SODIM
Société de développement de l'industrie maricole inc.

Évaluation du monitorage de la croissance du naissain de la moule bleue mytilus spp. basé sur les bouées de navigation

Rapport final

Dossier nº 710.147

Rapport commandité par la SODIM

Décembre 2010

UNIVERSITÉ DU QUÉBEC À RIMOUSKI

ÉVALUATION DU MONITORAGE DE LA CROISSANCE DU NAISSAIN DE LA MOULE BLEUE *MYTILUS* SPP. BASÉ SUR LES BOUÉES DE NAVIGATION

Mémoire présenté
dans le cadre du programme de maîtrise en océanographie en vue de l'obtention du grade de maître ès sciences

PAR

© IHSÈNE BEN SALAH

Décembre 2010

UNIVERSITÉ DU QUÉBEC À RIMOUSKI

ÉVALUATION DU MONITORAGE DE LA CROISSANCE DU NAISSAIN DE LA MOULE BLEUE *MYTILUS* SPP. BASÉ SUR LES BOUÉES DE NAVIGATION

Mémoire présenté
dans le cadre du programme de maîtrise en océanographie en vue de l'obtention du grade
de maître ès sciences

PAR

© IHSÈNE BEN SALAH

Décembre 2010

Composition du jury :

Réjean Tremblay, président du jury, Université du Québec à Rimouski

Marcel Fréchette, directeur de recherche, Institut Maurice-Lamontagne

Jocelyne Pellerin, codirectrice de recherche, Université du Québec à Rimouski

Bruno Myrand, examinateur externe, Ministère de l'Agriculture, des Pêches et de l'Alimentation du Québec

Dépôt initial le 13 Août 2010 Dépôt final le 10 Décembre 2010

REMERCIEMENTS

J'aimerais remercier mon directeur, monsieur Marcel Fréchette de m'avoir fait confiance tout au long de cette maîtrise, d'avoir été toujours disponible et de m'avoir ouvert de précieuses portes dans le monde de la recherche en aquaculture. Je souhaite également remercier ma codirectrice, madame Jocelyne Pellerin, pour ses conseils et son soutien.

Je souhaite remercier Linda Girard et Myriam Lachance-Bernard pour leur aide précieuse dans les analyses menées au laboratoire. Je tiens à remercier Florent Garnerot, Helmi Hamdi, et Hichem Dhif.

Mes remerciements au Réseau Aquacole du Québec (RAQ), au ministère des Pêches et Océans Canada (MPO), au Programme Coopératif de Recherche et Développement en Aquaculture (PCRDA), à la société de Développement de l'Industrie Maricole (SODIM) et au Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) pour leur support financier.

Je voudrai aussi remercier mon mari, mes parents, tous les membres de ma famille à Montréal ainsi que mes amis tunisiens qui n'ont jamais cessé de m'encourager.

Je remercie toute personne qui, d'une façon ou d'une autre, a contribué à la réalisation de ce travail et qui m'échappe en ce moment. À tous merci!

RÉSUMÉ

Le choix de zones à potentiel mytilicole se heurte dans beaucoup de cas à un manque de connaissances préalables sur le recrutement et la croissance en conditions d'élevage. Vu la grande étendue des côtes entourant le golfe du Saint-Laurent, la mise au point de stratégies efficaces et économiques pour l'étude des variations géographiques de la croissance du naissain de moule bleue est essentielle. Habituellement, la croissance du naissain s'étudie en installant des collecteurs et en examinant la taille des moules à intervalles réguliers. La présence de nombreux sites éloignés les uns des autres pose des contraintes logistiques très importantes. Une façon de minimiser ces contraintes serait d'utiliser les bouées de navigation à la place des collecteurs. Représentant un substrat favorable à la fixation, ces bouées sont ramenées à quai à chaque automne. Or la question de la variabilité de la taille du naissain, qui pourrait être liée à une différence d'âge, pose un problème dans l'estimation des paramètres de croissance. Un pigment qui s'accumule avec le temps, la lipofuscine, a été utilisé pour séparer les différents groupes de taille. Des moules provenant de cinq sites choisis pour leur proximité des sites d'aquaculture ont été échantillonnées en 2007. La relation entre l'accumulation de la lipofuscine et la taille a été testée. En ce qui concerne les paramètres de croissance, l'échantillonnage a eu lieu en 2007 et 2008. Deux types de collecteurs ont été utilisés: les bouées de navigation et les collecteurs standards.

Aucune corrélation significative entre la taille des moules et le taux d'accumulation de la lipofuscine n'a été démontrée. Aux Îles de la Madeleine, l'accumulation de ce pigment a été inversement corrélée avec la taille des moules. D'autre part, l'accumulation de ce pigment semble être contrôlée par les paramètres abiotiques des sites d'élevage tout au moins pour les moules juvéniles. La masse moyenne individuelle ne varie pas de façon significative selon le substrat. Ce résultat nous permet de conclure que les processus contrôlant la croissance agissent de la même façon au niveau des deux substrats (bouées versus collecteurs) malgré qu'ils représentent deux surfaces géométriquement différentes. Toutefois, la masse du naissain varie selon le site de prélèvement des échantillons sur les bouées (corps, chaine ou colonne). Ceci montre l'importance de tenir compte de la position d'origine des échantillons sur les bouées. La relation biomasse-densité ne montre aucun effet de compétition entre les moules, durant les cinq premiers mois de croissance, malgré la variabilité de taille observée au sein de la population. La comparaison de la croissance entre les différents sites révèle que ce paramètre suivait la même tendance que la température. En effet, les sites de la Côte Nord représentés par Natashquan, Sept-Îles et Havre St-Pierre ont une croissance inférieure à celle observée à Gaspé, Paspébiac et aux Îles de la Madeleine. De telles données pourraient servir d'indicateur pour le choix des sites d'élevage pour la moule bleue. Les résultats de cette étude plaident en faveur de la mise sur

pied d'un programme de monitorage de la croissance du naissain en se basant sur les bouées de navigation. Cependant, il est utile de noter que le calendrier de récupération des bouées peut varier d'une année à l'autre de façon imprévisible. D'autre part, les bouées de navigation ne sont pas nécessairement installées à proximité des sites mytilicoles potentiels. D'où la présence de contraintes supplémentaires à considérer dans le cadre d'un tel programme.

Mots clés : moule bleue, croissance, bouées, lipofuscine, naissain.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 IMPORTANCE ÉCOLOGIQUE ET ÉCONOMIQUE DE LA MOULE

Répartie dans la plupart des eaux polaires et tempérées à travers le monde entier, la moule bleue est une espèce caractérisée par une grande importance écologique et économique (Commito, et al., 2006). Elle est considérée parmi les espèces clé dans le maintien des écosystèmes benthiques (Borthagaray & Carranza, 2007; Commito, et al., 2008; Buschbaum, et al., 2009). L'activité de filtration des moules de particules de petite taille et leur activité de biodéposition influence les apports en seston, qu'il s'agisse de phytoplancton, de détritus ou de seston inorganique, de la colonne d'eau vers le benthos à travers sa production de fèces et de pseudo-fèces (Hatcher, et al., 1994; Jones, et al., 1997; Beadman, et al., 2002). Ce processus semble significatif, puisque des travaux menés sur l'effet de la biodéposition des moules d'élevage sur la communauté benthique ont révélé une augmentation de l'abondance et de la diversité de la macrofaune benthique près de ces élevages (Callier, et al., 2007, 2008; Weise, et al., 2009). Néanmoins, l'introduction massive d'organismes et d'infrastructures conchylicoles peut entraîner des effets négatifs sur le milieu benthique. En effet, la dégradation de la matière organique déposée sur le fond implique une demande en oxygène expliquée par l'activité microbienne. Si l'apport en matière organique est trop important, la demande benthique en oxygène peut être accrue. Cette situation pourrait aboutir à la création d'un environnement anaérobique (Hatcher, et al., 1994; Chamberlain, et al., 2001).

Les élevages commerciaux de moules par le monde ont connu une croissance importante au cours des dernières décennies. En effet, la production mondiale de moules d'élevage était estimée à 1 700 871 tonnes en 2002 et atteignait 1 860 249 tonnes en 2004 (FAO, 2007). Au Québec, la production des moules était de 370 tonnes en 2004, pour une valeur de 481000 dollars. Elle est passée à 610 tonnes en 2008, correspondant à une valeur de 800000 dollars ("Statistique Canada: Statistiques d'aquaculture," 2008). Ces données

reflètent la demande croissante des consommateurs. Il est possible que de nouveaux promoteurs se montrent intéressés à vouloir développer la mytiliculture dans des endroits différents de ceux qui ont été exploités jusqu'à maintenant. Ce qui implique la nécessité d'identifier de nouveaux sites d'élevage.

1.2 FACTEURS CONTRÔLANT LA CROISSANCE ET LA SURVIE DES MOULES

La croissance et la mortalité de la moule bleue en conditions d'élevage sont affectés par les caractéristiques du site ainsi que le stock (Mallet, et al., 1987a, 1987b). Parmi les caractéristiques du site, la qualité et la quantité de la nourriture disponible sont cruciales pour maximiser la croissance (Page & Hubbard, 1987). Cette nourriture est fortement influencée par l'hydrodynamisme en favorisant l'approvisionnement en particules en suspension compensant ainsi la déplétion en nourriture à proximité des bancs de moules (Newell, et al., 2001). La marée pourrait avoir un rôle important dans les apports en nutriments par le contrôle qu'elle exerce sur le renouvellement de la masse d'eau (Mallet, et al., 1990). D'autre part, les caractéristiques génétiques peuvent générer des variations intraspécifiques de la croissance. En effet, Hawkins et al. (1989) et Myrand et al. (2009) ont démontré que les individus hétérozygotes sont caractérisés par une meilleure stabilité métabolique comparativement aux homozygotes. D'autre part, Pearsons (2007) a prouvé que la stabilité métabolique est fortement corrélée avec la croissance dans les populations naturelles.

Un facteur important contrôlant la croissance est la température de la colonne d'eau entourant les moules. Ce facteur influence la reproduction, la croissance et la survie de ces bivalves. L'augmentation de la température favorise l'assimilation de la nourriture (Kimbro, et al., 2009), quoi que dans certains cas des niveaux létaux soient atteints (Tremblay, et al., 1998; Myrand, et al., 2000). Cependant, Page et Hubbard (1987)

concluent que le contrôle de la croissance dépend davantage de la nourriture que de la température.

De même que la température, la salinité constitue aussi un facteur limitant la croissance et la survie de la moule. Des réductions de la salinité peuvent affecter négativement la croissance tout en réduisant la biomasse ainsi que la densité de la population de moules (Kautsky, 1982; Westerbom, et al., 2008).

La présence de prédateurs pourrait aussi affecter la croissance et la survie des moules. Les prédateurs principaux des moules juvéniles font appel à diverses stratégies de prédation. Les principaux prédateurs de la moule sont les oiseaux (Guillemette, et al., 1996; Rail & Savard, 2003), les crustacés (tel que le crabe commun) et les étoiles de mer (Dolmer, 1998). D'autre part, une étude menée par Côté et Jelnikar (1999) a montré que les moules réagissent négativement aux prédateurs même sans contact physique.

Aussi, les polluants semblent affecter la croissance de la moule. En présence d'hydrocarbures, de PCBs ou de DDT, une diminution de la croissance due à une réduction de la respiration et de l'alimentation a été démontrée (Widdows, et al., 1995; Widdows, et al., 1997; Halldòrsson, et al., 2005).

1.3 PROBLÉMATIQUE ET HYPOTHÈSES DE TRAVAIL

En contexte aquacole, la croissance du naissain est habituellement étudiée en installant des collecteurs et en examinant la taille des moules à intervalle régulier. À titre d'exemple, Thomas et al. (2004) ont étudié la distribution de *M. edulis* et *M. trossulus* dans les régions maritimes du Québec en se basant sur cette méthode. Cette dernière nécessite une logistique relativement difficile à mettre en œuvre. En effet, elle requiert plusieurs

missions sur le terrain afin d'aboutir à des données fiables. Cartier et al. (2004) et Lemaire et al. (2006) ont utilisé un indice basé sur la masse de l'hépatopancréas (IHS) de moules bleues provenant de différents sites du golfe du Saint-Laurent afin d'évaluer la qualité nutritionnelle des sites de culture. Ce type d'analyse pourrait indiquer les sites les plus favorables pour l'élevage de cette espèce en raison du lien étroit entre la concentration de la nourriture disponible et la croissance (Freeman & Dickie, 1979; Mallet, et al., 1987b). Malgré que les effets des paramètres environnementaux aient été détectés, ils n'ont pas pu expliquer entièrement les variations de l'IHS. Par ailleurs, la présence de nombreux sites éloignés les uns des autres présente la principale contrainte pour la mise en place d'une telle approche. D'autre part, des études visant la caractérisation des différents sites pour l'élevage du pétoncle japonais *Mizuhopecten yessoensis* et de la palourde *Mercenaria* spp. utilisaient des méthodes basées sur les systèmes d'information géographique (SIG) (Arnold, et al., 2000; Radiarta & Saitoh, 2009). Ces systèmes intègrent des cartes thématiques en rapport avec les caractéristiques physiques, chimiques et biologiques. Ils permettent la localisation automatique des aires géographiques qui répondent à la fois aux différents critères prédéfinis caractérisant les sites potentiels d'élevage. Bien que cette approche permette l'étude de territoires étendus, il n'en demeure pas moins que les résultats obtenus doivent être validés par les observations *in situ*. Vu la vaste étendue du golfe du Saint-Laurent, la mise au point de stratégies plus adaptées pour l'étude des variations géographiques de la croissance du naissain de moules est essentielle.

Réparties partout dans l'estuaire et le golfe du Saint-Laurent, les bouées de navigation représentent un substrat favorable à la fixation de la moule. Contrairement aux différentes approches utilisées pour étudier la croissance des bivalves nécessitant plusieurs sorties en mer, les bouées de navigation sont recueillies chaque année par la garde côtière. De notre côté, on a qu'à aller à un ou deux endroits pour cueillir les échantillons au lieu de se déplacer sur des distances considérables. Ceci permettrait l'approvisionnement en échantillons provenant de différentes régions en peu temps, ce qu'il n'est pas possible d'envisager en utilisant les collecteurs standards. Depuis des années, les bouées ont été

utilisées dans l'étude de la distribution et de l'abondance de l'épifaune benthique (Fradette & Bourget, 1980; Ardisson & Bourget, 1991). De telles structures permettaient d'avoir une vue synoptique à grande échelle. En utilisant les bouées au lieu des collecteurs, on postule que les processus contrôlant la croissance à l'échelle des deux substrats (bouées versus collecteurs) agissent de la même façon malgré qu'ils représentent deux surfaces géométriquement différentes (une surface plane versus une corde). Des travaux réalisés par Plew et al. (2009) ont démontré l'effet des collecteurs de moules sur l'hydrodynamisme, mais n'ont mis en évidence aucun effet de l'activité de filtration des moules sur l'écoulement. Dans ce contexte, des travaux réalisés à proximité des bancs de moules ont montré une déplétion du phytoplancton au dessus de ces structures (Fréchette & Bourget, 1985; Jonsson, et al., 2005). Soulignons également que van Duren et al. (2006) ont montré que les moules modifient l'écoulement au voisinage du fond contrairement à ce qu'on observe avec les collecteurs (Plew, et al., 2009). Ceci pourrait influencer la dynamique de déplétion du phytoplancton, s'il y a lieu, au voisinage des bouées et de remise en suspension des particules. Des études menées par Newell (1990) ont démontré que le taux de croissance de *M. edulis* était significativement plus élevé à la périphérie qu'au milieu des bancs de moules de 2 à 10 m de diamètre. Les moules situées au bord ont une meilleure accessibilité aux ressources alimentaires expliquant ainsi les différences de croissance. Par conséquent, l'échelle spatiale des effets de bordure est généralement plus petite que la taille habituelle des bouées de navigation dont le diamètre varie de 2 à 4 mètres. Ainsi, les processus évoqués pour les bancs naturels pourraient avoir un impact similaire sur les bouées.

D'après des travaux réalisés par Fréchette et al. (soumis) en rapport avec les collecteurs autogérés (des collecteurs n'ayant subi aucun traitement d'élagage avant la récolte), la compétition n'affecte pas la croissance de façon significative au cours des 27 premiers mois d'élevage à Carleton. Donc, on peut postuler que ce phénomène est absent au niveau des collecteurs standards vu que la durée du suivi du naissain est de cinq mois seulement. Cet effet pourrait biaiser les résultats avec les bouées, dont la croissance du

naissain peut être contrôlée par la compétition (Ardisson & Bourget, 1991). Un moyen de représenter l'effet de la compétition sur la croissance d'individus est le diagramme biomasse-densité.

1.3.1 La relation biomasse-densité (B-N)

Cette relation a été depuis longtemps utilisée pour les plantes comme outil de gestion (Westoby, 1984). Par la suite, Fréchette et al. (1996) ont proposé son application en conchyliculture.

Schématiquement, la relation biomasse-densité est obtenue en représentant la biomasse en fonction de la densité d'un groupe observé à différents moments. On peut aussi représenter la biomasse en fonction de la densité pour plusieurs groupes observés en un même moment (Fig. 1.1).

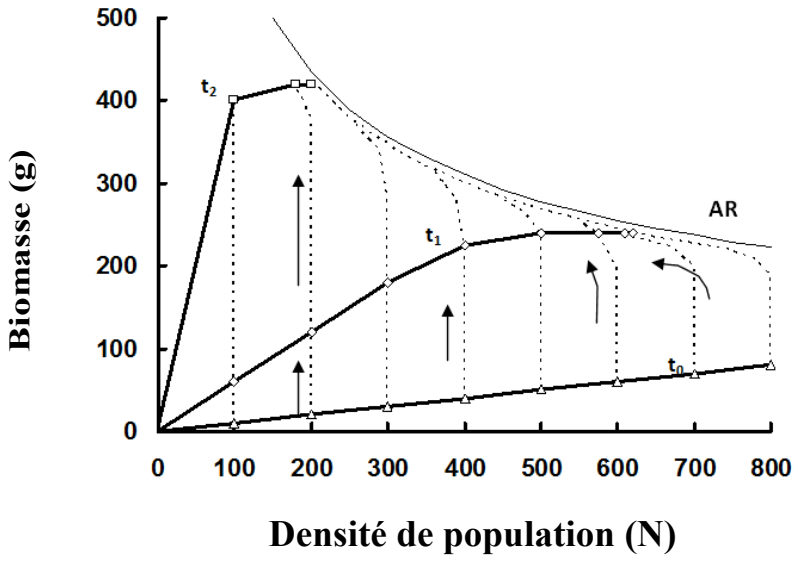


Figure 1.1: Exemple d'un diagramme B-N typique où la biomasse est exprimée en fonction de la densité de population (Westoby, 1984). Plusieurs groupes de densité initiale variant de 100 à 800 individus sont échantillonnés à intervalles de temps successifs (t_0 à t_2). Les flèches montrent le déplacement des groupes au fil du temps

Ce diagramme peut être analysé en suivant les courbes B-N au cours du temps. En se basant sur cette dernière, on peut déduire la présence ou l'absence de la compétition. La courbe de la relation B-N est formée de trois régions (Fig. 1.1). Une première région où la biomasse augmente linéairement avec la densité, indiquant l'absence du phénomène de compétition. Ainsi, la croissance est indépendante de la densité. Une deuxième région qui est une zone de densité intermédiaire caractérisée par un ralentissement du taux de croissance avec l'augmentation de la densité, reflétant la présence de compétition (la relation à t_1 pour une densité comprise entre 400 et 600). Une troisième région caractérisée par l'ajout du phénomène de mortalité lié à la compétition des individus, connu sous le nom de «self-thinning» ou «d'autoréduction» (AR). C'est la relation au temps t_1 à partir d'une densité égale à 700. Plus la biomasse des moules est importante, plus la densité induisant le phénomène de compétition diminue, comme l'indique la courbe B-N obtenue au temps t_2.

Grâce à la possibilité de détecter les effets sur la croissance à partir d'un échantillonnage unique, ce diagramme nous sera donc utile dans la détection d'éventuels effets de la compétition sur la croissance des moules fixées aux bouées de navigation. Toutefois, la variabilité de la taille du naissain de moule pourrait biaiser la relation B-N en cas de présence de plusieurs vagues de recrutement de moules. En effet, l'un des postulats requis pour l'application de la relation B-N est que les spécimens soient de même âge (Yoda, et al., 1963). Or l'âge du naissain des bouées ne peut pas dépasser cinq mois en raison de leur récupération à l'automne. Ceci empêche l'estimation de l'âge du naissain par les anneaux de croissance annuels (Seed, 1976). D'autre part, en présence de compétition, la relation entre la taille et l'âge serait biaisée. En effet, des petites moules pourraient avoir le même âge que des moules plus grosses. Pour pallier la situation, une méthode histochimique a été utilisée dans le but de vérifier la possibilité de détecter plus qu'un groupe d'âge au moyen de la lipofuscine, un pigment qui s'accumule avec le temps. En principe, ce pigment pourrait servir à l'estimation de l'âge des spécimens. Ceci permettrait de s'assurer que les courbes B-N ne sont pas biaisées par la présence de plus d'une cohorte.

1.3.2 La lipofuscine et la séparation des groupes d'âge

La lipofuscine est une substance non dégradable produite par auto-oxydation de lipides et de lipoprotéines et qu'on peut trouver chez les vertébrés et les invertébrés dans différents tissus et organes (Brunk & Terman, 2002; Terman & Brunk, 2004). Des travaux réalisés sur les crustacés (le crabe, le homard, l'écrevisse) et les mollusques (la palourde et la moule) ont démontré que ce pigment peut être détecté par le test de Schmorl, la coloration PAS et le noir soudan B (Sheehy, 1989; Belchier, et al., 1998; Lomovasky, et al., 2002). C'est un pigment composé de granules autofluorescents de coloration jaune-brune à orange de taille comprise entre 1 et 20 μm de diamètre (Sheehy, 1992). Cette autofluorescence est détectée par l'intermédiaire d'un microscope à épifluorescence avec une excitation comprise entre 365 et 514 nm (Belchier, et al., 1994; Sheehy, et al., 1998; Lomovasky, et al., 2002). Son émission est comprise entre 450 et 630 nm (Seehafer & Pearce, 2006). Des études faites sur les deux espèces *M. galloprovincialis* et *M. edulis* ont montré que ce pigment s'accumule dans les cellules de la glande digestive. Son accumulation augmente avec l'âge, mais aussi avec l'exposition aux polluants (Dimitriadis, et al., 2004; Akaishi, et al., 2007). Lomovasky et al. (2002) ont établit une relation basée sur le modèle de von Bertalanffy permettent la prédiction de l'âge en fonction de la concentration de la lipofuscine chez la palourde *Eurhomalea exalbida*. Dans le cadre de ce travail, l'approche utilisée sera basée sur la distribution des valeurs de l'accumulation de la lipofuscine en vue de vérifier la présence éventuelle de plus qu'un mode. Cette dsitribution reflètera le nombre de groupes d'âge présents dans notre échantillon.

1.4 BUT ET OBJECTIFS SPÉCIFIQUES

Ce projet vise la comparaison de la croissance des moules des collecteurs standards avec celle des moules des bouées de navigation. Le but de ce travail est de tester une nouvelle approche de suivi de la croissance du naissain basé sur les bouées de navigation.

Dans le premier chapitre de ce mémoire, on a testé la possibilité de mettre en évidence différents groupes d'âge de naissain car plusieurs pontes sont possibles au cours d'un même été. Pour ce faire, nous avons mesuré l'accumulation de la lipofuscine en fonction de la taille du naissain dans le but d'établir une relation entre ces deux paramètres. Le site d'élevage a été pris en compte en échantillonnant des moules provenant de cinq régions différentes.

Dans le deuxième chapitre, on a étudié les paramètres de croissance du naissain en tenant compte du type de substrat (bouées versus collecteurs) et du site d'élevage. Une attention particulière était portée aux courbes B-N des peuplements de moules colonisant les bouées, dans le but de détecter si ces courbes seraient indicatrices d'effets dépendants de la densité sur la croissance.

CHAPITRE II

EFFECT OF BODY SIZE AND SITE ON LIPOFUSCIN ACCUMULATION IN THE DIGESTIVE GLAND OF BLUE MUSSEL *MYTILUS* SPP. SPAT ON NAVIGATION BUOYS

Ben Salah, I., Pellerin, J., Daigle, G., Fréchette, M.

ABSTRACT

Blue mussel *Mytilus* spp. spat settled on navigation buoys may prove useful for monitoring spatial variability of mussel growth in large coastal systems. Growth estimates, however, may be blurred by age variability if spat populations originate from separate spawning events. In order to test whether age may contribute to size variability within samples, a pigment that accumulates with time, lipofuscin, was used as an age marker. Mussels were collected from seven different stations located in five sites in the Gulf of St. Lawrence. Correlations between mussel size and lipofuscin accumulation were not statistically significant. Most likely, the accumulation of this pigment requires longer time intervals to discriminate age groups. Variations in pigment accumulation among sites, however, were highly significant. Nevertheless, species effects could not be ruled out. Monitoring for longer durations could be required for accurate detection of age structure in mussel populations.

2.1 INTRODUCTION

Monitoring spatial variability of growth of bivalve spat in large coastal systems is hindered by logistic constraints inherent to the size of such systems. The development of cost-effective strategies for the study of geographical variation recruitment and growth of mussel spat is therefore essential. One possible way to deal with this situation is to study spat from artificial collectors deployed in various locations. Navigation buoys provide a standardized substratum for blue mussel spat settlement and may act as efficient spat collectors. Indeed, studies of benthic communities colonizing navigation buoys have been useful in revealing the biogeographical structure of hard-bottom benthos in the St. Lawrence system (Fradette & Bourget, 1980; Ardisson & Bourget, 1991). Alternatively, remote-sensing may provide a convenient strategy for assessing synoptic geographical variability of environmental variables controlling bivalve growth (Longdill, et al., 2008; Radiarta & Saitoh, 2009). However, field data are still needed for ground-truthing purposes, hence the use of mussels colonizing buoys or other standard substrates. According to this framework, mussels are sampled once buoys are returned to dock. This implies endpoint sampling only. To ensure that growth rate estimates are not biased by density-dependent interactions on buoys (Ardisson & Bourget, 1991), Ben Salah et al. (in prep.) used the absence of curvilinearity of body size-density curves as a criterion for density-independent growth as proposed by Fréchette et al. (submitted). This criterion, however, is based on the assumption that populations are even-aged (Westoby, 1984). Preliminary sampling in 2006 suggested that size spat distribution was bimodal, pointing out the need for an independent age marker. In the present context, a physiological age marker such as lipofuscin might be interesting for age estimation to separate the different mussel groups.

Lipofuscin is a non-degradable substance made of residues of proteins, lipids, carbohydrates and trace amounts of metals (Brunk & Terman, 2002; Seehafer & Pearce, 2006) which accumulates in lysosomes (Terman & Brunk, 2004; Moore, et al., 2007; Kurz, et al., 2008). Studies have been mainly performed on crustaceans for age monitoring (Odonovan & Tully, 1996; Sheehy, et al., 1998; Maxwell, et al., 2007). For bivalvs, most of studies focused on the effect of environmental stress (such as heavy metals) on the accumulation of this pigment (Hole, et al., 1993; Dimitriadis, et al., 2004; Akaishi, et al., 2007; Koukouzika, et al., 2009). However, some investigations were made on mollusks for age estimation using lipofuscin and proposed a model to predict age from the concentration of this pigment (Lomovasky, et al., 2002; Sukhotin, et al., 2002). To our knowledge, however, lipofuscin has never been used as an age marker to separate mussel cohorts as an alternative to the well known technique of shell marks, which is highly influenced by environmental stress factors (Seed, 1976; Abele, et al., 2009). Furthermore, the use of the conventional method is not reliable with a few months old mussels.

The aim of this study was to evaluate if the density of lipofuscin detected by autofluorescence and different staining techniques, is a valid indicator for the determination of the age of the blue mussel *Mytilus* spp. in the Gulf of St. Lawrence. Hence, we verified if this pigment is effective in separating cohorts of mussels according to their age, taking into account the potential effect of site.

2.2 MATERIALS AND METHODS

2.2.1 Sampling

We took advantage of the fact that in the Gulf of St. Lawrence, the Canadian Coast Guard retrieves all navigation buoys from their respective mooring sites during the fall (Fradette & Bourget, 1980; Ardisson & Bourget, 1991). The buoys are subsequently landed

in Gaspé and Cap-aux-Meules for cleaning. This provides an opportunity for sampling populations within hours after buoy retrieval.

Samples were taken from seven navigation buoys moored in five sites in the Gulf of St. Lawrence (Fig. 2.1): Gaspé, Paspébiac, Sept-Îles, Magdalen Islands and Natashquan, represented respectively by HD8 and HD9, AP2, BASQO and D6, YE2 and CN02. The buoys were sampled in November 2007. We haphazardly sampled about 100 mussels per buoy in the size interval ranging from about 0.5 cm to 2.5 cm shell length. All specimens were frozen in liquid nitrogen and kept at -80 ^{o}C until processing. We measured shell length and total wet mass of all individuals. Two mussel species are found along the Gaspé Coast and Sept-Îles (*M. edulis* and *M. trossulus*), in contrast with the Magdalen Islands where only *M. edulis* is present (Thomas, et al., 2004; Moreau, et al., 2005). Morphological similarity between the two species is such that taxonomic discrimination based on morphology only is unreliable (McDonald, et al., 1991). In the absence of specific information, we identified all mussels as *Mytilus* spp.

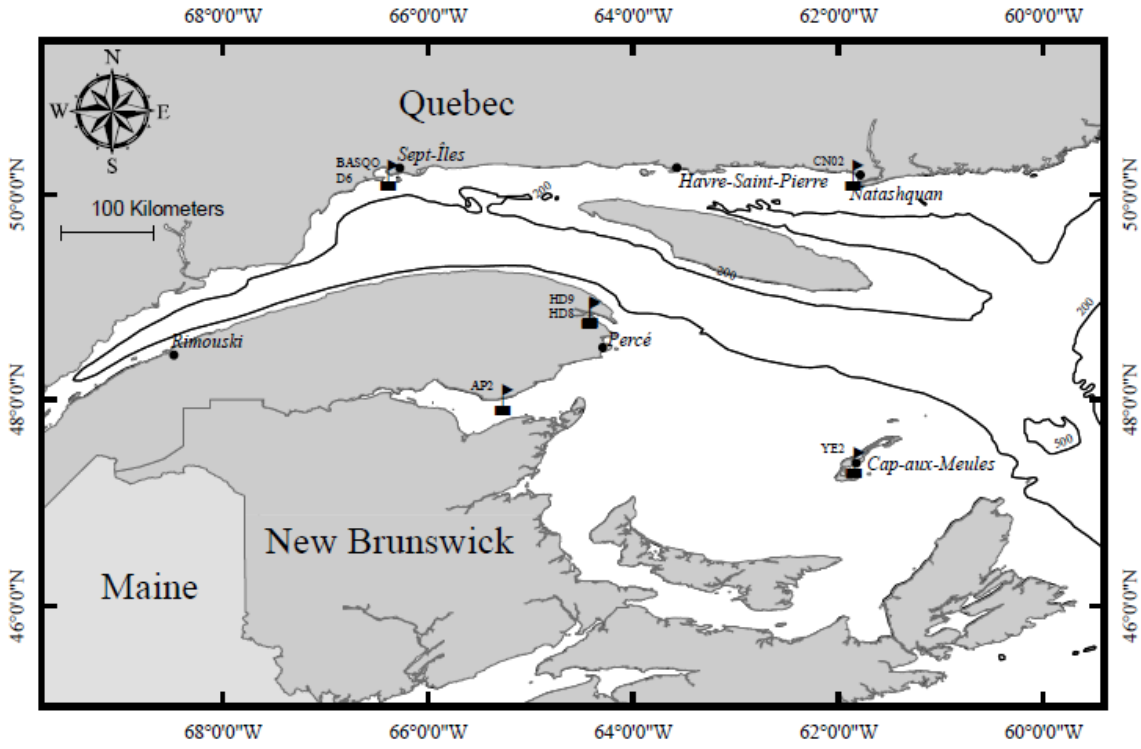


Figure 2.1: Location of buoy mooring sites. Projected coordinate system used was NAD1983MTM7

2.2.2 Mussel preparation

All samples were fixed in Bouin solution during 24 hours (Encarnação & Castro, 2001; Lomovasky, et al., 2002) and dehydrated in increasing concentration of ethanol, cleared in xylene and embedded in paraffin according to standard methods (see annex I). Embedded tissue was cut to a thickness of 5 µm using a Zeiss microtome. For each mussel, we performed a transverse section of the digestive gland which is a storage organ of lipofuscin (Viarengo, et al., 1991; Moore, et al., 2007). This affirmation was confirmed by observations made in our laboratory.

2.2.3 Validation of methods

We used only 30 specimens to verify the extent to which the observed fluorescent granules in the digestive gland were actually attributable to lipofuscin. Therefore, we analysed the digestive gland of these specimens using three stains: Sudan Black III, PAS and the ferric ferricyanid test (Schmorl test). Positive reaction of these stains suggests that these granules were lipofuscin (Lazorthes, et al., 1990; Sheehy, et al., 1998; Lomovasky, et al., 2002). We measured the individual variability of lipofuscin in the digestive gland on 10 mussels using 20 sections 5 µm thick (corresponding to 20 levels in the gland) for each mussel. Therefore, we were able to assess lipofuscin variability within the mussel digestive gland.

2.2.4 Lipofuscin detection and quantification

The tissue sections were excited using a TRITC filter (Tetramethyl Rhodamine Isothiocyanate) at 540 nm and emission was detected at 605 nm. Depending on the size of the digestive gland, 5 to 25 images (generally 15 or more per specimen; about 70 mussels per buoy) covering 65785 µ² were captured in order to cover the entire surface of the individual glands. We used an Olympus DP70 camera fitted to an Olympus BX51 microscope employing Image Pro Plus 5.1 for Windows. By a combination of automatic discrimination between grey levels and manual editing, binary images (black and white only) were produced. The autofluorescence in the digestive gland was photographed using a 60X oil immersion objective. The area fraction of lipofuscin (AF_L; also termed recovery rate) in each image was calculated as follows (Sheehy, 1992):

$$AF_L = \frac{A_L}{A_T} \cdot 100 \qquad (1)$$

Where A_L is the area covered by the lipofuscin granules and A_T is the total area analyzed. In this context, the recovery rate reflects the accumulation of this pigment.

2.2.5 Statistical analysis

We compared results obtained by autofluorescence with those obtained with the PAS coloration using a paired *t*-test. A randomized complete block ANOVA was used to verify among images the homogeneity of lipofuscin accumulation. A regression model with buoys and within-site variable residual variance as random effects was used to quantify the degree of association between body size and lipofuscin accumulation. A nested ANOVA with site as a fixed factor and buoys nested within sites as a random factor allowed us to compare the lipofuscin accumulation between sites. The angular transformation was applied to the dependent variable in order to satisfy the normality assumption. Normality was tested using the Shapiro-Wilk statistic and the homogeneity of variances was tested using Bartlett's test. However, data were hereroscedastic, so an heterogeneous ANOVA model was used with a residual variance specific to each site. The analysis was done using the MIXED procedure in SAS (SAS Institute, 2008).

The DIP test was used to test the unimodality in the distribution of the accumulation of lipofuscin for all sites. This test is based on the maximum difference, over all sample points, between the empirical distribution function and the unimodal distribution function that minimizes that maximum difference (Hartigan & Hartigan, 1985). It was used to test whether the distribution of the accumulation of this pigment was related to spatial variability in species composition. It was computed using R.

2.3 RESULTS

The buoys were landed in Gaspé and Cap-aux-Meules in November 2007. The samples were collected from the different buoys within hours after docking. Size distribution was similar among all sites, with mussel length ranging from 0.5 cm to 2.5 cm, except on buoy CN02 (Natashquan) where shell size ranged from 0.2 cm to 0.8 cm (see Ben Salah et al. (in prep.)). Because of the small size of mussels, lipofuscin tests were not performed for this site.

2.3.1 Detection of lipofuscin and its accumulation in the digestive gland

We examined 420 mussels and found spherical yellow-orange pigments following excitation at 540 nm and emission at 605 nm using TRITC as fluorochrome. These granules reacted positively to the Schmorl test, the PAS staining and Sudan Black III (Fig. 2.2).

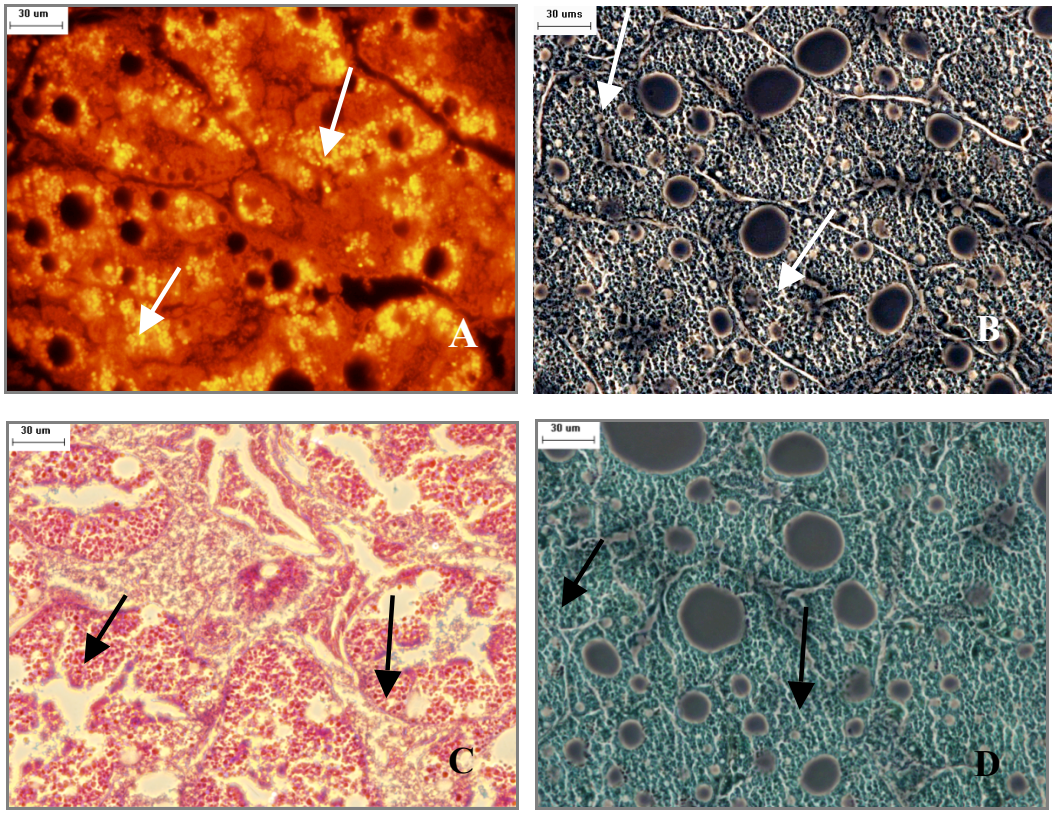


Figure 2.2, A-D: Identification of lipofuscin granules in the digestive gland of blue mussel spat *Mytilus* spp. through different techniques (600X magnification). A, fluorescent granules in the digestive gland; B, black coloration of granules with Sudan Black III; C, coloration of granules in red-purple with PAS; D, stained granules in green-blue with the Schmorl test. Arrows show lipofuscin granules

A comparative analysis between the lipofuscin accumulation found with autofluorescence and PAS shows that differences between the two methods were not significant (paired *t*-test; T_{28}=-0.76, P=0.45). Indeed, lipofuscin accumulation in the different sections of the digestive gland was homogeneous ($F_{19,171}$=0.94, P=0.54).

2.3.2 Variability of the accumulation of lipofuscin with mussel size and among sites

The recovery rate of lipofuscin, which reflects its accumulation, ranged from 22% to 32% for buoys BASQO, D6, AP2, HD8 and HD9, with mussel size ranging from 0.7 cm to 3 cm length. For the Magdalen Islands, it was about 4% for mussels of the same size range (Fig. 2.3). Apparently, slopes of the relation between recovery rate and mussel size were positive for HD8, HD9 and D6, opposite to BASQO, AP2 and YE2, which had negative slopes.

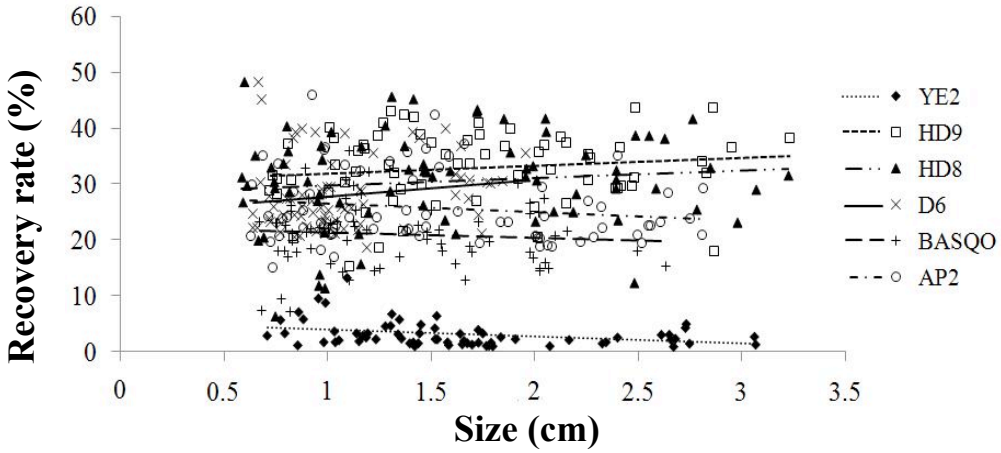


Figure 2.3: Recovery rate of lipofuscin as a function of size in all sites

A mixed regression model with heterogeneous error variances specific to sites was adjusted to the data in order to study the relationship between lipofuscin accumulation and mussel size (Table 1). In fact, the error variance was not homogeneous from one site to the other (χ^2=17.9, P=0.0005). In this model, buoys were considered as a random effect.

Table 2.1: Effect of site on the relationship between mussel size and lipofuscin accumulation. Mixed regression model with buoys considered as a random effect and variable residual variances within sites

Effect	DF	F value	Pr > F
Site	3	12.71	0.0738
Buoy(Site)	2		
Size	1	1.15	0.2837
*Size*Site*	3	3.62	0.0132
Error	413		
Corrected total	422		

*Error=Mussel(Site*Buoys)*

The interaction between site and mussel size was significant (Table 2.1). This interaction was probably caused by a weak, but significant relationship between mussel size and lipofuscin accumulation in the Magdalen Islands sample (Table 2.2). In all other cases, the lipofuscin accumulation-mussel size relationship was not significant.

Table 2.2: Estimation of the slope of the relation between lipofuscin accumulation and mussel size for the different sites. Regression model with buoys as a random effect and variable residual variances among sites

Slope within site	Estimate	Standard	DF	t Value	Pr > |t|
Gaspé	0.0016	0.0010	413	1.56	0.1197
Paspébiac	-0.0016	0.0013	413	-1.26	0.2069
Sept-Îles	0.0004	0.0014	413	0.32	0.7493
Magdalen Islands	-0.0031	0.0011	413	-2.82	0.0051

The effect of site on lipofuscin accumulation was barely significant ($F_{3,2}$=21.66, P=0.0444). Least squares method was applied to estimate the recovery rate for the different

sites. In Magdalen Islands, the recovery rate was less than one third of that in all other sites (Table 2.3) and presumably was responsible for the overall effect of site. This is supported by contrasts between individual sites (Table 2.4). Indeed, there were no significant differences in the accumulation of lipofuscin between sites with the exception of the Magdalen Islands, which were significantly different from all other sites.

Table 2.3: Estimation of the recovery rate of lipofuscin for the different sites. Least squares means method was applied for this analysis

Site	Estimate	Standard Error	DF	t Value	Pr > |t|
Gaspé	0.5942	0.0316	2	18.80	0.0028
Paspébiac	0.5294	0.0443	2	11.94	0.0069
Sept-Îles	0.5142	0.0313	2	16.41	0.0037
Magdalen Islands	0.1698	0.0440	2	3.85	0.0612

Table 2.4: Multiple comparisons of the accumulation of lipofuscin between the different sites. Least squares means differences of the recovery rate of lipofuscin between the different sites

Slope within site	Estimate	Standard Error	DF	t Value	Pr > |t|
Gaspé vs Paspébiac	0.0648	0.0544	2	1.19	0.3562
Gaspé vs Sept-Îles	0.0799	0.0445	2	1.80	0.2143
Gaspé vs Magdalen Islands	0.4243	0.0542	2	7.83	0.0159
Paspébiac vs Sept-Îles	0.0151	0.0543	2	0.28	0.8067
Paspébiac vs Magdalen Islands	0.3595	0.0625	2	5.75	0.0289
Sept-Îles vs Magdalen Islands	0.3444	0.0540	2	6.37	0.0238

2.3.3 Distribution of lipofuscin recovery rate

The frequency distributions of lipofuscin recovery rate are shown in Fig. 2.4. Distributions appeared to vary among sites. Lipofuscin recovery rate ranged between 1% and 17% in the Magdalen Islands and appeared less variable than in the other sites. It

ranged between 9% and 37% for BASQO, between 17% and 49% for D6, between 17% and 49% for AP2, between 10% and 46% for HD8 and between 19% and 46% for HD9.

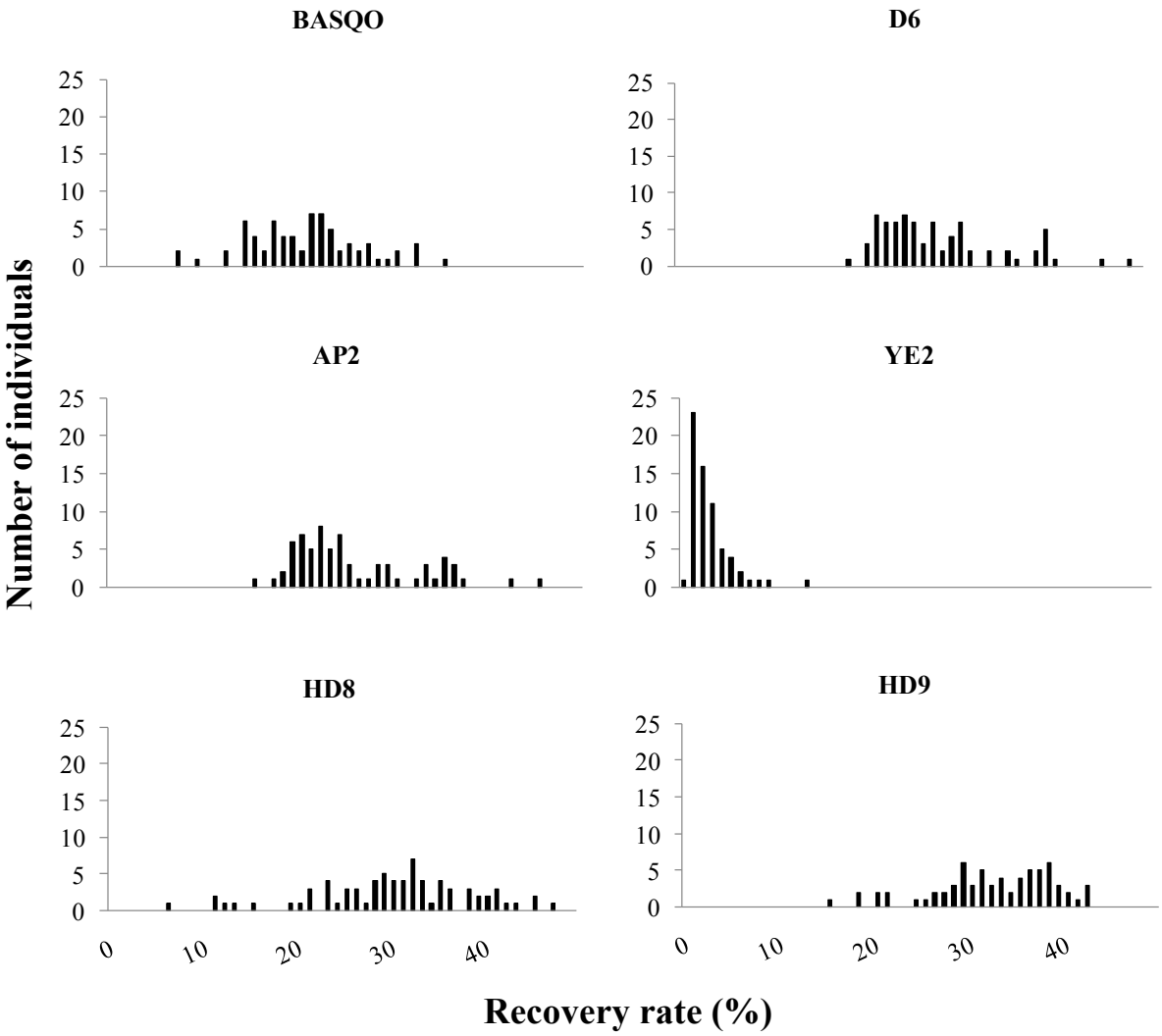


Figure 2.4: Frequency distributions of the recovery rate of lipofuscin. BASQO and D6: Sept-Îles; AP2: Paspébiac; YE2: Magdalen Islands; HD8 and HD9: Gaspé

Visual examination of Fig. 2.4 suggests that the frequency distribution of lipofuscin recovery rate was multimodal for some of the buoys, especially AP2 and HD9. On the opposite, the frequency distribution for the Magdalen Islands clearly was unimodal. We

tested these patterns using the DIP test (Hartigan & Hartigan, 1985). The null hypothesis of the DIP test is that distributions are unimodal. The hypothesis of multimodality was not supported by statistical evidence, as the DIP test failed to reject the hypothesis of unimodal distributions for each of the buoys (Table 2.5).

Table 2.5: Results of the DIP test of multimodality of recovery rate distributions for the different buoys

Buoys		*D6*	*AP2*	*YE2*	*HD8*	*HD9*
DIP test	0.0675	0.0590	0.0513	0.0763	0.0326	0.0508
P value	0.2480	0.4640	0.6060	0.1980	0.9680	0.7220

2.4 DISCUSSION

The autofluorescent granules found in this study correspond to lipofuscin granules. In fact, these granules showed resistance to solvent extraction during histological processing. Positive staining with PAS, Sudan black and the Schmorl test at the same position (Fig. 2.2) were considered indicative of lipofuscin (Sheehy, 1989; Lomovasky, et al., 2002). According to studies done on crustaceans and molluscs, lipofuscin accumulates with time (Sheehy, 1992; Bluhm, et al., 2001; Lomovasky, et al., 2002; Maxwell, et al., 2007). Our data didn't show any relation between the lipofuscin accumulation and mussel size in Gaspé, Paspébiac and Sept-Îles (Fig. 2.3). This result suggests that our group was homogenous in terms of mussel age. Likewise, the DIP test results revealed that the frequency distributions of the recovery rate of lipofuscin were unimodal for the different buoys, again supporting the conclusion that mussels were even-aged. Spawning may span less than two weeks or as much as a month or more. Therefore, polymodal distribution of size structure may reflect different spawning events and spat age in cases of protracted spawning seasons. It may be also related to the metamorphosis delay (Lane, et al., 1985). Such age differences that might exist between different spat groups may range between two

weeks to two months (François Bourque, STMIM, Cap-aux-Meules, Quebec, pers. comm). Such short amounts of time might not be long enough to reveal differences in accumulation of lipofuscin.

Surprisingly, in our data, the recovery rate was inversely correlated with size in the Magdalen Islands. The reasons of this pattern are unclear. A possible explanation for this finding would be the presence of a mixture of heterozygous and homozygous individuals in the Magdalen Islands (Tremblay, et al., 1998). Heterozygous individuals with higher metabolic stability are characterised by their superiority in fitness traits compared to homozygous ones as demonstrated by Hawkins et al. (1989) and Myrand et al. (2009). Indeed, Pearsons (2007) demonstrated the positive correlation between fitness and metabolic stability inducing rapid growth in natural populations. On the other hand, lipofuscin accumulation is negatively correlated with metabolic stability (Koukouzika, et al., 2009). This could explain the smaller accumulation of this pigment within the fast-growing, heterozygous individuals and hence, the negative correlation between mussel size and lipofuscin accumulation encountered in the Magdalen Islands.

Variations in pigment accumulation between the Magdalen Islands and the other sites, however, were highly significant. Tremblay et al. (1998) and Thomas et al. (2004) showed that Magdalen Islands mussels are composed in the majority by the species *M. edulis* as opposed to Gaspé, Paspébiac and Sept-Îles which are composed by a mixture of *M. edulis* and *M. trossulus* (Moreau et al., 2005). This leads to the hypothesis that each species has its own kinetics for the accumulation of lipofuscin. If this holds true, spatial variability in species distributions might have impacted the distributions in Fig. 4 in two possible ways. In fact, sites with both species would have bimodal lipofuscin frequency distributions and sites with a single species would have unimodal distributions. Apparently this was not the case as the DIP test was not significant anywhere. Second, assuming overlapping distributions, the variance of lipofuscin accumulation would increase with

species evenness or the distributions would be platykurtic. Visually, variance of lipofuscin accumulation in the Magdalen Islands was less variable than in the other sites. Knowing that the Magdalens samples are mainly composed by the species *M. edulis*, species-specific effects remain possible.

Lipofuscin accumulation increases with temperature through its effect on metabolic processes (Ju, et al., 1999; Kodama, et al., 2006). This implies a decrease of lysosomal stability, inducing the production of lipofuscin (Riveros, et al., 2002; Moore, et al., 2007). Sea surface temperature is typically higher around the Magdalen Islands than in the other sites (Habbane, et al., 1997; Myrand, et al., 2000). This general pattern was corroborated by observations of Ben Salah et al. (in prep.). Unexpectedly, spatial variability in lipofuscin accumulation did not correlate with temperature, as the lowest concentrations were found near the Magdalen Islands (Figs.2.3, 2.4).

Estuaries and bays are subject to pronounced salinity fluctuations because of inflow from rivers, rainfall and evaporation (Gosling, 2003). Moore et al. (1980) demonstrated that lysosomal stability is responsive to raising salinity from 15 to 33 as well as lowering salinity from 33 to 15 for the species *Mytilus edulis*. In fact, Gaspé, Paspébiac and Sept-Îles are more appropriate for these changes due to the presence of local freshwater sources near these sites (Koutitonsky & Bugden, 1991). Therefore, it is likely that lipofuscin accumulation was higher in these sites compared to the Magdalen Islands where salinity is more constant. Nevertheless, it's clear that small-scale variations are present, but the information needed to quantify them is not available yet. This remains a possible explanation, however, the accumulation of this pigment may be also controlled by other factors.

Castro et al. (2002) demonstrated the effect of diet on the accumulation of lipofuscin in the shrimp *Penaeus japonicus*. In fact, individuals fed with high vitamin levels had a

smaller area fraction covered with lipofuscin, and smaller and lower lipofuscin granule density. It is likely that spatial variability in lipofuscin accumulation in our samples was related to trophic properties of sites. Fuentes-Yaco et al. (1997) showed the importance of freshwater runoff and wind forcing on the spatio-temporal variability of phytoplankton in the Gulf of St. Lawrence. A physical–biological model developed by Le Fouest et al. (2006) demonstrated a regional East–West gradient in chlorophyll *a* concentration with higher values in the Northwestern Gulf. Cartier et al. (2004), for instance, used the digestive gland of the blue mussels to rate the nutritional quality of different sites. They found that the number of phytoplankton cells varied greatly between the Magdalen Islands and Gaspé. In the same context, Trottet et al. (2007) showed that the Grande-Entrée lagoon (Magdalen Islands) is characterized by the dominance of heterotrophic protists suggesting that the Magdalen Islands are biologically different from the other sites. Such investigation favors the effect of mussel diet on the accumulation of lipofuscin.

2.5 CONCLUSION

In summary, we find that lipofuscin failed in separating the different age groups, probably because of the short time intervals separating them. Other alternatives will be investigated to resolve the question. On the opposite, site effects were clear-cut and constituted the major factor controlling the accumulation of this pigment.

2.6 ACKNOWLEDGEMENTS

Financial support was provided by the Aquaculture Collaborative Research and Development Program (ACRDP), Réseau Aquaculture Québec (RAQ), Société de Développement de l'Industrie Maricole (SODIM) and an NSERC Discovery grant attributed to Jocelyne Pellerin. We would like to thank Afif Ben Salah for his comments on

the manuscript, Linda Girard for helping in sampling, Bilel Chalghaf for his invaluable assistance with lipofuscin determination and mapping, Suzanne Roy, Michel Gosselin, Karine Lemarchand for their advice and opinions and Mélanie Simard for her kind support in microscopic analyses.

CHAPITRE III

MONITORING LARGE SCALE SPATIAL VARIABILITY OF BLUE MUSSEL GROWTH IN THE GULF OF ST. LAWRENCE: TESTING FOR DENSITY DEPENDENT EFFECTS IN POPULATIONS SETTLED ON NAVIGATION BUOYS

Ben Salah, I., Pellerin, J., Bourque, F., Goaziou, Y., Guay, M., Thomas, B., Daigle, G., Fréchette, M.

ABSTRACT

Large geographical systems such as the Gulf of St. Lawrence have been associated to strong variability in mussel growth. Therefore, potential culture sites within such systems may yield quite different results. This implies that site-specific information is required to assess cultured mussel growth. One possible way to deal with this situation is to study spat from standard collectors deployed in various locations. Logistic constraints, however, may arise because potential sites are located far from one another. Navigation buoys represent a possible alternative to standard collectors since they provide a standardized substratum and are readily colonized by mussel spat. In the present study, a new approach of monitoring growth of mussels on navigation buoys was tested. Blue mussel spat from different sites in the Gulf of St. Lawrence chosen for their proximity to aquaculture sites was sampled in November 2007 and 2008. We tested the hypothesis that growth did not vary among two types of substrates, navigation buoys and standard collectors. Mean individual mass varied significantly from site to site, but not among substrates within sites. Spatial variability in growth correlated with temperature, as growth in the Southern Gulf sites was faster than in the North Shore sites. Therefore we conclude that navigation buoys provide a convenient alternative to standard collectors for monitoring spatial variability in growth of mussel spat. However this approach is hampered by several constraints such as the inconsistency of the collection program of coastal guard between seasons as well as their heterogeneous distribution in the Gulf of St. Lawrence. Despite these limitations, spatial variability assessment remains possible. Prospective studies should take into account the sampling area on the buoys because of variation among its parts.

3.1 INTRODUCTION

The blue mussel, *Mytilus* spp., is a worldwide species in the sublittoral zone. Growth rate and population density vary considerably from place to place. Studies done by Mallet et al. (1987b) and Myrand et al. (2000) showed that site explained a large proportion of the variance in growth. In fact, growth rates and survival reflect the interaction between the individual's heredity, physiology and the environment (Mallet & Carver, 1993). Generally, growth increases with increasing food concentration and temperature (Page & Hubbard, 1987; Pechenik, et al., 1990). Large geographical systems such as the Gulf of St. Lawrence encompass various biogeographic entities (El Sabh, 1976; Fradette & Bourget, 1981; Ardisson, et al., 1990). These areas have been associated to strong variability in mussel growth (Ardisson & Bourget, 1991; Cartier, et al., 2004). Therefore, potential culture sites within such systems may yield quite different results. This implies that site-specific information is required to assess cultured mussel growth. The application of models based on GIS for aquaculture site selection could be an effective tool (Nath, et al., 2000; Longdill, et al., 2008). It allows selection of appropriate areas by overlapping different layers containing pertinent informations related to aquaculture sites (such as physical, chemical and biological attributes). Nevertheless, ground truthing remains compulsory for the validation of GIS results. Given the size of the system, however, the sampling burden involved may be simply daunting. Clearly adapted sampling strategies are required.

Monitoring spatial variability of growth of bivalve spat in large coastal systems is hindered by logistic constraints. One possible way to deal with this situation is to study spat from standard collectors deployed in various locations. The presence of many sites far from each other poses many logistical problems. The development of cost-effective strategies for the study of geographical variation in recruitment and growth of mussel spat is therefore essential. Navigation buoys represent a possible substrate which can replace these

collectors. Studies of benthic communities colonizing navigation buoys have been useful for direct comparisons in different environments especially because buoys provide a standardized substratum for blue mussel spat settlement (Fradette & Bourget, 1980; Ardisson, et al., 1990; Ardisson & Bourget, 1991; Bourget, et al., 2003). They are generally moored in May and retrieved in early November. Because mussels spawn around mid-May in the Gulf of St. Lawrence, this provides roughly a five month growth period before sampling.

Using buoys instead of standard collectors assumes that the processes controlling growth act similarly on both substrates although they represent two geometrically different surfaces. We assume that growth after 5-6 months on standard spat collectors is density-independent as Fréchette et al. (submitted) found no evidence of intraspecific competition between mussels cultured on spat collectors, 27 months after settlement. On the other hand, Ardisson and Bourget (1991) reported self-thinning in mussel populations colonizing navigation buoys. This raises the possibility that growth on buoys may be controlled by competition. In agreement with this hypothesis, Wildish and Kristmanson (1984) suggested that sea water hydrodynamic factors may be critical for food supply to suspension-feeding animals. In this context, Fréchette & Bourget (1985) showed phytoplankton depletion above a natural mussel bed, which exerted direct control on mussel growth. So the benthic boundary layer had an important role in mussels feeding as demonstrated by Fréchette et al. (1989). In the same context, van Duren et al. (2006) demonstrated the effect of mussel filtration activity on exchange processes at the sediment-water interface. Newell (1990) demonstrated that growth rates of *M. edulis* were significantly higher at the edge than in the middle of mussel beds 2 to 10 m in diameter. Accordingly, the spatial scale of edge effects is typically smaller than the usual size of buoys and therefore the same processes observed with natural mussel bed could exist with mussel populations colonizing navigation buoys.

The study of competition usually involves treatment groups or plots with controlled population density. It is unrealistic to expect that mussel population density can be adjusted and maintained to desired levels on buoys because of recruitment occurring subsequently to the adjustment of experimental plots and because of the small size of the individuals. Therefore, an alternative strategy must be adopted. One way to represent the effect of competition on the growth of individuals is to plot biomass as a function of population density (Westoby, 1984). Such plots may reveal density-dependent mortality as inferred from self-thinning (ST) curves. They may also reflect density-dependent growth, which translates into curvilinear (concave) B-N curves, as opposed to density-independent situations where B-N curves are linear (Alunno-Bruscia, et al., 2000). The combination of ST curves and B-N curves yields a B-N diagram, which has been extensively used in plant sciences (Westoby, 1984). Because competition in buoy mussel populations can only be assessed in end-point observations, we chose to base our decision criterion on B-N curves, as judged from the presence/absence of curvilinearity in the curves. In this study we test the hypothesis that growth of mussel spat on navigation buoys is a valid estimate for growth on standard collectors. More specifically, we test that intraspecific competition does not bias comparisons among substrates.

3.2 MATERIALS AND METHODS

3.2.1 Field work

Navigation buoys and standard collector ropes with blue mussel spat from different sites in Gulf of St. Lawrence (Fig. 3.1) were sampled in November 2007 and 2008. Buoys were chosen for their proximity to aquaculture sites. Samples were taken from seven navigation buoys moored in five sites near Gaspé (buoys HD8 and HD9), Paspébiac (AP2), Sept-Îles (BASQO and D6), the Magdalen Islands (YE2) and Natashquan (CN02) in 2007. In 2008, we sampled the buoys YE6 and YE9 from the Magdalen Islands (instead of YE2) and we added buoys CM5 from Havre St-Pierre and CK1 from Natashquan. Thus, it was

not possible to use replicate buoys on all sites each year. The case arising, the replicates were selected in order to minimize the distance between the buoys.

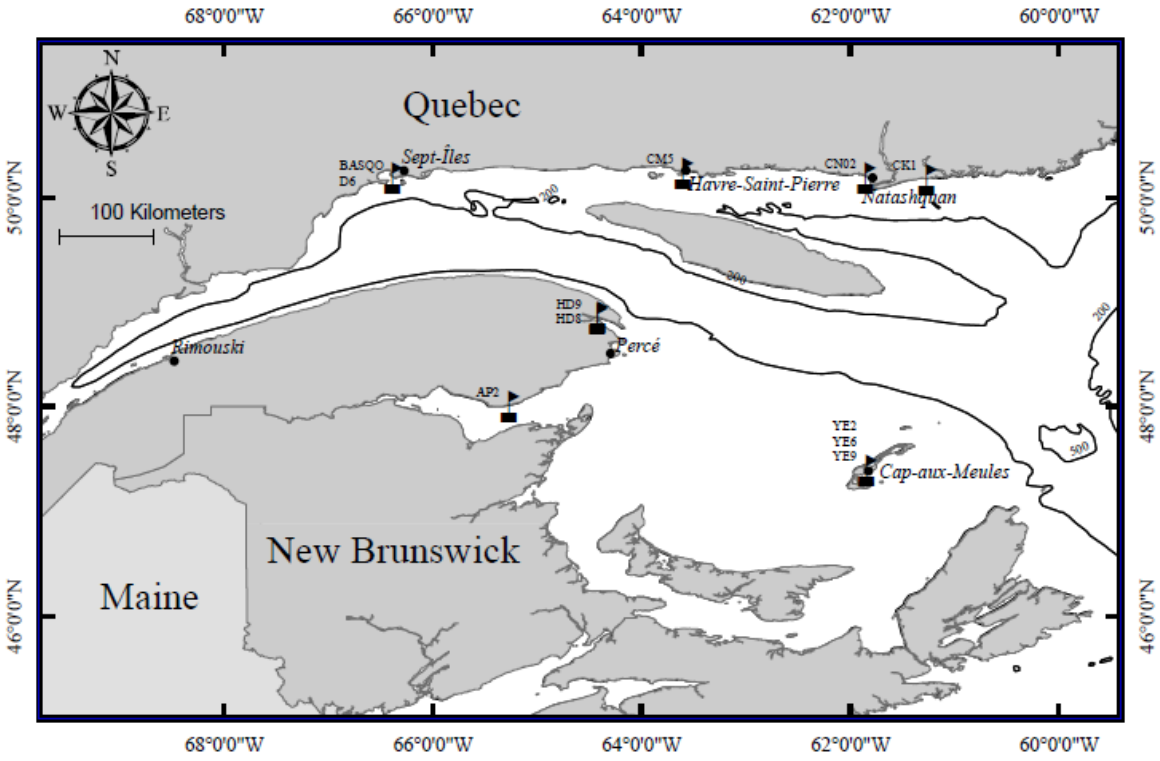


Figure 3.1: Location of buoy mooring sites. Projected coordinate system used was NAD1983MTM7

Sampling on buoys was done to collect the largest possible variability of biomass of mussels, provided that percent mussel cover was 100 %. Circular cutters (100 cm^2 surface area) were used to collect 20 samples from the body and 5 samples from the chain in 2007. In 2008, we collected 30 samples from each buoy, 10 from the body, 10 from the leg and 10 from the chain (Fig. 3.2).

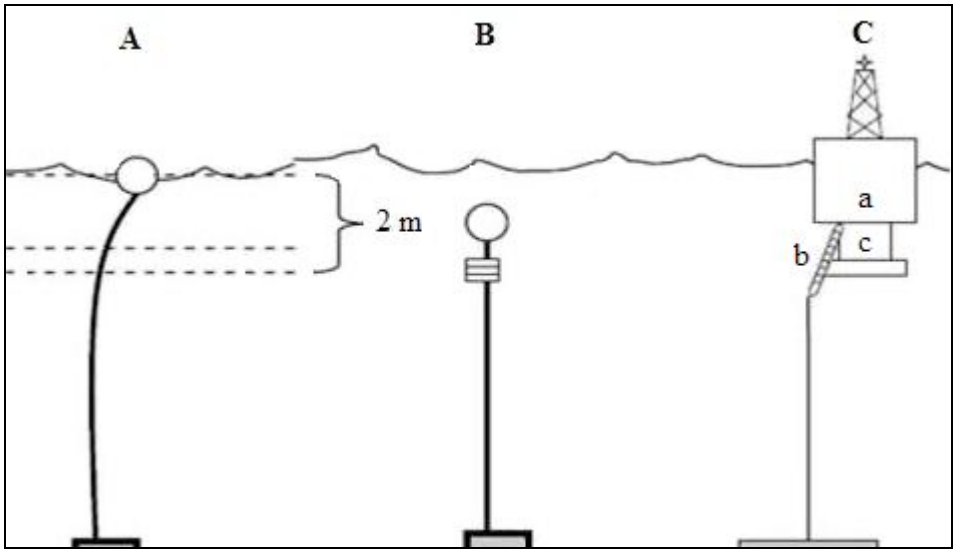


Figure 3.2: Schematic view of experimental setup. A, standard collector; B, Cage; C, Buoy made of three parts: body (a), chain (b) and leg (c). Cages were installed near the collectors to monitor mussel growth in the absence of competition. The distance between the buoy and the collector was about 500 m

For each site, there were three standard collectors. They were installed in mid-July. From the third week of August, they were visited every 15 days until mid-November. Three cages with 50 mussels per cage were installed near the collectors in mid-August and acted as controls to ensure that growth on collectors was density-independent. The size of caged mussels was at least 0.5 cm (shell length). Growth of cage mussels was monitored according to the same schedule than mussels from collectors. The experimental design is sketched in Fig. 3.2. We postulate that mussel fixation occurred at the same time within the two substrates (collectors vs buoys) in spite of the delay separating their installation.

Two sections of 10 cm each were sampled from the collectors at the level of 1 and 2 m. All samples were cleaned with seawater and sieved through 2 mm mesh. For each sample, we measured density (N) and total biomass (B).

3.2.2 Size structure and length-mass relationship

For each buoy, we haphazardly chose four samples from the body and two samples from the chain to examine size structure. We picked two samples, one from the body of the buoy and another from the chain to estimate the length-mass relationship ($W = a\ell^b$, where W is individual whole mussel mass, ℓ is shell length, a and b are adjusted parameters). We measured 100 individuals from each sample. Individual mussel length (distance from umbo to posterior margin) was measured with a vernier calliper and mussels were weighed after thawing and blotting to remove excess water.

3.2.3 Temperature

Temperature was monitored in each site from July to the end of November. VEMCO (Halifax, Nova Scotia) minilog temperature-depth recorders were attached to two collectors and two cages at 1-2 m depth. Sampling interval was 2h.

3.2.4 Fluorescence and Particulate Organic Matter (POM)

Preliminary sampling in 2006 suggested that spat size may vary according to location on buoys, with spat on the chain being larger than on the body of buoys. This pattern correlated with depth. In order to test whether the effect of depth might be related to the vertical distribution of seston, we monitored the change in abundance of phytoplankton and particulate organic matter (POM) with depth. We collected water samples at 1 m, 2 m and 4 m depth for 10 h on two consecutive days near Gaspé and Havre-aux-Maison (Magdalen Islands), near buoys HD8 and YE2, respectively. Sampling time step was every 20 minutes for fluorescence and every 4 hours for particulate organic matter. Fluorescence was measured after Phinney & Yentsch (1985). POM was estimated following Conover (1966).

3.2.5 Determination of growth parameters

Buoy mussels

Mussel size at time *t* may be inferred from the B-N relationship. The B-N curve is represented by the equation described by Fréchette et al. (submitted):

$$B=Nm_0-kN^2 \quad (1)$$

where *B* is biomass, *N* is population density, m_0 is density-independent individual mussel mass and *k* is an adjusted parameter. Growth is density-independent if parameter *k* in Eq. 1 is not significantly different from 0. In this case, the B-N curve is a straight line. On the other hand, a non linear (concave) relationship implies the presence of competition. In this case, Eq. 1 is required to estimate density-independent mean individual mass. Akaike's information criterion (AIC) proposed by Akaike (Akaike, 1974) was used to test the goodness of fit of the two models (linear versus non-linear model). It is defined as:

$$AIC=2k-2Ln(L) \quad (2)$$

where *k* is the number of parameters in the statistical model, and *L* is the maximized value of the likelihood function for the estimated model. Two measures can be used to compare models: the delta AIC and Akaike weights (Mazerolle, 2006). In the present study, we used the delta AIC (Δ_i) which represents the difference between the AIC of the alternative model and the AIC of the best model. As a rule of decision, a $\Delta_i < 2$ suggests little evidence for a single « best » model. Values between 3 and 7 indicate that the alternative model assessed has considerable less support, whereas a $\Delta_i > 10$ rejects the alternative model (Burnham & Anderson, 2002).

In this study, we encountered cases where the relationship between biomass and populations density was not significant. In such instances, mean individual mass was computed as the mean of average mussel mass of each sample.

Collector and cage mussels

For collectors and cages, growth rate of mussels was estimated from the temporal trend of mean mass. Mollusc growth is generally thought to be curvi-linear, in accordance with von Bertalanffy (1938) growth dynamics. Quince et al. (2008), however, developed a theory of biphasic somatic growth in fish, based on the distinction between pre- and post-maturation growth. Pre-maturation growth was linear. In our case, growth was studied during the five first months after settlement. Based on studies done by Mallet and Carver (1991; 1995) and Cartier et al. (2004), it is clear that sexual maturity is not attained. So we assume that linear growth applies to our mussel group. Growth of cage mussels was measured in terms of shell length. Length data were transformed to individual mass using the length-mass relationship for every site (see annex IV for length-mass relationship parameters). Growth was assessed from the increment in mean mussel mass from date to date. It is noteworthy that the last sampling date of standard collectors was 2-5 days earlier than buoys. Nevertheless, in 2008, this delay was about one month in the Magdalen Islands. So mean individual mass was estimated by linear extrapolation.

3.2.6 Statistical analyses

Temperature

Because of missing data for 2007 for the North Shore due to a loss of thermographs, a first analysis was conducted with a 4-way mixed ANOVA including one random effect (year) and three fixed effects (site, substrate and month). This analysis was made for Gaspé, Paspébiac and the Magdalen Islands for both systems (cages and collectors) and included

only four months (from August to November) in 2007 and 2008. In 2008, thermographs were installed in Île St-Charles to ensure the provision of data from the North Shore in case of loss of some thermographs. This allowed a second analysis to be made for six sites (Gaspé, Paspébiac, Magdalen Islands, Havre St-Pierre, Sept-Îles and Île St-Charles), using instruments from collectors only. The data series spanned four months, from August to November of 2008. An ANOVA was used with two fixed classification criteria (site and month).

Fluorescence

Each site was analyzed separately. Because fluorescence was sampled as a time series, we used 2-way repeated measures ANOVA with time and depth as fixed effects. The raw data were not normally distributed. A square root was the best transformation to meet the assumptions of ANOVA. To validate the findings of the analysis, we also tested the data using a nonparametric analysis.

Comparison of mean individual mass between buoys and collectors

The analysis aimed at comparing the mean individual mass between substrates, sites and years. The substrates tested were the standard collectors and the navigation buoys. Mussel biomass varied between the different parts of the buoy (body, chain and leg). Therefore, every part was considered as a substrate. So, we had four different substrates represented by collector, body, chain and leg. This analysis was done for 2007 and 2008, allowing the study of the effect of annual variability on spat growth. The analysis was made taking into account the possible dependence among substrates within a single buoy (body, chain and leg). A mixed model of ANOVA was therefore used for data analysis with four factors: substrates, sites and years as fixed factors and the buoy as random factor. The best transformation to satisfy the basic assumptions of the ANOVA was the power 0.15

(biomass$^{0.15}$). However, the log transformation was chosen because it was very close to the optimum transformation and it is generally used in the literature for mass data analysis.

Comparison of mass growth rate between cages and collectors

In order to ensure that growth on collectors was density-independent, mass growth rate was compared between cages and collectors among the different sites. A repeated measures ANCOVA model was applied with "site" and "rearing system" as fixed effects and with "year" and "site*rearing system" as random effects. Integrating these sources as random allowed taking into account the dependency between measurements in time on the same rearing system.

3.3 RESULTS

3.3.1 Environmental variables

Temperature

In the Magdalen Islands, temperature averaged 21-22°C until early August and subsequently decreased to roughly 7-8°C by early November (Fig. 3.3). In Gaspé, temperature averaged 15-17°C until mid-September and afterwards decreased to about 5°C by the end of the series. During the decreasing phase, temperature appeared to be systematically 1-2°C lower in Gaspé than in the Magdalen Islands. On the North Shore, temperature averaged between 10-15°C until early August and decreased to about 2°C in November.

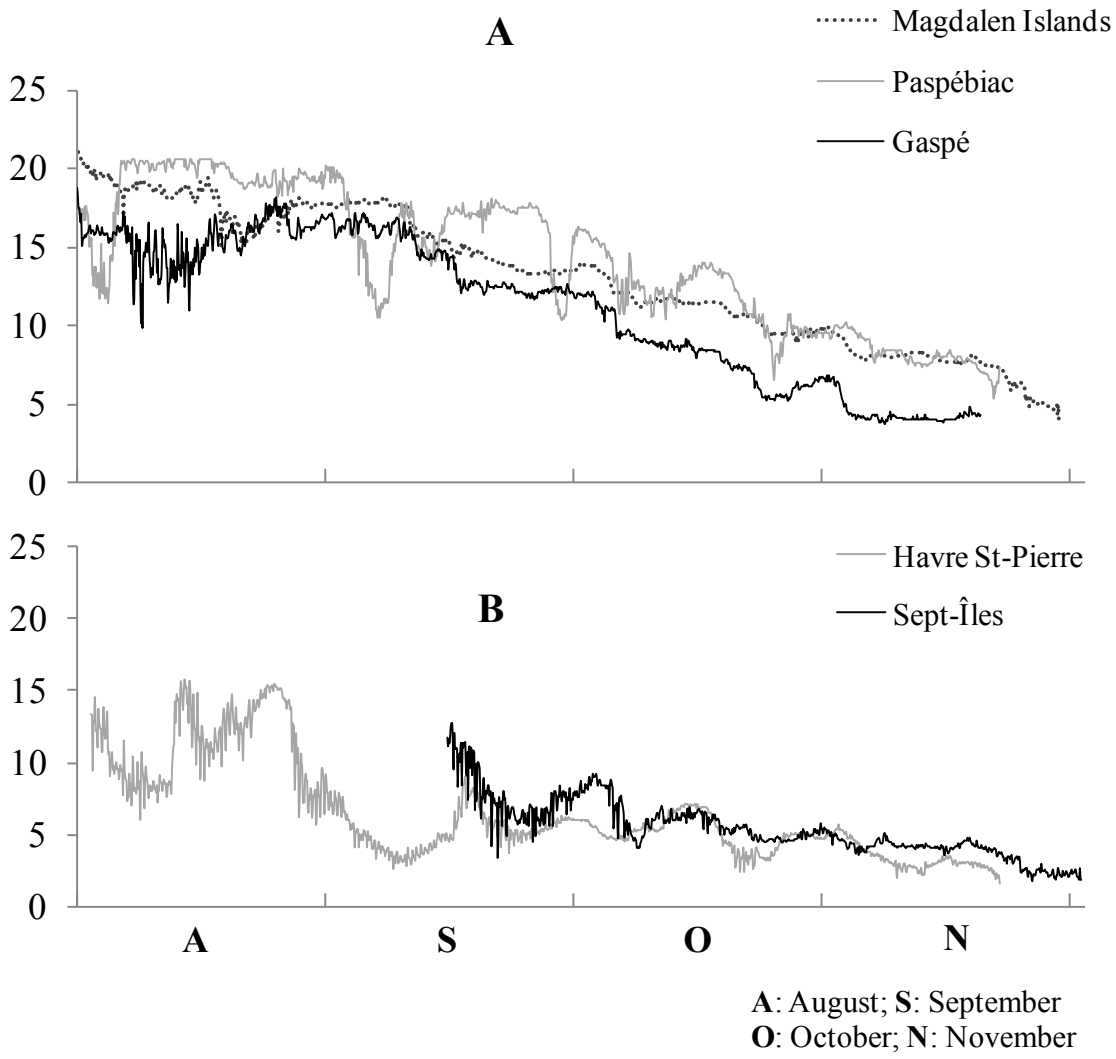


Figure 3.3, A-B: Temperature (°C) time series in 2008. A, Magdalen Islands, Gaspé and Paspébiac; B, Sept-Îles and Havre St-Pierre

Monthly averages were calculated to facilitate the analysis of temperature variation among rearing sites. The analysis of the 2007 records revealed the presence of a triple interaction between sites, rearing systems and months (Table 3.1). This means that the difference observed between the two rearing systems was not the same for all sites and months.

Table 3.1: Effect of site, rearing system and month on temperature in 2007. ANOVA with three fixed factors (site, rearing system and month) and one random factor (year)

Effect	Num DF	Den DF	F value	Pr > F
Site	2	65	72.61	<.0001
Rearing system	1	65	1.45	0.2330
*Site*Rearing system*	2	65	4.99	0.0096
Month	3	65	2438.66	<.0001
*Site*Month*	6	65	13.83	<.0001
*Rearing system*Month*	3	65	4.15	0.0094
*Site*Rearing system*Month*	6	65	2.84	0.0160

The least squares means of temperature are shown in Table 3.2. Differences among rearing systems, within sites, were tested. Temperature was higher at the collectors than at cages in Paspébiac. The opposite was observed in Gaspé. These results reflect intra-site differences. However, biologically, we can not consider that difference since it was between 0.3 and 1°C. On the other hand, temperature was significantly higher in the Magdalen Islands than in Paspébiac and Gaspé.

Table 3.2: Least squares means of temperature within sites for the two rearing systems (First analysis)

Site	Substrate	Estimate	Standard Error
Magdalen Islands	*Cage*	12.7069	0.2952
	Collector	12.6419	0.2952
Paspébiac	*Cage*	10.5995	0.2984
	Collector	11.6038	0.2984
Gaspé	*Cage*	10.2245	0.2984
	Collector	9.9287	0.2952

Table 3.3: Effect of site and month on temperature **3.3:** in 2008. ANOVA with site and month as fixed factors

Effect	Num DF	Den DF	F value	Pr > F
Site	5	24	102.44	<.0001
Month	3	24	800.75	<.0001
*Site*Month*	15	24	11.91	<.0001

There were significant differences in temporal variability of temperature in 2008, as shown by the significant Site*Month interaction (Table 3.3).

Fluorescence

Generally, fluorescence increased with time. Patterns along the depth of the water column were unclear (Fig. 3.4). In addition, fluorescence appeared higher in the Magdalen Islands than in Gaspé (between 1 and 8 units/L in Magdalen Islands and between 0 and 5 units/L in Gaspé).

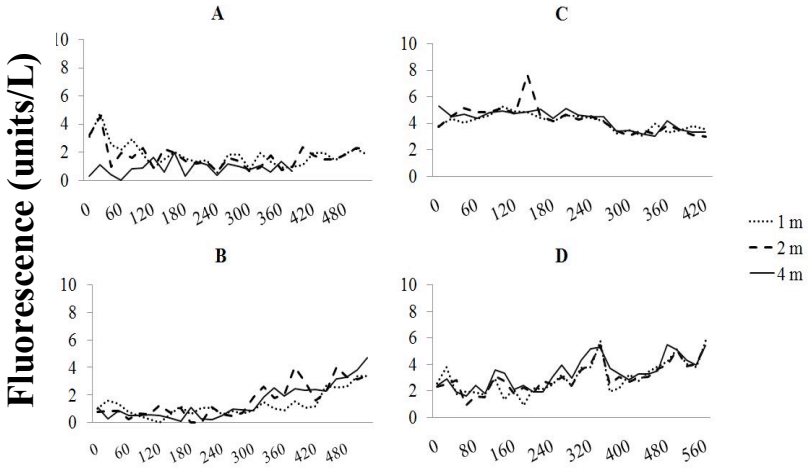


Figure 3.4, A-D: Time series of fluorescence data. A, first sampling day in Gaspé; B, second sampling day in Gaspé; C, first sampling day in Magdalen Islands; D, second sampling day in Magdalen Islands

The data were heteroscedastic and non-normally distributed. Therefore, they were transformed using the square root transformation. Since the basic assumptions of the ANOVA were not satisfied, a nonparametric analysis on the raw data was produced. Both analyses led to the same conclusions.

For Gaspé day 1 and day 2 samplings and the Magdalen Islands day 2, there was a significant interaction between time and depth ($P<0.05$) (Table 3.4). This implies that differences among depths varied through time. On day 1 in the Magdalen Islands, the Time*Depth interaction was not significant. However, the effect of depth alone was not significant ($P=0.5077$).

Table 3.4: Variability of fluorescence according to site, depth and sampling day. ANOVA with time and depth as fixed effects. Each day was tested separately

Site	Day	Effect	Num DF	Den DF	F value	Pr > F
Gaspé	1	*Time*	26	78	3.49	< .0001
		Depth	2	3	26.10	0.0127
		*Time*Depth*	52	78	1.59	0.0321
	2	*Time*	26	77	28.96	< .0001
		Depth	2	3	2.98	0.1938
		*Time*Depth*	52	77	4.35	< .0001
Magdalen Islands	1	*Time*	21	63	7.95	< .0001
		Depth	2	3	0.86	0.5077
		*Time*Depth*	42	63	1.24	0.2158
	2	*Time*	28	84	20.06	< .0001
		Depth	2	3	6.18	0.0127
		*Time*Depth*	56	84	1.60	0.0321

Particulate organic matter (POM)

Patterns in particulate organic matter concentration are shown in Fig. 3.5.

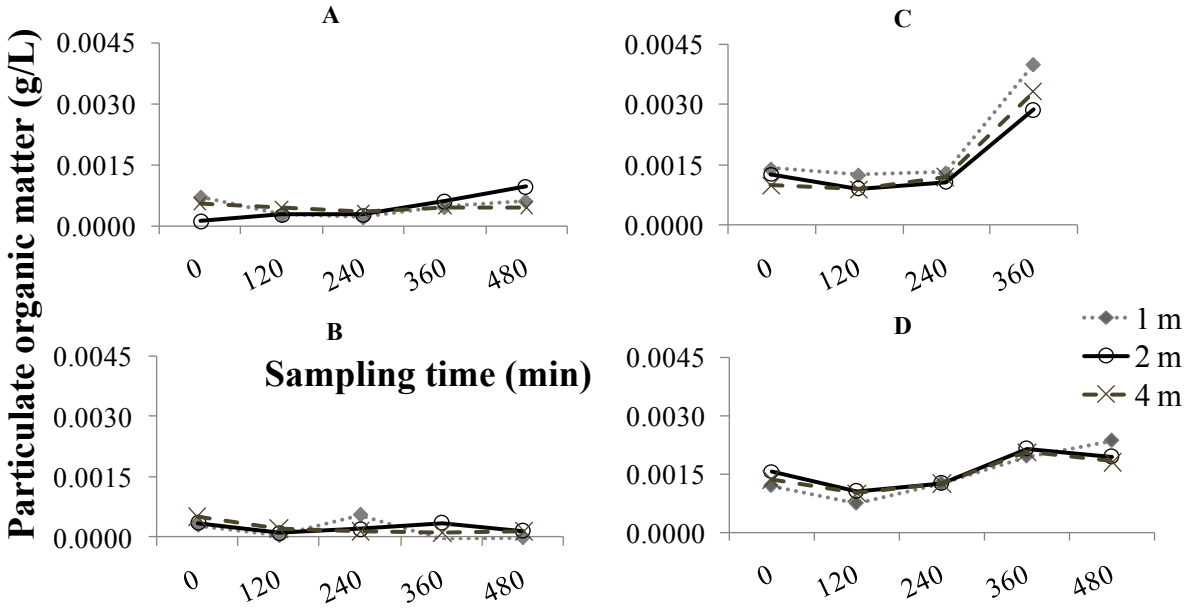


Figure 3.5, A-D: Particulate organic matter (POM) for the two sampling days in Gaspé and Magdalen Islands. A, first sampling day in Gaspé; B, second sampling day in Gaspé; C, first sampling day in Magdalen Islands; D, second sampling day in Magdalen Islands

There was no obvious trend in particulate organic matter concentration along the the depth of the water column. There was an increasing trend through time in three of the four series, but during day 2 in Gaspé, there was no obvious trend through time (Fig. 3.5B). Furthermore, it appears that POM was higher in the Magdalen Islands than in Gaspé (between 0.0010 and 0.0016 g/L in Magdalen Islands and between 0.0001 and 0.0006 g/L in Gaspé). These patterns were not tested statistically given the small number of data.

3.3.2 B-N relationships for buoys

The relationships between total fresh biomass (B) and population density (N) of live mussels obtained for samples collected from each buoy in 2007 and in 2008 are illustrated in Figs. 3.6 and 3.7. Figure 3.6 suggests that in general, the relationship between biomass and density for the body of the buoys was linear. However, for most chains, the relationships were not as tight as for the body. Although most appeared generally linear despite only 5 samples had been collected for each chain in 2007. A possible exception was AP2, where the B-N relationship was not quite clear for the chain. In 2008 (Fig. 3.7), we also found linear relationships for the different parts of buoys in most sites. Yet in other cases population density appeared to have too small scatter to allow any pattern to emerge (e.g., HD8, chain samples and HD9, body and leg samples). On the other hand, population density on the body was the lowest compared to the chain and the leg, especially in the Magdalen Islands.

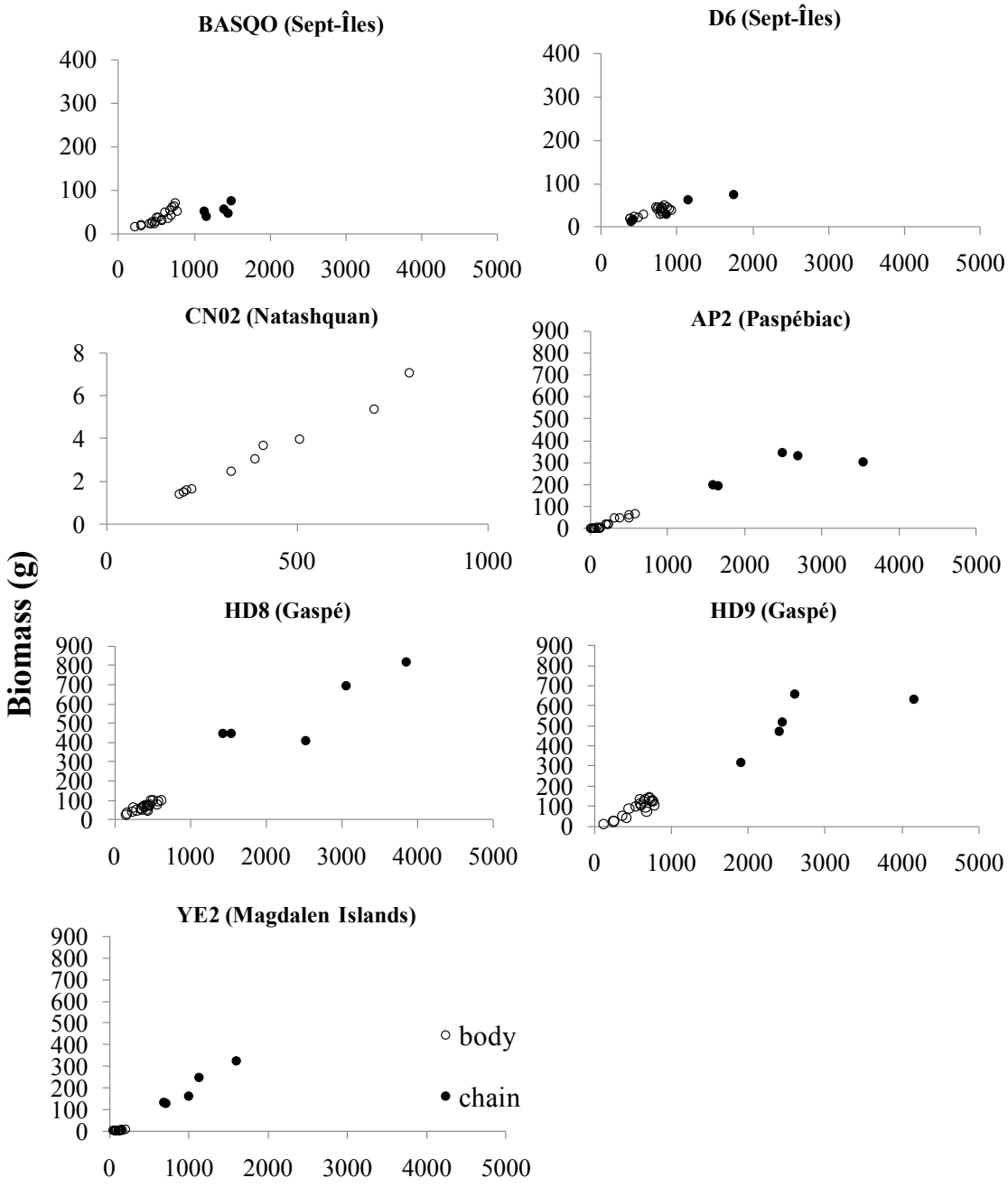


Figure 3.6: *Mytilus* spp. biomass-density relationships for all sizes of mussels for the different buoys sampled in 2007. Scale is not the same for all figures

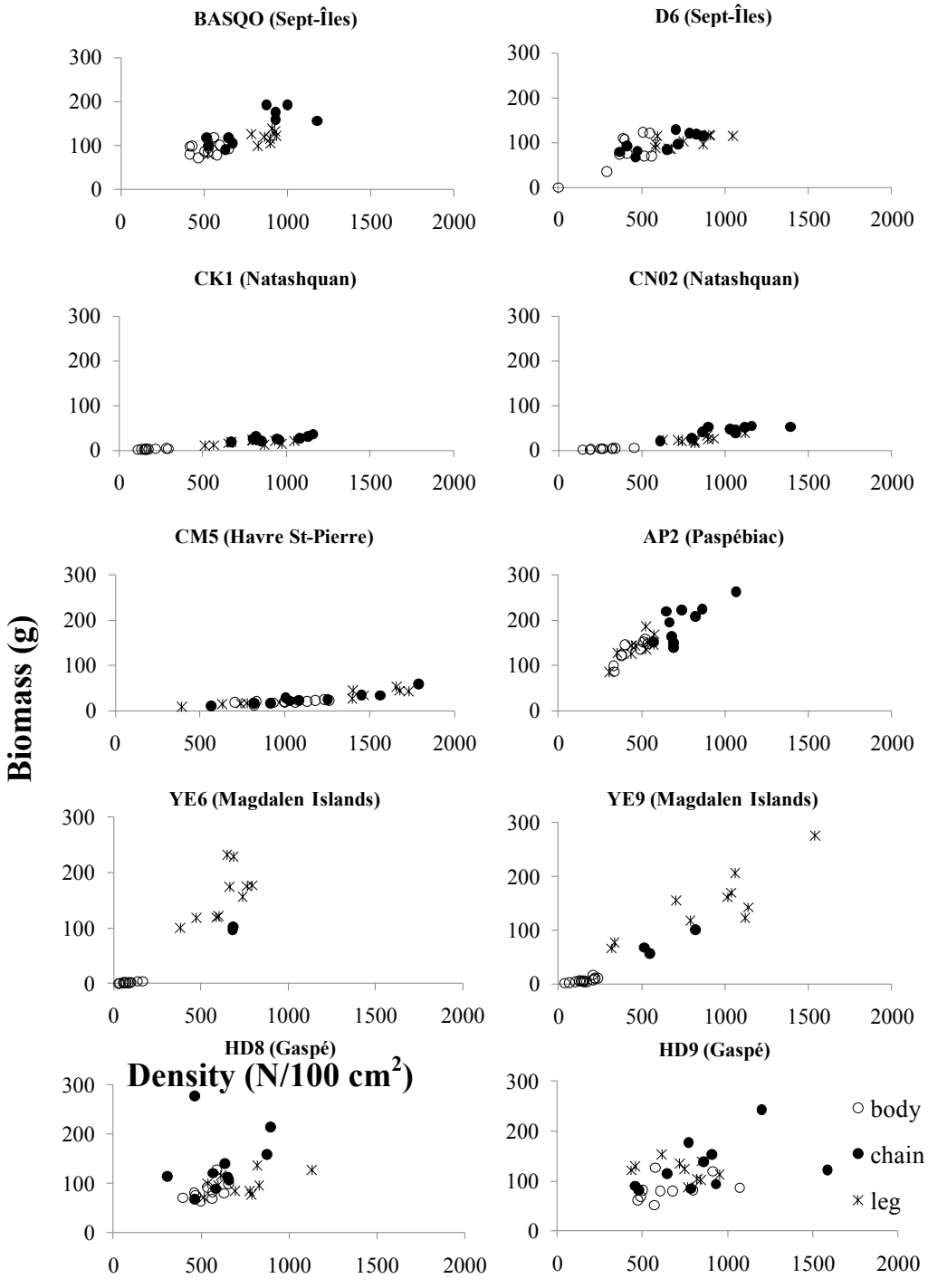


Figure 3.7: *Mytilus* spp. biomass-density relationships for all sizes of mussels for the different buoys sampled in 2008

The strength of the B-N curves was measured by Kendall's τ_b (Tables 3.5 and 3.6). Linearity of the B-N relationships was verified by the delta AIC (Δ_i).

Table 3.5: Correlation between biomass and density and delta AIC (Δ_i) for the different parts of buoys in 2007. Nonsignificant Kendall's τ_b was not represented

Substrate	Kendall's τ_b	Prob > r	Δ_i	Preferred model
CN02-body	1.0000	< .0001	2.0320	Linear
HD8-body	0.7789	< .0001	0.7210	Linear
HD9-body	0.5368	0.0009	2.0000	Linear
YE2-chain	0.8000	0.0500	2.0010	Linear
YE2-body	0.7651	< .0001	2.0010	Linear
AP2-body	0.8736	< .0001	2.0010	Linear
BASQO-body	0.8315	< .0001	2.0020	Linear
D6-chain	0.5111	0.0397	2.0010	Linear

B-N relationships were linear as demonstrated by the value of delta AIC which was inferior or equal to 2 for the two sampling seasons. However, two exceptions were noted in 2008 (Table 3.6) for AP2-body (Paspébiac) and D6-chain (Sept-Îles) where non-linear model was more appropriate.

Table 3.6: Correlation between biomass and density and delta AIC (Δ_i) for the different parts of buoys in 2008. Nonsignificant Kendall's τ_b was not represented

Substrate	Kendall rank	Prob > r	Δ_i	Preferred model
CK1-chain	0.5555	0.0253	1.2340	Linear
CK1-body	0.7333	0.0032	1.7600	Linear
CM5-chain	0.777	0.0017	2.0040	Linear
CM5-leg	0.733	0.0032	2.0050	Linear
CM5-body	0.6727	0.0040	0.2110	Linear
CN02-chain	0.5843	0.0196	1.7180	Linear
CN02-leg	0.4045	0.1060	2.0020	Linear
CN02-body	0.7333	0.0032	2.0000	Linear
YE6-leg	0.6000	0.0157	1.8060	Linear
YE6-body	0.8807	0.0002	2.0010	Linear
YE9-leg	0.6000	0.0157	1.9740	Linear
YE9-body	0.5865	0.0025	2.0000	Linear
AP2-chain	0.4222	0.0892	1.6000	Linear
AP2-leg	0.6292	0.0119	1.5160	Linear
AP2-body	0.7222	0.0067	5.4000	Non-linear
BASQO-chain	0.4045	0.1060	0.3160	Linear
BASQO-leg	0.4666	0.0603	1.9690	Linear
D6-chain	0.5111	0.0397	2.8150	Non-linear

3.3.3 Size structure

In order to avoid redundancies, only the most pertinent frequency distributions of mussel size were inserted in the present paper (Fig. 3.8), the remaining can be found in annex II and annex III.

Generally speaking, the distribution patterns were similar for Gaspé, Paspébiac and Magdalen Islands buoys. Mussel size ranged from 0.5 cm to 3 cm (with the exception of mussels from the body which did not exceed 1.1 cm in the Magdalen Islands). For Sept-Îles, mussel size ranged from 0.5 to 1.7 cm. On the North Shore, maximum size was 1.5 cm.

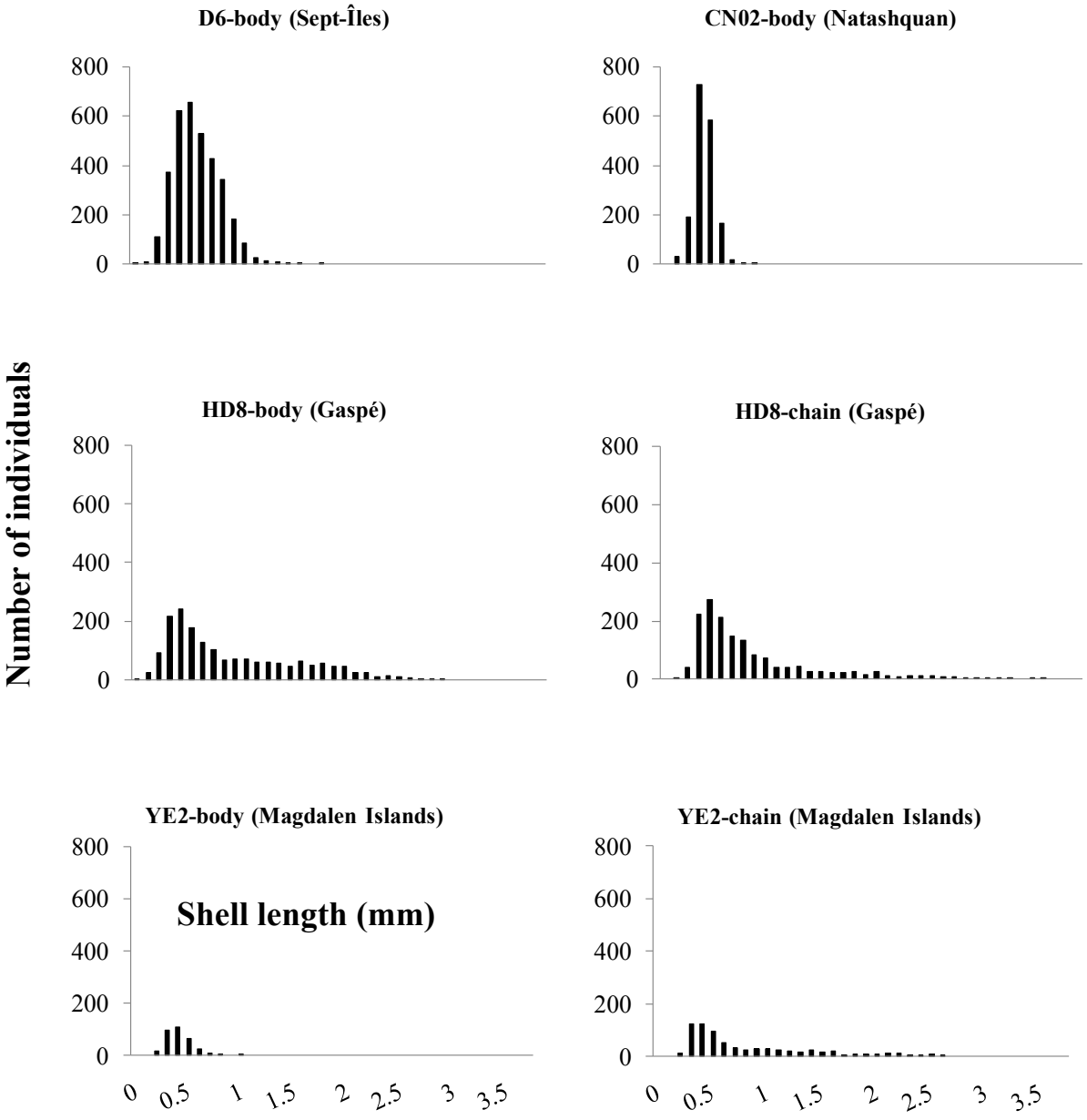


Figure 3.8: Representative size structure distribution for some buoys sampled in 2007

As a rule, size distributions were skewed to the right both in 2007 and 2008. Size structure appeared unimodal in Sept-Îles (BASQO and D6), Natashquan (CN02) and Magdalen Islands (YE2-body) in 2007 (see annex II). The situation was less clear for Paspébiac (AP2) and Gaspé (HD8 and HD9), however, as a rather long tail appeared at the right of the plots, indicating the presence of large but rare animals, as we noticed for the chain in the Magdalen Islands. In 2008, size structure on the leg showed the same trends as on the chain. A unimodal distribution appeared in the North Shore (CK1, CN02, CM5) and in Sept-Îles (BASQO, D6), suggesting the presence of a single cohort on each buoy. For Gaspé (HD8, HD9), Paspébiac (AP2) and Magdalen Islands (YE6, YE9), several modes were detected either at the leg, the body and/or at the chain revealing the co-existence of several size groups in the same mussel population (see annex III).

3.3.4 Comparison of growth between the two substrates (buoys versus collectors)

The measure of the mean individual mass of spat was estimated from the slope of the B-N relationships in case of straight line. When the relationship between biomass and populations density was not significant, mean individual mass was computed as the mean of average mussel mass of each sample. For the collectors, mean individual mass at the time of buoy retrieval was estimated by linear extrapolation. We recall that the different parts within a buoy (body, chain and leg) were considered as substrates. Results of the mixed model of ANOVA are shown in Table 3.7. Substrates, sites and years were considered as fixed factors and buoys as a random factor.

Table 3.7: Comparison of the mean individual mass of mussel spat between the different substrates (body, chain and leg of buoys, and collector), sites and years (2007 and 2008). Mixed model of ANOVA with substrates, sites and years as fixed factors and buoys as a random factor

	Num DF	Den DF	F value	Pr > F
Site	4	7	4.49	0.0411
Substrate	3	499	26.65	< .0001
*Site*Substrate*	11	499	9.53	< .0001
Year	1	499	25.47	< .0001
*Site*Year*	4	499	22.74	< .0001
*Substrate*Year*	2	499	10.76	< .0001
*Site*Substrate*Year*	6	499	17.34	< .0001

The significant Site*Substrate*Year interaction shows that the final mean individual mass varied significantly with the substrate, the site and the year (P<0.0001; Table 3.7). Results of the pairwise comparisons between the different substrates, sites and years of the mean individual mass are inserted in Table 3.8.

Table 3.8: Multiple comparisons of the mean individual mass of mussel spat between substrates within site and year. Letters represent the different substrates: A, body; B, chain; C, leg; D, collector. Underline indicates that mean individual mass is not significantly different among substrates

	Minganie	Gaspé	Magdalen Islands	Paspébiac	Sept-Îles
2007		A D B	A D B A D B	A D B A D B	A D B A D B
2008	A B C	A C D B A C D B	A B D C A B D C	A B C D	A B C D

No significant difference was detected between the collectors and the different parts of the buoy for any of the sites and in 2007 and 2008. However, in Paspébiac (2007), mean individual mass of mussel spat was significantly different between the chain and the body. However all comparisons appeared to be non significant in 2008 for this site. Likewise no significant difference was detected in Sept-Îles in both years except between chain and body in 2007 ($P=0.0100$). In Minganie, in 2008, pairwise comparisons between the different substrates were all significant. In Gaspé, only the chain vs body interaction was significant in 2008 ($P=0.0372$). In the Magdalen Islands, while the chain vs body interaction was significant in 2007 ($P<0.0001$), the comparisons became significant for chain vs leg ($P=0.0043$) and leg vs body ($P=0.0005$) in 2008. In conclusion, the most consistent significant difference appeared to be between body and chain in different sites during the two observation years.

3.3.5 Comparison of mass growth rate between cages and collectors among sites

The results of the ANCOVA comparing growth rates between cages and collectors are illustrated by the Table 3.9.

Table 3.9: Comparison of mass growth rate between cages and collectors among sites. Repeated measures ANCOVA with "Site'' and "Rearing system" as fixed effects and "year" as random effect, with sampling day as covariate

Effect	**DF**	**F value**	**Pr > F**
Site	3	8.14	0.0011
Rearing system	1	58.16	<.0001
*Site*Rearing system*	3	8.14	0.0011
Between subjects Error	19		
Sampling day	1	485.85	<.0001
*Sampling day*Site*	3	3.97	0.0104
*Sampling day*Rearing system*	1	0.39	0.5346
*Sampling day*Site*Rearing system*	3	7.20	0.0002
Within subjects Error	92		
Corrected total	126		

Sampling day*Site*Rearing system shows that mass growth rate of mussel spat varied significantly with the sampling day, the site and the rearing system ($F_{7,20}$=7.20, P=0.0002). The model revealed that all factors (site, rearing system and sampling day) included have significant individual effect.

Table 3.10 shows the estimation of log-mass growth rate for all sites and substrates. The multiple comparisons demonstrated that there was a difference between the two systems in the growth rate for all the sites except for Gaspé (Table 3.11). The growth rate was significantly greater on collectors than in cages for Magdalen Islands and Paspébiac, while the inverse occurred in Sept-Îles (Table 3.10).

Table 3.10: Estimation of log-mass growth rate (g/day) for all sites and rearing systems

Site	Substrates	Estimate	Standard Error
Magdalen Islands	*Cage*	0.0280	0.0028
	Collector	0.0367	0.0028
Gaspé	*Cage*	0.0321	0.0027
	Collector	0.0301	0.0041
Paspébiac	*Cage*	0.0213	0.0027
	Collector	0.0387	0.0039
Sept-Îles	*Cage*	0.0284	0.0044
	Collector	0.0108	0.0047

Table 3.11: Effect of rearing system on mass growth rate. Within-site comparisons

Label	Num DF	Den DF	F value	Pr > F
Magdalen Islands	1	92	4.71	0.0325
Gaspé	1	92	0.17	0.6769
Paspébiac	1	92	12.99	0.0005
Sept-Îles	1	92	7.36	0.0080

Table 3.12: Effect of site on mass growth rate of collector mussels

Label	Num DF	Den DF	F value	Pr > F
Magdalen Islands vs Gaspé	1	92	1.80	0.1826
Magdalen Islands vs Paspébiac	1	92	0.16	0.6895
Magdalen Islands vs Sept-Îles	1	92	22.14	< .0001
Gaspé vs Paspébiac	1	92	2.30	0.1325
Gaspé vs Sept-Îles	1	92	9.43	0.0028
Paspébiac vs Sept-Îles	1	92	20.51	< .0001

Table 3. 13: Multiple comparisons of mass growth rate between sites for cages

Label	Num DF	Den DF	F value	Pr > F
Magdalen Islands vs Gaspé	1	92	1.05	0.3079
Magdalen Islands vs Paspébiac	1	92	2.83	0.0960
Magdalen Islands vs Sept-Îles	1	92	0.00	0.9515
Gaspé vs Paspébiac	1	92	7.51	0.0074
Gaspé vs Sept-Îles	1	92	0.52	0.4714
Paspébiac vs Sept-Îles	1	92	1.82	0.1811

Within the rearing system "collector", the growth rate in Sept-Îles was significantly lower than in other sites, the latter not being significantly different from one another (Table 3.12). Table 3.13 shows that within the rearing system "cage" the only significant difference was between Gaspé and Paspébiac, the former having a higher growth rate than the latter (F=7.51; P=0.0074).

3.4 DISCUSSION

Blue mussel populations differ frequently in growth rate and individual size (Mallet, et al., 1987b). In the present study, mean individual mass at the moment of buoy retrieval did not vary among collectors and buoys. In spite of obvious physical differences between substrates (rope versus surface), spat growth was comparable. This result, coupled with the linearity of the B-N relationships, leads to the conclusion that the approach of the monitoring of spat mussel growth based on navigation buoys was not biased by density-dependent growth on buoys although self-thinning was sometimes reported on buoys (Ardisson & Bourget, 1991). In the present study, the B-N relationships were used to assess the presence of competition between mussels, which is considered as a source of biais in the estimation of growth parameters. The B-N curves were generally linear as demonstrated by the delta AIC (Δ_i). This finding suggests the absence of density-dependent growth on the

buoys. Nevertheless, some exceptions were noted for Paspébiac and Sept-Îles in 2008. Yet, the delta AIC was inferior to 10 indicating that the linear model is less supported but not very unlikely (Burnham & Anderson, 2002). Fréchette et al. (submitted) showed that in cases where populations are composed of more than one cohort, biomass-density curves are either concave or convex depending on the relationship between the abundances of the cohorts. In cases where the B-N curve is not linear, this could mistakenly lead to conclude that density-dependant growth is occurring. Therefore, samples with mixed cohorts can yield linear, concave or convex biomass-density curves without density-dependent growth. On the other hand, mass growth rate was significantly greater in collectors than in cages for Magdalen Islands and Paspébiac. This result indicates the absence of competition knowing that this phenomenon did not occur in cages due to the reduced number of mussels. An exception was found in Sept-Îles where mass growth rate was greater in cages. This finding is unclear especially since Fréchette et al. (submitted). found no evidence of intraspecific competition between mussels cultured on spat collectors, 27 months after settlement.

Differences observed in mussel size within a sample could be explained by the fact that mussels located at the surface have easier access to food than mussels located deep in the mussel bed. Indeed, Myrand et al. (2009) observed a positive correlation between mussel growth and multi-locus heterozygosity (MLH). This means that heterozygous individuals have better growth and survival than homozygous ones. Such result may be explained by the fact that heterozygous mussels are more active in maintaining their position at the periphery of the sleeves as proved by Myrand et al. (2009). So they have better access to food. In the same context, Ben Salah et al. (in prep.) showed that correlations between mussel size and a pigment which accumulates with time, lipofuscin, were not significant in any of the present sites except for a small-amplitude trend in Magdalen Islands. This trend was not consistent with age-related differences in size. Therefore, lipofuscin analysis rejected the hypothesis of the presence of more than one cohort. The presence of small animals near the surface of buoys was probably related to the variability of the species performance.

Mean individual mass varied within the different parts of the buoy. Population density on the body was the lowest compared to the chain and the leg (Figs. 3.6 and 3.7). Knowing that growth is highly correlated with food abundance (Page & Hubbard, 1987), we assessed whether vertical structure may explain such pattern. So we monitored the small-scale variability of fluorescence and particulate organic matter (POM) (Figs. 3.4 and 3.5). Analysis of fluorescence showed a significant interaction between time and depth. This means that vertical structure in fluorescence was not stable over time and therefore may be an unlikely explanation for the effect of depth on growth. The same conclusion probably holds for POM, as no clear differences emerged among depths. Therefore the variability observed in the growth within the different parts of the buoy was not necessarily related to variability in food abundance along the water column. Nevertheless, we could not rule out this possibility because of the short period of our experience (two sampling days). This variability could be related to wave action as a direct cause of stress to mussel spat. Our field observations allowed us to note that dislodgement of mussels colonizing the body required more strength compared to mussels from the leg and the chain. Therefore, mussels probably responded to the risk of dislodgment by increasing their attachment strength at wave exposed substrates (Lachance, et al., 2008; Carrington, et al., 2009). Studies done on the species *M. galloprovincialis* colonizing the west coast of South Africa demonstrated that growth rate declined at sites with extreme wave action (Steffani & Branch, 2003). Knowing that the body is the most exposed to the wave action, we can suggest wave effect to explain mussel growth variability between the body and the other parts of the buoy. Ardisson and Bourget (1991) used navigation buoys to estimate the production of the blue mussel *M. edulis* . In accordance with the method of Fradette and Bourget (1980, 1981) and Ardisson et al. (1990), they sampled one quadrat on each buoy. Results of this investigation showed that Gaspé Peninsula and the Western North Shore zones are the more productive in the Estuary and Gulf of St. Lawrence. Knowing that mean individual mass of mussel spat varied within the different parts of the buoy, we can suggest that the variability of mussel growth may be related to the variability of the sampling area of the buoy in addition to site effects.

Bourget et al. (2003) showed that biomass of epibenthic assemblages is influenced by environmental factors. Indeed, water temperature, salinity, current velocity and primary production are the main factors influencing mussel biomass. The dependence of mussel growth on water temperature has been extensively investigated (Kautsky, 1982; Page & Hubbard, 1987; Sukhotin & Kulakowski, 1992). In the present study, temperature was monitored during two experimental seasons. In fact, temperature was higher in Paspébiac, Magdalen Islands and Gaspé than along the North Shore sites. The presence of upwellings as well as the Labrador Current may explain the lowest temperature in these areas (Koutitonsky & Bugden, 1991). On the other hand, mean individual mass of mussel spat was the lowest in the North Shore sites. These findings sugestets that water temperature, probably, controlled mussel spat growth. On the other hand, food availability has been found to influence growth rate more than temperature (Page & Hubbard, 1987). Food supply is generally considered as a limiting factor for mussel growth (Smaal & van Stralen, 1990; Perez-Camacho, et al., 1991). Lemaire et al. (2006) reported lower phytoplankton concentration near Havre St-Pierre than near Gaspé and Carleton. Such results permitted to suggest that the higher growth rate of mussel spat observed in Gaspé, Paspébiac and the Magdalen Islands (Table 3.10) could be also related to food supply.

Although navigation buoys provide a convenient alternative to standard collectors for monitoring spatial variability in growth of mussel spat, this approach is hampered by several constraints. In fact, their heterogeneous distribution in the Gulf of St. Lawrence doesn't necessarily meet the location of the potential sites of aquaculture. However, given the large area encompassed by the buoys, they may allow the exploration of new sites. In addition, the comparison of mussel growth among years could also be affected by variability of collection schedule of coastal guard between seasons.

3.5 CONCLUSION

Our findings do not support the evidence for density-dependent growth as proven by linear relationship in the B-N curves. In addition, we found no differences between predicted mass on the collectors at the time of buoy retrieval and mean mussel mass on buoys. On the other hand, variability of mean individual mass within the same buoy confirms the importance of consideration of the substrate in prospective studies. Such results are very useful for a future program of monitoring the growth of blue mussel spat based on navigation buoys and might help understanding the aquaculture potential of the different regions of the Gulf of St. Lawrence.

3.6 ACKNOWLEDGEMENTS

Financial support was provided the Aquaculture Collaborative Research and Development Program (ACRDP), the Société de Développement de l'Industrie Maricole (SODIM) and the Réseau d'Aquaculture du Québec (RAQ). The authors also wish to thank Linda Girard, Myriam Lachance-Bernard, Mélanie Boudreau, Marilène Larocque, Nicolas Bouchard, Marine de Roumefort, Josianne Caron, Dorothée Mitchell and Bilel Chalghaf for their assistance.

CHAPITRE IV

CONCLUSION GÉNÉRALE

La grande étendue géographique du territoire aquacole potentiel du Québec constitue un puissant frein à l'acquisition d'informations critiques sur la croissance du naissain de moule. Pour pallier la situation, la croissance du naissain fixé aux bouées de navigation provenant de différents sites du Golfe du Saint-Laurent a été comparée avec celle du naissain des collecteurs standards. Contrairement à l'approche classique basée sur les collecteurs standards nécessitant plusieurs sorties en mer, ces bouées sont débarquées par la Garde Côtière en fin de saison, en un nombre de sites réduit. Dans la perspective d'un programme de monitorage, il pourrait en résulter une diminution sensible des contraintes logistiques liées à l'échantillonnage. La présente étude a révélé des résultats très comparables en termes de croissance du naissain de moule entre les bouées de navigation et les collecteurs standards. Aucune différence significative n'a été observée entre les deux substrats au niveau de la masse moyenne individuelle finale. Ceci mène à la conclusion que les bouées pourraient remplacer les collecteurs dans le suivi de la croissance du naissain. Ainsi l'approche du monitorage de la croissance du naissain de moule en se basant sur les bouées de navigation s'est révélée aussi efficace que l'approche classique, quoique des précautions s'imposent.

La masse moyenne individuelle variait d'une façon significative entre les différentes parties de la bouée, et en particulier entre d'une part le corps et d'autre part la chaîne et la colonne. Cette différence pourrait être expliquée par le mouvement des vagues induisant un stress aux moules du corps. Bien que cet effet n'ait pas été démontré dans le cadre de ce travail, cette hypothèse reste une explication possible de ces observations. Les bouées de navigation ont été utilisées dans des travaux antérieurs pour l'estimation de la production de *Mytilus edudis* à partir d'observations sur le recrutement, la croissance et les abondances. La Péninsule Gaspésienne et la région ouest de la Côte Nord représentaient les zones les plus productives de l'estuaire et du golfe du Saint-Laurent. De tels résultats pourraient être biaisés par le substrat échantillonné étant donné la variabilité de la croissance entre les

différentes parties de la bouée. La prise en considération du substrat est très recommandée dans des études futures basées sur les bouées de navigation.

L'étude du diagramme biomasse-densité (B-N) a permis de mettre en évidence l'absence du phénomène de compétition en se basant sur la linéarité des relations B-N. Le critère d'information d'Akaike (AIC) a été utilisé pour la validation du modèle linéaire. D'autre part, l'analyse de la tendance de la structure de taille a montré une distribution unimodale au niveau de la Côte Nord, révélant l'existence d'une population homogène. Toutefois, en Gaspésie et aux Îles de la Madeleine, la distribution unimodale n'était pas évidente. Cette variabilité en taille pourrait être expliquée par la présence de plusieurs cohortes. Afin de vérifier cette hypothèse, un pigment qui s'accumule avec le temps, la lipofuscine, a été utilisé comme marqueur d'âge. Nous avons démontré la variabilité de l'accumulation de ce marqueur en fonction du site d'élevage. Par contre, ce pigment ne s'est pas avéré très sensible pour départager finement les moules selon leur âge en termes de semaines. Ainsi, l'hypothèse de la présence de plus d'un groupe d'âge au sein de notre population de moules n'a pas été vérifiée. La variabilité de la taille du naissain était probablement liée à la performance de l'espèce.

En conclusion, cette étude nous permet de conclure que la variabilité spatiale de la croissance en biomasse du naissain suit le même patron que la température. La masse moyenne individuelle était plus importante en Gaspésie et aux Îles de la Madeleine suivies par les sites de la Côte Nord. Ces résultats montrent tout l'intérêt à caractériser les sites avant la mise en place d'un programme de monitorage de la croissance du naissain de la moule bleue basé sur les bouées de navigation. De plus, l'échantillonnage des bouées dépendra du calendrier de la garde côtière qui peut varier ce qui risque de compromettre la comparabilité de la croissance du naissain d'une année à l'autre. Afin de remédier à cet inconvénient, il est recommandé de coordonner avec la garde côtière la date de collecte des bouées. Un protocole d'entente avec la garde côtière est indispensable dans le cadre d'un

tel genre de programme. Et malgré que les bouées couvrent une grande étendue du golfe du Saint-Laurent, celles-ci ne sont pas réparties en fonction des sites potentiels de mytiliculture. Certes que ceci représente un défaut, mais cette approche demeure intéressante vu qu'elle permet l'exploration de nouveaux sites. De plus, elle pourrait être complémentaire de l'approche basée sur les systèmes d'information géographique (SIG). En effet, l'identification de nouveaux sites aquacoles est un problème complexe nécessitant une connaissance spatiale approfondie du milieu marin. L'application de modèles basés sur les SIG pour le choix de sites pourrait constituer un outil efficace. Cependant, la validation in situ des données acquises par les SIG est essentielle. Vu que les bouées représentent un substrat favorable à la fixation des moules, elles pourraient être utilisées pour cet effet.

RÉFÉRENCES BIBLIOGRAPHIQUES

Abele, D., Brey, T., & Philipp, E. (2009). Bivalve models of aging and the determination of molluscan lifespans. *Experimental Gerontology, 44*(5), 307-315

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control, 19*(6), 716-723

Akaishi, F. M., St-Jean, S. D., Bishay, F., Clarke, J., Rabitto, I. D., & Ribeiro, C. A. D. (2007). Immunological responses, histopathological finding and disease resistance of blue mussel (*Mytilus edulis*) exposed to treated and untreated municipal wastewater. *Aquatic Toxicology, 82*(1), 1-14

Alunno-Bruscia, M., Petraitis, P. S., Bourget, E., & Fréchette, M. (2000). Body size-density relationship for *Mytilus edulis* in an experimental food-regulated situation. *Oikos, 90*(1), 28-42

Ardisson, P.-L., & Bourget, E. (1991). Abundance, growth, and production estimation of the blue mussel *Mytilus edulis* on moored navigation buoys in the estuary and northwestern Gulf of St-Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(12), 2408-2419

Ardisson, P.-L., Bourget, E., & Legendre, P. (1990). Multivariate approach to study species assemblages at large spatiotemporal scales: The community structure of the epibenthic fauna of the estuary and gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences, 47*, 1364-1377

Arnold, W. S., White, M. W., Norris, H. A., & Berrigan, M. E. (2000). Hard clam (*Mercenaria* spp.) aquaculture in Florida, USA: geographic information system applications to lease site selection. *Aquacultural Engineering, 23*(1-3), 203-231

Beadman, H. A., Willows, R. I., & Kaiser, M. J. (2002). Potential applications of mussel modelling. *Helgoland Marine Research, 56*(1), 76-85

Belchier, M., Edsman, L., Sheehy, M. R. J., & Shelton, P. M. J. (1998). Estimating age and growth in long-lived temperate freshwater crayfish using lipofuscin. *Freshwater Biology, 39*(3), 439-446

Belchier, M., Shelton, P. M. J., & Chapman, C. J. (1994). The identification and measurement of fluorescent age-pigment abundance in the brain of a crustacean (*Nephrops norvegicus*) by confocal microscopy. *Comparative Biochemistry and Physiology, 108B*(2), 157-164

Ben Salah, I., Pellerin, J., Bourque, F., Goaziou, Y., Guay, M., Thomas, B., et al. (in prep.). Monitoring large scale spatial variability of blue mussel growth in the Gulf of St. Lawrence: testing for density dependent effects in populations settled on navigation buoys.

Ben Salah, I., Pellerin, J., Daigle, G., & Fréchette, M. (in prep.). Effect of body size and site on lipofuscin accumulation in the digestive gland of blue mussel *Mytilus* spp. spat on navigation buoys

Bluhm, B. A., Brey, T., & Klages, M. (2001). The autofluorescent age pigment lipofuscin: key to age, growth and productivity of the Antarctic amphipod *Waldeckia obesa* (Chevreux, 1905). *Journal of Experimental Marine Biology and Ecology, 258*(2), 215-235

Borthagaray, A. I., & Carranza, A. (2007). Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. *Acta Oecologica, 31*(3), 243-250

Bourget, E., Ardisson, P.-L., Lapointe, L., & Daigle, G. (2003). Environmental factors as predictors of epibenthic assemblage biomass in the St. Lawrence system. *Estuarine Coastal and Shelf Science, 57*(4), 641-652

Brunk, U. T., & Terman, A. (2002). Lipofuscin: Mechanisms of age-related accumulation and influence on cell function. *Free Radical Biology and Medicine, 33*(5), 611-619

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information theoretic approach*. (Second edition[e] éd.). New York.

Buschbaum, C., Dittmann, S., Hong, J. S., Hwang, I. S., Strasser, M., Thiel, M., et al. (2009). Mytilid mussels: global habitat engineers in coastal sediments. *Helgoland Marine Research, 63*(1), 47-58

Callier, M. D., McKindsey, C. W., & Desrosiers, G. (2007). Multi-scale spatial variations in benthic sediment geochemistry and macrofaunal communities under a suspended mussel culture. *Marine Ecology-Progress Series, 348*, 103-115

Callier, M. D., McKindsey, C. W., & Desrosiers, G. (2008). Evaluation of indicators used to detect mussel farm influence on the benthos: two case studies in the Magdalen Islands, Eastern Canada. *Aquaculture, 278*, 77-88

Carrington, E., Moeser, G. M., Dimond, J., Mello, J. J., & Boller, M. L. (2009). Seasonal disturbance to mussel beds: Field test of a mechanistic model predicting wave dislodgment. *Limnology and Oceanography, 54*(3), 978-986

Cartier, S., Pellerin, J., Fournier, M., Tamigneaux, E., Girault, L., & Lemaire, N. (2004). Use of an index based on the blue mussel (*Mytilus edulis* and *Mytilus trossulus*) digestive gland weight to assess the nutritional quality of mussel farm sites. *Aquaculture, 241*, 633-654

Castro, M., Encarnacao, P., & Tully, O. (2002). The effect of dietary antioxidants on lipofuscin accumulation in the crustacean brain. *Journal of Experimental Marine Biology and Ecology, 269*(1), 53-64

Chamberlain, J., Fernandes, T. F., Read, P., Nickell, T. D., & Davies, I. M. (2001). Impacts of deposits from suspended mussel (*Mytilus edulis*) culture on the surrounding surficial sediments. *ICES Journal of Marine Science, 58*, 411-416

Commito, J. A., Como, S., Grupe, B. M., & Dow, W. E. (2008). Species diversity in the soft-bottom intertidal zone: Biogenic structure, sediment, and macrofauna across mussel bed spatial scales. *Journal of Experimental Marine Biology and Ecology, 366*(1-2), 70-81

Commito, J. A., Dow, W. E., & Grupe, B. M. (2006). Hierarchical spatial structure in soft-bottom mussel beds. *Journal of Experimental Marine Biology and Ecology, 330*(1), 27-37

Conover, R. J. (1966). Assimilation of organic matter by zooplankton. *Limnology and Oceanography, 11*(3), 338-345

Côté, I. M., & Jelnikar, E. (1999). Predator-induced clumping behaviour in mussels (*Mytilus edulis* Linnaeus). *Journal of Experimental Marine Biology and Ecology, 235*, 201-211

Dimitriadis, V. K., Domouhtsidou, G. P., & Cajaraville, M. P. (2004). Cytochemical and histochemical aspects of the digestive gland cells of the mussel *Mytilus galloprovincialis* (L.) in relation to function. *Journal of Molecular Histology, 35*(5), 501-509

Dolmer, P. (1998). The interactions between bed structure of *Mytilus edulis* L. and the predator *Asterias rubens* L. *Journal of Experimental Marine Biology and Ecology, 228*(1), 137-150

El Sabh, M. I. (1976). Surface circulation pattern in the Gulf of St. Lawrence. *Journal of the Fisheries Research Board of Canada, 33*, 124-138

Encarnação, P., & Castro, M. (2001). The effect of fixatives in the quantification of morphological lipofuscin as an age index in crustaceans. *Hydrobiologia, 449*(1-3), 301-305

FAO. (2007). The state of world fisheries and aquaculture 2006. Fisheries and Aquaculture Department, Rome, 108 p.

Fradette, P., & Bourget, E. (1980). Ecology of benthic epifauna of the estuary and Gulf of St. Lawrence: Factors influencing their distribution and abundance on buoys. *Canadian Journal of Fisheries and Aquatic Sciences, 37*, 979-999

Fradette, P., & Bourget, E. (1981). Groupement et ordination appliquées à l'étude de la répartition de l'épifaune benthique de l'estuaire maritime et du golfe du Saint-Laurent. *Journal of Experimental Marine Biology and Ecology, 50*, 133-152

Fréchette, M., Bergeron, P., & Gagnon, P. (1996). On the use of self-thinning relationships in stocking experiments. *Aquaculture, 145*, 91-112

Fréchette, M., & Bourget, E. (1985). Food-limited growth of *Mytilus edulis* L. in relation to the benthic boundary layer. *Canadian Journal of Fisheries and Aquatic Sciences, 42*, 1166-1170

Fréchette, M., Butman, C. A., & Geyer, W. R. (1989). The importance of boundary layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. *Limnology and Oceanography, 34*(1), 19-36

Fréchette, M., Lachance-Bernard, M., & Daigle, G. (submitted). Body size, population density and factors regulating suspension-cultured blue mussel (*Mytilus* spp.) populations. *Aquatic Living Resources*

Freeman, K. R., & Dickie, L. M. (1979). Growth and mortality of the blue mussel *Mytilus edulis* in relation to environmental indexing. *Journal of the Fisheries Research Board of Canada, 36*, 1238-1249

Fuentes-Yaco, F., Vézina, A. F., Larouche, P., Gratton, Y., & Gosselin, M. (1997). Phytoplankton pigment in the Gulf of St. Lawrence, Canada, as determined by the Coastal Zone Color Scanner-Part II: multivariate analysis *Continental Shelf Research, 17*(12), 1441-1459

Gosling, E. (2003). *Bivalve Molluscs: Biology, Ecology and Culture*. Oxford.

Guillemette, M., Reed, A., & Himmelman, J. H. (1996). Availability and consumption of food by common eiders wintering in the Gulf of St Lawrence: Evidence of prey depletion. *Canadian Journal of Zoology-Revue Canadienne de Zoologie, 74*(1), 32-38

Habbane, M., El-Sabh, M. I., & Dubois, J.-M. (1997). Potentiel aquacole par télédétection passive et SIG: application aux eaux côtières de la Baie des Chaleurs (Est du Canada). *International Journal of Remote Sensing, 18*, 3439-3457

Halldòrsson, H. P., Svavarsson, J., & Granmo, A. (2005). The effect of pollution on scope for growth of the mussel (*Mytilus edulis* L.) in Iceland. *Marine Environmental Research, 59*(1), 47-64

Hartigan, J. A., & Hartigan, P. M. (1985). The DIP test of unimodality. *The Annals of Statistics, 13*(1), 70-84

Hatcher, A., Grant, J., & Schofield, B. (1994). Effects of suspended mussel culture (*Mytilus* spp.) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. *Marine Ecology-Progress Series, 115*, 219-235

Hawkins, A. J. S., Rusin, J., Bayne, B. L., & Day, A. J. (1989). The metabolic / physiological basis of genotype-dependent mortality during copper exposure in *Mytilus edulis*. *Marine Environmental Research, 28*, 253-257

Hole, L. M., Moore, M. N., & Bellamy, D. (1993). Age-related cellular reactions to copper in the marine mussel *Mytilus edulis*. *Marine Ecology-Progress Series, 94*(2), 175-179

Jones, C. G., Lawton, J. H., & Shachak, M. (1997). Positive and negative effects of organisms as physical ecosystem engineers. *Ecology, 78*(7), 1946-1957

Jonsson, P. R., Petersen, J. K., Karlsson, O., Loo, L. O., & Nilsson, S. F. (2005). Particle depletion above experimental bivalve beds: In situ measurements and numerical modeling of bivalve filtration in the boundary layer. *Limnology and Oceanography, 50*(6), 1989-1998

Ju, S. J., Secor, D. H., & Harvey, H. R. (1999). Use of extractable lipofuscin for age determination of blue crab *Callinectes sapidus*. *Marine Ecology-Progress Series, 185*, 171-179

Kautsky, N. (1982). Growth and size structure in Baltic *Mytilus edulis* population. *Marine Biology, 68*, 117-133

Kimbro, D. L., Largier, J., & Grosholz, E. D. (2009). Coastal oceanographic processes influence the growth and size of a key estuarine species, the Olympia oyster. *Limnology and Oceanography, 54*(5), 1425-1437

Kodama, K., Shiraishi, H., Morita, M., & Horiguchi, T. (2006). Verification of lipofuscin-based crustacean ageing: seasonality of lipofuscin accumulation in the stomatopod *Oratosquilla oratoria* in relation to water temperature. *Marine Biology, 150*(1), 131-140

Koukouzika, N., Raftopoulou, E. K., & Dimitriadis, V. K. (2009). Seasonal differences of lysosomal, lipid and lipofuscin parameters in the digestive gland of the mussel *Mytilus galloprovincialis*. *Journal of Molluscan Studies, 75*(3), 261–267

Koutitonsky, V. G., & Bugden, G. L. (1991). The physical oceanography in the Gulf of St. Lawrence: A review with emphasis on the synoptic variability of the motion. In: The Gulf of St. Lawrence: small ocean or big estuary? Therriault, J.-C. (ed.). *Can. Spec. Publ. Fish. Aquat. Sci*, 57-90

Kurz, T., Terman, A., Gustafsson, B., & Brunk, U. T. (2008). Lysosomes and oxidative stress in aging and apoptosis. *Biochimica et Biophysica Acta-General Subjects, 1780*(11), 1291-1303.doi:10.1016/j.bbagen.2008.01.009

Lachance, A. A., Myrand, B., Tremblay, R., Koutitonsky, V., & Carrington, E. (2008). Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. *Aquatic Biology, 2*(2), 119-129

Lane, D. J. W., Beaumont, A. R., & Hunter, J. R. (1985). Byssus drifting and the drifting threads of the young post-larval mussel *Mytilus edulis*. *Marine Biology, 84*, 301-308

Lazorthes, G., Guilhem, A., & Zadeh, J. (1990). Lipofuscine et vieillissement du neurone d'après l'étude de fragments d'écorce prélevés en cours d'opération. Dans A. Bès & G. Geraud (Dir.), *Circulation cérébrale et vieillissement* (pp. 57-59). Paris: John Libbey Eurotext Ltd.

Le Fouest, V., Zakardjian, B., Saucier, F. J., & Çizmeli, S. A. (2006). Application of SeaWIFS- and AVHRR-derived data for mesoscale and regional validation of a 3-D high-resolution physical-biological model of the Gulf of St. Lawrence (Canada). *Journal of Marine Systems, 60*, 30-50

Lemaire, N., Pellerin, J., Fournier, M., Girault, L., Tamigneaux, E., Cartier, S., et al. (2006). Seasonal variations of physiological parameters in the blue mussel *Mytilus* spp. from farm sites of eastern Quebec. *Aquaculture, 261*(2), 729-751

Lomovasky, B. J., Morriconi, E., Brey, T., & Calvo, J. (2002). Individual age and connective tissue lipofuscin in the hard clam *Eurhomalea exalbida*. *Journal of Experimental Marine Biology and Ecology, 276*(1-2), 83-94

Longdill, P. C., Healy, T. R., & Black, K. P. (2008). An integrated GIS approach for sustainable aquaculture management area site selection. *Ocean and Coastal Management, 51*, 612-624

Mallet, A. L., & Carver, C. E. (1991). An assessment of strategies for growing mussels in suspended culture. *Journal of Shellfish Research, 10*, 471-477

Mallet, A. L., & Carver, C. E. A. (1993). Temporal production patterns in various size groups of the blue mussel. *Journal of Experimental Marine Biology and Ecology, 170*(1), 75-89

Mallet, A. L., & Carver, C. E. A. (1995). Comparative growth and survival patterns of *Mytilus trossulus* and *Mytilus edulis* in Atlantic Canada. *Canadian Journal of Fisheries and Aquatic Sciences, 52*, 1873-1880

Mallet, A. L., Carver, C. E. A., Coffen, S. S., & Freeman, K. R. (1987a). Mortality variations in natural populations of the blue mussel, *Mytilus edulis*. *Canadian Journal of Fisheries and Aquatic Sciences, 44*, 1589-1594

Mallet, A. L., Carver, C. E. A., Coffen, S. S., & Freeman, K. R. (1987b). Winter growth of the blue mussel *Mytilus edulis*: importance of stock and site. *Journal of Experimental Marine Biology and Ecology, 108*, 217-228

Mallet, A. L., Carver, C. E. A., & Freeman, K. R. (1990). Summer mortality of the blue mussel in eastern Canada: spatial, temporal, stock and age variation. *Marine Ecology-Progress Series, 67*, 35-41

Maxwell, K. E., Matthews, T. R., Sheehy, M. R. J., Bertelsen, R. D., & Derby, C. D. (2007). Neurolipofuscin is a measure of age in *Panulirus argus*, the Caribbean spiny lobster, in Florida. *Biological Bulletin, 213*(1), 55-66

Mazerolle, M. J. (2006). Improving data analysis in herpetology: using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. *Amphibia-Reptilia, 27*, 169-180

McDonald, J. H., Seed, R., & Koehn, R. K. (1991). Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. *Marine Biology, 111*, 323-333

Moore, M. N., Koehn, R. K., & Bayne, B. L. (1980). Leucine aminopeptidase (aminopeptidase-1), N-acetyl-β-hexosaminidase and lysosomes in the mussel *Mytilus edulis* L., in response to salinity changes. *Journal of Experimental Zoology, 214*, 239-249

Moore, M. N., Viarengo, A., Donkin, P., & Hawkins, A. J. S. (2007). Autophagic and lysosomal reactions to stress in the hepatopancreas of blue mussels. *Aquatic Toxicology, 84*(1), 80-91

Moreau, V., Tremblay, R., & Bourget, E. (2005). Distribution of *Mytilus edulis* and *Mytilus trossulus* in the Gaspé Coast in relation to spatial scale. *Journal of Shellfish Research, 24*(2), 545-551

Myrand, B., Guderley, H., & Himmelman, J. H. (2000). Reproduction and summer mortality of blue mussels *Mytilus edulis* in the Magdalen Islands, southern Gulf of St. Lawrence. *Marine Ecology-Progress Series, 193-207*

Myrand, B., Tremblay, R., & Sevigny, J. M. (2009). Impact of suspension culture using mesh sleeves on genetic characteristics of *Mytilus edulis* L. in Canada. *Aquaculture, 291*, 147-153

Myrand, B., Tremblay, R., & Sévigny, J. M. (2009). Decreases in multi-locus heterozygosity in suspension-cultured mussels (*Mytilus edulis*) through loss of the more heterozygous individuals. *Aquaculture, 295*, 188-194

Nath, S. S., Bolte, J. P., Ross, L. G., & Aguilar-Manjarrez, J. (2000). Applications of geographic information systems (GIS) for spatial decision support in aquaculture. *Aquacultural Engineering, 23*, 233-278

Newell, C. R. (1990). The effects of mussel (*Mytilus edulis*, Linnaeus, 1758) position in seeded bottom patches on growth at subtidal lease sites in Maine. *Journal of Shellfish Research, 9*, 113-118

Newell, C. R., Wildish, D. J., & MacDonald, B. A. (2001). The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. *Journal of Experimental Marine Biology and Ecology, 262*(1), 91-111

Odonovan, V., & Tully, O. (1996). Lipofuscin (age pigment) as an index of crustacean age: Correlation with age, temperature and body size in cultured juvenile *Homarus gammarus Journal of Experimental Marine Biology and Ecology, 207*(1-2), 1-14

Page, H. M., & Hubbard, D. M. (1987). Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. *Journal of Experimental Marine Biology and Ecology, 111*(2), 159-179

Pearsons, P. A. (2007). Energetic efficiency under stress underlies positive genetic correlations between longevity and other fitness traits in natural populations. *Biogerontology, 8*, 55-61

Pechenik, J. A., Eyster, L. S., Widdows, J., & Bayne, B. L. (1990). The influence of food concentration and temperature on growth and morphological differenciation of blue mussel *Mytilus edulis* larvae. *Journal of Experimental Marine Biology and Ecology, 136*(1), 47-64

Perez-Camacho, A., Gonzalez, R., & Fuentes, J. (1991). Mussel culture in Galicia (N.W. Spain). *Aquacultue, 94*, 263-278

Phinney, D. A., & Yentsch, C. S. (1985). A novel phytoplankton chlorophyll technique: toward automated analysis. *Journal of Plankton Research, 7*, 633-642

Plew, D. R., Enright, M. P., Nokes, R. I., & Dumas, J. K. (2009). Effect of mussel bio-pumping on the drag and flow around a mussel crop rope. *Aquacultural Engineering, 40*, 55-61

Quince, C., Abrams, P. A., Shuter, B. J., & Lester, N. P. (2008). Biphasic growth in fish I: Theoretical foundations. *Journal of Theoretical Biology, 254*, 197-206

Radiarta, I. N., & Saitoh, S.-I. (2009). Biophysical models for Japanese scallop, *Mizuhopecten yessoensis*, aquaculture site selection in Funka Bay, Hokkaido, Japan, using remotely sensed data and geographic information system. *Aquaculture International, 17*, 403-419

Rail, J.-F., & Savard, J.-P. L. (2003). Identification des aires de mue et de repos au printemps des macreuses (*Melannita* sp.) et de l'Eider à duvet (*Somateria mollissima*) *dans l'estuaire et le golfe du Saint-Laurent.* Série de rapports techniques, 408 p.

Riveros, A., Zuniga, M., Hernandez, A., & Camano, A. (2002). Cellular biomarkers in native and transplanted populations of the mussel *Perumytilus purpuratus* in the intertidal zones of San Jorge Bay, Antofagasta, Chile. *Archives of Environmental Contamination and Toxicology, 42*(3), 303-312

Seed, R. (1976). Ecology. Dans B. L. Bayne (Dir.), *Marine mussels: their ecology and physiology* (pp. 13-65). Cambridge.

Seehafer, S. S., & Pearce, D. A. (2006). You say lipofuscin, we say ceroid: Defining autofluorescent storage material. *Neurobiology of Aging, 27*, 576-588

Sheehy, M. R. J. (1989). Crustacean brain lipofuscin: an examination of the morphological pigment in the fresh-water crayfish *Cherax cuspidatus*. *Journal of Crustacean Biology, 9*(3), 387-391

Sheehy, M. R. J. (1992). Lipofuscin age-pigment accumulation in the brains of aging field- and laboratory-reared crayfish *Cherax quadricarinatus* (von Martens) (Decapoda:Parastacidae). *Journal of Experimental Marine Biology and Ecology, 161*(1), 79-89

Sheehy, M. R. J., Caputi, N., Chubb, C., & Belchier, M. (1998). Use of lipofuscin for resolving cohorts of western rock lobster (*Panulirus cygnus*). *Canadian Journal of Fisheries and Aquatic Sciences, 55*(4), 925-936

Smaal, A. C., & van Stralen, M. R. (1990). Average annual growth and condition of mussels as a function of food source. *Hydrobiologia, 195*, 179-188

Statistique Canada: Statistiques d'aquaculture. (2008). *no 23-222-X*

Steffani, C. N., & Branch, G. M. (2003). Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. *Marine Ecology-Progress Series, 246*, 197-209

Sukhotin, A. A., Abele, D., & Portner, H. O. (2002). Growth, metabolism and lipid peroxidation in *Mytilus edulis*: age and size effects. *Marine Ecology-Progress Series, 226*, 223-234

Sukhotin, A. A., & Kulakowski, E. E. (1992). Growth and opulation dynamics in mussels (*Mytilus edulis* L.) cultured in the White Sea. *Aquacultue, 101*, 59-73

Terman, A., & Brunk, U. T. (2004). Molecules in focus: Lipofuscin. *The International Journal of Biochemistry and Cell Biology, 36*, 1400-1404

Thomas, B., Tremblay, R., & Turcotte, M.-P. (2004). Distribution de *Mytilus edulis* et *Mytilus trossulus* dans les régions maritimes du Québec entre 1996-1998 et avec références à la situation dans les provinces maritimes. MAPAQ, 38, 61 p.

Tremblay, R., Myrand, B., Sevigny, J. M., Blier, P., & Guderley, H. (1998). Bioenergetic and genetic parameters in relation to susceptibility of blue mussels, *Mytilus edulis* (L.) to summer mortality. *Journal of Experimental Marine Biology and Ecology, 221*(1), 27-58

Trottet, A., Roy, S., Tamigneaux, E., & Lovejoy, C. (2007). Importance of heterotrophic planktonic communities in a mussel culture environment: the Grande Entrée lagoon, Magdalen Islands (Québec, Canada). *Marine Biology, 151*, 377–392

van Duren, L., A., Herman, P. M. J., Sandee, A. J. J., & Heip, C. H. R. (2006). Effects of mussel filtering activity on boundary layer structure. *Journal of Sea Research, 55*(1), 3-14

Viarengo, A., Canesi, L., Pertica, M., Livingstone, D. R., & Orunesu, M. (1991). Age-related lipid-peroxidation in the digestive gland of mussels: The role of the antioxidant defence systems. *Experientia, 47*(5), 454-457

von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth laws. II). *Human Biology, 10*, 181–213

Weise, A. M., Cromey, C. J., Callier, M. D., Archambault, P., Chamberlain, J., & McKindsey, C. W. (2009). Shellfish-DEPOMOD: Modelling the biodeposition from suspended shellfish aquaculture and assessing benthic effects. *Aquaculture, 288*, 239- 253

Westerbom, M., Mustonen, O., & Kilpi, M. (2008). Distribution of a marginal population of *Mytilus edulis*: responses to biotic and abiotic processes at different spatial scales. *Marine Biology, 153*(6), 1153-1164

Westoby, M. (1984). The self-thinning rule. *Advances in Ecological Research, 14*, 167-225

Widdows, J., Donkin, P., Brinsley, M. D., Evans, S. V., Salkeld, P. N., Franklin, A., et al. (1995). Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. *Marine Ecology-Progress Series, 127*, 131-148

Widdows, J., Nasci, C., & Fossato, V., U. . (1997). Effects of pollution on the scope for growth of mussels (*Mytilus galloprovincialis*) from the Venice Lagoon, Italy *Marine Environmental Research, 43*, 69-79

Wildish, D. J., & Kristmanson, D. D. (1984). Importance to mussels of the benthic boundary layer. *Canadian Journal of Fisheries and Aquatic Sciences, 41*, 1618-1625

Yoda, K., Kira, T., Ogawa, H., & Hozumi, K. (1963). Self-thinning in over crowded pure stands under cultivated and natural conditions (Intraspecific competition among higher plants XI). *Journal of Biology Osaka City University 14*, 107-129

ANNEXES

ANNEXE I

Protocole de détection de la lipofuscine dans la glande digestive de la moule.

1. Préparation des tissus

Collecte des moules

Inclusion dans l'azote liquide pendant 5 minutes

Conservation à -80 ^{o}C

2. Fixation

Fixation de la chair de moule dans du Bouin pendant 24 heures

Lavage de la chair dans de l'éthanol 70%

3. Déshydratation et éclaircissement

- *Déshydratation*

Bain d'éthanol à 30% de 2 heures

Bain d'éthanol à 70% de 2 heures

Bain d'éthanol à 95% de 1 heure

- *Éclaircissement*

Un bain dans un mélange éthanol-butanol (50/50) pendant 1 heure

Un bain de butanol absolu de 1 heure ou plus (il ne faut pas dépasser une semaine)

4. L'inclusion dans la paraffine

Bain de paraffine mélangée avec du butanol (50/50) pendant 24 heures à 60 ^{o}C

Bain de paraffine pure pendant 24 heures à 60 ^{o}C

Un deuxième bain de paraffine pure pendant 24 heures à 60 ^{o}C

Mise en bloc de la chair et refroidissement à l'air libre pendant 24 heures

5. Faire des coupes de 5 microns moyennant un microtome

6. Déparaffinage des lames

Xylène (10 minutes)

Xylène (3 minutes)

7. Hydratation

Éthanol 100% (5 minutes)

Éthanol 95% (3 minutes)

Éthanol 80% (3 minutes)

Éthanol 65% (3 minutes)

Éthanol 50% (3 minutes)

Eau distillée (5 minutes)

8. Coloration des lames (voir dans Hould (1984) pour les détails)

Trois colorations différentes ont été utilisées:

- *Coloration PAS*

Bain d'acide périodique à 0.5% (10 minutes)

Eau distillée (lavage puis passage)

Réactif de Schiff (15 minutes)

Eau courante (10 minutes)

Harris (5 minutes)

Eau courante (5 minutes)

Alcool-Acide passage

Eau courante (5 minutes)

- *Coloration Noir Soudan III*

Plonger les lames pendant 5 minutes dans la coloration Noir Soudan III

Rinçage 3 fois ou plus à l'éthanol 70% jusqu'à disparition totale de la coloration

Eau courante (5 minutes)

- *Test de Schmorl*

Plonger les lames pendant 5 à 10 minutes dans la solution de Schmorl

Eau courante (5 minutes)

9. Déshydratation et montage des lamelles

Éthanol 100% (1 minutes)

Éthanol 100% (1 minutes)

Xylène (5 minutes)

Ajouter une goutte de Cytoseal et appliquer la lamelle pour couvrir les tissus

Laisser sécher pendant 24 à 48 heures à l'air

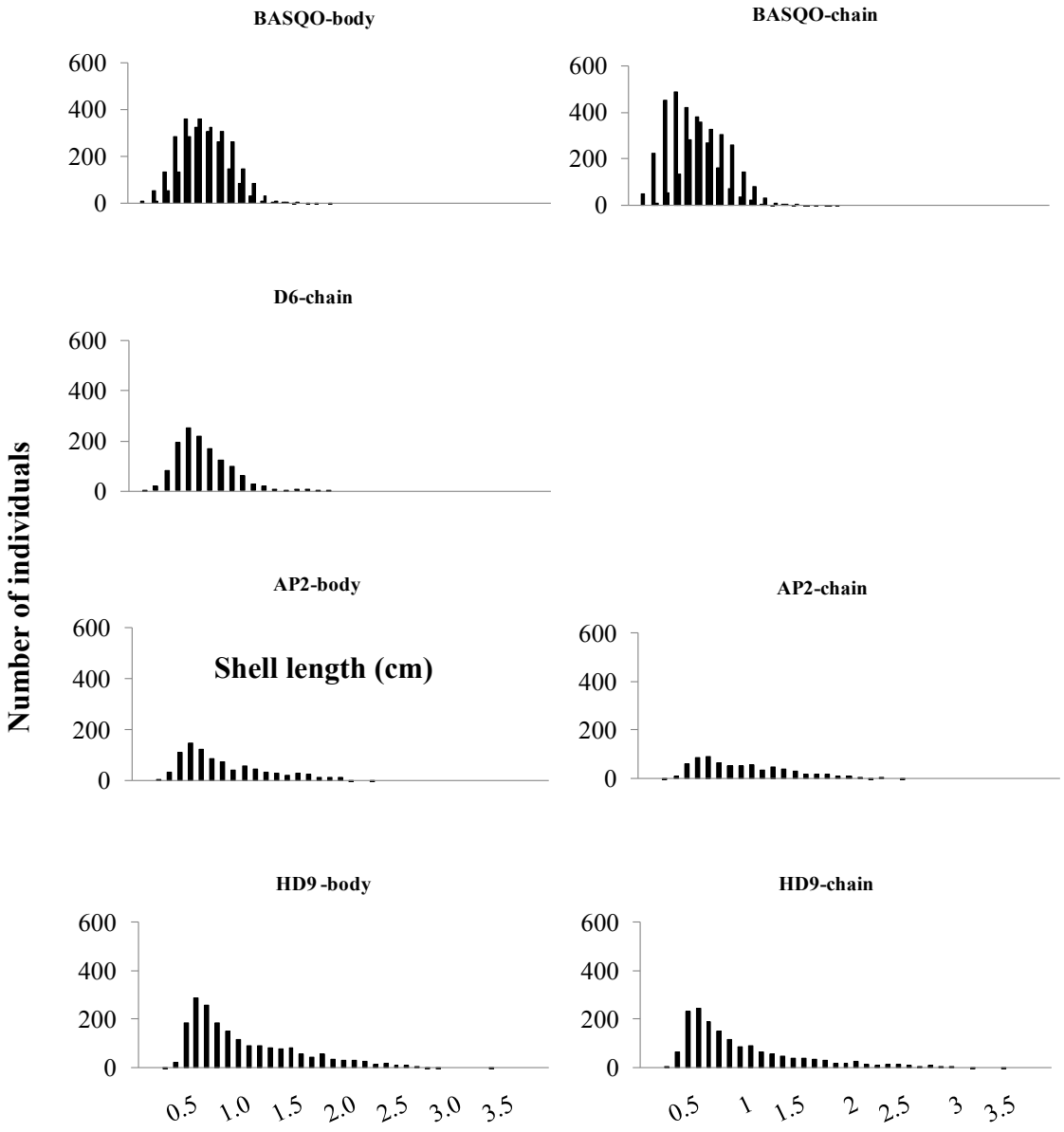
N.B: pour préparer des lames pour l'observation de la fluorescence, on applique les lamelles après le déparaffinage en utilisant le cytoseal et on laisse sécher à l'air pendant 24 à 48 heures.

10. Observation des lames au microscope

Les lames à autofluorescence étaient excitées par le Tetramethyl Rhodamine Isothiocyanate (TRITC) à 540 nm. L'émission a été détectée à 605 nm. Une caméra Olympus DP70 était reliée à un microscope BX51 pour prendre les photos. Le logiciel Image Pro Plus 5.1 a été utilisé pour le traitement des images. Toutes les lames étaient observées avec un grossissement de 60x.

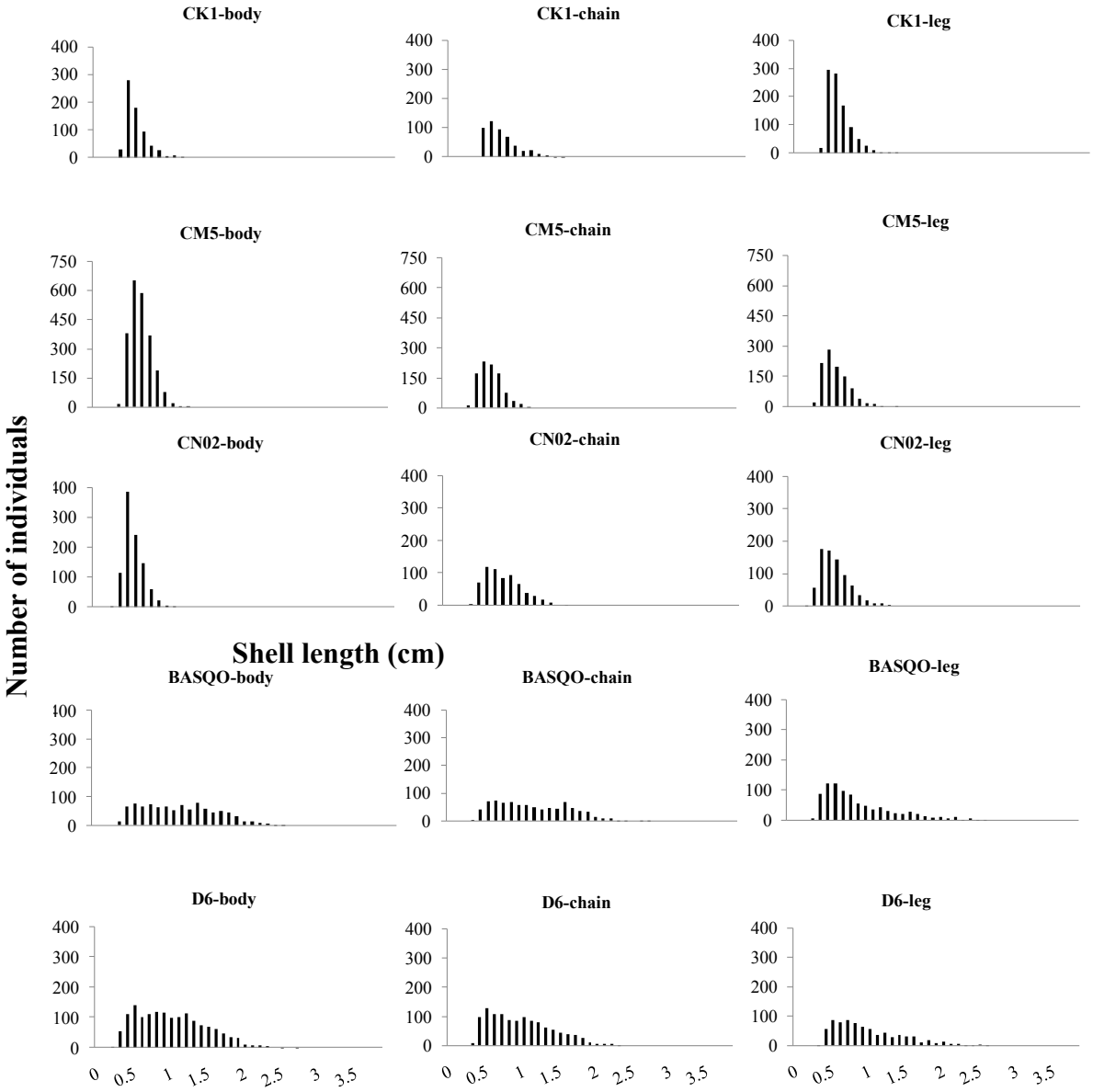
ANNEXE II

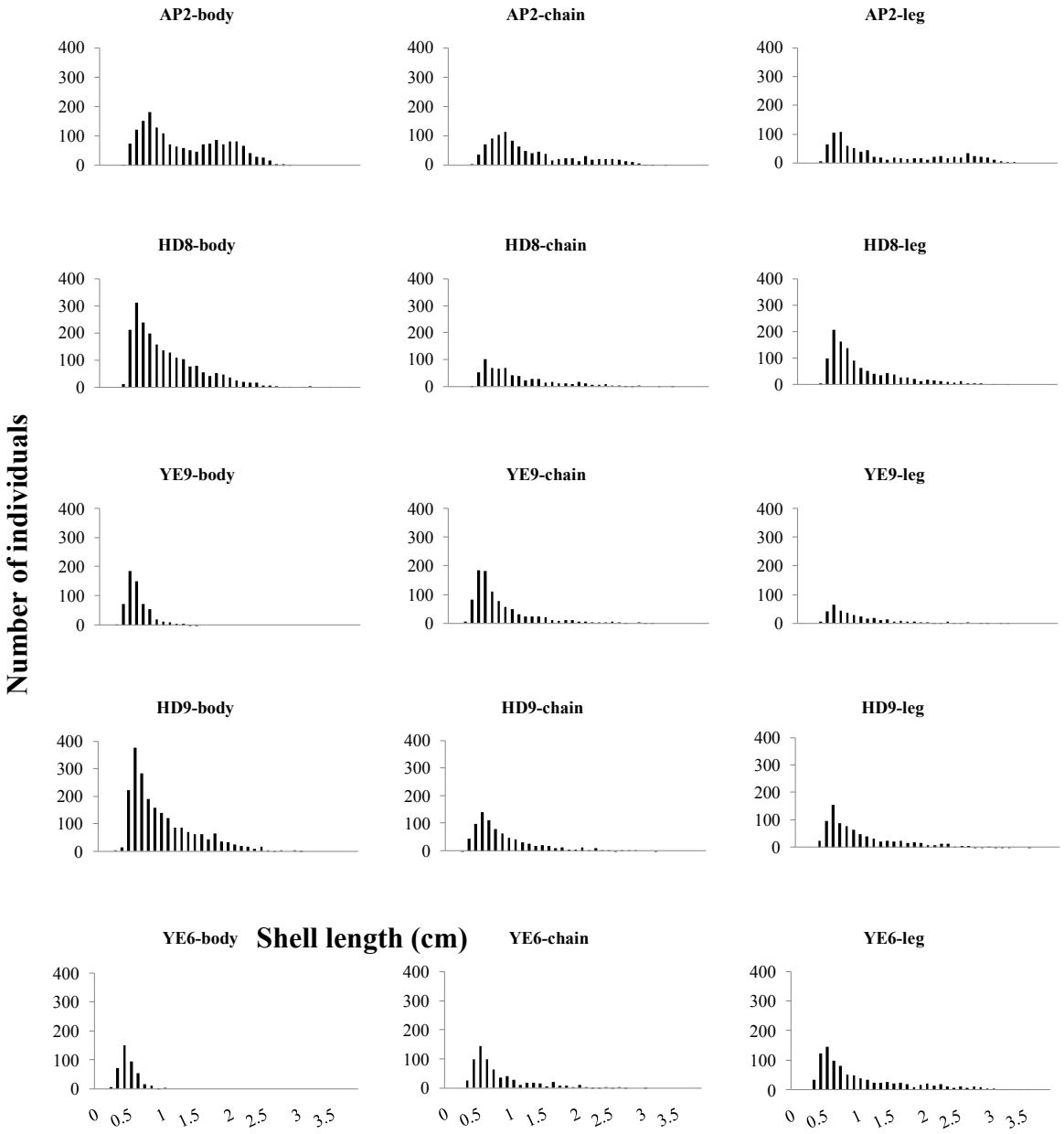
Shell length distributions for mussels *Mytilus* spp. for the different substrates of the buoys sampled in 2007.



ANNEXE III

Shell length distributions for mussels *Mytilus* spp. for the different substrates of the buoys sampled in 2008.




AP2-body
AP2-chain
AP2-leg
HD8-body
HD8-chain
HD8-leg
YE9-body
YE9-chain
YE9-leg
HD9-body
HD9-chain
HD9-leg
YE6-body
YE6-chain
YE6-leg
Number of individuals
Shell length (cm)
0
100
200
300
400
0 0.5 1 1.5 2 2.5 3 3.5

ANNEXE IV

Length-weight relationship for different buoys used to transform the data of cages for 2007 and 2008 ($W = a\ell^b$, where W is the individual whole mass, ℓ the shell length and a and b are adjusted parameters).

Buoy	Year	Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
AP2	2007	a	0.000414	0.000078	0.000260	0.000567
		b	2.433500	0.065500	2.304400	2.562700
	2008	a	0.000410	0.000056	0.000299	0.000520
		b	2.419400	0.042400	2.335900	2.503000
BASQO	2008	a	0.000247	0.000026	0.000196	0.000298
		b	2.590900	0.035400	2.521200	2.660500
HD8	2007	a	0.000194	0.000028	0.000140	0.000248
		b	2.662200	0.046200	2.571000	2.753300
	2008	a	0.000076	8.437E-6	0.000059	0.000092
		b	2.954900	0.034000	2.888100	3.021800
YE2	2007	a	0.000293	0.000024	0.000246	0.000341
		b	2.586400	0.025400	2.536400	2.636400
YE6	2008	a	0.000153	0.000020	0.000114	0.000193
		b	2.788900	0.039800	2.710400	2.867400

UNIVERSITÉ DU QUÉBEC À RIMOUSKI

ÉVALUATION DU MONITORAGE DE LA CROISSANCE DU NAISSAIN DE LA MOULE BLEUE *MYTILUS* SPP. BASÉ SUR LES BOUÉES DE NAVIGATION

Mémoire présenté
dans le cadre du programme de maîtrise en océanographie en vue de l'obtention du grade
de maître ès sciences

PAR

© IHSÈNE BEN SALAH

Décembre 2010

UNIVERSITÉ DU QUÉBEC À RIMOUSKI

ÉVALUATION DU MONITORAGE DE LA CROISSANCE DU NAISSAIN DE LA MOULE BLEUE *MYTILUS* SPP. BASÉ SUR LES BOUÉES DE NAVIGATION

Mémoire présenté
dans le cadre du programme de maîtrise en océanographie en vue de l'obtention du grade
de maître ès sciences

PAR

© IHSÈNE BEN SALAH

Décembre 2010