Université du Québec
à Rimouski

RÔLE NUTRITIONNEL DU PICOPHYTOPLANCTON POUR LES BIVALVES D'ÉLEVAGE

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Composition du jury :

Gesche Winkler, présidente du jury de thèse, Université du Québec à Rimouski

Réjean Tremblay, directeur de recherche, Université du Québec à Rimouski

Luc Comeau, codirecteur de recherche, Pêches et Océans Canada (Région du golfe)

Frédéric Olivier, codirecteur de recherche, Muséum National d'Histoire Naturelle (Paris, France)

Laurent Chauvaud, examinateur externe, CNRS, UMR6539, LEMAR (Brest, France)

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À ma mère qui a quitté ce monde…

…et à ma fille qui est venue au monde.

À ma douce Isabelle, mon beau Sébastien et belle Maëlle, je vous adore.

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RÉSUMÉ

L'aquaculture des bivalves illustre une forte croissance de la biomasse produite au Canada. Cette augmentation est notamment due à l'innovation constante des techniques d'élevage et une meilleure compréhension des interactions de cette industrie avec l'environnement. Ceci étant dit, la compétition inter- et intra spécifique sur la matière organique disponible comme source de nourriture demeure un enjeu pouvant avoir des répercussions sur la croissance des bivalves d'élevage, telles que la moule bleue (*Mytilus edulis*) et l'huître américaine (*Crassostrea virginica*). De fortes densités de bivalves, possédant une capacité de filtration importante, peuvent efficacement induire un certain contrôle sur les communautés phytoplanctoniques d'un estuaire tout en réduisant considérablement la concentration de matière organique en suspension (seston). La disponibilité de la nourriture n'est toutefois pas uniquement limitée à la biomasse présente dans le système, mais aussi à la taille des particules. L'efficacité de rétention de celles-ci par les bivalves augmente généralement en fonction de la taille des particules. Inversement, la rétention des petites cellules phytoplanctoniques, tel que le picophytoplancton (0,2–2,0 μm), est souvent perçue comme étant moindre et sans intérêt nutritionnel. Ce picophytoplancton est pourtant très abondant en milieux riches en nutriments dissouts et domine parfois la biomasse phytoplanctonique des estuaires. Les baies aquacoles du Canada Atlantique, comme à l'Île-du-Prince-Édouard (Î.-P.-É), ne dérogent pas à cette situation, mais la production de coquillages à l'intérieur de ces estuaires ne semble pas être affectée pour autant. Est-ce que *M. edulis* et *C. virginica* ont la capacité d'utiliser cette ressource abondante comme source nutritionnelle?

Ainsi, cette thèse est constituée de trois axes de recherche dans lesquels nous explorons le potentiel nutritionnel des cellules picophytoplanctoniques en relation avec les bivalves en élevage intensif. Le tout est regroupé en trois chapitres distincts cherchant à:

i) Déterminer si le picophytoplancton est une source majeure de l'alimentation des moules d'élevages (*M. edulis*);
ii) Analyser la capacité des huîtres d'élevage (*C. virginica*) à filtrer, ingérer et assimiler le picophytoplancton comme source de nourriture;
iii) Vérifier le potentiel de compétition entre *M. edulis* et le tunicier envahisseur (*Styela clava*) en l'ingestion et l'assimilation du picophytoplancton.

Dans un premier temps, nous avons testé la contribution potentielle du picophytoplancton à la croissance des moules d'élevage dans la baie de St. Peters (Î.-P.-É.) en complétant un suivi sur le terrain du phytoplancton (biomasse fractionnée), de la croissance de deux cohortes de moules (1 an et 2 ans), ainsi qu'un volet expérimental où nous avons analysé la capacité de rétention du picophytoplancton naturel par *M. edulis* qui démontra une efficacité de rétention de 20±2 % de ce petit phytoplancton. Nous avons par la suite intégré ces données de biomasses phytoplanctoniques, de croissances des moules et d'efficacité de rétention de picophytoplancton à l'intérieur d'un modèle numérique de type « Dynamic Energy Budget (DEB) ». Les simulations DEB excluant le picophytoplancton

(rétention de 0 % par les moules) prédirent une diminution de la croissance des moules de 14 à 29 %. Cette contribution du picophytoplancton (< 2,0 µm) au budget énergétique de l'aquaculture de la moule est donc non négligeable et devrait être considérée lors des études d'interactions entre l'aquaculture des bivalves et la dynamique du phytoplancton.

En second lieu, grâce à des méthodes analytiques utilisant des traceurs diététiques (profilage des isotopes stables et des acides gras), ainsi que l'utilisation de ^{13}C comme marqueur de traçabilité du picophytoplancton, nous avons exploré les sources nutritionnelles distinctes des huîtres cultivées (*C. virginica*) dans la baie de Foxley (Î.-P.-É.) en plus d'examiner leur capacité d'assimilation du picophytoplancton. Les huîtres cultivées en suspension démontrèrent une concentration en lipides significativement supérieure à celles provenant de la culture sur le fond. Les microalgues demeurent la source principale de nutrition des huîtres. De plus, malgré leur proximité au benthos, les huîtres de fond ne présentèrent aucun lien significatif envers les sources détritiques ou bactériennes. Les résultats d'enrichissement (^{13}C) démontrèrent concrètement une assimilation du picophytoplancton par *C. virginica*, le tout en intégrant ce carbone isotopiquement marqué directement dans leurs tissus, et ce, même lorsque nourris d'une diète ne comprenant que 20 % de picophytoplancton. De surcroît, les huîtres montrèrent même un enrichissement en ^{13}C de l'acide gras spécifique 22:2 (ou NMI), un acide gras uniquement bio synthétisé par les bivalves.

En terminant, des manipulations expérimentales à l'intérieur de notre laboratoire mobile (*in situ*) ont permis de déterminer le potentiel d'assimilation du picophytoplancton par les moules (*M. edulis*) et, par le fait même, de déterminer si celles-ci sont compétitrices face à l'une des espèces envahissantes retrouvées sur l'Î.-P.-É., soit le tunicier solitaire *Styela clava*. Utilisant le marquage isotopique (^{13}C) du picophytoplancton, les résultats obtenus nous ont permis de démontrer clairement une assimilation importante, et ce pour les deux espèces de filtreurs. La moule sembla toutefois présenter un avantage allométrique associé au taux d'assimilation du picophytoplancton comparativement au tunicier. Une compétition semble effectivement présente entre l'espèce cultivée et son envahisseur face à cette source distincte de nourriture. La proximité de ces deux espèces dans le contexte aquacole nous a incités à mener à terme une dernière comparaison expérimentale afin de vérifier si un transfert secondaire d'énergie (^{13}C) est possible. Ce processus fut démontré via l'ingestion des fèces provenant d'individus avoisinants. Pour les deux espèces, les individus ingérant les particules fécales produites par *S. clava* s'enrichissent de niveaux supérieurs en ^{13}C.

Grâce à cette étude, ces résultats apportent de nouvelles pistes de réflexion quant à l'importance des petites cellules phytoplanctoniques, telle que le picophytoplancton, en tant que source de nutrition non négligeable pour les bivalves d'élevage. Ce projet de doctorat présente des conclusions très pertinentes nous permettant d'améliorer nos connaissances sur les interactions entre les bivalves d'élevages et les communautés phytoplanctoniques.

Mots clés **:** picophytoplancton, *Mytilus edulis*, *Crassostrea virginica*, *Styela clava*, aquaculture, assimilation, biomarqueurs.

ABSTRACT

Bivalve aquaculture is an industry in constant growth in Canada. This growth can be attributed to thanks to the innovation of husbandry techniques, which translates to a slight increase in biomass production annually. This said, inter- and intra-specific competition for food sources is a concern which can have important repercussions on cultured bivalve species in the area such as the blue mussel (*Mytilus edulis*) and the eastern oyster (*Crassostrea virginica*). It is known that high densities of filter feeders, with their important filtration pressure, can control phytoplankton communities and deplete significantly suspended organic matter (seston) from the water column. Although important, food availability is not the only limiting factor to be considered in research on bivalve particle selection. Particle size is also an important parameter often dictating the retention efficiency of variable sources of organic matter during the filter-feeding process. Since, the retention efficiency is generally positively correlated with particle size, thus the retention of small phytoplankton cells such as picophytoplankton (0.2–2.0 µm) is notably low. Picophytoplankton is often abundant in nutrient rich estuaries and occasionally dominates the overall phytoplankton biomass of those productive ecosystems. Shellfish aquaculture areas in Atlantic Canada are not the exception, even though the bivalve production in those estuaries does not display any negative trends in relation to the presence of picophytoplankton. Are *M. edulis* and *C. virginica* benefiting from the strong availability of picophytoplankton as an energy source to thrive in these estuaries?

In this context, the present thesis is founded on three research questions relating to the nutritional contribution of picophytoplankton in intensive bivalves'culture settings. The information provided is divided in three distinct chapters focusing on the following specific themes:

i) Determine the potential contribution of picophytoplankton is an important food source for cultured mussels (*M. edulis*).
ii) Analyze the capacity of oysters (*C. virginica*) to filter, ingest and assimilate picohytoplankton as an energy source.
iii) Explore the potential competition between *M. edulis* and its invader, the tunicate (*Styela clava*), for the ingestion and assimilation of picophytoplankton.

To begin with, we verified the contribution of picophytoplankton towards the growth of cultured mussels from St. Peters Bay (P.E.I.) using field monitoring data, specifically phytoplankton size-fractionated biomass and mussel growth from two cohorts (1- and 2-year old crop). Experimental data based on natural seston was exploited to assess the efficiency of mussels to retain picophytoplankton cells; *M. edulis* demonstrated a retention efficiency of 20±2%. We then integrated phytoplankton biomass, mussel growth and picophytoplankon retention data in a Dynamic Energy Budget (DEB) numerical model. Simulations excluding picophytoplankton (retention efficiency adjusted to 0% by mussels) predicted a reduction in mussel growth of 14–29%. Consequently, the contribution of

picophytoplankton (< 2.0µm) to the energy budget of mussel aquaculture is not negligible and should be considered within studies on shellfish aquaculture interactions with phytoplankton dynamics as well as the environment.

Subsequently, using analytical methods such as dietary tracers (stable isotopes and fatty acids profiling) on the field, as well as ^{13}C for picophytoplankton traceability *in situ* laboratory experiments, we investigated the different nutritional sources for cultivated oysters (*C. virginica*) in the Foxley River system (P.E.I.). Oysters cultivated in suspension contained significantly higher lipids in their tissues than their bottom cultured counterparts. Microalgae remained the principal food source for oysters independent of culture method, and bottom cultured oysters did not demonstrate significant relations with bacterial and detrital sources. The enrichment experiment with isotopically labelled (^{13}C) picophytoplankton showed important assimilation of picophytoplankton carbon in oysters' tissues, even when fed a diet containing as low as 20% of picophytoplankton cells. Moreover, the fatty acid 22:2 (or NMI) biosynthesized by oysters showed ^{13}C enrichment. The latter fatty acid is only biosynthesized by bivalves, thus demonstrate enhance complexity of usage of integrated carbon from picophytoplankton.

To conclude, one last series of experimental trials were conducted using natural sea water to help us determine the potential of picophytoplankton assimilation by cultured mussels (*M. edulis*) and invasive tunicates (*S. clava*). This enabled us to determine if there is inter-specific competition for those small phytoplankton cells between the later cultured and invasive species. Our results using ^{13}C as dietary tracer clearly demonstrated an assimilation of picophytoplankton by both filter-feeder species and consequently indicated competition for that specific food source. This said, cultured mussels demonstrated a superior allometric relation in regards to the assimilation rates of picophytoplankton in comparison to their invader. In the context of aquaculture, the close proximity of both species on cultivation structures initiated our last series of trials looking at potential secondary transfer of energy through feces ingestion. We concluded that indeed, there is a transfer originating from picophytoplankton assimilation through feces ingestion of individuals in close proximity. For both species, individuals ingesting *S. clava* feces acquired important levels of enrichment (^{13}C).

Results in the present thesis bring new reflexions on the contribution of small phytoplankton cells, such as picophytoplankton, as an important food source for cultured bivalves. This doctoral project presented significant conclusions and novel information which will help better understand the complex relations between shellfish aquaculture and phytoplankton assemblages.

Key words: picophytoplankton, *Mytilus edulis*, *Crassostrea virginica*, *Styela clava*, aquaculture, assimilation, biomarkers.

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INTRODUCTION GÉNÉRALE

AQUACULTURE DES BIVALVES AU CANADA ATLANTIQUE

L'aquaculture, en général, est perçue comme étant une industrie importante puisqu'elle subvient à la demande grandissante en produits de la mer, et ce, à l'échelle du globe (Costa-Pierce 2002; Dumbault et al. 2009). Depuis près de 20 ans, la production aquacole (toutes espèces confondues) surpasse la production associée aux pêcheries (FAO, 2016). L'aquaculture des bivalves, communément appelée conchyliculture, est un secteur relativement limité au Canada comparativement aux activités piscicoles (toutes espèces de poissons confondues). Cela dit, la culture des bivalves démontre toutefois une forte croissance, tant pour de la biomasse produite annuellement que pour l'innovation des techniques d'élevage (Howlett et Rayner 2004). Le développement accéléré de la culture des bivalves au cours des dernières décennies (FAO 2012) a su mettre en valeur l'important rôle économique de cette industrie au sein des zones côtières à l'échelle planétaire. La conchyliculture est en constante expansion dans les provinces de l'Atlantique (Canada) (MacRae et al. 2005), passant d'une production annuelle de 1 463 tonnes en 1986 (Comeau et al. 2006) à 36 343 tonnes en 2015 (Pêches et Océans Canada, Statistiques, 2015). Deux espèces de mollusques bivalves dominent l'industrie conchylicole au Canada Atlantique, soient l'huître américaine (*Crassostrea virginica*) et la moule bleue (*Mytilus edulis*).

L'huître américaine, *Crassostrea virginica*

La distribution de *C. virginica* sur la côte Est atlantique s'étend du Golfe du Mexique au sud (Baynes 2017) jusqu'au golfe du Saint-Laurent au nord (Niles et al. 2014), représentant la limite nordique où la reproduction de l'espèce est toujours possible en raison des eaux demeurant froides durant la saison estivale. L'industrie ostréicole (culture de l'huître) de l'Est canadien dépend en majorité d'une collecte naturelle comme source de naissains comparativement en un approvisionnement via les écloseries commerciales présentement en nombre très limité. À la fin des années 1990, l'ostréiculture canadienne a

vu ses techniques d'élevage et de grossissement évoluer d'un épandage des naissains directement sur le fond vers la culture en suspension (dans la colonne d'eau). Ceci étant dit, malgré une production de l'huître en suspension très courante depuis longtemps au Nouveau-Brunswick, l'Île-du-Prince-Édouard demeure au stade de développement de l'aquaculture de l'huître (Lavoie 1996). La première technique explorée par les ostréiculteurs est la culture sur tables, très connue déjà en France, notamment dans le bassin d'Arcachon et Marennes-Oléron (Figure 1). On y cultive notamment l'huître japonaise, *Crassostrea gigas*. Cette méthode de grossissement offre un accès facile à la biomasse cultivée par les aquaculteurs ainsi qu'au contrôle des salissures (macroalgues, fixation indésirable de naissains d'huîtres, de moules ou de balanes) pouvant s'accumuler sur les poches Vexxar©. Ce retrait du compartiment épibenthique et l'utilisation des poches protègent également les cohortes d'huîtres des prédateurs benthiques (crabes, homards, étoiles de mer, certaines espèces de gastropodes). Un inconvénient majeur associé aux latitudes nordiques du golfe du Saint-Laurent est la formation d'une couche de glace sur une importante superficie des estuaires aquacoles durant la saison hivernale. Par conséquent, la totalité des structures (tables et poches) doit être déplacée dans une zone plus profonde ou retirée de l'eau avant chaque hiver, ce qui est beaucoup moins rentable sur le plan économique. De plus, l'utilisation des tables comme technique d'élevage limite l'aquaculteur à travailler sur son cheptel, uniquement lors des marées basses ou descendantes.

Figure 1 : Filières de tables de culture (Marennes-Oléron, France)
(Luc Comeau - MPO, Région du golfe)

À la lumière des contraintes présentées ci-haut, l'industrie de l'huître évolue maintenant vers une utilisation beaucoup plus efficace de la colonne d'eau, surtout en exploitant les masses d'eaux de surface où la température ainsi que la disponibilité et le renouvellement en phytoplancton sont supérieurs. Les huîtres filtrent en moyenne 3,47 ± 0,49 L g^{-1} h^{-1} (Shumway 2011). La qualité de la nourriture proche du fond est reconnue pour être moindre pour les bivalves et est souvent susceptible d'être remise en suspension à tout moment (Smaal et Haas 1997). La technique en poches flottantes est toujours très utilisée en Atlantique (Figure 2).

Figure 2 : Longues lignes (filières doubles) de poches flottantes d'huîtres.
(Luc Comeau - MPO, Région du Golfe)

Avec un tirant d'eau de seulement 15 cm, cette technique en poches permet un déploiement d'huîtres pour fin de grossissement à l'intérieur de zones peu profondes et abritées. Au cours de la dernière décennie, en eau un peu plus profonde et ayant une stabilité plus importante lors de périodes de grands vents, la technique de culture en cages (Figure 3) occupe maintenant une place de choix dans l'industrie ostréicole. Une seule cage peut contenir une biomasse équivalente de 4 à 6 poches mentionnées ci-haut.

Figure 3 : Longues lignes de cages flottantes contenant chacune six poches d'huîtres.
(Rémi Sonier - UQAR)

Ces deux dernières techniques aquacoles en suspension (poches et cages) utilisent communément la dessiccation comme protocole de contrôle des salissures contre le captage d'autres espèces filtreurs indésirables, ainsi que la colonisation par les macroalgues marines. Les poches sont retournées régulièrement afin de permettre au soleil de sécher les épibiontes qui croissent sur la poche. Dans ce contexte, les huîtres demeurent immergées en tout temps, contrairement aux cages qui, lorsque retournées, sortent complètement les bivalves de l'eau pour une période de séchage qui doit être surveillée de près afin de limiter le stress de température élevée sur ces bivalves. Lors de l'hivernage, les grosses bouées arrimées aux cages se remplissent d'eau et sont utilisées comme base surélevée lors du dépôt des cages sur le fond avant l'arrivée des glaces. Cette méthode diminue la possibilité d'envasement des huîtres et génère une meilleure survie au printemps lors de la remise en suspension pour une nouvelle année de grossissement.

La moule bleue, *Mytilus edulis*

L'aquaculture de la moule est une des pratiques aquacoles les plus anciennes. À cet égard, des vestiges de structures d'élevage ont été retrouvés en Espagne datant de 400 av. J.-C. (Caceres-Martinez et Figueras, 1997). Quelques-uns des principaux producteurs mondiaux sont la Chine, l'Espagne, les Pays-Bas et la France, pour en nommer que quatre (FAO 2012). Le plus grand producteur de moules bleues (*M. edulis*) en amérique du Nord est l'Île-du-Prince-Édouard, qui fournit à elle seule 80 % du marché nord-américain (Ministère des Pêches et Océans Canada, 2006) avec une production annuelle supérieure à 18 500 tonnes. Cela dit, l'industrie de la moule de l'Î.-P.-É. demeure présentement en *statu quo* au niveau de l'expansion puisque près de 90% des sites propices à la mytiliculture sont actuellement occupés par des baux aquacoles (actifs ou inactifs) (Lauzon-Guay et al. 2005). L'aquaculture de la moule, ou mytiliculture, y est effectuée en boudins de trois mètres de longueur, déployés sur des longues lignes en suspension (Figure 4). Cette méthode permet l'utilisation optimale de la colonne d'eau, tout en demeurant dans la zone très productive des baies mytilicoles de l'Î.-P.-É propice à l'accès à la nourriture ainsi qu'aux températures idéales de l'eau (Garen et al. 2004). La capacité moyenne de filtration de la moule bleue est

d'environ 2,98 ± 0,23 L g^{-1} h^{-1} (Shumway 2011). Contrairement à l'aquaculture des salmonidés qui requière l'ajout de moulée comme source nutritionnelle primaire, la mytiliculture est considérée comme une industrie extractive par l'utilisation du seston (principalement le phytoplancton) présent dans le milieu naturel comme source primaire de nourriture. Les bivalves en général sont considérés comme des ingénieurs de nos écosystèmes côtiers (Officer et al. 1982; Gutiérrez et al. 2003), en présentant certains processus bénéfiques, entre autres le déploiement de structures tridimensionnelles favorisant le dévelopement de refuges, en réduisant la turbidité de l'eau et, par la suite, en augmentant la sédimentation des particules par la production de fèces (Mugg Pietros et Rice 2003). De surcroit, la conchyliculture a certainement un rôle important à jouer sur l'utilisation de surplus de nutriments souvent présents dans nos estuaires, ainsi que le retrait de quantités non négligeables d'azote par l'entremise des récoltes annuelles importantes (sortie de l'eau de tissus mous riches en azote) (Rose et al. 2014; Guyondet et al. 2015).

Figure 4 : Longues lignes de boudins de moules à l'I.-P.-É.
(Pêches et Océans Canada)

Les espèces envahissantes

Un problème intrinsèque à la culture des bivalves en suspension offrant des structures tridimensionnelles, telles que les longues lignes, cages et poches, est l'augmentation du potentiel de recrutement de salissures ou d'espèces envahissantes (Adams et al. 2011). L'arrivée et l'établissement d'espèces exotiques sont définis par l'introduction délibérée ou accidentelle d'une espèce dans un habitat dont elle n'est pas native (Carlton et Geller

1993). Une espèce dite exotique obtient le statut d'espèce envahissante lorsque la population peut subvenir elle-même à tous les stages de son cycle de vie (reproduction, fixation, maturation) et entre alors en compétition avec les espèces marines indigènes, tant au niveau nutritionnel que spatial (Binggeli 1994). Malgré l'importance et la prospérité de l'industrie mytilicole à l'Î.-P.-É., celle-ci fait face à des infestations d'espèces envahissantes importantes depuis les 20 dernières années, dont quatre espèces de tuniciers : *Styela clava* (en 1997), *Ciona intestinalis* (automne 2004), *Botryllus schlosseri* et *Botrylloides violaceus* (tous deux au printemps 2005) (MacNair 2005; Locke et al. 2007; Ramsay et al. 2008).

Styela clava

Ciona intestinalis

Botryllus schlosseri

Botrylloides violaceus

Figure 5 : Les quatre espèces de tuniciers envahissants présentement présents à l'Î.-P.-É. (Pêches et Océans Canada)

L'aquaculture de la moule bleue nécessite l'utilisation de plusieurs structures submergées dans la colonne d'eau (boudins, câbles, bouées, ancres, etc.) offrant une quantité importante de substrats rigides aptes à la colonisation par les tuniciers (Locke et al. 2007) (Figure 6). Outre le fait d'être des compétiteurs pour l'espace, ces espèces de tuniciers solitaires (*S. clava* et *C. intestinalis*) ou coloniaux (*B. schlosseri* et *B. violaceus*) se nourrissent aussi principalement de phytoplancton (Lambert et Lambert 1998).

À l'Î.-P.-É., ces tuniciers se reproduisent annuellement (Bourque et al. 2007), parfois même à plusieurs occasions durant l'année, causant des invasions subséquentes dues aux recrutements multiples (Boothroyd et al. 2012). Les mytiliculteurs utilisent plusieurs méthodes afin de contrôler ces espèces nuisibles, dont l'utilisation de traitements chimiques (solutions d'acide acétique ou d'hydroxyde de calcium) ou physiques (lavage des boudins avec buses à haute pression).

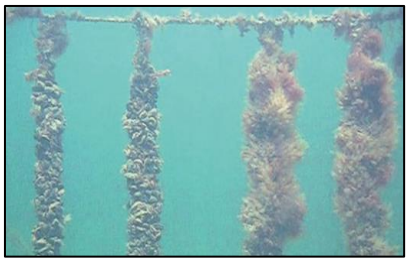

Figure 6 : Infestation de *Styela clava* sur des boudins de moules ainsi qu'une cage de monitorage à l'Î.-P.-É. (Pêches et Océans Canada, Province Î.-P.-É.)

FILTRATION ET SÉLECTION DES PARTICULES PAR LES BIVALVES

Les bivalves ont plusieurs mécanismes physiologiques adaptés à la filtration et à l'ingestion des particules. Deux grands groupes les distinguent : 1) les espèces utilisant des muqueuses (filet ou ficelle), normalement externes ou internes à la cavité du manteau, et 2) et celles utilisant des structures ciliées (proboscices, cténidies) afin de capturer, transporter et finalement faire une sélection des particules à ingérer (Ward et Shumway 2004). Les mollusques utilisant les filets muqueux sont surtout de la classe des gastéropodes, incluant des familles de mésogastéropodes et opisthobranches. Ceux qui utilisent des structures ciliées incluent les bivalves Protobranches, qui sont des suspensivores ayant une paire de palpes proboscites, ainsi que les bivalves Lamellibranches utilisant leurs cténidies (branchies) afin de se nourrir sur la matière en suspension ou en déposition (Ward et Shumway 2004). La classe des Lamellibranches comprend les huîtres et les moules, ainsi que les palourdes, pétoncles et de nombreuses autres familles de coquillages. Les mollusques bivalves dépendent principalement du phytoplancton naturellement produit dans les estuaires (Trottet et al. 2008) et acquiert leur nourriture en filtrant la matière organique en suspension qui les entoure à l'aide de leurs branchies (Møhlenberg and

Riisgård 1978). La capacité de filtration des bivalves est très importante, et son impact sur l'environnement l'est tout autant, particulièrement lorsque les densités de bivalves sur le fond ou dans la colonne d'eau dépassent les plusieurs centaines d'individus par m^2 ou m^3 (Shumway 2011). La compétition intraspécifique pour la nourriture disponible peut avoir un impact négatif sur la croissance de ces bivalves d'élevage (Filgueira et al. 2014; Guyondet et al. 2015), plus particulièrement à l'intérieur des fermes conchylicoles où les densités sont élevées (Grant 1996; Petersen et al. 2008). Ces densités de bivalves en suspension réduisent considérablement le seston pélagique (Dame 1996; Strohmeier et al. 2005) et imposent un fort contrôle sur les communautés phytoplanctoniques (Newell 2004; Maar et al. 2007; Cranford et al. 2009; Strohmeier et al. 2012). Dans certains cas, cette diminution de la biomasse phytoplanctonique causée par le broutage des filtreurs influence négativement la performance des bivalves d'élevage (Smaal et al. 2013). Outre la biomasse de phytoplancton disponible dans le milieu, la taille des particules est un paramètre très important pouvant modifier l'efficacité de rétention de la nourriture par les bivalves (Strohmeier et al. 2012). La capacité de rétention de la nourriture sélectionnée est souvent spécifique à l'espèce et varie beaucoup entre les individus (Møhlenberg et Riisgård 1978; Ward et Shumway 2004). Cela dit, la rétention des particules par les bivalves augmente normalement avec la taille de la nourriture (Cranford et al. 2016). De nombreuses études démontrent que les bivalves tels que *M. edulis* et *C. virginica* n'ingèrent pratiquement pas ou très peu de cellules picophytoplanctoniques (< 2 µm) (Riisgård 1988; Langdon et Newell 1990; Le Gall et al. 1997). Il y a plus de deux décennies, certains travaux démontrèrent chez l'huître une rétention de 86–98% pour des particules de 8–10µm (Møhlenberg et Riisgård 1978), contrairement à celles de 1µm de diamètre n'ayant été retenus qu'à 50% (Ward et Shumway 2004) le tout en utilisant souvent des algues de cultures ou des particules inertes. Plus récemment, respectivement Rosa et al. (2015) et Cranford et al. (2016) démontrèrent une efficacité de capture variable de particules inertes (< 20%) ainsi que de seston naturel (30-40%) de 2 µm de diamètre par *M. edulis*. En s'appuyant sur des conclusions plutôt contrastées dans la littérature présente, il est

déterminant de comprendre l'importance et le potentiel nutritionnel du picophytoplankton pour les bivalves d'élevage à l'intérieur de baies aquacoles très productives.

L`étude des caractéristiques du seston est de nature très complexe étant donné la possibilité pour celui-ci de changer régulièrement (saisons) et rapidement (tempêtes, marées, intensité lumineuse). En parallèle, les systèmes de filtrations des bivalves et leur comportement de nutrition ont fait l'objet de nombreuses études au cours des dernières années, et ce, en utilisant divers techniques comme l'histologie (Beninger 1991), la microscopie électronique (Silverman et al. 1999), et plus communément, les compteurs de particules (Riisgård 1988, Riisgård 2001, Newell et al. 2001, Strohmeier et al. 2009) ainsi que la cytométrie en flux (Cucci et al. 1985; Shumway et al. 1985; Rosa et al. 2013). Le compteur de particules (« coulter counter ») est majoritairement utilisé lors d'expériences d'ingestion de cellules phytoplanctoniques de culture où l'apport en détritus est minimal. Afin de limiter le biais des comptes de particules par la présence de matière détritique et allochtones lors de l'échantillonnage sur le terrain (*in situ*), l'utilisation de cytométrie en flux a été favorisée, ciblant uniquement les cellules ayant des pigments fluorescents. L'ingestion de particules par les bivalves fut investiguée par plusieurs auteurs en utilisant diverses sources nutritionnelles telles que des monocultures algales ajoutées à de l'eau de mer (Møhlendberg and Riisgård 1979; Palmer and Williams 1980), voire même des simulations de conditions naturelles dans une mixture de microalgues, des bactéries, matière inorganique en suspension et détritus, le tout incorporé à de l'eau de mer filtrée (Cucci et al. 1985; Shumway et al. 1985; Newell and Langdon 1986; Newell and Jordan 1986; Bayne et al. 1989). Au fil du temps, les études semblent promouvoir plutôt l'aspect d'importance écologique quant à l'utilisation des diètes naturelles afin d'expérimenter sur la physiologie nutritionnelle des bivalves (Cranford et al. 1998; Cranford et al. 2005; Strohmeier et al. 2009; Strohmeier et al. 2012). Cette approche est maintenant la plus commune pour l'étude de la filtration des mollusques bivalves et englobe notamment les variations naturelles de particules en suspension (disponibilité, composition chimique, distribution de taille, texture, etc.) (Strohmeier et al. 2012), justifiant l'utilisation du seston naturel pour nos expériences.

COMMUNAUTÉS PHYTOPLANCTONIQUES À l'ÉCHELLE DU GLOBE

Le réchauffement de la planète est un phénomène maintenant reconnu au niveau global (Richardson et Schoeman 2004) avec un rythme tout de même alarmant, équivalent à une augmentation moyenne de 0,6°C depuis les 100 dernières années (IPCC 2001b cité par Wiltshire et al. 2008). Les effets des changements climatiques sur les écosystèmes terrestres sont bien documentés, mais il y a un manque de connaissances sur l'impact du changement du climat sur les biotopes marins (Gaedke et al. 2010). À l'heure actuelle, nos océans subissent des changements majeurs. D'ailleurs, une étude ayant utilisé des données compilées au cours de cinquante années conclut que la masse d'eau océanique a emmagasiné vingt fois plus de chaleur que l'atmosphère entre 1955 et 2005 (Levitus et al. 2005). Toutefois, les effets associés aux changements climatiques le milieu pélagique marin doivent être étudiés davantage (Richardson et Schoeman, 2004). Certaines études récentes prédisent une altération de la productivité biologique océanique à la suite des changements climatiques en cours, sans toutefois en reconnaître les impacts réels sur la productivité de nos océans (Henson et al. 2010). En leur sein, de façon omniprésente, on retrouve des phototrophes microscopiques (phytoplancton) qui comptent, à eux seuls, approximativement la moitié de la production de matière organique sur la Terre (Boyce et al. 2010). Des biologistes estiment que la biomasse de phytoplancton présente en tout temps dans nos océans est d'un milliard de tonnes de ces organismes unicellulaires, en plus d'une production annuelle d'environ 45 milliards de tonnes. Ceci équivaut à un renouvellement complet de la biomasse du globe pratiquement de façon hebdomadaire (Falkowski 2012). À ce qui a trait aux communautés phytoplanctoniques, l'augmentation de la température des océans a un impact sur la taille du phytoplancton, favorisant les plus petites cellules comme le picophytoplancton (Sommer et Lengfellner 2008; Klauschies et al. 2012).

COMMUNAUTÉS PHYTOPLANCTONIQUES DES BAIES AQUACOLES

Le golfe du Saint-Laurent est situé à l'est du Canada à la limite sud de la région subarctique, en plus d'être une importante zone de pêche des provinces maritimes, produisant environ 25% des débarquements (poids) provenant des pêches commerciales au pays (Chadwick et Sinclair 1991). Après le Mississippi, la rivière Saint-Laurent est la plus importante en termes d'apport en eau de toute l'Amérique du Nord, en plus de démontrer des variations saisonnières de débit très importantes allant d'un maximum de 15 000 m^3s^{-1} (avril à juin) à 11 000 m^3m^{-1} durant le reste de l'année (Bourgault et Koutitonsky 1999). L'apport important en eau douce des multiples tributaires affecte considérablement la circulation des courants (Koutitonsky et Bugden, 1991) ainsi que la dynamique du phytoplancton (Fuentes-Yaco et al. 1997). L'abondance et la productivité du phytoplancton, producteur primaire dominant dans plusieurs estuaires de la planète, jouent des rôles critiques sur la qualité de l'eau, les pêches, l'utilisation du carbone et des nutriments (Paerl et al. 2014). La filtration des particules en suspension, dont le phytoplancton, par les bivalves peut avoir un impact direct sur le contrôle des communautés phytoplanctoniques ainsi que l'utilisation des nutriments à l'échelle même de l'écosystème (Asmus et Asmus 1993; Norkko et al. 2001). Nielsen et Maar (2007) introduisent dans l'une de leurs publications que la présence de grandes densités de bivalves (assemblages, bancs, fermes ou cohortes) force un changement des communautés de phytoplancton vers les espèces de petite taille ayant une croissance rapide. Les plus petites cellules phytoplanctoniques, procaryotes ou eucaryotes, se nomment picophytoplancton (Magazzu et Decembrini 1995; Raven 1998) et sont définies dans la littérature par une taille variant de 0,2µm à 2,0µm (Bec et al. 2008; Winder 2009; Lesser et Slattery 2015) ou même à la limite 3,0µm (Gaulke et al. 2010; Jacobs et al. 2015) sans consensus définitif entre ces deux limites maximales. Le picophytoplankton comprend majoritairement des cyanobactéries ainsi que des eucaryotes (Kamiyana et al. 2009), et ces organismes peuvent atteindre des biomasses importantes, voir même dominer la biomasse phytoplanctonique d'estuaires riches en nutriments dissouts (Agawin et al. 2000) démontrant des conditions optimales (Cuvelier et al. 2010; Grob et al. 2011; Kirkham et al.

2013). Pendant que ces dernières études s'atardent principalement au monitorage phytoplanctonique des océans et estuaires, certaines études démontrent des liens étroits entre l'aquaculture des bivalves et une dominance du picophytoplankton à l'intérieur de ces estuaires aquacoles. Par exemple, dans la baie de Tracadie (Î.-P.-É.) (« TR » dans la figure 7), où les moules sont cultivées sur longues lignes à des densités maximales en plus d'être la baie la plus productive de cette province, le picophytoplankton représente parfois plus de 70% de la biomasse phytoplanctonique (Cranford et al. 2009) (Figure 7). De surcroit, cette dernière étude présente aussi un indice de déplétion de la biomasse phytoplancton basé sur relation entre le temps de filtration du volume d'eau d'une baie par les bivalves d'élevages (CT) et le temps de renouvellement total (RT) de ce même volume d'eau par les voies hydrographiques. Un indice < 1 suggère donc une déplétion du phytoplancton sous la capacité de support de la baie. Les baies les plus productives en termes de production aquacole semblent aussi présenter une biomasse phytoplanctonique plus importante selon Cranford et al. 2009.

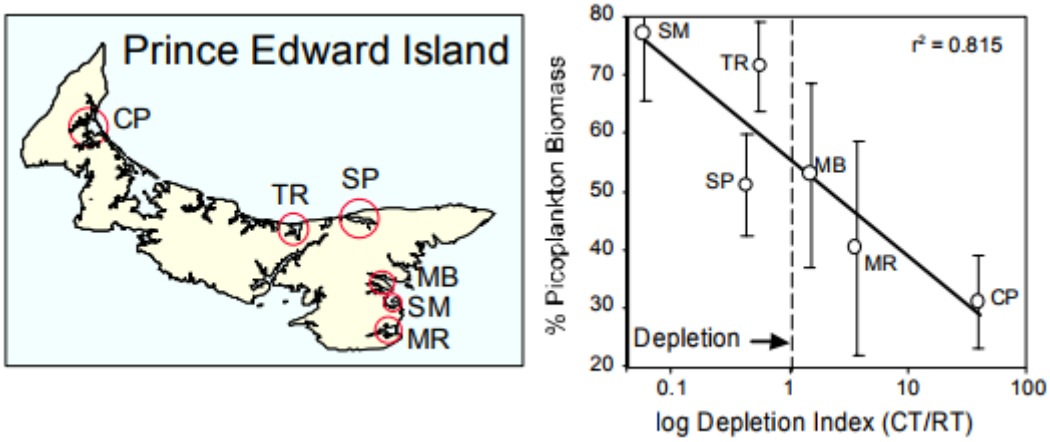

Figure 7 : Provenant de Cranford et al. 2009. « Mean contribution of picophytoplankton in six PEI embayments containing different levels of mussel culture (August 18-22, 2008). The picophytoplankton contribution (fpico) is plotted against a phytoplankton depletion risk index that compares bay flushing characteristics (RT = residence time) with the biofiltering capabilities of the resident mussel farms (CT = clearance time). Depletion index levels below 1 indicate bay-scale depletion ».

UTILISATION DE BIOMARQUEURS

Isotopes stables, lipides et acides gras

Dans plusieurs systèmes côtiers, l'analyse des isotopes stables est largement utilisée pour étudier les liens trophiques entre les producteurs primaires et les niveaux trophiques supérieurs en indiquant l'utilisation des différentes sources nutritionnelles autant par les invertébrés benthiques que pélagiques (Fry, 2007). Ce type d'analyse est basé sur le postulat que, par exemple, les valeurs de carbone ($\delta^{13}C$) du consommateur reflètent normalement les valeurs de carbone provenant de la diète incluant des différences minimes (normalement à l'intérieur de 1‰). Les isotopes stables ont déjà été utilisés afin de caractériser les sources de nourritures utilisées par des bivalves benthiques dans différents écosystèmes côtiers à l'échelle mondiale (Rossi et al. 2004; Lefebvre et al. 2009; Allan et al. 2010).

Les acides gras (FA) sont une composante majeure des lipides provenant des organismes vivants et faisant essentiellement partie des lipides neutres et polaires (Gaillard et al. 2015). Les lipides sont essentiels à la formation des cellules et tissus chez les animaux et constituent aussi une source principale en énergie pour le métabolisme (Bergé et Barnathan 2005). L'utilisation des FA comme marqueurs trophiques est basée sur la prémisse que, en observant les producteurs primaires en milieux marins, plusieurs patrons en AG semblent être transférables à l'intérieur des chaînes trophiques aquatiques (Bergé et Barnathan 2005). Les animaux ont la capacité de synthétiser des FA saturés et mono-insaturés. Cependant, leur capacité à produire des FA polyinsaturés est très réduite en raison d'une activité enzymatique limitée servant à la conversion par élongation (élongases) ou désaturation (désaturases) en FA essentiels (EFA) tels que : l'acide arachidonique (AA, 20:4ω6), l'acide eicosapentanoïque (EPA, 20:5ω3) et l'acide docosahexanoïque (DHA, 22:6ω3) (Chu et Greaves 1991; Pirini et al. 2007). Ces processus d'élongation et de désaturation des précurseurs en EPA, DHA et AA sont considérés comme étant rares chez les organismes marins (Glencross 2009) et à peu près inexistants chez les bivalves (Soudant et al. 1998; da Costa et al. 2015). Conséquemment, ces bivalves ont donc recours à leur

alimentation, principalement le phytoplancton, constitué de sources importantes de EFA. Les EFA tels que l'EPA et le DHA sont considérés comme étant importants au niveau de la croissance, du développement et de la survie des bivalves (Sargent et al. 1995), surtout aux niveaux larvaires et juvéniles (Waldock et Holland 1984). Étant les producteurs primaires de l'environnement marin, la composition en lipides et FA des microalgues (phytoplancton) est étudié depuis longtemps et maintenant plusieurs patrons en FA peuvent être utilisés comme indicateurs taxonomiques de certains groupes d'algues distincts (Gaillard et al. 2015). Cette approche est largement utilisée pour différencier les diatomées des dinoflagellés (Zhukova et Aizdaicher 1995) car les diatomées sont riches en EPA (et C16 insaturés tels que la production de 16:4ω1 à partir du 16:0) tandis que les dinoflagellés sont plutôt riches en DHA et AA (et C18 polyinsaturés tels que 18:4ω3) (Budge et Parrish 1998; Dalsgaard et al. 2003). Les FA ayant un nombre impair de carbones sont souvent spécifiques des bactéries (Wilson et al. 2010). Ceci étant dit, certains FA peuvent associés à plusieurs sources, donc comprendre le contexte biologique et écologique des sites étudiés est essentiel. La glande digestive des bivalves est le site principal de digestion extra- et intracellulaire, emmagasinant normalement de grandes quantités de lipides neutres (Pernet et al. 2007). Chez les invertébrés benthiques, tels que les huîtres et les moules, la composition en FA des leurs tissus est surtout dictée par l'alimentation plutôt que sur la biosynthèse de précurseurs (Chu et Greaves 1991). Par le fait même, la disponibilité de la ressource trophique (proies) influence la composition lipidique des prédateurs marins (Narvaez et al. 2008). Ainsi, la présente étude utilise l'analyse des isotopes stables (mentionné plus haut) parallèlement avec les profils en acides gras des glandes digestives des bivalves. Ces processus seront utilisés autant au niveau de la détermination des sources alimentaires des bivalves cultivés que pour les expériences en milieux contrôlés sur l'assimilation du picophytoplancton comme source énergétique.

SITES À L'ÉTUDE

La baie de St. Peters, au nord-est de l'Île-du-Prince-Édouard, est plutôt de morphologie allongée (13 x 2 km), limitée à une extrémité par une embouchure (100m de largeur et 3,5m de profondeur) et de l'autre d'un chenal sinueux (longé de bancs de sable) donnant sur le Golfe du Saint-Laurent. Cette baie mytilicole (*M. edulis*) est relativement peu profonde (3–5m) sans aucune stratification de la colonne d'eau. Le gradient de salinité (ppt) est faible, de 26 à l'embouchure diminuant à 24 en amont. Près de 38% de la surface totale (1,712 hectares) de cette baie est présentement utilisée pour la culture de la moule sur longues lignes. En 2009, une entente à l'amiable entre les mytiliculteurs fut conclue, restreignant le nombre de boudins à 988 par hectare. Cette décision fut prise à la suite d'inquiétudes soulevées par l'industrie quant à la capacité de support de la baie St. Peter`s. Les travaux de terrains du premier chapitre de cette thèse portant sur la moule furent entrepris à ce site de l'I.-P.-É. Dans le cadre du deuxième chapitre de cette étude portant sur l'huître, la récolte d'échantillons sur le terrain furent entrepris dans la baie de Foxley, aussi à l'Î.-P.-É. Cette baie est l'un des seuls estuaires ayant uniquement des baux alloués à la culture de l'huître (*C. virginica*). Ce système est peu profond avec une profondeure maximale de près de 3m et la colonne est très homogène (pas de stratification). Le troisième et dernier chapitre fut entrepris dans un laboratoire mobile situé aux abords du quai de Georgetown (Î.-P.-É.) et les filtreurs utilisés provenèrent de la baie de Malpèque (Î.-P.-É.).

OBJECTIFS DE LA THÈSE

L'objectif principal de ce projet est de comprendre la potentielle contribution du picophytoplancton dans le contexte de l'aquaculture des bivalves au Canada atlantique. Plus précisément et dans un premier temps, il s'agit d'investiguer la contribution de ces petites cellules phytoplanctoniques dans les écosystèmes côtiers utilisés pour l'élevage des bivalves. De plus, nous explorerons la capacité de ces filtreurs à retenir ces picoparticules en milieux naturels ainsi qu'en conditions expérimentales (laboratoire). Et finalement, nous allons vérifier la capacité d'assimilation du picophytoplancton comme source de nourriture par les bivalves et même une espèce envahissante que l'on retrouve dans la région. Les trois expériences comprises dans ce projet doctoral se sont déroulées en se partageant des composantes *in situ*, c'est-à-dire sur le terrain dans le laboratoire mobile du Ministère des Pêches et Océans Canada à l`Î.-P.-É., la station aquicole de l'Institut des sciences de la mer (ISMER) à Rimouski, ainsi que les laboratoires du Centre d'appui à l'innovation par la recherche (CAIR) de l'Université du Québec à Rimouski (UQAR). Le chapitre 1 fut publié dans le journal « Marine Biology » en février 2016 et le chapitre 2 dans le journal « Aquatic Living Resources » en aout 2017. Finalement, lors du dépôt final de cette thèse, le chapitre 3 sera en soumission au journal « Aquatic Living Resources » dans le cadre d'une édition spéciale affiliée à la conférence Physiomar 17 qui eut lieu à la « Cambridge University » (Angleterre) en septembre 2017.

CHAPITRE 1

CONTRIBUTION DU PICOPHYTOPLANCTON À LA CROISSANCE DE LA MOULE BLEUE, *MYTILUS EDULIS*, EN CULTURE INTENSIVE

Objectif

Déterminer si le picophytoplancton est une source majeure de l'alimentation des moules d'élevages (*Mytilus edulis*) à l'Î.-P.-É.

Hypothèse

Le picophytoplancton contribue à la croissance des moules (*Mytilus edulis*) d'élevage à l'Î.-P.-É.

CHAPITRE 2

HUÎTRES D`ÉLEVAGE, *CRASSOSTREA VIRGINICA* : RÉTENTION ET ASSIMILATION DU PICOPHYTPLANCTON UTILISANT UNE APPROCHE DE BIO-MARQUEURS MULTIPLES

Objectif

Analyser la capacité des huîtres d'élevage (*Crassostrea virginica*) à ingérer et assimiler le picophytoplancton comme source de nourriture

Hypothèse

Les huîtres d'élevage (*Crassostrea virginica*) ingèrent et assimilent le picophytoplancton comme source de nourriture et d'énergie.

CHAPITRE 3

***MYTILUS EDULIS* ET *STYELA CLAVA*) : PARTENAIRES EN L`ASSIMILATION DU PICOPHYTOPLANCTON (FRAIS OU PRÉINGÉRÉ)**

Objectif

Vérification du potentiel de compétition entre l'espèce cultivée (*Mytilus edulis*) et le tunicier envahisseur (*Styela clava*) en l'ingestion et l'assimilation du picophytoplancton.

Hypothèse

La moule et le tunicier tous deux ingèrent et assimilent le picophytoplankton comme source de nourriture de façon équivalente.

CHAPITRE 1

CONTRIBUTION DU PICOPHYTOPLANKTON À LA CROISSANCE DE LA MOULE BLEUE, *MYTILUS EDULIS*, EN CULTURE INTENSIVE

1.1 RÉSUMÉ

Les autotrophes picoplanctoniques (≤ 2,0 µm) sont considérées comme étant la catégorie de phytoplancton le plus abondant dans les écosystèmes marins. La contribution du picophytoplancton en relation avec la croissance de la moule bleue (*Mytilus edulis*) d'élevage de l'est de l'Île-du-Prince-Édouard fut investiguée. Cette province produit la majorité de la production canadienne de moules. Des expériences sur le terrain, utilisant de la cytométrie en flux, furent complétées afin de déterminer la capacité des moules à retenir le picophytoplancton (0,2–2,0 µm) ainsi que le nanophytoplancton (2,0–20,0 µm). La biomasse fractionnée (chl-a) ainsi que la croissance des bivalves (longueur de la coquille, poids des tissus) furent mesurées. L'efficacité de rétention (ER) du picophytoplancton du picophytoplancton et nanophytoplancton par la moule fut évaluée à 20 ± 2.0% et 60 ±3.5% respectivement. Ces estimations de ER ainsi que les biomasses de phytoplancton furent intégrées à un modèle numérique de type « Dynamic Energy Budget (DEB) » afin d'investiguer la contribution du picophytoplancton sur la croissance des moules durant les périodes postbloom du printemps. Lorsque les simulations DEB excluent le picophytoplancton (ER = 0%), les prédictions présentent une diminution de la croissance des moules entre 14 et 29 %. En conclusion la contribution du phytoplancton < 2,0 µm au budget énergétique de l'aquaculture de la moule est non-négligeable et devrait être considéré lors de l'étude des études d'interactions entre l'aquaculture des bivalves et la dynamique du phytoplancton.

1.2 PICOPHYTOPLANKTON CONTRIBUTION TO *MYTILUS EDULIS* GROWTH IN AN INTENSIVE CULTURE ENVIRONMENT

Rémi Sonier [ab*], Ramon Filgueira [a], Thomas Guyondet [a], Réjean Tremblay [b], Frédéric Olivier [c], Tarik Meziane [c], Michel Starr [d], Angeline R. LeBlanc [a], Luc A. Comeau [a]

[a] *Department of Fisheries and Oceans Canada, Gulf Fisheries Centre, Science Branch, P.O. Box 5030, Moncton, NB, E1C 9B6, Canada*

[b] *Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 allée des Ursulines, C.P. 3300, Rimouski, QC, G5M 1L7, Canada*

[c] *Unité Mixte de Recherche Biologie des organismes et écosystèmes aquatiques (BOREA UMR 7208), Sorbonne Université, Muséum National d'Histoire Naturelle, Université Pierre et Marie Curie, Institut de Recherche pour le Développement, Université de Caen-Normandie, Université des Antilles, 61 rue Buffon 75005 Paris, France*

[d] *Department of Fisheries and Oceans Canada, Maurice-Lamontagne Institute, 850 Route de la Mer, Mont-Joli, QC, G5H 3Z4, Canada*

1.3 ABSTRACT

Autotrophic picoplankton (≤ 2.0 µm) is one of the most abundant phytoplankton components in marine ecosystems. The contribution of picophytoplankton to blue mussel (*Mytilus edulis*) growth was investigated in eastern Prince Edward Island (PEI), where the bulk of the Canadian suspended mussel industry is located. Field trials using flow cytometry were used to determine the ability of mussels to retain picophytoplankton (0.2–2.0 µm) and nanophytoplankton (2.0–20.0 µm). Size-fractionated phytoplankton biomass (chl-*a*) and mussel growth (shell height and tissue weight) were also measured. Mussel retention efficiencies (RE) for picophytoplankton and nanophytoplankton averaged 20 ± 2.0% and 60 ± 3.5%, respectively. Estimates of RE and phytoplankton biomass were integrated into a Dynamic Energy Budget (DEB) model to investigate the contribution of picophytoplankton to mussel growth during the post-spring bloom period. When DEB simulations excluded picophytoplankton (RE = 0%), the predicted reduction in mussel growth ranged between 14% and 29%. It is concluded that the contribution of < 2.0-µm phytoplankton to the mussel's energy budget is non-negligible and should be taken into account when investigating interactions between bivalve farming and phytoplankton dynamics.

Keywords: Dynamic Energy Budget model, DEB, picophytoplankton, shellfish aquaculture, Mytilus edulis growth

1.4 INTRODUCTION

Intra-specific competition for food resources can substantially impact bivalve growth (Fréchette 2005; Lachance-Bernard et al. 2010; Filgueira et al. 2014a; Guyondet et al. 2015), particularly at high densities on shellfish farms (Grant 1996; Petersen et al. 2008). Localized seston depletion has been observed at levels as high as 50% for *Mytilus edulis* longline systems (Strohmeier et al. 2005) and up to 80% for *Mytilus galloprovincialis* raft systems (Petersen et al. 2008). In such cases phytoplankton depletion has a direct negative effect on shellfish performance (Smaal et al. 2013). Dense populations of bivalves have the filtration capacity to effectively reduce suspended seston concentrations (Dame 1996; Strohmeier et al. 2005; Guyondet et al. 2013) and impose a strong control on phytoplankton populations (Dame 1996; Prins et al. 1998; Newell 2004; Maar et al. 2007; Cranford et al. 2009; Strohmeier et al. 2012; Filgueira et al. 2014b). Food availability, however, is not only a function of particle concentration, but also particle size characteristics and the suspension feeder's capacity to retain these particles (Strohmeier et al. 2012). The particle retention efficiency (RE) of filter-feeders increases with particle size until it reaches a maximum, but the rate at which efficiency changes is species-specific and may vary widely among individuals (Møhlenberg and Riisgård 1978; Ward and Shumway 2004). Typically, under laboratory conditions, *M. edulis* retains particles larger than 4 µm with a nominal efficiency of 100%, but RE rapidly declines as particle size decreases below this threshold (Møhlenberg and Riisgård 1978; Riisgård 1988).

Picophytoplankton (PPP) includes the smallest photosynthetic free-living cells (Raven, 1998) and is defined in the literature as organisms ranging from 0.2 µm to a maximum of 2.0 µm (Raven 1998; Bec et al. 2008; Winder 2009; Lesser and Slattery 2015) or 3.0 µm (Gaulke et al. 2010; Bec et al. 2011; Jacobs et al. 2015) with no formal consensus. PPP consists mostly of picocyanobacteria and picoeukaryotes (Kamiyama et al. 2009). These organisms can attain very high concentrations and may dominate the phytoplankton biomass in relatively nutrient-rich estuaries under certain conditions (Badylak and Phlips 2004; Richardson and Jackson 2007; Cuvelier et al. 2010; Grob et al.

2011; Kirkham et al. 2013). Moreover, Seychelles et al. (2009) demonstrated that some PPP species (such as *Nannochloropsis* sp.) are rich in essential fatty acids necessary for bivalves' growth and survival (namely EPA). During the last decade, studies on the feeding behaviour of *M. edulis* have revealed a range of RE values for PPP cells. Using a mesocosm approach, Trottet et al. (2008) determined a RE of 0.04 – 3.20% for *M. edulis* feeding on PPP communities (< 2.0 µm). At two experimental sites in Norway, Strohmeier et al. (2012) observed a RE of 14 – 64% for the same species feeding on 1µm particles. However, specific *in situ* information about the contribution of PPP cells to the energetic balance of mussels in suspended culture is, to our knowledge, not available.

Understanding the relationships between phytoplankton size-fraction dynamics and the filtration/retention efficiency of cultured bivalves is crucial to understanding ecosystem functioning and modelling shellfish aquaculture production. The aim of this research is to investigate the effects of mussel farming on picophytoplankton (PPP) and nanophytoplankton (NPP) at the ecosystem-scale. For that, a bay-scale seasonal sampling of seston, PPP and NPP has been performed in St. Peters Bay, a densely cultured bay in Eastern Canada. In order to investigate the mussel-plankton trophic relationship, *in situ* experiments on retention efficiency (RE) of mussels for PPP (0.2–3.0 µm) and NPP (>3.0 µm) using natural seston (natural sea water) as a food source were performed. A Dynamic Energy Budget (DEB) model was also developed to determine whether consumption of PPP can substantially contribute to the growth of *M. edulis* in suspended culture.

1.5 MATERIAL AND METHODS

1.5.1 Study area

St. Peters Bay is an elongated (13×2 km) coastal inlet located on the north-east coast of Prince Edward Island (PEI) in eastern Canada (Figure 8). It opens onto the Gulf of Saint Lawrence through a 100-m wide, 3–4-m deep channel meandering between sandy intertidal banks. It is shallow (depth 3–5 m) and well mixed (no vertical stratification) with a weak salinity gradient extending along the main axis of the bay decreasing from 26 ± 3.0 ppt at

the mouth to 24 ± 3.4 ppt at the head. About 38% of its total surface area (1712 ha) is dedicated to mussel farming. Mussels are reared in 2-m polypropylene sleeves that are suspended from longlines. In 2009, growers collectively developed a bay management plan that limits the stocking density to 988 sleeves per leased hectare. A small area at the head of the bay is restricted to the deployment of spat collectors.

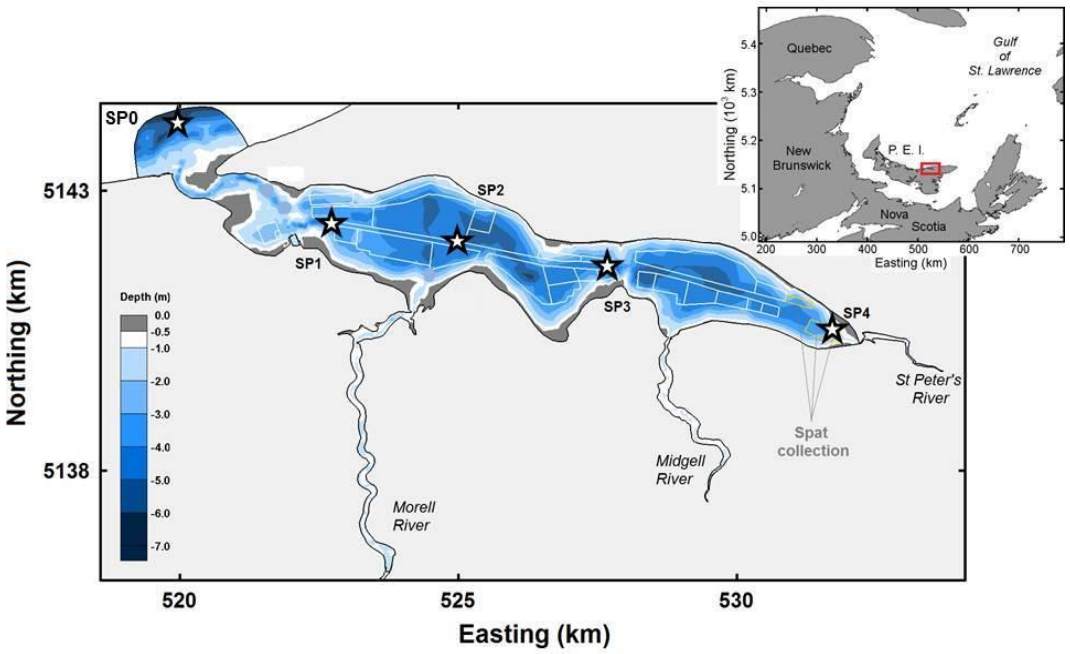

Figure 8: Map of St. Peters Bay (Prince Edward Island, Atlantic Canada) showing bathymetry (blue scale), aquaculture leases (polygons) and sampling stations (SP0, SP1, SP2, SP3 and SP4).

1.5.2 Seston characteristics

During the ice-free period, from early June to late November 2011 and mid-May to late June 2012 (16 sampling missions), duplicate water samples were obtained from five fixed stations: SP0, SP1, SP2, SP3 and SP4 (Figure 8). Note that SP0 is located 1 km

offshore from the mouth of the bay in the Gulf of St. Lawrence and 3.3 km away from SP1. Consequently SP0 is not likely to be impacted by the St. Peters Bay tidal regime or mussel farming activities. Samples were collected at two different depths (1 m below surface and 1 m off bottom) using a 5-L Niskin© bottle. Subsamples (4 ml) were pre-filtered at 40 µm (Falcon Cell Strainer©), stabilized in 5-ml cryovials containing 20 µl of 0.1% gluteraldehyde and kept frozen (-80°C) until counted with a flow cytometer. Size fractions computed by the flow cytometer were limited to 0.2–2.0 µm for PPP and 2.0–20.0 µm for NPP (FCM, Becton Dickinson, San Jose, CA, equipped with a 488-nm laser beam of 15 mW) as described in Belzile et al. (2008).

Phytoplankton biomass measurements were based on size-fractionated chlorophyll-*a* (henceforth chl-*a*). Samples were filtered through 3.0-µm and 0.2-µm polycarbonate filters (25 mm, GE Water and Process Technologies, Trevorse, PA) to yield size fractions of > 3.0 µm and 0.2–3.0 µm. Filters were frozen directly in the field at -40°C (Stirling Ultracold portable freezer, Athens, OH, USA) and kept frozen until analysed. Chl-*a* extractions were performed in 20-ml scintillation vials with 10 ml of acetone (90% final concentration) for a minimum of 24 h at -40°C. Chl-*a* readings were obtained using a laboratory fluorometer (Trilogy®, Turner Designs, Sunnyvale, CA) equipped with a chl-*a* Acidification Fluorescent Module as described in the EPA Method 445.0. Note that chl-*a* determination was not performed during the last four missions, and our sampling program did not cover the spring bloom period in April due to technical constraints. This bloom is typically of short duration and occurs soon after ice-breakup in this area.

Suspended particulate matter (SPM) and particulate organic matter (POM) were also analyzed during 14 of the 16 sampling missions. SPM (mg l^{-1}) samples were obtained in duplicate at two sampling depths by filtering 1 L of water onto a pre-weighed GFF filter (25 mm, glass fiber, Whatman™) that was subsequently rinsed with 0.5-M ammonium formate to dissolve residual salts. Filters were subsequently dried at 70°C for a minimum of 24 h. After being weighed to estimate SPM, the samples were burned in a ceramic

convection oven at 500°C for 5-6 h and then re-weighed to compute the organic content (mg l^{-1}) of the seston (POM) following Aminot (1983).

1.5.3 *M. edulis* retention efficiency (RE)

Cultured mussels (mean size 53.3 ± 6.4 mm) were collected at station SP2 in September 2011 and transferred to a portable aquatic laboratory. They were held in a large tank (250 L) continuously supplied with *in situ* (St. Peters Bay) natural seawater (15.6 ± 2.1°C, 27.6 ± 1.1 ppt, n=5) pumped from close proximity (<0.25 km) to the mussel leases at 5 m below the surface at a rate of approximately 1500 L h^{-1} (Tsurumi Titanium 50TM2.4S submersible pump, semi-vortex/propeller). The high flow rate ensured that mussels did not become food-depleted during the acclimation phase. After a one-week acclimation period, 16 mussels were transferred to individual acrylic chambers (1100 ml) supplied with the same seawater as the holding tank. Metabolic chambers, tanks or containers are commonly used in particle selection, clearance rates or retention efficiency studies with bivalves (Poulsen et al. 1982; Riisgård 1988; Strohmeier et al. 2009; Rosa et al. 2013; Comeau 2013; Comeau et al. 2015). Additional chambers (n=4) containing cleaned shells served as controls to account for gravitational settling of particles. Particle mixing was promoted by fine bubble aeration, introduced in such a way as to minimize the resuspension of faeces. Each chamber was equipped with a fluorometer (CYCLOPS-7® submersible sensor, Turner Designs, Sunnyvale, CA) connected to a data acquisition controller with software (Microlink 751, Windmill Software Ltd, Manchester, UK) that provided a quasi-real time (5-s delay) graphical display of fluorescence. Following a 1-hour adaptation period, water flow was halted and the decline in fluorescence over time was monitored on the computer screen. Any individual that expulsed chlorophyll-concentrated material into its chamber, creating major spikes in the graphical fluorescence display, was excluded from the experiment. Following the protocol in Comeau et al. (2015), only individuals from chambers that showed a continuous exponential decrease in fluorescence over time were included in the final analysis. Each chamber was sampled in duplicate for particle counts (PC) before the input of natural water was turned off and again after 1 h of

static incubation. Each water sample consisted of 4 ml pre-filtered at 40 µm (Falcon Cell Strainer©), stabilized in 5-ml cryovials containing 20 µl of 0.1% gluteraldehyde and kept frozen (-80°C) until counted (# viable cells ml^{-1}) with a flow cytometer. Size characterization of PPP vs NPP was completed by flow cytometry (FCM, Becton Dickinson, San Jose, CA, equipped with a 488 nm laser beam of 15 mW) as described in Belzile et al. (2008). Size fractions computed by the flow cytometer were limited to 0.2–2.0 µm for PPP and 2.0–20.0 µm for NPP. RE for the two size fractions was calculated based on PC measurements at the end of the incubation and the following equation (Comeau et al. 2015):

$$RE = [(PC_{ctrl} - PC_{exp}) / PC_{ctrl}] \times 100$$

where PC_{ctrl} is the particle count in the control chambers, which corrects for the effect of deposition, and PC_{exp} is the particle count in the experimental chambers containing the feeding mussels.

Particular attention was given to the possibility that RE could be positively related to the number of times the animal processed (filtered) the chamber volume. Conceivably, an elevated RE may be attributable to an animal re-filtering the chamber volume many times and accumulating PPP and NPP over time. Such artifacts in the methodology would result in amplification of RE only in those trials where re-filtration occurred, thereby rendering inter-trial comparisons impossible. Available data were used to assess the potential effect of re-filtration of water by individual mussels on the value of RE, and a re-filtration factor (RF) was calculated using the equation below (Comeau et al. 2015):

$$RF = (Inc_T \times CR_{ind} / 60) / V_{ch}$$

where Inc_T is the incubation time (min) in relation to the clearance rate ($l\ h^{-1}$) of individual mussels (CR_{ind}) in each chamber. V_{ch} is the total volume of the chamber (l). CR_{ind} has been measured using the indirect method described in Riisgard (1988, 2011) and Petersen et al. (2004) where water samples (10 ml) were collected at 10-min intervals. Aliquots (100 µl) were processed using a Beckman Coulter Counter Z2 ™ fitted with a 100-µm aperture tube.

The instrument range was set for particles of 5–19 µm which are known to be efficiently retained by mussels (Ward and Shumway 2004). Only mussels that had their valves open and chambers that showed a continuous exponential decrease in particle counts were included in the final analysis. Additionally, only the 10-min interval exhibiting the greatest depletion of particles was used for the calculation of CR_{ind} (Comeau et al. 2015). RF values are equivalent to the number of times the entire volume of the chamber was filtered; an RF value > 1 confirmed that the chamber volume was processed at least once before the end of the feeding trial. In addition, the relationship between RF and RE was examined through a regression analysis in order to determine whether RF effectively augmented RE.

1.5.4 Mussel growth

First and second year-class mussel sleeves (n=40) were deployed at SP2 in November 2010 and thereafter monitored for shell (mm) and tissue (g) growth until June 2012. Approximately 50 mussels from each year-class were randomly sampled from these sleeves by SCUBA divers on six different occasions. At the laboratory the mussels were cleaned of any epifauna and their shell length determined using electronic calipers (Mitutoyo®, ± 0.01mm). Mussels were opened using a scalpel and their tissues extracted from the valves. Tissues were then placed in pre-weighed aluminum boats (60-mm diameter) and dried for 24 h in a convection oven set at 70°C (Fisher Scientific, Mississauga, ON). Dry weights were measured using a digital scale (± 0.0001 g, Mettler Toledo, Mississauga, ON) equipped with glass enclosures. Temperature loggers (Minilogs, Vemco®, Bedford, NS) were deployed with a continuous logging interval of 1 h. Data from the same location (SP2) as the mussel growth assessment were averaged to obtain daily temperature values (°C).

1.5.5 DEB-model

The mussel model is based on DEB theory (Kooijman, 2000), which describes the individual in terms of three state variables: reserve(s), structure(s), and maturity/reproduction. The energy assimilated from food is stored as reserves; a fixed fraction of this energy (κ) is directed towards maintenance and growth of the structural

body, and the remainder (1-κ) is directed towards maturation, gamete production and/or maintenance of the reproductive system depending on the life cycle stage of the organism. DEB theory has been favourably compared to other modelling approaches that explore bivalve bioenergetics (e.g. Filgueira et al. 2011, Larsen et al. 2014).

The DEB model was constructed in Matlab® (http://www.mathworks.com) using a mathematical formulation based on Rosland et al. (2009). A brief description of the model is presented in Table 1 and a more thorough presentation of the model and corresponding equations is given in Pouvreau et al. (2006) and Rosland et al. (2009). The only modification from the original DEB model used in the Pouvreau et al. (2006) study was to the ingestion function module. Specifically, this function was modified as follows so as to incorporate picoplankton and nanoplankton as potential food sources. The notation follows Kooijman (2000), where braces {} denote quantities expressed as per unit surface area of the structural volume and first derivatives with respect to time are indicated with overdots. The energy ingestion rate $\dot{p}_X$ (J d^{-1}) is proportional to the surface area of the mussel:

$$\dot{p}_{\bar{x}} = \{\dot{p}_{Xm}\} T_D f V^{2/3} \qquad \text{Eq. 1}$$

where $\{\dot{p}_{Xm}\}$ is the maximum ingestion rate per unit surface area (J cm^{-2} d^{-1}), T_D is the Arrhenius temperature function, f is the dimensionless mussel ingestion function, and $V^{2/3}$ is proportional to the surface area of the mussel expressed by the structural volume V. The dimensionless mussel ingestion function, f, scales the ingestion rate to food concentration following a Michaelis-Menten term:

$$f = \frac{RE_p \times X_p + RE_n \times X_n}{RE_p \times X_p + RE_n \times X_n + X_K} \qquad \text{Eq. 2}$$

where RE_p and RE_n are the retention efficiency of PPP and NPP, respectively, X_p and X_n are the chlorophyll biomass (µg chl-*a* l^{-1}) of PPP and NPP, respectively, and X_K is the half-saturation coefficient (µg chl-*a* l^{-1}), or the food concentration when ingestion rate reaches half the maximum rate.

In our study, two sets of DEB parameters for mussels were tested, Rosland et al. (2009) and Saraiva et al. (2011). These sets were used strictly to verify their capacity to successfully simulate mussel growth using their respective parameters. However, some DEB parameters were calibrated in this study as well as other parameters that refer to the specific initial set up of the model (Table 1).

Table 1: Differential equations and parameters of DEB model. See references for parameter values discussion

Differential equation	Parameter	
$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C$	E	energy storage (J)
	$\dot{p}_A$	assimilation rate (J d^{-1})
	$\dot{p}_C$	mobilization rate of reserve energy (J d^{-1})
$\dot{p}_A = \{\dot{p}_{Am}\} T_D f V^{2/3}$	$\{\dot{p}_{Am}\}$	maximum surface-area-specific assimilation rate (J cm^{-2} d^{-1})
	f	Michaelis-Menten term (see text)
	V	structural volume (see text)
$T_D = \exp\left(\frac{T_A}{T_1} - \frac{T_A}{T_K}\right)$	T_D	Arrhenius temperature function
	T_A	Arrhenius temperature (K)
	T_1	reference temperature (K)
	T_K	observed temperature (K)
$\dot{p}_C = \frac{[E]}{[E_G] + \kappa [E]} \left(\frac{[E_G]\{\dot{p}_{Am}\} V^{2/3}}{[E_m]} + \dot{p}_M \right)$	κ	fraction of utilized energy to somatic maintenance and growth
	$[E_G]$	volume-specific costs for structure (J cm^{-3})
	$[E_m]$	maximum storage density (J cm^{-3})
$\dot{p}_M = [\dot{p}_M] V$	$\dot{p}_M$	maintenance rate (J d^{-1})
	$[\dot{p}_M]$	volume-specific maintenance costs (J cm^{-3} d^{-1})
$\frac{dV}{dt} = (\kappa \dot{p}_C - \dot{p}_M) / [E_G]$		
$\frac{dE_R}{dt} = (1-\kappa)\dot{p}_C - \left(\frac{1-\kappa}{\kappa}\right) \cdot \min(V_P, V) \cdot [\dot{p}_M]$	E_R	energy allocated to reproductive buffer (J)
	V_P	structural volume at sexual maturity (cm^{-3})
$\frac{dE_R}{dt} = \kappa \dot{p}_C - \dot{p}_M \mid \kappa \dot{p}_C - \dot{p}_M < 0$		reproductive buffer dynamics when energy storage is too low
$L = \frac{V^{1/3}}{\delta_M}$	L	mussel shell length (cm)
	δ_M	dimensionless shape coefficient

The calibration of these parameters followed the protocol described by Duarte et al. (2010). A population of 100,000 mussels was simulated, in which each individual has a specific parameter set randomly selected within certain ranges (Table 2). In the case of PPP and NPP retention efficiency (RE), the values were randomly selected following a normal distribution according to the mean and standard deviation values calculated in this study (Table 2).

Table 2: Ranges of calibrated parameters (DEB model).

Parameter	Minimum	Maximum	Mean±s.d.
Shape coefficient (dimensionless)	0.195	0.3	
Fraction of initial non structural energy to E_R (%)	0	100	
Gonado-somatic index threshold for spawning (%)	15	40	
Reproductive buffer released during spawning (%)	0	100	
X_K (µg Chl a L^{-1})	0	20	
Phytoplankton absorption efficiency (%)	70	90	
Picoplankton retention efficiency (%)			20±2.0
Nanoplankton retention efficiency (%)			60±3.5

The agreement in mussel length and weight between simulated and observed values has been computed for each mussel as deviation (F, Rosland et al. (2009)):

$$F = \frac{100}{2T}\left(\sum_{t=1}^{T}\frac{|M_m(t)-M_o(t)|}{M_o(t)} + \sum_{t=1}^{T}\frac{|L_m(t)-L_o(t)|}{L_o(t)}\right) \quad \text{Eq. 3}$$

where t is the time index, T the total number of observations in the dataset, M_m and M_o the modeled and observed tissue mass, and L_m and L_o the modeled and observed shell length. The parameter sets that produced an acceptable fit between predicted and observed mussel growth were selected according to the following criteria:

- Deviation was less than 9%.
- Final deviation ($t = T$ in Eq. 3) was less than 9%.
- Simulated weight and length were within the standard deviation of the observations.

A new simulation was run for each successful set of parameters in which picoplankton retention efficiency was parameterized as 0% in order to investigate the picoplankton contribution to mussel growth.

1.5.6 Statistics

Generalised Additive Mixed Models (GAMMs) were used to investigate potential relationships between chl-*a* and cell counts by sampling station over time. The ‘‘mixed GAM computation vehicle’’ (mgcv) library version 1.7–29 (Wood, 2006) of the R environment (version 3.0.1, R Core Team, 2013) was used to model the data. Phytoplankton size fractions (PPP vs NPP) and sampling stations (spatial) were used as factors while thin plate splines were used for the smooth functions for time. Models that included smooth functions for each phytoplankton size fraction by station combination were compared to models that included a different smooth function for each PP type and/or station. Significance of factors was determined by comparing models with and without the factor using likelihood ratio (L.ratio) tests and Akaike’s information criteria (AIC). When a significant difference between models was found ($\Delta AIC > 2$ and $p < 0.05$), the best model was chosen based on the AIC where the model with the lowest AIC is generally the best. The significance (p-value) of the chosen model was assessed using the L.ratio analyses and presented in tables. When differences were found in the parametric terms, multiple comparisons were carried out using the *glht* function of the *multcomp* library version 1.2-18 (Hothorn et al 2008). GAMMs were not used as a prediction tool, but rather as descriptive statistics to assess the state of St. Peters Bay in terms of phytoplankton size and biomass over space and time. GAMMs outputs included parametric terms (means variance, in tables), as well as multiple comparisons (integrated in figures, grouping means with letters) with a set significance threshold of $p<0.01$. Smooth functions for each data set (chl-*a* and cell counts) were presented graphically to show temporal trends in relation to a significant factor (i.e. sampling station or phytoplankton size fraction). A single-factor ANOVA was used to calculate if there were significant differences in the phytoplankton community ratio (PPP vs. NPP) between sampling stations using phytoplankton biomass (chl-*a*) data. Linear

regression was used to evaluate the effect of RE on RF and assess the relationship between SPM and POM versus phytoplankton biomass.

1.6 RESULTS

1.6.1 Seston characteristics

Residual analysis of the phytoplankton biomass (chl-*a*) data indicated heterogeneity in the data. Consequently, a variance covariate was included in the models testing for a relationship with phytoplankton size and sampling station. The GAMM model with one smooth (time) per sampling station had the lowest AIC (Table 3) and therefore was retained as the best model. Accordingly, the partial effects of time on phytoplankton biomass (independent of phytoplankton size) are presented for each sampling station in Figure 9. The L.ratios of the GAMM analysis show that the temporal patterns were similar at SP0 and SP4 as well as at SP2 and SP3, while SP1 was significantly different from all the other stations ($p<0.001$). The parametric terms of the GAMM model for phytoplankton biomass, which represent the means of all the phytoplankton size and sampling stations averaged over time, are presented in Table 3. Means (showed in Figure 10) were significantly different between phytoplankton size fractions and sampling stations and there was a significant interaction between the two factors (Table 3).

Table 3: GAMM results showing AIC and L.ratio (with p-value) for best model choice and variance analyses for chlorophyll-*a* in relation to the phytoplankton size classes and sampling station.

Model definition	**df**	**AIC**	**L-Ratio**	**p-value**
Smooth (time)/phytoplankton size x sampling stations	37	1215.3		
Smooth (time) /phytoplankton size	21	1239.8		
Smooth (time) /sampling stations	27	**1159.6**	**35.6**	**< 0.001**
One overall smooth (time only)	19	1217.2		

Chlorophyll-a	**df**	**F**	**p-value**
Phytoplankton size	1	64.6	*8.4e-15***
Sampling station	4	13.7	*1.5e-10***
Phytoplankton x Station	4	6.9	*1.9e-05***

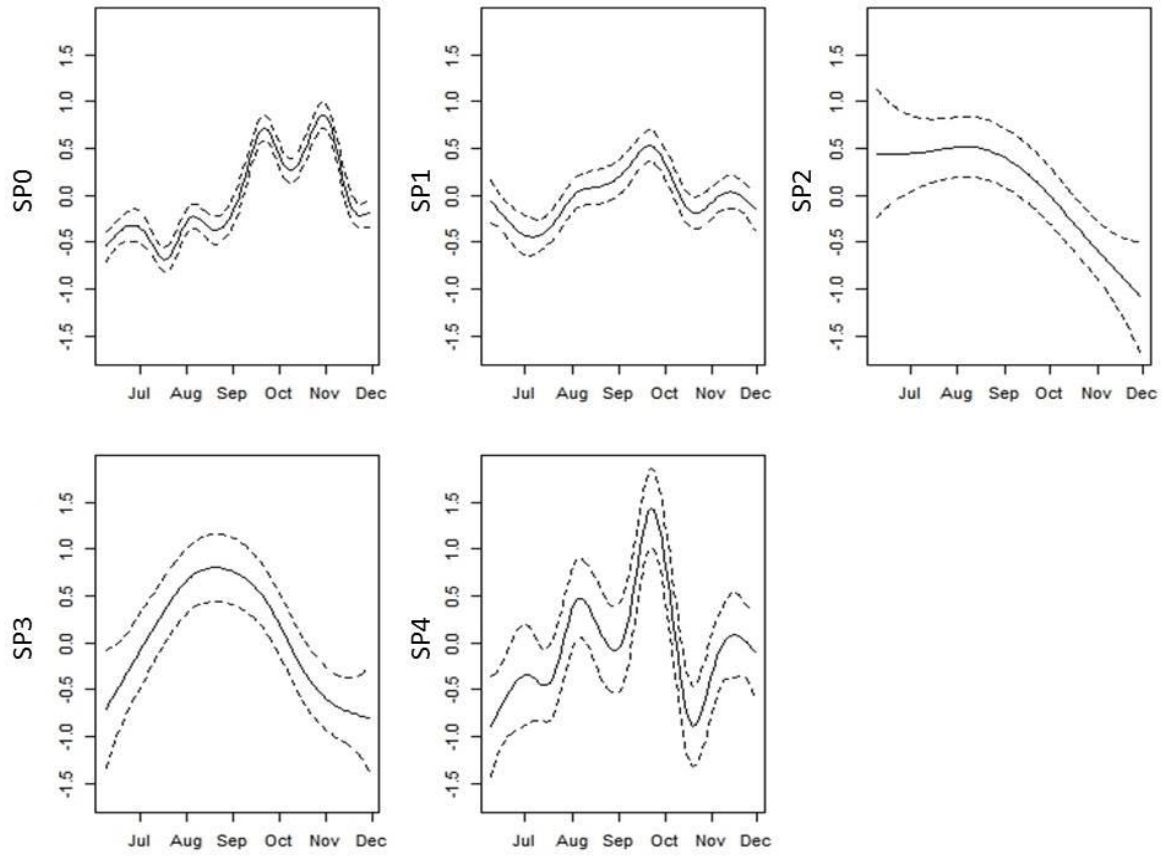

Figure 9: Chlorophyll-*a* biomass time series (smooths , ±95% confidence intervals) with sampling stations (SP0, SP1, SP2, SP3 and SP4) for 2011.

Multiple comparison results (Figure 10) showed that NPP biomass at SP0 was similar to SP1, but both were significantly lower than at the inner stations (SP2, SP3 and SP4). Similar results are observed for PPP biomass where SP0 and SP1 were different but both were significantly lower than the three upstream sampling stations (SP2, SP3 and SP4). Estimates for the latter three sampling stations were similar. These results reveal an increasing gradient in NPP and PPP biomass from outside towards the head of St. Peters Bay. However, there was no significant difference (Table 4) in the PPP:NPP ratio (%) (Figure 10) among sampling stations; values were similar for SP0 (1 km outside the bay) and SP4 at the head of the bay.

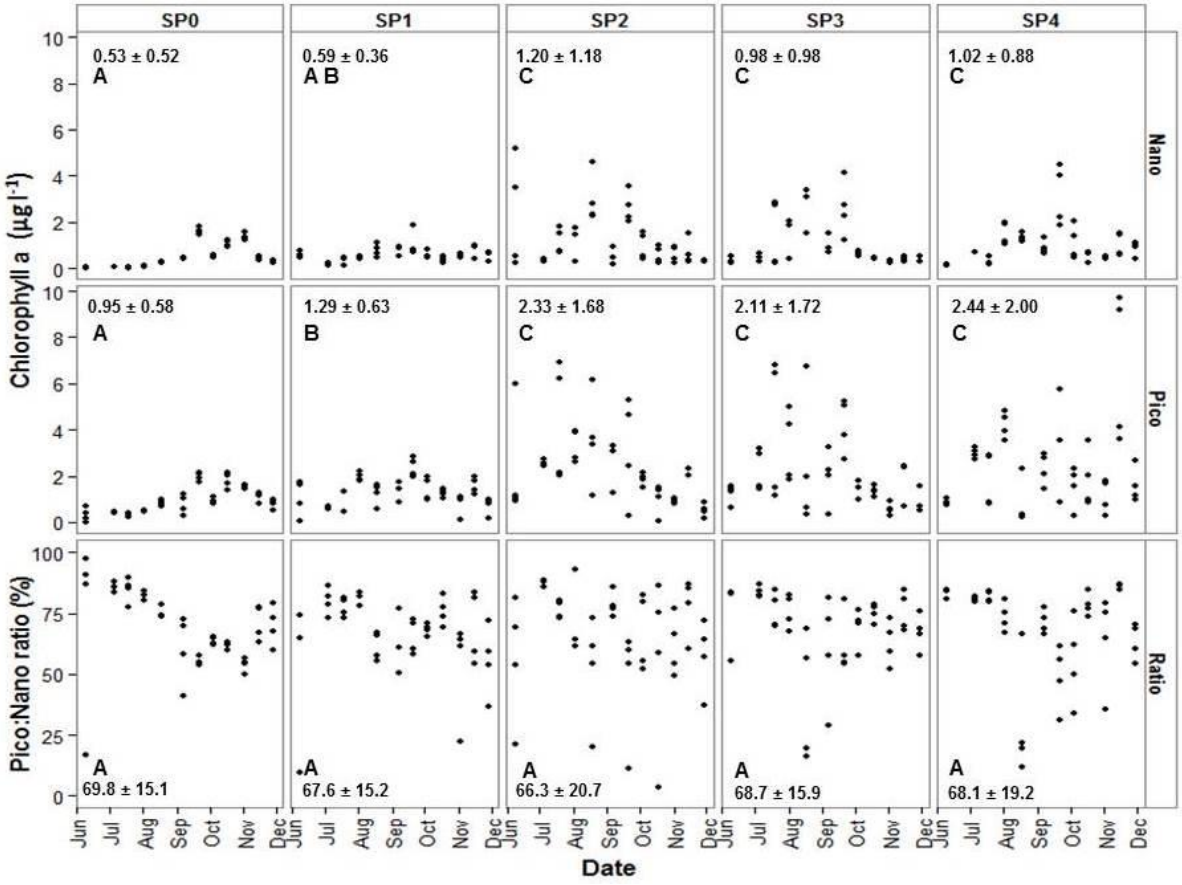

Figure 10: Chlorophyll-a (µg l^{-1}) (means ± SD) associated to NPP (>3µm) and PPP (0.2<3µm) including the PPP ratio (%) at each station in relation to sampling dates in 2011. Station sequence is from the mouth of the bay (SP0) to the head of the bay (SP4). Significant multiple comparisons results presented with A, B and C lettering.

Table 4: Single-factor ANOVA for mean PPP:NPP biomass ratio (%) in relation with sampling stations.

Sampling stations	**df**	**MS**	**F**	**p-value**	**F crit**
Between groups	4	0.007809	0.259345	0.90375	2.410058
Within groups	235	0.03011			
Total	239				

As noted for phytoplankton biomass, residual analysis of the cell counts showed heterogeneity in the data. A variance covariate was also included when testing for the effect of phytoplankton size and sampling station. The GAMM model with one smooth (time) per phytoplankton size had the lowest AIC (Table 5) and was used as the best statistical model.

Table 5: GAMM results showing AIC and L.ratio (with p-value) for best model choice and variance analyses for cell counts in relation to the phytoplankton size classes and sampling station.

Model definition	**df**	**AIC**	**L-Ratio**	**p-value**
Smooth (time) /phytoplankton size x sampling stations	40	4788.9		
Smooth (time) /phytoplankton size	24	**4698.6**	**58.3**	**< 0.0001**
Smooth (time) /sampling stations	30	4800.2		
One overall smooth (time only)	22	4795.5		
Counts	**df**	**F**	**p-value**	
Phytoplankton size	1	168.1	*<2e-16***	
Sampling station	4	22.0	*2.9e-15***	
Phytoplankton x Station	4	0.8	0.512	

Therefore, the partial effects of time on cell counts (independent of sampling station) are presented for each phytoplankton size fraction in Figure 11. The L.ratios of the GAMM analysis show a temporal pattern for the two phytoplankton size fractions ($p<0.001$), where NPP counts follow a depressed normal curve whilst PPP counts show a high variability. Parametric terms of the GAMM model for cell counts, which represent the means of all the phytoplankton size fraction by sampling station combinations averaged over time, are presented in Table 5. Cell counts means in Figure 12 are different between sampling stations as well as phytoplankton size fractions with no significant interaction between the two factors (Table 5).

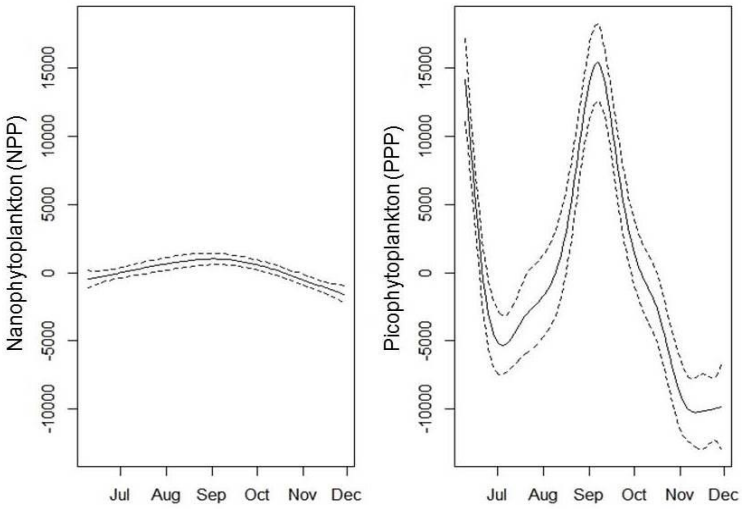

Figure 11: Cell counts time relation (smooths, ± 95% confidence intervals) with phytoplankton size classes for 2011.

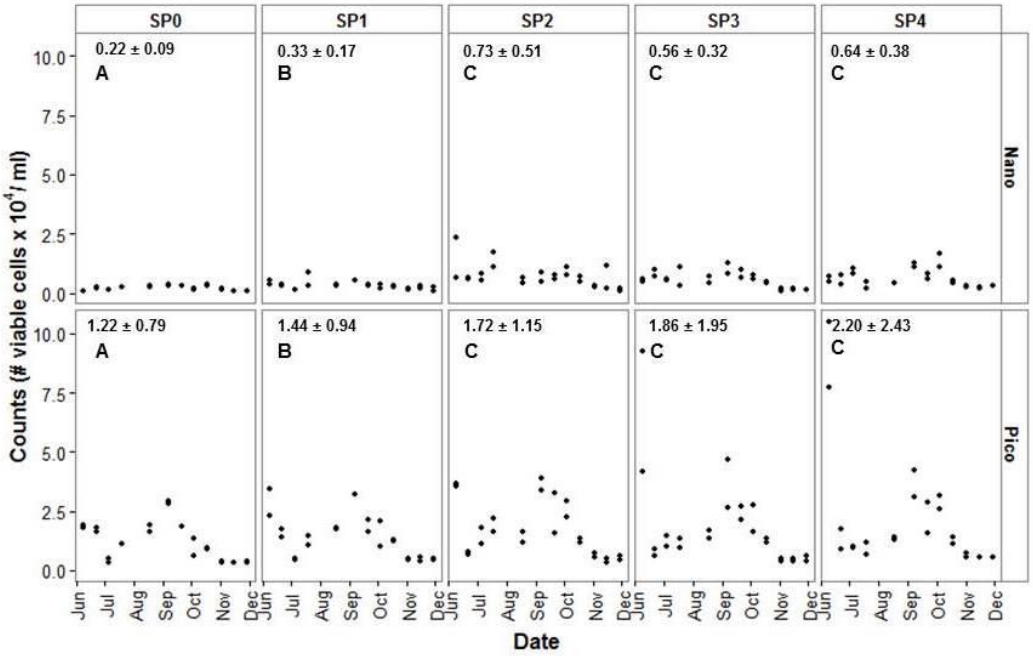

Figure 12: Counts (# viable cells ml^{-1}) (means ± SD) of NPP (2 < 20µm) and PPP (0.2<2µm) at each station in relation to sampling dates (2011-2012). Station sequence is from the mouth of the bay (SP0) to the head of the bay (SP4). Significant multiple comparisons results presented with A, B and C lettering.

Subsequent multiple comparisons showed that, for both phytoplankton size fractions (NPP and PPP), SP0 was different and lower than all other stations. SP1 also differed from SP0 as well as SP2, SP3 and SP4. The latter three sampling stations were similar in terms of cell counts. Consistent with the phytoplankton biomass results, cell counts data revealed an increasing gradient from outside towards the inside of the bay.

Maximum SPM content (Figure 13) was observed in late September and mid-November (21.1 and 44.4 mg l^{-1}, respectively). The latter value may coincide with a wind storm on November 16th, 2011. POM data were also relatively high for the same sampling date. Total chl-*a* values (sum of chl-*a* data presented in Figure 3) showed significant relationships with SPM ($p=0.047$, $R^2=0.409$ $n=10$) and POM ($p=0.039$, $R^2=0.433$ $n=10$). Water temperature reached a maximum 20.0°C in early September, whilst the lowest value recorded prior to data logger retrieval was 4.3°C in late November (Figure 13).

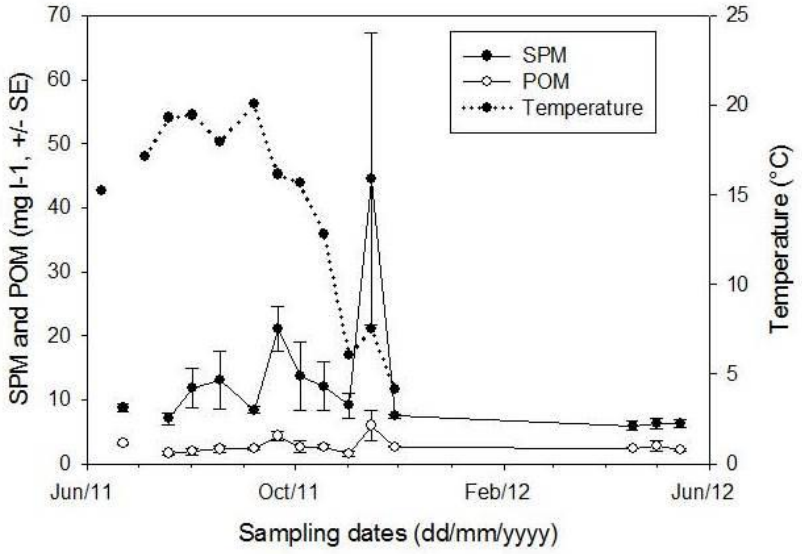

Figure 13: Mean (± SE) SPM (mg l^{-1}) and POM (mg l^{-1}) at each sampling date (n=14).

1.6.2 *In situ* retention efficiency (RE) of *M. edulis*

Mytilus edulis exhibited significantly different RE values for PPP and NPP (t-test, $p<0.001$, df=29, n=19). In trials using natural seston estimates of RE for PPP (0.2–2.0 μm) ranged from 3% to 37% and averaged 20 ± 2% (SE). Estimates of RE for NPP (2.0–20.0 μm) ranged from 19% to 81% with a mean of 60 ± 3.5% (SE). Re-filtration or RF values for the feeding trials ranged from 1.5 to 6.5 with an average of 3 ± 0.3 (SE). Linear regressions (Figure 14) indicated that RF had no significant effect on the estimates of RE for PPP and NPP ($p>0.05$, n=16).

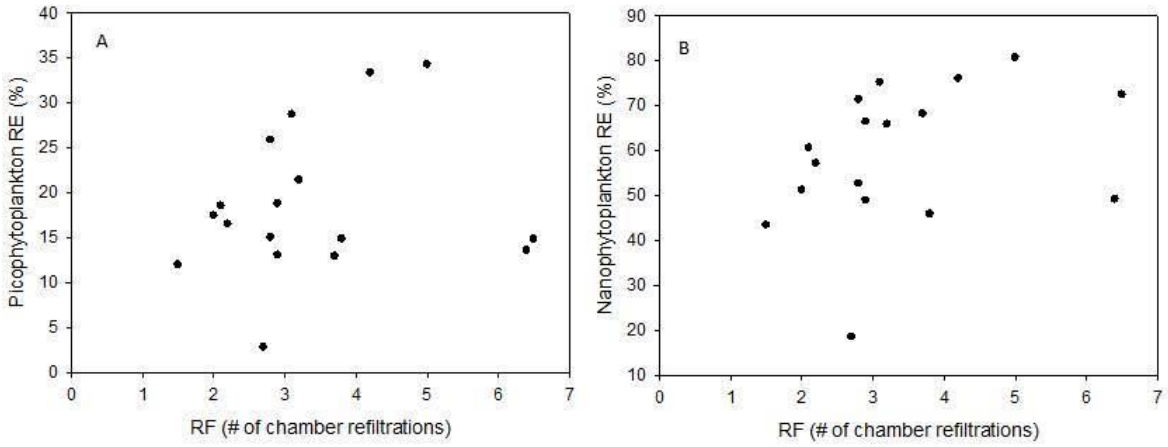

Figure 14: Regressions (not significant) between RE and RF for A) picophytoplankton and B) nanophytoplankton.

1.6.3 DEB model

Both core sets of DEB parameters, Rosland et al. (2009) and Saraiva et al. (2011), were found to simulate bivalve growth successfully (Figure 15). The main difference between the two sets was related to weight predictions and specifically to spawning. Rosland's parameters required two spawning events while only one was needed with Saraiva's parameters (Figures 15c and 15d, respectively). Regarding the calibrated parameters, statistically similar values (Kruskal-Wallis, $p>0.05$) were obtained for both sets

of parameters and years (Table 6) with two exceptions: (1) 'Fraction of initial non-structural energy to E_R (%)' in the 2009 cohort for Saraiva's parameters, which was statistically higher than the 2010 cohort, as well as higher than the values calibrated using Rosland's parameters; (2) 'X_K' which was different between cohorts for both sets of parameters. In addition, the impact of PPP on predicted growth was also statistically different, ranging between 14% and 29% for the 2009 and 2010 cohorts, respectively, using Rosland's set of parameters versus 27% and 16% using Saraiva's set.

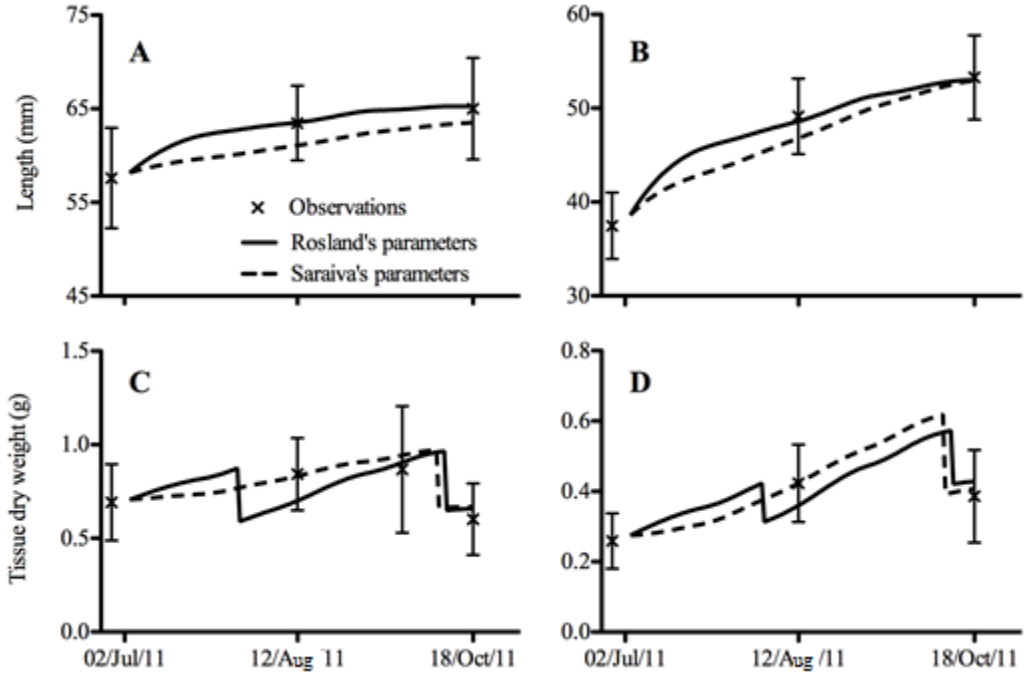

Figure 15: Simulated and observed mussel shell length (mm) and tissue dry weight (g) for the 2009 (A and C) and 2010 cohorts (B and D), including error bars (±SD).

Table 6: Calibrated parameters, *** represents significant statistical differences ($p<0.001$) between 2009 and 2010 cohorts and consequently averaged values were not calculated.

Parameter	Rosland's parameters				Saraiva's parameters			
	2009	**2010**	**Mean±sd**	**CV (%)**	**2009**	**2010**	**Mean±sd**	**CV (%)**
Shape coefficient (dimensionless)	0.21±0.01	0.21±0.01	0.21±0.00	1.6	0.22±0.01	0.22±0.01	0.22±0.00	1.0
Fraction of initial non structural energy to E_R (%)	27.7±16.6	29.8±15.5	28.7±1.5	5.2	57.6±22.2	29.0±14.3	***	46.8
Gonado-somatic index threshold for spawning (%)	32.0±4.2	29.9±4.1	31.0±1.5	4.9	35.0±4.7	31.9±5.6	33.5±2.2	6.5
Reproductive buffer released during spawning (%)	59.6±26.4	62.6±22.7	61.1±2.1	3.4	89.2±12.2	77.4±20.7	83.3±8.3	10.0
X_K (µg Chl a L^{-1})	2.62±0.55	1.59±0.15	***	34.6	3.28±0.34	1.52±0.16	***	52.0
Phytoplankton absorption efficiency (%)	79.1±5.7	77.4±5.3	78.2±1.2	1.6	77.0±5.3	74.6±3.8	75.8±1.7	2.2
Picoplankton retention efficiency (%)	19.8±2.0	19.9±2.0	19.9±0.1	0.4	19.8±2.7	20.0±1.3	19.9±0.14	0.7
Nanoplankton retention efficiency (%)	60.2±2.5	59.1±2.1	59.6±0.8	1.4	59.5±2.5	59.4±1.9	59.5±0.1	0.1
Growth reduction without picoplankton (%)	13.7±13.0	28.6±3.4	***	49.5	27.0±8.7	16.4±1.5	***	34.4
Successful cases	206	38			13	11		

1.7 DISCUSSION

In this study, we determined *M. edulis* RE and estimated the potential contribution of PPP to the growth of mussels in an intensive culture environment. To our knowledge, the contribution of PPP to mussel growth and therefore aquaculture production has never been previously assessed. Thus, for the first time, our results based on DEB modelling indicate that PPP could contribute between 13.7% and 28.6% of the energy to support mussel growth (shell and tissues). The following sources of uncertainty should be considered when interpreting these results. It is important to emphasize that organic detritus has not been included as a potential food source in the model and consequently the contribution of PPP to mussel growth could be overestimated. Another potential source of uncertainty in the model estimations is the nutritional value of PPP and NPP, which is assumed to be similar in this numerical exercise. In addition, the estimated contribution of PPP to mussel growth is also dependent on the RE values integrated into the DEB model. Due to technical constraints, the PPP size range for RE analysis (0.2–2.0 µm) differed from the size range used for biomass assessment (0.2–3.0 µm). This may have lead to an underestimate of the mussels' ability to retain the PPP biomass component used in the DEB model (0.2–3.0 µm), thus underestimating the contribution of PPP to *M. edulis* growth. Given these sources of uncertainty and the fact that a model is based on modeller's assumptions, this numerical exercise should be considered as a scoping exercise on the contribution of PPP to mussel growth.

In situ feeding trials indicated that mussels have an RE (± SE) of 20 % (± 2.0) for PPP (0.2–2 µm) and 60% (± 3.5) for NPP (2–20 µm). These results are comparable to those obtained for cultivated *M. edulis* in Norway; specifically, Strohmeier et al. (2012) found REs of 15–45% for 2-µm particles and 35–90% for 20-µm particles. Regarding PPP, our mussel RE value of 20% falls within the range of 14–64% reported for Norway (Strohmeier et al. 2012) and is consistent with an average value of 20% recently reported in the Netherlands for juvenile mussels (Jacobs et al. 2015). Kach and Ward (2008) suggested that mussels have a RE lower than 50% for PPP. However, it should be noted that RE is

variable and species specific (Shumway et al. 1985; Riisgård 1988; Ward and Shumway 2004). For instance, Strohmeier et al. (2012) reported that particle capture by *M. edulis* is subject to temporal and spatial variations, which suggests that the species has the capacity to modulate RE in response to the seasonal availability of trophic resources. On the other hand, there is no obvious physical mechanism that can explain RE modulation (Riisgård et al. 2013, 2015), and there are possible confounding factors and artifacts (e.g., shifts in phytoplankton shape) when experiments rely on natural seston over an extended period of time (Strohmeier et al. 2012; Rosa et al. 2015). While a better understanding of mussel-phytoplankton interactions can be achieved by constructing ecosystem models, these models require robust food biomass and RE parameterization. Such efforts will not only help understand the net effect of bivalve filtration on pelagic/planktonic communities, but also contribute to refining estimates of production and ecological carrying capacity of coastal systems.

The DEB simulations suggested a reduction in mussel growth when the RE for PPP was set at 0%. Using our *in situ* RE values and mussel growth (shell and tissue) data, such simulations imply that PPP could account for approximately 20% of mussel growth during the post-spring bloom period. This result is also consistent with reports that *M. edulis* can assimilate PPP, as recently demonstrated by the use of isotopically-labelled PPP (Leblanc et al. 2012). The main difference between Rosland's and Saraiva's parameters is related to the different predictions for reproductive behavior. Rosland's set of parameters required two spawning events to match the observed mussel weight, while Saraiva's parameters only needed one. This is directly related to the different values of one of the key DEB parameters, κ. Rosland's κ is lower than Saraiva's, 0.45 vs 0.67, and consequently, 1-κ, the energy allocated towards reproduction is higher in Rosland's parameters. Therefore, the higher energy invested in reproduction using Rosland's parameters implies a higher release of gametes than Saraiva's. The two spawning events predicted by Rosland`s parameters are consistent with the behavior of mussels in St. Peters Bay (Smith and Ramsay, 2012). The other parameter that differs significantly is X_K, a parameter that has been commonly used to calibrate DEB models of *M. edulis* (e.g. Rosland et al. 2009; Filgueira et al. 2011). A

thorough comparison of parameters was not the objective of this study and specific experiments involving simultaneous feeding, respiration, excretion, structural body weight and gonadosomatic index are required to elucidate which parameter set better represents mussel energetics.

Shellfish aquaculture is an industry dependent on natural resources (mainly phytoplankton) and the ability of the marine system to replenish itself in a sustainable way (McKindsey et al. 2006). It is also well documented that shellfish aquaculture applies important grazing pressure on available phytoplankton biomass and communities (Dame 1996; Prins et al. 1998; Strohmeier et al. 2012; Cranford et al. 2014). Given size-selective feeding, filtration pressure could exert a differential effect on PPP and NPP populations. For instance, NPP is significantly curtailed compared to PPP in the Thau Lagoon (France), a small embayment (75 km^2) where over 20,000 t of oysters are produced annually (Vaquer et al. 1996). By contrast, in our study, the PPP: NPP ratio was stable across sampling stations, including the reference SP0 station outside the bay. Therefore, we found no indication that intensive mussel culture impacts the composition of the phytoplankton community in St. Peters Bay (PEI). The discrepancy with Vaquer et al. (1996) could be related to (1) the bivalve biomass in St. Peters might be not enough to alter the PPP:NPP ratio through grazing pressure; (2) the effect of nutrient dynamics on phytoplankton dynamics; or (3) different horizontal advection patterns, which can affect water renewal time and consequently food replenishment. For example, it is known that bivalves can excrete important amounts of ammonium ion in the water column which favors the growth of PPP (Chisholm, 1992; Courties et al. 1994). A similar relationship has been recently demonstrated with bacteria by Jacobs et al. (2015) looking at the impact of juvenile mussel filtration on the microbial community. In the case of NPP, Fang et al. (2006) demonstrated that NPP production is accelerated at high phosphate levels. Agricultural land covers 42.5% of PEI, most of which (69%) is used to grow various types of crops (Statistics Canada 2014). St. Peter's Bay is surrounded by agricultural land and supplied by three major rivers, all of which are potential sources of anthropogenic nitrate (Bugden et al. 2014) and phosphate which may benefit NPP. Therefore, excreted ammonia and riverine nutrients

such as phosphate may act synergistically to maintain the PPP:NPP ratio despite heavy bivalve grazing pressure.

1.8 CONCLUSION

In conclusion, our ecosystem-based study provides new information on the value of PPP in the context of mussel aquaculture. We found that PPP dominated the autotrophic biomass within one of the most intensive mussel culture embayments in eastern Canada. However, the PPP:NPP ratio remained stable along the longitudinal axis of the bay including a reference site located 1 km outside the bay. Thus it seems that the composition of the phytoplankton community was not dictated by grazing pressure impacts, such as size-selective feeding or ammonia excretion. Although the underlying factors controlling PPP biomass dynamics remain unclear, we conclude that PPP may be responsible for sustaining up to 14–29 % of the observed mussel growth during the post-spring bloom period. While further field experiments are needed to refine these estimations, this finding promotes our understanding of mussel aquaculture production and carrying capacity in coastal ecosystems.

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CHAPITRE 2

HUÎTRES D`ÉLEVAGE, *CRASSOSTREA VIRGINICA* : RÉTENTION ET ASSIMILATION DU PICOPHYTPLANCTON UTILISANT UNE APPROCHE DE BIO-MARQUEURS MULTIPLES

2.1 RÉSUMÉ

Comprendre les interactions environnementales dont les organismes filtreurs font face est essentiel afin de gérer la ressource conchylicole de façon durable. Peu d'études ont investiguées l'impact de culture des huîtres sur, simultanément, la biomasse phytoplanctonique et la composition de leurs diètes. La présente recherche explore les sources de nourriture importantes pour l'huître, *Crassostrea virginica*, cultivée au Canada Atlantique. L'analyse de profils isotopiques (^{13}C et ^{15}N) ainsi que l'évaluation de profils d'acides gras furent mises de l'avant afin d'identifier les sources nutritionnelles dans des conditions *in situ* en comparant deux techniques d'élevage (suspension et sur le fond). En milieu naturel, la matière organique particulaire représente la source principale de nourriture pour les bivalves incluant une majeure contribution en phytoplancton et microalgues. Une concentration en lipide significativement ($p < 0.05$) plus importante fut détectée dans les tissus des huîtres cultivées en suspension comparativement à celles cultivées sur le fond. Ces dernières, malgré leur proximité du benthos, ne démontrent aucune préférence pour la matière organique issue de sources détritiques ou bactériennes. Les échantillons d'eau (1m sous la surface) démontrent une biomasse de picophytoplancton (PPP, 0.2–2 μm, $1.93 \pm 0.16 \mu g l^{-1}$) significativement ($p < 0.001$) plus importante que le nanophytoplancton (NPP, >2 μm, $1.05 \pm 0.15 \mu g l^{-1}$). Le potentiel d'ingestion de cellules picophytoplanctoniques par *Crassostrea virginica* fut vérifié en laboratoire utilisant des diètes comportant différents ratios PPP / NPP (20%, 50% et 80%). Le PPP utilisé (*Nannochloropsis oculata*) fut enrichi isotopiquement (^{13}C) tandis que le NPP (*Tisochrysis lutea*) resta non marqué. L'analyse d'enrichissement (^{13}C) des acides gras provenant des

différents tissus d'huîtres (glande digestive, branchies, manteau et muscle abducteur) démontre une assimilation du carbone provenant du PPP et ce même en utilisant une diète faible en PPP (20%). De surcroît, l'enrichissement en ^{13}C de l'acide gras 22:2 (NMI) démontre que les précurseurs de NMI, acide gras uniquement bio synthétisé par le bivalve, ont bénéficié du carbone provenant du PPP dans ce processus de biosynthèse. *Crassostrea virginica*, assimile donc la matière organique en suspension, incluant le PPP, qui parfois domine les communautés phytoplanctoniques dans les eaux de surface de nos estuaires. En conclusion, *C. virginica* a la capacité d'exploiter le PPP comme source de nourriture et d'énergie, en utilisant même son carbone dans la bio synthèse d'acides gras dans ses tissus.

2.2 CULTURED OYSTERS, *CRASSOSTREA VIRGINICA*: RETENTION AND ASSIMILATION OF PICOPHYTOPLANKTON USING A MULTI-BIOMARKER APPROACH

Rémi Sonier [ab*], Réjean Tremblay [b], Frédéric Olivier [c], Tarik Meziane [c] and Luc A. Comeau[a]

[a] *Department of Fisheries and Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Science Branch, Moncton, NB, E1C 9B6, Canada*

[b] *Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 allée des Ursulines, C.P. 3300, Rimouski, QC, G5M 1L7, Canada*

[c] *Unité Mixte de Recherche Biologie des organismes et écosystèmes aquatiques (BOREA UMR 7208), Sorbonne Université, Muséum National d'Histoire Naturelle, Université Pierre et Marie Curie, Institut de Recherche pour le Développement, Université de Caen-Normandie, Université des Antilles, 61 rue Buffon 75005 Paris, France*

*Corresponding author: Remi.Sonier@dfo-mpo.gc.ca;

2.3 ABSTRACT

In this study, we investigated the food sources of eastern oysters *Crassostrea virginica* cultivated in Atlantic Canada. Stable isotopes (^{13}C and ^{15}N) and fatty acid biomarkers were used to identify these sources under *in situ* conditions for suspended (~ 0.5 m below surface) and bottom (~ 2 m) culture stocks. It was found that particulate organic matter represented the main food source, with major contributions from live phytoplankton. Higher lipid contents were detected in the digestive glands of suspended oysters compared to bottom oysters ($p < 0.05$). Bottom oysters did not show significant preference for detrital or bacterial organic matter. Near-surface waters contained an elevated picophytoplankton biomass (PPP, 0.2–2 μm, 1.93± 0.16 $\mu g l^{-1}$, mean ± SEM) compared to nanophytoplankton biomass (NPP, > 2 μm, 1.05 ± 0.15 $\mu g l^{-1}$, mean ± SEM). To determine whether the small size PPP was captured and assimilated by *C. virginica*, feeding trials were conducted in the laboratory using three PPP/NPP diets (20%, 50%, and 80% PPP), consisting of isotopically-labelled (^{13}C) PPP cells (*Nannochloropsis oculata*) and non-labelled NPP cells (*Tisochrysis lutea*). An isotopically-labelled fatty acids analysis indicated PPP assimilation in various tissues (digestive gland, gills, mantle, and abductor muscle), including from oysters fed the reduced (20%) PPP diet. Isotopic enrichment (^{13}C) in the FA 22:2 (non-methylene-interrupted or NMI) showed that precursors of NMIs utilized PPP carbon in its biosynthesis process. In conclusion, *C. virginica* assimilated primarily particulate organic matter (POM), including PPP, which dominated the phytoplankton community in near surface waters. *C. virginica* can exploit PPP carbon during fatty acid production and further biosynthesis.

Keywords: *Crassostrea virginica*, picophytoplankton, fatty acids, stable isotopes, aquaculture, shellfish

2.4 INTRODUCTION

The Eastern oyster, *Crassostrea virginica*, has a broad latitudinal distribution along the Northwest Atlantic seaboard (Comeau 2013). Suspension-feeding bivalves are important components of many coastal ecosystems; however, feeding pressure impacts plankton dynamics, biodeposition, and nutrient cycling (Prins et al. 1991). This said, shellfish aquaculture also provides important ecological services to the environment such benthic-pelagic coupling, creation of refuges for species from higher trophic levels and control on suspended particles concentration (Coen et al. 2007). Recently, it has been demonstrated that bays with large rivers and high exchange with the open ocean will be more resilient under climate change when bivalve aquaculture is present (Filgueira et al. 2016). Bivalves primarily rely on naturally produced phytoplankton and other particulate organic matter as food sources (Trottet et al. 2008). Therefore, the filtration capacity of bivalves could effectively reduce seston concentrations (Guyondet et al. 2013), imposing strong control on phytoplankton assemblages at the base of the aquatic food chain (Prins et al. 1998; Cranford et al. 2009; Filgueira et al. 2014b). In conjunction with particle sorting before ingestion, the size and nutritional quality of food particles are two important factors explaining differences in filtration and retention capacities by bivalves (Barillé et al. 1997; Cresson et al. 2016). The retention efficiency (RE) of filter-feeders usually increases with particle size and may be species-specific (Møhlenberg and Riisgård 1978; Ward and Shumway 2004). Large particles can be captured with high efficiency, based on reported absolute efficiencies of 86 to 98% for 8–10 μm particles for a variety of bivalves (Møhlenberg and Riisgård 1978). In comparison, the retention of 1 μm diameter particles is 50% (Shumway et al. 1985; Riisgård 1988).

A multi-biomarkers approach, using stable isotopes and fatty acid (FAs) trophic marker methods, increases the potential to identify food sources from different origins (Perez et al. 2013). Stable isotopes analyses are widely used in food webs studies, food source reliance and trophic position estimation (Peterson and Fry 1987; Gaillard et al. 2017) also as a tool to determine the contribution of various food items to a given organism

diet (Fry 2007). Since secondary consumers are enriched in ^{13}C and ^{15}N relative to their food supply or prey, stable isotopes, such as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), are useful to determine the long-term assimilation of food (Peterson and Fry, 1987; Post 2002). Because the relationships of these two isotopes differ with the origin of organic matter, measuring the isotopic ratios of the digestive gland of oysters allows the major contributing nutritional sources to be identified (Cresson et al. 2016). Prominent FA biomarkers of primary producers may be tracked within consumers, as they remain mostly unchanged through trophic pathways (Kelly and Scheibling, 2012). Omega-3 (ω3) and omega-6 (ω6) poly-unsaturated FAs (PUFA) are synthesized almost exclusively by phytoplankton, macrophytes and plants (Dalsgaard et al. 2003) and phytoplankton community composition is a strong predictor of FAs content in nature (Lowe et al. 2014). In fact, it has been presented that divisions such Chlorophyta (green algae eukaryote), Dinophyta (dinoflagellate) and diatoms are rich in ω3 FAs with 31%, 40% and 17% respectively (Galloway and Winder 2015). Some FAs are also attributed as dietary tracers for specific sources such as eicosapentanoic acid (EPA 20:5ω3 and 16:4ω1) for diatoms, docosahexanoic acid (DHA 22:6ω3 and 18:4ω3) for dinoflagellates as well as numerous tracers for zooplankton (20:1ω11, 20:1ω9, 22:1ω11, 22:1ω9) and bacteria (*i*-15:0, 15:0, *i*-17:0, 17:0) (Parrish 2013; Gaillard et al. 2015). In bivalves, FAs from the digestive gland contribute to the storage of metabolic reserves, and may be profiled to obtain information on recent food ingestion (Pérez et al. 2013).

The current study used stable isotopes and FAs' methods in parallel to provide information on the food sources of oysters cultured under different conditions: on the bottom and in suspension. Most research on the trophic dynamics of oysters focuses on efficiently retained (80–100%) autotrophic nanophytoplankton (NPP, 2–20 µm) (Barillé et al. 1993; Ward and Shumway 2004; Kach and Ward 2008; Comeau 2013), as higher biomass often leads to greater feeding activity by shellfish (Prins et al. 1998; Newell 2004). To our knowledge, few studies have investigated how oyster culture is jointly impacted by phytoplankton biomass (quantitative) and diet composition (qualitative) (Pernet et al. 2012). Furthermore, most studies focus namely on *Mytilus edulis* (Trottet et al. 2008;

Strohmeier et al. 2012) and *Crassostrea gigas* (Barillé et al. 1993,1997) with little attention on the implications of smaller particles, such as picophytoplankton (PPP) cells which can reach high biomass in nutrient-rich estuaries under certain conditions (Richardson and Jackson 2007; Kirkham et al. 2013). In the Gulf of St. Lawrence (Canada), numerous PPP species are present such as; *Micromonas pusilla, Bathycoccus prasinos, Ostreococcus lucimarinus, Nannochloropsis* sp., *Picochlorum* sp. and *Aureococcus anophagefferens* (Péquin et al. 2017). Thus, this present study aimed to: 1) assess the availability of size-fractioned phytoplankton (NPP and PPP) in the Foxley River system in Prince Edward Island (Canada); 2) identify the major food sources of cultured oysters (bottom versus suspension); and 3) determine the potential of retention and assimilation of PPP cells by *C. virginica*. We tested two hypotheses, 1) bottom cultured oysters use more bacterial and microphytobenthos food compared to suspended cultured oysters and 2) PPP is a dominant food source in this oyster culture system, and is significantly ingested and assimilated by oysters.

2.5 MATERIAL AND METHODS

2.5.1 Field experiments

Phytoplankton biomass

During the ice-free period (May to November 2014), water samples were collected (duplicates) twice a week from five sampling stations in Foxley River (Prince Edward Island, Canada), where oyster aquaculture is predominant (Figure 15). This area is quite shallow (max depth ~3 m) and well mixed (no stratification). Water was collected at 1 m below the surface (5-l Niskin® bottle). Size-fractioned phytoplankton biomass measurements were obtained by extracting chlorophyll-*a* (henceforth chl-*a*). Sub-samples were filtered on 0.3 µm (50 ml) pore size glass fiber filters (GF75) to obtain total biomass (>0.3 µm), and on 2 µm (150 ml) a pore size polycarbonate filter (25 mm, GE Water and Process Technologies, Trevorse, PA®) for the NPP fraction (> 2 µm). The difference between these two size fractions yields PPP biomass (0.3 > 2 µm). The filters were frozen

directly in the field to -40 °C (Stirling Ultracold portable freezer, Athens, OH, USA), and were kept frozen until analysis. Chl-*a* extractions were performed in 20-ml scintillation vials with 10 ml acetone (90% final concentration) for a minimum of 24 h at -40 °C. Final data were obtained using a bench top fluorometer (Trilogy®, Turner Designs, Sunnyvale, CA) equipped with a chl-*a* (with acidification) module.

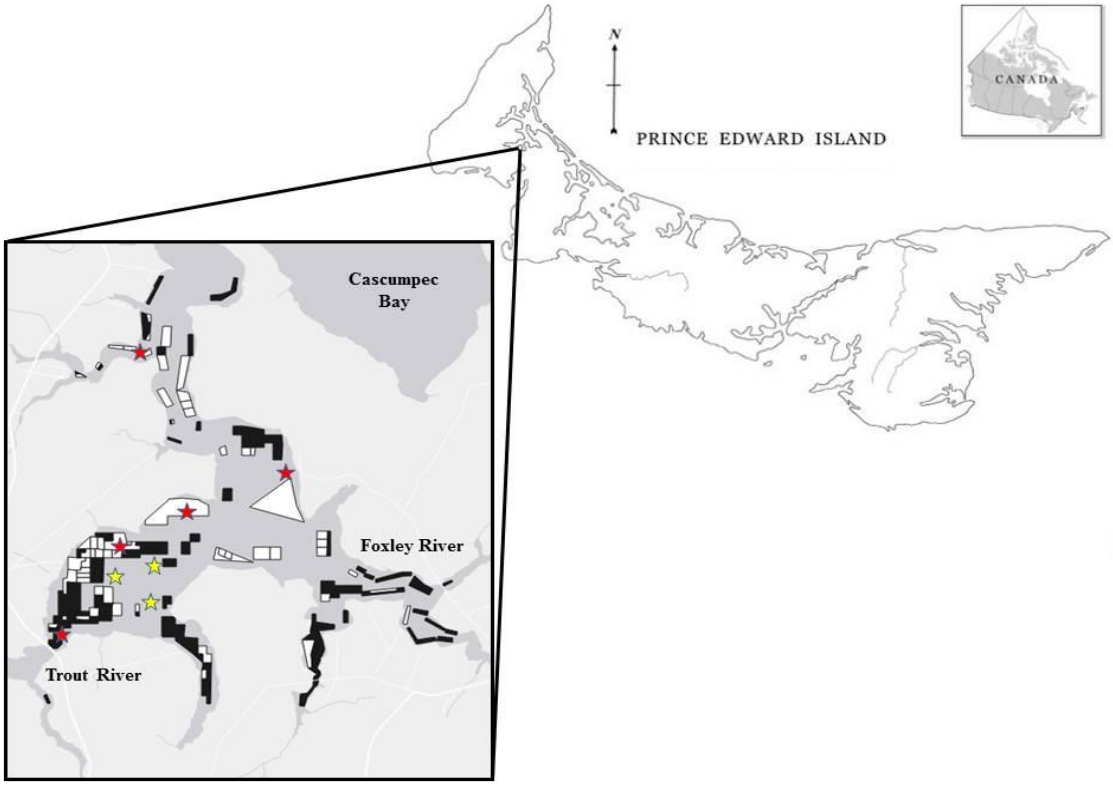

Figure 16: Foxley River system in Prince Edward Island (Canada). Shellfish leases allocated for oyster (*C. virginica*) aquaculture are shown by polygons (white and black areas), in addition to sampling stations for phytoplankton biomass (red) and stable isotope (food sources and oysters) (yellow).

Stable isotopes

To assess the isotopic signature of organic matter in the water column (n = 12), three sampling stations were established (Figure 16) where the water samples were collected (1 m below sea-surface) in June, July, and August 2014. The samples (1-L) were filtered on

GF/F filters (47 mm), dried for a 48-h, and sent to an accredited laboratory facility to analyze (GG Harch Isotope Lab, Dept. of Earth Sciences, University of Ottawa, Canada) carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes. GF/F filters were fumigated with 10% HCl for 4 h to remove inorganic carbon. Samples and standards were weighed in tin capsules, and loaded into an elemental analyzer (Isotope Cube, Elementar, Germany) interfaced to an isotope ratio mass spectrometer. Separated gases were sent to an IRMS (interface Conflo III with unit Delta Advantage, Thermo, Germany). The results are expressed in standard delta notation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard})\text{-}1] \times 1000,$$

where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$ of the sample or standard (Peterson and Fry 1987). All mean values or stable isotopes are expressed as mean ± standard deviation (S.D.).

The isotopic signature ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of oyster tissues was determined at the end of July, from both the bottom (n = 40) and suspension (n = 40) cultures at the three sampling stations (Figure 16). After dissecting half of the digestive gland from each bottom or suspended cultured oyster, it was frozen (-80 °C) in 4.5 ml cryovial, fumigated with 10% HCl for 4 h to remove inorganic carbon and sent for stable isotope analysis. The isotopic ratio (*R*) values of the dried digestive glands from the *C. virginica* samples were determined by the methods developed at the IsoEnvironmental facility (Department of Botany, Rhodes University, Grahamstown, South Africa), using a Europa Scientific 20–20 IRMS interfaced to an ANCA SL Europa Analyser. All $\delta^{13}C$ and $\delta^{15}N$ values were reported as ‰ vs Vienna PeeDeeBelemnite (VPDB) and air, respectively, and were normalized to internal standards calibrated to the International Atomic Energy reference materials (IAEA-CH6 for $\delta^{13}C$ and IAEA-N2 for $\delta^{15}N$). Also samples of other potential food sources were collected by SCUBA divers and sent out for analysis. Samples included macroalgae (*Ulva sp.*), marine plants (*Zostera marina*), and the first layer (3 mm) of sediment (n = 12) where oysters are bottom cultured.

Fatty acids and lipid biomarkers

Half of the digestive gland tissue from oysters from both cultured technique (bottom and suspended) were sampled at five time periods (June, beginning of July, end of July, August and September) for fatty acid (FAs) analysis. Each FAs data point consist of five digestive gland samples pooled together to assure sufficient material for proper extractions. Samples were freeze dried and ground to a homogeneous powder. Lipids were extracted using a modified Folch procedure as described in Parrish (1999). Fatty acid methyl esters (FAME) were prepared according to the method described by Lepage and Roy (1984) using sulfuric acid and methanol (2:98, v/v) at 100 °C for 10 min, and were analyzed in the full scan mode (ionic range: 50–650 m/z) on a Polaris Q ion trap coupled to a multichannel gas chromatograph "Trace GC ultra" (Thermo Scientific) equipped with an autosampler model Triplus, a PTV injector, and a mass detector model ITQ900 (Thermo Scientific®). The separation was performed with an Omegawax 250 (Supelco) capillary column with high purity helium as the carrier gas. Data were treated using Xcalibur v.2.1 software (Thermo Scientific®). Methyl nonadecanoate (19:0) was used as an internal standard. FAME were identified by comparing retention times with known standards (Supelco 37 Component FAME Mix and menhaden oil; Supleco), and were further confirmed by mass spectrometry (Xcalibur v.2.1 software). In all samples, unknown peaks were identified according to their mass spectra, with emphasis on the FA trophic markers described in Table 1.

Table 7: Common fatty acid (FA) biomarkers that were investigated and discussed in the present study.

Source	**FATMs**	**References**
Diatoms	EPA, EPA/DHA >1, 16:1ω7 / 16:0 >1	Dalsgaard et al. 2003, Cabrol et al. 2015, Gaillard et al. 2015
Dinoflagellates	DHA, DHA/EPA >1	Søreide et al. 2008, Kelly and Scheibling 2012
Picoeukaryotes	16:4ω3 + 18:3ω3	Moynihan et al. 2016
Zooplankton	20:1ω9, 20:1ω11, 22:1ω9	Gaillard et al. 2015
Terrestrial plants or seagrasses	18:2ω6 + 18:3ω3 >2.5	Budge and Parrish 1998
Detritus and bacteria	16:1ω7, i15:0, 15:0, i17-0, 17:0	Pernet et al. 2012, Parrish 2013, Cabrol et al. 2015, Gaillard et al. 2017

** EPA: eicosapentanoic acid, 20:5ω3 ** DHA: docohexaenoic acid, 22:6ω3

2.5.2 Laboratory experiments

Retention efficiencies (RE)

Adult oysters (n = 15, dried tissue mass 1.31 ± 0.17 g, mean ± SD) were collected in February 2013 (water temperature of -2°C), and were transferred to the marine station of the Institut des Sciences de la Mer de Rimouski (Pointe-au-Père, Québec, Canada). Equal numbers of oysters were placed in three 180-l tanks with flow-through seawater (filtered through a 1µm filter and UV disinfected) and a gradually increasing temperature (3°C/day) to reach 21 °C for optimal filtration rates (based on Loosanoff 1958). Oysters were acclimatized for one month and were fed daily with a 1:1:1 mixture (v/v/v) of *Tisochrysis lutea* (CCMP 1324)*, Diacronema lutheri* (CCMP 459)*,* and *Chaetoceros neogracile* (CCMP 1317) microalgae at a ratio of 5% oyster tissues dried mass. Algal strains originated from Bigelow National Center for Marine Algae and Microbiota (Maine, USA). Trials were conducted to assess the retention efficiency (RE) of each individual for targeted micro algal species. To assess the RE of *C. virginica* towards PPP cells (<2 µm) in relation to different phytoplankton assemblages, three distinct regimes were used in a controlled environment. These regimes consisted of three different size fraction ratios: 1) 20:80, 2) 50:50, and 3) 80:20 between PPP and NPP (%). Diets were adjusted at a total cell concentration equivalent in volume to 40 cells μl^{-1} of *T. lutea,* which corresponds to approximately 1.72×10^6 μm^{-3} ml^{-1}. Cell counts were determined with a coulter counter (Z2 Beckman Coulter, California, USA) equipped with a 70 µm aperture tube, with 0.1 ml subsample being collected per reading. Algal species considered were *Nannochloropsis oculata* (PPP) (CCMP 525), a spherical unicellular flagellate of 1–2 µm in diameter and *T. lutea* (NPP), which is a golden brown flagellate with a spherical to pear shape of 3–5 µ in diameter (Bigelow Laboratory for Ocean Sciences, Maine, USA). The species were batch-cultured in f/2 medium at 20 °C, under continuous illumination, in 20-l carboys that was supplied continuously with CO_2 to maintain a pH of ~8. Each oyster was acclimatized for 1 h in a 1.2-l metabolic chamber with a circular stream of aeration along the chamber's wall to provide good water homogenization without promoting the resuspension of feces and

pseudo-feces. After oysters were fed their respective experimental regime, subsamples were collected from each chamber every 15 min to assess cell depletion over time. Five oysters, or chambers, were allocated per experimental regime (N = 15). The retention efficiency (RE) was computed as presented in Comeau et al. 2015:

$$RE = [\ (C_{control}-C_{live})/C_{control}\ ] \times 100$$

where RE is percent retention efficiency of phytoplankton (PPP or NPP), $C_{control}$ is the phytoplankton counts (cells per ml) in control chambers, and C_{live} is the phytoplankton concentration (cells per ml) in live specimen chambers.

Refiltration factor (RF)

Particular attention was given to the possibility that RE is positively related to the number of times the animal processed (filtered) the chamber volume during RE trials. Conceivably, an elevated RE might be attributable to an animal re-filtering the chamber volume numerous times, and accumulating PPP and NPP over time (Sonier et al. 2016). This artefact in the methodology would only amplify RE in trials where re-filtration occurred. Available data were used to assess RE versus the re-filtration factor (termed RF), which was calculated using the equation below (Comeau et al. 2015, Sonier et al. 2016):

$$RF = (IncT \times CRind/60)/Vch$$

where Inc_T is the incubation time (min) in relation to the clearance rate (l h^{-1}) of individual oysters (CR_{ind}) in each chamber. V_{ch} is the volume of the chamber (l). CR_{ind} was measured using the indirect method described by Riisgard (1988), with water samples (10 ml) being collected at 15-min intervals. RF values are equivalent to the number of times that the entire volume of the chamber is filtered; RF > 1 confirms that the chamber was processed at least once before the end of the feeding trial. When RF values >1 were recorded, the relationship between RF and RE was further examined through regression analysis to determine whether RF effectively augmented RE. For CR_{ind} values, the first and last particle counts were omitted from the calculations to eliminate potential artifacts, such as

changes in feeding behavior due to the addition of food (experimental regimes) in each chamber or low particle counts at the end of incubation.

Picophytoplankton assimilation

PPP assimilation by oysters was conducted at a larger scale using bigger tanks (180 l) with flow through natural sea water (28 ppt salinity, heated to 21 °C). Each feeding regime (treatment tanks) contained five oysters, for which the food supply was adjusted accordingly. For one week before the experiment, oysters were fed the three respective regimes (%PPP:%NPP of 20:80, 50:50, and 80:20) at a ratio of 5% of oyster tissue dry mass daily for acclimatization. The experimental trials involved three days of feeding, during which regular PPP cells (*N. oculata*) were substituted by isotopically labelled cells (with ^{13}C). PPP was labelled using the protocol of Arnold et al. (2015), which involves supplementing the sea water medium with 1 mM sodium [^{13}C]-bicarbonate ($NaH^{13}CO_3$, 99%) (Cambridge Isotope Laboratories, MA, USA). The experiment ended after the oysters were subject to fasting for three complete days, to allow complete digestion and to purge the digestive system. Each oyster was dissected, and four tissue types were kept: 1) gills, 2) digestive gland, 3) mantle, and 4) abductor muscle. Each tissue was freeze-dried and homogenized using the classic glass mortar and pestle grinding method (sterilized between each sample with ethanol). Five grams of material for each tissue sample was weighed for hydrolysis (100 µl KOH 12.5% to the tissue sample) and subsequent fatty acids (FAs) extraction. In parallel, extraction from cultured and isotopically marked *N. oculata* algal stocks were obtained using direct hydrolyze combined with 50 µl concentrated (centrifuged) algal suspension and 50 µl KOH 25%. For both oyster tissues and algae solutions, the hydrolyzed mixture was then placed in a thermostat controlled dry-bath (60 °C) for 30 min. Following hydrolysis incubation, 900 µl of extraction solvent (1/3 15 mM ammonium acetate and 2/3 acetonitrile, pH 4) was incorporated to the hydrolyzed solution and homogenized (vortex) for fatty acid extraction. Extracted samples were then centrifuged (10 000 rpm, 3 min) to purify lipid extracts by allowing residual proteins to sediment on the bottom of the Eppendorf. After centrifugation, 500 µl of clear supernatant

was collected and transferred to 5 ml amber vials (with PVC septums) for fatty acid characterization. The analysis was performed using Continuous-flow Isotope Ratio Mass Spectrometry (CF-IRMS) with a Deltaplus XP mass spectrometer (ThermoScientific) coupled to an elemental analysis (EA) COSTECH 4010 (Costech Analytical). Analytical error (n = 50) of the measurements was 0.2‰ and 0.4‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. System suitability was evaluated before analysis using a standard deviation of zero reference gas (nitrogen and carbon dioxide) over 10 measurements. Maximum acceptable variation was set to 0.06‰. For each analysis sequence, 10 secondary standards were used to calibrate the obtained values. The certified sediment standard (MicroElemental analysis) had reference values of -4.40 and -26.10 for $\delta^{15}N$ and $\delta^{13}C$, respectively. The two mobile phases consisted of A) 10 mM ammonium acetate and B) 0.01% acetic acid in acetonitrile (ACN). The ingestion and assimilation of PPP by *C. virginica* were estimated using mass spectrometry outputs. More specifically, looking at the enrichment in ^{13}C of specific fatty acids from different food regimes containing isotopically ($\delta^{13}C$) marked PPP cells. Four fatty acids were considered: 1) 20:5ω3 (EPA), which is a dominant fatty acid in the composition of *N. oculata* (Seychelles et al. 2009), 2) 22:6ω3 (DHA), which is a dominant fatty acid in *T. lutea* (then *Isochrysis galbana*) (Seychelles et al. 2009), 3) 20:1, a precursor for NMI and usually synthetized by the organism and are absent in algae used in feeding trials (Zhukova 1991; Da Costa et al. 2015) and 4) 22:2 (Zhukova and Svetashev 1986) typical for most bivalves.

2.5.3 Statistical analysis

The relationship between size fraction (PPP and NPP) biomass over time was tested for autocorrelation and heterogeneity using the "gls" function (library-nlme) in the R environment (version 3.0.1, R Core Team, 2013). Pearson's correlation tests were used to detect correlations between time series. Linear analysis of covariance (ANCOVA) was performed using the "lm" function with one covariate in the R environment. ANCOVA outputs were presented textually (Likelihood Ratio Test = LRT, df, *p* values), and as correlation coefficients (ρ). For the comparison of FA profiles, distance-based

permutational multivariate analysis of variance was used to compare multivariate variables between oysters from the two culture methods. The assumption of homoscedasticity was verified with a PERMDISP test, no data transformations were needed, and then a PERMANOVA (9999 permutations) was performed. A posteriori comparisons were completed using a PERMANOVA pairwise test. To analyze the similarity between profiles, SIMPER (SIMilarity PERcentages) procedures were used to identify variables responsible for dissimilarities (PRIMER®6 software module and PERMANOVA 1.02). Multivariate analyses were performed with PRIMER®6 software and univariate tests using R software (version 3.0.1, R Core Team, 2013). The linear regression analyses was used to evaluate the effect of RE on RF. Differences between culture methods (bottom vs surface) for stable isotopes data ($\delta^{13}C$ and $\delta^{15}N$) were tested using two samples t-tests, with single-factor ANOVAs being used to assess how sampling dates impacted the time series results.

2.6 RESULTS

2.6.1 Phytoplankton biomass

Phytoplankton fractioned (NPP and PPP) biomass (chl-*a* µg/l) (Figure 17) showed autocorrelation ($\rho = 0.49$) and a significant interaction between phytoplankton size and sampling date (LRT = 8.01, df = 1, $p < 0.001$). Both NPP and PPP biomass significantly changed over time (One-way ANOVA, df = 12, $p < 0.0001$). Differences between size classes (Tukey posteriori tests, $p < 0.05$) were attributed to the lower biomass of NPP in late-May and peaks in mid-June and at the end of August. In comparison, PPP biomass had significant peaks in mid-May and late-June, with low concentrations in mid-June and September to November (fall) (Figure 17). Sub-surface water samples showed that PPP (1.93 ± 0.16 µgl^{-1}) biomass was significantly higher compared to NPP (1.05 ± 0.15 µgl^{-1}) biomass ($p < 0.001$). The PPP:NPP biomass ratio (%) ranged between 44 and 89%, and was significantly different between sampling dates (df = 12, $p < 0.0001$) (Figure 17). Significantly lower ratios of PPP were observed in mid-August and September to November (fall) (Tukey posteriori tests, $p < 0.05$).

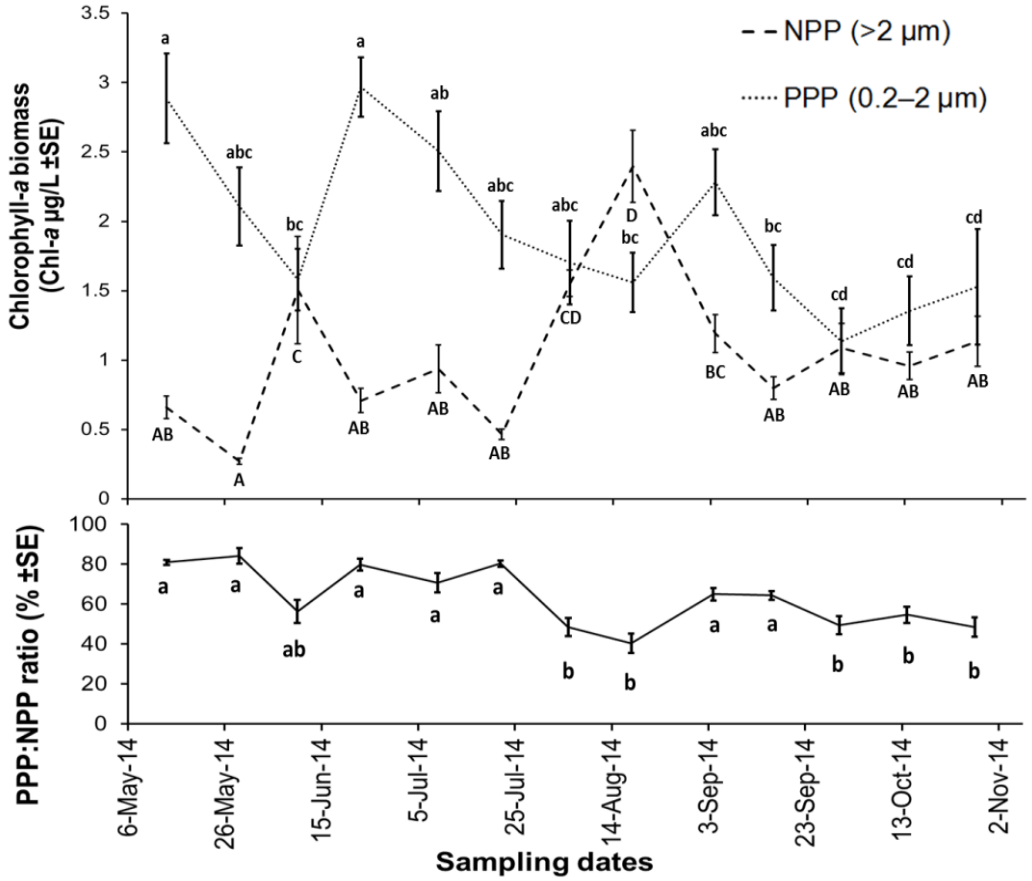

Figure 17: Size-fractioned phytoplankton biomass (average ± SE, averaged from seven sampling stations, in duplicates) including PPP ratio (average % ± SE) in Foxley River (PEI). Tukey posteriori tests results ($p < 0.001$) are presented, with different letters representing significant differences, NPP (uppercase) and PPP (lowercase).

2.6.2 Stable isotopes

In June, July, and August 2014, surface water POM $\delta^{13}C$ and $\delta^{15}N$ values ranged between -24.75 ± 1.53‰ and -12.96 ± 2.60‰ and 7.23 ± 0.94‰ to 10.44 ± 0.29‰, respectively (Figure 18). A significant difference (two-sample t-test, $t = -6.38$ $p < 0.001$, $n = 40$) between $\delta^{13}C$ ratios of suspended cultured oysters (-21.32 ± 1.02‰) and bottom cultured oysters (-19.78 ± 1.14‰) was obtained, whilst no difference was found between $\delta^{15}N$ ratios (9.90‰ ± 0.56 and 9.55‰ ± 1.08, respectively, $n = 40$) (Figure 18). Oysters'

shell length (mean ± SD) was similar at 74.9±7.2 mm and 70.1±7.7 mm for bottom and suspension cultured oysters respectively. Regardless of the culture method used *C. virginica* mostly fed on POM where the nitrogen (δ^{15}N) values of oysters sampled in July were closely related to water POM sampled in June, with a typical enrichment of 2–4‰ (Peterson and Fry, 1987; Post 2002) between the primary consumer and its diet (Figure 18). However, δ^{15}N enrichment results > 4‰ also indicated that the sediments, macroalgae (*Ulva sp.*), and marine plants (*Z. marina*) were not detected as suitable food inputs in cultured oysters from our system.

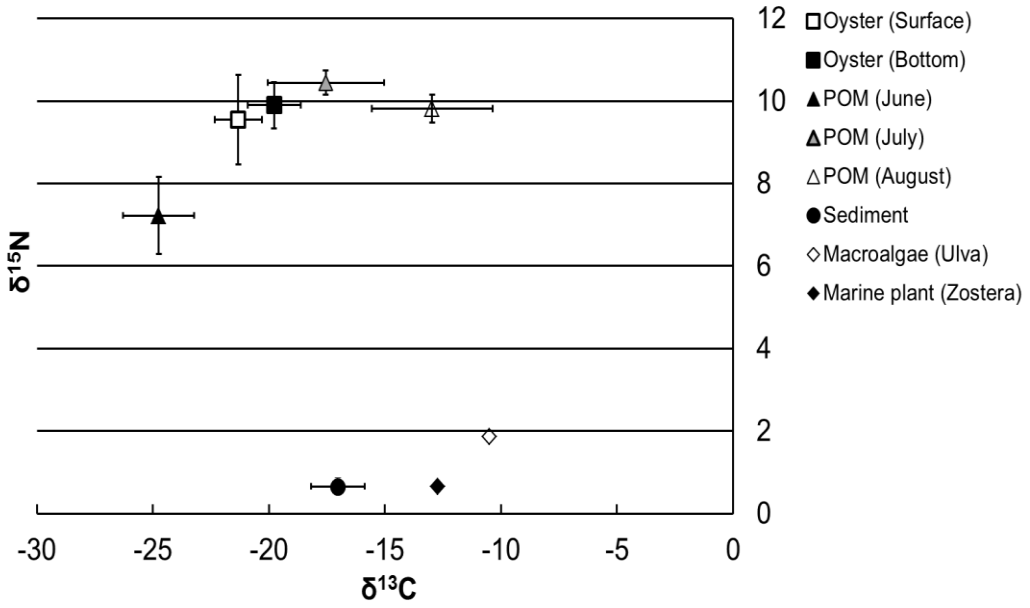

Figure 18: Mean (‰ ± standard deviation) δ^{13}C and δ^{15}N of the digestive glands from surface (n = 40) and bottom (n = 40) cultured oysters (*C. virginica*), in addition to the δ^{13}C and δ^{15}N from different sources: POM (n = 12), sediment (n = 12), a macroalgae (*Ulva sp.*) (n=1), and a marine plant (*Zostera marina*) (n=1).

2.6.3 Fatty acids and lipid biomarkers

Total FA concentration in the digestive glands of oysters was significantly affected by sampling dates (Pseudo-F = 3.73, df = 4, $p < 0.01$) and culture methods (Pseudo-F = 3.86, df = 1, $p < 0.05$) without interaction (Pseudo-F = 0.61, df = 4, $p > 0.05$) between these

two factors (Table 8) (PERMANOVA analyses). Thus, oysters cultured in suspension accumulate more fatty acids in their digestive glands compared to bottom cultured oysters. Total FA concentration was significantly lower in July compared to all other months (pair-wise tests on sampling dates, $p < 0.05$) (Table 8). FA composition (%) (Table 8) differed significantly in relation to sampling date (Pseudo-F = 9.14, df = 4, $p < 0.001$) and culture method (bottom vs suspension) (Pseudo-F = 2.76, df = 1, $p < 0.05$), without any interactions between the two factors (Pseudo-F = 0.70, df = 4, p = 0.88) (PERMANOVA analyses). Pairwise test results showed significant differences between all sampling dates ($p < 0.05$, df = 12) and culture methods ($p < 0.05$, df = 30). The 7% depletion (Table 8) of EPA (20:5ω3) contributed to most (16%) of the dissimilarity between sampling dates. In August and September, EPA levels returned to similar levels as June. Regardless of the culture method used, specific FA contribution was similar in June, August, and September. Statistically discrepancies (SIMPER analyses with Bray Curtis similarity, pooled data from all sampling stations) between culture methods were mainly explained by microalgal biomarkers (Table 8) in bottom cultured oysters, which were represented by slightly superior values of EPA and inferior values of DHA, explaining 11.4% and 8.5% of differences, respectively. However, biologically interpreting EPA and DHA as proxies our results suggest that oysters fed more on dinoflagellates (DHA/EPA > 1), with a marked input in late July when DHA levels significantly increased. The opposite is apparent in the fall (September) where diatoms biomarker (EPA/DHA>1) is predominant. Even though less important than dinoflagellates, diatoms might represent a minimal food source, especially in early June and late September, due to increasing EPA values. Detritus and bacteria biomarkers (16:1ω7, 15:0,15:0*i*+*ai*, 17:0, 17:0*i*+*ai*) did not present differences between bottom vs suspension cultured oysters. Food sources derived from terrestrial plants or seagrasses are shown as contributing to the diet of oysters (18:2ω6+18:3ω3 >2.5) independent of the culture method used.

Table 8: Relative contribution (%) of fatty acids (FA) in the digestive glands of bottom and suspension-cultured oysters, *C. virginica*.

	Suspended culture					Bottom culture				
	June 3rd	**July 3rd**	**July 25th**	**Aug 14th**	**Sept 28th**	**June 3rd**	**July 3rd**	**July 25th**	**Aug 14th**	**Sept 28th**
Saturated (SFA)										
14:0	3.66 ± 0.93	3.94 ± 1.00	1.72 ± 0.54	3.88 ± 0.80	5.07 ± 1.19	3.65 ± 0.28	3.80 ± 0.53	1.46 ± 0.31	3.48 ± 0.86	4.04 ± 0.89
15:0	0.90 ± 0.10	0.75 ± 0.07	0.73 ± 0.07	0.85 ± 0.08	0.99 ± 0.05	0.84 ± 0.11	0.80 ± 0.09	0.86 ± 0.08	0.87 ± 0.06	0.86 ± 0.05
16:0	22.88 ± 2.11	24.35 ± 0.38	25.14 ± 1.76	26.10 ± 0.73	21.09 ± 3.10	21.99 ± 1.70	24.22 ± 1.98	24.07 ± 2.26	24.23 ± 1.80	21.06 ± 1.38
17:0	1.30 ± 0.11	1.27 ± 0.15	1.45 ± 0.14	1.18 ± 0.09	1.18 ± 0.12	1.30 ± 0.22	1.31 ± 0.12	1.47 ± 0.17	1.17 ± 0.08	1.13 ± 0.05
18:0	4.18 ± 0.63	5.01 ± 0.72	5.72 ± 0.34	3.37 ± 0.40	2.65 ± 0.19	4.60 ± 0.81	5.38 ± 0.63	5.38 ± 0.75	3.50 ± 0.47	3.37 ± 0.47
20:0	0.12 ± 0.03	0.15 ± 0.05	0.12 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.12 ± 0.05	0.17 ± 0.08	0.17 ± 0.05	0.10 ± 0.05	0.11 ± 0.04
15:0*i+ai*	0.21 ± 0.06	0.12 ± 0.05	0.18 ± 0.07	0.11 ± 0.08	0.19 ± 0.08	0.17 ± 0.05	0.14 ± 0.03	0.16 ± 0.06	0.15 ± 0.04	0.22 ± 0.09
17:0*i-ai*	0.24 ± 0.03	0.40 ± 0.24	0.27 ± 0.03	0.57 ± 0.39	0.53 ± 0.42	0.29 ± 0.10	0.31 ± 0.19	0.29 ± 0.08	0.30 ± 0.14	0.37 ± 0.16
Sum%	*33.51 ± 2.42*	*35.99 ± 0.88*	*35.33 ± 1.70*	*36.09 ± 0.75*	*31.76 ± 2.34*	*32.96 ± 2.21*	*36.14 ± 2.00*	*33.87 ± 2.45*	*33.81 ± 2.06*	*31.16 ± 1.52*
Monounsaturated (MUFA)										
14:1	0.07 ± 0.01	0.12 ± 0.03	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.09 ± 0.02	0.12 ± 0.05	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
16:1ω7	5.78 ± 1.45	3.91 ± 1.48	3.00 ± 1.10	6.94 ± 1.86	9.27 ± 2.20	5.33 ± 0.73	3.36 ± 1.09	3.17 ± 0.63	6.91 ± 1.58	8.38 ± 1.61
18:1ω9	3.72 ± 0.36	4.76 ± 0.19	4.55 ± 0.59	3.48 ± 0.17	3.21 ± 0.34	3.60 ± 0.14	4.76 ± 0.97	4.60 ± 0.61	3.74 ± 0.93	3.35 ± 0.73
20:1ω9	0.66 ± 0.22	0.94 ± 0.27	0.64 ± 0.18	0.31 ± 0.07	0.49 ± 0.43	0.66 ± 0.04	0.76 ± 0.21	0.67 ± 0.13	0.73 ± 0.75	0.80 ± 0.70
Sum%	*10.23 ± 1.24*	*9.72 ± 1.29*	*8.24 ± 0.82*	*10.77 ± 1.70*	*13.00 ± 2.21*	*9.67 ± 0.60*	*9.00 ± 0.50*	*8.49 ± 0.93*	*11.42 ± 0.72*	*12.58 ± 1.62*
Polyunsaturated (PUFA)										
18:2ω6	2.14 ± 0.21	3.65 ± 0.35	2.23 ± 0.15	2.15 ± 0.17	1.81 ± 0.16	2.15 ± 0.11	4.20 ± 0.56	2.36 ± 0.23	2.12 ± 0.23	1.70 ± 0.10
18:4ω3	0.18 ± 0.05	0.15 ± 0.02	0.16 ± 0.04	0.25 ± 0.06	0.28 ± 0.06	0.17 ± 0.02	0.17 ± 0.04	0.21 ± 0.03	0.27 ± 0.02	0.29 ± 0.03
18:3ω3	2.29 ± 0.27	4.17 ± 0.51	4.50 ± 0.68	2.75 ± 0.21	2.00 ± 0.48	2.38 ± 0.54	3.82 ± 0.98	3.71 ± 1.34	2.40 ± 0.81	1.58 ± 0.38
20:2	0.34 ± 0.05	0.56 ± 0.10	0.31 ± 0.05	0.20 ± 0.03	0.18 ± 0.03	0.38 ± 0.04	0.53 ± 0.09	0.31 ± 0.03	0.24 ± 0.04	0.19 ± 0.02
20:3ω6	0.25 ± 0.03	0.23 ± 0.03	0.24 ± 0.05	0.26 ± 0.06	0.28 ± 0.05	0.26 ± 0.03	0.26 ± 0.04	0.27 ± 0.05	0.30 ± 0.02	0.32 ± 0.04
20:4ω6	0.13 ± 0.02	0.21 ± 0.04	0.53 ± 0.45	0.14 ± 0.03	0.09 ± 0.02	0.16 ± 0.07	0.26 ± 0.19	0.19 ± 0.06	0.12 ± 0.02	0.21 ± 0.16
20:3ω3	3.34 ± 0.28	2.58 ± 0.31	3.36 ± 0.38	2.71 ± 0.47	2.93 ± 0.41	3.58 ± 0.37	3.68 ± 0.37	5.51 ± 1.25	4.67 ± 1.38	5.15 ± 1.06
20:5ω3 (EPA)	22.38 ± 1.44	15.99 ± 1.25	16.08 ± 3.14	20.30 ± 1.88	24.06 ± 2.66	23.20 ± 0.44	16.14 ± 2.30	16.85 ± 1.40	20.77 ± 1.35	24.86 ± 1.11
22:6ω3 (DHA)	24.60 ± 1.88	26.20 ± 1.79	28.02 ± 1.72	23.76 ± 3.56	22.98 ± 2.76	24.49 ± 2.16	25.11 ± 1.62	27.72 ± 2.19	23.21 ± 2.27	21.32 ± 1.33
Sum%	*55.65 ± 2.97*	*53.74 ± 1.38*	*55.44 ± 1.91*	*52.52 ± 2.21*	*54.61 ± 1.42*	*56.76 ± 2.69*	*54.16 ± 1.88*	*57.13 ± 3.24*	*54.10 ± 2.36*	*55.61 ± 1.70*
EFA	47.11 ± 2.65	42.39 ± 1.26	44.63 ± 2.22	44.20 ± 1.72	47.13 ± 1.13	47.85 ± 2.33	41.51 ± 3.21	44.76 ± 3.24	44.10 ± 2.23	46.38 ± 1.07
EPA/DHA	0.91 ± 0.08	0.61 ± 0.09	0.58 ± 0.14	0.88 ± 0.19	1.06 ± 0.22	0.95 ± 0.08	0.64 ± 0.09	0.61 ± 0.04	0.90 ± 0.13	1.17 ± 0.11
DHA/EPA	1.10 ± 0.10	1.65 ± 0.23	1.79 ± 0.36	1.19 ± 0.31	0.97 ± 0.23	1.06 ± 0.09	1.57 ± 0.19	1.65 ± 0.11	1.12 ± 0.15	0.86 ± 0.08
18:2ω6+18:3ω3	4.43 ± 0.09	7.82 ± 0.64	6.73 ± 0.80	4.90 ± 0.14	3.81 ± 0.63	4.53 ± 0.60	8.02 ± 1.37	6.06 ± 1.56	4.52 ± 0.99	3.28 ± 0.46
PUFA/SFA	1.69 ± 0.21	1.51 ± 0.07	1.57 ± 0.15	1.48 ± 0.08	1.77 ± 0.15	1.75 ± 0.20	1.52 ± 0.14	1.72 ± 0.22	1.63 ± 0.17	1.83 ± 0.12
16:1ω7/16:0	0.25 ± 0.06	0.16 ± 0.06	0.12 ± 0.05	0.27 ± 0.08	0.45 ± 0.15	0.24 ± 0.02	0.14 ± 0.05	0.13 ± 0.03	0.29 ± 0.06	0.40 ± 0.09
Total μg mg^{-1}	3.93 ± 1.41[a]	2.60 ± 1.10[ab]	2.48 ± 0.72[ab]	4.37 ± 0.93[a]	7.40 ± 3.36[a]	3.58 ± 1.30[c]	2.02 ± 1.05[cd]	1.93 ± 0.85[cd]	3.71 ± 1.31[c]	3.81 ± 2.75[c]

2.6.4 Retention efficiency (RE) and refiltration factor (RF)

Crassostrea virginica exhibited significantly different RE values for PPP and NPP (two-sample *t* test = 2.26, $p < 0.001$, df = 9). RE was significantly higher ($p < 0.05$) in PPP cells (92.1 ± 2.7%) for oysters in the regime with the lowest concentration (20%) of *N. oculata* cells (Table 9). Inversely, the lowest RE (71.8 ± 8.2%) was associated with PPP from the regime with the highest (80%) concentration of *N. oculata*. RE values in NPP were not significantly different between experimental regimes, and averaged between 92.9 ± 6.9% and 99.1±1.0% (Table 9). For all RE trials (1 h incubation time with experimental food regimes), all RF values were ≤ 1, indicating that individual oysters did not have the opportunity to refilter the whole chamber`s volume (1.25 l). No further regression analysis was needed based on confidence that RE values were not overestimated by refiltration.

Table 9: Retention efficiencies of *C. virginica* (Mean % ± SD) on PPP (*Nannochloropsis occulata*) and NPP (*Tisochrysis lutea*) in relation to the three experimental regimes (%PPP: %NPP ratios). Two significantly different values are shown in bold.

Regime ratios	**Retention efficiency (%)**	
	PPP (*N. oculata*)	**NPP (*T. lutea*)**
1) 20:80	***92.1 ± 2.7****	99.1 ± 1.0
2) 50:50	74.2 ± 12.8	92.9 ± 6.9
3) 80:20	***71.8 ± 8.2****	93.6 ± 6.1

**p value* < 0.05

2.6.5 Picophytoplankton assimilation

The polyunsaturated fatty acid EPA was naturally present at high concentrations in *N. oculata* (22.7 ± 1.58%), whereas DHA was the dominant FA in *T. lutea* (Table 10). The results of the enrichment trials (labelled regimes) against the controls (unlabelled regimes) confirmed that PPP was assimilated in the tissues of *C. virginica*, especially for 20:1, EPA, 22:2 (NMI), and DHA (Table 11). The most abundant FA present in oysters was EPA from the labelled PPP species. This FA showed isotopic (^{13}C) enrichment in oyster tissues. This FA was particularly detected in the digestive gland, confirming the ingestion of PPP cells.

Bivalves have no, or very limited, capacity to biosynthesize EPA and DHA. Therefore, the ^{13}C enrichment of EPA in certain organs (such as gills, mantle, and abductor muscle) suggests that PPP was assimilated, with carbon subsequently being transferred to other tissues. The lack of DHA isotopic enrichment, which was highly abundant in the NPP species (*T. lutea*) (Table 10), confirmed that ^{13}C enrichment in oysters (Table 11) originated solely from the ingestion of labelled PPP cells. Enrichment of 22:2 (NMI) was also detected using 20:1 as a precursor.

Table 10: Lipid composition (% of total lipid classes, % of specific fatty acids) of fresh PPP (*Nannochloropsis oculata*) and NPP (*Tisochrysis lutea*) in relation to total dried weight. Null contribution is represented by a blank data cell.

	Nannochloropsis oculata	***Tisochrysis lutea***
Saturated (SFA)		
14:0	**0.24** ± 0.12	**18.75** ± 3.13
15:0	0.57 ± 0.03	0.62 ± 0.03
16:0	**34.76** ± 1.50	**20.5** ± 0.81
17:0		0.61 ± 0.07
18:0	0.15 ± 0.17	3.00 ± 0.39
20:0	0.22 ± 0.02	0.81 ± 0.29
21:0	0.10 ± 0.01	0.15 ± 0.30
22:0		1.46 ± 0.62
24:0		1.13 ± 0.48
Sum%	*36.04 ± 1.54*	*47.03 ± 2.79*
Monounsaturated (MUFA)		
14:1	0.18 ± 0.09	0.55 ± 0.11
15:1		
16:1ω7	**30.72** ± 1.39	**9.12** ± 2.48
17:1		0.81 ± 0.11
18:1ω9	**3.12** ± 1.04	**10.43** ± 6.98
20:1	0.09 ± 0.02	0.81 ± 0.18
Sum%	*34.11 ± 1.85*	*21.74± 4.99*
Polyunsaturated (PUFA)		
18:3ω3	0.18 ± 0.02	4.49 ± 1.73
18:3ω6	0.22 ± 0.01	1.22 ± 0.82
18:4ω3		
18:2ω6	3.07 ± 0.19	2.38 ± 2.79
20:4ω6	3.10 ± 0.92	0.93 ± 0.16
20:5ω3 (EPA)	**22.71 ± 1.58**	**5.38** ± 1.09
20:3ω6	0.50 ± 0.04	
20:3ω3		0.76 ± 0.24
22:6ω3 (DHA)		**14.17 ± 1.71**
22:2	0.06 ± 0.09	
22:5ω3	1.90 ± 0.07	
Sum%	*29.84 ± 2.25*	*31.23 ± 2.48*

Table 11: Results (%^{13}C) for specific fatty acids of *C. virginica* tissues (gills, digestive gland = DG, mantle, and abductor muscle = AM) from regimes with different PPP/NPP ratios. Bold numbers represent enrichment in ^{13}C compared to the unlabelled regime (control).

Food regime	Tissue		20:1	20:5n3 (EPA)	22:2 (NMI)	22:6n3 (DHA)
Unlabelled	Gills	(n=3)	1.03	1.05	1.13	1.03
(Control)	DG	(n=2)	1.03	1.05	1.13	1.03
	Mantle	(n=3)	1.04	1.07	1.14	1.03
	AM	(n=5)	1.03	1.05	1.12	1.05
Regime 1	Gills	(n=6)	**1.17**	**1.54**	1.14	1.03
20% PPP	DG	(n=6)	**2.03**	**3.31**	**1.27**	1.05
80% NPP	Mantle	(n=5)	**1.08**	**1.26**	1.13	1.04
	AM	(n=6)	1.05	**1.18**	1.13	1.03
Regime 2	Gills	(n=5)	1.05	**1.16**	1.14	1.04
50% PPP	DG	(n=6)	**1.34**	**2.23**	**1.16**	1.04
50% NPP	Mantle	(n=6)	1.03	**1.13**	1.13	1.03
	AM	(n=6)	1.04	**1.14**	1.13	1.04
Regime 3	Gills	(n=5)	1.04	1.07	1.13	1.03
80% PPP	DG	(n=6)	**1.18**	**2.02**	**1.17**	1.04
20% NPP	Mantle	(n=5)	1.03	1.06	1.13	1.03
	AM	(n=7)	1.03	1.07	1.13	1.03

2.7 DISCUSSION

Using stable isotopes, we demonstrated that the main food sources of farmed oysters, regardless of culture methods, are particulate organic matter (POM) with no apparent contribution from sediments (which could include microphytbenthos), a green macroalgae (*Ulva sp.*) and a seagrass (*Zostera marina*). This said, future studies should consider further extensive surveys of food sources such as the potential implication of microphytobenthos (MPB) to benthic filter feeders communities. The MPB is characterized by high spatial variability at microscale (Spilmont et al. 2011) with fairly stable biofilms at a large scale in estuaries (Brito et al. 2013). Those general isotopic results are in agreement with numerous studies on oyster's food sources discrimination (Pernet et al. 2012; Moynihan et al. 2016). Stable isotopes analysis can provide time-integrated information (long term) on food

sources, averaging the natural environment variability in dietary components (Pernet et al. 2012). Whereas FAs are incorporated largely unaltered into the lipid reserves of the primary consumers (like oysters) generally reflecting the FA profiles of the food consumed (short term) (Dalsgaard et al. 2003). Our FA biomarkers results indicated that dinoflagellates (DHA or DHA/EPA>1) may be considered a main food source, with no significant discrepancies between bottom and suspension cultured oysters while diatoms (EPA or EPA/DHA>1) may contribute minimally to cultured oysters diet. Also, high DHA/EPA ratio in July seems to also correspond to an increase in PPP biomass as well, which may translate to important dinoflagellates numbers in the PPP communities. This said, other diatom biomarkers may be used such as (16:1ω7/16:0) >1, and from our results indicating no evident contribution from diatoms in the oysters diets with low ratios ranging from 0.12±0.05 to 0.45±0.15. Although not statistically significant, superior EPA contribution can be observed in bottom cultured oysters compared to their suspension-cultured counterparts. This small discrepancy might arise from microphytobenthos found normally in the first millimeters of sediment and includes numerous unicellular phototrophic microorganisms dominated by marine diatoms (up to 95%) (Cognie et al. 2001). In estuaries, such as the Foxley system, microphytobenthos could easily be resuspended by tidal currents which reach up to 20cm/s; thus, contribute to the food supply of filter-feeders (Underwood and Kromkamp 1996). However, it is noteworthy to mention that only our FAs profiles suggest a potential microphytobenthos/diatoms interaction whereas bulk isotopic results showed no contribution of sediment-derived material in the oyster's diet. Similar discrepancies between analytical methods were observed where FAs profiles suggested that terrestrial plants may contribute to the oyster's diet due to the presence of 18:2ω6 + 18:3ω3 >2.5 in their tissues (Budge and Parrish, 1998) since terrestrial plants (as well as their respective pollen) contain large amounts of those two FAs (Ackman 1986; Parrish et al. 1995). This relation could not be captured using solely our stable isotopes analyses with the seagrass, *Z. marina* which is a marine plant. Using FAs and stable isotopes analyses to assess the nutritional value of vascular plants for bivalves has been explored extensively and recent studies presented macroalgae as a marginal

contributor to diet of the bivalve *Astarte elliptica* (Gaillard et al. 2016) and *Venus verrucosa* (Perez et al. 2003). However, not exclusive to plants and seagrass species, 18:3ω3 is also present in some picoeukaryotic prasinophytes (Chlorophyta) (Dalsgaard et al. 2003; Moynihan et al. 2016) and is sometimes significantly correlated with dinoflagellates abundance (Lower et al. 2014). Overall, techniques that assess both long term (stable isotopes) and recent (FAs profiles of the digestive glands) food assimilation by bivalves should be used together because detection thresholds might differ for the same food source.

Phytoplankton biomass in the Foxley River system is very variable, with PPP being a major contributor to total phytoplankton biomass of this oyster culture area. Based on Comeau (2013), even though calculations do not take into account natural oyster reefs, this system does not exert a dominant top-down control on phytoplankton abundance in relation to the abundance of cultured bivalves. The present study showed that oysters cultured in suspension had higher fat content compared to those cultured on the bottom. This outcome may be attributed to different parameters such as enhanced food fluxes at the surface layer, important primary production in the photic layer, and high surface temperatures. However, Comeau (2013) showed that the grazing ability (clearance rates) of oysters, assessed by the surface of gills per unit of dry tissue, is lower for suspended cultured oysters. Such relation reinforces our inference that food availability in the surface layers of water is not a limiting factor for suspended cultured oysters. Such direct link between food availability and bivalve's growth has been demonstrated by Grangeré et al. (2010) in France. During our surveys, we noticed that total FAs in the digestive glands significantly declined in July, which we attributed to the reproductive cycle involving spawning events during the summer. Indeed, the Atlantic Canada region is the most northerly distribution area where *C. virginica* are able to reproduce, with spawning events generally occurring when the water temperature exceeds 20°C, which is a threshold value that is commonly surpassed in July (Filgueira et al. 2014a). It has been demonstrated that a decrease in EPA (20:5ω3) is namely associated with stressful or energetically expensive situations in bivalves such as gametogenesis and spawning (Stanley and Howard, 1998).

Nano- and pico-sized phytoplankton assemblages are significant global primary producers (Moynihan et al. 2016). Bivalves exhibit high degrees of trophic plasticity, retaining a large amount of food from other sources of different sizes (Cresson et al. 2016). Our study showed that *C. virginica* retains PPP cells and further assimilates its carbon in its tissues. To our knowledge, this study was the first to use isotopically (^{13}C) labelled PPP to confirm its assimilation by Eastern oysters. Results suggest that PPP's carbon constitutes a food source assimilated sometimes through biosynthesis processes such as the production of NMI (22:2), which is intrinsic to mollusks. Noticeable amounts of NMI have been reported in Mytilidae (*Mytilus edulis*, 4.6%) (Paradis and Ackman, 1977) and Ostreidae (*C. virginica*, 5.8%) (Paradis and Ackman, 1975). Using *Mytilus edulis* Zhukova (1991) showed that NMI is one of the only polyunsaturated acids synthesized by marine mollusks. Monoenoic acids, such as 20:1, are present in significant quantities in mollusks (Ackman et al, 1971) and are precursors of NMI (Zhukova and Svetashev, 1986). The complex biosynthesis of NMI (22:2) FAs is *de novo* (Pogoda et al. 2013) and resumed by the desaturation of 16:0 and 18:0 to produce 16:1ω7 and 18:1ω9. Followed by subsequent elongation to 18:1ω7 and 20:1ω9 (or aforementioned 20:1), further desaturation to 18:2 and 20:2, and a final elongation for the production of 20:2 and 22:2 (NMI) (Monroig et al. 2013). Therefore, complex use of PPP carbon was clearly demonstrated through the production of isotopically (^{13}C) labelled NMI. No statistical analysis was performed for the enrichment results of the experimental regimes because feeding (3 days) and fasting (3 days) periods during the feeding trials might act as an artefact. Enrichment rates may vary between experimental regimes and longer digestion times could be appropriate in the future. This study PPP assimilation trials focused on four major fatty acids; one NMI precursor (20:1), the 22:2 NMI, EPA, and DHA. EPA is a typical marine PUFA. Both EPA and DHA are important and conservative elements of bio-membranes (Pogoda et al. 2013). DHA is resistant to pressure changes, allowing it to function independently of a fluctuating environment (Rabinovich and Ripatti, 1991). However, Pernet et al. (2007) demonstrated that DHA is sensitive to temperature notably during early stages of warming where

bivalves, such as *Mytilus edulis* and *C. virginica,* experience a remodeling of membrane phospholipids.

Picophytoplankton abundance increases from spring to summer in temperate areas, when nutrient concentrations decrease and the system switches from "new" to "regenerated" production and when water temperature reaches its annual maximum (Caroppo, 2000). Major classes of PPP have been presented as Prasinophyceae (division Chlorophyta), Pelagophyceae (division Heterokontophyta) and Prymnesiophyceae in numerous marine systems (Thomsen and Buck, 1998; Moon-van der Staay et al., 2000). Smaller phytoplankton like PPP, tends to have a preference for dissolved ammonium, while their nitrate uptake is positively related to cell size (Stolte et al. 1994). In relation to bivalve aquaculture, Mugg Pietros and Rice (2003) hypothesized that ammonia generated by oysters is utilized by rapidly regenerating phytoplankton in the water column, such as PPP. Potential picoeukaryote biomarkers have been demonstrated recently by Moynihan et al. (2016), in which FAs ratio 16:4ω3 +18:3ω3 /Σω3 had a strong correlation with cells abundance. Our study did not assess 16:4ω3, as we could not use this recently developed PPP biomarker. However, before the current study, knowledge was limited on PPP species assimilation by mature or commercially sized oysters. The PPP:NPP biomass ratios (20%, 50% and 80%) used in the retention efficiency trials are representative of the Foxley system as shown from field data in Figure 2. Similar ratios were also reported by Sonier et al. (2016) in a mussel culture bay in PEI. A number of natural processes might contribute to the capacity of bivalves to capture and ingest PPP cells. In fact, Kach and Ward (2008) showed that aggregation significantly enhances *C. virginica* ingestion of pico-sized particles such as 0.5 and 1µm fluorescent beads, as well as bacteria (greatest cell dimension of 0.6 µm). If bivalves could ingest all particles in an aggregate, they would be able to access a highly concentrated, rapidly sinking source of picoplankton that could represent an important food source (Waite et al. 2000). Flocculation or aggregation might partly explain differential RE values on PPP in our study, showing higher retention in the regime with the lowest PPP cell availability. In the natural environment, particle agglomeration is enhanced by the presence of transparent exopolymeric particles (TEP) that are formed from

mucopolysaccharides, which are released in abundance by bacteria, phytoplankton, and benthic suspension-feeders (Li et al., 2008). Other confounding factors also exist, such as surface charge and wettability of particles in suspension (Rosa et al. 2013) and chemical interactions between lectins in the mucus of the pallial organs and carbohydrates present on the surface of microalgal cells (Pales Espinosa et al. 2009). The exact mechanisms used by suspension-feeding bivalves to determine which particles are ingested and which are rejected as pseudofeces, however, have yet to be elucidated (Rosa et al. 2013). In the context of shellfish aquaculture, especially in modelling exercises and sustainable aquaculture research, it is important to have reliable data on feeding capabilities and rates of cultured bivalves on available food sources including PPP (Sonier et al. 2016).

2.8 CONCLUSION

Our study provided important information on the preferred food sources of oysters cultured on the bottom and in suspension. Stable isotope analyses confirmed that all oysters mainly feed on pelagic resources, such as particulate organic matter (POM). FA biomarker methods confirmed that lipid reserves were mostly associated with microalgal sources, but also showed that oysters cultured in suspension have more total FAs in their digestive glands compared to those cultured on the bottom. Further analysis using specific FAs allowed us to infer that, regardless of the culture method used, oysters may feed mainly on dinoflagellates, with minimal contribution from diatoms. While not dominant, biomarkers associated to terrestrial (vascular) plants (which include marine plants) were also detected, and might minimally contribute to the diet of oysters. Discrepancies between analytical methods (stable isotopes and fatty acids profiles) were observed and should be addressed and verified when used in parallel in food web research. Over the course of this study, PPP and NPP biomass differed significantly along the ice-free period when the PPP fraction usually dominates the autotrophic biomass of the Foxley River system (PEI). Through controlled experiments, independent of the micro algal tested treatments, *C. virginica* showed high RE on PPP (*N. oculata*) and NPP (*T. lutea*) species, with ranges of 71.8–92.1% and 92.9–99.1%, respectively. Unexpectedly, the RE of PPP in oysters increased

when the availability of PPP was low when compared to a regime with 80% PPP biomass. We suggest that flocculation or aggregation processes between *N. oculata* and *T. lutea* explain these results. Through labelling experiments on pico-sized algae, we demonstrated that mature *C. virginica* assimilate PPP as a food source by incorporating carbon into all the sampled tissues (digestive gland, gills, mantle, and abductor muscle). Our work also confirms that carbon from ingested PPP cells is used in an internal biosynthesis process, possibly by the elongation and desaturation of the precursor 20:1 into the final NMI (22:2) (Da Costa et al. 2015), based on the isotopic enrichment of 22:2 (NMI) FA in the digestive glands after the PPP assimilation trials. While further experiments are needed, especially in the field context, our study advanced our knowledge on the feeding physiology of the Eastern oyster (*C. virginica*). This information is important in the context of aquaculture production, particularly to establish the carrying capacity in coastal ecosystems.

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CHAPITRE 3

MYTILUS EDULIS ET *STYELA CLAVA* : PARTENAIRES EN L`ASSIMILATION DU PICOPHYTOPLANCTON (FRAIS OU PRÉINGÉRÉ)

3.1 RÉSUMÉ

La moule bleue (*Mytilus edulis*) ainsi que le tunicier (*Styela clava*) sont tous deux des filtreurs ayant un impact sur la diminution de la matière organique en suspension dans la colonne d'eau. Contrairement à la moule, *S. clava* utilise un filet muqueux pour la capture de particules lors de la filtration. En utilisant de l'eau de mer non filtrée (seston naturel), des études récentes démontrent une efficacité de rétention normalisée de 59 ± 2% du picophytoplancton (PPP, cellules de 0,2–3,0µm) par ce tunicier solitaire comparativement à la moule retenant que 20 ± 2% de particules similaires (0,2–2,0 µm). Ceci étant dit, autre l'étude de la filtration et l'efficacité de rétention, très peu d'études s'attardent à démontrer le potentiel d'assimilation du PPP comme source nutritionnelle pour les filtreurs tels que les moules et les tuniciers. Notre étude utilise l'enrichissement isotopique (^{13}C) comme outil quantitatif de l'assimilation de PPP. Nous avons validé la possibilité d'un transfert secondaire de carbone organique provenant du PPP par la refiltration de fèces de filtreurs déployés à proximité l'un de l'autre. Nos résultats démontrent que *M. edulis* et *S. clava* ont la capacité d'ingérer et assimiler le PPP comme source de nourriture en emmagasinant son carbone dans leurs tissus. La moule présente une relation allométrique plus importante en relation avec le taux d'assimilation du PPP. Lorsque les deux espèces sont incubées ensemble, une compétition pour le PPP est apparente. De plus, un transfert secondaire de la matière organique fut démontré par la refiltration des fèces, et significativement plus par l'entremise des fèces produites par *S. clava*. À la lumière de ces résultats, il est clair que

l’espèce cultivée (moule) ainsi que son envahisseur (tunicier) se nourrissent activement de la production primaire de petite taille provenant d’estuaires riches en nutriments. Nous pouvons suggérer que ceux-ci rendent un service écologique à l’écosystème en retirant la biomasse phytoplanctonique en excès.

3.2 *MYTILUS EDULIS* AND *STYELA CLAVA*: PARTNERS IN THE ASSIMILATION OF FRESH AND PRE-DIGESTED PICOPHYTOPLANKTON

R. Sonier [ab*], R. Tremblay [b], F. Olivier [c], T. Meziane [c], B. Genard[bd] and L.A. Comeau[a]

[a] *Department of Fisheries and Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Science Branch, Moncton, NB, E1C 9B6, Canada*

[b] *Institut des Sciences de la Mer, Université du Québec à Rimouski, 310 allée des Ursulines, C.P. 3300, Rimouski, QC, G5M 1L7, Canada*

[c] *Muséum National d'Histoire Naturelle, UMR CNRS 7208 BOREA, CP 53, 61 rue Buffon, 75231, Paris Cedex 5, France*

d Les laboratoires Iso-BioKem inc., 367, rue Gration-Gélinas, Rimouski, QC, G5M 0V1, Canada

Remi.Sonier@dfo-mpo.gc.ca;

*Corresponding author: 1-506-851-2977

3.3 ABSTRACT

Blue mussels (*Mytilus edulis*) and sea squirt (*Styela clava*) are efficient filter-feeders, capable of depleting suspended particles efficiently in the water column. In this study, we used isotopic enrichment (^{13}C) to quantify picophytoplankton (PPP) ingestion by filter-feeders and examined the subsequent carbon assimilation into their tissues. We also examined a possible secondary transfer (recycling) of organic carbon through filtration of feces and pseudofeces by filter feeders. Our results show that both *M. edulis* and *S. clava* ingest and assimilate picophytoplankton, but *M. edulis* exhibit faster assimilation. When both species were held together, they both competed for PPP. Isotopic labelling (^{13}C) clearly demonstrated that carbon originating from picophytoplankton is recycled between individuals in close proximity via the ingestion of feces and pseudofeces. Both species consumed predigested particles, particularly the significantly larger fecal particles produced by *S. clava.* Because both cultured and invasive species actively feed on small phytoplankton, primary producers dominating nutrient-rich estuaries, we conclude that this situation may provide an ecological service to the ecosystem by removing the excess of phytoplankton biomass and their associated nutrients.

Keywords: picophytoplankton, isotopic labelling, aquaculture, feces, carbon cycling, blue mussel, ascidian

3.4 INTRODUCTION

Solitary ascidians, such as the clubbed sea squirt *Styela clava*, are recognized as nuisance species in many aquaculture settings, since they may cause considerable economic losses (Carver et al. 2003; Ramsay 2014). They reproduce seasonally (Bourque et al. 2007), producing free-swimming larvae that settle on mussel socks, where they grow rapidly (Boothroyd et al. 2002). Solitary tunicates are also efficient filter-feeders capable of depleting suspended particles or even controlling phytoplankton communities in the water column (Thompson and MacNair, 2004). For instance, solitary tunicates in longline mussel farms may increase filtration rates by 30–47% compared to non-infested scenarios (Comeau et al. 2015). Unlike mussels, which operate using a ciliated gill system, tunicates rely on a mucus net to capture food particles. This mucus net is produced in the endostyle (Goodbody 1974; Riisgård and Larsen, 2000) and is shaped like an elongated rectangular mesh consisting of transverse and longitudinal filaments (Flood and Fiala-Medioni 1981). Various retention experiments have suggested that the mucus net of *S. clava* is 100% efficient in retaining 1–2 µm particles (Randløv and Riisgård 1979; Bak et al. 1998), although a lower efficiency (59 ± 2%) has been reported for solitary ascidians feeding on natural particles ranging between 0.2 and 3.0 µm (Comeau et al. 2015). Bivalve retention efficiencies (RE) are much lower. Sonier et al. (2016) for instance reported a RE of 20 ± 2% for cultivated blue mussels (*Mytilus edulis*) feeding on 0.2–2.0 µm picophytoplankton (PPP). The fate of PPP following its retention by gills or mucus nets remains undocumented. Quantifying the extent to which cultivated mussels and invasive ascidians assimilate PPP was of particular interest in our study. Furthermore, assuming that the energy (or carbon) from PPP ingestion is not totally assimilated by *M. edulis*, we looked at further transfer and utilization of PPP via feces. Excreted organic matter represents another potential food source for which assimilation rates are poorly quantified. In suspended shellfish aquaculture, feces produced by mussels and ascidians are a component of organic matter that is concentrated, aggregated, and sink to the seabed. Bivalves actively excrete fecal or pseudofecal particles, whereas ascidians mostly squirt water through their siphons to rid themselves of undesirable waste particles (Carlisle 1966; Robbins 1984). Fouling

filter-feeder communities associated with bivalve shellfish farms may biodeposit as much as two times more biomass onto the seabed than they do in mussel farms not heavily infested (McKindsey et al. 2009). In addition, bivalves and invasive sea squirt fouling them (such as cultured *M. edulis* and the invasive, clubbed ascidian, *S. clava*) live in such close physical proximity in an aquaculture environment that interactions with excreted feces and pseudofeces may influence the nutritional intake of both organisms.

In the present study, we investigate the competitive interactions between *M. edulis* and *S. clava*, specifically in regards to the species' respective abilities to assimilate and retain PPP carbon. Both species were exposed to natural seston under a common environment. Isotopic enrichment (^{13}C) was applied as a diet-tracing method to follow PPP ingestion and subsequent assimilation of carbon into tissues. The same isotopic approach was exploited to determine the magnitude at which transfers of organic matter are mediated through feces production and ingestion. We test the hypothesis that both species compete for PPP and exploit the ingestion/excretion processes of other species to increase their rate of PPP carbon assimilation.

3.5 MATERIAL AND METHODS

3.5.1 Clearance rates

Two-year old cultured *M. edulis* (mean shell length 52.6 ± 4.9 mm, n = 250) and invasive *S. clava* of various sizes (length 73.5 ± 17.6 mm, n = 250) were collected from Malpeque Bay in Prince Edward Island (PEI) and transferred to a nearby laboratory in Georgetown PEI. Individuals were held in two large (250 L) tanks continuously supplied with natural seawater (12.5 ± 1.4 °C, 30.8 ± 0.6 ppt). Water was pumped to the lab using a submersible, semi-vortex/propeller pump (Tsurumi Titanium 50TM2.4S), at a rate of approximately 1500 L h^{-1}, positioned at 5 m below the sea surface. The high flow rate avoid animal food-depletion. After a 2-week acclimatization period, acrylic metabolic chambers (1.9 L, n =12) were installed and supplied with the same seawater input as the holding tanks. Metabolic chambers, tanks, or containers are commonly used in examining

particle selection, clearance rates, and retention efficiency in bivalves and solitary ascidians (Riisgård 1988; Strohmeier et al. 2009; Comeau 2013; Rosa et al. 2013; Comeau et al. 2015; Sonier et al. 2016).

Experimental trials were first conducted using one *M. edulis* or *S. clava* per chamber; thereafter, species were paired. Pairing was conducted using various sizes of *S. clava* (42.4–118.8 mm) to assess competition for PPP. Mussel shells and dead ascidians, previously treated in acetic acid (Forrest et al. 2007), were used as controls in each trial to estimate the extent at which particles naturally settle to the bottom via gravity. Fine aeration (bubbling) was provided in a manner that mixed the water in the chamber thoroughly, but minimized fecal and detrital resuspension. To confirm that individuals were actively feeding during our experiments, feces (or pseudofeces production in the case of mussels) was verified, as was the clearance rate (CR) by animals in the experimental chambers. For CR, the animals were allowed an hour to acclimate to the natural seawater water flowing through their chambers. Measurements started when the water flow in the chambers was stopped. The decline of particles was monitored over time in each chamber by taking water samples (10 ml water filtered through a 63 µm sieve to remove macroalgae and zooplankton) at regular (10 min) intervals. Aliquots (100 µl) were processed with a particle-counting instrument (Beckman Coulter Counter Z2TM) fitted with a 100-µm aperture by counting particles within the 5–19 µm size range, which is the size known to be efficiently retained by *M. edulis* (Ward and Shumway 2004) and *S. clava* (Petersen 2007). Only individuals that had their valves or siphons opened were included in the final analysis. The interval exhibiting the greatest depletion of particles was used to determine the CR of each chamber (CR_{ch}):

$$CR_{ch} = V/t \times ln\ (C_0 / C_t) \quad (1)$$

where V is the volume of the chamber (1.9 L), t is elapsed time between measurements (10 min), and C_0 and C_t are particle concentrations at times 0 and t respectively. An exponential line was fitted to the decline in particle concentration over time and only experiments with $r^2 > 0.90$ were considered for further analysis.

It is possible that retention or assimilation rates of PPP by mussels and tunicates are related to the number of times the incubated animals processed (filtered) the entire volume of the chamber (i.e., the turnover rate of the chambers' waters). Thus, turnover rate would affect biomass assimilation in trials where turnover is high. Available data were used to assess potential effects of re-filtration of water on the assimilation value, by calculating a re-filtration factor for each chamber (RF_{ch}), using the equation below (Comeau et al. 2015; Sonier et al. 2016):

$$RF_{ch} = (Inc_T \times CR_{ch}/60)/V_{ch} \quad (2)$$

where Inc_T is the incubation time (min) in relation to the clearance rate (per hour) of individuals (mussels and/or tunicates) in each chamber (CR_{ch}). V_{ch} is the total volume of the chamber (1.9 L). RF values are equivalent to the number of times that the entire volume of the chamber is filtered; RF >1 assumes that the chamber is processed at least once before the end of the feeding trial. When RF values >1 are recorded, the relationship between RF and other experimental parameters were further examined regression analysis.

3.5.2 Picophytoplankton assimilation

At the beginning of each assimilation trial (one-hour duration), 10 ml of cultured *Nannochloropsis oculata* (a spherical, unicellular flagellate 1–2 µm in diameter) was introduced in suspension into each metabolic chamber to supplement the PPPs already in the unfiltered seawater (natural seston). Before introducing *N. oculata* into the chambers, they were isotopically labeled with ^{13}C using the protocol of Arnold et al. (2015). This labelling procedure involved supplementing the sea water medium with 1 mM sodium [^{13}C]-bicarbonate (99% $NaH^{13}CO_3$) (Cambridge Isotope Laboratories, MA, USA). After running the feeding experiments, all animals were immediately frozen at -40 °C (portable digital freezer) for further analysis. In subsequent laboratory manipulations, each individual was then dissected with separate sterile tools to avoid cross contamination. All tissues were collected in 20 ml cryotubes (Corning©), lyophilized at -40 °C and grinded with a mortar and pylon disinfected with 90% alcohol to produce a homogenate powder. For each tissue

sample, a subsample of 0.8–1.2 g of powder was collected in a tin capsule (6 × 4 mm) and mounted into an automated carousel for organic carbon and nitrogen analyses by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Deltaplus XP mass spectrometer (Thermo Scientific), coupled with an elemental analyzer (COSTECH 4010, Costech Analytical). Carbon dioxide and nitrogen gases were analyzed for isotopic abundance and total organic carbon and nitrogen. Results are reported as $\delta^{13}C$ and $\delta^{15}N$ values relative to PDB (Pee Dee Belemnite) and ambient air, respectively. Analytical error (n = 50) for measurements was ±0.4‰ for δC and 0.2‰ for δN. Prior to our analysis, system suitability was evaluated using reference gas (nitrogen and carbon dioxide) with a standard deviation of zero over 10 measurements; maximum acceptable variation was set to 0.06‰. Since the PPP cells we used were carbon-labeled, only carbon ($\delta^{13}C$) data was used in further analyses. Relative abundance (%) of assimilated ^{13}C in each tissue ($\%C_{sample}$) was calculated by deconstructing the carbon ratio (R) using the following formulas provided by the manufacturer:

$$R = {}^{13}C/{}^{12}C \tag{3}$$

$$\delta^{13}C_{sample} = [(R_{sample}/R_{reference})\text{-}1] / 1000 \tag{4}$$

$$\%\,{}^{13}C_{sample} = [(0.0112372 \text{ x } (\delta^{13}C_{sample} / 1000+1)) / (1+0.0112372 \text{ x } (\delta^{13}C_{sample} / 1000+1))] \times 100 \tag{5}$$

where the universal standard ratio ($R_{reference}$) is represented by 0.0112372 (from Pee Dee Belemnite), R_{sample} represents the sample's carbon ratio ($^{13}C/^{12}C$), and $\delta^{13}C_{sample}$ is the sample's carbon isotopic ratio generated by the IRMS output. Dried tissue weights (in grams) of each individual (DTW_{total}) were obtained and coupled with the sample's relative abundance of total carbon ($\%^{13}C_{sample}$). This enabled us to assess total carbon biomass of each animal ($^{13}C_{total}$) using the equation:

$$^{13}C_{total} = (\%^{13}C_{sample} / 100) \times DTW_{total} \tag{6}$$

Because assimilation trials were time-based for CR purposes, an estimate of individual assimilation rates in mg ^{13}C ($^{13}C_{ind\ h\text{-}1}$) for mussels and ascidians were assessed using the following equation, where T is time of the incubation (h):

$$^{13}C_{ind\ h\text{-}1} = {}^{13}C_{total} / T \quad (7)$$

3.5.3 Assimilation of predigested particles

Our experimental design allows us to explore the transfer of labelled (^{13}C) PPP carbon that moved from one individual to another through the filtration and assimilation of feces particles. Our microcosm experiment was conducted using a "group stacking trial", enabling one species to produce feces in close proximity and suspended above another species. Two acrylic tanks (19 L) containing mussels and two other tanks containing ascidians were used to acclimate the test organisms for seven days in continuously-flowing, natural seawater. Isotopically-labeled individuals were conditioned in a separate acrylic tank and fed with isotopically-labelled *N. oculata* cells every 30 min for 9 h. An upper Vexar© platform (10-mm mesh size) was then used to support those isotopically-labelled (^{13}C) feces producers. Control individuals highly enriched in ^{13}C via PPP assimilation were transferred to the upper platforms and exploited as ^{13}C-labelled feces and pseudofeces producers for the secondary assimilation trials. This experimental design (Figure 19) ensured that all species combinations would be assessed, both as isotopically-labelled feces producers and as unmarked, filter-feeders receiving feces released from above. Four combinations were monitored: (1) *M. edulis* suspended above *M. edulis*, (2) *M. edulis* suspended above *S. clava*, (3) *S. clava* suspended above *M. edulis*, and (4) *S. clava* suspended above *S. clava*. Feces production trials were conducted for 16 h, followed by the rinsing and freezing experimental animals. A comparison of unmarked animals (n = 10) and marked feces producers (*M. edulis,* n = 6, tunicates n = 10) were used to demonstrate that our ^{13}C labelling technique worked properly. *Mytilus edulis* and *S. clava* tissues were analyzed for degree of isotope enrichment (^{13}C) using the same procedures provided above (see methods for PPP assimilation trials).

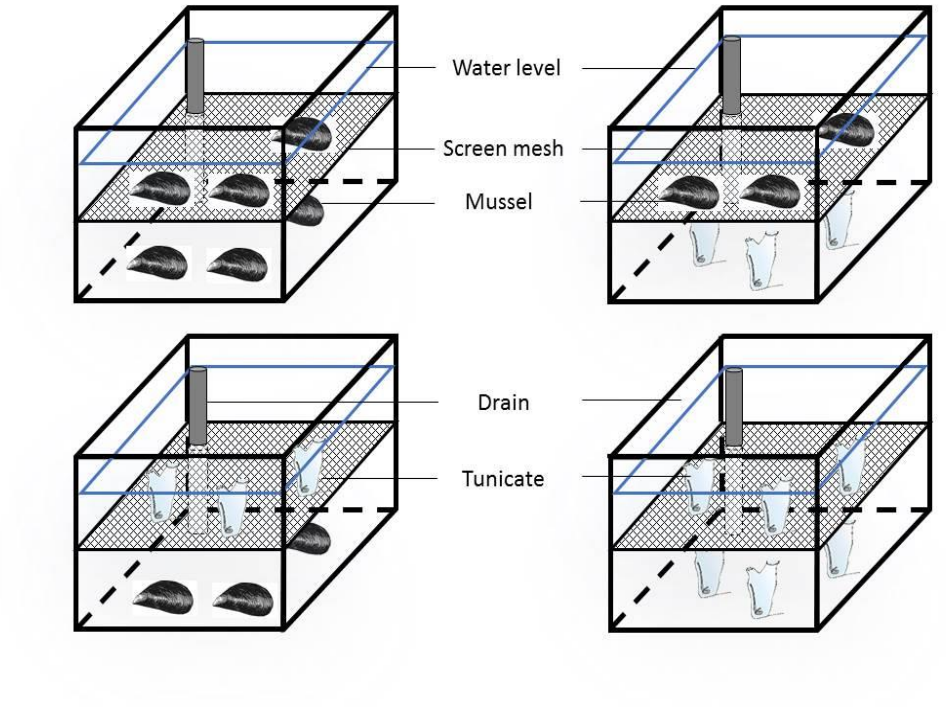

Figure 19: Experimental design used to assess the assimilation rates of predigested particles related to four species combinations (mussels over mussels or ascidians and ascidians over mussels or congeners). The numbers of individuals in the diagram are reduced for clarity.

3.5.4 Chatacterizing feces

To assess the morphometric characteristics of feces produced by *M. edulis* and *S. clava*, both species were acclimatized for one month with 1µm filtered and UV sterilized seawater and then fed daily with a 1:1:1 mixture (v/v/v) of the microalgae *Tisochrysis lutea* (CCMP 1324), *Diacronema lutheri* (CCMP 459), and *Chaetoceros neogracile* (CCMP 1317) at a ratio of 3% dry biomass (of filter feeder tissues). Algal strains were obtained from the Bigelow National Center for Marine Algae and Microbiota (Maine, USA). Mussels (n = 4) and ascidians (n = 4) were disposed in 2-L metabolic chambers (one organism per chamber) receiving gentle aeration (bubbling) to mix the entire water volume

without promoting feces resuspension. Furthermore, four mussels and four sea squirts were used to release feces after the digestion of two different food sources, (1) nanophytoplankton *Tisochrysis lutea* and (2) the picophytoplankton species *Nannochloropsis oculata*. Incubations were stopped when an adequate amount of feces particles had been produced for sampling. For both algal food sources, mussels had 4-h incubation periods for proper feces production, whereas ascidians needed a 24-h incubation periods. Sampling of feces was done using wide-tipped pipettes to minimize particle deformation and destruction. Observations were made under a compound microscope (40× power) in celled-slides (Sedgwick-Rafer cell). Three feces segments were measured per individual (n = 4) for every filter-feeding species and microalgal treatment. Since produced feces were often elongated, one length and several width measurements (6 to 8) were performed on each particle to obtain accurate mean length data.

3.5.5 Statistical analysis

Clearance rate data from commercial-sized mussels and variously-sized ascidians were compared using a two-sample t-test (assuming equal variances). Then, phytoplankton (PPP) assimilation was established per individual (one animal per chamber) and for paired animals (mussel and ascidian in the same chamber) by determining the linear relationship between PPP assimilation rate ($mg\ ^{13}C_{ind\ h\text{-}1}$) and total dry tissue weight (DTW_{total}). Power functions were used to determine the allometric relationship between PPP assimilation and dry tissue weight ($y = ax^b$, with r^2 values), where a is the value for a standardized animal (DTW_{total} = 1 g) and the exponent is the allometric correction applied to additional PPP assimilation rate estimates. Differences between groups fed with enriched ^{13}C feces was assessed using single-factor ANOVAs and t-tests (two-sample, assuming equal variances). Differences in feces between species were compared using a t-test (assuming equal variances). All statistical analyses were run with SPSS and Sigmaplot software. Statistical significance was set at $p < 0.01$. In parallel with the predigested-particle experimental trials, qualitative characterizations of feces particles (size and shape) examined under a microscope were made of *M. edulis* and *S. clava* feces.

3.6 RESULTS

3.6.1 Clearance rates

Clearance rates (CR) from incubations using single *M. edulis* and *S. clava* were significantly different (t-test = 5.02, df = 28, $p < 0.0001$). Mussels had higher average CRs than tunicates: mean 3.8 ± 0.2 L h^{-1} (±SE) for mussels and 1.5 ± 0.3 L h^{-1} for tunicates. Corresponding dry tissue weight (mean ± SE) also differed significantly ($t\text{-test}_{(28)} = 6.53$, $p < 0.0001$) between mussels (0.38 ± 0.02 g) and ascidians (0.05 ± 0.02 g). Refiltration (RF > 1) of the entire water volume was detected in more than 90% of metabolic chambers, mostly in the chambers that had both species in them. Linear regressions between PPP assimilation rates ($^{13}C_{ind\ h\text{-}1}$) showed no significant relationship (Figure 20) with the RF in chambers with a single mussel , a single, or paired individuals. Therefore, no corrections for RF were needed.

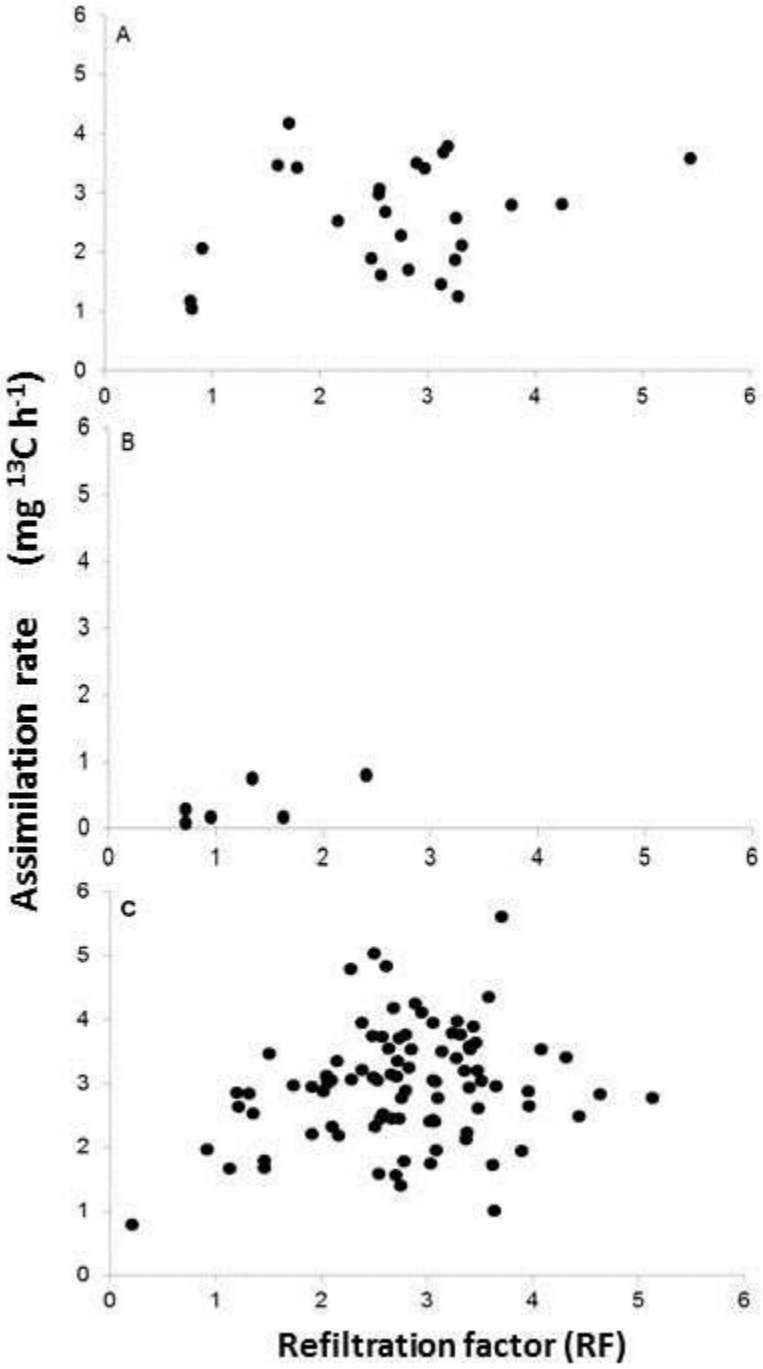

Figure 20: PPP assimilation rate (mg ^{13}C h^{-1}) as a function of the refiltration factor (RF) for feeding chambers containing (a) a single *M. edulis,* (b) a single *S. clava*, and (c) both species together.

3.6.2 Picophytoplankton assimilation

By supplementing natural seston with ^{13}C-labeled PPP (*N. oculata*) in treatment water, we demonstrated that both cultured *M. edulis* and invasive *S. clava* were able to filter, ingest, and assimilate PPP carbon into their tissues, whereas individuals in the control treatment (unlabeled natural seston) differed significantly (single-factor ANOVA, $F_{(5,246)} = 4.98$, $p < 0.001$) (Table 12). There were no significant differences ($p > 0.01$) in PPP assimilation between single or paired treatments for each species (Table 12). Mussels in the control treatment (non-labeled food source) showed no ^{13}C enrichment ($p<0.0001$) in comparison with experimental groups using isotopically labelled PPP namely single *M. edulis* (t-test$_{(39)} = 4.81$, $p < 0.0001$) as well as *M.edulis* incubated with *S. clava* (t-test$_{(102)} = 6.99$, $p <0.0001$) treatments. Because the rates of ^{13}C-enrichement varied widely for sea squirt incubated with a mussel, only enrichment results from the single ascidian treatments were significantly higher than unlabeled control tunicates controls (t-test$_{(24)} =3.31$, $p < 0.01$) (Table 12). Linear regression of the allometric relationship between PPP assimilation rate (mg ^{13}C h^{-1}) and dry tissue weight (g) of single individuals showed that *M. edulis* and *S. clava* assimilated 6.92 and 4.73 mg ^{13}C h^{-1} from PPP (respectively) per gram of body weight (Figure 21). Power function regression equations describing the assimilation rate (AR) of PPP were $6.92 \times DTW^{1.01}$ ($r^2 = 0.82$, $n = 26$, $p < 0.0001$) for *M. edulis* and $4.73 \times DTW^{0.86}$ ($r^2 = 0.86$, $n = 6$, $p < 0.001$) for a *S. clava*. The exponent describes how quickly AR increased relative to body weight.

Table 12: Tissue isotopic ratios ($\delta^{13}C/^{12}C$) in *M. edulis* and *S. clava*. Values represent means ± SE.

Species	$\delta^{13}C/^{12}C$	**N**
Single *M. edulis*	200.1 ± 25.9	31
Single *S. clava*	238.7 ± 61.3	16
M. edulis (competing with a *S. clava*)	165.4 ± 8.7	93
S. clava (competing with a *M. edulis*)	108.3 ± 29.4	93
Control *M. edulis*	-21.6 ± 0.07	10
Control *S. clava*	-20.6 ± 0.11	10

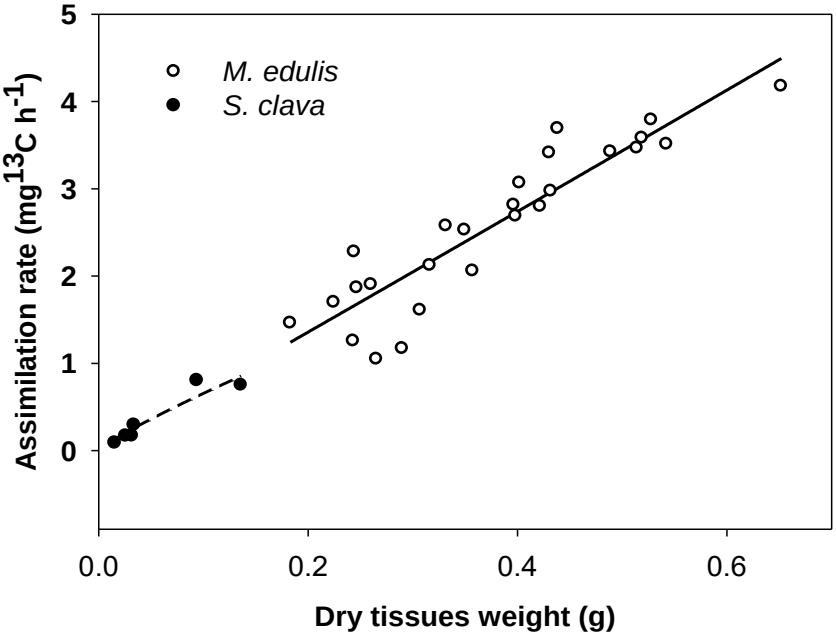

Figure 21: PPP assimilation rate (mg $^{13}C\ h^{-1}$) as a function of dry tissue mass of a single *M. edulis* (n = 26) or a single *S. clava* (n = 6).

Competition trials in metabolic chambers were conducted by pairing a commercial-sized mussel with ascidians of various sizes. The allometric linear regression between the AR of PPP (mg $^{13}C\ h^{-1}$) and dry tissue weight of single mussels relative to paired individuals (competing mussel and sea squirt) showed higher AR from single mussels than when they were paired with their filter-feeding competitor (Figure 22). The AR for *M. edulis* (without a competitor) was 6.92 mg $^{13}C\ h^{-1}$ per gram of dry tissue weight, while the AR for *M. edulis* with a competitor (paired with a single *S. clava*) was 5.61 mg $^{13}C\ h^{-1}$ per gram of dried tissue weight ($5.61 \times DTW^{0.85}$, $r^2 = 0.67$, n = 91, $p < 0.0001$).

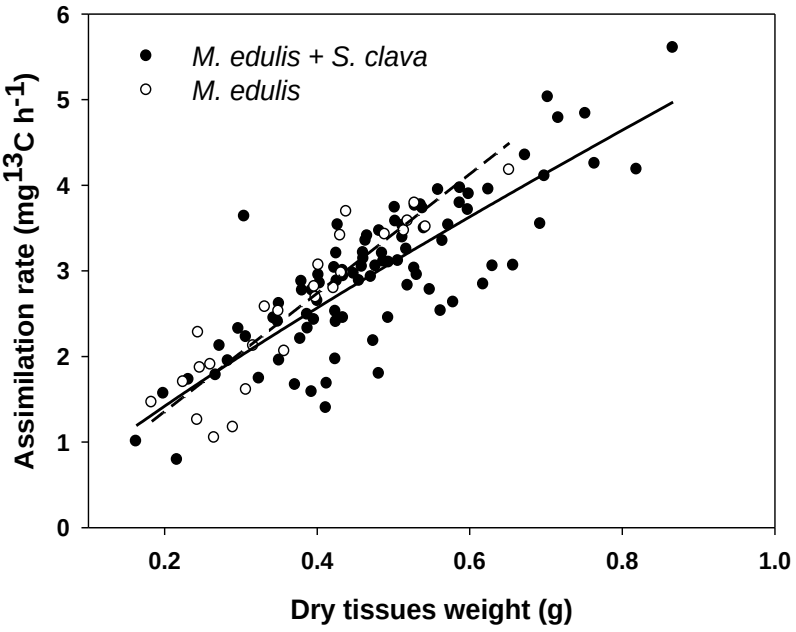

Figure 22: PPP assimilation rate ($mg^{13}C\ h^{-1}$) as a function of *M. edulis* dry tissue mass for single mussels (n =26, dotted line) and single mussels paired with a single ascidian (*M. edulis* + *S. clava*) (n = 91, solid line).

3.6.3 Assimilation of pre-ingested particles

Isotopically-labeled control animals were thoroughly conditioned by supplementing their natural seston diet with labeled (^{13}C) PPP, demonstrating an exceptional intake of ^{13}C (Table 12). Parallel (baseline) control values were also been obtained, showing natural ^{13}C intake from *in-situ* water from the bay (Table 12). Results for PPP assimilation of predigested particles (Figure 23) showed that mussels filter-feeding beneath ^{13}C-labeled mussels (AR = $0.12 \pm 3.44 \times 10^{-5}$: t-test $_{(23)} = 2.07$, $p < 0.001$) and ^{13}C-labelled ascidians (AR = $0.12 \pm 4.77 \times 10^{-4}$: t-test $_{(23)} = 2.07$, $p < 0.01$) acquired significant isotopic enrichment, similar to that of sea quirts feeding beneath their ^{13}C-labeled congeners (AR = $1.22 \pm 8.61 \times 10^{-4}$: t-test $_{(23)} = 2.07$, $p < 0.01$). However, ascidians did not significantly feed on mussel biodeposits as PPP assimilation rates were similar to the AR of control individuals.

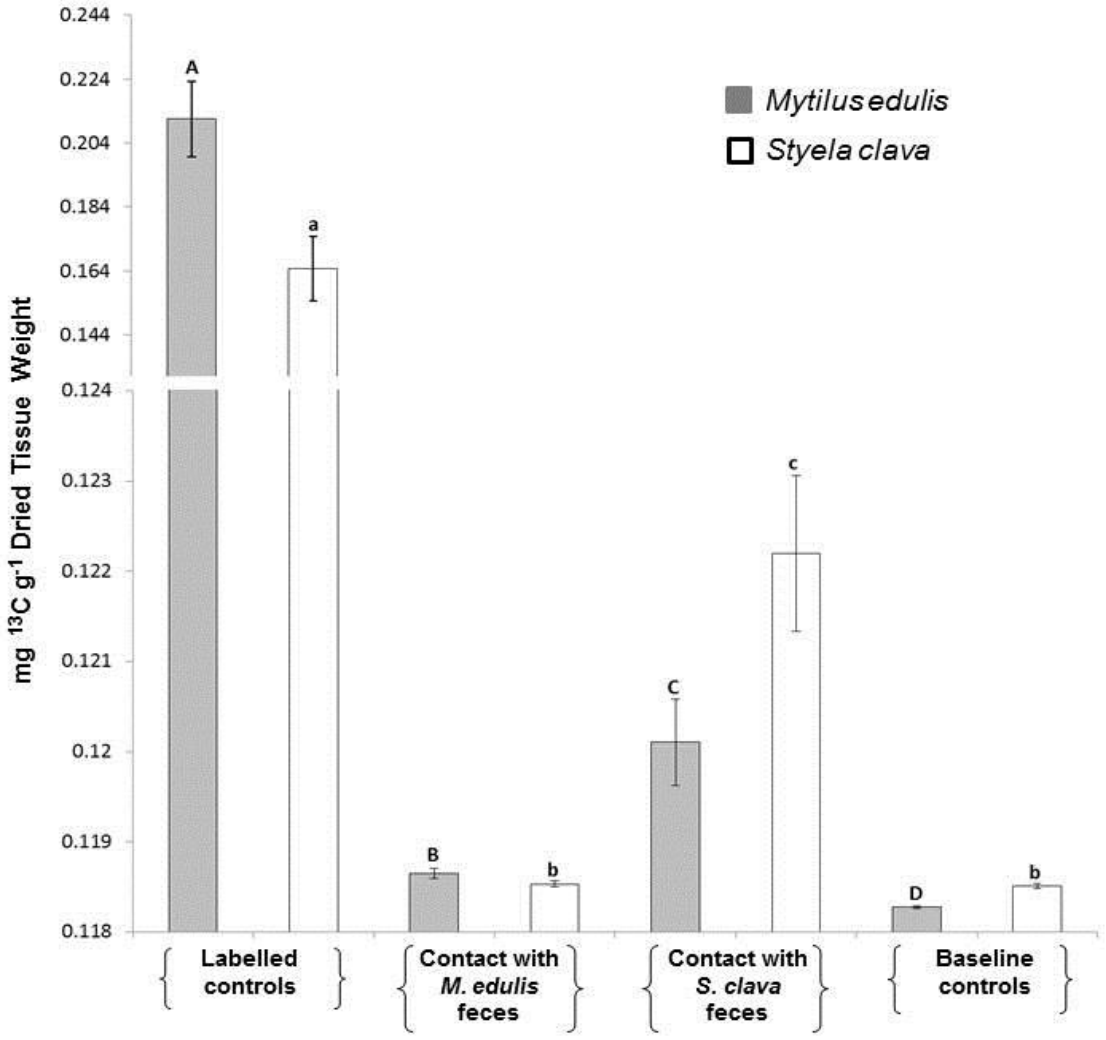

Figure 23: Picophytoplankton assimilation (mg ^{13}C per gram of dry tissue weight, mean ± SE) through ingestion of feces or pseudofeces produced by isotopically labelled (and labelled control) mussels (*M. edulis*) and ascidians (*S. clava*). Statistical differences ($p < 0.01$) are represented by different upper case letters (*M. edulis*) and lower case letters (*S. styela*).

3.6.4 Feces characteristics

Feces segments produced by *M. edulis* and *S. clava* were characteristically elongated. Independent of the microalgal food source provided (the picophytoplankton *Nannochloropsis* sp. or the nanophytoplankton *Tisochrysis* sp.), ascidian fecal pellets were significantly longer (t-test$_{(45)}$ =13.83, p < 0.0001) and larger (t-test $_{(270)}$ = 6.4, p < 0.0001) than those of mussels (Figure 6). Both species produced fecal pellets with larger diameters (*M. edulis*: t-test $_{(124)}$ = 1.98, p < 0.05; *S. clava:* t-test$_{(143)}$ = 2.21, p < 0.05) when fed with larger phytoplankton cells such as *Tisochrysis* sp. in comparison with being fed PPP (*Nannochloropsis* sp). However, lengths of fecal particles did not differ significantly relative to the size of the phytoplankton provided as food (Figure 24).

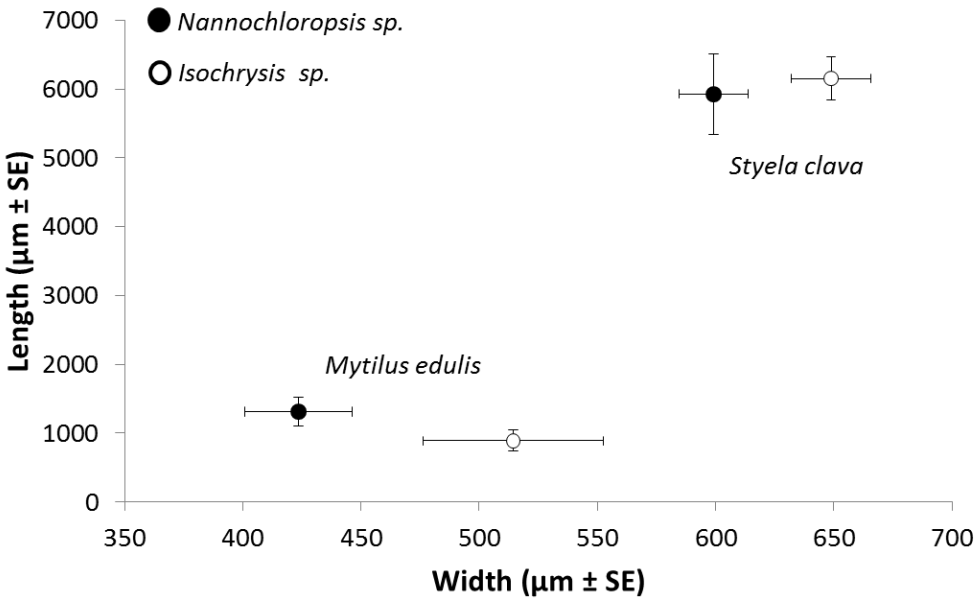

Figure 24: *M. edulis* and *S. clava* feces size features (width and length, mean ± SE) following the ingestion of two different-sized microalgal food resources (*Nannochloropsis* sp. and *Tisochrysis* sp.).

3.7 DISCUSSION

The retention of particles by *M. edulis* has been extensively explored under a variety of methodological approaches: closed systems in laboratory settings (Møhlenberg and Riisgård, 1978; Shumway et al. 1985; Riisgård 1988; Clausen and Riisgård, 1996), closed *in situ* mesocosms using natural seston (Trottet et al. 2008; Jacobs et al. 2015), open *in situ* systems using natural seston (Strohmeier et al. 2012), *in situ* mapping in shellfish farms (Cranford et al. 2014), and stand-alone ecosystem models (Fréchette, 2012). However, there have been few studies focusing on the importance of PPP as a food source for filter-feeders. Recent numerical modelling results suggest that PPP may contribute up to 29% of the energy budget of commercial-sized mussels (Sonier et al. 2016). In the present study, we demonstrated that PPP carbon effectively accumulates in *M. edulis* tissues, thus supporting the fact that PPP represents a food source for cultivated mussels. Furthermore, assimilation rates were species-specific, suggesting that competition for food occurs between *M. edulis* and *S. clava*. Interestingly, *M. edulis*' maximum assimilation rate was not reached when a *S. clava* competitor was present. *Styela clava* is an efficient filter-feeder capable of extracting suspended particles at a rate of 5.8 L h^{-1} (Petersen et al. 2007). *Styela clava* can also effectively retain small particles when seston concentration is low (Kang et al. 2011). It can do so through physiological adjustments, namely by pumping water in through its buccal siphon, straining the food through its branchial filter, and then pumping the filtered water out through its atrial siphon (Cohen 2005). Particle straining occurs in the mucus sheet or the net covering its branchial filter (pore size approximately 0.2µm), which should theoretically remove small particles, such as PPP, and larger organisms, including large bacteria (Lambert and Lambert 1998). Therefore, this ability of *S. clava* to remove variable sizes of particles from its surrounding water may induce the observed competition for PPP when incubated with *M. edulis* individuals.

Some discrepancies between PPP retention and carbon assimilation rates by competing filter-feeders in *in situ* experimental trials may be influenced by biological or physical factors, such as flocculation of suspended particles. Flocculated particles are

referred as micro aggregates (<500 μm) or marine snow (>500 μm), both formed when particles collide and stick together (Thornton 2002). Aggregates sinking from the water column towards the seabed can be beneficial to epibenthic species, such as scallops (*Placopecten magellanicus*), which respond by slightly elevating their clearance rates and absorption efficiencies (Cranford et al. 2005). Laboratory results of Heinonen et al. (2007) showed that over a five-hour filtering period, actively-pumping blue mussels, bay scallops, slipper snails, and some solitary species of ascidians (including *S. clava*) can boost the amount of transparent exopolymer particles (TEPs) significantly above background levels. Both unicellular and multicellular organisms produce exopolymers, which consist largely of polysaccharides that hydrate rapidly by contact with water and coalesce to form gels (Wotton 2004). This link may contribute significantly to the production of aggregates in estuarine systems, where shellfish are cultured and ascidians are well established. Mckee et al. (2005) demonstrated that oysters also enhance TEP concentrations in coastal marine ecosystems. If TEPs produced by suspension feeders enhance aggregation and particle deposition rates, then these processes may also strengthen the intensity of benthic-pelagic coupling (Heinonen et al. 2007) in marine ecosystems. Because all of our *S. clava* specimens were acclimatized in a flow-through system using natural seawater from the bay prior to starting our experiments, we reduced the risk of limiting mucus net production, which could have resulted in lower clearance rates for some ascidian species in food-limited scenarios (Petersen and Riisgård 1992). For some bivalves, it is possible to determine the intensity of filtration by measuring the extent to which valve gapes are open (Jørgensen,1990; Riisgård et al., 2003; Comeau and Babarro, 2014). However, for ascidians, it is not easy to determine if individuals are filtering at an optimal rate by simply observing them. Indeed, ascidians are sensitive to disturbance and may squirt in response to high particle concentrations or when subjected to severe disturbance (Armsworthy et al. 2001). It has been demonstrated through numerical modelling that fouling communities dominated by filter-feeding ascidians can limit productivity in bivalve farms because they compete for food resources and increase biodeposition rates (Guyondet et al. 2016). Our study provides important insights into potential carbon and nutrient cycling dynamics in

high-density aquaculture settings, enhanced by the ingestion of fecal particles by filter-feeders.

Our results indicate that *M. edulis* and *S. clava* compete for PPP food resources and exploit predigested particles. *Mytilus edulis* assimilated carbon from feces produced by other *M. edulis* and *S. clava*; however, *S. clava* only assimilated feces produced by congeners (i.e., we did not detect assimilation of mussel feces in ascidians). The latter discrepancies may originate from feces characteristics such as size, where *S.* clava produced significantly larger particles than *M. edulis.* Also, because ^{13}C concentration remaining in feces particles from each filter-feeding species was not quantified, enrichment levels could vary. Suspension feeders alter the composition and transfer mechanisms of organic matter, mostly by collecting and ingesting small particles and subsequently ejecting larger pellets (feces or pseudofeces) onto which many small particles become bound (Wotton and Warren, 2007). By consolidating carbon of small cells (such as PPP) into larger carbon aggregates (i.e. feces or pseudofeces), the predigested organic material may then be formed into a size that can be optimally retained by filter-feeders. We demonstrated that fecal particles can be refiltered and assimilated by filter-feeders growing in close proximity to one another. In Atlantic Canada, some bays supporting mussel aquaculture are infested by invasive ascidians, such as *S. clava.* If a mussel sleeve is left untreated (without mitigation measures), *S. clava* density can attain as many as 3136 individuals per 2–m mussel sleeve (A. Ramsay, Prince Edward Island Department of Agriculture, Fisheries and Aquaculture, pers. com.). If fouling is periodically removed, the density of live *S. clava* can be reduced to approximately 627 individuals per mussel sleeve (Ramsay et al. 2014). Since *M. edulis* grows in very close proximity of *S. clava* on these sleeves, often with overlapping fixation points, it is reasonable to assume that *S. clava* feces are readily accessible to *M. edulis*. The contribution of such feces-derived carbon to the energy budget of cultivated mussels remains undocumented, however this association between cultured and invasive species may be presented as an early level commensalism.

3.8 CONCLUSION

Our study provides new insights into assimilation and cycling of PPP carbon by *M. edulis* and *S. clava*. It is concluded that both species are able to ingest and assimilate PPP as a food resource, with *M. edulis* assimilating PPP more rapidly than *S. clava*. However, *S. clava* out-competes *M. edulis* for PPP when both species co-exist, such as within infested mussel farm environments. It is also concluded that secondary assimilation occurred through ingestion of feces or pseudofeces, thus enabling the transfer of carbon from PPP among individuals living in close proximity. Therefore both cultured mussels and invasive ascidians are able to actively feed on PPP in nutrient-rich estuaries, where PPP production is typically elevated.

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DISCUSSION GÉNÉRALE

L'objectif général de cette thèse est d'approfondir notre compréhension envers les petites cellules phytoplanctoniques (picophytoplancton, < 2μm) comme étant une source potentielle de nourriture importante pour les bivalves d'élevage cultivées au Canada Atlantique notamment l'huître américaine (*Crassostrea virginica*) ainsi que la moule bleue (*Mytilus edulis*). Le tout, en investiguant tout d'abord la disponibilité (biomasse) du picophytoplancton à l'intérieur d'estuaires ostréicoles et mytilicoles de l'Île-du-Prince-Édouard où la culture de ces bivalves est importante. En deuxième lieu, nous nous sommes attardés à la physiologie d'alimentation des bivalves en présence de ces petites particules particulièrement au niveau de la rétention, l'ingestion et l'assimilation dans leurs tissus mous du carbone provenant de picophytoplancton. Pour conclure, dû à la présence de nombreuses espèces envahissantes présentes dans cette région, une composante de compétition alimentaire fut explorée, en autre relatif à l'espèce de tunicier solitaire, soit *Styela clava*. Afin d'y arriver nous avons utilisé de multiples techniques allant de la modélisation écosystémique et l'expérimentation sur le terrain (*in situ*) vers l'enrichissement isotopique et l'utilisation de biomarqueurs comme traceurs diététiques, tels que les isotopes stables et le profilage des acides gras.

DISPONIBILITÉ ET RÉTENTION DU PICOPHYTOPLANCTON PAR LES BIVALVES

L`eutrophisation côtière est un enjeu écologique important dans plusieurs régions du littorale à l'échelle de la planète. L'Île-du-Prince-Édouard n'est malheureusement pas à l'abri de ce phénomène, souvent amplifié par les activités humaines, car l'apport en nutriments allochtones provenant en autre de l'industrie de la pomme de terre, l'agriculture en générale et même le tourisme (terrains de golf) pourrait effectivement contribuer aux renouvellements accélérés des biomasses phytoplanctoniques (Paerl 1995). Ces productions massives d'algues en milieux estuariens sont souvent suivies de périodes anoxiques à l'échelle locale (Cooper 1995). Cette relation étroite entre la biomasse de phytoplancton (mesurée souvent par la concentration du pigment chlorophylle-*a*) et les nutriments (totaux)

à l'intérieur d'une multitude d'estuaires de l'Î.-P.-É. fut présentée il y a près de 20 ans (Meeuwig et al. 1998). Pour l'industrie conchylicole toujours en expansion et en développement dans les provinces de l'Atlantique, plusieurs baies aquacoles ont vu les densités de bivalves augmenter. La biomasse de filtreurs dans l'écosystème est donc passée à des niveaux nettement supérieurs. Dans certains cas, ces biomasses importantes de bivalves se retrouvent parfois mêmes en marge de la capacité de support maximale offerte par l'environnement. Cette situation précaire stimule beaucoup d'intérêt en recherche, vers l'étude de la physiologie de filtration des bivalves d'élevage ainsi que leurs interactions avec l'environnement, plus précisément leurs sources de nourritures (phytoplancton), et ce, tant au niveau académique que gouvernemental.

Quant à la potentielle capacité des bivalves à moduler la sélection des particules, Strohmeier et al. (2012) avança l'hypothèse que la capture de particules par *M. edulis* est sujette à des changements spatiaux-temporels suggérant une capacité de modification de la rétention de la nourriture dépendamment de la disponibilité et de la qualité de la ressource nutritionnelle présente dans le milieu. Cet énoncé corrobore les résultats déjà présentés par Cranford et Hill en 1999, cette fois-ci chez une autre espèce commerciale, soit le pétoncle géant, *Placopecten magellanicus*. Ceci dit, aucun mécanisme évident pouvant expliquer une telle capacité de modulation du comportement de sélection des particules ne fut présenté jusqu'à présent dans la littérature (Riisgård et al. 2013, 2015). La rétention de picophytoplancton par les bivalves peut aussi dépendre de l'environnement influençant les différentes biomasses et communautés phytoplanctoniques disponibles. En méditerranée, plus précisément dans l'étang de Taux (France), l'huître japonaise, *Crassostrea gigas,* ne présente supposément aucune rétention de picophytoplancton. En contrepartie, dans cette région ces petites particules ne représentent qu'une source nutritionnelle appauvrie dans un système où les diatomées dominent (Dupuy et al. 2000). Néanmoins, l'ingestion de picoplancton par *C. gigas* fut démontrée de façon indirecte par l'intermédiaire d'un protozoaire cilié, prédateur du picophytoplancton, qui par la suite peut être capturé par les bivalves lors de la filtration. Ce transfert d'énergie par plusieurs niveaux trophiques remet

en question l'importance des picoparticules à l'intérieur de la chaîne alimentaire estuarienne (Le Gall et al. 1997).

L'utilisation de biomarqueurs, tels que les isotopes stables ou l'utilisation des profils d'acides gras, comme processus de détermination des sources alimentaires ingérées et assimilés par les bivalves devient maintenant très intéressante comme concept analytique. Ces techniques ne s'attardent pas à la physiologie de la filtration ni à la capacité des rétentions des particules par les bivalves, mais bel et bien aux sources d'énergie (sources alimentaires) digérées et assimilées directement dans leurs tissus. Les chapitres 2 et 3 de cette thèse ont sus utilisé ces différents marqueurs diététiques dans plusieurs contextes différents.

PROCESSUS BIOTIQUES ET ABIOTIQUES FAVORISANT LA RÉTENTION

Exopolymères en suspension

Les particules d'exopolymères que l'on retrouve dans nos estuaires sont normalement composées de mucopolysaccharides provenant des milieux aquatiques (marins ou dulcicoles). Ces polysaccharides sont souvent relâchés par des microorganismes (phytoplancton, bactérie) sous forme d'exopolymères extracellulaires (Passow 2002a) agissant souvent comme protection pour la cellule (Geun Goo et al. 2013). Récemment, une étude canadienne dans le golfe du Saint-Laurent conclut qu'une présence de particules d'exopolymères transparents pourrait contribuer de façon significative au transport du carbone notamment au niveau estuarien (Annane et al. 2015). Une relation étroite semble présente entre la présence de blooms phytoplanctoniques et l'augmentation d'exopolymères dans la colonne d'eau, surtout dans les estuaires (Nixon et al. 1995; Wetz et al. 2009). Ces particules démontrent souvent des propriétés apparentées à un gel, ayant l'habilité de gonfler/rétrécir, une grande flexibilité et tendance à être très collant, le tout à des degrés variables tout dépendamment de leur environnement (Passow 2002b). La production d'exopolymères par certains phytoplanctons semblent souvent agirent comme agent déclencheur de processus d'agrégation abiotique des particules en suspension (Kerner et al.

2003). C'est le cas notamment pour la diatomée *Skeletonema costatum* (Radić et al. 2005; Urbani et al. 2005; Schenk et al. 2008), une espèce dominante à l'intérieur des estuaires de l'I.-P.-É. (Bates et al. 2006) et reconnue pour sa contribution importante en exopolymères dans la colonne d'eau, surtout lors des périodes de floraisons (bloom) printanières (Engel 2000).

L'assimilation de particules synthétiques, ayant la même taille que certains picophytoplancton, fut démontrée par l'entremise de la production d'agrégats (Kach et Ward 2008), et ce chez plusieurs espèces de bivalves notamment la palourde *Mercenaria mercenaria*, le pétoncle *Argopecten irradians*, la crépidule *Crepidula fornicata*, et bien sûr *M. edulis* et *C. virginica*. Ceci étant dit, cette expérience ne fut pas répétée en utilisant des cellules phytoplanctoniques vivantes. La production d'agrégats peut aussi engendrer la bioaccumulation de pathogènes, en autre de genres; *Vibrio, Aeromonas, Pseudomonas, Escherichia et Mycobacteria*. (Lyons et al. 2007), devenant par la suite accessible à la filtration des bivalves (Kramer et al. 2016). Ce type de résultats à des implications importantes sur la surveillance des pathogènes aquatiques de nos zones côtières, incluant les zones de culture de bivalves ainsi que de baignade. La formation d'agrégats accélère aussi la déposition vers le fond de déchets comme les microplastiques. Certaines microalgues ont la capacité d'adhérer aux particules de plastiques, augmentant la masse totale, et accélérant le processus de déposition vers le benthos (Long et al. 2015).

Floculation et agrégation des particules en suspension

Plusieurs études sur le terrain et en laboratoire ont étudié les effets de l'abondance (biomasse) ainsi que la composition de la nourriture relativement aux taux de filtration et d'ingestion chez les bivalves filtreurs sauvages et cultivés. Or, peu de travaux s'attardent sur les processus d'agrégation ou floculation des particules en milieu estuarien (Cranford et al. 2005). Les agrégats sont omniprésents dans la colonne d'eau des systèmes marins et ont aussi une importance écologique pour 1) leur fonction comme agents de concentration et de transport de particules et 2) la création de microenvironnements dans la colonne d'eau (Thornton et Thake 1998). Ceux-ci sont aussi classifiés selon leur taille et présentés

notamment comme microagrégats (<500 µm) ou de la neige marine (« marine snow », > 500 µm). Ces dernières sont très présentes dans le milieu océanique et se forment lorsque des particules se frappent et collent ensemble (Shanks 2002; Thornton 2002; Lyons et al. 2007). La sédimentation suivie d'une remise en suspension des agrégats marins connecte les réseaux trophiques pélagiques et benthiques dans les environnements aquatiques. Ces processus ont une importance en autre dans la boucle microbienne qui participe au flux d'agrégats associés aux pico- et nanoorganismes disponibles pour les organismes de niveaux trophiques supérieurs. Certains de ces organismes supérieurs sont notamment capables de se nourrir de petites particules uniquement lorsqu'elles sont associées à des agrégats (Zimmerman-Timm 2002), sinon cette source de nourriture serait trop de petite taille afin d'être capturée efficacement. À titre d'exemple, il a été démontré que le pétoncle géant (*Placopecten magellanicus*) aurait la capacité d'ingérer des agrégats entiers (parfois appelés flocons) lors de la période de déposition suivant un bloom phytoplanctonique printanier (Cranford et al. 2005). Newell et al. (2005) ont démontré des résultats similaires présentant la neige marine comme étant une source potentielle de nourriture pour les populations sauvages de moules, *M. edulis*, toutefois en soulignant que ces agrégats semblent être souvent de piètre qualité nutritionnelle. En bout de ligne, très peu de recherche sur la physiologie des bivalves considèrent l'effet de la floculation ou agrégation des particules dans leurs travaux, et ce, même lors de situations floraisons (bloom) phytoplanctonique ou même de périodes de remise en suspension des sédiments (Thornton 2002). Karlsson et al. (2003) ont démontré que la taille limite supérieure de rétention des particules par les bivalves pourrait se situer entre 0,5 et 6 mm. Il est donc raisonnable de spéculer une contribution importante des flocons ou agrégats, incluant la neige marine, comme une source nutritionnelle potentiellement importante chez les bivalves d'élevage.

Le principe de floculation est aussi beaucoup utilisé dans la production commerciale de micro algues, entre autres dans l'industrie des biocarburants. Plusieurs méthodes de récoltes sont explorées lors de la production de micro algues, combinant une étape de floculation suivie d'une mise en suspension (flottaison) ou une sédimentation (Schenk et al. 2008). Durant la floculation les cellules micro algales dispersées forment des agrégats de

tailles supérieures ayant un taux de sédimentation plus élevé (Salim et al. 2011). Plusieurs facteurs externes, de sources naturelles ou induites, peuvent provoquer la floculation des algues. En autres, l'utilisation de pH extrêmes, une diminution des nutriments disponibles, des changements de température ou d'oxygène dissout (Salim et al. 2011). Certains utilisent l'ajout de sels contenant certains métaux (aluminium, fer ou zinc) afin de coaguler certaines espèces de micro algues tel que *Chlorella minutissima* (Papazi et al. 2011). Ceci étant dit, la majorité des méthodes présentées ci-haut ne sont pas souhaitables, car elles peuvent engendrer des modifications indésirables à la composition et la structure cellulaire des algues (Benemann et Oswald 1996). D'autres chercheurs démontrent la possibilité de créer des agrégats phytoplanctoniques sans additifs chimiques en utilisant qu'un facteur physique, soit la turbulence (brassage). En laboratoire, Kach et Ward (2008) eurent du succès à produire des agrégats de >100 µm à quelques mm de diamètre uniquement en brassant des bouteilles contenant de l'eau de mer (filtré sur un tamis ayant des pores de 210µm pour retirer le zooplancton), quelques billes inertes de différentes tailles et la bactérie *Escherichia coli*.

Vidéoendoscopie

L'utilisation de la vidéoendoscopie est une technique initialement développée pour l'étude des invertébrés (Heinzel et al. 1993) et par la suite adaptée pour l'observation des structures de filtration des bivales (Ward et al. 1991, 2003; Beninger et al. 2008). Bref, une petite section de la coquille au niveau de la chambre inhalante doit être retirée afin de pouvoir voir branchies (Figure 25). Cette incision se doit d'être suffisante pour y insérer la tête de la fibre optique provenant du système endoscopique. Celle-ci comprend une source lumineuse ainsi qu'une caméra. Ayant une coquille robuste et épaisse, l'huître est souvent une espèce de choix pour ce type de manipulation (Ward et al. 1993; Beninger et al. 2008), toutefois d'autres espèces furent aussi étudiées comme la moule *Mytilus chilensis* (Navarro et al. 2011) et la palourde *Mercenaria mercenaria* (Robbins et al. 2010). L'utilisation de la vidéoendoscopie nous permet de visuellement investiguer différents processus physiologiques interne liés à la filtration, par exemple le mouvement de particules

capturées par les branchies vers les palpes labiaux (Ward et al. 1991). Dans le cadre du stage international relié au curriculum académique de cette thèse, l'apprentissage de la méthode endoscopique pour les bivalves fut exploré (Figure 25).

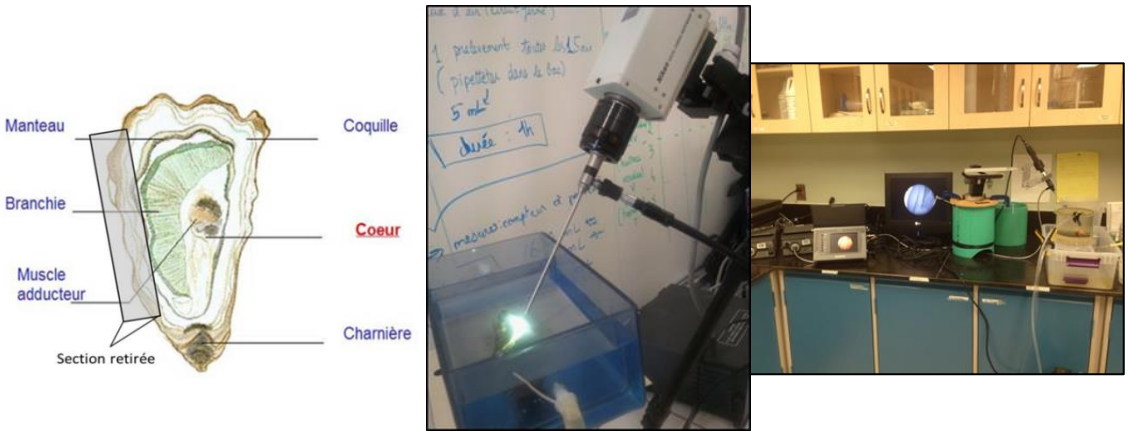

Figure 25 : Schéma de la section de coquille à retirer pour l'insertion de la fibre optique du vidéoendoscope (Bruno Cognie, U. de Nantes et Réjean Tremblay. ISMER)

Deux espèces furent investiguées sous vidéo endoscopie, soient *Crassostrea gigas* dans les installations de l'Université de Nantes en France (laboratoire du Dr. Bruno Cognie) ainsi que *C. virginica* à la station aquicole de l'Université du Québec à Rimouski (laboratoire du Dr. Réjean Tremblay). Comme représenté par le schéma ici-bas, une section de la frange de la coquille au niveau postérieure de l'huître doit être retirée afin d'avoir accès au système de filtration (branchies) et parfois même de sélection des particules (palpes labiaux). L'incision se doit d'être un peu plus grande que la fibre optique de l'endoscope.

Durant le court de ces manipulations endoscopiques, des cultures d'algues ont été utilisées comme sources nutritionnelles de maintenance durant l'observation de la filtration. Étant donné l'intérêt lié au picophytplancton omniprésent dans cette thèse, nous avons utilisé deux scénarios nutritionnels contenant tous deux le picophytoplancton *Nannochloropsis oculata* et un autre micro algue entre, 1) la diatomée *Skeletonema costatum* ou 2) *Tisochrysis lutea*.

Le rationnel nous ayant portés à utiliser la diatomée *S. costatum* est basé sur les caractéristiques autoflocullantes de celle-ci en plus d'être une espèce ayant une importante biomasse dans les estuaires de l'Î.-P.-É (Bates and Strain 2006). Cette dernière est très utilisée pour des fins d'affinage de l'huître (*C. gigas*) dans les claires de la façade atlantique française (Méléder et al. 2001) mais aussi comme espèce auto floculant utilisée à la récolte des microalgues. La floculation de cette diatomée peut être en autre induite par un changement de pH vers des valeurs plutôt acides (Pérez et al. 2017) ainsi que part une augmentation de la température en présence d'une importante biomasse phytoplanctonique (Thornton et Thake 1998). Lors d'essais en laboratoire, simplement en combinant une espèce de picophytoplanctonique (*N. occulata*) avec une quantité minimale de *S. costatum* nous avons pu confirmer les formations d'agrégats. Ceux-ci sont filtrés par les huîtres et comme l'indique l'image ici-bas (Figure 26), il est facilement possible de les observer (même à l'œil nu) lors de leur trajet sur les branchies vers les palpes labiaux. À noter que malgré les résultats des études mentionnées ci-haut, aucun traitement physique ou chimique n'a été appliqué aux cultures alguaires durant ces manipulations avec *C. gigas.*

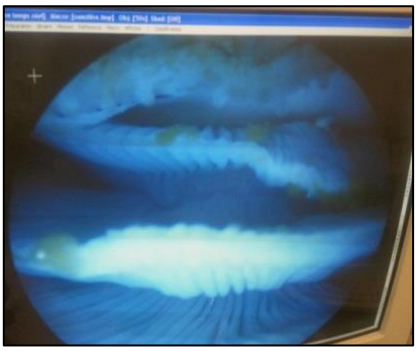

Figure 26 : Présence de flocons sur les branchies (*C. gigas*) observée en vidéoendoscopie.

Dans le cadre de l'expérimentation ayant comme source de nourriture un mélange de diatomées (*S. costatum*) ainsi que du picophytoplancton (*N. oculata*), non seulement la formation d'agrégats fut évidente, mais nous avons pu en échantillonner directement sur la gouttière de *C. gigas* ainsi que les pseudofèces (Figure 27). Cet échantillonnage est possible facilement à l'aide d'une pipette Pasteur, car le comportement de fermeture des valves de cette espèce d'huître semble beaucoup moins rapide que *C. virginica.*

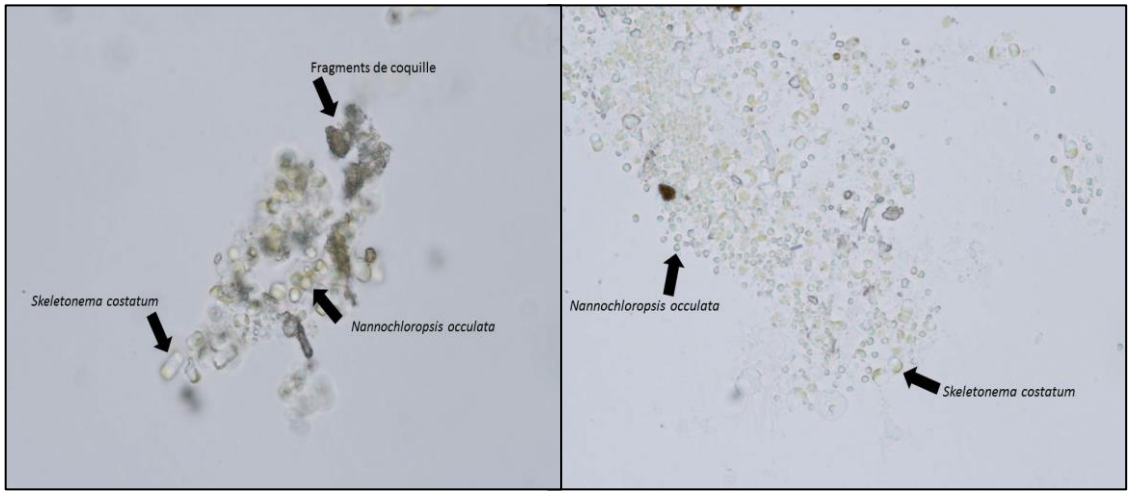

Figure 27 : Agrégats échantillonnés dans les pseudosfèces (gauche) ainsi qu'au niveau de la gouttière (droite) de *Crassostrea gigas.*

La figure ci-dessus (Figure 27) nous démontre très bien le contenu d'un petit agrégat comprenant; des fragments de coquille due la création de l'incision pour insérer l'endoscope, des cellules de *N. oculata* ainsi que *S. costatum*. Nous avons aussi vérifié le contenu des pseudoféces, qui à leur tour, nous démontrent la présence des mêmes types de cellules, toujours sous forme d'agrégats. Or, lors des manipulations en endoscopie, cette fois-ci en utilisant l'huître américaine *C. virginica* et en omettant d'utiliser une diatomée autoflocculante, des résultats similaires furent observés. Malheureusement, il fut impossible de déceler le moment ou à quelles étapes des manipulations la production d'agrégats algaux fut produit. En échantillonnant directement la source de nourriture utilisée pour ces expériences, une présence d'agrégation des cellules phytoplanctoniques à même la solution mère fut aussi observée sous le microscope. Une hypothèse mise de l'avant face à cette

observation est la tendance aux veilles cultures de la microalgue *N. oculata* à former plus facilement des dépôts (potentiellement sous forme de flocons ou agrégats).

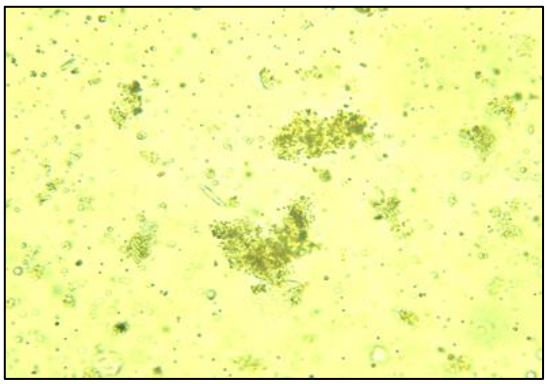

Figure 28 : Agrégats présents dans la solution mère (âgée) contenant les microalgues *N. oculata* et *Tisochrysis lutea*.

Rôles des fèces dans la rétention du picophytoplancton (< 2 µm)

Les espèces filtreurs ont la capacité de modifier la composition et les mécanismes de transport du seston vers le benthos, notamment par l'ingestion de petites particules suivie d'une concentration en particules de taille plus imposante (fèces et pseudofèces) (Wotton et Warren 2007). En modifiant la taille et la forme de petites cellules, telles que le picophytoplancton, il est fortement probable, que les fèces et pseudo fèces produites fassent maintenant partie d'une classe de taille optimale à la rétention pour certaines espèces de bivalves ou tuniciers. Nos résultats présentés dans le chapitre 3 démontrent clairement une assimilation de fèces isotopiquement marquée (^{13}C) par *M. edulis* et *S. clava* incubés à proximité l'une de l'autre. Les structures d'élevage en suspension de la moule bleue et des huîtres à l'Î.-P.-É. consistent souvent à maintenir de hautes densités de bivalves dans un espace restreint. Cette pratique augmente forcément les pressions associées à la proximité entre individus filtreurs, autant intra- que interspécifique. Dans les baies mytilicoles aux prises avec des infestations importantes de tuniciers, tant solitaires que coloniaux, ces interactions s'intensifient significativement. Les courants estuariens forcés par les marées, les rivières et les vents (Wotton et Malmqvist 2001) permettent aussi aux particules fécales de se déplacer horizontalement, les rendant à nouveau disponibles aux fermes de bivalves

cultivés à proximité. En se déposant éventuellement sur le fond, la filtration et la production de fèces par les bivalves convertit notamment de l'azote particulaire d'un format inaccessible à un format utilisable par la végétation aquatique, en autre au niveau de la rhizosphère (Peterson et Heck Jr. 1999).

UTILISATION DE BIOMARQUEURS

La sélectivité des particules par les bivalves peut être influencée au niveau de plusieurs processus internes tels que : 1) rétention différentielle au niveau des branchies, 2) sélection par les palpes des particules capturées par les branchies (production de pseudo fèces) et 3) digestion sélective du phytoplancton ingéré (Rouillon et Navarro 2003; Beninger et al. 2008). Due à ces paramètres physiologiques qui semblent chacun induire un certain degré de variabilité, l'utilisation de marqueurs biologiques est maintenant de plus en plus favorisée afin de démontrer de façon concrète l'assimilation du carbone des différences sources de nourritures directement dans les tissus des filtreurs. L'utilisation des isotopes stables, communément le carbone ($\delta^{13}C$) et l'azote ($\delta^{15}N$), ainsi que le profilage des acides gras des tissus mous des bivalves sont des méthodes analytiques très communes dans les recherches portant surtout sur la discrimination nutritionnelle chez les organismes marins. Cela dit, chacune de ces méthodes comporte des forces et des lacunes en ce qui concerne la robustesse ou la capacité d'interprétation de l'information générée. Par exemple, l'utilisation des isotopes stables de déterminer les sources de nourriture ingérée à long terme se modifiant avec la variabilité naturelle des composantes diététiques de l'environnement (Cloern et al. 2002; Malet et al. 2008; Pernet et al. 2012). D'autre part, les acides gras sont intégrés directement à l'intérieur des lipides de réserve du consommateur primaire (bivalves), reflétant normalement le profile en acides gras des proies ingérées (court terme). Les marqueurs lipidiques, tels que les acides gras, fournissent de l'information supplémentaire, surtout au niveau du type et de la qualité de la ressource assimilée (Dalsgaard et al. 2003). Utilisés en complémentarité, ces deux techniques analytiques démontrent une capacité intéressante à résoudre certaines questions écologiques à l'intérieur d'écosystèmes aquatiques complexes (Pernet et al. 2012).

Le profilage des acides gras est un type d'analyse dans les résultats se doivent d'être interprétés avec précautions et nuances. Une connaissance approfondie de l'environnement et des sources de nourritures potentielles au site étudié se doit d'être mise de l'avant, car un seul acide gras peut être utilisé comme biomarqueur diététique de plusieurs sources nutritionnelles. Ceci est facilement percevable en regardant certaines publications présentant un tableau (Table 13) de marqueur en acides gras tel qu'ici-bas où l'on remarque que l'acide gras 20:5ω3 (EPA) est utilisé à la fois comme marqueur de diatomée ainsi que les Phéophycées (algues brunes). La même observation peut être faite pour l'acide gras 18:4ω3 (acide stéaridonique) qui est associé aux dinoflagellés ainsi qu'aux chlorophytes, une division comprenant une bonne proportion des algues vertes.

Table 13 : Tableau provenant de Gaillard et al. 2017. « Table 1. Selected fatty acids trophic markers (FATMs) used as dietary tracers in the study of food resources of *Astarte elliptica* in Kobeefjord, southwest Greenland ».

Source	FATM	References
Bacteria	Σ*i*-FA + *ai*-FA, 18:1ω7, 16:1ω7	Viso & Marty (1993), Budge & Parrish (1998), Stevens et al. (2004)
Diatoms	16:4ω1, 16:1ω7, 20:5ω3	Viso & Marty (1993), Napolitano et al. (1997), Reuss & Poulsen (2002), Kelly & Scheibling (2012)
Dinoflagellates	22:6ω3, 18:4ω3, 18:1ω9	Napolitano et al. (1997), Mansour et al. (1999), Kelly & Scheibling (2012)
Copepods	Σ20:1ω9 + 22:1ω11	Dalsgaard et al. (2003), Lee et al. (2006)
Chlorophyta (green macroalgae)	18:3ω3, 18:4ω3, 16:4ω3	Graeve et al. (2002), Khotimchenko et al. (2002), Kelly & Scheibling (2012), this study
Phaeophyceae (brown macroalgae)	18:2ω6, 20:4ω6, 20:5ω3, 18:1ω9	Graeve et al. (2002), Kelly & Scheibling (2012), Wessels et al. (2012), this study

Plus notre compréhension des interactions entre les acides gras provenant des proies ainsi que les prédateurs s'améliore, la fiabilité de ces biomarqueurs augmente à leur tour. Certaines études suggèrent de nouvelles modifications pouvant être apportées à l'interprétation des profils d'acides gras, par exemple en intégrant des ratios de plusieurs acides gras. Dans l'optique de cette thèse portant sur le picophytoplancton, tout récemment, Moynihan et al. (2016) démontrèrent une corrélation significative entre les concentrations de picoeucaryotes aux stations d'échantillonnage (Chausey, Normandie) et cette relation en acides gras (16:4ω3 + 18:3ω3)/Σω3. Cette équation fut en autre proposé comme nouveau biomarqueur des picoeucaryotes pour cette région aquacole.

Finalement, l'un des obstacles rencontrés durant les travaux de cette thèse portant sur les profiles en acides gras se situe au niveau de l'interprétation quantitative des résultats. En autres mots, à savoir quelles quantités d'un acide gras donné doit être interprété comme une bioaccumulation due à l'alimentation ou simplement une augmentation relative à un métabolisme de base. Pour les huîtres, cette réponse semble absente pour le moment. Les baux aquacoles de nos régions (Canada atlantique) pourraient offrir des qualités de bons sites contrôles, particulièrement durant la saison hivernale. Durant cette période froide, en plus de la couverture de glace à la surface, la température de l'eau tombe sous les 4°C, atteignant normalement les -2°C. Il est connu que les huîtres réduisent leur métabolisme durant à ces températures en ouvrant leurs valves que quelques fois afin de renouveler la masse d'eau intervallaire. C'est à cette période que des profils d'acides gras de l'huître seraient envisageables sans l'impact de l'alimentation sur le bivalve. Toutefois, il faut garder en tête que la température de l'eau semble être le facteur principal du réveil des huîtres au printemps dont la majorité des individus ouvrent leurs valves activement lorsque celle-ci atteint les 2,61 ± 0,66°C (Comeau 2014).

IMPORTANCE POUR LA MODÉLISATION ET LA PRISE DE DÉCISIONS

La modélisation numérique de processus physiques, chimiques et biologiques est maintenant un outil très utile lors de la gestion de l'aquaculture et des zones côtières. Cet axe de recherche est constamment en développement et la demande de données variées et de qualité est toujours en demande (Hawkins et al. 1998). La productivité d'une ferme de bivalve est largement déterminée par la disponibilité du seston organique (parfois limitant dans l'environnement) ainsi que le niveau de compétition inter- ou intraspécifique sur ce dernier (Guyondet et al. 2013, 2016). Ceci dit la présence de filtreurs envahissants, tels que les tuniciers, rend la productivité d'une ferme conchylicole très vulnérable tant au niveau biologique que socio-économique. L'intégration d'information sur la capacité de rétention et ingestion du phytoplancton par des espèces nuisibles à l'intérieur des modèles numériques est très importante afin de présenter une image juste et actuelle de l'industrie aquacole, mais aussi prédire certains scénarios futurs. La possibilité de produire des

modèles de manières efficaces et dans des délais raisonnables est nécessaire afin de faciliter les dialogues entre les conseillers scientifiques, l'industrie aquacole et les gestionnaires des instances gouvernementaux (Guyondet et al. 2013). Tout récemment, un nouvel article en modélisation numérique des écosystèmes marins fut publié où les auteurs proposent l'intégration du concept de résilience du fonctionnement des écosystèmes face aux pressions liées à l'aquaculture des bivalves (Kluger et al. 2017). Ce type de modélisation peut grandement bénéficier des résultats présentés dans cette thèse afin de générer des estimations fiables à l'intérieur des discussions courantes sur la gestion durable de l'aquaculture des mollusques.

IMPLICATIONS FUTURES

Il est important de noter que l'aquaculture des bivalves participe aussi à la fertilisation des communautés phytoplanctoniques en excrétant des nutriments sous forme dissoute (Nielsen et Maar 2007). De plus, certaines tendances à long terme indiquent qu'une augmentation de l'oligotrophie des systèmes marins lors d'événement de réchauffement de type El Niño se traduiront par une diminution totale du phytoplancton avec une dominance des particules picoplanctoniques (Kostadinov et al. 2010). Dans l'Arctique canadien, l'abondance du picophytoplancton peut atteindre jusqu'à 18 400 cellules ml^{-1}, majoritairement des eucaryotes comptant ainsi pour 70% de la biomasse phytoplanctonique totale du système (Tremblay et al. 2009). Les écosystèmes côtiers, incluant les baies aquacoles, sont tous vulnérables aux pressions causées par les changements climatiques, tels que le réchauffement des océans, l'augmentation du niveau de l'eau, l'acidification et la modification des régimes de précipitations (Filgueira et al. 2016). Tous ces paramètres auront, de près ou de loin, des implications importantes sur la physiologie des bivalves et la possibilité d'en faire la culture (ou non) au même rythme de développement tel que nous le voyons en ce moment. L'aquaculture des bivalves est de plus en plus importante comme méthode production de protéine marine alternative face à l'exploitation des ressources naturelles. L'approche écosystémique devient primordiale afin de maintenir une production soutenable et durable pour les années à venir. L'intégration du

concept de résilience à l'intérieur d'approches écosystémiques sera aussi un atout indéniable à considérer dans les recherches et prises de décisions futures (Kluger et al. 2017).

Très peu de recherches s'attardent présentement sur les relations entre la forme des cellules phytoplanctoniques et la capacité de rétention des bivalves d'élevage. Dans des conditions où le seston pourrait être dominé par un phytoplancton de forme allongée plutôt qu'ovoïde par exemple, la variable « orientation de la particule » devient un facteur important à considérer (Cranford et al. 2016) surtout lorsque l'instrument de comptage (compteur de particules) n'a pas la capacité de détecter la morphologie des cellules algales. Pour terminer, l'étude du mucus produit par les bivalves est présentement à nouveau sur la sellette scientifique. Son importance dans le processus de filtration des bivalves fut confirmée, par video endoscopie, il y a plus de 20 ans (Ward et al. 1993). Par la suite, nombreux sont les articles portant sur la physiologie des mollusques bivalves ont su à leur tour démontrer une production de certains mucus (Jørgensen 1996) et exopolymères lors de la filtration. Les espèces étudiées comptent entre autres, la moule bleue (*M. edulis*), le pétoncle de baie (*Argopecten irradians*) ainsi que la crépidule (*Crepidula fornicata*) (Heinonen et al. 2007).

CONCLUSION GÉNÉRALE

En conclusion, l'ensemble de la présente étude présente des informations novatrices face à l'implication des petites cellules phytoplanctoniques (picophytoplancton) face aux bivalves d'élevages. La mise en évidence de l'assimilation du picophytoplancton par l'huître américaine (*Crassostrea virginica*) et la moule bleue (*Mytilus edulis*) est une conclusion importante afin de mieux comprendre nos systèmes conchylicoles côtiers. De plus, après la présentation de comportement de compétition d'un tunicier invasif (*Styela clava*) contre les moules relance le débat sur les interactions interspécifiques entre les espèces natives de l'Î.-P.-É. et les espèces envahissantes présentes dans leurs systèmes estuariens. La pensée populaire nous laissant présumer l'inaptitude aux bivalves d'élevages d'ingérer et assimiler le picophytoplancton comme source de nourriture, et ce sur des bases strictement physiques, doit être vue avec un grain de sel. Plusieurs mécanismes biotiques (mucus, production de fécès ou pseudoféces) ou abiotiques (floculation, production d'agrégats) peuvent rendre ces petites cellules phytoplanctoniques sous des formats en facilitant la filtration et l'absorption. La notion d'incorporer le picophytoplancton comme source importante de nourriture pour les bivalves d'élevages apporte un nouveau questionnement au niveau de la physiologie de filtration et ingestion par les bivalves, des notions de prédations et compétions sur le phytoplancton tel nous le connaissons présentement, des variables alimentaires utilisées à l'intérieur de modèles écosystémiques (notamment de capacité de support des baies aquacoles) ainsi que nos modes de gestions des bivalves afin d'offrir une ressource durable.

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