elevation (m) and clam shell length (mm). Distances from the Leyre River were calculated with the Arcview 3.2 GIS software.

3. Results

3.1. Trophic-enrichment of $\delta^{13}C$ and $\delta^{15}N$

During the course of the diet-switch experiment, adult Manila clams exhibited a positive, but small growth in shell length (+3.3 ± 1.1 mm); however, they increased by 3.2- and 2.6-fold in total tissue and adductor muscle dry weight, respectively. At the start of the feeding experiment, carbon and nitrogen isotopic ratios of adductor muscles averaged –16.7 ± 0.3 and 9.2 ± 0.4‰ (n = 5), respectively. Both $\delta^{13}C$ and $\delta^{15}N$ values of adductor muscles changed toward more depleted values and converged on asymptotic values reflecting the incorporation of the algal diet (Fig. 11.2). *Skeletonema costatum* had stable carbon and nitrogen isotopic ratios of –28.5 ± 0.8 and 5.4 ± 0.6‰ (n = 45), respectively. The exponential decay model provided a good fit to changes in both $\delta^{13}C$ and $\delta^{15}N$ muscle values with significant correlations ($\delta^{13}C$: $r = 0.96$ $p < 0.001$; $\delta^{15}N$: $r = 0.63$, $p < 0.01$,). The trophic

enrichment for adductor muscles was +3.5‰ for $\delta^{13}C$ and +3.0‰ for $\delta^{15}N$. The half-life values were calculated as 26.8 and 13.3 d for $\delta^{13}C$ and $\delta^{15}N$, respectively.



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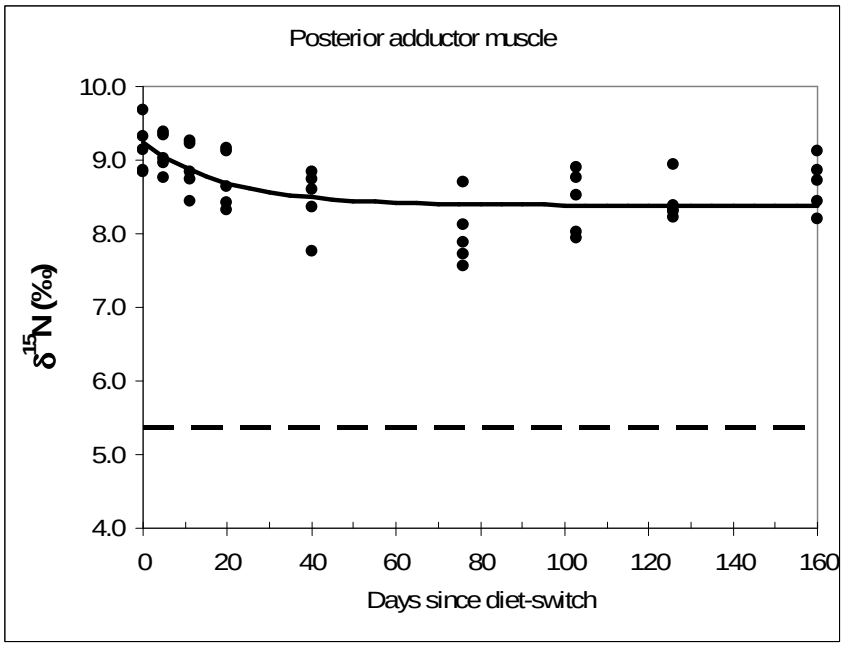

B

Figure 11.2. *Ruditapes philippinarum*. Changes in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) of the posterior adductor muscle of adult clams during the diet-switch experiment. The mean $\delta^{13}C$ and $\delta^{15}N$ values for the algal diet (dashed lines) and exponential decay curves (solid lines) are indicated.

3.2. Isotopic signatures

Carbon isotopic ratios of muscle tissue sampled at the 50 stations in the bay ranged from –20.2 to –16.4‰ (Fig. 11.3), owing to significant $\delta^{13}C$ differences between stations (nested ANOVA, $p < 0.001$). However, most of the values were scattered around –17.4 ± 0.6‰. Spatial dispersion in δ13C values seemed heterogeneous with no evidence of a consistent spatial pattern (Fig. 11.3). No significant relationships were thus found between $\delta^{13}C$ and the distance to the Leyre River or the clam shell length ($p > 0.05$) (Table 11.1). However, a significantly positive correlation was found between $\delta^{13}C$ and tidal elevation ($p < 0.05$) (Table 11.1).

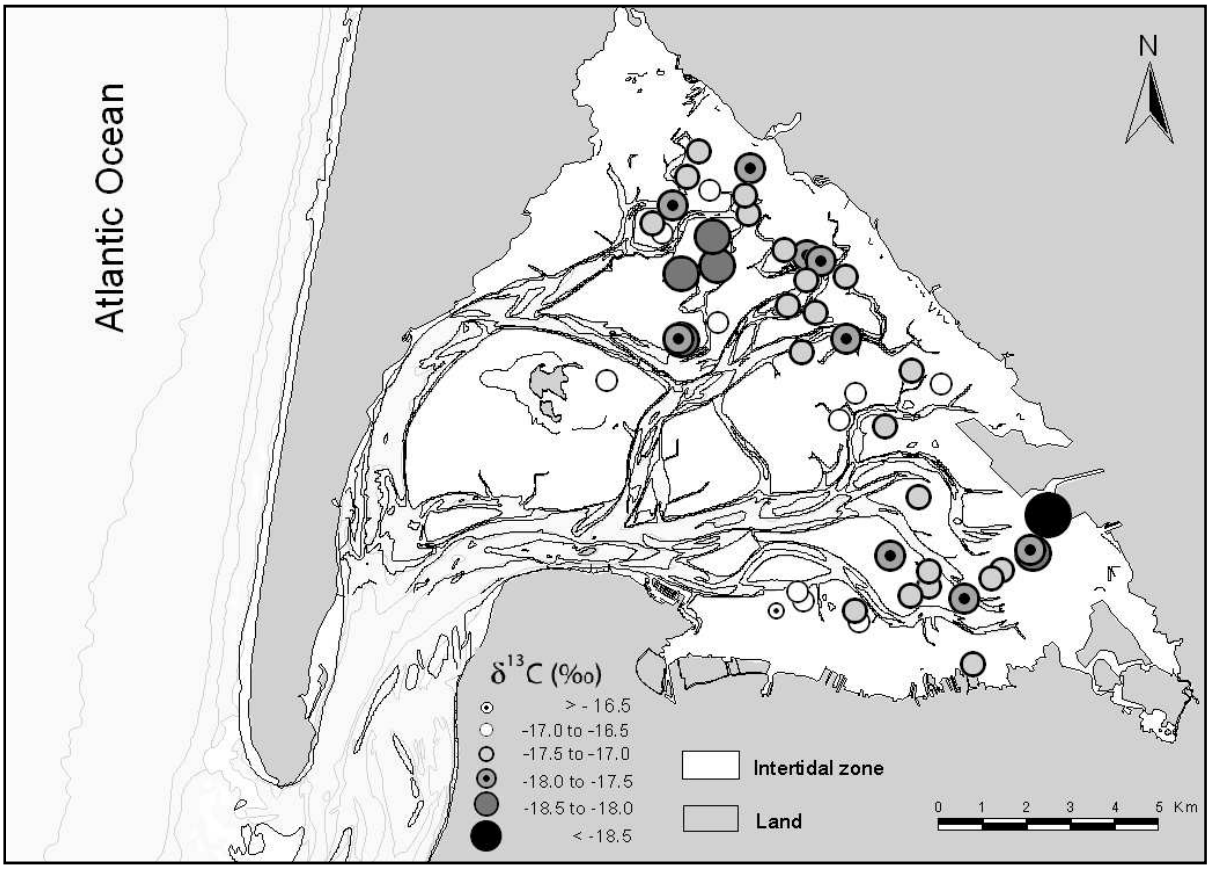

Figure 11.3. *Ruditapes philippinarum*. Spatial variation in carbon isotopic ratios ($\delta^{13}C$) of posterior adductor muscle of adult clams sampled at 50 stations in Arcachon Bay in May-June 2006.

Table 11.1. Single regression results between $\delta^{15}N$, $\delta^{13}C$ and distance to the Leyre River, tidal level and clams shell-length.

	$\delta^{15}N$		$\delta^{13}C$	
	R^2	P	R^2	P
Leyre distance	0.40	< 0.01	0.01	0.58
Tidal level	< 0.01	0.80	0.15	0.01
Clam shell-length	< 0.01	0.35	< 0.01	0.41

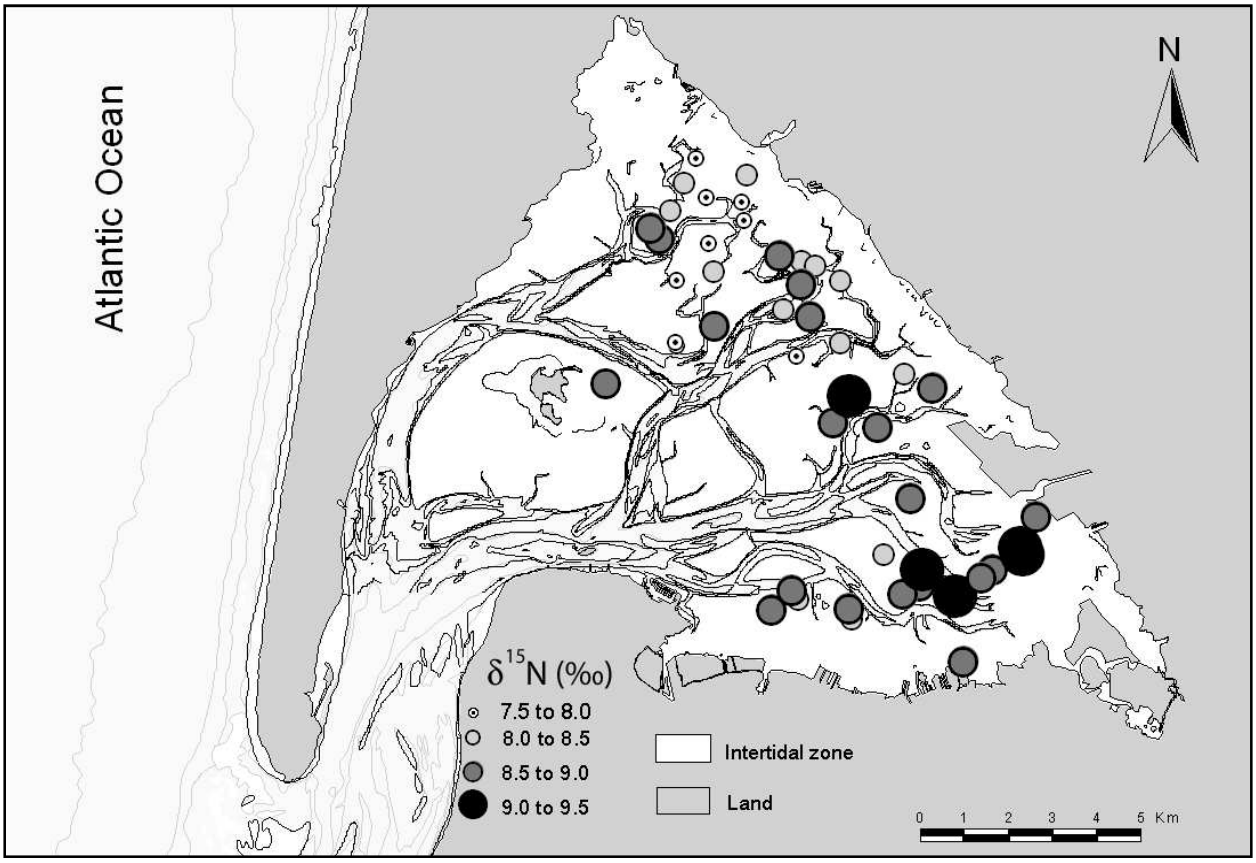

Figure 11.4. *Ruditapes philippinarum*. Spatial variation in nitrogen isotopic ratios ($\delta^{15}N$) of posterior adductor muscle of adult clams sampled at 50 stations in Arcachon Bay in May-June 2006.

Nitrogen isotopic ratios of muscle tissues ranged between 7.4 and 9.8‰ with a mean value of 8.5 ± 0.5‰ (Fig. 11.4). Significant differences in $\delta^{15}N$ were observed among stations (nested ANOVA, $p < 0.001$). The highest clam muscle $\delta^{15}N$ values were located at the southeastern part of the bay along the Teychan channel, whereas almost all the low $\delta^{15}N$ values were located to the north in the bay (Fig. 4). A negative correlation was observed between $\delta^{15}N$ and the distance to the Leyre River ($p < 0.05$) (Table 11.1). No significant correlations were found with tidal elevation or clam shell length ($p > 0.05$) (Table 11.1).

3.3. Isotopic signature of primary producers and sediment

Stable isotopic ratios of primary producers and sediment in Arcachon Bay are presented in Table 11.2. Phytoplankton $\delta^{13}C$ was –21.0 ± 1.2 and –20.6 ± 0.4‰ in the outer and inner bay, respectively. POM sampled in the Leyre River was highly depleted in ^{13}C (–28.2 ± 0.1‰) compared to the phytoplankton of the bay. Outer and inner bay phytoplankton and riverine POM $\delta^{15}N$ were 5.6 ± 1.4, 4.7 ± 0.3 and 5.2‰, respectively (Table 11.2). Microphytobenthos had much lighter $\delta^{13}C$ values than riverine POM and bay phytoplankton (–16.8 ± 2.3‰) but a similar $\delta^{15}N$ value (4.7 ± 0.5‰) compared to inner bay phytoplankton. SOM averaged –18.4 ± 0.6‰ in $\delta^{13}C$ and 3.9 ± 0.7‰ in $\delta^{15}N$. Living leaves of *Zostera noltii* displayed a C isotopic ratio of –11.8 ± 1.1‰ and N values of 4.0 ± 1.2‰. The scatterplot of stable isotope values of clam muscle tissues corrected for trophic enrichments ($\delta^{13}C$: 3.5‰; $\delta^{15}N$: 3‰) and potential trophic sources is presented in Fig. 11.5. With the exception of one station located close to the Leyre River mouth which was significantly different in $\delta^{13}C$ (–23.6 ± 0.1‰), isotopic values of other stations appeared as a single cluster with $\delta^{13}C$ ranging from –21.7 to –19.5‰ and $\delta^{15}N$ from 4.6 to 6.3‰. The scatterplot also highlights the close correspondence between the clam isotopic values corrected for trophic enrichment and those of outer and inner bay phytoplankton.

Table 11.2. Carbon and nitrogen stable isotope ratios for primary producers and sediment in Arcachon Bay. $\delta^{15}N$ analysis from Pothier and Savoye (unpub. data).

Potential source	**$\delta^{13}C$** Mean ± 1 SD	**n**	**$\delta^{15}N$** Mean ± 1 SD	**n**
Phytoplankton (outer bay)	-20.96 ± 1.23	4	5.62 ± 1.39	5
Phytoplankton (inner bay)	-20.60 ± 0.42	4	4.75 ± 0.33	11
POM Leyre	-28.24 ± 0.10	2	5.24	1
Microphytobenthos	-16.81 ± 2.29	2	4.76 ± 0.55	2
Sedimentary organic matter (SOM)	-18.40 ± 0.26	7	3.94 ± 0.66	7
Zostera noltii leaves	-11.77 ± 1.07	7	3.99 ± 1.18	7

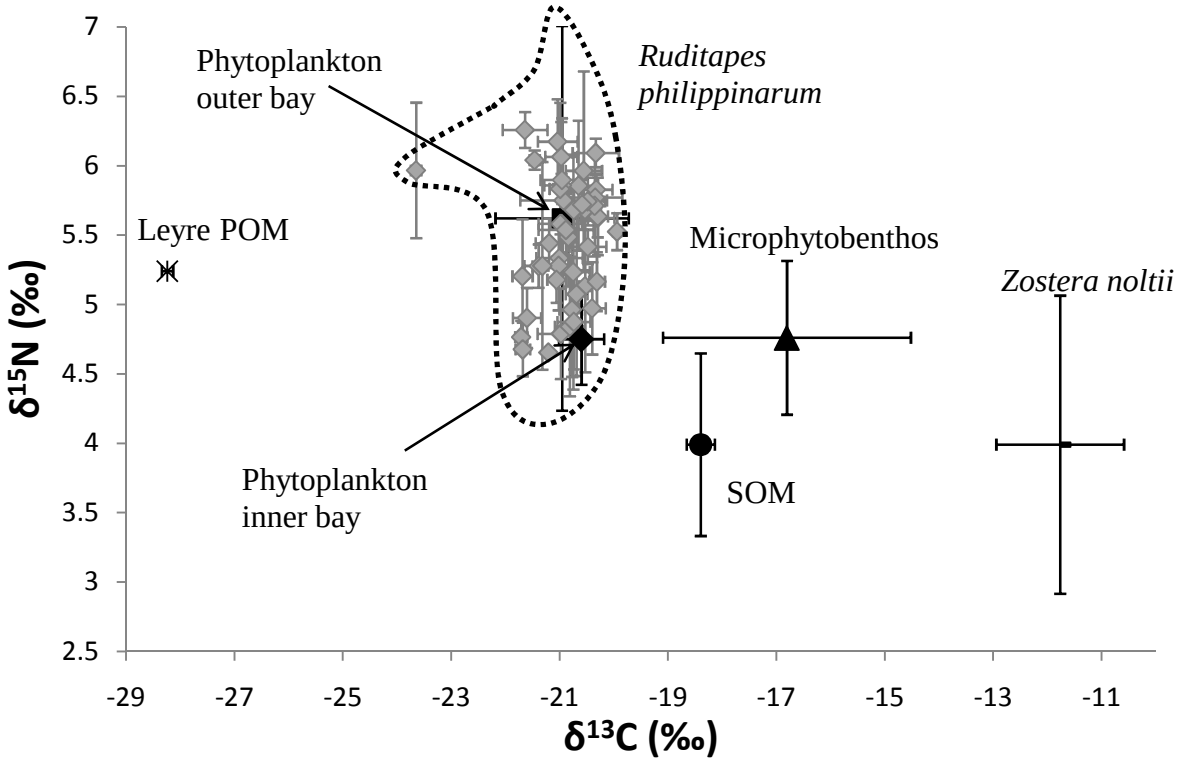

Figure 11.5. Dual plots of nitrogen *vs* carbon stable isotope ratios (mean ± standard deviation) of posterior adductor muscles of *Ruditapes philippinarum* sampled in 50 stations (◆) and trophic sources potentially consumed as inner bay phytoplankton-dominated POM (◆), outer bay phytoplankton-dominated POM (■), Leyre POM (*), microphytobenthos (▲), sediment (●) and *Zostera noltii* (▂).The area enclosed by the dotted line corresponds to the expected $\delta^{13}C$ and $\delta^{15}N$ of the food resources incorporated by the Manila clam within its adductor muscle as deducted from trophic-shifts of 3.5‰ and 3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

3.4. Food source analysis: IsoSource

The IsoSource mixing model estimated that, at the bay scale, phytoplankton contributed most (63% on average) to the diet of Manila clams. Outer bay phytoplankton contributed the most (50 ± 27%), followed by riverine POM (16 ± 9%) and inner bay phytoplankton (14 ± 10%). The proportions of microphytobenthos, SOM and *Zostera noltii* to the clam diet were 9 ± 5, 7 ± 5 and 5 ± 3%, respectively. Excluding *Zostera noltii* as a potential food source from the mixing model does not affect these values since contributions of outer bay phytoplankton, riverine POM, inner bay phytoplankton, microphytobenthos and SOM were 52 ± 25, 12 ± 8, 15 ± 8, 12 ± 6 and 9 ± 6%, respectively.

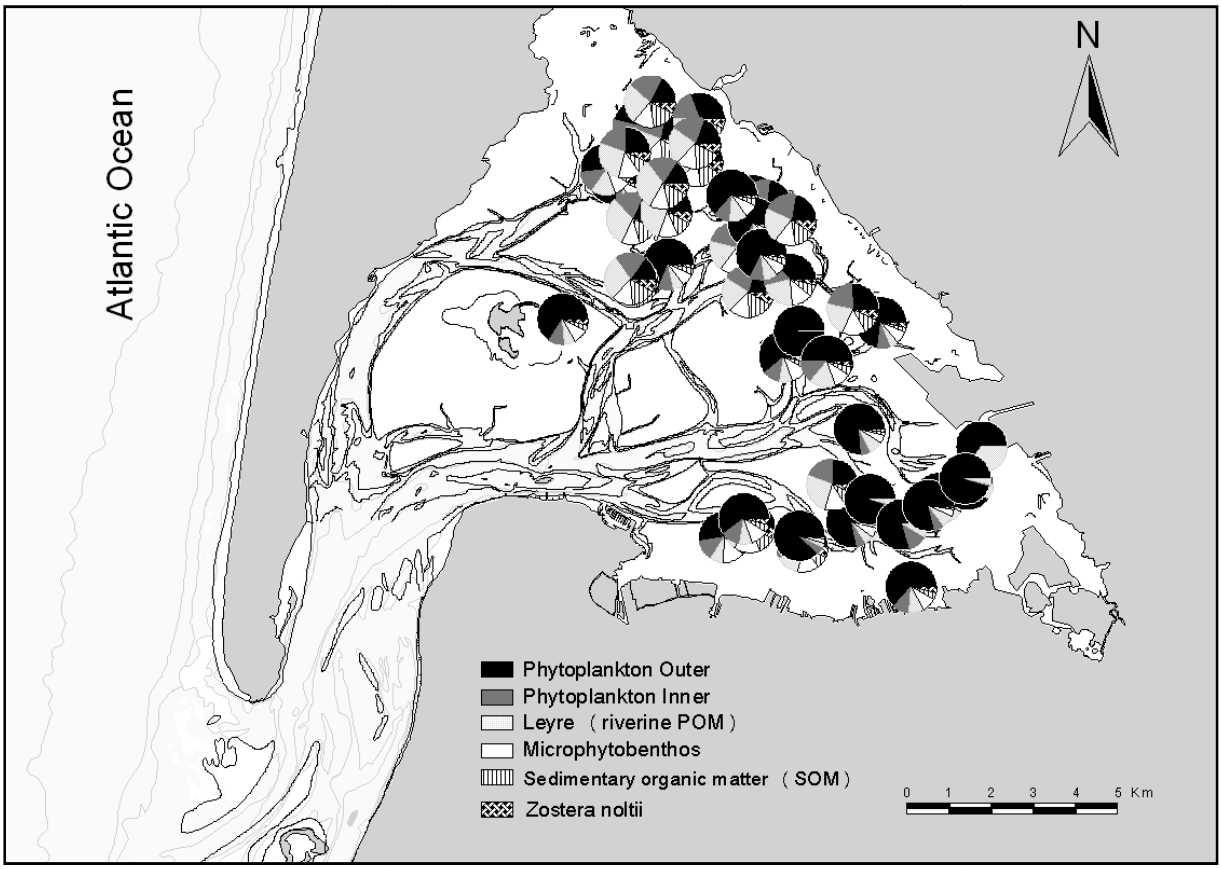

Figure 11.6. *Ruditapes philippinarum*. Predicted spatial variation in the proportion of trophic sources for adult clams within the Arcachon Bay. Trophic sources considered are outer-bay phytoplankton, inner-bay phytoplankton, riverine particulate organic matter (POM; Leyre River), microphytobenthos, sedimentary organic matter and *Zostera noltii*.

Modelling of the Manila clam food sources yielded different proportions of each food source, depending on geographical location (Fig. 11.6).Clams located in the southern part of the bay along the Teychan channel assimilated much higher outer bay phytoplankton than clams located to the north. In the northeastern end of the bay, trophic sources of clams were the most diverse: contributions of riverine POM, inner bay phytoplankton, microphytobenthos and SOM were higher than for clams collected in the south. The riverine POM contribution was sometimes higher than that of outer bay phytoplankton, and the contribution of *Zostera noltii* to the clam diet was low and never exceeded 9%.

4. Discussion

4.1. Trophic-enrichment in Manila clam muscle

Trophic enrichment in ^{13}C and ^{15}N of consumers relative to their diet is consistently reported in stable isotope analyses (DeNiro and Epstein 1978, 1981, Peterson and Fry 1987). The trophic enrichment of 3.5‰ for $\delta^{13}C$ experimentally found from algal diet to adductor muscle of Manila clams was much higher than the average values of 0.8 ± 1.1 and 0.47 ± 1.23‰ given for whole body organisms by DeNiro and Epstein (1978) and Vander Zanden and Rasmussen (2001), respectively. However, variations existed among species as $\delta^{13}C$ trophic enrichment values ranged from –3 to +3.5‰ (Post 2002). Yokoyama et al. (2005a) reported an experimentally determined trophic enrichment of 0.6‰ for $\delta^{13}C$ in whole body of *Ruditapes philippinarum* juveniles. Highly significant variations have also been reported among tissues within species, and McCutchan et al. (2003) reported a higher trophic shift for consumers analysed as muscle (+1.3 ± 0.3‰) than for consumers analyzed whole (+0.3 ± 0.1‰). Tissue-specific differences in trophic enrichment are linked to biochemical compounds (DeNiro and Epstein 1978) and pathways by which compounds are metabolized in and/or routed to different tissues (Gannes et al. 1997). Lipids are depleted in 13C compared to carbohydrates and proteins (DeNiro and Epstein 1978). Since lipid contents are much lower in muscle tissues than in gonads and digestive glands of bivalves (Lorrain et al. 2002, Malet et al. 2007), muscles typically are enriched in 13C relative to other lipid-rich organs.

To our knowledge, tissue-specific $\delta^{13}C$ trophic enrichment has never been estimated for marine bivalves, despite recent experimental studies by Yokoyama et al. (2005a), Paulet et al. (2006) and Dubois et al. (2007). Our experimental results are in accordance with Paulet et al. (2006) and field studies which analyzed tissue-specific trophodynamics in bivalves (Stephenson and Lyon 1982, Lorrain et al. 2002, Malet et al. 2007). In these studies, differences in $\delta^{13}C$ between adductor muscles and digestive glands ranged from +1.8 to +5.5‰, depending on the species and season. A similar range (+1.4 to +2.5‰) was reported by Page and Lastra (2003) between adductor muscle and gut contents of *Ruditapes decussatus*. As $\delta^{13}C$ in digestive glands reflects the assimilated diet with a low trophic enrichment, dietary reconstruction from adductor muscle of bivalves should use new $\delta^{13}C$ trophic enrichment values instead of average values. However, new estimates need to be further confirmed for bivalves other than *R. philippinarum*. The weight-to-weight proportion of clam adductor muscles versus whole body was constant over the feeding experiment and averaged 15.8 ± 2.3% (authors' unpubl. data, diet-switch experiment). Assuming that the weighted average of the isotopic composition of all organs equals the isotopic composition of the whole body (see equation in Lorrain et al. 2002), the utilization of the trophic enrichment of 3.5‰ for carbon in muscle with a trophic enrichment of 0.5‰ for carbon in whole body Manila clams (Yokoyama et al. 2005a) would indicate a 0.1 to 0.3‰ trophic enrichment for carbon in all remaining tissues. This mass-balance analysis suggests that a >3‰ muscle-specific $\delta^{13}C$ trophic enrichment may be consistent with <1‰ $\delta^{13}C$ trophic enrichment for whole body in Manila clams.

In contrast to carbon, the trophic enrichment of 3‰ in $\delta^{15}N$ from the diatom *Skeletonema costatum* to Manila clam during the diet-switch experiment was in the range of the 3 to 4‰ given by Peterson and Fry (1987). This was consistent with the average values provided by Post (2002) (3.4 ± 1‰) and McCutchan et al. (2003) (2.9 ± 1.2‰) for muscle tissues of a large variety of consumers.

4.2. Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ of Manila clams

Variation in carbon isotopic ratios of clam adductor muscles were lower than 4‰ and ranged from –23.7 to –19.9‰ for values corrected for trophic enrichment. Contrary to what could be expected from depleted $\delta^{13}C$ Leyre River POM (Table 11.1) and the spatial extent of its low salinity plume on most of the clam habitat during winter (Bouchet 1993), lack of

significant correlation between $\delta^{13}C$ clam muscle and distance to the Leyre River suggested no or low contributions of riverine materials to the diet of clams. Clam muscles, as slow growing tissues, should reflect the isotope composition of food assimilated during their growth season, from April to July in Arcachon Bay (Robert et al. 1993). Since clams in the present study were sampled from mid-May to mid-June, and the half-time of $\delta^{13}C$ was estimated at ca. 1 mo, this suggests that clam muscles have integrated food variability for the end of winter. However, the period between early April and June was characterized by low Leyre River runoffs with restricted spatial extension of the river plume and high pelagic primary production in the inner bay (Glé et al. 2008), explaining the spatial patterns in $\delta^{13}C$ of clam muscles. The hypothesis of selective feeding or selective assimilation against riverine compounds cannot, however, be excluded because it is consistent with the conclusions of Kasai et al. (2004). They indicated that in Japanese coastal habitats, *Ruditapes philippinarum* consumed mainly marine POM despite the dominance of terrestrial material in the water column. Similar conclusions were given for *R. decussatus* from the Ria Formosa lagoon, Portugal (Machás et al. 2003), and Ria de Arosa (Page and Lastra 2003).

In contrast, small-scale variability was exemplified by the significant positive correlation between $\delta^{13}C$ values of clam muscle and tidal elevation. Two hypotheses may explain this: (1) local variation in the contributions of benthic food sources, i.e. microphytobenthos, SOM and associated bacteria, which may vary according to tidal elevation; and/or (2) higher contribution of pelagic sources to the diet of clams located at low tidal levels, since pelagic sources were less ^{13}C-enriched than benthic sources (Table 11.1) (Kang et al. 1999, Kasai et al. 2004, Kanaya et al. 2005, Schaal et al. 2008). Both mechanisms can operate simultaneously because resuspended bacteria may constitute a notable contribution to *Ruditapes philippinarum* diet in *Zostera noltii* meadows, despite a dominant contribution of phytoplankton (Kharlamenko et al. 2001).

$\delta^{15}N$ values of muscle tissue from clams varied by 2.4‰ across the bay, exhibiting a south to north decreasing trend with enriched ^{15}N values in the southeast, near the Leyre River mouth. A similar trend was reported between inner and outer parts of the Ria Formosa lagoon for both muscles and digestive glands of *Mytilus galloprovincialis* and *Ruditapes decussatus* (Machás et al. 2003). In contrast, McKinney et al. (2001) found a significant correlation between $\delta^{15}N$ in *Geukensia demissa* mussels and nitrogen derived from anthropogenic activities. As suspension-feeders, Manila clams incorporate diet components linked to primary production, which depend on nutrient inputs within coastal systems. Thus 2

hypotheses could explain $\delta^{15}N$ variations in relation to changes in $\delta^{15}N$ of nitrogen-loadings in Arcachon Bay: (1) inputs of heavier nitrogen by rivers and streams compared to oceanic waters and (2) spatial variability within the bay in the nitrogen recycling by microbial organisms in the water column and/or sediments. First, rivers and streams running into Arcachon Bay are fuelled by different nitrogen sources, but relative proportions among atmospheric depositions, fertilizers and wastewaters depend on land use and management policies in the catchment. Intensive agriculture occupies only 9% of the land-catchment surface but contributes 78% of the total nitrogen load entering the lagoon, mainly in the form of nitrates (De Wit et al. 2005, Deborde et al. 2008). To prevent eutrophication in the lagoon, all paper mill effluents and human wastewaters have been collected since the 1970s by a sewage collector which diverts treatment plant effluents to the open ocean, 8 km south of the entrance to the lagoon (De Wit et al. 2005). This resulted in a negligible influence of wastewater inputs on the nitrogen-cycling within Arcachon Bay. The influence of fertilizers appeared low or spatially restricted to small stream inputs located in the northwestern bay since $\delta^{15}N$ of both Leyre River POM and inner bay phytoplankton (Table 11.1) were similar to those reported in other coastal systems (e.g. Kang et al. 1999, Machás et al. 2003, Page and Lastra 2003, Kasai et al. 2004). Secondly, mineralization processes are intense in Arcachon Bay, reflected by the composition of intertidal flats, which consist of muddy sediments enriched in organic matter with pore-water containing higher recycled nutrient concentrations (Deborde et al. 2008). These authors estimated that during one year, nutrient exports to the pelagic system by tidal pumping provided about 5 times more ammonium inputs than the freshwater fluxes, which predominantly carried nitrates (De Wit et al. 2005, Deborde et al. 2008). According to Glé et al. (2008), seasonal and spatial variation in pelagic primary production in Arcachon Bay is driven by the relative balance between nutrient levels. The highest pelagic primary production rates are observed in spring in the whole bay (outer and inner) following winter nutrient inputs by freshwater runoffs. In contrast, mid-spring to fall primary production is much lower in the outer than the inner bay. During the period of nitrogen-limitation, inner bay production is sustained by ammonium pulses coming from benthic remineralisation. In this context, light $\delta^{15}N$ values recorded during the present study in inner bay phytoplankton during spring (Table 11.1) indicated the uptake of ammonium during a period of nitrate depletion (Cifuentes et al. 1988). At the whole bay scale, these processes are indeed constrained by tidal hydrodynamics and residence time of water masses (Glé et al. 2008) and they may contribute to the spatial variability observed in $\delta^{15}N$ clam muscle through the incorporation of spatially distinct labelled $\delta^{15}N$ phytoplankton.

4.3. Spatial variation in dietary regime of Manila clams

The spring dietary regime of *Ruditapes philippinarum* in Arcachon Bay was dominated by phytoplankton with minor contributions of *Zostera noltii* and intermediate proportions of SOM. Dominance of phytoplankton in the dietary regime of Manila clams has been shown by other studies (Kharlamenko et al. 2001, Kasai et al. 2004, Kanaya et al. 2005, Yokoyama et al. 2005b). The present study, however, highlighted spatial variation in the dietary regime of *R. philippinarum* within its 70 km^2 distribution area in Arcachon Bay. Numerous stable isotope studies of coastal food webs have similarly suggested that spatial changes in trophic structure are common in heterogeneous environments (Stephenson and Lyon 1982, Deegan and Garritt 1997, Cloern et al. 2002). Although variability in food resources was buffered by the time-integrated response of muscle tissues (Tieszen et al. 1983), spatial changes in stable isotope ratios of Manila clams in Arcachon Bay were much lower than those recorded in bivalves inhabiting the estuarine to ocean gradient (Riera and Richard 1996, Cloern et al. 2002, Page and Lastra 2003). The present study suggests the presence of major differences between the southern and northern parts of the bay in terms of diversity of trophic sources. The proportion of trophic sources gradually changed from almost entirely outer phytoplankton in the south along the Teychan channel to a more diversified diet regime along the Piquey channel (Fig. 11.1). This spatial pattern may be mainly explained by water mass circulation within the bay, as 60% of oceanic inputs entering the lagoon followed the Teychan channel versus 15% for the Piquey channel (Plus et al. 2006). Both $\delta^{13}C$ and $\delta^{15}N$ values of clams located at the South in the vicinity of Leyre River mouth indicated a major contribution of outer bay phytoplankton during periods of low Leyre River discharge. The network of channels lowered marine inputs to the North Bay, the outer bay phytoplankton availability decreased and clams have to diversify their trophic sources to guarantee their metabolic requirements. Higher contributions of inner bay phytoplankton, riverine POM, microphytobenthos, SOM and *Z. noltii* in the food regime of clams located in the northern bay were also consistent with longer residence times of water masses (Plus et al. 2006), higher numbers of riverine diffuse sources (De Wit et al. 2005) and larger *Zostera noltii* meadow surfaces (Auby and Labourg 1996) compared to the southern bay. This is in agreement with numerous studies (e.g. Deegan and Garritt 1997) which have suggested that benthic primary consumers rely on locally produced food sources in estuarine food webs.

Chapitre 12

Performances de croissance de *Ruditapes philippinarum* dans le bassin d'Arcachon : le rôle des ressources trophiques

Growth performance of Manila clam (*Ruditapes philippinarum*) in Arcachon Bay (France): the role of trophic sources

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In preparation.

Vue du banc d'Arguin depuis la dune du Pyla

Abstract

The Manila clam *Ruditapes philippinarum* was introduced for culture purposes in 1980 into Arcachon Bay. This species is intensively exploited by fishermen; production and biomass of clams in Arcachon Bay has ranked first in France. Previous studies revealed length-frequency distributions with a lack of large clams and a low growth in Arcachon Bay as compared to other sites. In light of this, the present study focused on various factors controlling clam growth. A field experiment of tagging-recapture was performed at five sites during two years in order to obtain growth increment data. Then, Von Bertalanffy Growth Function (VBGF) parameters K and L_∞ were determined through Appeldoorn's method using FISAT II software. At the same time, trophic sources of clams were evaluated via carbon and nitrogen stable isotopic ratios and the IsoSource mixing model. Environmental parameters like temperature, granulometry and seagrass coverage were determined for each of the five sampled sites. L_∞ was highest at Arguin, at 46.9 mm, as compared to 38.2-42.4 mm inside the bay. K was between 0.51 and 0.97 yr^{-1}. Diet varied over time with differences between sites. Clams from Arguin consumed the greatest proportion of phytoplankton from the outer bay, particularly during winter. Sources were more diversified inside the bay at Andernos and Ile aux Oiseaux than at Gujan and Lanton. Differences in L_∞ between sites were mostly due to the proportion of phytoplankton consumed by clams and to a lesser extent to seagrass cover and physical factors (temperature, salinity).

Keywords: clam, *Ruditapes philippinarum*, growth, diet, environmental factors

1. Introduction

The Manila clam *Ruditapes philippinarum* is one of the most widely fished and farmed mollusks. It contributes to more than half of the global yield of clams (Gosling 2004). This species is indigenous to the Indo-pacific region (Ponurovsky and Yakovlev 1992) and was introduced in 1972 to France for culture purposes. *R. philippinarum* was first planted in Arcachon Bay (SW France) in 1980 (Goulletquer et al. 1999) and rapidly became naturalized in beds of seagrass (*Zostera noltii*). This clam species suffers an intensive exploitation by fishermen in this area. The production and biomass of Manila clams in Arcachon Bay are ranked highest in France, at 1028 mt and 4870 mt, respectively (Caill-Milly et al. 2008).

Stock assessment studies were conducted in 2003, 2006 and 2008 (Caill-Milly et al. 2003, 2006, 2008) and displayed an unbalanced size structure, with a lack of juveniles and a deficit of clams larger than 38 mm. Especially since a legal minimum shell length (40 mm before January 2008 and 35 mm after) is used as a primary tool of fishery management in Arcachon Bay, understanding growth of *R. philippinarum* in Arcachon Bay was fundamental. A recent population dynamics study revealed low growth performances in the bay (Dang et al. in revision-a). Growth in Arcachon Bay was lower than in other parts of the world, like British Columbia (Bourne 1982), Japan (Goshima et al. 1996) and England (Humphreys et al. 2007). Many studies have reported that water temperature and food availability are the principal factors determining bivalve growth rates (Lelong and Riva 1976, Maître-Allain 1982, Page and Hubbard 1987, Goulletquer and Bacher 1988, Harvey and Vincent 1990, Laing and Child 1996).

Von Bertalanffy Growth Function (VBGF) parameters (K, L_∞) were determined at five sites in Arcachon Bay. These sites were selected because they are representative of different conditions encountered in the bay, in terms of temperature and salinity variations, sediment grain size, immersion duration, seagrass cover, and potential trophic sources. To evaluate the assimilated trophic sources, carbon and nitrogen isotopic ratios were determined in the clam adductor muscle.

Stable isotope analyses (SIA) are widely used to investigate trophic food webs in the marine environment (Peterson and Fry 1987, Michener and Schell 1994). Nitrogen isotopic ratios enable the determination of the trophic level of consumers (DeNiro and Epstein 1981), whereas carbon isotopic ratios allow the investigation of primary food sources assimilated by

consumers (Fry and Sherr 1984). A recent work (Dang et al. 2009-c) highlighted differences in clam signatures in terms of isotopes ($\delta^{13}C$, $\delta^{15}N$) within Arcachon Bay.

Consequently, the objective of the present study was to investigate possible relationships between growth parameters (L_∞, K) and environmental parameters, but also with the nature of potential trophic sources within the clam production area.

2. Materials and methods

2.1. Studied sites

Arcachon Bay is a 180-km^2 semi-sheltered lagoon on the southwest coast of France (Fig. 12.1). The inner part is composed of 110-km^2 of muddy flats colonized by a vast *Zostera noltii* seagrass bed. The dominant species of these tidal flats, in term of biomass, is the Manila clam *Ruditapes philippinarum* (Blanchet et al. 2004). Arcachon Bay receives marine water through two channels situated in the south-west end of the lagoon and freshwater inputs mainly from the Leyre River and other little streams located around the bay. These oceanic and freshwater inputs as well as the slow renewal of water by tides induce salinity and temperature gradients within the bay (Bouchet 1968). Consequently, water salinity and sediment temperature of the Manila clam habitats vary with tide and seasons from 4 to 35 psu and from -2 to 44°C, respectively (Dang et al. 2008). Sediments are heterogeneous, ranging from mud to muddy sand. Four sites were sampled in the inner part of the bay, Ile aux Oiseaux, Andernos, Gujan and Lanton, and another site, Arguin, was sampled from the oceanic part of Arcachon Bay (Fig. 12.1). Tidal level of these sampling sites ranged from 1.46 to 2.63 m.

2.2. Environmental parameters

At the five studied sites, sediment grain-size, sediment organic matter, sediment temperature, water salinity, tidal level, and seagrass cover have been determined (Dang et al. in revision-a). *Z. noltii* seagrass cover was rated through indices from 0 (no seagrass) to 4 (100% coverage). This was performed in autumn, before grazing by Brent geese.

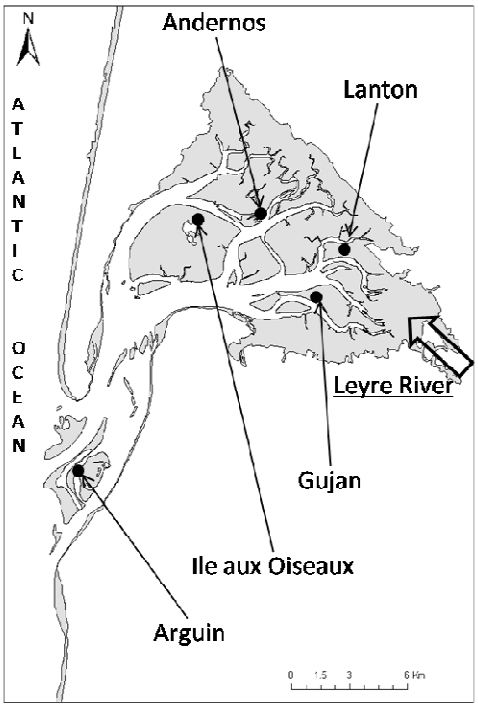

Figure 12.1. Position of the 5 sampling sites, Arguin, Andernos, Ile aux Oiseaux, Gujan and Lanton, in Arcachon Bay.

2.3. Manila clam growth

2.3.1. Sampling strategy and processing

Hundreds of clams across a wide size range (6 – 44 mm shell length) were collected at Ile aux Oiseaux in November 2005. Each individual was labeled with a numbered tag and its shell length was measured. Clams were rapidly planted in enclosures, previously cleared of macrofauna, in each of the five sampled sites in Arcachon Bay: Arguin, Ile aux Oiseaux, Andernos, Gujan and Lanton. Each cage was composed of a 50 x 50 x 25 cm topless metal

frame meshed with 2-mm plastic net, buried 15 cm and projecting 5 cm above the sediment, and anchored with iron bars. The density and biomass of introduced clams in each cage were 320 ind. m^{-2} and 24.2 g shell-free dry weight, respectively. Arguin is considered to be the reference site in terms of maximal growth because of its outer position and its healthy clams (high condition index, few pathogens) (Dang et al. in preparation). *R. philippinarum* were seasonally recaptured and their lengths measured over a total period of two years. The obtained size measurements were used for the estimation of growth parameters.

2.3.2. Data analysis

Growth increment data were analyzed in order to estimate Von Bertalanffy growth function (VBGF) parameters L_∞ and K through the Appeldoorn's method using FISAT II software (version 1.2.2, FAO-ICLARM). The VBGF equation
$Lt = L\infty\ [1 - \exp(-K \times t)]$ predicts length as a function of age and is commonly used in bivalve growth studies (Bachelet 1980, Bourne 1982, Pouvreau and Prasil 2001, Fiori and Morsan 2004). L_∞ is the asymptotic shell length (mm), and K is the growth coefficient (yr^{-1}).

2.4. Trophic sources

2.4.1. Sampling strategy and processing

Five specimens of Manila clams (30-35 mm) were collected every other month at each of the five stations from January 2007 to November 2007. Clams were opened and their posterior adductor muscles were dissected, frozen at -20°C for storage, and then at -80°C before freeze-drying. Muscle samples were reduced to a homogeneous fine powder using a mortar and a pestle.

2.4.2. Stable Isotopic Analysis (SIA)

Isotopic analyses were performed using an elemental analyser (Carlo Erba 2500) in line with an isotope ratio mass spectrometer (VG Isoprim). Data were corrected and calibrated against home-made working standards (casein and glycin) and against certified standards

(acetanilide, IAEA-N2, USGS-24), which allowed an analytical precision of 0.2‰ to be calculated. Isotopic values are calculated following:

$$\delta^{A}X = \left[\left(R_{sample}/R_{ref}\right) - 1\right] \times 1000$$

where A is the atomic mass of the heavy stable isotope of the element X, and R_{sample} and R_{ref} are the ratios of heavy to light isotope for carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Reference (Ref) is Vienna Pee Dee Belemnite (PDB) for $\delta^{13}C$, and atmospheric nitrogen for $\delta^{15}N$.

2.4.3. Model of food web interaction: IsoSource

Isotopic variations among organs reflect their differing metabolic activity as well as their turnover rates. Because of its long turnover time, adductor muscle is commonly used in trophic studies of clams (Page et al 2003, Kasai et al. 2004, Kanaya et al. 2005, Kharlamenko et al. 2001). To determine the relative contribution of food source to the mixed signature of adductor muscle of clams in the five stations along time, IsoSource software was used (Phillips and Gregg 2003, Phillips et al. 2005). Potential trophic sources considered in this study for Manila clam were the same as in Dang et al. (2009-c) *i.e.* inner (-20.60‰, 4.75‰) and outer bay phytoplankton (OBP) (-20.96‰, 5.62‰), Leyre River POM (-28.24‰, 5.24‰), microphytobenthos (-16.81‰, 4.76‰), sedimentary organic matter (SOM) (-18.40‰, 3.94‰) and *Zostera noltii* (-11.77‰, 3.99‰). *Zostera noltii* source was considered in the mixing model, according to its quantitative prevalence within the bay. At Arguin, which is situated in the oceanic part of the bay, Leyre River POM and inner bay phytoplankton were not considered to be potential trophic sources. The fractionation factor used for carbon and nitrogen was determined in a diet switch experiment between a monospecific algal diet (*Skeletonema costatum*) and the adductor muscle of adult *R. philippinarum* (30-36 mm) from Arcachon Bay (Sauriau et al. in preparation). This study revealed an increase of 3.5‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$ between the algae and the adductor muscle of the clam.

IsoSource calculates all possible combinations of dietary sources using an iterative approach, and then determines the predicted isotopic signature of the mixture. This model provides the distribution of source proportions that are consistent with isotopic mass balance. A distribution of feasible solutions and dietary source proportions was generated within a defined increment of 5% and a tolerance of 0.5‰.

2.5. Statistical procedure

A two-way analysis of variance (ANOVA) was performed to compare carbon and nitrogen isotopic ratios considering the five sites and the seven months as independent factors (Sokal and Rohlf 1981). Statistical analyses were performed using Statistica software. Maximum type I error rates were set at $\alpha = 0.05$. Prior to analysis, homogeneity of variance was checked using Cochran test.

Normalized principal component analysis (PCA) was used to investigate the relationships among environmental conditions and VBGF parameters for the five sampled sites and to determine which variables differed the most between stations. All variables were projected on factorial axes. It was decided that average values over one sampling year be used, the ultimate aim being to determine which factors were correlated with K and L_∞, two parameters that integrate over a long period of time (the clam life spam). Environmental factors included granulometry parameters (e.g. grain size (SGS), % silt and clay (SSC), % organic matter (SOM), tidal level, mean sediment temperature for the year 2007, and the seagrass (*Z. noltii*) cover index. Annual means (January-November 2007) of trophic source proportion consumed by Manila clam, including outer bay phytoplankton (OBP), inner bay phytoplankton (IBP), sedimentary organic matter (SOM), microphytobenthos, riverine particulate organic matter (POM) and *Zostera noltii*, were also considered in the PCA analysis. VBGF parameters considered here were K and L_∞.

Finally, correlations between growth parameters (K, L_∞) and environmental and trophic variables were performed.

3. Results

3.1. Environmental parameters

The five sites displayed contrasting environmental parameters in terms of sediments (grain size ranged from 69 µm to 360 µm), tidal level (1.5 to 2.6 m), and seagrass cover (0 to 4) (Table 12.1). Lanton and Gujan displayed the greatest variability in salinity and the lowest mean (26.7). Sediment temperature variability was high everywhere (maximum at Gujan) but the annual mean temperature was lowest at the oceanic site (Arguin) where annual mean salinity was the highest (34.2).

3.2. Growth

L_∞ was higher at Arguin (46.9 mm) than at sites located inside the lagoon (Fig. 12.2). L_∞ was similar at Gujan and Lanton (42.4 and 42.1 mm, respectively) and higher than L_∞ at Andernos (39. 5 mm) and Ile aux Oiseaux (38.2 mm). K was the lowest at Lanton (0.51 yr^{-1}) and Gujan (0.52 yr^{-1}), followed by Arguin (0.55 yr^{-1}), Ile aux Oiseaux (0.68 yr^{-1}), and Andernos (0.97 yr^{-1}).

Table 12.1. Environmental variables characterizing sampling sites: sediment grain size (median in µm), organic matter of sediment (%), sediment silt (%), sediment temperature (°C), water salinity, tidal level (m), immersion rate (%) and seagrass coverage index (0: no seagrass to 4: 100% coverage).

Site	Sediment grain size	Sediment organic matter	Sediment silt	Sediment temperature			Water salinity			Tidal level	Immersion rate	Seagrass coverage
				Min	Max	Mean	Min	Max	Mean			
Andernos	163	3.3	14.5	-1.0	35.4	15.8	18.5	34.5	30.0	2.11	60	3
Lanton	78	10.1	41.0	-1.7	37.8	16.0	4.8	34.4	26.7	1.89	71	1
Ile aux Oiseaux	97	13.0	42.4	0.2	37.9	16.1	12.1	34.8	29.6	2.63	45	4
Gujan	69	5.6	47.2	-0.2	43.7	16.1	4.8	34.4	26.7	1.46	75	0
Arguin	360	1.0	3.5	-0.2	30.0	15.1	31.2	35.4	34.2	2.07	65	1

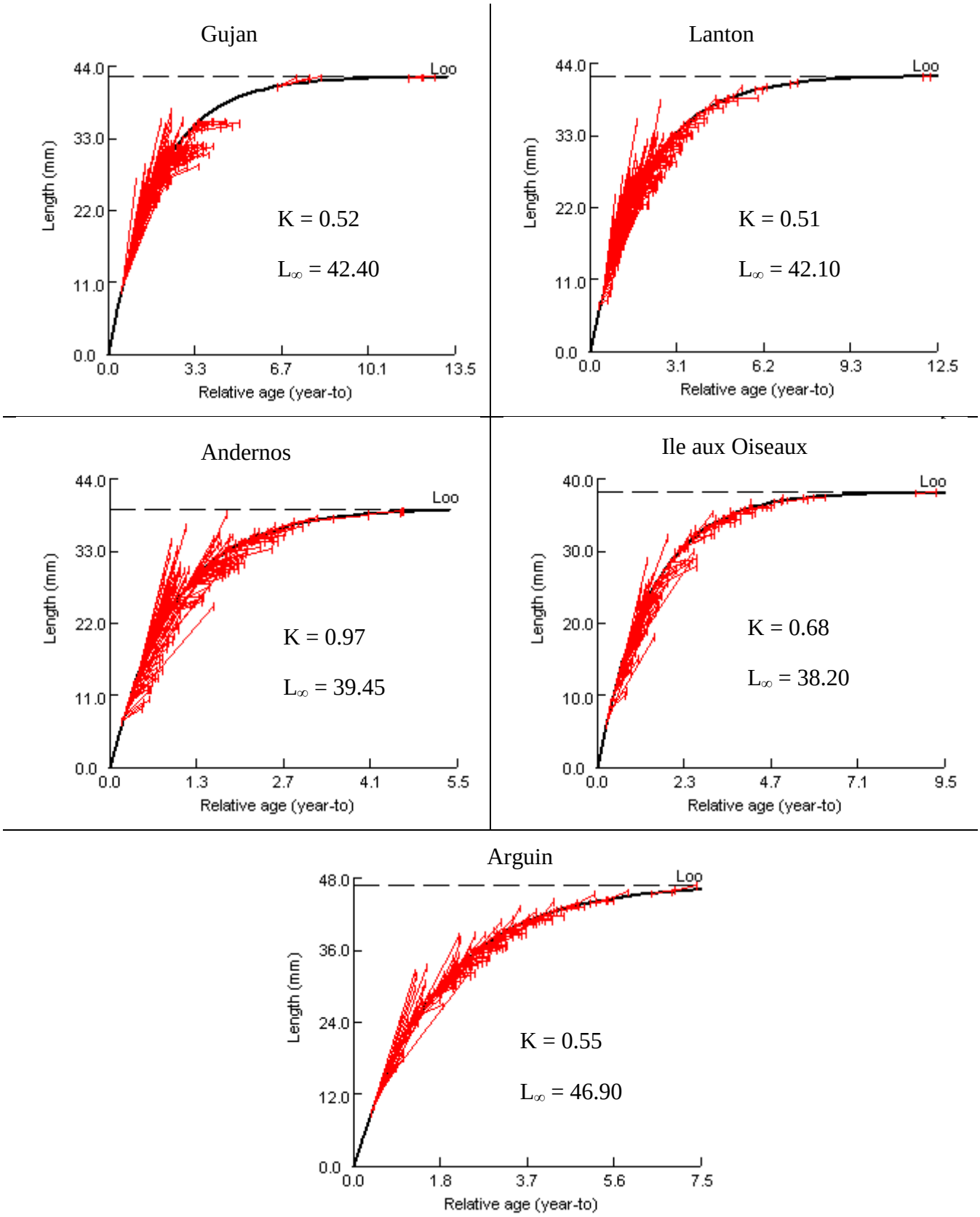

Figure 12.2. Growth curves for *R. philippinarum* at the five sampling sites obtained by Appeldoorn's method in FISAT II software. On graphs, growth segments for all individuals that were measured seasonally are represented. Appeldoorn's method generates Von Bertallanffy parameters K (yr^{-1}) and L_∞ (mm).

3.3. Stable isotopes analysis

Values of nitrogen isotopic ratios of *R. philippinarum* adductor muscle were between 7.7 and 9.2‰ (Fig. 12.3). Two-way ANOVA revealed strong differences between sites ($P < 0.001$) and months ($P < 0.001$), with a significant interaction ($P < 0.001$). An increasing gradient was observed between the outer site of Arguin (annual mean: 8.06‰) and the inner site of Gujan (annual mean: 8.76‰). A gradient appeared between January 2007 and January 2008, especially at the inner stations (Gujan and Lanton), with no obvious seasonal trend (Fig. 12.3).

Values of carbon isotopic ratios ranged between -18.3 and -15.6‰ (Fig. 12.3). Two-way ANOVA also displayed a significant effect of sites ($P < 0.001$) and months ($P < 0.001$), and a significant interaction between these ($P = 0.01$). The inner sites of Gujan and Lanton had the lowest values (means: -17.91 and -17.69‰, respectively, *vs* -16.57 to 17.06‰ in the outer sites). Gujan had the lowest seasonal variability (0.86‰ *vs* 0.95 to 1.69‰ for the other stations).

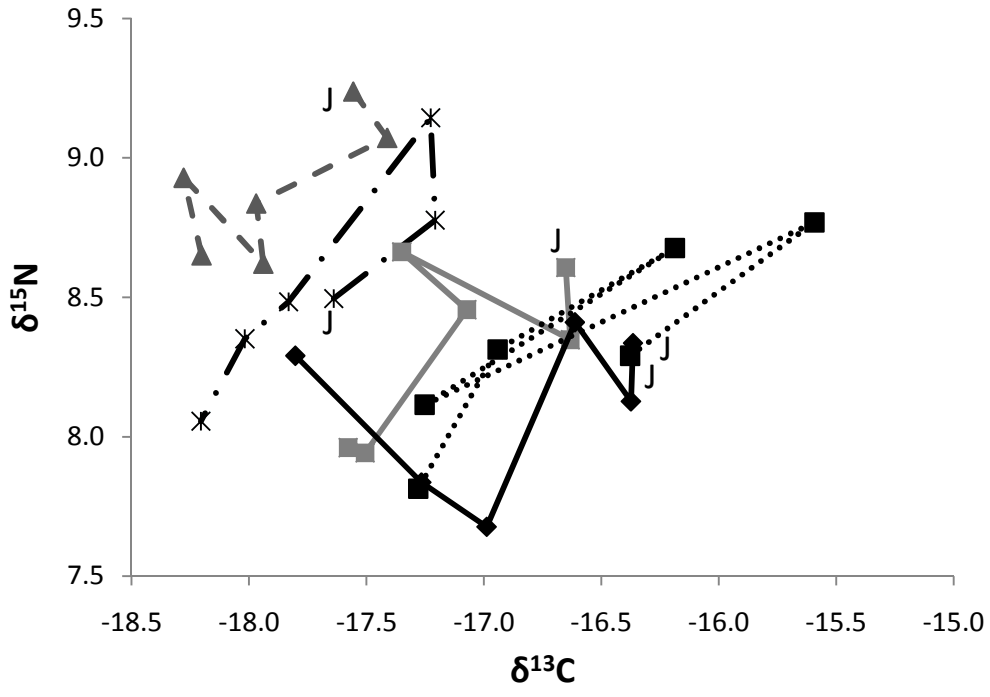

Figure 12.3. Dual plot of nitrogen *vs* carbon isotopic ratio of *R. philippinarum* adductor muscle for the five sampling sites: Arguin (——), Andernos (——), Ile aux Oiseaux (.....), Gujan (– – –) and Lanton (— . —) from January 2007 to November 2007. J, January 2007.

The dual nitrogen and carbon isotopic ratios plot showed a distribution pattern from the oceanic area on the right to the most inner site in the bay, i.e. Arguin, Ile aux Oiseaux, Andernos, Lanton and Gujan (Fig. 12.3).

3.4. Model of trophic sources interactions

As previously reported (Dang, in revision-b), *R. philippinarum* mainly fed upon "OBP" at all sampled sites (Fig. 12.4). The second dominant source depended on the site and the period within the year. The site where the consumption of outer bay phytoplankton was most important was Arguin (mean: 77.6%), followed by Gujan (66.8%), Lanton (46.1%), Ile aux Oiseaux (38.7%) and Andernos (38.4%). *Z. noltii* source was modest, with a global mean of 4.8%. Inner bay phytoplankton and Leyre River POM were at an equivalent level at inner sites (between 7 and 19%). Clams from Arguin consumed a constant proportion of outer bay phytoplankton along the year with a peak in November 2007 (97.5%). Conversely, the four sites located inside the lagoon displayed more variability over the year, the proportion of OBP ranging from 19% to 96%.

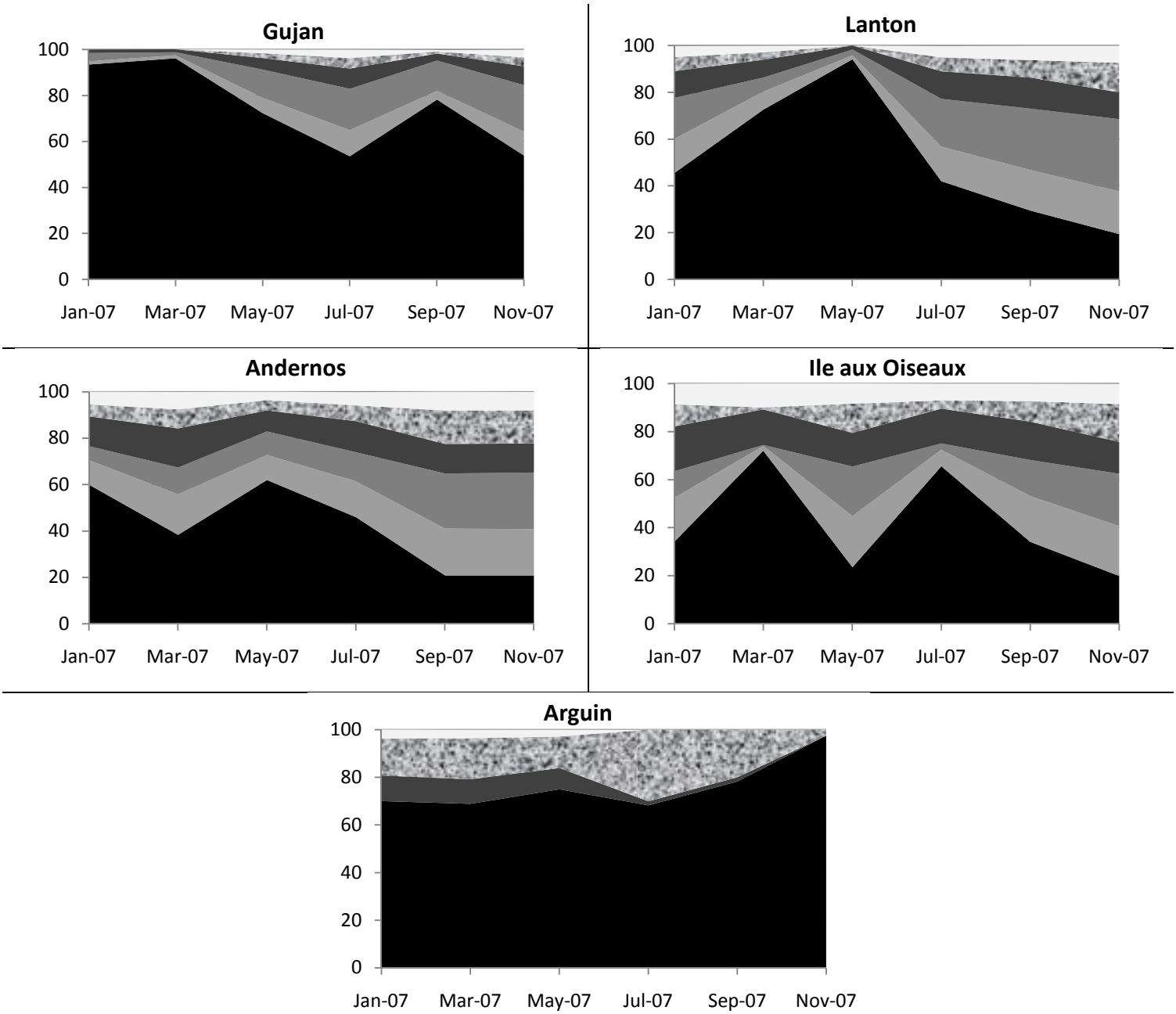

Figure 12.4. Percentage of potential trophic sources i.e. outer bay phytoplankton (■), inner bay phytoplankton (□), riverine POM (■), microphytobenthos (■), sedimentary organic matter (□) and *Zostera noltii* (□) that contributed to the Manila clam diet from January 2007 to November 2007.

3.5. PCA data analysis

Principal component analysis of the environmental parameters, trophic sources and VBGF parameters revealed that the stations could be separated into three distinct groups: 1) Arguin; 2) Ile aux Oiseaux and Andernos; 3) Lanton and Gujan (Fig. 12.5a). 88.3% of the total variation was explained by the two first axes, and consequently the 2-dimensional PCA ordination provided a correct explanation of the relationship between stations. Factor 1 discriminated Arguin (outer station) from the four inner stations. Arguin contributed to 64.4% of this factor and Ile aux Oiseaux 28.1%. Variables which most contributed to the separation of Arguin from other stations were: L_∞ (11.5 %), IBP (11.1%), OBP (9.6%), Zostera noltii (9.1%), MPB (8.6%) and riverine POM (8.5%) (Fig. 12.5b).
Factor 2 separated Ile aux Oiseaux, Andernos and Arguin from Lanton and Gujan. It was mostly explained by Gujan (52.6 % of this factor). Variables which contributed to the separation of stations along this axis were tidal level (18.9%), SOM (trophic source, sediment organic matter) (16.2%), and *Z. noltii* cover (10.8%). K did not appear as a consistent variable to explain differences among stations, in contrast with L_∞. L_∞ was highly correlated with OBP (and anti-correlated with IBP).

When a correlation matrix including all average variables was generated, significant correlations were observed only between L_∞ and OBP ($r = 0.85$, $p = 0.05$) and between L_∞ and IBP ($r = -0.93$, $p = 0.022$).

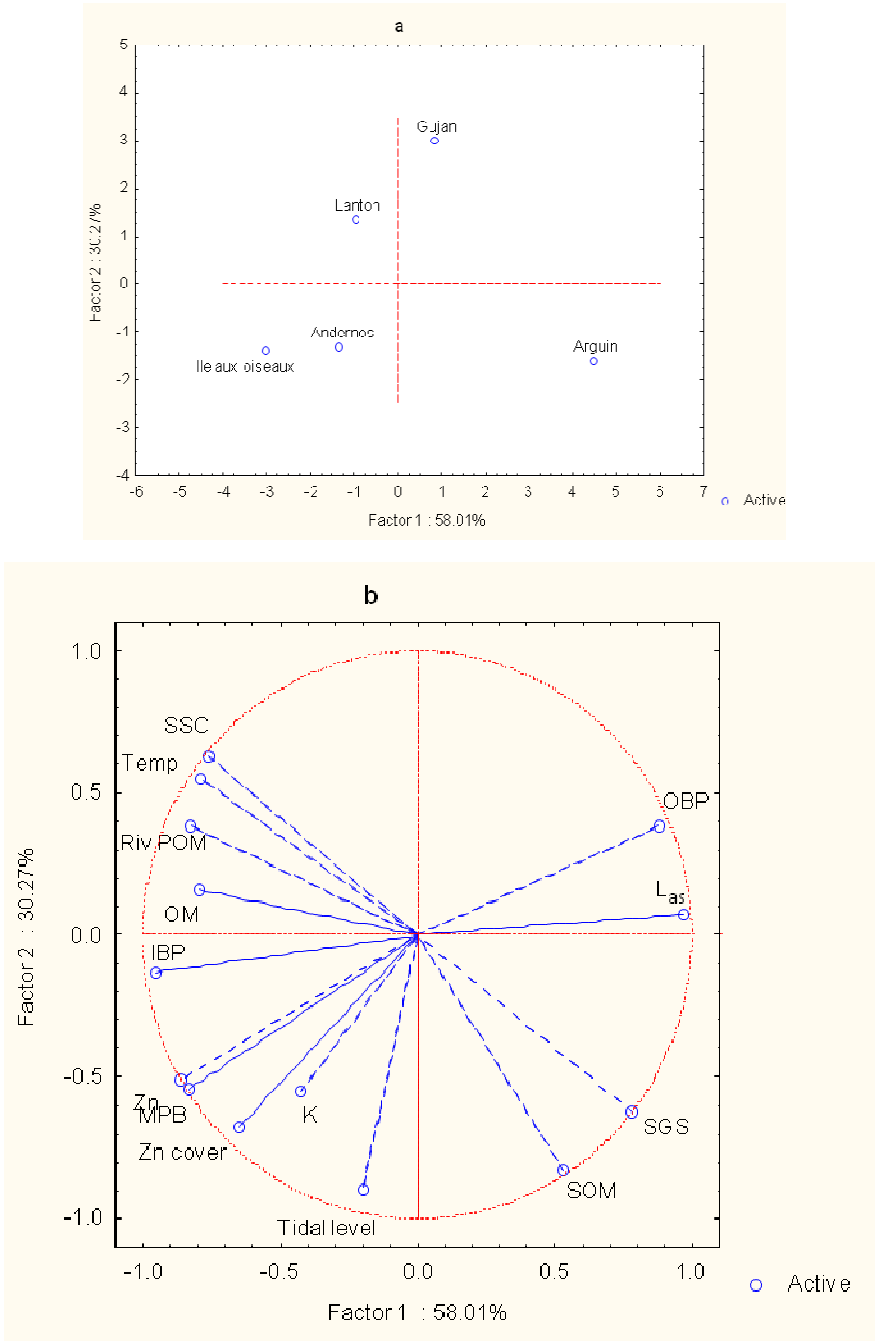

Figure 12.5. Results of ACP. (a) Projection of the stations on the factor-plane. (b) Projection of the variables on the factor-plane.

OBP, outer bay phytoplankton. IBP, inner bay phytoplankton. MPB, microphytobenthos. SOM, sediment organic matter. Zn, *Zostera noltii*. Riv POM, riverine particulate organic matter. Zn cover, *Zostera noltii* cover. SSC, sediment silt and clay (%). OM, sediment organic matter (%). SGS, sediment grain size (µm). Temp, sediment temperature (°C). K and L_{as}, growth parameters of Von Bertalanffy model.

4. Discussion

This study revealed that the apparent landscape homogeneity of the 70-km² seagrass flat at Arcachon Bay hid different habitats. Previous studies had already indicated that benthic and pelagic communities differed within this lagoon (Blanchet 2004, Glé 2007). This study showed that the heterogeneity is reflected at the five sampled stations, through trophic and environmental differences. Among investigated factors, those that best explained these differences were trophic source characteristics, tidal level, and seagrass cover. Surprisingly, this heterogeneity had just a modest effect on *Ruditapes philippinarum* growth since only L_∞ was involved in explaining site differences. Environmental characteristics differed in terms of food quality, immersion time and *Zostera noltii* cover. Consequently, this heterogeneity induced growth differences in clams, especially in the asymptotic length reached by Manila clams. A previous study in Arcachon Bay underlined homogeneity of the K parameter in the entire bay and a heterogeneity of the asymptotic length between stations (Dang et al. in revision-a). Arguin was characterized by a much higher L_∞ (46.9 mm) than for *R. philippinarum* in the inner lagoon. Including this oceanic outer station in the data analysis gave a better understanding of parameters involved in stunted/stimulated growth. This could be explained by a higher consumption of outer bay phytoplankton (OBP) at Arguin as well as a higher stability of environmental parameters: sediment temperatures were higher in winter and lower in summer than at inner stations. Differences in growth rates (L_∞) of *R. philippinarum* between inner and outer sites could consequently also be explained by differences in temperature (Mann 1979). As observed in the bay of Marennes-Oléron (Bacher and Goulletquer 1989), the more oceanic position of Arguin in Arcachon Bay protects organisms from the stress of extreme temperatures. Temperature was not correlated with growth parameters in our study because of using annual means. However, temperatures are considered as key factors in explaining growth (Lelong and Riva 1976, Thompson 1984, Bacher and Goulletquer 1989, Chew 1989, Nakamura et al. 2002), *R. philippinarum* preferring rather high temperatures (Humphreys et al. 2007, Dang and de Montaudouin 2009).

Asymptotic length was correlated with OBP and inner bay phytoplankton (IBP), showing the importance of phytoplankton in clam growth (Lelong and Riva 1976). The lack of correlation between other environmental factors and *R. philippinarum* growth performance was also due to a general deficit of growth within the lagoon as already underlined by Dang et al. (in revision-a). Of course, food supply is a major contributing factor to growth (Bodoy and Plante-Cuny 1984, Thompson 1984, Bacher and Goulletquer 1989, Chew 1989, Robert et al.

1993, Nakamura et al. 2002), but it was not quantitatively measured in the present study. In Arcachon Bay, the phytoplankton dynamics show important seasonal and spatial (i.e. external *vs* internal waters) variations (Glé et al. 2008). For example, during the winter period, phytoplankton biomass (dominated by diatoms) in the external waters is higher than in internal waters. This observation is mainly explained by the entry of oceanic phytoplankton coming from the Bay of Biscay (Glé et al. 2008). Our analysis was restricted to food quality and gave a new insight on trophic source quality, variability, and its role in explaining *R. philippinarum* growth differences between sites.

The OBP was the dominant trophic source for *R. philippinarum* during the major part of the year at all sampling stations, except in winter. During this period, the contribution of OBP in clam diet decreased by proportions that varied by station. *R. philippinarum* from Arguin consumed up to 60% of OBP all year long, which represented the highest proportion of phytoplankton during winter months, in comparison with other stations. However, the observed seasonal patterns of potential trophic sources in clams (Fig. 12.4) could be biased by the fact that the input of sources in IsoSource software did not take into account seasonal variation of these sources. They were considered to be stable over the year. Perhaps the greater consumption of OBP in winter at Arguin may explain its higher L_{∞}. The interesting feature is that the contributing factor is not the quality of the trophic source alone but its variability. Two factors that explained variations between sites were tidal level and seagrass cover. They could also be linked with trophic characteristic variations.

Additionally, a low tidal level equates to a higher immersion time, an increase of feeding time, and consequently a higher growth rate. However, no relationship was found here between tidal level and growth parameters. The lack of influence of immersion time on *R. philippinarum* growth was not consistent with the literature concerning most filter-feeders, which indicates that lower the tidal level is, higher the growth rate should be (Glock and Chew 1979, Griffiths 1981, Fréchette and Bourget 1985, Goulletquer et al. 1987, Harvey and Vincent 1990, de Montaudouin 1996, Beal et al. 2001). In the case of *R. philippinarum*, however, previous studies either showed that tidal level was not the principal factor explaining growth variability between different sites (Bacher and Goulletquer 1989, Masu et al. 2008) or did not find any correlation at all (Nakamura et al. 2002). With a larger set of stations in Arcachon Bay (twenty), Dang et al. (in revision-a) did not find any correlation between these two variables.

The presence of a seagrass bed is also reputed to promote a faster growth of suspension feeders living buried in the sediment (Irlandi and Peterson 1991, Irlandi 1996). Three hypotheses were proposed by Irlandi and Peterson (1991) to explain the seagrass effect on bivalve growth: 1) effects on the food supply; 2) effects on the activity of a biological disturbance agent as the presence of seagrass reduced clam predators (Irlandi and Peterson 1991); and 3) effects on the stabilization of sediments. Indeed, suspension feeders often grew more slowly when exposed to high suspended sediment loads due to the energy expenditure required to process inorganic particles in order to obtain their food (Turner and Miller 1991). The root-rhizome of seagrass plants binds and stabilizes sediments and the blades attenuate wind waves and reduce sediment transport and resuspension beneath the seagrass canopy (Gambi et al. 1990, Fonseca and Cahalan 1992). These changes in the sediment dynamics with the presence of seagrass may have profound effects on suspension-feeder growth (Irlandi 1996). In the present case, *Z. noltii* did not alter *R. philippinarum* growth. Conversely, seagrass beds could have played a role in the observed seasonality of trophic source quality absorbed by *R. philippinarum*. Indeed, *Zostera noltii* seagrass cover influences the quality and the diversity of food sources available for *R. philippinarum* through its protective effect on the interface sea water/sediment, *i.e.* by the availability of fine sedimentary organic matter and/or microphytobenthos. Several studies showed that chlorophyll *a* concentrations and variations were greater in a seagrass bed than in the adjoining unvegetated habitat (Irlandi and Peterson 1991, Judge et al. 1993). In the same way, bivalves that were feeding within seagrass meadows experienced different food sources than those feeding in unvegetated areas (Judge et al. 1993). Within the seagrass, the current speed also decreases and this begets a vertical gradient of chlorophyll *a*, as compared with unvegetated bottom where the chlorophyll *a* is uniformly distributed (Judge et al. 1993). Up to 90 % of the near-bottom microalgal count in the seagrass bed can be epibenthic or epiphytic diatoms (Irlandi and Peterson 1991, Fonseca and Cahalan 1992, Judge et al. 1993). Consequently, the seagrass provides a locally-generated near-bottom food resource for *R. philippinarum* and could explain why the diet of *R. philippinarum* at Andernos and Ile aux Oiseaux (higher cover index) was more diversified than at Gujan, Lanton and Arguin.

Finally, the most important variable affecting Manila clam asymptotic length was trophic source, and especially the consumption of phytoplankton. However, trophic sources could vary in quality and quantity, depending for instance on the seagrass cover. The physical

parameters (salinity, temperature) also play a role, evidenced here by their higher stability at Arguin, correlated with higher L_{∞}.

Troisième partie :

Gestion des populations exploitées

Chapitre 13

Modèle de gestion de la pêche à la palourde japonaise dans le bassin d'Arcachon

A dynamic modelling approach for the management of Manila clam fishing in Arcachon Bay

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Will be submitted in *Aquatic Living Resources.*

Platiers intertidaux recouvert d'herbier à *Zostera noltii*

Abstract

Stock assessment studies have shown that the Manila clam (*Ruditapes philippinarum*) stock in Arcachon Bay considerably decreased between 2003 and 2008. This sign of clam decline as well as the slow growth and the low recruitment observed within the bay prompted this study. The objective was to provide a tool that makes quantitative predictions extending ten years based on different management strategies, in order to balance harvest with stock sustainability. Current management policies for the Manila clam population in Arcachon Bay include the restriction of fishermen numbers by limiting licenses to 70, the closing of certain areas (reserves) that represent 18.5% of the total habitat occupied by the clam population, and the allowance of fishing all year long (12 months). Fishing efforts considered in the model were estimated at 40, 50 and 10% for 21-34, 35-39 and > 40 mm clam shell length categories, respectively. Simulations began in 2003. Of management strategies tested, the best measure for increasing the stock was to decrease the capture season duration to 11 months, to increase the surface of the reserve areas to 22%, and to decrease the number of fishing licenses to 55. Adopting only on one of these measures did not sufficiently restore and enhance the clam stock. This model also showed that if recruitment alone increased, the Manila clam population in Arcachon Bay would be restored and enhanced. Even if this model was realistic (same estimated and measured stock in 2008), it did not take into account the recruitment variability, the food resources and the diseases.

Keywords: clam, population dynamic, model, stock, management, Arcachon Bay

1. Introduction

The Manila clam *Ruditapes philippinarum* was introduced in the 1980's into Arcachon Bay for cultivation purposes. Reproduction occurred and this species became naturalized in muddy habitats throughout the bay. Intensive fishing soon followed. Currently, clam production and biomass in Arcachon Bay are highest among all French sites for which stock assessment studies are conducted. Management measures include limiting the number of fishing licenses at 70, imposing a minimum legal shell length at 40 mm (until 2008), and closing certain areas. The effect of the reserve areas is probably not significant, given that the closed areas are very small and the local fisheries commission changes the locations every year. In Arcachon Bay, the mean shell length of the population has been low and clams hardly reached the legal size of 40 mm (Dang, in revision-a). At most sites, clams never attain this shell length, or if they do it is only after more than ten years of growth (Dang, in revision-a). The poor growth performance of *R. philippinarum* in Arcachon Bay recently led to a new regulation setting the minimum legal size at 35 mm in 2008.

In marine fisheries, stock assessment is one of the most important scientific tasks due to its role in ensuring the sustainability of marine resources for human consumption. The Manila clam stock of Arcachon Bay was assessed in 2000, 2003, 2006 and 2008 (Bertignac et al. 2001, Caill-Milly et al. 2003, 2006, 2008). From 2001 to 2006, these studies demonstrated a relative stable stock, around 7000 – 8000 mt. However, a drastic decrease of the total stock from 7307 mt to 4457 mt was observed between 2006 and 2008. Mean densities dropped from 24 to 18.6 ind. m^{-2} and mean biomass fell from 159 to 98.4 g m^{-2} from 2006 to 2008. These indicators predict the decline of the *R. philippinarum* stock in Arcachon Bay, i.e. they indicate that the balance between fishing activities and capability of stock renewal was maintained. Reasons involved are the succession of poor recruitment years, the ineffective management measures previously described, and the fact that not all fishermen respect the legal catch size. Indeed, anonymous capture reports and authority controls note that fishermen collect clams below 35 mm.

Consequently, it is important to devise new fishing strategies to maintain Manila clam populations in Arcachon Bay and to restore the economic efficiency of the fishing industries. Effective management of mollusk fishing is of crucial importance for both socio-economic and ecological reasons, as intensive clam harvesting is the second most important local industry related to the bay, after oyster culture. Extensive clam fishing in Arcachon Bay is

currently performed at the expense of long-term environmental and economic sustainability of the fishing industries. New management policies are thus necessary to make compatible the achievement of harvesting activities with the long-term conservation of the natural environment. In this context, building reliable management models is fundamental to developing efficient and sustainable exploitation strategies for coastal ecosystems. Models may allow quantitative prediction of future scenarios of clam population dynamics under alternative management strategies and help managers to choose the strategy that would balance harvest with sustainability. Several management models permit simulation of clam population dynamics over the year, to describe the relationship between seasonal fluctuations of temperature and clam growth rate, to assess the effect of different seeding densities on final yield, and to identify the optimal timing for seeding and for harvesting, to maximize the economic value of rearing activities (Borja and Bald 2000, Melià et al. 2003, Solidoro et al. 2003, Melià et al. 2004). The majority of the models that attempted to describe clam growth necessitated the estimation of a large number of parameters (Solidoro et al. 2000, Pastres et al. 2001).

The population dynamics model developed by Borja and Bald (2000) is user-friendly and was first applied to modeling the management of *Ruditapes decussatus* in estuaries of the Basque Country (Spain). Later, this model was adapted for the natural population of Manila clam *R. philippinarum* in Arcachon Bay (Sinquin 2006, Bald et al. 2009). These studies utilized data from the Manila clam standing stock assessment of 2003, e.g. length frequency distributions (Caill-Milly et al. 2003). However, the other parameters were extracted from the literature (Solidoro et al. 2000, Melià et al. 2003, Solidoro et al. 2003, Melià and Gatto 2005). This resulted in an inaccurate model of uncertain predictive value. Since this study, parameters required by the model have been determined in natural *R. philippinarum* populations within Arcachon Bay (Dang et al. in revision-a). Therefore, it became relevant to run the model with more realistic parameters.

The aim of this study was thus to compare stock evolution over ten years predicted by the model under different policies of renewable resource exploitation. Different scenarios of management policies with environmental conditions were defined and compared.

2. Materials and methods

The model used was that developed by Borja and Bald (2000) in the Spanish research institute of AZTI Tecnalia. The model was constructed using Ventana Simulation Environment software (Vensim ® 5). Details about the model are reported in Bald et al. (2009) (Annexe 1). Hereafter, the main characteristics are summarized.

2.1. Basic concept and state variables

The clam size frequency distribution was divided into four length classes: 1) the juvenile size class from 0 to 20 mm. Sexual maturation might occur but clams were unable to spawn efficiently; 2) the non-exploited adults from 21 to 34 mm; and two classes of exploited adults 3) 35-39 mm, and 4) > 40 mm (Fig. 13.1) (Bald et al. 2009).

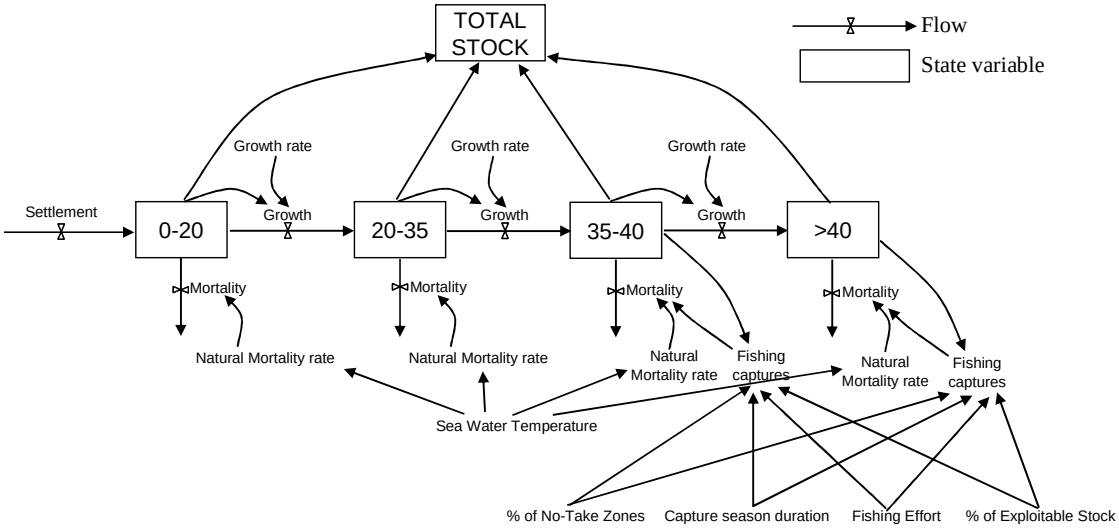

Figure 13.1. Schematic diagram of the Manila clam population dynamics model for Arcachon Bay (France). Shell length ranges are given in mm.

The separation between the two exploited classes was established because before 2008, 40 mm was the minimum legal length for capture in Arcachon Bay and it is now the legal minimum size in many places in France. Since 2008, the size allowed in Arcachon Bay has been 35 mm. In spite of legislation, it seems that fishermen exploit all size classes above 30 mm (anonymous pers. com.).

The length frequency distribution obtained by Caill-Milly et al. (2003) allowed calculation of density (D_i, ind. m^{-2}) and mean length (ML_i, mm) for each size class (Bald et al. 2009). The following length-weight relation was provided in Caill-Milly et al. (2003):

$$Weight\ (g) = 0.2162\ \times\ Length^{3.0469} \quad (r^2 = 0.94)$$

Temperature is a major environmental variable regulating both growth and survival of *R. philippinarum* (Mann 1979, Goulletquer et al. 1989b, Melià et al. 2004). Consequently, seasonal temperature variations were simulated using the sinusoidal formulation suggested by Melia et al. (2004) for Arcachon Bay and used by Bald et al. (2009). Temperature T (°C) at time t (days) is given by the relation:

$$T\ (t) = f\ \times sin\left(\frac{2\pi}{365}\ (t\ + h)\right) + g$$

where f = 6.54°C (maximum temperature variation), h = -115.1 (phase), and g = 15.85°C (mean annual temperature). Coefficients f and h were determined by Bald et al. (2009) using an iterative modeling until achieving a "good fit" between the sea water temperature data set provided by IFREMER Arcachon and the modeled data, following regression analysis (r^2 = 0.91).

Clams in a length class i (LC_i, ind. m^{-2} $month^{-1}$) increase their number by the input of individuals from the previous LC through the growing process (G_i, ind. m^{-2} $month^{-1}$) and decrease their number by the mortality (Mi, ind. m^{-2} $month^{-1}$) and by the transfer of individuals to subsequent LC (Bald et al. 2009):

$$LC_i = G_{i-1} - M_i - G_{i+1}$$

The transfer of individuals between the different length classes (G_i) was due to growth at the growth rate (GR_i, dimensionless):

$$G_i = LC_i\ \times GR_i$$

Growth rate was calculated using the growth model developed by Melia et al. (2004) for the Sacca di Goro Lagoon (Italy), using temperature formulation previously described and VBGF parameters obtained experimentally by Dang et al. (in revision-a), *i.e.* K = 0.51 yr^{-1} and L_∞ = 44.6 mm for all studied stations within Arcachon Bay. The model developed by Melia et al. (2004) allows calculation, for each size class, of the minimum clam length to pass to the next size class in a month. The proportion of clams that grow and pass to the subsequent class (GR_i) was evaluated, based on size frequency distribution of Caill-Milly et al. (2003) study. Calculated proportions were tuned by iterative simulations until stable values were found.

For exploited clams, total mortality was the sum of natural (n_i, ind. m^{-2} $month^{-1}$) and fishing mortality (fishing captures, FC_i, ind. m^{-2} $month^{-1}$). It equaled natural mortality alone for non-exploited clams:

$$M_i = n_i + FC_i$$

In contrast with Bald et al. (2009) who calculated natural mortalities using the model developed by Solidoro et al. (2000, 2003), natural mortalities of each class were estimated from field enclosure experiments (Dang et al. in revision-a). Values were 6.9% for length class 0-20 mm, 3.9% for 21-34 mm, 1.8% for 35-40 mm and 1.2% for size class > 40 mm. Fishing mortality (%) was fixed by the user and adjusted following fishermen captures.

Fishing captures for a length class *i* are calculated by multiplying LCi by fishing effort (FE_i, %), the percentage of exploitable stock (PES, %), the capture season duration (CSD, month), and the percentage of reserve areas (PRA, %):

$$FC_i = LC_i \times FE_i \times PES \times CSD \times PRA$$

In 2004, total capture represented 14-21% of the total exploitable stock, which corresponded to fished individuals. The PRA corresponds to the percentage of the fishing reserves within the total surface of Arcachon Bay.

Finally, all this system is sustained by clam recruitment (R, ind. m^{-2} $month^{-1}$) that enters into the 0-20 mm length class. The density of new recruits was determined by iterative modeling until achieving stability of the model, *i.e.* 3.3 ind. $month^{-1}$ m^{-2}, when the sea water temperature ranged between 15 and 22°C. Recruitment depends on spawning efficiency and consequently on clam gametogenesis that mainly occurs after two years of life (Holland and

Chew 1974). In Arcachon Bay, intensity and number of spawning periods depend on the site (Dang et al. in revision-a), but clams generally spawn two times per year, in summer and in autumn. The two spawning periods are entered in the model.

Finally, the stock (SC_i, Kg) was obtained by multiplying LC_i by the mean weight (MW_i, g) and by the total surface area occupied by clam populations within Arcachon Bay (46.4 km^2).

$$SC_i = LC_i \times MW_i \times Surface\ area\ within\ Arcachon\ Bay$$

Consequently, the total stock (t) corresponds to the sum of the stock of each length class.

2.2. Simulations

The model was used to illustrate the possible evolution of the Manila clam populations within Arcachon Bay under the current policy of management, *i.e.* 70 fishing licenses, fishing all year long (12 months), and with a total reserve area of 18.5% where fishing is forbidden. Fishing efforts were estimated at 40%, 50% and 10% for 21-34 mm, 35-39 mm and > 40 mm length classes, respectively.

The clam stock was simulated under eight different management scenarios: (1) No supplementary management measure was applied and results will show the evolution of the clam stock if no measure is taken; (2) The second simulation considers that fishermen stop their fishing activity one day per week; (3) The reserve area where fishing is forbidden is extended to 22%, an increase relative to the current situation (18.5%); (4) The reserve area where fishing is forbidden is extended to 24%; (5) The number of fishing licenses decreases from 70 to 55; (6) The capture season duration is fixed at 11 months, the reserve area at 22% and the fishing licenses at 60; (7) No supplementary measure is applied but recruitment naturally increases by 10%; (8) No supplementary measure is applied but recruitment naturally increases by 20% (Table 13.1).

Simulations began in July 2003, according to the first available data on size frequency distribution in the whole Arcachon Bay, provided by Caill-Milly et al. (2003). In 2003, the total stock has been estimated at 7992 mt (Caill-Milly et al. 2003).

Results for each scenario were obtained when the stock was stabilized, and considered after 120 months (10 years).

3. Results

By running the model under different initial conditions, we are able to estimate the stock evolution associated with different management policies. Decision variables were: the capture season duration (CSD), the percentage of reserve areas (RA) and the number of fishing licenses.

The total stock, the exploitable stock, and the captures per month and per license for each simulation corresponding to various scenarios are presented in Table 13.1.

Table 13.1. Results of total stock (mt) and captures (mt. fishing license^{-1} month^{-1}) obtained following different simulated management options after 120 months: fishing efforts (FE, %), capture season duration (CSD, month), reserve areas (RA, %), number of fishing licenses and recruitment (%).

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**	**Case 8**
FE [> 40 mm]	10	10	10	10	10	10	10	10
FE [35-39 mm]	50	50	50	50	50	50	50	50
FE [21-34 mm]	40	40	40	40	40	40	40	40
CSD	12	10.3	12	12	12	11	12	12
RA	18.5	18.5	22	24	18.5	22	18.5	18.5
Fishing licenses	70	70	70	70	55	60	70	70
Recruitment	0	0	0	0	0	0	+ 10%	+20%
Total stock	1538	7938	2236	8505	8377	9567	2040	8287
Captures	8.35	43.10	12.14	46.14	35.72	44.41	11.07	44.97

Case 1 represents the actual situation without new management measures and with a fishing effort that applies pressure on all clams of length > 30 mm. Ten years later, clam populations have strongly decreased with a total stock of around 1538 mt and monthly captures per license of 8.35 mt (Table 13.1, Fig. 13.1). In 2006, the model does not fit well with a predicted stock of 8403 mt whereas it was estimated at 7306 mt (Table 13.2). The

percentage of deviation was 15% and consequently above the maximum advised deviation percentage for model prediction (10%). However, the model fits well with the reality concerning the clam stock in 2008, as the predicted stock corresponds to the measured stock with a low percentage of deviation (0.4%) (Table 13.2).

Table 13.2. Comparison between the measured and the predicted *Ruditapes philippinarum* stock in 2003, 2006 and 2008 (Caill-Milly et al. 2003, 2006, 2008) in Arcachon Bay.

Year	Measured stock	Predicted stock	Deviation (%)
2003	7992	8311	4.0
2006	7306	8403	15.0
2008	4615	4632	0.4

Case 2 corresponds to a closing of fishing for one day per week, *i.e.* the CSD was reduced to 10.3 months per year. This measure allows improvement over the actual situation with a total stock of 7938 t after ten years and monthly captures per licenses of 43.10 mt, *i.e.* 35 mt more than scenario 1 without new management measures (Table 13.1, Fig. 13.1).

In case 3, the percentage of reserve areas (fishing forbidden) is extended to 22% but this measure does not ameliorate the situation. The model total simulates a decrease of the total stock which tends to stabilize at 2236 mt (Table 13.1, Fig. 13.1). Monthly captures per license increase by 3.8 mt related to situation 1 (Table 13.1).

In case 4, when the reserve areas are more extended than case 3 (24%), the stock is improved compared to situations 1 and 3 with a stabilization at 8505 mt (Table 13.1, Fig. 13.1). Furthermore, captures increase after ten years with 46.14 mt. $month^{-1}$ license $^{-1}$ (Table 13.1).

In case 5, the fishing effort is reduced by decreasing the number of fishing licenses from 70 to 55. This measure has a positive effect on the stock, which increases to 8377 mt (Table 13.1, Fig. 13.1). Captures (35.72 mt. $month^{-1}$. $license^{-1}$) are higher than cases 1 and 3 (Table 13.1).

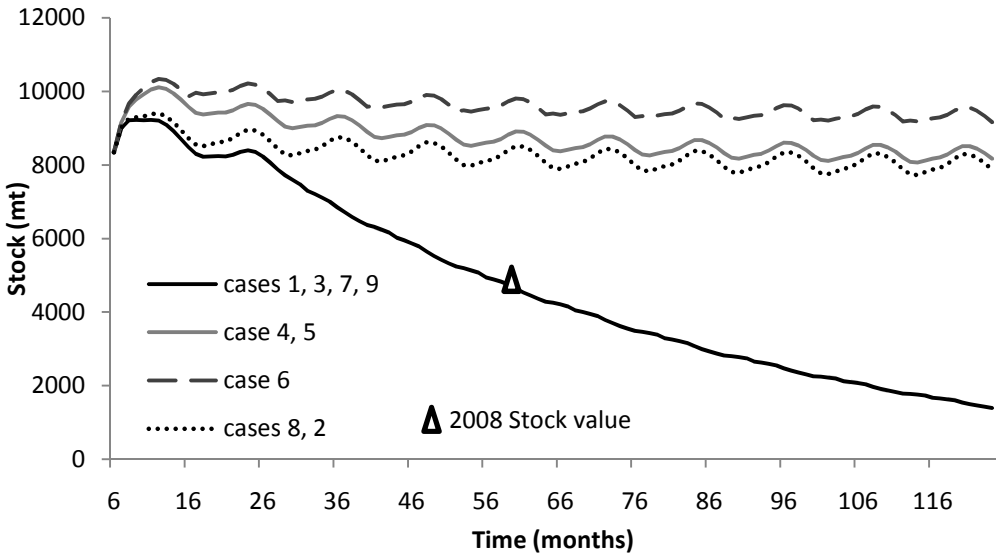

Figure 13.1. Simulated evolution of the *Ruditapes philippinarum* stock over a 120-month period for cases 1 to 9.

Case 6 is a trade off between the different policies of management: the number of fishing licenses is 60, reserve areas represent 22% and the capture season duration is 11 months. These measures lead to an enhancement of the clam stock to 9497 mt and maximum captures of 44.41 mt month^{-1} license^{-1} (Table 13.1, Fig. 13.1).

Case 7 corresponds to the case 1, i.e. without new policies management measures, but with a natural increase in recruitment of 10% in comparison to the actual situation. This increase in recruitment does not improve the situation relative to case 1. The total stock remains low after ten years at 2040 mt and maximum captures slightly increase (11 mt month^{-1} license^{-1}).

However, an increase in recruitment of 20% (case 8) allows restoration of the stock to 8287 mt after ten years. It also enhances captures (45 mt month^{-1} license^{-1}).

4. Discussion

4.1. Report of stock decline and potential causes

From the beginning of the stock assessment in 2003 to that of 2006, the total Manila clam stock in Arcachon Bay remained unchanged, at 7992 mt in 2003 (Caill-Milly et al. 2003) and 7306 mt in 2006 (Caill-Milly et al. 2006). In 2008, the stock assessment study revealed a drastic decrease (4457 mt) (Caill-Milly et al. 2008), with no evolution of management policies since 2003. The number of licenses was 70, situation of reserve areas (18.5% of the total fishing area) where fishing was forbidden was established every year, and the minimum catch length was 40 mm until 2008. Since the beginning of January 2008, the minimum catch size in France was fixed at 35 mm, a result of fishermen complaints that poor growth prevented clams from rapidly reaching 40 mm shell length. Consequently, these results threatened both the sustainability of *R. philippinarum* populations in Arcachon Bay and their exploitation by fishermen.

In other countries like Japan and Italy, certain stocks of Manila clams showed some signs of decline. In Italy, the production of Manila clams in Sacca di Goro lagoon (Po River Delta, Northern Adriatic Sea), one of the major areas of intensive clam rearing in Italy, increased to 16000 mt at the beginning of the 1990s, but a decline in productivity occurred in recent years and production in 2005 was about 10000 mt (Solidoro et al. 2000, Melià and Gatto 2005). This phenomenon has been ascribed to dystrophic events caused by extensive macroalgal blooms (Melià and Gatto 2005). Moreover, clam density in Sacca di Goro lagoon was very high and in some case exceeded 2000 ind. m^{-2}. Eutrophication also was detrimental in producing hypoxia in the water column (Melià et al. 2003, Melià and Gatto 2005). The Manila clam is one of the two most exploited bivalves along the coast of Tokyo Bay in Japan (Toba 2004). Annual production of *R. philippinarum* was very high and reached a peak of 70000 mt in late 1960s (Toba 2004). As in Europe, Manila clam production declined (8000 mt in 1999) but in this case it was due to coastal development, poor recruitment of juvenile clams, and high mortality of the adult population (Toba 2004). Adult mortality was caused by blue tide, river floods, low temperatures and wave action, and predation by birds (Toba 2004).

In Arcachon Bay, the decline of the stock certainly resulted from: 1) a deficient gametogenesis as well as 2) a bad recruitment, and 3) a high fishing effort.

1) As described previously (Dang et al. in revision-a), gametogenesis of Manila clams in Arcachon Bay was low compared to other sites (Laruelle et al. 1994). The amplitude of the condition index (CI), which is representative of spawning efficiency, varied from 60 to 131‰ in the Bay of Brest, from 60 to 142‰ in the Morbihan Gulf (Laruelle et al. 1994) and only from 40 to 66‰ in Arcachon Bay (Dang et al. in revision-a). A factor that could contribute to this situation was the mean shell length of the population evaluated at 25.1 mm in 2008 (Caill-Milly et al. 2008). Indeed, it is well known in bivalves that an exponential relationship exists between the size of the animal and the number of eggs that are produced (Ivell 1981). The management strategy of closing certain areas to fishing in order to conserve the reproductive potential of the population did not appear efficient because reserve areas were too small and the locations changed every year.

Another management measure that is effective in many bivalve populations elsewhere in the world is to fix a minimum catch size (Narvarte et al. 2007) in order to protect the reproductive capacity of the population and thus to ensure the productivity (Gosling 2004). However, anonymous information revealed that the allowed legal minimum size limit was not respected.

2) Different studies like the stock assessments in 2003, 2006 and 2008 (Caill-Milly et al. 2003, 2006), a survey in 2002 of 49 stations (Blanchet et al. 2004), a two-year monitoring of two stations (Dang et al. in revision-a), and other studies (Dang 2005, Lavesque et al. 2009) revealed a lack of clam recruitment at Arcachon Bay. Interannual variability in shellfish recruitment is clearly evident for all bivalve species (Hartill et al. 2005). Nevertheless, clam recruitment at Arcachon Bay has remained low for several years, at least the last six.

3) Annual capture data showed a regular increase of clam production from 500 mt in 2005 to 1028 mt in 2007. Fishing statistics pointed out a higher number of boats and a more intensive exploitation of each license. Consequently, pressure applied to the stock by fishermen also contributed to the decline of the stock in Arcachon Bay. The high level of harvesting coupled with a poor recruitment has rapidly led to a clam abundance deficit.

4.2. Output of the model

According to stock decline, some effective measures of management need to be adapted over the long-term. The role of the model was to propose alternative solutions for management. Simulations of the model that began in 2003 did not fit well in 2006 with the real stock, estimated by stock assessment (Caill-Milly et al. 2006), because the model needs four years to stabilize (Bald et al. 2009). However, in 2008, the predicted stock was

equivalent to the estimated stock (Caill-Milly et al. 2008) and consequently this model can be considered as valid. When the effects of the different management decisions related to stock evolution and captures are compared (Table 13.1), the best management decisions are those which are able to maximize, at the same time, the captures and the number of fishermen (licenses), and to minimize the stock losses (Bald et al. 2009).

The model showed that if management measures are implemented, the stock can return to its initial state, which was around 7000-8000 mt from 2001 to 2006 (Table 13.1). The best management strategy to restore the stock and even increase the total stock in comparison to 2006 was case 6. In this scenario, the capture season duration decreases from 12 (current situation) to 11 months, the reproductive reserve area is increased from 18.5 to 22%, and the number of fishing licenses is decreased from 70 to 60. With these measures, fishermen captures would be increased to 44 mt fishing license^{-1} month^{-1} in ten years instead of 8 mt fishing license^{-1} month^{-1} without management modification. Case 6 seems the best trade off of all cases tested here. The second best case was to increase the surface of the reproductive reserve areas (case 4). However, given the high variability of reproduction and recruitment by bivalves (Hartill et al. 2005, Maurer et al. 2007), this management strategy remains hazardous if applied alone. Indeed, it is also relevant to reduce the fishing effort on Manila clam populations. The third best case is case 5, i.e. a decrease of the number of fishing licenses to 50. After ten years, the total stock is estimated at 8377 mt with fishing capture of 36 mt fishing license^{-1} month^{-1}. However, this case is not practical due to socio-economic reasons. Nevertheless, a 25% decrease of the fishing effort on *R. decussatus* from Galicia produced a 30% increase in performance (Fernández Cortéz et al. 1987). The fourth best case (case 2) was to reduce the fishing effort by decreasing the capture season duration from 12 to 10.3 months per year, for example by closing the fishery one day per week. In this situation, the stock is stabilized over ten years. On the other hand, slightly increasing the area of the reserves (case 3) did not influence the evolution of the stock relative to the actual situation.

However, and in contrast with case 6 which allows an enhancement of the total stock of Manila clam, cases 2, 4 and 5 only support a stabilization of the stock. Simulations began in 2003 and were based on a stock evaluated at 7000-8000 mt. Given the current depleted status of the stock in 2008 (4457 mt), simply maintaining this stock is insufficient and the stock needs to be enhanced.

This model also underlines the fact that recruitment is a strong factor controlling population dynamics. When recruitment is increased, the Manila clam population in Arcachon Bay remained preserved with no change of management strategy. However, as previously

mentioned, recruitment in bivalves is highly variable, depends significantly on environmental conditions, and cannot be human-mediated and stimulated or controlled.

4.3. Criticism of the model

This model seems very realistic as the predicted stock and the estimated stock in 2008 are similar. However, as other models, it presents some problems and notably, it does not take into account recruitment variability, food resources, diseases, and also leisure fishing. This model considered a constant arrival of recruits to the population although annual recruitment in bivalves is highly variable, and dependent on environmental conditions (Hartill et al. 2005). In fact, the production of newly settled juveniles was established by iterative modeling until stabilization of the population. The model did not consider the food supply and availability which are the key influences on population dynamics. Indeed, food supply controls clam growth and reproduction (Flye-Sainte-Marie et al. 2007a). Bivalve growth is strongly influenced by temperature and food supply (Thompson 1984, Bacher and Goulletquer 1989, Chew 1989, Nakamura et al. 2002). Chapter 12 demonstrated that the maximum length of *R. philippinarum* in Arcachon Bay mostly depends on phytoplankton abundance. Some trophic mass-balance models that have been developed include primary production estimates, species trophic level and diet composition (Pauly et al. 2000, Vasconcellos and Gasalla 2001, Duarte and García 2004, Gasalla and Rossi-Wongtschowski 2004, Jiang and Gibbs 2005), which are not considered in our model. The model does not take into account predation, although it is included in the natural mortality rate obtained in field experiment. In Arcachon Bay, the Manila clam could be prey for the green crab *Carcinus maenas*, the grey triggerfishes *Baliste carolinensis*, and the oyster drill *Ocenebra erinacea* (Robert and Deltreil 1990). The model did not consider diseases that can affect clam reproduction (Ngo and Choi 2004), growth and mortality (Paillard 2004, Dang et al. 2008, Dang and de Montaudouin 2009). However, studying the impact of diseases is particularly difficult, given the high spatial variability of these pathologies within the bay, e.g. brown muscle disease (Dang et al. 2008, Dang and de Montaudouin, 2009) or perkinsosis (Dang et al. 2009-c, in preparation). To consider disease in the model, the bay has to be divided into different sectors in order to proceed to different simulation runs.

5. Conclusion

A population dynamics model for the Manila clam has been applied to Arcachon Bay using experimental field data for the estimation of growth and mortality. According to the urgent need to design alternative management strategies for the Manila clam population from Arcachon Bay, we outlined the main line of action that in our opinion could allow a sustainable and efficient use of these resources in this system. We believe that the model presented here provides useful guideline for the development of effective management fishing strategies in *Ruditapes philippinarum* populations in Arcachon Bay. Furthermore, if in the future this model is coupled with a trophic mass-balance model, it should provide crucial information about the sustainability of clam populations and also about the sustainability of intensive clam fishing.

Chapitre 14

Discussion générale

Vol de bernaches (*Branta bernicla*) au dessus de l'Ile aux Oiseaux

1. Rappel des objectifs

Le sujet de cette thèse a des origines locales (bassin d'Arcachon) et a été bâti dans un contexte socio-économique préoccupant. Les pêcheurs professionnels ont été confrontés à de fortes proportions de palourdes de tailles inférieures à la taille légale ainsi qu'à un poids de chair relégant les prises à des produits de qualité inférieure. La palourde japonaise (*Ruditapes philippinarum*) dans le bassin d'Arcachon constituait en 2006 plus de 95% du stock de palourdes, faisait l'objet de 70 licences de pêche sur un stock considéré comme le plus important de France (Caill-Milly et al. 2006). L'objectif général de cette thèse était d'étudier la dynamique de population de cette palourde, dans son habitat naturel (herbiers intertidaux à *Zostera noltii*) du bassin d'Arcachon et notamment de proposer une explication à l'absence de grands individus (mauvaise croissance ? surpêche ?). Le but *in fine* était d'améliorer la gestion de l'activité de pêche à travers un modèle qui serait incrémenté des valeurs obtenues dans ce travail. De plus, au vu des structures en taille fortement déséquilibrées de cette espèce (très peu à la fois de juvéniles et d'adultes de taille supérieure à 38 mm) observées depuis 2001, de nombreuses interrogations demeuraient en terme de maintien à court/moyen terme des populations de palourdes dans le bassin d'Arcachon et donc de l'activité halieutique. La forte décroissance du stock observée entre 2006 et 2008 (Caill-Milly et al. 2008) a également largement nourri les inquiétudes des scientifiques et des pêcheurs. S'il est classique chez un bivalve d'afficher une forte variabilité interannuelle du recrutement, un "bon recrutement" n'avait pas été observé dans le bassin d'Arcachon depuis au moins cinq ans (au début de la thèse).

2. Synthèse de l'étude

2.1. Dynamique des populations

Les paramètres de dynamique de population montrent une croissance relativement faible à l'intérieur de la lagune avec une taille asymptotique maximale de 43.9 mm, par comparaison au site océanique du banc d'Arguin (46.9 mm) qui n'est pas considéré comme un gisement (trop faible stock) ou à d'autres zones géographiques (Japon, Colombie Britannique). La constante représentative du taux de croissance, K, est homogène dans tout le

bassin, contrairement à la longueur asymptotique, L_∞, qui varie suivant le site. Les mortalités observées présentent des valeurs classiques, à l'exception des bas niveaux tidaux où la palourde subit une forte prédation par le bigorneau perceur *Ocenebra erinacea* et de certaines zones fortement touchées par la maladie du muscle marron (BMD). De plus, cette étude met en évidence une gamétogénèse peu intense (faibles amplitudes de variation de l'indice de condition), largement inférieure aux palourdes bretonnes par exemple. Les problèmes liés à cette mauvaise reproduction sont vraisemblablement responsables du déficit en recrutement, cela pouvant être dû en partie aux petites tailles des individus rencontrés à Arcachon. En effet, il existe classiquement une relation exponentielle entre le nombre d'œufs produits et la longueur des bivalves. Cependant, d'autres facteurs tels que la température, la salinité, les ressources trophiques et les pathologies peuvent aussi interférer sur les paramètres de croissance et de mortalité des individus.

2.2. Facteurs de contrôle des populations

2.2.1. Pathologies

Les "grandes" pathologies (helminthose, perkinsose et maladie de l'anneau brun) affectant la palourde japonaise ont été suivies durant deux ans dans le bassin d'Arcachon. Cette étude a révélé une résistance (sans doute en partie mécanique) des palourdes face aux trématodes digènes et en conséquence, des prévalences de cette maladie très faibles dans la lagune. La maladie de l'anneau brun, connue pour avoir entraîné de sévères mortalités dans l'ouest de la France (Bretagne) vers la fin des années 1980 est faiblement prévalente dans les palourdes japonaises du bassin d'Arcachon. De plus, l'intensité de la maladie est à son plus faible niveau d'intensité et uniquement visible sous loupe binoculaire. Le caractéristique "anneau brun" sur la face interne des coquilles n'a jamais été observé durant cette étude.

En revanche, *Ruditapes philippinarum* est fortement infestée par le protozoaire *Perkinsus olseni*, présent en moyenne entre 10^4 et 10^5 cellules par gramme de tissu frais et des prévalences souvent supérieures à 90%. Cette maladie n'entraîne pas d'effets évidents sur la croissance, en dépit des symptômes macroscopiques observables à la surface des différents organes des palourdes (nodules blancs). Les abondances de *Perkinsus* rencontrées dans le bassin sont cependant à la limite d'exercer des effets néfastes, voire délétères sur leur hôte en termes de croissance, mortalité ainsi que de gamétogénèse, comme c'est le cas actuellement

en Corée. A Mundaka, où ce parasite est présent à des concentrations de 10^6 cellules par gramme de tissu frais, les effets sur la croissance ont été clairement établis lors de cette étude. *P. olseni* est un parasite sensible à la température de l'eau, ce qui explique sa moindre abondance dans l'ouest de la France (10^3 cellules par gramme de tissu frais) et ses forts effectifs en Espagne (Pays Basque, Galice), même si l'espèce étudiée n'est pas la même (*Ruditapes decussatus*). *Perkinsus* est également très sensible à la salinité, les plus faibles abondances étant rencontrées à l'embouchure de la rivière principale du bassin, la Leyre. C'est donc un parasite hautement sensible aux changements climatiques. En particulier, un réchauffement des eaux pourrait entraîner à long terme une augmentation des abondances parasitaires et donc des effets néfastes sur les populations de palourdes japonaises du bassin d'Arcachon. Il est possible que les populations de palourdes restent actuellement sensiblement "protégées" contre cette maladie grâce au débit des eaux douces dans le bassin et principalement par le panache de la rivière Leyre. Une année particulièrement sèche pourrait favoriser le développement de cette maladie.

Cette thèse a également permis de découvrir une nouvelle pathologie, la maladie du muscle marron (BMD) très inquiétante pour la santé des populations de palourdes japonaises du bassin d'Arcachon. Cette maladie est spécifique à la palourde japonaise et pour le moment, uniquement signalée à Arcachon. Elle provoque des symptômes particulièrement visibles et délétères pour l'hôte. Ils apparaissent tout d'abord sur le muscle strié puis s'étendent au muscle lisse, entraînant une importante calcification du muscle adducteur postérieur et la mort de la palourde à court terme. Cette pathologie entraîne des difficultés d'ouverture et de fermeture des valves, ce qui induit la remontée en surface des palourdes puis leur mort. Deux échelles permettant de quantifier cette maladie à l'œil nu et sous loupe binoculaire ont été établies durant la présente thèse. Les prévalences sont localement importantes puisqu'à certaines périodes de l'année, 50% de la population de palourdes enfouies (en position de vie normale) est atteinte. Nous nous sommes intéressés de près à la BMD sur le site de Lanton (partie interne du bassin) qui est notre site le plus infecté. Les palourdes de surface présentent en moyenne une prévalence de 67% contre 23% pour les palourdes enfouies dans le sédiment. En laboratoire 82% des palourdes de surface mourraient en quinze jours contre 12% pour les palourdes de profondeur. Le suivi au cours du temps d'une cohorte de 2003 a montré un taux de mortalité de 39 % en milieu naturel dont 95% directement imputé à cette maladie. La BMD est actuellement la pathologie la plus importante pour les palourdes japonaises du bassin

d'Arcachon. Il semblerait que cette pathologie soit plutôt estivale (fortes températures), donc également sensible aux éventuels changements climatiques.
Comme toute nouvelle maladie découverte, il est avant tout nécessaire de déterminer l'agent causal. Un agent infectieux a été suspecté étant donné que les autres bivalves vivant en sympatrie avec *R. philippinarum* n'étaient pas affectés. Les protozoaires et bactéries ayant été exclus (histologie et biologie moléculaire), l'hypothèse virale a ensuite été envisagée. En effet de nombreuses particules ont été observées dans les tissus musculaires en microscopie électronique à transmission. Plus le stade de la maladie était avancé, plus nombreuses étaient ces particules. De par leur morphologie, il semblerait qu'elles appartiennent aux picornavirus. Même si cela n'a pas été détaillé dans la présente thèse, plusieurs essais de transmission de la maladie (injection de broyat de muscle malade dans des individus sains) ont été réalisés en conditions expérimentales mais sans succès. Cependant, les températures dans nos aquariums n'étaient certainement pas assez élevées, nos travaux à cette époque n'ayant pas privilégié la période estivale comme la plus critique.

2.2.2. Ressources trophiques

Les ressources trophiques sont reconnues comme paramètre majeur influant sur la dynamique de population des bivalves : croissance, résistance aux différents stress… Ce volet a été abordé par l'outil isotopique (isotopes stables du carbone et de l'azote). Cette étude démontre une hétérogénéité spatiale des sources trophiques de *R. philippinarum*, avec une dominance de la nourriture phytoplanctonique. Ces sources mettent en évidence des différences inter-sites avec par exemple Arguin (site le plus océanique) dominé par une nourriture phytoplanctonique toute l'année, alors qu'une baisse de la consommation de phytoplancton en hiver est enregistrée dans les sites internes. Les isotopes stables de l'azote et du carbone identifient deux sous-bassins au sein de la lagune avec au sud, une dominance de la nourriture phytoplanctonique et au nord une nourriture beaucoup plus diversifiée. Cette thèse montre que la longueur maximale que peut atteindre la palourde est corrélée avec la fraction de phytoplancton ingérée, même si d'autres paramètres peuvent aussi interférer tels que la couverture en herbier qui entraîne une plus forte saisonnalité des ressources trophiques. En revanche, le niveau de marée n'a pas montré d'influence sur la croissance des palourdes.

2.2.3. Bilan

Les deux types de facteurs (pathologies et ressources trophiques) étudiés lors de cette thèse exercent donc un contrôle sur les paramètres de dynamique de population tels que la croissance et la mortalité. La BMD est actuellement la seule maladie dans le bassin d'Arcachon ayant un impact sur la dynamique de population (mortalité rapide des individus) mais la perkinsose reste cependant à surveiller et pourrait avoir une influence au moins sur la croissance et la reproduction si la charge en parasite venait à augmenter. Est-ce que le défaut de croissance peut être expliqué par ces facteurs ? De par la rapidité avec laquelle les individus infectés meurent, nous ne considérons pas que la BMD ait un impact sur la croissance mais uniquement sur la mortalité. Cependant, les individus malades sont détectés à l'œil nu, et sont déjà à un stade bien avancé de la pathologie. Peut-être que la maladie met longtemps à se déclarer sans qu'aucun symptôme ne soit visible. Dans ce cas, un effet sur la croissance est possible mais il faudrait disposer d'une méthode de détection plus précise, par exemple de biologie moléculaire comme évoqué dans les perspectives. Les ressources trophiques ont certainement une part importante dans le défaut de croissance observé dans le bassin d'Arcachon mais cela dépend aussi de beaucoup d'autres paramètres comme les conditions environnementales (températures, salinités). Par ailleurs, notre étude s'est restreinte à une analyse qualitative (% des sources de nourriture potentielle), l'estimation quantitative des ressources étant aussi fondamentale pour expliquer la croissance des individus.

3. Gestion des populations de palourdes japonaises

Le modèle employé dans le chapitre 13 incorpore les paramètres de dynamique de population étudiés dans le chapitre 2. Il permet de mesurer l'évolution possible du stock à long terme et de proposer des solutions de gestion. Etant donné le rapide déclin du stock entre 2006 et 2008 et les résultats de la présente thèse, des solutions d'urgence ont été adoptées en concertation entre les scientifiques, le Comité Local des Pêches Maritimes et des Elevages Marins d'Arcachon et le Comité Régional des Pêches Maritimes et des Elevages Marins d'Aquitaine. Pour essayer de maintenir la population de palourdes dans le bassin d'Arcachon, il a été adopté les trois solutions relatives au cas 6 du chapitre 13 : (1) Agrandir les zones de réserve et les fermer pour une durée de trois ans renouvelable. Auparavant, les zones de réserve étaient exiguës et déplacées chaque année. (2) Le modèle ayant montré l'effet

bénéfique sur le stock de réduire la durée de capture de 1,7 mois par an, il a été décidé d'interdire toute activité de pêche un jour par semaine. (3) Le nombre de licences attribuées va être progressivement diminué de 70 actuellement, à 55 (une entrée pour deux sorties). Ces mesures devraient permettre une restauration progressive du stock au fil du temps. Cependant, certains paramètres ne sont pas contrôlables. C'est le cas des facteurs environnementaux (températures, salinités, …) qui conditionnent à la fois la gamétogénèse, la survie des recrues et les ressources trophiques et les pathologies.
Le modèle montre bien qu'en cas d'année(s) à très bon recrutement, le stock pourrait se rétablir de lui-même.

4. Conclusion générale

La situation de la palourde japonaise dans le bassin d'Arcachon est assez inquiétante, compte tenu de la faible croissance et surtout du manque de recrutement. Cependant, au vu des mesures adoptées, la situation peut se rétablir surtout si une année à (très) bon recrutement venait à arriver. L'étude de stock menée en 2008 a montré un léger recrutement, qui était absent les autres années.

Cela est d'autant plus inquiétant qu'une nouvelle pathologie, la maladie du muscle marron, est apparue au sein du bassin, affectant sévèrement les populations de palourdes. En tant que pathologie totalement méconnue, il est impossible à l'heure actuelle de prédire une quelconque évolution. Cela peut aussi poser des problèmes en terme de contamination d'autres sites en cas de transfert de bivalves d'une zone contaminée vers une zone indemne. De plus, le réchauffement climatique pourrait favoriser le développement de cette maladie, de même que la perkinsose ou même l'émergence de nouvelles pathologies. En cas de refroidissement, la maladie de l'anneau brun pourrait être favorisée. Les parasites, comme les organismes libres, se partagent les niches écologiques, certains occupants la fenêtre hivernale d'autre la fenêtre estivale. Ces successions ont été observées chez les huîtres (Carnegie 2005).

5. Perspectives

Des perspectives concernant le modèle de gestion du stock peuvent être évoquées. Ce modèle semble plutôt réaliste, de par l'adéquation entre le stock estimé et mesuré en 2008 ; cependant il ne prend pas en compte les ressources trophiques, ni les pathologies. Certains modèles disponibles dans la littérature tiennent compte des ressources trophiques, il serait donc intéressant d'intégrer ces autres paramètres au sein de ce modèle.

Il serait également important d'arriver à mieux caractériser le recrutement de la palourde japonaise dans le bassin d'Arcachon, à travers un suivi plus détaillé de la gamétogénèse et de la ponte mais aussi de quantifier les larves dans la colonne d'eau.

Les paramètres de dynamique de population sont hautement dépendants des ressources trophiques mais seule la partie qualitative a été étudiée ici grâce aux isotopes stables de l'azote et du carbone. Il serait particulièrement intéressant de coupler cette étude qualitative à une étude quantitative grâce à un suivi dans la colonne d'eau des différentes populations phytoplanctoniques ainsi que des populations de microphytobenthos. L'exploitation de données de réseau de surveillance, type SOMLIT (INSU) ou REPHY (IFREMER) pourrait être bénéfique.

De nombreuses questions restent ouvertes concernant la maladie du muscle marron, que ce soit au niveau de la transmission de cette maladie, de son développement ainsi que de sa présence sur la côte atlantique française. Des suivis réguliers sur le terrain devront être effectués pour surveiller la progression de cette pathologie. L'étiologie virale est d'autant plus inquiétante que plusieurs espèces de bivalves se sont déjà éteintes dans le bassin d'Arcachon par le passé, suite à des maladies virales. Par exemple, l'huître plate *Ostrea edulis*, espèce dominante de la lagune jusqu'en 1910 a soudainement régressé en 1920 à cause d'une pathologie virale. Elle a été remplacée par l'huître portugaise *Crassostrea angulata*, qui elle aussi s'est éteinte au début des années 1970, suite à de très fortes mortalités provoquées certainement par un iridovirus. La connaissance des différents pathogènes infestant les bivalves d'importance commerciale est un préalable indispensable à une bonne gestion de leur stock et au maintien des activités de pêche.

Pour essayer de rechercher l'agent viral responsable de la BMD, des tentatives de clonage ont été également entreprises durant cette thèse mais sans résultat. Dernièrement, avec la collaboration d'un laboratoire de virologie (INRA, Jouy en Josas), ce virus a réussi à être

purifié et la présence d'ARN (picornavirus) a été confirmée. Les perspectives de recherches sur la BMD seraient d'essayer de cultiver le virus grâce à des cultures cellulaires, dans le but de disposer d'un grand nombre de particules, rendant ainsi plus facile les tentatives de caractérisation et de transmission. Le génome de ce virus devra être caractérisé pour (1) confirmer qu'il s'agisse bien d'un picornavirus ; (2) déterminer des amorces afin de détecter par PCR la BMD à un stade précoce. Il sera ainsi possible de rechercher si des palourdes provenant d'autres sites français présentent également cette pathologie. Cela pourra aussi servir à déterminer l'origine de cette maladie, c'est-à-dire par exemple un possible vecteur. Il est bien reconnu que les oiseaux sont porteurs de nombreux virus et le nombre croissant de bernaches cravant hivernant chaque année sur le bassin serait une piste à creuser…

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Annexe

A system dynamics model for the management of the Manila clam, *Ruditapes philippinarum* (Adams & Reeve, 1850) in the Bay of Arcachon (France)

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Abstract

The Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850) is one of the mollusc species that, driven mainly by the shellfish market industry, has extended throughout the world, far beyond the limits of its original habitat. The Manila clam was introduced into France for aquaculture purposes, between 1972 and 1975. In France, this venerid culture became increasingly widespread and, since 1988, this species has colonised most of the embayments along the French Atlantic coast. In 2004, this development resulted in a fishery of ca. 520 tonnes in Arcachon Bay.

Within this context, evaluation of the clam stock in Arcachon, as undertaken by the French Institute for the Marine Research (IFREMER) in 2000, 2003 and 2006, underlined: (a) the presence of disequilibrium in the population structure; and (b) the need for an improved knowledge of the population dynamics. In order to meet these requirements, a research project was initiated. The main objective of this study was to provide an improved knowledge of the biotic and abiotic parameters which control the dynamics of the clam population, within Arcachon Bay (growth, mortality, reproduction, trophic sources, etc).

With these data, the aim of the present study is to develop a system dynamics model, capable of predicting clam population evolution within the embayment, in response to different management measures. Once the model was constructed, the effect of diverse management measures and environmental scenarios on the stock response, over time, was simulated: (i) sensitivity to exceptional environmental changes (sea water temperature); (ii) changes in the minimum legal size for capture; (iii) changes in season capture duration; and (iv) an increase of the surface area of no-take zones.

Considering all the assumptions made here, the best management decisions are those which are able to maximise, at the same time, the captures and the number of shellfishers; likewise, minimise stock losses, as a consequence of this exploitation. Taking into account this approach, the best management decisions are, in order of efficiency: (i) the minimum legal size for capture maintenance; (ii) a reduction in the capture season; and (iii) an increase of no-fishing zones.

Key words: system dynamics model; management; Manila clam; *Ruditapes philippinarum*

1. Introduction

The decline of fisheries worldwide (Pauly et al., 1998; 2002; Sequeira et al., 2008), due to the over-exploitation of a large number of stocks (Fao, 1995), is associated with limited scientific knowledge and uncertainties in the evaluation and management methods (Mc Goodwin, 1990; Ludwig et al., 1993; Hilborn et al., 1995; Hartill et al., 2005). According to Freire and Garcia-Allut (2000), these problems are critical to the coastal ecosystems and traditional fisheries, due to their great complexity, i.e. a large number of human and technical factors, interacting in space and time.

The management of the exploitation of marine renewable resources integrates biological, economical and social aspects, in order to maintain both the exploited populations and fishery activity (Fernández Cortes et al., 1984; 1987a; 1987b; Nunes et al., 2004). In spite of their minor economic importance, traditional coastal fisheries, at a low scale, are of an higher social importance; this is in comparison with industrial fisheries, in spite of the higher economic value of the latter (Mc Goodwin, 1990; Fao, 1995; Orensanz and Jamieson, 1998). As stated by Freire (2005), a high number of invertebrate species, mainly benthic coastal organisms, has been over-exploited; in some cases, this has reached a situation of collapse.

The Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850) is native from the Pacific shores of Asia (Rodríguez-Moscoso et al., 1992). It is one of the mollusc species that, driven mainly by the shellfish market industry, has spread successfully throughout the world, far beyond the limits of its original habitat (Melià et al., 2004; Melia and Gatto, 2005). The Manila clam was introduced for aquaculture purposes, to France, between 1972 and 1975 (Flye-Sainte-Marie et al., 2007). This Venerid culture became increasingly widespread and, since 1988, natural populations have colonised most embayments along the French Atlantic coast, replacing the native populations of the European clam, *Ruditapes decussatus* (L.); this is the case for Arcachon Bay (Auby, 1993) shown in Figure 1, where Manila clam culture commenced between 1980 and 1986 (Robert and Deltreil, 1990). Conversely, high competition with the Spanish and Portuguese markets, together with high mortalities in summer and autumn, caused the abandonment of the aquaculture activity, at the end of the 1980's (Caill-Milly et al., 2003); this has lead to a fishing activity over most of the natural populations (520 tonnes in 2004, Caill-Milly et al., 2006; see Figure 2).

Within this context, the evaluation of clam stock in Arcachon Bay for management purposes and undertaken by IFREMER in 2000, 2003 and 2006 (Bertignac *et al.*, 2001; Caill-Milly *et al.*, 2003; 2006), underlined: (a) the presence of a disequilibrium in the population structure (a low proportion of juveniles, favouring individuals of 25-37 mm in length); and (b) the need for an improved knowledge of the population dynamics, for improved management of the resource. Within this context, a research project commenced in 2005, between IFREMER (Laboratoire Ressources Halieutiques d´Aquitaine Angelt, LRHA and Laboratorie Environnement Ressources d´Arcachon, LER), Unité Mixte de Recherche Environnements et Paléoenvironnements Océaniques (UMR-EPOC, University Bordeaux 1), the Applied Laboratory of Mathematics (Pau et des Pays de l´Adour University) and the Marine Research Division of AZTI-Tecnalia. The main objective was to obtain an enhanced understanding of the biotic and abiotic parameters, which control the dynamics of the clam population within Arcachon Bay (growth, mortality, reproduction, trophic sources, etc).

This enhanced understanding is used ultimately to develop a system dynamics model, able to predict the clam population evolution within the embayment, in response to different management measures. According to Sterman (1988), the best solution is the development of a system dynamics model, applied to the modelisation and understanding of complex systems (in the case of the present contribution, the population structure and dynamics of the Manila clam population, established by Caill-Milly et al., 2003; 2006, together with its relationship with the prevailing environmental conditions and potential human activity).

Hence, the model attempts to evaluate which management decisions would be appropriate for the sustainable exploitation of the resource, i. e. modification of the capture season, the minimum legal length for capture, increasing the “no take” zones in the embayment, etc. Similar approaches have been adopted elsewhere, in managing clam (Borja and Bald, 2000) and goose barnacle (Bald et al., 2006) exploitation in the Basque Country (northern Spain). Additionally, the response of the clam population to exceptional changes in environmental conditions, such as increases in the average sea water temperature, were evaluated.

2. Materials and methods

2.1 Basic concept and state variables

The model was constructed using the Vensim ® DSS, for Windows Version 5.7a software. Similar to the model developed by Borja and Bald (2000), for the management of the European clam in the Basque Country (Northern Spain), the Manila clam population in the Bay of Arcachon, was divided into four length classes as shown in Figure 3: (i) juveniles (shell lengths between 0 and 20 mm); (ii) non-exploited adults (shell lengths between 20 and 35 mm); and (iii) two classes of exploited adults (shell lengths between 35 and 40 mm and >40 mm). Each length class grows and passes to the next length class through a growth rate. Even if the minimum legal length for capture in Arcachon Bay is established at 40 mm, some tolerance exists; as such, some individuals below this length class are also exploited (the 35-40 mm length class, in particular). This is the reason why two length classes were established for the exploited adults, enabling modelling of the response of the population, to different exploitation patterns. The importance of this length class differentiation has been identified by several authors, such as De Leo and Gatto (2000) and Solidoro et al. (2003); this is due to differences in vital rates such as fertility, mortality, respiration, etc, depending upon the size of the individuals (Flye-Sainte-Marie et al., 2007).

The mortality for each length class is the result of natural mortality (for non-exploited adults and juveniles), together with a fishing mortality (for exploited adults, see Figure 3). The natural mortality depends upon the sea water temperature, according to the relationship established by Solidoro et al. (2000; 2003). In the case of juveniles and non-exploited adults, the fishing mortality is equal to zero. The exploitable stock, which corresponds to individuals above 35 mm in shell length (in 2004, this resulted in a fishery of ca. 520 tonnes, as shown in Figure 2), depends upon: the capture season duration; the percentage of the total stock which is exploited; the percentage of the total surface of the Arcachon Bay which is being considered as a “no-take” zone; and the fishing effort for each length class.

Finally, all of the system is fed trough the settlement of new individuals, which enter into the 0-20 mm length class.

2.2. Model development

2.2.1 Stock and density

The temporal evolution of the stock within a length class (LC_i; ind·m^{-2}·month^{-1}) within a generic *i*-th length class, corresponds to the transfer of individuals from a previous length class as a consequence of the growing process of individuals (G_i; ind·m^{-2}·month^{-1}), less the mortality (M_i; ind·m^{-2}·month^{-1}) and the transfer of individuals to the subsequent length class:

$$LC_i = G_{i-1} - M_i - G_{i+1} \quad (1)$$

The stock (SC_i; Kg) in a generic *i*-th length class can be estimated by multiplying the length class by the mean weigh (MW_i; g), together with the surface area of Arcachon Bay (46.4 km^2):

$$SC_i = LC_i \text{ x } MW_i \text{ x Arcachon Bay surface area} \quad (2)$$

Consequently, the total stock (t) in Arcachon Bay corresponds to the sum of the stock of each length class.

The density (D_i; ind·m^{-2}) and the mean length (ML_i; mm) for each length class, listed in Table 1, have been calculated from the population data obtained by Caill-Milly et al. (2003). The mean weight for each length class has been derived from the published length-weight relationship for the Manila clam population in Arcachon Bay (Caill-Milly et al., 2003):

$$\text{Weight} = 0.2162 \text{ x Length}^{3.0469} \ (r^2=0.94). \quad (3)$$

2.2.2 Mortality

The natural mortality rate *n* (ind·m^{-2}·month^{-1}) for each length class depends upon the sea water temperature (*T*; ºC), the density (*N*: ind·m^{-2}) and the length (*L*; mm) of individuals, according to the model developed by Solidoro et al. (2000; 2003):

$$n\,(L, T, N) = \exp(a + c \text{ x } L + d \text{ x } T + e \text{ x } N) \quad (4)$$

where a = -7.3, c = 0.041 mm^{-1}, d = 0.055ºC^{-1}, e = 0.0006 (individuals/m^2)$^{-1}$ (Table 2).

This empirical equation, developed for Venice Lagoon (Italy), was adapted to the present study by; (i) removing the influence of the salinity included in the model of Solidoro et al.

(2000; 2003); this was due to the difficulty in modelling this variable within Arcachon Bay, and; (ii) recalculating the coefficient a by iterative modelling, until achieving stability of the population.

The mortality (M_i; ind·m^{-2}·month^{-1}) for a length class$_i$ in a generic *i*-th length class, is the sum of the natural mortality (*n*) and the fishing captures (FC):

$$M_i = n_i + FC_i \quad (5)$$

2.2.3 Fishing captures

In the case of juveniles (length class 0-20 mm) and non-exploited adults (length class 20-35), the fishing captures are equal to zero. According to Caill-Milly et al. (2003), the total captures represent only 14-21% of the total exploitable stock (PEE, dimensionless), which corresponds to individuals above 35 mm in shell length (in 2004, this resulted in a fishery of ca. 520 tonnes, as shown in Figure 2). From the total captures, 30% of the fishing effort (FE, dimensionless) corresponds to the >40 mm length class and 70% to the 35-40 mm length class (Table 3). In addition, 11% of the total surface area of Arcachon Bay is considered as a "no-take" zone (Pntz, dimensionless). The fishing season extends throughout the year (CSD, 12 months) and the number of fishermen has remained constant, at 70 licenses since 1997, decreasing to 55 in 2001 and 2002 (Table 3 and Figure 2).

Accordingly, the fishing captures (FC_i; ind·m^{-2}·month^{-1}), for a length class$_i$ in a generic *i*-th length class, is the result of multiplying the length class (LC_i; ind·m^{-2} ·month^{-1}) by the fishing effort (FE_i; %) and the capture season duration (CSD; month):

$$FC_i = LC_i \text{ x } FE_i \text{ x } CSD \quad (6)$$

Then, the total captures correspond to the sum of all the FC_i, multiplied by the ratio of exploitable stock (PEE; dimensionless) and the percentage of "no take" zones (Pntz; dimensionless):

$$\text{Total Captures} = \sum FC_i \times PEE \times \left(1 - \left(\frac{Pntz}{100}\right)\right) \quad (7)$$

2.2.4 Environmental factors: sea water temperature

The sea water temperature was simulated using the sinusoidal curve proposed by Melià et al. (2004), for Arcachon Bay. Temperature T (ºC) at time *t* (in days):

$$T(t) = f \sin\left(\frac{2\pi}{365}(t-h)\right) + g \qquad (8)$$

with f (half of the maximum temperature variation) = 6.5 ºC, h (phase) = -115 days and g (mean annual temperature) = 15.8 ºC. The coefficient f was determined by Singular Value Decomposition (Press et al., 1989), for different values of h, estimating the difference between the sea water temperature data set provided by Gilles Trut (Ifremer, unpublished data) and the modelled data. The selected values of h correspond to those presenting the lower mean error between these two data sets, which can be observed in Figure 4.

2.2.5 Settlement

In the absence of adequate published information, the settlement of new individuals (S; ind·m^{-2}·month^{-1}) that feed the whole system has been determined here by iterative modelling, with a constant value of 3.3 ind.month^{-1}.m^{-2}, until achieving stability of the population (Table 2) when the sea water temperatures ranges between 15 and 22ºC. Elsewhere, other authors define this range as lying between 18 and 26ºC (Solidoro et al., 2003), or when sea water temperature reaches 12ºC (Flye-Sainte-Marie et al., 2007) or even 15, 18 and 21ºC, (Rodríguez-Moscoso et al., 1992). The sexual maturation of the Manila clam takes place after the second year of life, when the shell length reaches 20 mm (Robert et al., 1993; Laruelle et al., 1994). Whereas Robert et al. (1993) reported only one spawning event in Arcachon Bay, Laruelle et al. (1994) have described, for the Bay of Brest, two partial spawnings in spring and summer, with one total spawning event in autumn. The latter authors describe four spawning events for the Gulf of Morbihan, whilst Calvez (2003) has indicated that only one of the spawning events was efficient in terms of recruitment. Beninger and Lucas (1984), in Marennes-Oléron and Williams (1980), have described two spawning events for Puget Sound (USA). The data obtained during the study of biotic parameters in Arcachon Bay, within the research framework outlined above (see Introduction), appears to reveal two spawning events, in summer (June-August) and autumn (September-October) (unpublished data). Taking into

account the sea water temperature ranges established in the present model for the production of new recruits, these two spawning events are correctly being modelled.

2.2.6 Growth

The transfer of individuals between the different length classes (G_i; ind.month^{-1}.m^{-2}) is due to the growth process of clams through a growth rate (GR_i; dimensionless):

$$G_i = LC_i \times GR_i \quad (9)$$

The growth rates were calculated initially following the growth model developed by Melià et al. (2004), for the Sacca di Goro Lagoon (Italy). This model permits the calculation, for each length class, of the minimum length necessary to pass to the next length class, in a month. Subsequently, from data obtained by Caill Milly et al. (2003) for Arcachon Bay, the proportion of individuals for each length class, that grow and pass to the next class, was calculated. This proportion was considered to be the growth rate (GR_i; dimensionless), for each length class. As these growth rates were calculated following the model developed by Melià et al.(2004), for the Sacca di Goro Lagoon (Italy), the calculated proportions were tuned (by iterative simulations) until achieving stability of the population (Table 2).

As mentioned above, several parameters of the model have been tuned, until achieving stability of the population. However, as stated previously (Introduction), no evidence is provided that the Arcachon stock is presently in a equilibrium state. We look such equilibrium in the model, in order to be able to compare the results of different management policies on the clam population; this is until more ecological data on the Manila clam become available, as on-going studies in the Arcachon Bay are completed.

2.2. Simulations

The risk of the overexploitation of the Manila clam populations in Arcachon Bay leads to the application of different management measures, by the French Administration responsible for resource management of (i.e.) control of the number of fishermen, establishment of “no-take” zones, etc. In the present contribution, the effectiveness of 7 different management measures and cases are evaluated: (i) Case 1, fits the model results with the stock evaluation

of the Manila clam population undertaken by IFREMER in 2003 and 2006. In this particular case, no management measure was applied; (ii) Case 2, simulates a fishing effort upon individuals above the minimum legal capture length (40 mm); (iii) Case 3, simulates a fishing effort upon individuals above the minimum legal capture length (80% of fishing effort, over 40 mm), but applies also a 20% fishing effort over individuals below the minimum legal capture length (21-39 mm); (iv) Case 4, simulates a reduction in the capture season, to 3 months commencing in October; (v) Case 5, simulates a reduction in the capture season to 8 months commencing in October; and (vi) Case 6, simulates an increase in the surface area of the "no-take" zones, from 11% to 30% of the total distribution area of the Manila clam population, in Arcachon Bay. Additionally, the impact of extreme environmental conditions, such as an increase in the average sea water temperature over the summer months, which is suspected to produce high mortalities in the Bay, are evaluated. Finally, Case 7 simulates the effect of a 2ºC sea water temperature increase, during 1.5 months in July-August, over the clam stock evolution; this is within the range of the extreme temperatures observed in Arcachon Bay.

Modelling of each of the cases commenced in July 2003, when the first data related to the prevailing conditions of the population were obtained by Cail-Milly et al. (2003); and ends 10 years later. The result of each management measure was obtained when stabilisation of the stock evolution was observed.

3. Results

The results obtained for each of the (7) different management options over, the clam stock evolution simulated by the model, are summarised in Table 4. However, such management decisions have not been tested in the field and the results obtained are only a simulation of the model developed.

3.1 Case 1

Table 3 and Figure 5 shows the model output fits well with the stock results obtained by Caill-Milly et al. (2003; 2006), since the percentage of deviation between the modelled data (8,311 t in 2003 and 7,831 t in 2006), compared with the measured data of Caill-Milly et al.

(2003; 2006), is about 4% in 2003 and 5% in 2006 (Table 5). Such deviation lies below the maximum accepted deviation percentage for model prediction, established as 10% by Bald and Borja (2002) and Bald et al. (2006).

3.2 Case 2

Figure 5 shows that when only clams over the minimum legal length are captured, the model simulates an increase in the stock of up to 17,656 t, with an average capture volume of 880 t, per season (approximately 5% of the total stock, Table 4). Such an increase in the clam stock and captures leads to a (shellfisher) profit of about 1,050 kg·shleffisher^{-1}·month^{-1}, during the fishing season (Table 4). This represents an increment in profit of about 38%, with respect to Case 1, indicating the importance of respecting the minimum legal length for capture. The result of this management measure can be observed about 4 years, since the application of the management action.

3.3 Case 3

In this case, the main fishing effort remains above the minimum legal capture length (80%), but "slight" fishing effort is allowed below the minimum legal length (10%, between 21 and 39 mm, Table 4). The model simulates a decrease in the stock, which tends to stabilise around 7,257 t. The annual captures increase up to 1,322 t (approximately 18% of the total stock), due to the "catchability" of most of the clam population (Table 4). The shellfisher profit increases up to 1,573 kg·month^{-1}, throughout the fishing season (Table 4), duplicating the profit obtained in Case 1. However, in contrast, the stock decrease is about 13%, in comparison with Case 1.

3.4 Case 4

This case relates to limiting the fishing season to 3 months, commencing in October (similar to the legal regulations in the Basque Country, in northern Spain). The model simulates a considerable increase in the clam stock, up to 18,000 t and the shellfisher profit (3,019 kg·shellfisher^{-1}·month^{-1}). However, the annual captures remain the same as in Case 1, at 633 t (approximately 3.5% of the total stock), due to the severe reduction in the extent of the capture season (Table 4).

3.5 Case 5

Similar to Case 4, the fishing season in this case is reduced to 8 months, commencing in October. This approach permits an increase in the clam stock of up to 9,859 t, together with the shellfisher profit to 1,132 kg·shellfisher^{-1}·month^{-1}. As in Case 4, the annual captures remain similar to those in Case 1, at 633 t (approximately 6% of the total stock, Table 4); this is in response to the increasing capture season and the consequent reduction in the clam stock.

3.6 Case 6

In this case, the "no-take" zones are increased from 11% to 30% of the total surface area of distribution of the species in Arcachon Bay; this leads to results similar to those in Case 1. The clam stock increases up to 9,232 t, which represent an increment of about 11%, in comparison with the Case 1. In contrast, the annual captures and the shellfisher profit remain very similar to those described in Case 1: 634 t (approximately 7% of the total stock) and 755 kg·shellfisher^{-1}·month^{-1}, respectively (Table 4).

3.7 Case 7

An increase in sea water temperature can lead to high mortality rates in clam populations. In this case, an increase of 2ºC in annual sea water temperature, during the summer months (July to August), has been modelled. The stock decreases to 6,587 t during the years when these events are detected (Table 4), representing a decrease of about 20% in the clam stock. Consequently, the annual captures decrease to 530 t (approximately 8% of the total stock) and the shellfisher profit to 631 kg·shellfisher^{-1}·month^{-1} (Table 4), representing a decrease of about 24%, in comparison with Case 1.

4. Discussion

In the particular case of the Manila clam populations in Arcachon Bay, the need for an improvement in the knowledge of population dynamics and the development of a management tool was concluded by Caill-Milly et al. (2003), during the field surveys undertaken by IFREMER, for management purposes. This tool should be able to consider different management decisions: from the complete conservation of the system, to the sustainable exploitation of the resource. Such an approach should integrate different social and biological factors, permitting the protection of the stock and development of the fishery (Bald et al., 2006).

For this purpose, one of the best solutions is the development of a "system dynamics" model applied to the understanding and modelling of complex systems, together with their relationships with the surrounding environment and potential human activity (Sterman, 1988). Establishing reliable management models is fundamental to developing efficient and sustainable exploitation strategies, for coastal ecosystems (Nunes et al., 2004; Melia and Gatto, 2005). Such models have been developed broadly for the management of the fishery of natural populations (Bertignac et al., 1998; De Leo and Gatto, 2000; Savina, 2004; Gertseva and Gertsev, 2006; Arendse et al., 2007), or the management of populations for aquaculture purposes (Bacher et al., 1998; 2003; Solidoro et al., 2000; 2003; Gangnery et al., 2001; 2004a; 2004b; Melia and Gatto, 2005; Bacher and Gangnery, 2006; Ferreira et al., 2007).

As summarised below, the model developed in this contribution incorporates some important assumptions which represent their main weaknesses.

(i) The lack of knowledge on larval transport and settlement in Arcachon Bay has led to the development of a model which takes into account the whole of the embayment, instead of a more spatially-adjusted approach. The processes of physical transport have been described as one of the key factors in the recruitment of coastal invertebrates (Botsford et al., 1994; Pineda, 1994, 1999, 2000; Pineda and Caswell, 1997; Wing et al., 1998; Pineda and López, 2002). Due to the high phenotypic plasticity of bivalves, the parameters related to the population dynamics are influenced in particular by environmental factors (De Montaudouin, 1996), e.g. mortality can vary considerably from one site to another (Ohba, 1959; Goulletquer et al., 1987; Cigarria and Fernández, 1998; Solidoro et al., 2000). "Spatially-explicit" management could take advantage of dispersal information, combined with benthic

population dynamics, to determine areas that are more or less likely to be origins of larvae; likewise, to vary the harvesting accordingly (Botsford, 2001).

(ii) The natural predation of Manila clam populations is not taken into account in the model, due to the absence of reliable data, even if predation by green crabs (*Carcinus maenas*), grey triggerfishes (*Baliste carolinensis*) and oyster drills (*Ocenebra erinacea*) is considered to be important in Arcachon Bay (Robert and Deltreil, 1990).

(iii) The effect of food availability, in comparison with biological parameters of the population (growth, mortality, reproduction, etc), is not taken into account; this is due to the lack of information, in the particular case of Arcachon Bay. Food supply controls clam growth and reproduction (Flye-Sainte-Marie et al., 2007), which are one of the key processes of the population dynamics. Thus, the explicit compartment limitation in the mass-balance models, with the explicit consumption of food resources, such as those developed by Pauly et al. (2000), Vasconcellos and Gasalla (2001), Duarte and Garcia (2004), Gasalla and Rossi-Wongtschowski (2004) and Jiang and Gibbs (2005), amongst others, is not available in this model. Therefore, the population regulation mechanisms must accommodate such a limitation. In this sense, a combination of physical and biogeochemical models is a very promising research subject area, for the management of shellfish resources (Ferreira et al., 1998; 2007; Duarte et al., 2003; 2008).

(iv) The effects of diseases, which can be the cause of high mortalities, such as the Brown Ring Disease (BRD, (Paillard, 2004) and perkinsosis (Villalba et al., 2008) are not taken into account; this is even if data have been obtained, in studies carried out in Arcachon Bay, provide evidence of its presence (Lassalle et al., 2007; Dang et al., 2008).

(v) The production of newly-settled juveniles has been established by iterative modelling until stabilisation of the population; this is due to the lack of information of settlement processes and larval mortality rates, in Arcachon Bay. The production of newly-settled juveniles is constant, throughout the spawning season; it is related to the range in sea water temperatures; this has been identified as a key variable in the processes controlling clam populations, as highlighted by Flye-Sainte-Marie et al. (2007). This means that there is no linkage between the stock and the recruitment, which can be a quite unrealistic hypothesis, unless density-dependent mechanisms are strongly damping down all of the recruitment fluctuations. In particular, this is

likely to determine a “dangerous” bias in the assessment of long-term effects of fishing policies, on stock abundance.

(vi) Growth rates of juveniles and adults are constant throughout the year; these have been established by a iterative modelling until stabilisation of the population, related to the absence of information of growth rates, in Arcachon Bay. Melià et al. (2004) have shown that clam growth can vary over time, depending upon sea water temperature. Subsequently, an increase in the temperature should subsequently not have effects only on mortality. Furthermore, a second indirect impact of variation in temperature can be a modification of the available trophic resource. Since the effect of the trophic resource is not taken into account in this model, this indirect effect cannot be predicted by the model. Then, an increase of temperature may have complex ecological impacts and may affect population dynamics in an indirect way i.e. modifying the available trophic resource or the competition between species, etc. Consequently, it is necessary to take care about the results of Case 7. Likewise, the model demands an improvement of these aspects that will be undertaken in the future.

Despite these problems, the fitting of the model with real data, provided by Caill-Milly et al. (2003, 2006), can be considered to be adequate; this is because the deviation is below the maximum accepted deviation percentage for model prediction, established at 10% (Borja and Bald, 2000; Bald et al., 2006). Nevertheless, it is necessary to point out that the “model fitting” has been undertaken with only two (real) datasets, which could be considered as being insufficient. This holds, in particular, when the model is used to assess the economic, social and conservation consequences of different management policies. Under such circumstances, a sensitivity analysis is highly recommend, to assess to what extent the results are influenced by the specific values assumed for the parameters.

The remainder of the initial conditions for modelling are based upon the results obtained by Caill-Milly et al. (2003; 2006), related to population structure and stock availability in Arcachon Bay. These conditions are combined with different bibliographic sources, related to population dynamics of the Manila clam, together with data obtained from the present study. As more ecological data on the Manila clam become available, as on-going studies in the Arcachon Bay are completed, revision of the model parameters is recommended. This approach is to determine if the values obtained, during the model calibration for population stability, lie within the same range as those derived from recent ecological studies. Meanwhile, use of the present model should be considered as a preliminary approach.

Nonetheless, the model results obtained are consistent and (at least, qualitatively) robust, so that the interpretations arising from comparison of the results are reliable.

When the effects of the different management decisions related to stock evolution and captures are compared (Table 2), the best management decisions are those which are able to maximise, at the same time, the captures and the number of shellfishermen; likewise, minimise the stock losses, as a consequence of this exploitation (Bald et al., 2006). On this basis, the most important management decision derived is in relation to the minimum legal length for capture (Case 2). This measure permits a considerable increase in clam stock and, consequently, an increase in the annual captures and fisherman profit. This Maximum Sustainable Yield (MSY) approach can lead often to overexploitation of the resource, when the current spawning stock is below the level associated with MSY (Ye et al.); therefore, it can be inappropriate when conservation issues are also important. Conversely, the time needed for the stabilisation of the model, following the management decision being taken, is about 4 years. Such a time period can provide an estimation of the time needed for a fully-efficient management decision, i.e. this may provide an answer to the important question "if we take a management decision, such as limiting the minimum length for capture to 40 mm today, how long do we have to wait to reconstruct the Manila clam stock?" In the other hand, the parameters of the model have been tuned under the hypothesis that the stock is at equilibrium. The studies undertaken by Bertignac (2001) and Caill-Milly et al. (2003, 2006) demonstrated an unchanging stock, around 7,000 – 8,000 tonnes from 2001 to 2006. However, as stated in the Introduction, the same authors underline the presence of disequilibrium in the population structure. Due to this inconsistency it is possible that the time needed for the stabilisation of the model in simulations can’t be taken as a measure of the time management decision take to be effective, as a different parameter set might indeed increase or decrease the time needed for the system to approach equilibrium.

The second and third management decisions relate to a reduction in the capture season, to 3 and 8 months, respectively (Cases 4 and 5). In such cases, the clam stock and fisherman profit increase, but the annual total captures remains similar to that in original situation (Case 1). On the other hand, this management measures encompasses a social concern, derived from an important reduction in the capture season.

The fourth management decision relates to an increase in the “no-take” zones, from 11% to 30% of the total area of the distribution of the clam population (Case 6). The main interest of this measure relates to the demonstration of how the protection of some areas can permit

the conservation of others; they act as spawning pools of larvae, nourishing the exploited areas (Nakagawa, 1994; Bald et al., 2006). This approach produces a slight increase in the clam stock, but annual captures and fisherman profit remain the same, as in original situation (Case 1). This result can be explained in relation to the fact that settlement is assumed to be constant.

The fifth management decision relates to a reduction in minimum legal length, for capture, permitting a 10% of fishing effort over 20-35 mm and 35-40 mm length classes. Such a measure permits a considerable increase in the annual captures and fisherman profit, but the clam stock decreases (to approximately in 1,000 t); however this situation is not sustainable.

Finally, the modelling of unusual environmental conditions, such as a 2ºC increase in sea water temperature in summer (Case 7), reveals a considerable decrease in the clam stock; consequently, in the annual captures and shellfishers profit, during the years when these events are detected. Nevertheless, the potential positive effect of temperature, on growth and reproduction, may counteract the effect of mortality. As mentioned above, since the effect of temperature over the growth and reproduction is not taken into account in the model, this result has to be considered with care. Melià et al. (2004) and Flye-Saint-Marie (2007), in describing the dependence of growth and mortality rates upon seasonal temperature fluctuations, have revealed the importance of temperature as a key variable in these vital processes.

The increase in clam stock observed, in relation to the best (5) management decisions are probably overemphasised, due to the fact that food limitation is not included in the model (as mentioned above). Another explanation of this behaviour can be the differences in growth rates, established by progressive approximation in the present contribution, in relation to the real growth parameters in Arcachon Bay. Consequently, the stock values obtained may exceed the carrying capacity of the system (*sensu* Dame and Prins, 1998) and could be lower. In any case, comparison between the different cases is possible and a decision related to the best management measure can be undertaken.

According to Solidoro et al. (2003), the “economically more profitable” strategies coincide with, an “ecologically more conservative” policy. If fishing is preferred, only larger individuals should be caught. The establishment of a minimum landing length, greater than the size of first maturation, is a common measure applied in other invertebrate resources (González-Gurriarán and Freire, 1994). Appropriate minimum legal length limits protect part of the reproductive capacity of a population, thus ensuring future productivity (Gosling, 2004;

Hartill et al., 2005). Similarly, strategies focused upon reducing the capture season duration, establishing closed seasons, maintaining a minimum sustainable biomass (Borja and Bald, 2000), together with the implementation of “no-take” zones (Freire et al., 2002), are the best options. The key challenges for fisheries involve a reduction in fishing effort, removing excess fishing capacity; likewise, establishing the institutional arrangements needed to restore spawning biomass to more productive levels, to reverse degradation of the supporting habitats (Bell et al., 2006).

Failure to manage a fishery appropriately can have disastrous effects on social and economic conditions (Maunder et al., 2006). The reasons for failure can be attributed to many factors, including: inadequate or erroneous scientific information; poor management decisions; and the inability of policy-makers to act (Maunder et al., 2006). In this sense, the role of management models, based upon reliable biological information as advising tools for scientist and managers, can be a key factor in the modern sustainable management of bivalve fisheries.

5. Conclusions

Considering the assumptions made in this study, the most appropriate management decision for the sustainable exploitation of Manila clam populations in Arcachon Bay is maintaining the minimum legal length for capture (40 mm), together with the limitation of fishing effort, through a reduction in the duration of the capture season.

The model developed incorporates some important assumptions, which could be subject to improvement; this is mainly in relation to the improved understanding of the planktonic phase behaviour of the Manila clam (growth, mortality, transport and settlement). Changes in this phase have important consequences on the subsequent juvenile and adult structure. Improved knowledge is needed also in those aspects related with the growth process, together with the natural mortality of juveniles and adults, in relation to the environment in Arcachon Bay. For such an improvement in knowledge, future studies are required on: (i) resource stock and catches monitoring; (ii) the growth and natural mortality estimation of individuals; (iii) food sources; and (iv) evaluation of the total bivalve biomass supported by Arcachon Bay, as a function of the water residence time, primary production and bivalve ecophysiological dynamics.

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