UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

en association avec

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LES LARVES DE LA MOULE ZÉBRÉE (*DREISSENA POLYMORPHA*) DANS LA ZONE DE LA TRANSITION ESTUARIENNE DU FLEUVE SAINT-LAURENT : DISTRIBUTION SPATIO-TEMPORELLE, IMPACTS ET SOURCES DE CARBONE

THÈSE

PRÉSENTÉE

COMME EXIGENCE PARTIELLE

DU DOCTORAT EN SCIENCES DE L'ENVIRONNEMENT

PAR

CHRISTINE BARNARD

SEPTEMBRE 2006

Remerciements

C'est un grand plaisir pour moi de mettre fin à un travail si colossal et surtout de remercier tous les gens qui m'ont porté main forte dans l'accomplissement de ce défi. Les premiers remerciements vont à mon directeur et mon co-directeur de thèse les Professeurs Jean-Jacques Frenette et Warwick F. Vincent. D'abord à Jean-Jacques avec qui j'ai tissé des liens d'amitié et qui m'a accompagné à travers les hauts et les bas de ma recherche. Tu as toujours su bien m'écouter et me conseiller aux moments opportuns. Merci pour ton esprit scientifique aiguisé et ton côté humain si chaleureux. Ensuite, merci à Warwick pour les heures de travail très stimulantes et amusantes et pour m'avoir fait confiance. Tu continues à m'étonner par ta passion inépuisable, ton optimisme infroissable et ton talent de rédacteur. Je tiens aussi à remercier les membres de mon jury de thèse les Drs. James H. Thorp, Dolores Planas et Ladd E. Johnson qui ont généreusement accepté de lire mes travaux et me suggérer des corrections.

Je tiens aussi à remercier les chercheurs Ladd E. Johnson, Connie Lovejoy et Paul Hamilton pour leur aide à différentes étapes de ma recherche. Sur le terrain et dans le laboratoire, je tiens à remercier les personnes suivantes qui se sont succédées au fil des années et avec qui le travail fût fort agréable : Andrea Bertolo, Hugues Boulanger, Andy Casper, Julie Harnois, Kim Huggins, Brigitte Laberge, Marie-Audrey Livernoche, Didier M'Radamy, Carl Martin, Christine Martineau, Pedro Peres-Neto, Leira Retamal, Sébastien Roy, Geneviève Trudel, Gesche Winkler et l'équipe du Willie DeRaspe et du Marie-Karine D. Je remercie aussi les gens de l'INRS qui m'ont aidé lors des analyses, Martine Savard, Anna Smirnoff et Lise Rancourt. Pour l'aide financière, je remercie le CRSNG (BESC).

Au plan personnel, je dois d'abord remercier Hugues qui est resté à mes côtés malgré mes absences mentales de si longue durée et qui me supporte au jour le jour et qui comprend tout. Merci à Juliette qui me fait sourire et qui m'ancre les deux pieds sur terre. Sans Hugues et mes amis les plus chers, Binette, Kim, Claudie, Isabelle V.,

ma sœur Sandra, mon frère James, Isabelle T., Karen, Phil, Pat et Alex, ce travail n'aurait jamais vu le jour. Merci pour les moments de joie intense si nombreux et les soirées qui sont toujours beaucoup trop courtes. Je remercie aussi ma famille, Pierre, Louise, James et Sandra Barnard, Giska Laflamme, Stéphane Girard, mes quatre neveux (Ludvic, Justin, Walker et Lawson) et la famille Boulanger qui m'encouragent (et m'endurent!) depuis toujours dans tous les projets que j'entreprends.

Pour ceux que j'oublie il faudra excuser l'épuisement et soyez certains que l'oubli n'est que temporaire…

Cette thèse est dédiée à ma petite famille, Hugues, Juliette, le bébé dans mon ventre et Merlot.

Résumé

La zone de transition estuarienne (ZTE) du fleuve Saint-Laurent est reconnue pour sa grande productivité, tel que le démontre son rôle de pouponnière de larves de poissons. Depuis 1994, les larves véligères de *Dreissena polymorpha* ont envahi le plancton de cette zone pour atteindre des densités allant jusqu'à 260 individus L^{-1}. L'invasion par les larves *Dreissena* a le potentiel de modifier les ressources disponibles aux organismes de niveaux trophiques supérieurs et de changer la structure de la communauté microbienne par l'effet combiné de leur forte abondance et de leur alimentation. Cette étude documente l'impact de *Dreissena* sur la communauté microbienne, les facteurs environnementaux contrôlant la distribution des véligères dans la ZTE, leur position trophique ainsi que les sources de carbone qu'elles assimilent. Les variations observées dans la structure de la communauté microbienne ne peuvent pas être attribuées uniquement à l'arrivée des véligères. La ZTE semble assez productive pour supporter ces envahisseurs et malgré des changements mineurs dans le spectre en taille des protistes, la communauté microbienne est demeurée dominée par les autotrophes, particulièrement le picophytoplancton et le nanophytoplancton. De plus, les concentrations de cellules microbiennes sont semblables à celles obtenues avant l'invasion. Le broutage du zooplancton, la salinité et la rétention hydrodynamique constituent les facteurs environnementaux responsables de la structure et de la composition de cette communauté microbienne. Selon le concept lotique de continuum longitudinal des caractéristiques biotiques et abiotiques des rivières, la ZTE joue un rôle de discontinuité vu les changements abrupts observés dans la structure de la communauté microbienne et des variables environnementales. En ce qui concerne les véligères, leur distribution horizontale était limitée par la salinité avec une diminution abrupte de leur abondance à 2 ‰. L'observation d'un grand nombre de leurs proies enveloppées dans des agrégats pourrait impliquer une interférence mécanique au niveau de la taille et de la biodisponibilité pour les véligères. En effet, un déclin simultané de la biodisponibilité de leurs proies à des salinités >2 ‰ fut observé; cette chute de biodisponibilité pourrait constituer un stress additionnel à l'effet de la salinité. Leur distribution était homogène dans toute la colonne d'eau, même lors de la présence d'un gradient vertical de densité. Une analyse de redondance a révélé que les densités de véligères étaient positivement corrélées à la température et à la turbidité, mais négativement corrélées à la salinité et au phosphore total. Les densités de véligères sont positivement corrélées à leurs proies, i.e., à la biomasse de chlorophylle *a*, au picophytoplancton et au nanoplancton, impliquant qu'elles ne semblent pas avoir d'impacts sévères sur leurs proies planctoniques. Les véligères étaient positivement corrélées au ratio sestonique du phosphore particulaire au phosphore total, ce qui indique un lien direct avec une nourriture de bonne qualité alimentaire. Les véligères semblent donc n'avoir aucun impact négatif sur l'ensemble de la communauté planctonique de la ZTE. Dans la portion amont de la ZTE, leur

distribution est restreinte aux conditions favorables à leur survie i.e. des eaux de faible salinité et un seston de bonne qualité alimentaire. Nous avons établi au moyen des isotopes stables de carbone et d'azote ($\delta^{13}C$ et $\delta^{15}N$) la structure trophique du réseau alimentaire de la ZTE et le rôle trophique des véligères au sein de ce réseau. Les valeurs de $\delta^{13}C$ variaient de -31.2 ‰ (seston) à -16.1 ‰ (poisson adulte) et celles de $\delta^{15}N$ variaient de 2.6 ‰ à 17.4 ‰. Le réseau trophique semble être largement supporté par la matière autochtone (production primaire *in situ*) plutôt que par la matière allochtone (d'origine terrestre), malgré les importantes quantités de subsides terrestres présentes dans le système. L'examen des relations trophiques démontre que les véligères utilisent des sources de carbone semblables à celles qu'utilisent d'autres consommateurs primaires tels que les cladocères et les copépodes, mais dans des proportions différentes. Les valeurs isotopiques du poisson n'indiquent pas une contribution significative des véligères à leur diète alimentaire. Les valeurs de $\delta^{13}C$ indiquent que les véligères s'alimentent de bactéries libres, de carbone organique dissous (COD) et d'algues d'eau douce incubées *in situ*. Afin de tester la possibilité d'une assimilation de COD par les véligères, nous les avons exposées aux lysats d'algues marquées au ^{14}C. Nous avons démontré qu'elles peuvent assimiler rapidement le COD et incorporer ce carbone dans leur biomasse à un taux équivalent à 6% de leur poids sec en tissus mou par heure. Les véligères ont donc la capacité d'utiliser directement le COD autochtone comme source alternative dans leur alimentation sans avoir recours au réseau alimentaire microbien. Elles occupent ainsi une position trophique unique dans le réseau alimentaire de la ZTE, leur permettant de minimiser les interactions directes avec d'autres composantes du réseau trophique. Cette position trophique unique pourrait expliquer l'absence d'impacts sévères des véligères sur la communauté planctonique.

Abstract

The St. Lawrence estuarine transition zone (ETZ) is a productive ecosystem supporting a larval fish nursery. Since 1994, zebra mussels (*Dreissena polymorpha*) have invaded this region and their larval veligers have become the dominant zooplankton during the summer (up to 260 individuals L^{-1}). Their seasonal dominance of the zooplankton community caused much concern about their potential impacts on the ETZ food web. Much information is available on the impacts of adults on aquatic food webs; however there is a paucity of information on their larval veliger stage, particularly concerning their distribution, the carbon sources they use, their impacts on food webs and their capacity to use dissolved organic carbon (DOC) as adults have been shown to do. Consequently, the overarching objectives of this research were to determine: 1) their impacts on the structure of the pelagic microbial community through their dominance and grazing pressure; 2) the role the ETZ plays in the context of a large river system; 3) the environmental factors controlling the distribution of veligers across the ETZ (freshwater to saltwater); 4) the carbon sources assimilated by the veligers *in situ*; 5) the trophic role they play in the ETZ food web; and 6) the veligers' capacity to directly assimilate DOC and use this carbon source in the ETZ.

The microbial variations observed in the microbial community structure cannot be ascribed to the veliger invasion. The ETZ appears productive enough to support these dominant invaders and despite minor changes in the biovolume size spectrum, the microbial community remains dominated by autotrophs, in the picoplankton and nanoplankton size classes, and the cell concentrations of microbial components have remained similar to pre-invasion values. Grazing by the abundant ETZ zooplankton, salinity, and hydrodynamic retention are the environmental factors controlling the structure and composition of the microbial community from freshwater to saltwater across the transition zones. According to the traditional view of rivers as longitudinal continua, the ETZ inserts itself as a discontinuous segment within the St. Lawrence River corridor.

The sharp gradients in the environmental variables incur abrupt changes in the structure of the planktonic community. The veligers' longitudinal distribution was limited by salinity, with maximum decreases in concentration at 2 ‰. A sharp decline in prey availability at > 2 ‰ may be a secondary stressor for the veligers, in addition to the direct effects of salinity. Their vertical distribution was homogeneous throughout the water column, even in the presence of a pycnocline. Redundancy analysis revealed that veliger concentrations were positively correlated with temperature and turbidity and negatively correlated with salinity and total phosphorus. Veligers were also positively correlated with chlorophyll *a* and picophytoplankton concentrations, suggesting little effect on their phytoplankton prey. Moreover, the veligers were positively correlated with the sestonic ratio of particulate to total phosphorus, indicating their positive association with good food

quality. The veligers appear to have no severe negative impacts on the ETZ plankton community and are restricted to favourable conditions (high seston quality) for their survival in the upstream, low salinity region of the ETZ.

We evaluated by stable isotope analysis the trophic structure of the ETZ food web and the trophic role played by the veligers. $\delta^{13}C$ ranged from -31.2 ‰ (seston) to -16.1 ‰ (adult fish) and $\delta^{15}N$ ranged from 2.6 ‰ to 17.9 ‰. Isotopic analysis of samples indicated that the overall food web was largely supported by autochthonous phytoplankton rather than by allochthonous terrestrial carbon. Large differences among the isotopic signals of veligers, cladocerans and copepods suggested the use of different proportions of food items, and the isotopic values of fish larvae indicated no significant assimilation of veligers. The $\delta^{13}C$ signature of the veligers was in a range consistent with feeding on free-living bacteria and DOC or both, and freshwater algae incubated *in situ*. To investigate the possibility of DOC uptake by the veligers, veligers were incubated with ^{14}C-labelled algal lysates. There was rapid uptake of DOC and incorporation into biomass, equivalent to 6% of the soft tissue dry weight per hour. Zebra mussel veligers are likely using autochthonous DOC as an alternate food source, and they occupy an exotic trophic position in which there is little direct interaction with other major components of the ETZ food web.

Table des matières

Liste des tableaux

Chapitre 1

Chapitre 2

Chapitre 3

Chapitre 3a. Web Appendix

Liste des figures

Chapitre 1

Chapitre 2

Chapitre 3a. Web Appendix

Introduction générale

Les rivières ont longtemps été perçues comme un continuum longitudinal de caractéristiques physiques, chimiques et biologiques, selon le célèbre concept lotique *River Continuum Concept* (RCC; Vannote et al. 1980). Depuis ce concept, plusieurs auteurs ont questionné l'idée d'un continuum lorsque des discontinuités furent documentées le long des cours d'eau. Ces discontinuités peuvent être causées par plusieurs facteurs, tels la présence de barrages (Ward et Stanford 1983), des apports latéraux de matière organique découlant de plaines inondables (Junk et al. 1989) et des apports verticaux provenant de sédiments apportés par les tributaires qui modifient ainsi l'habitat physique à leurs embouchures (Rice et al. 2001). Depuis le RCC, plusieurs concepts qui introduisent les discontinuités à l'intérieur de systèmes fluviaux ont été élaborés, tels le *Serial Discontinuity Concept* (Ward et Stanford 1983), le *Flood Pulse Concept* (Junk et al. 1989), le *Riverine Productivity Model* (Thorp et Delong 1994; 2002), le *Catchment Hierarchy* (Townsend 1996) et le *Riverine Ecosystem Synthesis* (Thorp et al. 2006).

À notre connaissance, aucun concept lotique n'a encore intégré le rôle que joue l'interface eau douce / eau salée dans les modèles écosystémiques lotiques. Les modèles actuels se sont limités à l'eau douce et la littérature a documenté entre autre le rôle des barrages, des tributaires, des plaines inondables, des sédiments mais le rôle des zones de transition estuarienne (ZTE) n'a pas été abordé. Les études limnologiques étendent rarement leurs zones d'études jusqu'aux eaux saumâtres tandis que les études océanographiques inclut les eaux de faibles salinité mais considèrent rarement les secteurs d'eau douce. Selon nous, il est primordial de reconnaître que les ZTEs sont les extensions des grandes rivières et qu'elles servent de portail à la mer en régissant l'entrée et la sortie de matière organique et inorganique. Nous proposons que les ZTEs soient introduites dans les modèles lotiques vu qu'elles représentent des parties intégrales des corridors fluviaux et qu'elles jouent un rôle critique dans la connectivité entre les rivières et la mer.

L'estuaire moyen du fleuve Saint-Laurent est une zone où les eaux douces drainées du continent par le fleuve se mélangent aux eaux salées en provenance de l'océan. La ZTE du Saint-Laurent se situe entre l'Île d'Orléans et l'Île-aux-Coudres. Elle possède une hydrographie complexe se caractérisant par une salinité variant entre 0.1 et 10 psu (practical salinity units) et des propriétés physiques propres aux estuaires, telles que des patrons de recirculation, des zones de rétention de sédiments ainsi que des alternances de stratification et de mélange associées aux cycles de marées (Vincent et Dodson 1999). Le fort débit d'eau douce provenant de la section fluviale y rencontre le courant d'eau salée créant alors une advection verticale de particules organiques et inorganiques. Cette advection est responsable des fortes turbidités et de la formation d'agrégats qui caractérisent cette zone (Kranck 1979; Vincent et Dodson 1999). La ZTE est également habitée par de forts gradients biologiques et trophiques.

En plus de la resuspension verticale des particules organiques et inorganiques, l'advection amène une recirculation des eaux responsable de la rétention et de la forte abondance de plancton dans cette zone (Pace et al. 1992; Laprise et Dodson 1994; Frenette et al. 1995). Aussi y retrouve t-on une accumulation de cellules de grande taille et une communauté plus diversifiée que dans les zones adjacentes (Lovejoy et al. 1993; Frenette et al. 1995). La ZTE est d'ailleurs reconnue pour sa grande productivité et la présence de stocks importants de bactéries et de fortes biomasses de zooplancton et de phytoplancton (Painchaud et Therriault 1989; Lovejoy et al. 1993; Vincent et al. 1996). Elle est par exemple le site privilégié d'alimentation des larves d'espèces de poissons exploitées pour la pêche commerciale et sportive tels que l'éperlan arc-en-ciel (*Osmerus mordax*) et le poulamon Atlantique (*Microgadus tomcod*) (Laprise et Dodson 1989).

Au cour des deux dernières décennies, l'espèce exotique la plus remarquable a se répandre dans l'écosystème du fleuve Saint-Laurent est la moule zébrée, *Dreissena polymorpha*, qui a été introduite dans la région des Grands Lacs à la fin des années 1980s. La moule zébrée s'est aussi répandue très rapidement dans de nombreux cours

d'eau d'Amérique du Nord depuis son introduction (Hebert et al. 1989; Mellina et Rasmussen 1994; Costan et de Lafontaine 2000).

Cette espèce exotique est versatile et peut coloniser une grande variété d'habitats. Dans certaines régions, les adultes *Dreissena* ont atteint des densités s'élevant jusqu'à 175 000 individus par m² par année en l'espace de quelques années (Mellina et Rasmussen 1994). Compte tenu qu'une femelle peut relâcher plus d'un million d'œufs lors d'une période de reproduction, la colonne d'eau peut être véritablement envahie de gamètes de moule zébrée. Au plan écologique, l'impact des adultes est désastreux, pouvant modifier en profondeur la structure et le fonctionnement des écosystèmes. Compétitrice redoutable pour l'habitat et la nourriture, elle finit par dominer rapidement les espèces indigènes. Par son énorme pouvoir de filtration, elle change la transparence de l'eau, modifiant ainsi l'habitat physique (Ricciardi et al. 1995; Ricciardi et al. 1998). Par son alimentation, elle peut engendrer une diminution du phytoplancton, des flagellés et autre zooplancton (Holland 1993; Caraco et al. 1997; Findlay et al. 1998; Pace et al. 1998). Cette moule contamine également la chaîne alimentaire en accumulant des contaminants chimiques (e.g. métaux, BPC, HAP et composés organo-métalliques) qui se répandent dans tout le réseau trophique (de Kock et Bowmer 1993; Costan et de Lafontaine 2000). Compte tenu des impacts écologiques associés à l'invasion de la moule zébrée, l'identification de facteurs qui contrôlent sa distribution est primordiale.

Vu sa localisation à l'extrémité du corridor fluvial du fleuve Saint-Laurent, la ZTE est susceptible à toutes perturbations en amont, telles contaminants, matières organique et inorganique et introduction d'espèces exotiques. Aucune larve pélagique de moule zébrée n'a été recensée dans la ZTE lors de croisières effectuées en 1991 et 1992. Mais en 1995, cette situation devait changer dramatiquement puisqu'elles devenaient désormais dominantes dans le zooplancton (Vincent et Dodson 1999; Winkler et al. 2005). Les véligères sont maintenant la composante dominante du zooplancton pendant les mois de juin, juillet et août. Elles représentent entre 52 et

90% des décomptes zooplanctoniques (Winkler et al. 2005). Ces larves sont possiblement retenues et concentrées dans la ZTE par les mêmes processus physiques qui engendrent la forte turbidité et la rétention hydrodynamique (Frenette et al. 1995). Par leur état libre dans la colonne d'eau, les véligères peuvent se disperser à travers les cours d'eau avec une efficacité remarquable et parcourir de grandes distances.

Contrairement au stade adulte, les impacts des véligères sur les communautés planctoniques sont peu connus. Ces larves sont préférentiellement herbivores et se nourrissent de proies mesurant entre 1 et environ 12 µm (Sprung 1993; Bernier 2003). Sprung (1989; 1993) a fait plusieurs essais de culture avec plus de 50 espèces d'algues et de bactéries. Il n'a cependant pas réussi à déterminer la nourriture exacte des larves véligères en raison de problèmes reliés à la taille des espèces utilisées pour les expériences. En laboratoire, il a pu finalement établir que leur régime était principalement constitué d'algues, de bactéries, de cyanobactéries (*Synechococcus* sp.), de flagellées et de détritus organiques mais aucune expérience ne fut réalisée en milieu naturel. Les moules zébrées adultes ont la capacité d'assimiler du carbone organique dissout (COD) et jusqu'à 50% de leurs besoins métaboliques en carbone peuvent être comblés de cette façon (Roditi et al. 2000). Si les véligères *Dreissena* ont cette capacité d'assimiler du COD, elles pourraient être une source importante de carbone pour les niveaux trophiques supérieurs qui, dans les réseaux trophiques herbivores et hétérotrophes habituels, dépendent de la production primaire et/ou de l'activité bactérienne et des bactérivores pour l'assimilation du carbone.

Lors d'expériences en laboratoire, Wright et al. (1996) ont observé une diminution dans la densité des véligères due à la prédation des rotifères. À l'état larvaire, certaines espèces de poissons, telles *Osmerus esperlanus*, *Lucioperca lucioperca*, *Acerina arnua* et *Ruttilus ruttilus*, s'alimentent de véligères et celles-ci semblent être une composante importante de leurs régime alimentaire (Kornobis 1977, Wiktor 1958 tirés de Sprung 1993). Les gaspareaux (*Alosa pseudoharengus*) et les éperlans (*Osmerus mordax*) adultes semblent également prédateurs de ces larves (Mills et al.1995).

Leur invasion massive et leur comportement alimentaire ont le potentiel d'engendrer un changement dans la structure de communauté du réseau alimentaire en raison de leur forte abondance et de leur capacité importante de broutage sur des gammes de taille restreintes. L'altération de la communauté de protistes (phytoplancton et protozoaires) pourrait avoir un effet cascade sur les niveaux trophiques supérieurs. En tant que proies, les véligères pourraient influencer le régime alimentaire des prédateurs de zooplancton du système (e.g. larves de poissons, mysidacés). Dans l'éventualité qu'elles peuvent assimiler le COD, les véligères pourraient, si elles sont consommées, avoir une influence positive sur le transfert de carbone aux niveaux trophiques supérieurs de la ZTE. De plus, l'utilisation de cette source de carbone dissout pourrait diminuer l'impact direct de leur broutage sur la communauté microbienne.

L'invasion de la ZTE par les véligères *Dreissena* a donc généré de l'intérêt ainsi que soulevé plusieurs questions : Est-ce que les ZTEs devraient être intégrées aux modèles lotiques actuels, étant donné leur connectivité immédiate avec les grandes rivières? Est-ce que la structure de la communauté microbienne a été modifiée depuis leur invasion? Quelles variables environnementales influencent la structure de cette communauté de l'eau douce vers l'eau salée? Quelles sont les variables environnementales qui contrôlent la distribution spatiale et temporelle des véligères dans la ZTE? Quels sont les impacts des véligères sur la communauté microbienne? Quelles sources de carbone utilisent les véligères dans leur milieu naturel? Est-ce que les véligères utilisent des sources de carbone alternatives, telles le COD, ce qui leur permet de dominer l'écosystème sans affecter la disponibilité des proies en tant que ressources alimentaires de la ZTE? Afin de répondre aux questions soulevées, les objectifs sont divisés en 3 chapitres.

Le premier chapitre brosse un portrait général de la ZTE et de sa communauté microbienne et aborde les premiers tests de l'impact des véligères sur la structure de cette communauté. La ZTE y est décrite en tant qu'écosystème fluvial et en fonction du rôle qu'elle joue dans un contexte de continuité ou de discontinuité longitudinale.

Le deuxième chapitre examine de plus près les variables environnementales qui contrôlent la distribution spatiale et temporelle des véligères ainsi que leur impacts sur des composantes spécifiques de la communauté microbienne. Le troisième chapitre décrit le rôle trophique des véligères dans le réseau alimentaire de la ZTE ainsi que les sources de carbone qu'elles utilisent *in situ* et évalue l'hypothèse qu'elles assimilent le carbone organique dissout. Une brève introduction de chaque chapitre suit.

Chapitre 1 : Structure de la communauté microbienne dans une zone de transition estuarienne : résilience à l'invasion des véligères de la moule zébrée

Compte tenu de l'impact potentiel exercé par les véligères sur le réseau alimentaire microbien de la ZTE par leur abondance et leur alimentation sélective, le premier chapitre vise à décrire la communauté microbienne depuis cette invasion. Les véligères arrivent en masse dans la colonne d'eau de la ZTE en juin et dominent le zooplancton jusqu'en septembre. À la fin juin en 1991, avant l'invasion, le nanophytoplancton (2-20 μm) était largement responsable de l'augmentation considérable de la concentration en chlorophylle *a* (Chl *a*; Vincent et al. 1994). En mai, le nanophytoplancton contribuait en moyenne à 33% de la Chl *a* totale pour constituer jusqu'à plus de 69% en juin. La prédominance du nanophytoplancton en juin noté par Vincent et al. (1994) semblait coïncider avec l'explosion de larves véligères de moule zébrée dans le plancton depuis 1995, mais il reste à déterminer si cette transition dans le spectre en taille de plancton se maintient toujours suite à l'invasion des véligères. L'échelle de taille de plancton (<20μm) comprend la taille des proies des véligères (1 à environ 12 μm) (Sprung 1993; Bernier 2003) et de plusieurs herbivores zooplanctoniques (Fortier et al. 1994). L'arrivée massive de ces brouteurs dans le plancton a potentiellement changé la forte contribution du nanophytoplancton à la biomasse totale de la Chl *a*. Les protistes (phytoplancton et protozoaires) sont reconnus pour jouer un rôle critique d'intermédiaire dans les chaînes alimentaires aquatiques (Sherr et al. 1988; Lovejoy et al. 1993). Leurs rôles

incluent ceux de producteurs primaires, de brouteurs de bactéries et de picoplancton autotrophe, de proies pour les niveaux trophiques supérieurs (zooplancton) et d'agents de recyclage d'éléments nutritifs (Reid et al. 1991, tiré de Lovejoy et al. 1993). Des expériences sur des cultures d'enrichissement et de broutage ont démontré que les estuaires sont des environnements très riches en protistes.

Rogerson et Laybourn-Parry (1992) ont étudié l'utilisation que font les protistes d'agrégats en milieu estuarien. Les agrégats sont très nombreux dans ces milieux où le seston est dominé par la matière organique et inorganique en suspension. De plus, ces agrégats semblent être des niches importantes d'activités microbiennes et proto-zooplanctoniques (Zimmermann-Timm 2002). Ces auteurs ont démontré qu'une grande variété d'organismes peuvent co-exister sur ces agrégats en exploitant chacun une niche différente. La contribution de ces agrégats au réseau trophique est donc un aspect important à considérer lorsque nous étudions la dynamique des populations estuariennes. Les études de Frenette et al. (1995) et de Lovejoy et al. (1993) sont les seules à décrire les communautés de protistes dans la ZTE du fleuve Saint-Laurent. Toutefois, ces auteurs n'ont pas considéré l'aspect des agrégats dans leurs analyses.

Afin de déterminer l'impact de l'invasion récente sur les micro-organismes, la structure de la communauté de protistes, bactéries et picophytoplancton sera étudiée en fonction de l'abondance, la taille et le rôle fonctionnel des organismes (autotrophe ou hétérotrophe). La structure de communauté sera ensuite comparée à celle décrite par Lovejoy et al. (1993) et Frenette et al. (1995) avant l'invasion des véligères afin d'évaluer si la dominance des véligères dans le plancton a altéré la structure de cette communauté. Une anomalie dans la distribution en taille des proies pourrait suggérer une baisse de leur disponibilité aux niveaux trophiques supérieurs. Si l'observation d'un grand nombre de leurs proies enveloppées dans des agrégats pourrait impliquer une interférence mécanique au niveau de la biodisponibilité pour les véligères. Si une observation de proies sous forme d'agrégats concorde avec une diminution de l'abondance des véligères, la biodisponibilité des proies pourrait être un facteur

additionnel limitant leur abondance dans la ZTE. Ce chapitre a pour but de caractériser la communauté microbienne de la ZTE ainsi que les facteurs physiques, chimiques et biologiques qui contrôlent leur distribution et leur abondance.

Objectifs spécifiques du chapitre 1:

1.) Décrire les caractéristiques biologiques du réseau alimentaire microbien de la ZTE en fonction de la taille, de l'abondance et du groupe fonctionnel des organismes;

2.) Déterminer l'abondance d'agrégats en relation avec les variables biologiques et physiques;

3.) Tester l'hypothèse que les véligères ont occasionné des changements dans cette communauté microbienne;

4.) Décrire brièvement les facteurs environnementaux qui contrôlent la communauté microbienne;

5.) Tester l'hypothèse que les ZTEs devraient être intégrées aux modèles lotiques actuels, étant donné leur connectivité immédiate avec les grandes rivières et leur spécificité en terme d'hydrographie et de productivité.

Ce chapitre est présenté en anglais sous forme d'article co-écrit avec les Pr. Jean-Jacques Frenette et Warwick F. Vincent et le chercheur Paul B. Hamilton. Il sera soumis prochainement.

Chapitre 2 : Envahisseurs planctoniques de la zone de transition estuarienne du fleuve Saint-Laurent: variables environnementales contrôlant la distribution des larves véligères de moule zébrée

Les processus hydrodynamiques de la ZTE imposent des gradients de salinité et de turbidité importants en amplitude et conséquemment induisent des variations dans la structure de la communauté planctonique. Réputées comme espèces dulcicoles, les véligères semblent néanmoins tolérer des salinités allant jusqu'à 4 psu pour une courte période de temps selon la température de l'eau et la période

d'acclimatation (Strayer et Smith 1993; Kilgour et al. 1994; Wright et al. 1996). L'effet physiologique par lequel la salinité contrôle la distribution des véligères dans les estuaires nord américains est peu documenté. La façon par laquelle la turbidité affecte l'alimentation des véligères est aussi méconnue. Dans la ZTE, la turbidité semble être surtout causée par la remise en suspension des sédiments qui cause, avec l'influence de l'eau salée, une floculation de particules organiques et inorganiques (Kranck 1979). Cette floculation emprisonne les petits organismes dans des agrégats, changeant ainsi la taille des proies et par conséquent leur biodisponibilité. Puisque les véligères se nourrissent de proies de petite taille (1 µm à environ 12 µm), les processus de floculation retrouvés dans la ZTE pourraient altérer la biodisponibilité de leurs proies. Dans la ZTE du fleuve Saint-Laurent, l'agrégation de particules peut donc s'ajouter à la salinité et constituer un facteur de contrôle important de la densité de véligères.

Ce chapitre vise à caractériser spécifiquement la distribution spatiale et temporelle des véligères et d'identifier les variables environnementales qui contrôlent leur distribution. L'environnement physique et chimique sera représenté par la salinité, la température, la turbidité, la lumière ainsi que plusieurs éléments nutritifs dissouts et particulaires (carbone, azote et phosphore). La caractérisation des variables associées à la densité de larves véligères permettra d'évaluer si l'environnement favorise leur croissance. Leur relation avec leurs proies potentielles telles la biomasse totale de la Chl *a* et le picophytoplancton sera analysée afin d'évaluer leurs impacts sur ces composantes phytoplanctoniques.

Objectifs spécifiques du chapitre 2:

1.) Caractériser la distribution spatiale et temporelle des véligères dans la ZTE en fonction des variables physiques, chimiques et biologiques du milieu;

2.) Tester l'hypothèse que les variables environnementales contrôlant leur distribution sont la salinité et la turbidité;

3.) Évaluer si la ZTE est un environnement favorable à la croissance des véligères;

4.) Déterminer si les véligères ont un impact sur deux composantes spécifiques (Chl *a* et picophytoplancton) de la communauté microbienne.

Ce chapitre est écrit en anglais sous forme d'article et paraît dans la revue scientifique intitulée *Canadian Journal of Fisheries and Aquatic Sciences*. Il est co-écrit avec les Pr. Jean-Jacques Frenette et Warwick F. Vincent (Barnard et al. 2003).

Chapitre 3 : Rôle trophique des larves véligères de moule zébrée et leur assimilation de carbone organique dissout

Le COD et la matière organique particulaire (POM) jouent des rôles primordiaux dans les rivières caractérisées par des fortes concentrations de détritus (Findlay et al. 1992; Hullar et al. 1996). Dans la ZTE, la concentration de POM augmente approximativement d'un facteur dix de la zone d'eau douce à la zone de transition lors des marées basses et intermédiaires (Barnard et al. 2003). Findlay et al. (1992) ont observé que le COD et la POM sont des composantes fondamentales dans ces réseaux trophiques, supportant de fortes abondances de bactéries. Selon plusieurs études, dans les réseaux trophiques où l'influence du COD et la POM est proéminente, la biomasse bactérienne et les substrats organiques associés constituent la source majeure de carbone, plutôt que la biomasse des producteurs primaires (e.g. Hall et Meyer 1998; Pace et al. 2004).

Des expériences en laboratoire ont démontré que les véligères s'alimentent de protistes, de bactéries et de détritus (Sprung 1993; Wacker et al. 2002); cependant, aucune étude n'a documenté leurs sources de carbone en milieu naturel. De récentes recherches démontrent que les adultes pourraient obtenir entre 10–25% de leurs besoins métaboliques via l'assimilation du COD d'origine fluviale (Baines et al. 2005). Si les véligères *Dreissena* ont aussi cette capacité d'assimiler du COD, cette source de carbone pourrait constituer une source de carbone alternative. Ceci pourrait subséquemment expliquer l'absence d'impacts majeurs de la dominance des véligères sur la communauté planctonique de la ZTE (Barnard et al. 2003 (Chapitre 2); Winkler

et al. 2005). Ce chapitre vise à identifier les sources de carbone utilisées par les véligères dans la ZTE et à déterminer si elles ont la capacité d'assimiler le COD.

Une étude récente des signatures isotopiques de carbone ($\delta^{13}C$) du plancton de la ZTE (comprenant celles des véligères, le seston <5μm et <250μm) a révélé la dominance du processus de l'omnivorie dans le fonctionnement du réseau alimentaire (Martineau 2003). Ce comportement est typique des écosystèmes dans lesquels les consommateurs assimilent de l'énergie à la fois du COD et de la POM et à la fois des consommateurs microbiens (Hall et Meyer 1998). Cependant, lors de cette étude isotopique, la source d'alimentation des véligères n'a pas été clairement identifiée, ni la signature des algues *in situ*. La signature isotopique du seston total est souvent utilisée pour représenter celle des producteurs primaires (e.g. Forsberg et al. 1993). Par contre, la ZTE est un système qui reçoit des subsides importants de matière allochtone (d'origine terrestre) et la signature du seston n'est donc pas représentative de la matière autochtone (production primaire *in situ*), tel que démontré par Martineau et al. (2004). De ce fait, on a voulu identifier la signature de la matière autochtone en analysant la signature isotopique d'algues incubées *in situ* et d'évaluer le rôle que joue cette matière autochtone dans le réseau trophique.

Dans ce chapitre nous avons caractérisé la signature isotopique ($\delta^{13}C$ et $\delta^{15}N$) des proies potentielles des véligères (bactéries libres et en agrégats, seston, algues d'eau douce et estuariennes incubées *in situ* et COD). Le rôle trophique que jouent les véligères au sein de la communauté planctonique sera défini à l'aide d'isotopes stables de carbone et d'azote. Les signatures du COD pourront être comparées à celles des véligères préalablement établies par Martineau (2003). Ces déterminations isotopiques vont permettre d'illustrer les interactions qui caractérisent le réseau alimentaire de la ZTE depuis la base, en abordant les sources de carbone disponibles, jusqu'à leur cheminement vers les niveaux trophiques supérieurs.

Objectifs spécifiques du chapitre 3:

1.) Tester en laboratoire l'hypothèse que les véligères ont la capacité d'utiliser le COD comme source de carbone;

2.) Utiliser les analyses isotopiques pour déterminer si elles utilisent le COD dans la ZTE;

3.) Cerner les sources de carbone assimilées par les véligères dans la ZTE en utilisant une approche basée sur les analyses isotopiques du carbone et de l'azote;

4.) Établir le rôle trophique joué par les véligères dans la ZTE.

Ce chapitre est écrit en anglais sous forme d'article et paraît dans la revue scientifique intitulée *Limnology and Oceanography*. Il est co-écrit avec Christine Martineau, les Pr. Jean-Jacques Frenette, Warwick F. Vincent et Julian J. Dodson (Barnard et al. 2006).

Finalement, il est important de préciser mon apport à cette recherche dans la mesure des publications co-signées. En ce qui concerne les chapitres 1 et 2, l'ensemble des travaux de terrain et d'analyses de laboratoire (à l'exception des analyses d'éléments nutritifs, spécifié dans les articles) ainsi que l'encadrement des assistants impliqués ont relevé de ma seule responsabilité. Paul B. Hamilton m'a donné une formation en microscopie et en taxonomie. Dans le troisième chapitre, Christine Martineau (deuxième auteur) a obtenu dans le cadre de sa maîtrise les signatures isotopiques des véligères et des organismes de niveaux trophique supérieurs. Elle a donc généreusement accepté de partager ses données pour la réalisation de cette étude. J'ai cependant participé pleinement à l'interprétation des résultats et je suis responsable de leur intégration dans l'article. J'ai obtenu la signature des algues incubées *in situ*, des bactéries, du COD, du carbone inorganique dissout et du seston. Les expériences d'assimilation de COD par les véligères ont été entièrement effectuées par moi seule. La rédaction des articles et de la thèse a été

réalisée en étroite collaboration avec mon directeur de thèse, le Pr. Jean-Jacques Frenette, et mon codirecteur, le Pr. Warwick F. Vincent.

Références

Barnard, C., Frenette, J.-J., et Vincent, W.F. 2003. Planktonic invaders of the St. Lawrence estuarine transition zone: environmental factors controlling the distribution of zebra mussel veligers. Can. J. Fish. Aquat. Sci. **60**: 1245–1257.

Barnard, C., Martineau, C., Frenette, J.-J., Dodson, J.J., et Vincent, W.F. 2006. Trophic position of zebra mussel veligers and their use of dissolved organic carbon. Limnol. Oceanogr. **51**: 1473-1484.

Baines, S.B., Fisher, N.S., et Cole, J.J. 2005. Uptake of dissolved organic matter (DOM) and its importance to metabolic requirements of the zebra mussel, *Dreissena polymorpha*. Limnol. Oceanogr. **50**: 36-47.

Bernier, S. 2003. Efficacité et sélectivité alimentaire des larves de la Moule Zébrée : effets potentiels sur le réseau alimentaire de la zone de transition estuarienne du Saint-Laurent. Mémoire MSc, Université Laval, 43 pp.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S., et Fischer, D.T. 1997. Zebra mussel invasion in a large river, turbid river: phytoplankton response to increased grazing. Ecology **78**: 588-602.

Costan, G., et de Lafontaine, Y. 2000. Présence de la moule zébrée dans le Saint-Laurent: à suivre. Environnement Canada, Région du Québec. Conservation de l'environnement, Centre Saint-Laurent. Rapport scientifique et technique, No. de catalogue: En40-591/2000F.

de Kock, W.C. et Bowmer, C.T. 1993. Bioaccumulation, biological effects, and food chain transfer of contaminants in the zebra mussel (*Dreissena polymorpha*). *In* Zebra mussels: biology, impacts and control. *Edited by* T. Nalepa et D. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 503-533.

Findlay, S., Pace, ML., Lints, D., et Howe, K. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. Mar. Ecol. Prog. Ser. **89**: 147-153.

Findlay, S., Pace, M.L., et Fischer, D.T. 1998. Response of heterotrophic planktonic bacteria to the zebra mussel invasion of the tidal freshwater Hudson River. Microb. Ecol. **36**: 131-140.

Forsberg, B.R., Araujo-Lima, C.A.R.M., Martinelli, L.A., Victoria, R.L., et Bonassi, J.A. 1993. Autotrophic carbon sources for fish of the central Amazon. Ecology **74**: 643-652.

Fortier, L., Le Fevre, J., et Legendre, L. 1994. Export of biogenic carbon to fish and to the deep ocean, the role of large planktonic microphages. J. Plankton Res. **16**: 809-839.

Frenette, J.-J., Vincent, W.F., Dodson, J.J., et Lovejoy, C. 1995. Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar. Ecol. Prog. Ser. **120**: 99-110.

Hall, R.O. Jr. et Meyer, J.L. 1998. The trophic significance of bacteria in a detritus-based stream food web. Ecology **79**: 1995-2012.

Hebert, P.D.N., Muncaster, B.W., et Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. **46**: 1587-1591.

Holland, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island Area, Western Lake Erie since the establishment of the zebra mussel. J. Great Lakes Res. **19**: 617-624.

Hullar, M.A., Fry, B., Peterson, B.J., et Wright, R.T. 1996. Microbial utilization of estuarine dissolved organic carbon : a stable isotope tracer approach tested by mass balance. Appl. Environ. Microbiol. **62**: 2489-2493.

Junk, W., Bayley, P., et Sparks, R. 1989. The flood pulse concept in river-floodplain systems. In Proceedings of the international large river symposium. *Edited by* D. Dodge. Can. Spec. Publ. Fish. Aquat. Sci. pp. 110-127.

Kilgour, B., Mackie, G., Baker, M., and Keppel, R. 1994. Effects of salinity on the condition and survival of zebra mussels (*Dreissena polymorpha*) Estuaries **17**: 385-393.

Kranck, K. 1979. Dynamics and distribution of suspended particulate matter in the St. Lawrence estuary. Naturaliste Can. **106**: 163-173.

Laprise, R. et Dodson, J.J. 1989. Ontogeny and importance of tidal vertical migrations in the retention of larval smelt *Osmerus mordax* in a well-mixed estuary. Mar. Ecol. Progr. Ser. **55**: 101-111.

Laprise, R. et Dodson, J.J. 1994. Environmental variability as a factor controlling spatial patterns in distribution and species diversity of zooplankton in the St. Lawrence estuary. Mar. Ecol. Progr. Ser. **107**: 67-81.

Lovejoy, C., Vincent, W.F., Frenette, J.-J., et Dodson, J.J. 1993. Microbial gradients in a turbid estuary: application of a new method for protozoan community analysis. Limnol. Oceanogr. **38**: 1295-1303.

Martineau, C. 2003. Étude isotopique du réseau trophique de la zone de transition eau douce / eau salée de l'estuaire du fleuve Saint-Laurent : implication de l'invasion par la moule zébrée (*Dreissena polymorpha*). Mémoire MSc, Université Laval, 97 pp.

Martineau, C., W.F. Vincent, J.-J. Frenette, et J.J. Dodson. 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnol. Oceanogr. **49**: 1679-1686.

Mellina, E., et Rasmussen, JB. 1994. Occurrence of zebra mussel (*Dreissena polymorpha*) in the intertidal region of the St. Lawrence estuary. J. Freshwater Ecol. **9**: 81-84.

Mills, EL., O'Gorman, R., Roseman, EF., Adams, C., et Owens, RW. 1995. Planktivory by alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) on microcrustacean zooplankton and dreissinid (Bivalvia: Dreissenidae) veligers in southern Lake Ontario. Can. J. Fish. Aquat. Sci. **52**: 925-935.

Pace, M.L., Findlay, S.E.G., et Lints, D. 1992. Zooplankton in advective environments: the Hudson River community and a comparative analysis. Can. J. Fish. Aquat. Sci. **49**: 1060-1069.

Pace, M., Findlay, S., et Fischer, D. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshwater Biol. **39**: 103-116.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van de Bogert, M.C., Bade, D.L., Kritzberg, E.S., et Bastviken, D. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**: 240-243.

Painchaud, J. et Therriault, J.-C. 1989. Relationships between bacteria, phytoplankton and particulate organic carbon in the Upper St. Lawrence estuary. Mar. Ecol. Progr. Ser. **56**: 301-311.

Rice, S., Greenwood, M., et Joyce, C. 2001. Tributaries, sediment sources, and the longitudinal organization of macroinvertebrate fauna along river systems. Can. J. Fish. Aquat. Sci. **58**: 824-840.

Ricciardi, A., F.G. Whoriskey, et J.B. Rasmussen. 1995. Predicting the intensity and impact of *Dreissena* infestation on native unionid bivalves from *Dreissena* field density. Can. J. Fish. Aquat. Sci. **52**: 1449-1461.

Ricciardi, A., Neves, RJ., et Rasmussen, JB. 1998. Impending extinctions of North American freshwater mussels (Unionidae) following the zebra mussel (*Dreissena polymorpha*) invasion. J. Anim. Ecol. **67**: 613-619.

Roditi, H.A., Fisher, N.S., et Sanudo-Wilhelmy, S.A. 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407**: 78-80.

Rogerson, A. et Laybourn-Parry, J. 1992. Aggregate dwelling protozooplankton communities in estuaries. Arch. Hydrobiol. **125**: 411-422.

Sherr, B.F., Sherr, E.B., et Hopkinson, C.S. 1988. Trophic interactions within pelagic microbial communities: indications of feedback regulation of carbon flow. Hydrobiologia **159**: 19-26.

Sprung, M. 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: abundance, growth, mortality, and food demands. Arch. Hydrobiol. **115**: 537-561.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. *In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa et D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. pp. 39-53.

Strayer, D.L., et Smith, L.C. 1993. Distribution of the zebra mussel (*D. polymorpha*) in estuaries and brackish waters. *In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa et D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. pp. 715-725.

Thorp, J.H. et Delong, M.D. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos **70**: 305-308.

Thorp, J.H. et Delong, M.D. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos **96**: 543-550.

Thorp, J.H., Thoms, M.C., et Delong, M.D. 2006. The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Res. Appl. **22**: 123-147.

Townsend, C. 1996. Concepts in river ecology: pattern and process in the catchment hierarchy. Arch. Hydrobiol. Suppl. **113**: 3-21.

Vannote, R., Minshall, G., Cummins, K., Sedell, J., et Cushing, C. 1980. The River Continuum Concept. Can. J. Fish. Aquat. Sci. **37**: 130-137.

Vincent, W.F., Bertrand, N., et Frenette, J.-J. 1994. Photoadaptation to intermittent light across the St. Lawrence estuary freshwater-saltwater transition zone. Mar. Ecol. Prog. Ser. **110**: 283-292.

Vincent, W.F., Dodson, J.J., Bertrand, N., et Frenette, J.-J. 1996. Photosynthetic and bacterial production gradients in a larval fish nursery: the St. Lawrence River transition zone. Mar. Ecol. Prog. Ser. **139**: 227-238.

Vincent, W.F. et Dodson, J.J. 1999. The St. Lawrence River, Canada-USA: the need for an ecosystem-level understanding of large rivers. Jpn. J. Limnol. **60**: 29-50.

Wacker, A., Becher, P., et Elert, E.V. 2002. Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha*. Limnol. Oceanogr. **47**: 1242-1248.

Ward, J. et Stanford, J. 1983. The serial discontinuity concept of lotic ecosystems. In Dynamics of lotic ecosystems. *Edited by* T. Fontaine and S. Bartell. Ann Arbor Science Publishers, Ann Arbor pp. 29-42.

Winkler, G., Dodson, J.J., Bertrand, N., Thivierge, D., et Vincent, W.F. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. Mar. Ecol. Prog. Ser. **251**: 59-73.

Winkler, G., Sirois, P., Johnson, L.E., et Dodson, J.J. 2005. Invasion of an estuarine transition zone by *Dreissena polymorpha* veligers had no detectable effect on zooplankton community structure. Can. J. Fish. Aquat. Sci. **62**: 578-592.

Wright, DA., Setzler-Hamilton, EM., Magee, JA., Kennedy, V.S. et McIninch, S.P. 1996. Effect of salinity and temperature on survival and development of young zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels. Estuaries **19**(3): 619-628.

Zimmermann-Timm, H. 2002. Characteristics, dynamics and importance of aggregates in rivers - an invited review. Internat. Rev. Hydrobiol. **87**: 197-240.

CHAPITRE 1

STRUCTURE DE LA COMMUNAUTÉ MICROBIENNE DANS LA ZONE DE TRANSITION ESTUARIENNE DU FLEUVE SAINT-LAURENT: RÉSILIENCE À L'INVASION DE VÉLIGÈRES DE LA MOULE ZÉBRÉE

MICROBIAL STRUCTURE OF THE ST. LAWRENCE ESTUARINE TRANSITION ZONE: RESILIENCE TO INVASION BY ZEBRA MUSSEL VELIGERS

Résumé

Depuis 1994, les larves véligères de moule zébré, *Dreissena polymorpha*, sont devenues la composante dominante du zooplancton de la zone de transition estuarienne (ZTE) du fleuve Saint-Laurent. Cette zone est reconnue pour sa grande productivité et son hydrographie dynamique. L'invasion par les larves *Dreissena* a le potentiel de modifier la disponibilité des ressources disponibles aux organismes de niveaux trophiques supérieurs en modifiant la structure de la communauté microbienne par l'effet combiné de leur forte abondance et de leur alimentation. Cette étude décrit les variations observées dans la structure de la communauté microbienne depuis l'invasion de *Dreissena* et identifie les variables environnementales qui contrôlent la composition et la structure de cette communauté. De plus, la ZTE et sa communauté microbienne sont examinées dans le contexte d'un concept lotique qui décrit une rivière comme un continuum longitudinal. La ZTE semble suffisamment productive pour supporter ces envahisseurs. Malgré les changements mineurs observés dans le spectre en taille des protistes, la communauté microbienne est demeurée dominée par les autotrophes, particulièrement le picophytoplancton et le nanophytoplancton. Les abondances de cellules sont restées similaires à celles observées avant l'invasion. La structure et la composition de la communauté microbienne subissent des changements abrupts tout au long du gradient eau douce / eau salée et sont attribuables au broutage par le zooplancton, la salinité et la rétention hydrodynamique. La ZTE constitue une interruption majeure dans le continuum longitudinal lotique du fleuve Saint-laurent et rassemble une multitude de sources de matière organique.

Abstract

Since 1994, zebra mussel veligers have become the dominant zooplankton component in the St. Lawrence estuarine transition zone (ETZ), the dynamic frontal region where freshwater grades into saltwater. Given the known feeding preferences of veligers for small particles, the primary objective of this study was to determine whether grazing pressure by these invasive species could have had a major impact on this microbial food web. We analyzed the pelagic microbial community structure in terms of density, size class, functional group (autotrophic vs. heterotrophic), biovolume size spectrum, and its relation with veligers in terms of edible size and abundance. We also evaluated the ETZ and its microbial community across the freshwater-saltwater transition zone in the context of lotic concepts and the longitudinal river continuum. From comparisons with microbial data obtained prior to the veliger invasion of the ETZ, the observed variations cannot be ascribed to the presence of veligers. The ETZ appears sufficiently productive to support this large population of exotic invaders. Despite minor changes in the biovolume size spectrum, the microbial community remains dominated by autotrophs, in the pico- and nanoplankton size classes, and the cell concentrations of microbial components have remained similar to pre-invasion values. The freshwater microbial community structure and composition undergo substantial changes across the ETZ, underscoring the role of this zone as a critical interface and discontinuity in the river corridor.

Keywords: protist, zebra mussel veliger, estuary, microbial community, autotrophs, heterotrophs

1.1. Introduction

Large river ecosystems have been traditionally characterized as continuous, longitudinal physical, chemical and biological gradients from the headwaters to the downstream extent (Vannote et al. 1980; Minshall et al. 1985). In recent years, the continuity of river systems has been questioned as discontinuities have been noted across river segments. Interruptions in river continuum have been attributed to various processes, including water impoundments (Ward and Stanford 1983), lateral inputs from floodplains (Junk et al. 1989), and sediment recruitment from tributaries and related changes in the physical habitat (Rice et al. 2001). The river continuum concept (RCC; Vannote et al. 1980) predicted the biotic succession of organic matter consistent with patterns of upstream loading (e.g. terrestrial and nutrient inputs), transport, utilization and storage of organic matter (OM) along the length of the river. These downstream trophic dynamics were criticized from a lateral floodplain perspective (Junk et al. 1989) and from a perspective of the importance of autochthonous production within river segments (Thorp and Delong 1994; 2002). Concepts have evolved since the RCC and expanded the dimensions of exchanges to lateral, vertical, and temporal, in addition to longitudinal, with continuous and discontinuous patterns along river corridors. These multidimensional exchanges cause discontinuities in the traditional view of a river continuum, as sources and sinks of nutrients and OM may be found throughout a river's course (e.g. Tockner et al. 2000). In this perspective, lotic concepts examining ecosystem functioning which integrate ideas of discontinuities along a river continuum have emerged (Townsend 1996; Thorp et al. 2006).

To our knowledge, no lotic concept to date addresses estuaries, as though large river systems come to an end at the freshwater-saltwater interface. Yet estuaries are gateways to the sea via their exchanges of carbon and sediments and are intimately linked to both the fluvial and the marine realms. The longitudinal component is inherent, but estuaries are also strongly influenced by lateral

(exchanges with riparian zones, shoals and saltwater marshes), vertical (sediment resuspension, water column stratification and mixing), and temporal (seasonal and tidally induced) exchanges (e.g. Silverberg and Sundby 1979; Vincent and Dodson 1999). These multidimensional interactions in turn influence the biotic community. Finally, estuarine transition zones (ETZs) are regulators of the connectivity between the river and the sea, but it has yet to be determined whether they represent an important dimension of river continuity or discontinuity.

The St. Lawrence ETZ is representative of many ETZs with its dynamic nature as a frontal region where the fresh water draining from the continent first mixes with salt water. This ETZ is a biologically productive ecotone hosting organisms from the fluvial, estuarine, and marine sections of the river (Vincent and Dodson 1999 and references therein). Tidal influence and variable salinity produce dramatic changes in abiotic and biotic features, creating strong longitudinal gradients in chemical and physical characteristics including salinity, nutrient concentrations, and underwater light. These changes in the chemical and physical environment induce changes in the plankton community. For instance, the bacterial population shifts from planktonic to particle-attached (Painchaud et al. 1995; Vincent et al. 1996), changes are observed in the size distribution of the protist community (Frenette et al. 1995), and overall food web structure also changes substantially (Winkler et al. 2005 and references therein). Strong trophic coupling has also been described where grazing pressures play a pivotal role in controlling the relative proportion of autotrophs (with chlorophyll *a*) to heterotrophs (without chlorophyll *a*) in the plankton community across the freshwater to saltwater transition (Frenette et al. 1995; Winkler et al. 2003).

The St. Lawrence ETZ has recently been invaded by the veliger larval stage of zebra mussels, *Dreissena polymorpha* (Vincent and Dodson 1999; Barnard et al. 2003; Winkler et al. 2005). Veligers are the dominant zooplankton component during the summer, constituting between 52 and 90% of the total zooplankton counts (Winkler et al. 2005). These larvae are likely retained and concentrated in the ETZ by

the same physical mechanisms that cause the high turbidity and hydrodynamic retention of large particles (Frenette et al. 1995). Zebra mussel veligers are primarily herbivores with a preference for PUFA-rich algae but also consume picophytoplankton (0.2–2 μm), bacteria, flagellates and detritus (Sprung 1989, 1993; Vanderploeg et al. 1996; Wright et al. 1996). However, there are conflicting results on the veligers' capacity to feed selectively (Vanderploeg et al. 1996; Bernier 2003). According to these authors, the size spectrum of their prey varies between 1 and approximately 12 μm (for the D-stage veligers). Under favourable conditions, a veliger can increase roughly ten times its weight during its 1 to 3 week planktonic period (Sprung 1993). The impact of adult zebra mussels on the plankton through their filter feeding is well documented (Caraco et al. 1997; Findlay et al. 1998; Holland 1993; Pace et al. 1998); however, the impacts of the veligers' feeding on the microzooplankton remains poorly documented (cf. Barnard et al. 2003). The massive invasion may change the microbial community structure through its high abundance and its grazing activity and consequently affect food web dynamics.

Prior to the zebra mussel veliger invasion, the microbial community was comprised largely of autotrophs across the freshwater to the transition zone (Frenette et al. 1995) despite the ETZ's net metabolic state of heterotrophy where respiration exceeds production (Painchaud and Therriault 1989). Across the zones, autotrophic protist density dominated the microbial community, comprising between 51 and 78% of the community (Frenette et al. 1995). Vincent et al. (1994) found that nanophytoplankton (2-20 μm) contributed to more than 69% of the chlorophyll *a* concentrations in summer (June). This prevalence of autotrophs and the nanophytoplankton size fraction may no longer be as important since these correspond to the veligers' preferential prey items. Veliger grazing could also potentially change the importance of the nanophytoplankton's contribution to the total Chl *a* biomass. With the selective feeding of veligers, the proportion of autotrophs may have changed.

The seasonal omnipresence of veligers, their retention in the ETZ, and the grazing pressure they may exert led us to hypothesize that the invasion of this planktonic life cycle stage has resulted in significant modification of the ETZ microbial food web structure. The objectives of this research were therefore to define the microbial community structure of the St. Lawrence ETZ and to evaluate any changes in relation to earlier studies (e.g., Frenette et al. 1995). More specifically, the microbial community was examined in terms of: 1) the distribution of organisms according to their density, size class and functional group (autotrophic vs. heterotrophic) from the downstream freshwater reach of the river into the transition zone; 2) the biovolume size spectrum; and 3) its relation to veligers in terms of edible size and abundance. The biotic and abiotic factors controlling the composition of the microbial community are also discussed in the context of large river ecosystem continuity, or discontinuity, from the fluvial section to the transition zone.

1.2. Materials and Methods

1.2.1. Study site

The ETZ of the St. Lawrence River is situated approximately 50 km downstream from Québec City (Canada) between the islands of Île d'Orléans and Île-aux-Coudres (Figure 1.1). These waters are characterized by salinities ranging from 0.1 and 10 ‰. The freshwater discharge averages 10 000 $m^3 s^{-1}$, and current speeds can reach > 2.5 m s^{-1} (D'Anglejan and Smith 1973). The bottom morphology of the ETZ consists of three channels, one following the north shore, one short channel in the middle, and one following the south shore. Large mudflats are found on the south shore near Montmagny, while vast intertidal marshes (3 x 10^6 m^2) are located on the shores of the north channel at Cap Tourmente (Lucotte and d'Anglejan 1986).

In summer 2001, two cruises were undertaken during maximal veliger abundance (Winkler et al. 2005) to assess veliger and microbial community dynamics at fixed stations during different tidal states. Both cruises occurred during the spring

tides of June and July. Sampling was conducted at fixed stations over the period of 3 to 4 days. One fixed station was located in the freshwater but tidal zone (46° 52' 86" N, 70° 55' 60" W) and the other directly in the transition zone (47° 06' 71" N, 70° 42' 44" W) (Figure 1.1). A total of six high, six low and eight intermediate tides were sampled at each site during both cruises. For the microplankton community and chemical variables, water samples were collected using a 5 L Glo-Flo bottle at the surface (0-2 m) and at the bottom (1-2 m from the bottom).

1.2.2. Chemical and physical variables

For total dissolved nitrogen, the filtrate from samples filtered through pre-combusted GF/F filters was stored cool and in the dark until analysis. To obtain total nitrogen (TN), particulate organic nitrogen was added to the total dissolved nitrogen. For total phosphorus (TP), unfiltered water was acidified (1 mL 30% H_2SO_4 per 100 mL sample) and stored cool until analysis. Particulate organic carbon (POC) and nitrogen (PON) were obtained by filtering 20 - 100 mL (depending on turbidity) of water through pre-combusted and acid-washed GF/F filters. These were then stored frozen (–20 °C) in the dark until analysis. The chemical analyses were conducted at the National Laboratory for Environmental Testing in Burlington (Ontario) according to standard methods (National Laboratory for Environmental Testing 1994). Colorimetric and autoclave digestion methods were used for total dissolved nitrogen and TP; stannous chloride was also used for TP. PON and POC were determined with the use of a CHN analyser.

Water column salinity and temperature profiles were recorded using a CTD (Conductivity-Temperature-Depth meter, Sea logger SBE-19) which was lowered to 2 m from the bottom. To compensate for missing values of turbidity which were recorded using an HF Scientific Inc. nephelometer, model DRT15-CE in Nephelometric Turbidity Units (NTU), turbidity was also assessed by dry weight analysis of the total seston filtered onto preweighed GF/F glass fibre filters. Seston was filtered onto these filters which were then dried at 60°C for 48h. Filters were weighed for total (inorganic and organic) seston content. The filters were placed in an

oven at 450 °C for 12h to burn the organic content and obtain weight of the inorganic content. The organic content was calculated by subtracting the inorganic content from the total content. Correlation analysis between the log of NTU measurements and the log total seston concentrations was performed to validate the use of seston as a measure of turbidity ($R^2 = 0.96$, $n = 72$, $p < 0.0001$).

For the June cruise, a spectroradiometer (Model PUV-500, biospherical instruments, San Diego, USA) was used to measure the cosine-corrected downwelling underwater irradiance (E_d) at 320 nm (UVB), 340 nm (UVA) and cosine-corrected photosynthetically active radiation (PAR 400-700 nm). Data were corrected by subtracting the "dark irradiance" values (obtained when the instrument was fitted with a light-tight neoprene cap at *in situ* temperatures) from the $E_d(\lambda)$ readings. Diffuse vertical attenuation coefficients (K_d) were calculated by linear regression of the natural logarithm of E_d versus depth. The depth to which 1% of subsurface irradiance penetrated ($Z_{1\%}$) was calculated as 4.605 K_d^{-1} (Kirk 1994).

1.2.3. Veligers

The veliger densities at the times of sampling have been previously published (Barnard et al. 2003). In brief, the veligers were collected with two 5 L Go-Flo bottles. These bottles collected water at the surface (0 - 2 m), mid-column (depending on the column depth) and near the bottom (1 - 2 m from the bottom). The water was then filtered through a 63 μm screen to eliminate smaller particles and then preserved in denatured ethanol (95%) at a final concentration of approximately 80%. Veliger counts were conducted in the laboratory using cross-polarisation according to Johnson (1995). This method renders the veligers visible and easily distinguishable from other zooplankton and detritus. The volume of the counts had to correspond to a minimum of 10% of the total sample volume.

1.2.4. Chlorophyll *a*

Upon arrival at the laboratory, the water collected with the Go-Flo bottles was passed through GF/F Whatman 25 mm filters in duplicate. These filters were then

frozen (-20 °C) and kept in the dark until extraction. Samples were extracted in hot ethanol (95%), measured by fluorometry (Sequoia-Turner, Model 450) following Nush (1980) with calculations from Jeffrey and Welschmeyer (1997). Chlorophyll *a* (Chl *a*) from *Anacystis nidulans* (Sigma-Aldrich) was used for calibration.

1.2.5. Picophytoplankton (0.2-2 µm)

In a darkened room, immediately upon arrival in the laboratory, approximately 15-20 mL of water sample were filtered onto Anodisc Whatman (0.2 µm) filters which were mounted on slides using Aquapolymount (Polysciences). These slides were immediately stored in the dark at 4 °C for 24 h then stored frozen (–20 °C). Within days of the slide preparation, counting was undertaken with an Olympus epi-fluorescence microscope with exchangeable blue and green excitation filters and 1000 x oil immersion. To convert cell counts to total picophytoplankton cell volume, a value of 1.3 μm^3 was used, which was an average of 840 measurements of cell size across the freshwater and transition zones of the St. Lawrence River (Lovejoy et al. 1993). Three hundred individuals were counted with a minimum of 15 fields. When counts were low a minimum of 50 fields was counted.

1.2.6. Bacteria

Samples were immediately fixed with 2 % formaldehyde (1 mL 37% formaldehyde in 20 mL water) and kept in the dark at 4 °C. Samples were stained with the fluorochrome 4′-6-diamidino-2-phenylindole (DAPI) and filtered onto Whatman Anodisc filters (0.2 µm). Five hundred individuals were counted with a minimum of 15 fields (Kepner and Pratt 1994). A minimum of 50 fields was counted when counts were low. To convert counts to total biovolume, the densities were multiplied by a cell biovolume of 0.048 μm^3 per cell, a value obtained in a previous study of bacteria from the freshwater and transition zones of the St. Lawrence River (Lovejoy et al. 1993).

1.2.7. Protist community (3-150 μm)

Samples for protists (phytoplankton and protozoa) were prepared and examined using the procedures outlined by Lovejoy et al. (1993). Not all samples collected could be counted due to the lengthy time of analysis required for each sample because of the high concentrations of detritus. Two surface and 2 bottom samples were counted in the freshwater zone and 6 surface and 4 bottom samples in the transition zone for June and July cruises. These samples were fixed immediately with 1% gluteraldehyde and 0.1% paraformaldehyde (final concentrations) and stored in the dark at 4 °C. For sedimentation, only 1 to 3 mL of the sample was settled for transition zone samples in Utermöhl chambers due to the high concentrations of detritus. For the freshwater samples, between 7 and 15 mL was settled. The small protists (3-50 μm) were counted at magnification 1000x with a minimum of 100 fields and duplicate subsamples were counted when counts were low. For organisms >50 μm, the entire chamber was scanned at 100x. Natural and fluorochrome DAPI (final concentration 0.1 μg mL^{-1}) fluorescence allowed us to differentiate organisms from abiotic particles and to locate and characterize cells. Counting was undertaken with a Nikon Diaphot 300 epi-fluorescent microscope with exchangeable blue and green excitation filters and 1000x oil immersion. The organisms were grouped into two broad categories: autotrophs (containing photosynthetic pigments excited with the green filter, including mixotrophs) and heterotrophs (absence of photosynthetic pigments, DAPI-stained nuclei excited with the blue filter). All cells were measured, in addition to aggregate-dwelling organisms in which case the total size of the aggregate was also measured in order to obtain the edible size. To obtain the density of aggregates per sample, the number of aggregates within each field was counted.

Total biovolume of organisms was calculated from the microscopic determination of the mean length and width of all organisms counted from each sample. The shapes and the ensuing morphological volumes were determined using calculations from Hillebrand et al. (1999). The biovolume was then partitioned according to the following size classes ≤ 3, $3 <$ to ≤ 7.5, $7.5 <$ to ≤ 12, $12 <$ to ≤ 17.5,

17.5 < to ≤ 25, and > 25 μm which were defined by the maximal linear length of a given organism and expressed as biovolume size spectrum. These size classes were chosen to compare with data shown in Frenette et al. (1995). Phytoplankton were counted with the help of Algamica (Gosselain and Hamilton 2000), a computerized counting program for algae and other microscopic bodies. A MANOVA (SAS version 9.1) was conducted to compare the variation in the distribution of the percent total biovolume in each size class between the following factors: cruise (June vs. July), depth (surface vs. bottom) and zone (freshwater vs. transition).

A three-way ANOVA (SAS version 9.1) was conducted to assess the effects of cruise, depth and zone (as above) on the density of various size groups of autotrophs and heterotrophs (3-12, 12-25, and >25 μm). The following interactions were used in the model: zone x cruise, zone x depth, and depth x cruise. All densities were log transformed to meet the Shapiro-Wilk test of normality and residuals had homogeneous variance. Normality was not met for the 12-25 μm autotrophs due to the presence of outliers. These outliers were removed and the test revealed no significant effects of the variables on the densities. The same results were observed when the outliers were included; hence we chose to rely on the values from the test containing the outliers since the outliers were zero values obtained from the counts.

1.2.8. Relationships within the microbial community and with veligers

Given that no significant differences in abundance were observed between June and July for all protist size groups (see below), protist data for the two cruises were pooled into two groups depicted by the freshwater zone (n=8) and transition zone (n=20). Seeing as all chemical and physical variables had little variability in the freshwater zone and high variability in the transition zone (Figure 1.2), it was appropriate to separate the variables by zone for the analysis. Correlations between the densities of autotrophs and heterotrophs (size groups 3-12, 12-25, and >25 μm), picophytoplankton, free and aggregate bacteria, veligers, Chl *a* concentration, salinity, temperature, TN, TP, and seston concentrations were analyzed using nonparametric Spearman correlations.

1.3. Results

1.3.1. Physical and chemical data

Turbidity, as indicated by the seston concentrations, varied markedly in the transition zone (Figure 1.2). Inorganic matter accounted for much of the turbidity in the transition zone. For example, the mean ratios (± SD) of inorganic to organic matter at low tide were of 6.3 (2.2) and 12.6 (6.8) for June and July respectively. Salinity in the transition zone increased from low to high tide, whereas seston was highest during low tides, decreasing with increasing salinity. These observations are consistent with previous reports of the St. Lawrence ETZ (Kranck 1979; Vincent et al. 1994). Temperature increased slightly between the two cruises, but this increase was not significant (ANOVA, $n = 30$, $F = 0.95$, $p > 0.05$). Temperature and salinity were strongly negatively correlated (Pearson $r = -0.97$, $n = 30$).

The underwater light climate varied markedly between zones. The K_d values as well as the depth to which 1% of the surface irradiance penetrated ($Z_{1\%}$) differed substantially (Figure 1.3), consistent with the observed turbidity. The strongest light attenuation was consistent with the highest seston concentrations (Figure 1.2), observed during the low, ebb and flood tides of the transition zone. The euphotic depth (1% PAR) extended to 4.5 m in the freshwater zone but decreased to 0.5 m in the transition zone during low, ebb and flood tides, approximately a 90% decrease in light penetration. In the transition zone, less than 2.7% of the water column was in the euphotic zone during low, ebbing and flooding tides. As with PAR, 1% penetration depth decreased by 83% for UVA and UVB from the freshwater zone to the transition zone.

TN and TP concentrations varied little in the freshwater zone but were highest in the transition zone during the low and intermediate tides and decreased with increasing salinity (Figure 1.2). TP increased approximately 15 fold across the freshwater to the transition zone while TN increased by less than 3 fold. For the POC to PON ratios, expressed as C:N, the values obtained in the freshwater zone imply a seston without a nitrogen deficiency, i.e., values were below the estimated deficiency

threshold (see Barnard et al. 2003) (Figure 1.4). For the transition zone however, mean values were just below the critical threshold of deficiency (~15) and at intermediate tides the values for June represented a deficiency in nitrogen.

1.3.2. Chl *a*

In June, values for Chl *a* ranged between 0.72 and 21.85 μg L^{-1} with means (± SD) of 1.8 (1.6) and 5.1 (5.6) μg L^{-1} for the freshwater and transition zones respectively. In July, the means (± SD) were 1.9 (1.2) and 3.8 (2.6) μg L^{-1} for the freshwater and transition zones, respectively. The highest values were observed in the transition zone at low tide and then decreased with increasing salinity (Figures 1.2, 1.5). There was high variability in the Chl *a* concentrations in both zones but most markedly in the transition zone. The coefficient of variation (CV) averaged 65% with a peak of 74% at low tide in the transition zone, compared to 43% in the freshwater zone. Chlorophyll *a* concentrations did not vary significantly between cruises (Mann-Whitney Rank Sum Test, $T = 2430$, $p = 0.251$).

1.3.3. Picophytoplankton

Picophytoplankton communities are composed of eukaryotic algae and prokaryotic cyanobacteria. The highest concentrations of picophytoplankton were observed in the freshwater zone, with a gradual downstream decrease with increasing salinity (Figure 1.5). In the freshwater zone, cell density averaged (± SD) 7.10 (2.48) x 10^6 cells L^{-1} for June and July cruises, with a decrease to 3.68 (1.71) x 10^6 cells L^{-1} in the transition zone. These values are similar to those previously obtained in the ETZ (Bertrand and Vincent 1994).

1.3.4. Bacteria

Free bacteria decreased from a mean (± SD) of 20.5 (3.4) x 10^8 cells L^{-1} in the freshwater zone to 5.97 (9.21) x 10^8 in the transition zone. Free bacteria were more abundant than aggregate bacteria in the freshwater zone, but this difference reversed itself in the transition zone with aggregate bacteria dominating (Figure 1.5). Bacterial abundances were in the same range as previously observed in the ETZ (Painchaud and Therriault 1989).

1.3.5. Protist community

Autotrophic and heterotrophic proportions - Both the freshwater and the transition zone protist communities were dominated by autotrophs in June and July (Tables 1.1 and 1.2). The heterotrophic proportion did not exceed 29 and 13% in terms of total number and total biovolume respectively, with the exception of a high value of 42.3% for the heterotrophs in the transition zone for July which was marked by abundant ciliates (*Strombidium* sp. and various tintinnids). In total number the community did not shift towards heterotrophy from the freshwater to the transition zone. However, in terms of total biovolume, there was a tendency for the proportion of heterotrophs to increase substantially from the freshwater to the transition zone, with the exception of the July bottom samples where there was a slight decrease in heterotrophic biovolume (Table 1.2).

Biovolume size spectrum - In the freshwater zone, on average (± SE) 79.5 (5.4) % of the protist biovolume was represented by diatoms and this proportion decreased to 62.6 (5.1) % in the transition zone. This proportion was likely underestimated given that many of the small organisms (<10 μm) were not easily identifiable due to the presence of aggregates and detritus, and hence were classified as autotrophic unknowns. Ciliates dominated the heterotrophic biovolume in the transition zone representing 15.9 (4.6) % of the total biovolume but this proportion was lower in the freshwater zone with 5.1 (1.5) %.

For all depths, cruises and zones the >25 μm size group occupied the greatest percentage of the biovolume size spectrum (Figure 1.6). For June, the proportion of biovolume present in the > 25μm fraction decreased from 48 and 53% in the freshwater zone to 29 and 35% in the transition zone, for surface and bottom samples respectively. During July, the opposite was true in that this proportion increased from 54 and 40% in the freshwater zone to 58 and 57% in the transition zone for surface and bottom samples respectively. The >25μm fraction represented a slightly higher proportion of the biovolume at the bottom in June in both zones but the opposite was true in July when the >25 μm fraction represented a higher proportion of the

biovolume at the surface. According to the MANOVA results, the zone was the only variable which had a significant effect on the distribution of the biovolume percentages across the size classes ($n = 28$, $F_{5,17} = 4.87$, $p = 0.006$). Since the distribution of the percentages across the size classes varied significantly according to the zone, further analyses (one-way ANOVAs) were conducted to identify which size class(es) varied with the zone. Only the $3 <$ to ≤ 7.5 µm size class varied significantly from the freshwater to the transition zone ($F = 16.12$, $p = 0.0006$). The percentage of biovolume in this size class was consistently higher in the transition zone (least square means (LSM) = 9.49%) than in the freshwater zone (LSM = 3.49%).

Density of protist groups across the zones - For the density of 3-12 µm autotrophs, zone and depth had significant effects on density (3-way ANOVA, $n = 28$, $F = 33.55$, $p < 0.001$ and $n = 28$, $F = 4.48$, $p = 0.046$ respectively). The density was higher in the transition zone (depths and cruises combined) with a difference in LSM of 3.62×10^6 individuals L^{-1}. There were more individuals in the bottom, with a LSM difference of 1.25×10^6 individuals L^{-1}. For the 3-12 µm heterotrophs, only the zone had a significant effect on the density ($n = 28$, $F = 10.88$, $p = 0.003$). The LSM increased from 0.55 to 1.43×10^6 individuals L^{-1} from the freshwater to the transition zone. The increase for both the autotrophs and heterotrophs in this size group was also visually evident (Figure 1.5). There were no significant effects of depth nor zone on the densities of 12-25 µm autotrophs and heterotrophs, and > 25µm heterotrophs (data not presented). For the >25 µm autotrophic density, a significant effect of the interaction depth x cruise was observed ($n = 28$, $F = 8.73$, $p = 0.008$). A multiple comparisons test (Protected Fisher least significant difference) of the LSM showed that in June, the surface had significantly more individuals L^{-1} ($p = 0.005$) in both zones. For July, there was slightly more cells at the bottom, but the difference was not significant ($p = 0.43$). There was a very sharp, significant increase ($p = 0.01$) in >25 µm autotrophs at the bottom from June to July with the LSM increasing by more than an order of magnitude from 1.2×10^4 to 1.8×10^5 individuals L^{-1}.

1.3.6. Aggregates

The concentration of aggregates increased abruptly from the freshwater to the transition zone (Figure 1.7). Maximum abundance was observed at low tide in the transition zone, with a mean (± SD) density of 5.3 (3.6) x 10^8 aggregates L^{-1} and then decreased downstream with increasing salinity. In the freshwater zone, aggregate densities were lower with a mean of 1.5 (0.9) x 10^7 aggregates L^{-1}. Variability in aggregate densities was high, with CVs of 64 and 66% for the freshwater and transition zones respectively. Aggregate densities did not vary significantly between cruises (Mann-Whitney Rank Sum Test, T = 206, F=, p = 0.91).

Consistent with the increase in aggregate density, an increase in the concentration of aggregate-dwelling protists was also noted (Figure 1.8). Aggregate-dwelling protists comprised about 30% of the freshwater community but up to 80% in the transition zone. Autotrophs were more prone to aggregation than heterotrophs in the transition zone with a mean of 27% ± 12 SD aggregated in the freshwater zone compared to 76% ± 10 SD in the transition zone. Because of this aggregation, the edible size of the organisms increased, and this was most evident for the protists measuring 2-10 µm (data not shown).

1.3.7. Veligers

Veliger densities did not vary significantly between cruises (Mann-Whitney Rank Sum Test, T = 3168, p = 0.72). Maximum abundance was noted in the freshwater zone with peak abundances between 150 and 240 individuals L^{-1}. Abundance decreased abruptly with increasing salinity in the transition zone. Distribution analysis and details on the environmental variables controlling their distribution in the ETZ are documented in Barnard et al. (2003).

1.3.8. Relationships within the microbial community and with veligers

The number of samples varied between zones (see Methods, section 1.2.8.), which caused the statistical criteria to vary as well. The Spearman correlation coefficient *rho* required a value of ≥0.7 to be significant in fresh water, whereas in

the ETZ, a *rho* ≥0.45 was significant (Table 1.3). In the freshwater zone, physical and chemical variables varied little (Figure 1.2). Autotrophs 12-25 μm were positively correlated with Chl *a* whereas in the transition zone, Chl *a* was strongly correlated with autotrophs 3-12 μm and picophytoplankton in addition to autotrophs 12-25 μm. In the transition zone, where physical and chemical variables varied greatly (Figure 1.2), veligers were positively correlated with their potential prey, i.e., picophytoplankton, autotrophs and heterotrophs 3-12 μm and Chl *a* concentrations. Salinity was negatively correlated with all biological variables, except free bacteria and heterotrophs 12-25 and >25 μm. Conversely, the heterotrophic densities 12-25 and >25 μm increased with increasing salinity (Figure 1.5). Aggregate, seston, TN and TP concentrations were highest at low tide in the transition zone (Figure 1.2), and positively correlated with Chl *a*, aggregate bacteria, autotrophs 3-12 and 12-25 μm and heterotrophs 3-12 μm (Table 1.3).

1.4. Discussion

The St. Lawrence River undergoes abrupt changes in its physical and chemical properties upon entering the ETZ. Fundamental determinants of ecosystem functioning such as salinity, light climate, and nutrients change substantially over a short distance (approximately 10 nautical miles; Vincent et al. 1996). In the literature concerning lotic theory development, some factors introducing discontinuities in biotic and biotic river continua are water impoundments (Ward and Stanford 1983), the presence of tributaries (Rice et al. 2001), and floodplain inundation (Junk et al. 1989). ETZs should be incorporated within the concept of lotic ecosystems as they constitute integral parts of large river corridors and play a pivotal role in the connectivity between rivers and the sea. Lotic theory has concentrated on the origin and transformations of OM within river corridors, but the downstream fate should also be addressed by integrating ETZs. The ETZ should be considered a discontinuity

along the St. Lawrence River corridor. In the St. Lawrence ETZ, abrupt gradients induce abrupt changes in the microbial community (this study) and entire pelagic community (e.g. Winkler et al. 2003). The ETZ ecosystem dynamics are therefore inconsistent with the view of a longitudinal river continuum since sources of OM in the ETZ are multidimensional, in addition to the longitudinal inputs from upstream. Subsidies originate from lateral inputs from the adjacent salt marshes (Lucotte and d'Anglejan 1986), high *in situ* primary production (Vincent et al. 1994), vertical resuspension of sediments and OM (e.g. Lovejoy et al. 1993), and downstream longitudinal inputs due to tidal influence. Retention mechanisms which characterise this zone combine the longitudinal and vertical dimensions with upstream-downstream exchanges and vertical sinking and resuspension mechanisms (Frenette et al. 1995). Some of these abrupt changes in the ETZ ecosystem are discussed below.

1.4.1. Total Chl *a*

Chl *a*, all autotrophic densities and nano-heterotrophic densities showed sharp increases at the leading edge of the salinity front (<2 psu) followed by negative correlations with salinity. Salinity could thus be a controlling factor for Chl *a* concentrations in the transition zone with the negative correlation observed. Bernat et al. (1994) attributed the negative correlation between Chl *a* and salinity in the Elbe estuary to the dominance of phytoplankton by riverine species. However, in addition to salinity, the high density of macrozooplankton (Laprise and Dodson 1993) and subsequent grazing losses have accounted for the decline in autotrophic abundance across estuarine transition zones, including the St. Lawrence, in previous studies (Vincent et al. 1996; Jochem 2003; Winkler et al. 2003). Winkler et al. (2003) noted a negative exponential relation between salinity and Chl *a* biomass indicative that the phytoplankton produced *in situ* and advected from the upstream freshwater section provided a major food source for the zooplankton in the low salinity region of the ETZ. These authors also noted that the sharp decline in the Chl *a* biomass occurred at salinity >2 psu, the same salinity range where high zooplankton populations were found. Phytoplankton is known to be a more nutritional food source to zooplankton

than bacteria or detritus (Elser and Urabe 1999; Lehman 2000). ETZ phytoplankton are generally of high nutritional quality (Barnard et al. 2003), and the autotrophic community is dominated by diatoms (Winkler et al. 2005; this study), which are recognized as high quality food sources. Sobczak et al. (2002) noted that a large percentage of Chl *a* from a section of the San Francisco Bay Estuary is contained in cells smaller than 10 μm and generally consist of a high proportion of diatoms and cryptophytes of good nutritional quality, similar to our results. The concomitant increase in the heterotrophic abundance noted in this study lends support to the potential grazing pressures exerted with increasing salinity. Furthermore, Martineau et al. (2004) and Barnard et al. (2006) showed that autochthonous carbon was at the base of the St. Lawrence ETZ food web, indicative that autotrophs are heavily grazed upon and that this carbon is efficiently transferred to higher trophic levels. Similar results have been found in the San Francisco Estuary's Sacramento–San Joaquin River Delta, despite phytoplankton production being a small component of the ecosystem's organic matter mass balance (Sobczak et al. 2002).

With heavy seston loads and high light attenuation, it would be reasonable to assume that light is also a limiting factor for the ETZ autotrophs. Our results point to high autotrophic concentrations across the zones with peaks in abundance occurring during the highest seston concentrations, consistent with periods of highest light attenuation. Vincent et al. (1994) showed that photosynthetic rates per unit Chl *a* remained high across the zones despite the turbidity. This phenomenon was explained by low light penetration being offset by shallow mean mixing depths, and frequent exposure of all cells to bright, near-surface irradiances.

Retention mechanisms could also be responsible for the peaks in Chl *a* abundance observed in the transition zone at salinities similar to those in the freshwater zone where Chl *a* values were lower. These findings are comparable to previous findings in the Seine estuary (Bodineau et al. 1998), in the San Francisco Bay estuarine turbidity maximum (Hollibaugh and Wong 1999), and in the St. Lawrence ETZ (Frenette et al. 1995) with peak Chl *a* concentrations occurring at the

leading edge of the salinity front. Chl *a* maxima at low to mid-salinities have been attributed to active advection leading to packing of phytoplankton at density fronts (Franks 1992) and hydrodynamic retention mechanisms (Frenette et al. 1995).

1.4.2. Distribution of protist community

According to Frenette et al. (1995), most phytoplankton < 5μm are likely to be flushed through the system and exported downstream, without being retained whereas the bigger phytoplankton cells are likely to be retained by sinking, accumulation and resuspension mechanisms. Our statistical analyses revealed that the autotrophic abundance for >25μm cells was significantly higher at the bottom during the July cruise than at the surface compared to the June cruise, which could be evidence of retention. We noted highest mean densities in the abundance of autotrophic protists 3-12 μm in the transition zone at low tide. Franks (1992) found that in addition to large cells, weak swimmers also tended to have high concentrations near the front, where the vertical velocities are the greatest. Jochem (2003) also noted that picophytoplankton and nanoplankton exhibited highest abundances in the high-phytoplankton biomass region at the edge of the salt wedge. These results are similar to ours, where our nanoplankton, both heterotrophic and autotrophic, had highest abundances at low tide in the transition zone, possibly evidence of accumulation just upstream of the salt wedge. Furthermore, considering that 15-40% of the ETZ Chl *a* is contained within the <5 μm fraction (Martineau et al. 2004), it is consistent that peak nanophytoplankton abundances coincide with peak Chl *a* concentrations.

As for the larger autotrophic cells, their size renders them more susceptible to grazing by the abundant macrozooplankton in the transition zone as the grazing threshold for most herbivorous zooplankton is ca. 5 μm (Fortier et al. 1994). This could explain the observed decline in large autotrophic cell numbers (>25 μm) with increasing salinity, consistent with the increase in abundance of several phytoplankton-grazing zooplankton in the ETZ: *Eurytemora affinis* nauplii, copepodites, and adults, *Ectinosoma curticorne*, *Neomysis americana*, *Mysis*

stenolepis, *Microgadus tomcod* larvae and *Osmerus mordax* larval smelt (Winkler et al. 2003).

Other studies have noted that bacterioplankton, photosynthetic cyanobacteria (e.g. *Synechococcus* sp.) and small eukaryotic algae can also be heavily grazed upon by heterotrophic nanoflagellates with increasing salinity (Caron et al. 1991). Ciliates and phagotrophic dinoflagellates can also exert high grazing pressures on these populations; in fact, they removed over 80% of primary production in the high salinity Mississippi River plume (Strom and Strom 1996, from Jochem 2003), and their intestines were filled with *Synechococcus* sp. in the St. Lawrence ETZ (Lovejoy et al. 1993; Barnard, unpubl. data). This lends support to our observations of declining picophytoplankton and bacterial populations along the salinity gradient. A decrease in bacterial standing stocks downstream is common in estuaries (e.g., Painchaud and Therriault 1989; Hollibaugh and Wong 1999; Karrasch et al. 2003) and has been attributed to the 1) increased dilution with seawater of waters highly loaded with anthropogenic sources, 2) seawater containing less autochthonous matter compared to riverine inputs, 3) increases in salinity inducing stress and mortality, and 4) increased grazing with increasing heterotrophic communities in the frontal zone and downstream. Hence, in addition to grazing, salinity may control the longitudinal distribution of bacteria and picophytoplankton cells. In this study, the numbers of free bacteria decreased in the transition zone and in some samples, only aggregate bacteria were observed. Painchaud and Therriault (1989) also documented this phenomenon in the ETZ, and it appears common across estuarine frontal zones (Bernat et al. 1994; Crump et al. 1999; Hollibaugh and Wong 1999; Karrasch et al. 2003). Our estimates of bacterial densities are most likely, however, only approximate due to difficulties in the enumeration of bacterial cells caused by to the abundant particulate matter. This matter could have masked free bacteria, causing overestimates in the number of aggregated bacteria and underestimates in free cells. Although our results are similar to those previously obtained in the ETZ, future studies in turbidity maxima should

dilute samples with filtered sample prior to enumeration and count additional fields (see details in Hollibaugh and Wong 1999).

1.4.3. Biovolume size spectrum and total biovolume

Similar to Frenette et al. (1995), we observed an increase in total cell volume from the freshwater to the transition zone (Table 1.2) but no decline in cell numbers, rather an increase. These authors observed a marked accumulation of large-sized autotrophs near the bottom. We observed a sharp, significant increase or accumulation of large autotrophic cells at the bottom from June to July but not larger cells at the bottom of the water column, nor a higher percent biovolume of larger cells in the transition zone. In fact, the only size class which had a significantly higher percent biovolume in the transition zone was the 3-7.5 μm size class. This divergence in findings may be explained by our spring tide sampling and the possible resuspension of bottom-sedimented organisms in the well-mixed water column, perhaps influencing the distribution of large cells. Furthermore, our sampling period varied noticeably from that of Frenette et al. (1995) who sampled in May and June. We did not observe such marked variations in discharge from June to July.

In the total biovolume size spectrum, veliger biovolume contributed variably to the heterotrophic biovolume in the >25 μm size class for the freshwater zone. This contribution was negligible in the transition zone. During the June cruise, this contribution was equal to 11.3 and 30.6%, for the surface and bottom respectively. For the July cruise, this contribution averaged 46.5 and 11.5%, for the surface and bottom respectively. The >25 μm proportion of biovolume may therefore be overrepresented in the freshwater zone when comparing to pre-invasion data for the surface samples of the July cruise. For these samples only, a significant increase in this biovolume size class in the transition zone was observed when the veliger biovolume was removed, resulting in findings similar to Frenette et al. (1995). Nonetheless, removing the veliger component from the other stations did not alter the pre-eminent dominance of this size class.

1.4.4. Autotrophic and heterotrophic proportions

No shift towards a heterotrophic protist community in the transition zone was observed, but a slight increase in heterotrophic abundance was noted, similar to Frenette et al. (1995). Our data show a strongly autotrophic community across the freshwater and transition zones in terms of numerical abundance and biovolume (Tables 1.1 and 1.2). Contrary to these findings, Winkler et al. (2003) observed a community shift towards heterotrophy, perhaps indicative of the dynamic and rapidly changing nature of this system. Autotrophic dominance does not appear to have been affected by the omnipresence of veligers.

Given the differences in sampling periods and protocols, we are unable to fully compare our protist data with those of Winkler et al. (2003) and Frenette et al. (1995). Other ETZ studies (e.g. Lovejoy et al. 1993) have sampled at higher salinities and have noted significant decreases in the microbial community at higher salinities.

1.4.5. Aggregates

In the ETZ, aggregates were a prominent component of the microbial community, with densities exceeding 6 x 10^8 aggregates/L in the transition zone. These results are higher than those obtained by Wörner et al. (2002; 3.2 to 11.96 x 10^5 aggregates/L) in the Elbe estuary but similar to those from the River Danube (Berger et al. 1996, from Zimmermann-Timm 2002). ETZs are prime candidates for aggregation due to the pronounced physical and chemical gradients at the freshwater/saltwater interface that favour aggregation (Kranck 1979; Zimmermann-Timm 2002). In addition, intertidal mudflats and salt marshes on estuarine shorelines inevitably contribute to influxes of organic matter, debris and exopolysaccharide-rich biofilms covering the tidally exposed sediments. Aggregates can be composed of detritus, microorganisms, and organic and inorganic matter. Aggregates could be expected to be an important food source since microaggregate concentrations can be up to 100 times higher than those of auto- and heterotrophs in rivers and estuaries (Zimmermann-Timm 2002). Also, free-living bacteria are not efficiently captured by grazers because of their small size, and aggregation may be a mechanism which

enhances the transfer of bacterial carbon to higher trophic levels. In the Elbe Estuary, analyses of *Neomysis integer* revealed that up to 90% of the stomach content was composed of detritus, indicating that mysids actively feed on aggregates (Zimmermann-Timm 2002). Certain ciliates and copepods (particularly *Eurytemora affinis*) selectively feed on certain aggregates (see references in Zimmermann-Timm 2002). Aggregates may serve as niches for bacteria and protists, providing a nutrient-rich microenvironment, protection against conductivity and salinity gradients, as well as protection from grazers, or they could also be viewed as the negative outcome for trapped protists (Zimmermann-Timm 2002). In the ETZ, up to 80% of autotrophs were found within aggregates (Barnard et al. 2003; this study), or surrounded by exopolysaccharide-rich mucilage, implying that zooplankton must be feeding on aggregates and/or autotrophs which are embedded in a matrix. The ETZ and its associated hydrodynamics are packaging food resources as aggregates which are then used by higher trophic organisms such as zooplankton. Aggregation therefore induces important changes in the pelagic community from the freshwater to the transition zone and lends support to our proposition of a discontinuous patch within the river segment.

Our microbial counts may underestimate the true abundance of cells and biomass due to losses and changes in cell size during storage (Kepner and Pratt 1994 and references therein). Fixation is also a potential problem when counting aggregates as it could destroy aggregate structure, stimulate excretion of mucopolysaccharides and change the micro-habitat, leading to the disappearance of protozoans and resulting in an underestimation of aggregate-dwelling organisms (Zimmermann-Timm 2002). On the other hand, if the fixation breaks up larger aggregates into smaller ones, the total number of aggregates may be overestimated. Samples were, however, handled with care and were inverted gently by hand for 1 to 2 minutes prior to counting to avoid cell damage and aggregate break up.

1.4.6. TN, TP, and C:N

Large phytoplankton should require higher nitrogen concentrations than smaller cells, because of their low surface to volume ratio meaning that nitrogen could be a limiting factor for large cells in estuarine systems which are recognized as nitrogen depleted. In the ETZ, nitrate and phosphorus concentrations were at detectable levels and TP and TN concentrations were even highest in the transition zone (Vincent et al. 1994; Barnard et al. 2003). However, in the transition zone we noted a C:N value above 15 for the intermediate tide, which could be indicative of a cellular nitrogen deficiency for phytoplankton (Wetzel 2001). In the San Francisco Bay estuarine turbidity maximum, Hollibaugh and Wong (1999) also noted an increase of the C:N of total seston from <10 in fresh water to >12 in the turbidity maximum. POC increased up to two orders of magnitude from the freshwater to the transition zone whereas the variability in PON was considerably less significant, leading to higher C:N ratios (Figure 1.4). This potentially represented the high pulses of allochthonous organic matter from the littoral marshes (Wetzel 1990) and sediments and heterogeneous flocs from the aggregation of dissolved matter. This may lead to the C:N being no longer representative of the organismal characteristics but rather representative of the allochthonous particulate seston. Total seston in the ETZ was in fact characterized as allochthonous and decoupled in its isotopic $\delta^{13}C$ signature from autochthonous matter and the food web (Martineau et al. 2004). The increase in the C:N ratio may also be the result of retention since longer residence times enhance degradation and naturally increase the C:N ratio of particulate matter (Harvey et al. 1995). This would corroborate with the lower ratios observed upstream which are subjected to higher flow and shorter residence times. Selective degradation may also occur where N-rich labile compounds (such as amino acids and proteins) from the dissolved phase are degraded prior to aggregation, leading to particulate matter poorer in nitrogen (Harvey et al. 1995). At higher salinities and further downstream, the St. Lawrence estuary is periodically nitrate depleted during the

summer (Tremblay et al. 1997), and our higher C:N values may be representative of the mixture of the freshwater/littoral/estuarine assemblage. Lastly, Chl *a* and nutrient concentrations were significantly positively correlated, thus phytoplankton were not able to consume the large amounts of nutrients in the freshwater and transition zones, presumably due to relatively high nutrient concentrations and accumulation in the retention zone.

1.4.7. Veligers and associations with microbial community components

Similar to results found by Barnard et al. (2003), veliger abundance was positively correlated with their prey abundance in the transition zone. In this study however, we examined all of their potential prey items whereas Barnard et al. (2003) only addressed picophytoplankton and total Chl *a* biomass. The positive correlations are indicative that the potential prey were present with the veligers and show that the veligers and their prey covary, however, we cannot conclude that veligers were actively feeding on the available prey in the transition zone. In correlation analysis of estuarine data, biological variables are bound to covary because of their strong correlation with salinity, hence relations between biological variables could potentially result from a spatial gradient induced by the influence of salinity rather than from a functional relationship (Painchaud and Therriault 1989). In spite of this, using stable isotope analysis Barnard et al. (2006) demonstrated that carbon sources used by veligers were indeed free bacteria (no positive correlation in this study), freshwater algae and dissolved organic carbon. In sum, these results imply that veligers are not having a severe, negative impact on the microbial community in the transition zone.

In the freshwater zone, physical, chemical, and biological properties are relatively uniform (Figures 1.2 and 1.5). Veligers showed no positive correlation with their prey, perhaps indicative of their variable and sporadic abundance when no controlling variables such as salinity are present. Data from the ETZ were not collected on a daily basis but showed evidence of sporadic and distinct peaks in veliger abundance amongst five different cruises (Barnard et al. 2003). This is

consistent with the results obtained during the fixed station sampling of summer 2001, which showed large variation in veliger abundance between samples. Stoeckel et al. (1997) also noted the continual passage of pulses of individuals in a large river. Based on the numerical abundance of veliger prey items, it can be assumed that veligers are not prey limited in the freshwater portion of the ETZ. For the correlation analysis of this zone, the *n* of 8 may have influenced the power of the test and rendered the detection of significant correlations difficult.

Zooplankton abundance patterns have been shown to fluctuate over the years due to forcing by the physical environment and biological communities such as discharge, weather, prey and predator abundance, and water levels (Winkler et al. 2005). Variations in the zooplankton assemblage may induce changes in the microbial community and vice versa. This said, the observed divergence between our data and previously published ETZ data may be accounted for by natural variations in the planktonic community. Furthermore, dredging activities are frequent during the summer months in the ETZ and upstream. Such activities can have severe impacts on the plankton community by changing the physical and chemical environment with the resuspension of sediments and organic matter. Lastly, zebra mussels were introduced into the St. Lawrence waterway via the emptying of ballast waters into the Great Lakes. The St. Lawrence ETZ is sensitive to all upstream events and numerous shipping ports exist from the Great Lakes to the port of the city of Québec. In addition to zebra mussel veligers, many other plankton species have been identified as non-indigenous and may contribute substantially to the fluctuations observed in plankton species composition and abundance. A study sampling ballast waters from 48 ships in 14 Eastern Canadian maritime ports found that 25% of the phytoplankton were classified as non-indigenous, 44% were classified as indigenous, and 31% were considered of unknown geographic affiliation (Carver and Mallet 2002). From 1959 to 2000, five protist species were introduced in the St. Lawrence River via the emptying of ship ballast waters (Grigorovich et al. 2003). Fluctuations in the composition of plankton species are therefore susceptible to the physical and

chemical environment as well as anthropogenic activities such as dredging and exotic species introduction.

In summary, the St. Lawrence ETZ microbial community dynamics and the gradients in its abiotic characteristics illustrate how this zone inserts itself as a discontinuous segment along the St. Lawrence River corridor. The variations observed in the microbial community structure of the ETZ were small and seem unlikely due to the arrival of veligers. The ETZ appears productive enough to support these dominant invaders and despite minor changes in the biovolume size spectrum, the protist community remains dominated by autotrophs, specifically by picoplankton and nanophytoplankton, and the cell concentrations of the microbial community components have remained similar to pre-invasion values.

1.5. Acknowledgements

This research is a contribution to the programme of Québec-océan and CEN (Centre d'Études Nordiques). We thank Ladd Johnson, Connie Lovejoy and Paul Hamilton for assistance and support at various stages of the project. We also thank Hugues Boulanger, Andy Casper, Julie Harnois, Kim Huggins, Brigitte Laberge, Marie-Audrey Livernoche, Carl Martin, Sébastien Roy, Geneviève Trudel, Gesche Winkler, the crew of the Willie DeRaspe and of the Marie-Karine D. for their help in the field and in the laboratory. The first cruise of summer 2001, conducted on the Willie DeRaspe, was possible thanks to the support of the Department of Fisheries and Oceans (Canada). This study was funded by a strategic NSERC (Canada) grant led by Ladd E. Johnson, NSERC discovery grants to J-J.F. and W.F.V., and a NSERC (Canada) fellowship to C.B.

1.6. References

Barnard, C., Frenette, J.-J., and Vincent, W.F. 2003. Planktonic invaders of the St. Lawrence estuarine transition zone: environmental factors controlling the distribution of zebra mussel veligers. Can. J. Fish. Aquat. Sci. **60**: 1245–1257.

Barnard, C., Martineau, C., Frenette, J.-J., Dodson, J.J., and Vincent, W.F. 2006. Trophic position of zebra mussel veligers and their use of dissolved organic carbon. Limnol. Oceanogr. **51**: 1473-1484.

Bernat, N., Köpcke, B., Yasseri, S., Thiel, R., and Wolfstein, K. 1994. Tidal variation in bacteria, phytoplankton, zooplankton, mysids, fish and suspended particulate matter in the turbidity zone of the Elbe estuary; interrelationships and causes. Netherlands J. Aquat. Ecol. **28**: 467-476.

Bernier, S. 2003. Efficacité et sélectivité alimentaire des larves de la Moule Zébrée : effets potentiels sur le réseau alimentaire de la zone de transition estuarienne du Saint-Laurent. MSc Thesis, University Laval, 43 pp.

Bertrand, N. and Vincent, W.F. 1994. Structure and dynamics of photosynthetic picoplankton across the saltwater transition zone of the St. Lawrence River. Can. J. Fish. Aquat. Sci. **51**: 161-171.

Bodineau, L., Thoumelin, G., Béghin, V., and Wartel, M. 1998. Tidal time-scale changes in the composition of POM within the estuarine turbidity maximum zone in the macrotidal Seine Estuary in France: the use of fatty acid and sterol biomarkers. Estuar. Coast. Shelf S. **47**: 37-49.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S.E.G., and Fischer, D.T. 1997. Zebra mussel invasion in a large, turbid river: phytoplankton response to increased grazing. Ecology **78**: 588-602.

Caron, D.A., Lim, E.L., Miceli, G., Waterbury, J.B., and Valois, F.W. 1991. Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar. Ecol. Prog. Ser. **76**: 205-217.

Carver, C. E. and Mallet A. L. 2002. An Assessment of the Risk of Ballast Water-Mediated Introduction of Non-Indigenous Phytoplankton and Zooplankton into Atlantic Canadian waters. *Prepared for:* Transport Canada, Marine Safety, Dartmouth, N.S., B2Y 4K2. Mallet Research Services Ltd. 98 pp.

Crump, B.C., Armbrust, E.V., and Baross, J.A. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl. Env. Microb.**65**: 3192-3204.

d'Anglejan, B.F., and Smith, E.C. 1973. Distribution, transport, and composition of suspended matter in the St. Lawrence Estuary. Can. J. Earth Sci. **10**: 1380-1396.

Elser, J.J. and Urabe, J. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology **80**: 735-751.

Findlay, S., Pace, M.L., and Fischer, D.T. 1998. Response of heterotrophic planktonic bacteria to the zebra mussel invasion of the tidal freshwater Hudson River. Microbial Ecol. **36**: 131-140.

Fortier, L., Le Fèvre, J., and Legendre, L. 1994. Export of biogenic carbon to fish and to the deep ocean, the role of large planktonic microphages. J. Plankton Res. **16**: 809-839.

Franks, P.J.S. 1992. Sink or swim: accumulation of biomass at fronts. Mar. Ecol. Prog. Ser. **82**: 1-12.

Frenette, J.-J., Vincent, W., Dodson, J.J., and Lovejoy, C. 1995. Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar. Ecol. Prog. Ser. **120**: 99-110.

Gosselain, V. and Hamilton, P.B. 2000. Algamica: revisions to a key-based computerized counting program for free-living, attached, and benthic algae. Hydrobiologia **438** : 139-142.

Grigorovich, I.A., Colautti, R.I., Mills, E.L., Holeck, K., Ballert, A.G., and MacIsaac, H.G. 2003. Ballast-mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. Can. J. Fish. Aquat. Sci. **60**:

740-756.

Harvey, H.R., Tuttle, J.H., and Bell, J.T. 1995. Kinetics of phytoplankton decay during simulated sedimentation: changes in biochemical composition and microbial activity under oxic and anoxic conditions. Geochim. Cosmochim. Ac. **59**: 3367-3377.

Hillebrand, H., Dürselen, C.-D., and Kirschtel, D. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. **35**: 403-424.

Holland, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island Area, Western Lake Erie since the establishment of the zebra mussel. J. Great Lakes Res. **19**: 617-624.

Hollibaugh, J.T. and Wong, P.S. 1999. Microbial Processes in the San Francisco Bay Estuarine Turbidity Maximum. Estuaries **22**: 848-862.

Jeffrey, S.W. and Welschmeyer, N.A. 1997. Spectrophotometric and fluorometric equations in common use in oceanography. *In* Phytoplankton pigments in oceanography. *Edited by* Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. Unesco Publishing, Paris pp. 597-615.

Jochem, F.J. 2003. Photo- and heterotrophic pico- and nanoplankton in the Mississippi River plume: distribution and grazing activity. J. Plankton Res. **25**: 1201-1214.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* sp.) veligers using cross-polarized light microscopy. Hydrobiologia **312**: 139-146.

Junk, W., Bayley, P., and Sparks, R. 1989. The flood pulse concept in river-floodplain systems. *In* Proceedings of the international large river symposium. Edited by D. Dodge. Can. Spec. Publ. Fish. Aquat. Sci., pp. 110-127.

Karrasch, B., Ullrich S., Mehrens, M., and Zimmermann-Timm, H. 2003. Free and particle-associated extracellular enzyme activity and bacterial production in the lower Elbe estuary, Germany. Acta Hydrochim. Hydrobiologica **31**: 297-306.

Kepner, R.L. and Pratt, J.R. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol. Rev. **58**: 603-615.

Kilgour, B., Mackie, G., Baker, M., and Keppel, R. 1994. Effects of salinity on the condition and survival of Zebra Mussels (*Dreissena polymorpha*) Estuaries **17** : 385-393.

Kirk, J.T.O. 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press.

Kranck, K. 1979. Dynamics and distribution of suspended particulate matter in the St. Lawrence estuary. Can. Naturalist **106**: 163-173.

Laprise, R. and Dodson, J.J. 1993. Nature of environmental variability experienced by benthic and pelagic animals in the St. Lawrence Estuary, Canada. Mar. Ecol. Prog. Ser. **94**: 129-139.

Laprise, R. and Dodson, J.J. 1994. Environmental variability as a factor controlling spatial patterns in distribution and species diversity of zooplankton in the St. Lawrence estuary. Mar. Ecol. Prog. Ser. **107**: 67-81.

Lehman, P.W. 2000. Phytoplankton biomass, cell diameter, and species composition in the low salinity zone of the northern San Francisco Bay estuary. Estuaries **23**: 216-230.

Lovejoy, C., Vincent, W.F., Frenette, J.-J., and Dodson, J.J. 1993. Microbial gradients in a turbid estuary: applications of a new method for protozoan community analysis. Limnol. Oceanogr. **38**: 1295-1303.

Lucotte, M. and d'Anglejan, B. 1986. Seasonal control of the Saint-Lawrence Maximum Turbidity Zone by Tidal-Flat Sedimentation. Estuaries **9**: 84-94.

Martineau, C., Vincent, W.F., Frenette, J.-J., and Dodson, J.J. 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnol. Oceanogr. **49**: 1679-1686.

Minshall, G., Cummins, K., Petersen, R., Cushing, C., Bruns, D., Sedell, J., and Vannote, R. 1985. Developments in stream ecosystem theory. Can. J. Fish.

Aquat. Sci. **42**: 1045-1055.

National Laboratory for Environmental Testing. 1994. Manual of Analytical Methods, Volume 1, Major Ions and Nutrients. Burlington, Ontario. 84 p.

Nush, E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Arch. Hydrobiol. Beih. **14**: 14-36.

Pace, M.L., Findlay, S.E., and Fischer, D. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshwater Biol. **39**: 103-116.

Painchaud, J. and Therriault, J.-C. 1989. Relationships between bacteria, phytoplankton and particulate organic carbon in the Upper St. Lawrence estuary. Mar. Ecol. Prog. Ser. **56**: 301-311.

Painchaud, J., Therriault, J.-C., and Legendre, L. 1995. Assessment of salinity-related mortality of freshwater bacteria in the St. Lawrence Estuary. Appl. Env. Microb. **61**: 205-208.

Rice, S., Greenwood, M., and Joyce, C. 2001. Tributaries, sediment sources, and the longitudinal organization of macroinvertebrate fauna along river systems. Can. J. Fish. Aquat. Sci. **58**: 824-840.

Silverberg, N., and Sundby, B. 1979. Observations in the turbidity maximum of the St. Lawrence estuary. Can. J. Earth Sci. **16**: 939-950

Sobczak, W.V., Cloern, J.E., Jassby, A.D., and Müller-Solger, A.B. 2002. Bioavailability of organic matter in a highly disturbed estuary: The role of detrital and algal resources. Proc. Nat. Acad. Sci. **99**: 8101-8105.

Sprung, M. 1989. Field and laboratory observations of *Dreissena polymorpha* larvae: abundance, growth, mortality, and food demands. Arch. Hydrobiol. **115**: 537-561.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. 39-53.

Stoeckel, J.A., Schneider, D.W., Soeken, L.A., Blodgett, K.D., and Sparks, R.E. 1997. Larval dynamics of a riverine metapopulation : implications for zebra mussel recruitment, dispersal, and control in a large-river system. J. N. Amer. Benthol. Soc. **16**: 586-601.

Thorp, J.H. and Delong, M.D. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos **70**: 305-308.

Thorp, J.H. and Delong, M.D. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. Oikos **96**: 543-550.

Thorp, J.H., Thoms, M.C., and Delong, M.D. 2006. The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Res. Appl. **22**: 123-147.

Tockner, K., Malard, F., and Ward, J. 2000. An extension of the flood pulse concept. Hydrol. Process. **14**: 2861-2883.

Townsend, C. 1996. Concepts in river ecology: pattern and process in the catchment hierarchy. Arch. Hydrobiol. Suppl. **113**: 3-21.

Tremblay, J-É., Legendre, L., and Therriault, J-C. 1997. Size-differential effects of vertical stability on the biomass and production of phytoplankton in a large estuarine system. Estuar. Coast. Shelf S. **45**: 415-431.

Vanderploeg, H.A., Liebig J.R., and Gluck, A.A. 1996. Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha)*: the importance of size and polyunsaturated fatty acid content. J. Great Lakes Res. **22**: 36-45.

Vannote, R., Minshall, G., Cummins, K., Sedell, J., and Cushing, C. 1980. The River Continuum Concept. Can. J. Fish. Aquat. Sci. **37**: 130-137.

Vincent, W.F., Bertrand, N., and Frenette, J.-J. 1994. Photoadaptation to intermittent light across the St. Lawrence estuary freshwater-saltwater transition zone. Mar. Ecol. Prog. Ser. **110**: 283-292.

Vincent, W.F. and Dodson, J.J. 1999. The St. Lawrence River, Canada-USA: the

need for an ecosystem-level understanding of large rivers. Jpn. J. Limnol. **60**: 29-50.

Vincent, W.F., Dodson, J.J., Bertrand, N., and Frenette, J.-J. 1996. Photosynthetic and bacterial production gradients in a larval fish nursery: the St. Lawrence River transition zone. Mar. Ecol. Prog. Ser. **139**: 227-238.

Ward, J. and Stanford, J. 1983. The serial discontinuity concept of lotic ecosystems. In Dynamics of lotic ecosystems. *Edited by* T. Fontaine and S. Bartell. Ann Arbor Science Publishers, Ann Arbor pp. 29-42.

Wetzel, R.G. 1990. Land-water interfaces: Metabolic and limnological regulators. Verh. Internat. Verein. Limnol. **24**: 6-24.

Wetzel, R. G. 2001. Limnology: lake and river ecosystems. San Diego. 1006 p.

Winkler, G., Dodson, J.J., Bertrand, N., Thivierge, D., and Vincent, W.F. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. Mar. Ecol. Prog. Ser. **251**: 59-73.

Winkler, G., Sirois, P., Johnson, L.E., and Dodson, J.J. 2005. Invasion of an estuarine transition zone by *Dreissena polymorpha* veligers had no detectable effect on zooplankton community structure. Can. J. Fish. Aquat. Sci. **62**: 578-592.

Wright, D.A., Setzler-Hamilton, E.M., Magee, J.A., and Harvey, H.R. 1996. Laboratory culture of zebra mussel (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussel larvae using estuarine algae. J. Great Lakes Res. **22**: 46-54.

Wörner, U., Zimmermann-Timm, H., and Kausch, H. 2002. Aggregate-associated bacteria and heterotrophic flagellates in the River Elbe- their relative significance along the longitudinal profile from km 46 to km 583. Internat. Rev. Hydrobiol. **87**: 255-266.

Zimmermann-Timm, H. 2002. Characteristics, dynamics and importance of aggregates in rivers - an invited review. Internat. Rev. Hydrobiol. **87**: 197-240.

Table 1.1. Mean value (10^6 cells L^{-1}), SE in parentheses for the total number of autotrophic and heterotrophic protists sampled in the upstream freshwater zone and directly in the transition zone of St. Lawrence.

	Cruise 1 (June)		Cruise 2 (July)	
	Freshwater	Transition	Freshwater	Transition
Surface				
Autotrophs	1.87 (0.33)	3.94 (1.17)	1.19 (0.18)	5.17 (0.94)
Heterotrophs	0.75 (0.25)	0.99 (0.26)	0.28 (0.06)	1.38 (0.41)
% heterotrophs	28.48	20.15	19.05	21.09
Total	2.62 (0.37)	4.94 (0.72)	1.47 (0.27)	6.56 (0.75)
Bottom				
Autotrophs	2.97 (0.21)	10.71 (3.36)	1.38 (0.00)	7.53 (2.68)
Heterotrophs	0.9 (0.02)	2.38 (0.86)	0.33 (0.08)	1.91 (0.83)
% heterotrophs	23.26	18.19	19.22	20.22
Total	3.87 (0.60)	13.09 (2.06)	1.7 (0.31)	9.43 (1.68)

Table 1.2. Mean value (10^6 μm^3 L^{-1}), SE in parentheses for the total cell biovolume of autotrophic and heterotrophic protists sampled in the upstream freshwater zone and directly in the transition zone of St. Lawrence.

	Cruise 1 (June)		Cruise 2 (July)	
	Freshwater	Transition	Freshwater	Transition
Surface				
Autotrophs	2647 (2017)	710.0 (195.9)	844.0 (330.3)	647.0 (161.6)
Heterotrophs	191.0 (40.90)	278.0 (79.68)	113.0 (77.52)	473.0 (287.0)
% heterotrophs	6.73	28.15	11.84	42.25
Total	2838 (1087)	989.0 (120.0)	958.0 (252.4)	1120 (159.2)
Bottom				
Autotrophs	2883 (2580)	1010 (428.5)	885.0 (455.7)	1751 (693.5)
Heterotrophs	181.0 (58.26)	200.7 (82.64)	129.0 (29.95)	186.0 (121.4)
% heterotrophs	5.89	16.58	12.75	9.61
Total	3064 (1311)	1211 (253.4)	1014 (286.9)	1938 (440.1)

Table 1.3. Spearman *rho* correlation coefficients: a) freshwater zone, b) transition zone.
a)

n = 8	Aggr Bact	Vel	Pico	Chl.*a*	Auto 3-12	Auto 12-25	Auto >25	Hetero 3-12	Hetero 12-25	Hetero >25	Aggr	Sal	Temp	TP	TN	Turb
Free Bact	-0.38	-0.52	-0.17	-0.33	-0.17	-0.10	-0.21	-0.05	0.76*	0.24	0.50	0.05	-0.19	-0.46	-0.33	-0.64
Aggr Bact		0.69	-0.31	0.05	0.67	019	0.50	0.69	-0.19	-0.19	-0.05	0.62	-0.57	0.29	0.67	0.48
Veligers			0.24	0.45	0.67	0.40	0.48	0.74*	-0.12	-0.02	0.10	0.24	-0.36	0.68	0.69	0.69
Pico				-0.07	0.29	-0.40	-0.52	-0.03	-0.10	0.55	-0.12	-0.45	0.33	0.29	-0.36	0.17
Chl. *a*					-0.24	0.90*	0.69	0.14	0.14	0.05	0.21	-0.29	0.17	0.89*	0.33	0.50
Auto 3-12						-0.19	-0.10	0.79*	0.07	0.05	0.29	0.64	-0.67	0.29	0.52	0.50
Auto 12-25							0.83*	0.29	0.33	-0.14	0.40	0.00	-0.12	0.79*	0.52	0.36
Auto >25								0.21	0.10	-0.12	0.02	0.02	-0.05	0.50	0.50	0.17
Hetero 3-12									0.21	-0.19	0.52	0.69	-0.86	0.61	0.76*	0.62
Hetero 12-25										0.38	0.83*	0.14	-0.29	0.07	0.00	-0.17
Hetero >25											-0.02	-0.52	0.45	0.21	-0.62	-0.11
Aggr												0.48	-0.62	0.18	0.40	0.24
Sal													-0.93*	0.04	0.71*	0.33
Temp														-0.11	-0.74*	-0.36
TP															0.32	0.86*
TN																0.60
Turb																

b)

n = 20	Aggr Bact	Vel	Pico	Chl. *a*	Auto 3-12	Auto 12-25	Auto >25	Hetero 3-12	Hetero 12-25	Hetero >25	Aggr	Sal	Temp	TP	TN	Turb
Free Bact	-0.81*	-0.30	0.12	-0.35	-0.32	-0.14	-0.30	-0.27	-0.20	0.04	-0.36	0.18	-0.20	-0.22	-0.16	-0.28
Aggr Bact		0.56*	0.21	0.50*	0.44	0.35	0.34	0.45*	0.11	-0.04	0.52*	-0.45*	0.43	0.37	0.34	0.46*
Veligers			0.48*	0.72*	0.66*	0.56*	0.21	0.67*	0.22	-0.30	0.72*	-0.71*	0.68*	0.72*	0.63*	0.80*
Pico				0.72*	0.40	0.70*	0.25	0.53*	0.22	-0.35	0.42	-0.66*	0.63*	0.53*	0.52*	0.55*
Chl. *a*					0.81*	0.83*	0.35	0.84*	0.34	-0.28	0.85*	-0.76*	0.75*	0.81*	0.77*	0.90*
Auto 3-12						0.58*	0.24	0.91*	0.53*	-0.30	0.89*	-0.62*	0.68*	0.82*	0.78*	0.87*
Auto 12-25							0.21	0.65*	0.16	-0.21	0.65*	-0.66*	0.62*	0.66*	0.57*	0.72*
Auto >25								0.33	-0.01	-0.26	0.22	-0.45*	0.53*	0.15	-0.04	0.16
Hetero 3-12									0.47*	-0.37	0.91*	-0.64*	0.69*	0.85*	0.79*	0.92*
Hetero 12-25										-0.30	0.36	-0.14	0.19	0.33	0.18	0.40
Hetero >25											-0.28	0.21	-0.31	-0.28	-0.17	-0.35
Aggr												-0.52*	0.55*	0.90*	0.80*	0.96*
Sal													-0.98*	-0.60*	-0.59*	-0.61*
Temp														0.60*	0.59*	0.63*
TP															0.86*	0.93*
TN																0.84*
Turb																

1.7. List of figures

Figure 1.7. Aggregate densities for June and July. Values are means (± SE) for all tidal states and depths in each zone.

Figure 1.8. Percent of total protists within aggregates as a function of tidal state and zone. Values are means (± SE) for June and July. Values are similar to those obtained during summer 2000 (Barnard et al. 2003).

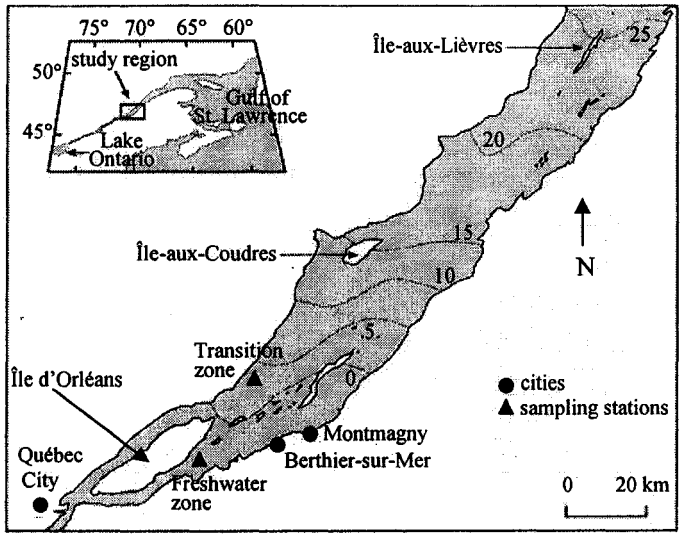

75° 70° 65° 60°
50°
45°
study region
Gulf of St. Lawrence
Lake Ontario
Île-aux-Lièvres
25
20
15
10
5
0
Île-aux-Coudres
N
Transition zone
Île d'Orléans
cities
sampling stations
Montmagny
Berthier-sur-Mer
Québec City
Freshwater zone
0 20 km

Figure 1.1.

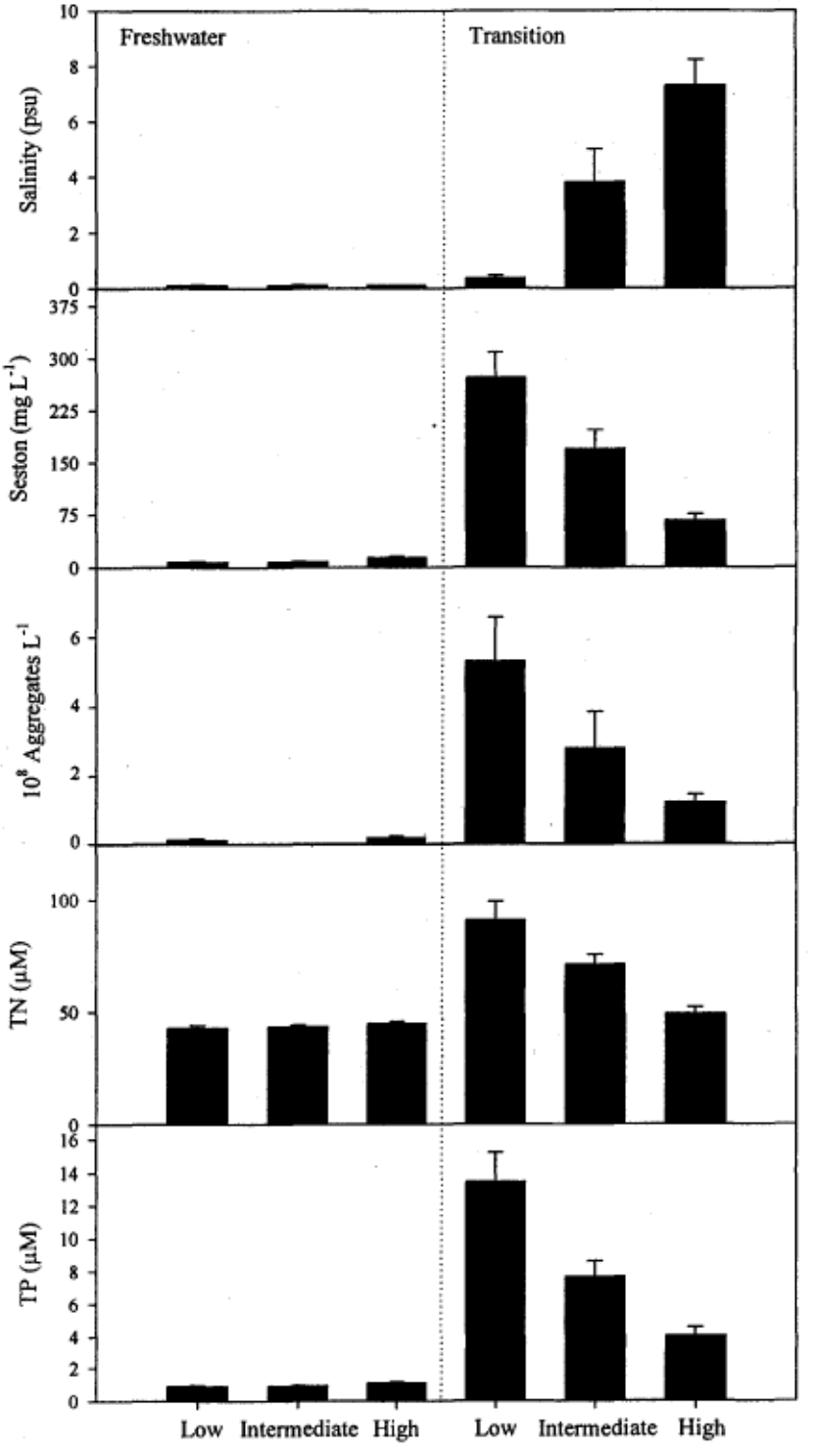

Freshwater
Transition
Salinity (psu)
Seston (mg L-1)
10^8 Aggregates L-1
TN (µM)
TP (µM)
Low
Intermediate
High
Low
Intermediate
High

Figure 1.2.

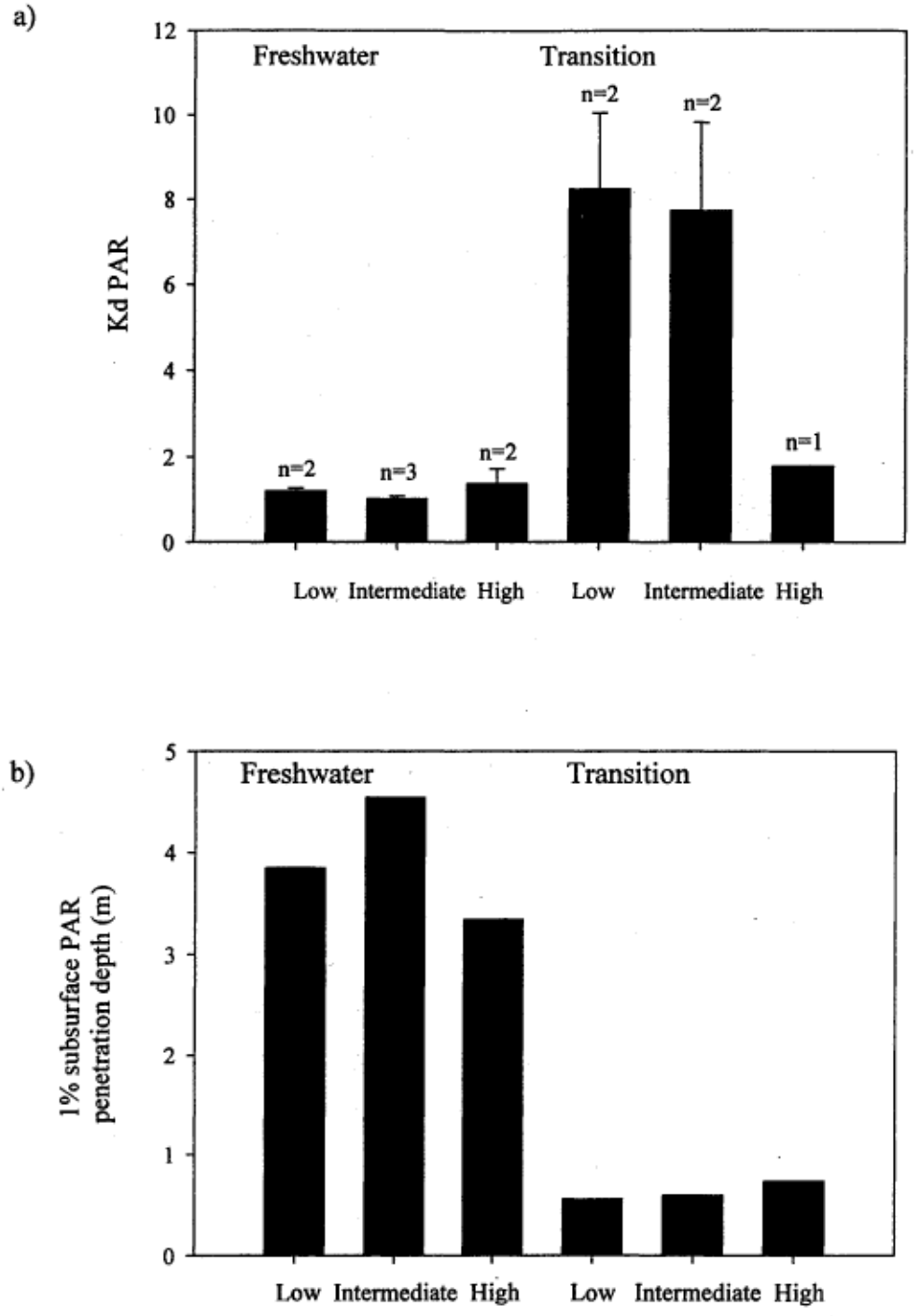

a)
Freshwater
Transition
Kd PAR
12
10
8
6
4
2
0
n=2
n=3
n=2
n=2
n=2
n=1
Low
Intermediate
High
Low
Intermediate
High
b)
Freshwater
Transition
1% subsurface PAR penetration depth (m)
5
4
3
2
1
0
Low
Intermediate
High
Low
Intermediate
High

Figure 1.3.

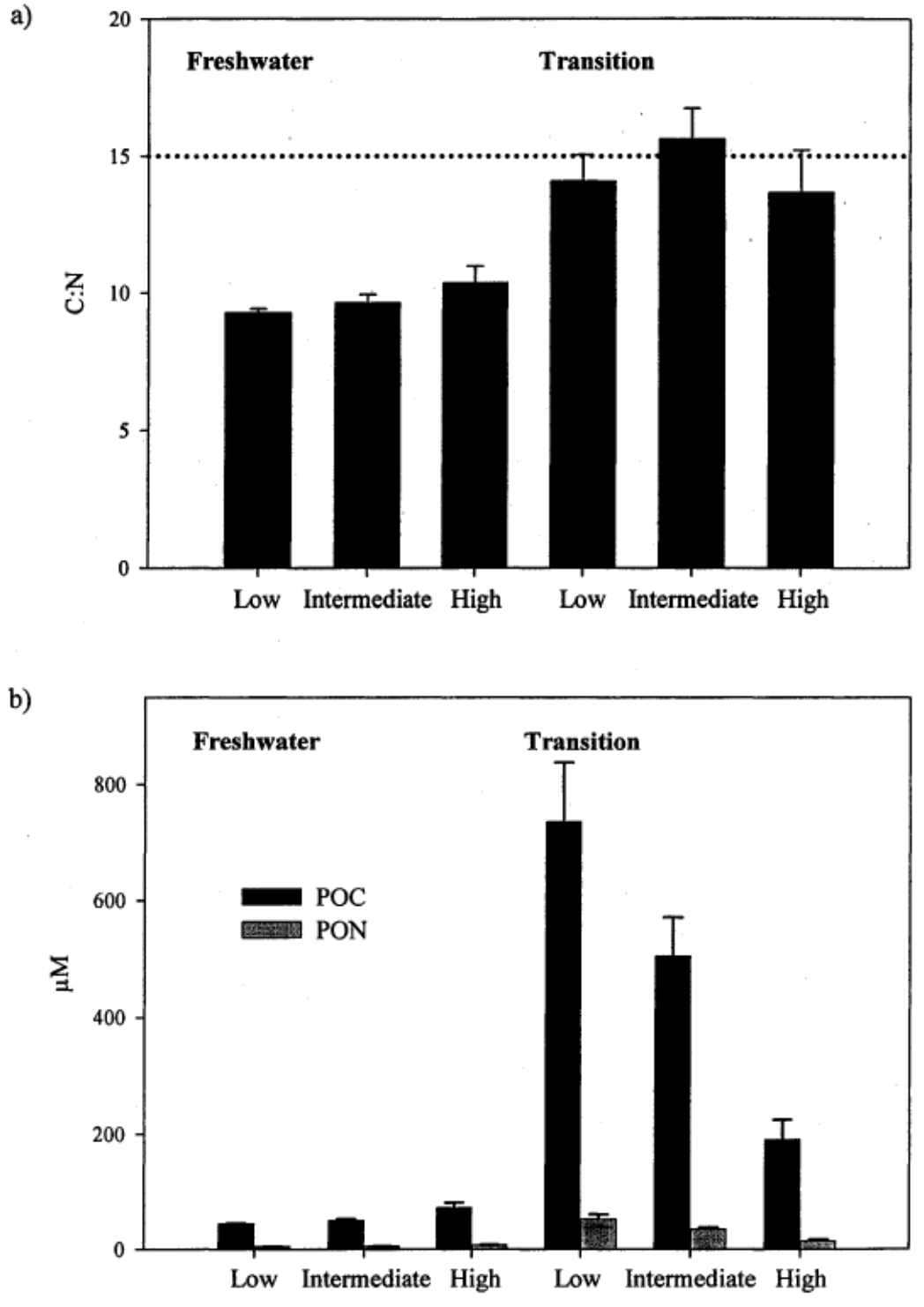

a)
Freshwater
Transition
C:N
20
15
10
5
0
Low
Intermediate
High
Low
Intermediate
High
b)
Freshwater
Transition
POC
PON
µM
800
600
400
200
0
Low
Intermediate
High
Low
Intermediate
High

Figure 1.4.

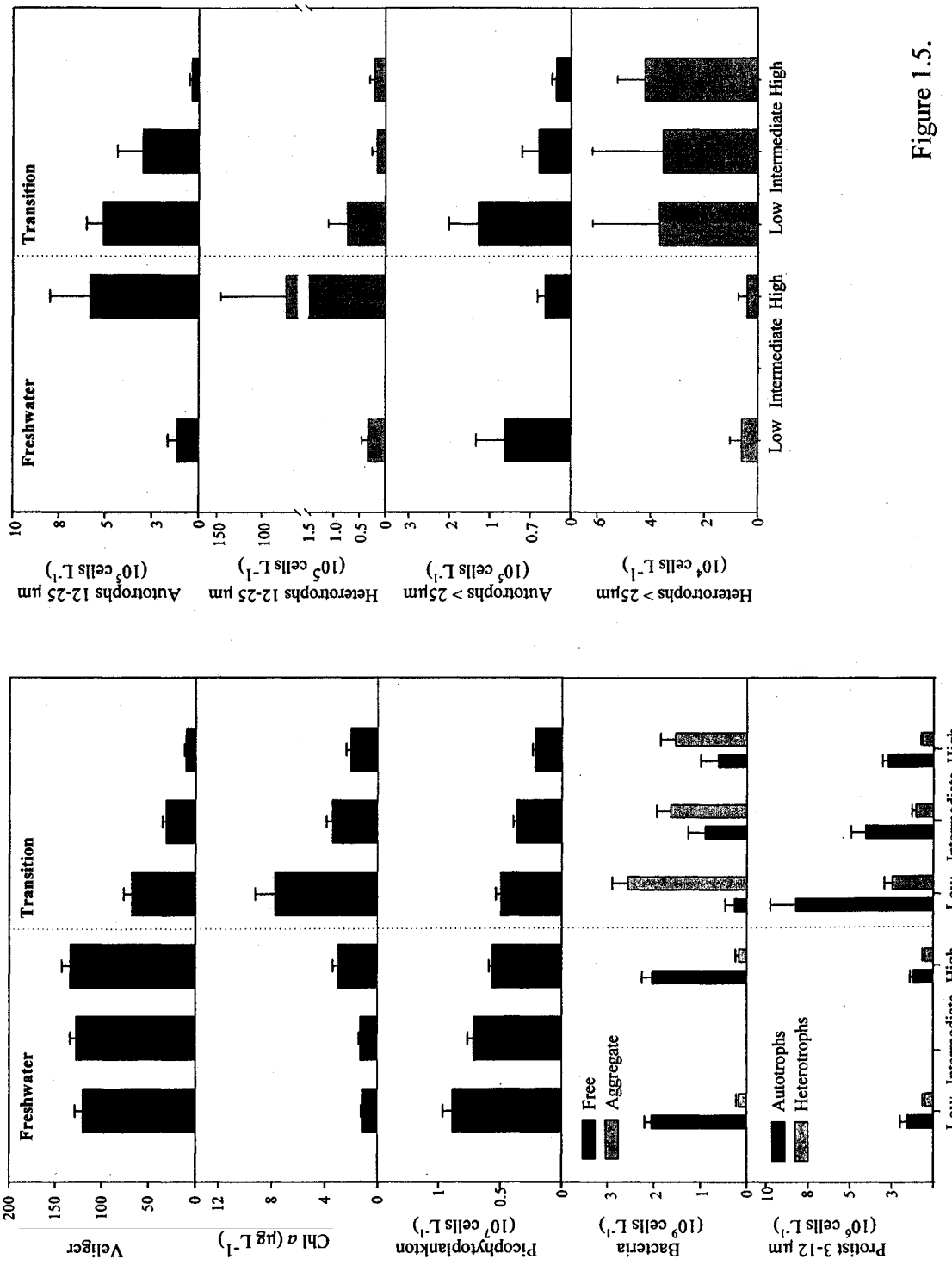

Freshwater
Transition
Low Intermediate High Low Intermediate High
Protist 3-12 µm (10⁶ cells L⁻¹)
Autotrophs
Heterotrophs
Bacteria (10⁹ cells L⁻¹)
Free
Aggregate
Picophytoplankton (10⁷ cells L⁻¹)
Chl a (µg L⁻¹)
Veliger
Heterotrophs > 25µm (10⁴ cells L⁻¹)
Autotrophs > 25µm (10⁵ cells L⁻¹)
Heterotrophs 12-25 µm (10⁵ cells L⁻¹)
Autotrophs 12-25 µm (10⁵ cells L⁻¹)

Figure 1.5.

a)

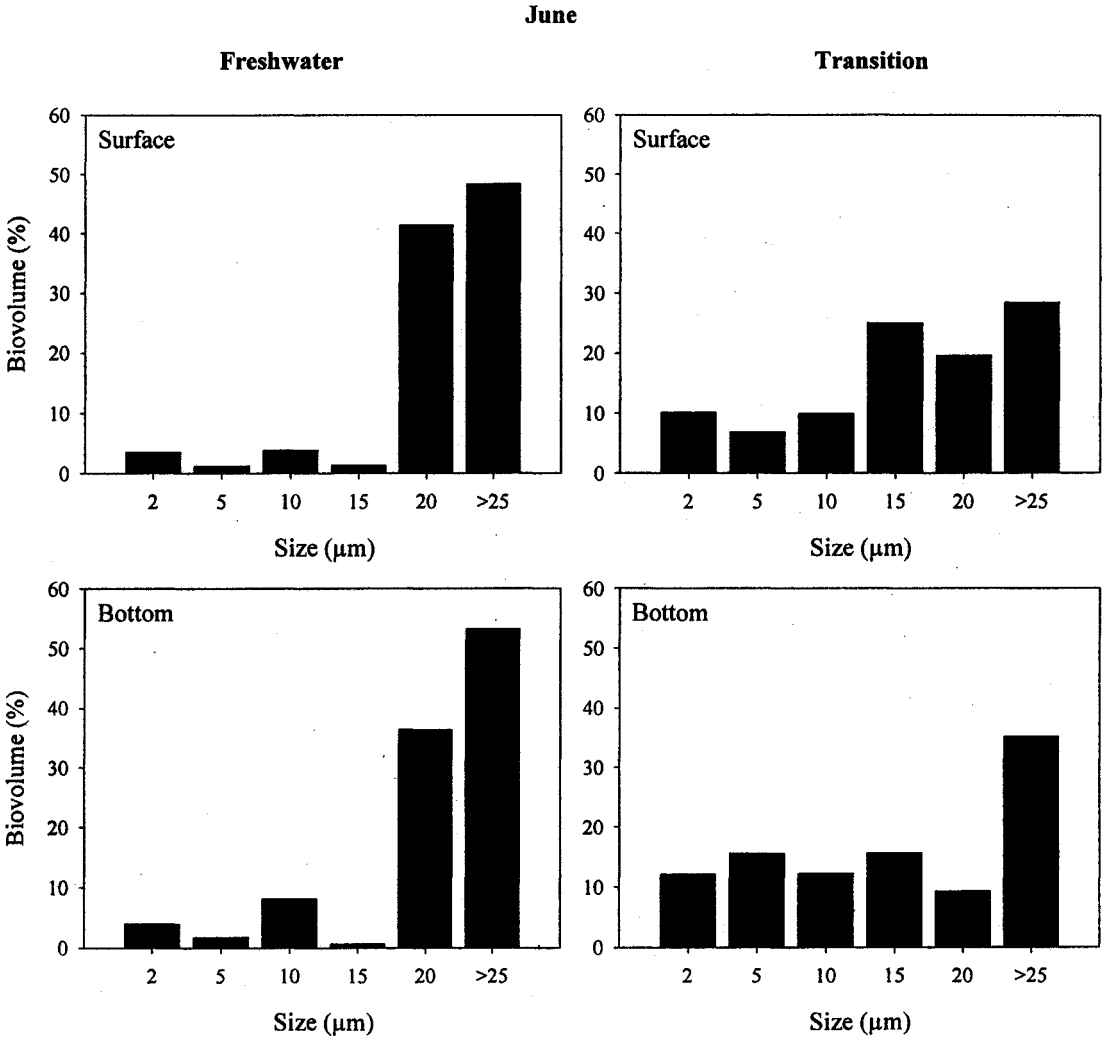
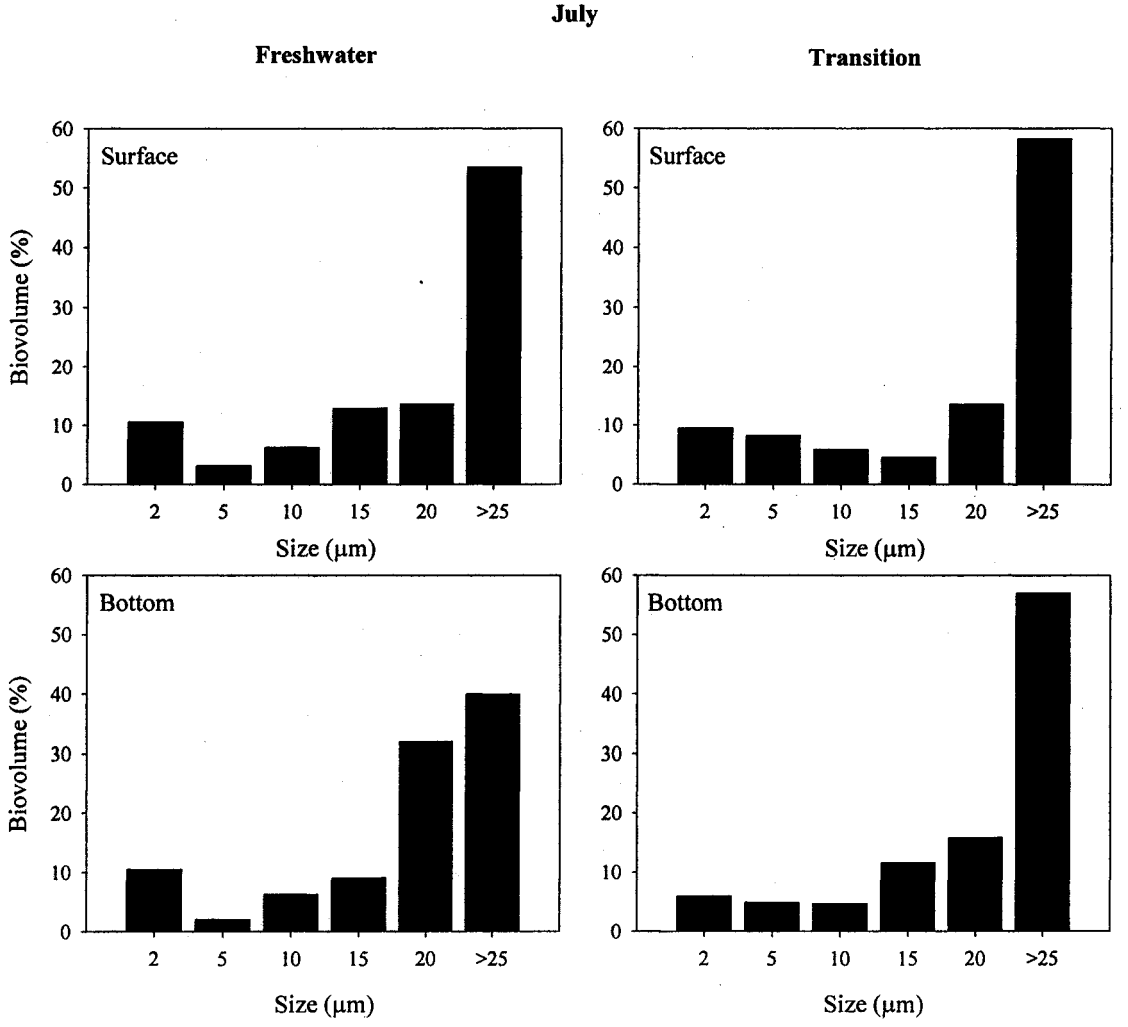

June
Freshwater
Transition
Surface
Surface
Bottom
Bottom
Biovolume (%)
Biovolume (%)
0
10
20
30
40
50
60
2
5
10
15
20
>25
Size (µm)
Size (µm)
Size (µm)
Size (µm)

Figure 1.6.

b)


July
Freshwater
Transition
Surface
Surface
Bottom
Bottom
Biovolume (%)
Biovolume (%)
0
10
20
30
40
50
60
2
5
10
15
20
>25
Size (µm)
Size (µm)
Size (µm)
Size (µm)

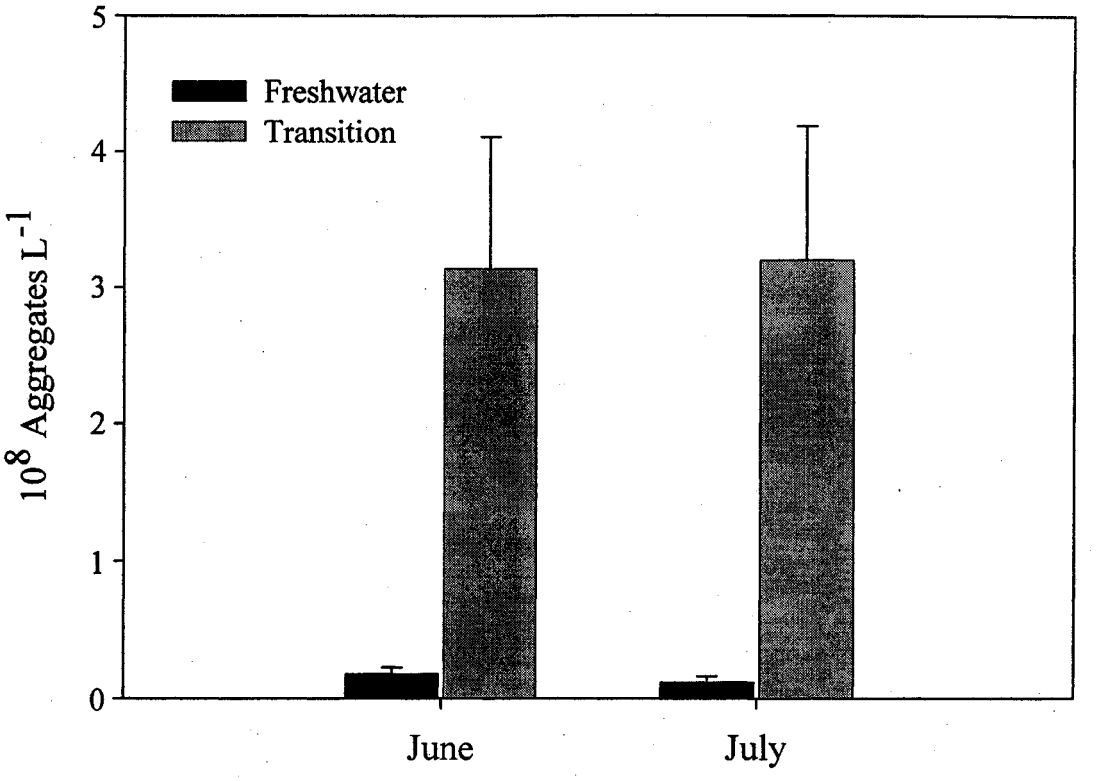


Figure 1.7.

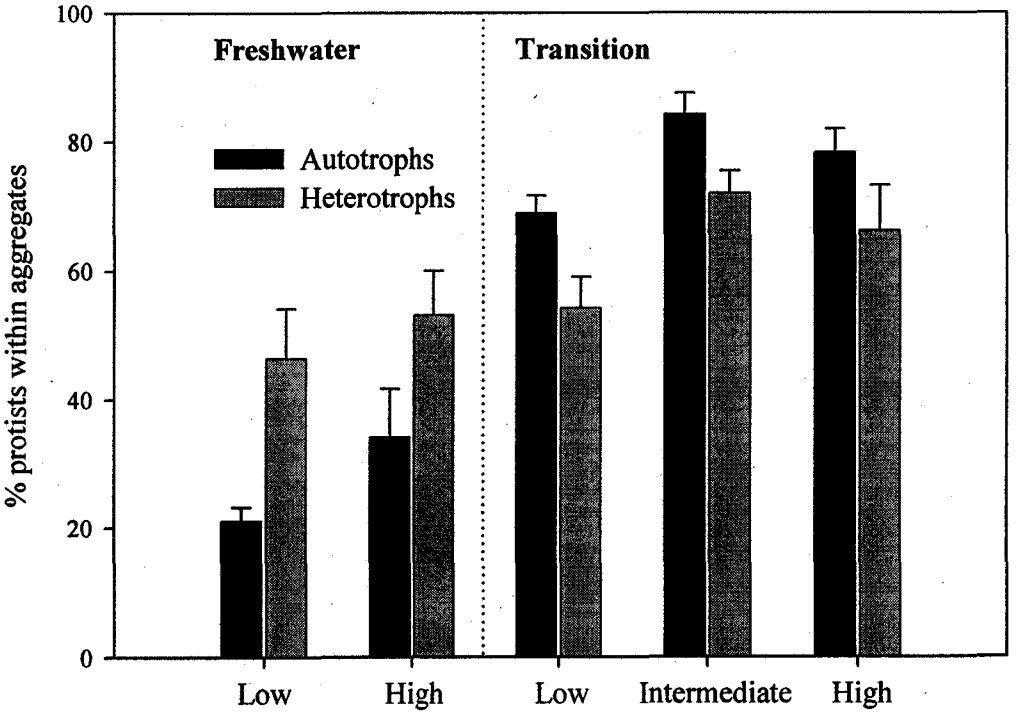

100
80
60
40
20
0
% protists within aggregates
Freshwater
Transition
Autotrophs
Heterotrophs
Low
High
Low
Intermediate
High

Figure 1.8.

CHAPITRE 2

ENVAHISSEURS PLANCTONIQUES DE LA ZONE DE LA TRANSITION ESTUARIENNE DUFLEUVE SAINT LAURENT: VARIABLES ENVIRONNEMENTALES CONTRÔLANT LA DISTRIBUTION DES LARVES VÉLIGÈRES DE LA MOULE ZÉBRÉE

PLANKTONIC INVADERS OF THE ST. LAWRENCE ESTUARINE TRANSITION ZONE: ENVIRONMENTAL FACTORS CONTROLLING THE DISTRIBUTION OF ZEBRA MUSSEL VELIGERS

Résumé

La zone de transition estuarienne (ZTE) du fleuve Saint-Laurent est reconnue pour sa grande productivité, tel que démontré par son rôle de pouponnière de larves de poissons. Depuis 1994, les larves véligères de *Dreissena polymorpha* ont envahi le plancton de cette zone (jusqu'à 260 individus L^{-1}). Cette étude documente les facteurs environnementaux contrôlant leur distribution dans la ZTE et leurs impacts sur la communauté phytoplanctonique. Leur distribution horizontale était limitée par la salinité avec une diminution abrupte de leur abondance à 2 ‰. Un déclin simultané de la biodisponibilité de leurs proies à des valeurs de salinité > 2 ‰ a été observé, impliquant que cette chute de biodisponibilité pourrait constituer un stress additionnel à l'effet de la salinité. Leur distribution était homogène dans toute la colonne d'eau, même lors de la présence d'un gradient vertical de densité. Une analyse de redondance a révélé que les densités de véligères étaient positivement corrélées à la température et à la turbidité, mais négativement corrélées à la salinité et au phosphore total. Les densités de véligères étaient positivement corrélées à leurs proies, i.e., à la biomasse de chlorophylle *a* et au picophytoplancton, impliquant qu'elles ne semblent pas avoir d'impacts sévères sur leurs proies phytoplanctoniques. Celles-ci étaient positivement corrélées au seston de bonne qualité alimentaire et associées aux conditions favorables à leur croissance qui caractérisent les eaux de faibles salinités de la ZTE.

Abstract

The St. Lawrence estuarine transition zone (ETZ) is a productive ecosystem supporting a larval fish nursery. Since 1994, *Dreissena polymorpha* veligers have become the dominant zooplankton (up to 260 individuals L^{-1}). The environmental factors controlling their distribution across the ETZ and their potential impact on the plankton were determined. Their horizontal distribution was limited by salinity, with maximum decreases in concentration at 2 ‰. A sharp decline in prey availability at > 2 ‰ may be a secondary stressor for the veligers, in addition to the direct effects of salinity. Their vertical distribution was homogeneous throughout the water column, even in the presence of a pycnocline. Redundancy analysis showed that veliger concentrations were positively correlated with temperature and turbidity and negatively correlated with salinity and total phosphorus. Veligers were also positively correlated with chlorophyll *a* and picophytoplankton concentrations, suggesting little effect on their phytoplankton prey. Moreover, the veligers were positively correlated with the sestonic ratio of particulate to total phosphorus, indicating their positive association with good food quality. The veligers appear to have no severe negative impacts on the ETZ plankton community and are restricted to favourable conditions for their survival in the upstream, low salinity region of the ETZ.

Keywords: Zebra mussels, veliger larvae, estuary, environmental variables, distribution, salinity

2.1. Introduction

Zebra mussels, *Dreissena polymorpha*, have spread throughout North American aquatic ecosystems since their introduction to the Great Lakes in the late 1980s (Hebert et al. 1989). This exotic species is remarkably versatile and adept at exploiting new niches. In some areas, *D. polymorpha* can reach densities of 175 000 individuals m^{-2} per year within 2 or 3 years of colonisation (Mellina and Rasmussen 1994). Unsurpassed competitors for habitat and food, they continually out-compete native bivalves (Ricciardi et al. 1998) and cause great physical disturbance. Their invasion is linked to a significant decrease in phytoplankton (Caraco et al. 1997), flagellated protozoa (Findlay et al. 1998) and zooplankton (Pace et al. 1998). Contamination of the food web by their bioaccumulation of chemicals has also been documented (de Kock and Bowmer 1993). A vast literature exists on the distribution, biology and ecological impacts of adult zebra mussels (Strayer and Smith 1993 and references therein). However, the environmental factors controlling the successful development of the veliger larval stage and its potential impacts on the microbial food web are poorly documented (Sprung 1993), despite the fact that veligers can reach extremely high densities in the plankton of North American freshwaters (> 400 individuals per litre, de Lafontaine et al. 1995).

Zebra mussels are well documented in North American rivers, and high proportions of veligers must be ultimately advected to the sea. However, little information is available on the physical factors limiting the horizontal and vertical distribution of veligers in estuaries. Although recognised as a freshwater species, the zebra mussel veligers are believed to tolerate salinities up to 4 ‰ for a short period of time, depending on water temperature and acclimation period (Strayer and Smith 1993; Kilgour et al. 1994; Wright et al. 1996). It remains unknown, however, how salinity may limit the downstream distribution of veligers in North American estuaries.

A few freshwater studies have found that veligers in lakes are situated within the upper few meters of the water column (Strayer and Smith 1993; Stoeckel et al.

1997). Fraleigh et al. (1993) found that their vertical distribution is controlled by water temperature, wind speed and mixing rate. During the warmer month of July, the veligers were found at slightly lower temperatures in the deeper part of the epilimnion. Stratification was observed only when the wind speed was below 8 km h^{-1} and 64% of the individuals were found in the epilimnion between 4 m and 6 m. In lakes, veligers are thus found slightly deeper in the water column when mixing rates are weak, probably due to passive sedimentation (Garton and Haag 1993). Kern et al. (1994) observed that veligers are distributed evenly throughout the water column in the Rhine River, probably due to higher mixing rates. *Dreissena* veligers have locomotive capacities, yet it is unknown if their vertical distribution is due to passive or active transport in estuaries where the water column may be stratified. A vertically stratified environment could prevent the veligers from crossing the density barrier (pycnocline) between the freshwater and saltwater, thereby resulting in a non-homogeneous distribution.

Zebra mussel veligers are primarily herbivores with a preference for algae rich in poly-unsaturated fatty acids (PUFA) (Vanderploeg et al. 1996) but also consume photosynthetic picoplankton, bacteria, flagellates and detritus (Sprung 1993; Wright et al. 1996). According to these authors, the size spectrum of their prey varies between 1 μm and 9 μm. In addition, Wright et al. (1996) observed that in laboratory experiments, the veligers also fed on the estuarine algae, *Isochrysis galbana* and *Pavlova lutheri*. Veligers tend to have a significantly higher survival rate when they consume plankton containing long-chained PUFAs, in particular algae containing the long-chained (n-3) PUFA, docosahexaenoic acid (Wacker et al. 2002). Under favourable conditions, a veliger can increase roughly ten times its weight during its 1 - 3 week planktonic period (Sprung 1993). The impact of adult zebra mussels on the plankton through their filter feeding is well documented, however little is known about how feeding by the veliger stage of this organism may affect phytoplankton abundance.

The St. Lawrence estuarine transition zone (ETZ) is situated between Île d'Orléans and Île-aux-Coudres and contains high concentrations of microplankton and macroplankton that support a productive fish nursery (Vincent and Dodson 1999; Winkler et al. 2003). The recent arrival of *Dreissena polymorpha* in this region has raised concerns about their potential competition with herbivorous zooplankton that provide the food supply to the larval fish community. Zebra mussel veligers were absent from the ETZ plankton samples obtained during cruises in 1991 to 1993. However, in 1994, 1995 and 2000 this situation changed dramatically as they were among the dominant zooplankton, contributing to more than 80% of the total number of animals and achieving densities > 200 individuals per litre (Vincent and Dodson 1999; G. Winkler, Université Laval, Département de biologie, Ste-Foy, Québec, G1K 7P4, unpublished data). The veligers are assumed to originate mainly from upstream and from the shores of the St. Lawrence, since adults are sparsely distributed on the fluvial bed of the ETZ (A. Casper, Département de biologie, Université Laval, Ste-Foy, Québec, G1K 7P4, unpublished data). Any impacts on the food web would therefore not be due to adult populations, but rather to the omnipresence of the veligers in the plankton. Hydrodynamic processes play a major role in controlling the distribution of organisms in this tidal estuary. For example, Frenette et al. (1995) documented the prolonged residence time for phytoplankton and protozoa in the ETZ and a combination of immigration and emigration pathways which favour larger particles and high taxon diversity. Hydrodynamic processes in combination with an abundant food supply from upstream similarly result in high populations of macrozooplankton in this region (Winkler et al. 2003). Zebra mussel veligers, in their most abundant stage, measure between 70 μm and 160 μm and also have the potential of being retained within the ETZ.

The overall aim of this study was to determine the role of several environmental variables in influencing the distribution of veligers in an estuarine transition zone. More specifically, the objectives were to determine the horizontal and vertical distribution of the veligers across the St. Lawrence ETZ, and to assess the

effects of salinity, depth, temperature, water column stratification, temporal and tidal variation. In addition, we evaluated the potential impact of veligers on communities at the base of the food web by examining their relationship with total phytoplankton biomass (chlorophyll *a*), picophytoplankton cell concentrations and the nutritional quality of the seston. The present study provides insights into the factors controlling the distribution and abundance of this invasive species in the upper estuary of a large river ecosystem.

2.2. Materials and methods

2.2.1. Sampling site

The estuarine transition zone (ETZ) of the St. Lawrence River is situated approximately 50 km downstream from Québec City (Canada) between the islands of Ile d'Orléans and Ile-aux-Coudres (Figure 2.1). These waters are characterized by salinities ranging from 0.1 ‰ and 10 ‰. The freshwater discharge averages 10 000 $m^3 s^{-1}$ and current speeds may reach > 2.5 m s^{-1} (d'Anglejan and Smith 1973).

The bottom morphology of the ETZ consists of three channels, one following the north shore, one short channel in the middle and one following the south shore. The north channel is used for maritime transport and is characterized by deeper waters and stronger currents. The middle channel consists of an archipelago of small islands with Ile d'Orléans and Ile-aux-Coudres being the largest islands marking its eastern and western boundaries. Finally, the south channel has slower currents and shallower waters than the north channel. Large mudflats are found on the south shore near Montmagny, while vast intertidal marshes (3×10^6 m^2) are located on the shores of the north channel at Cap Tourmente (Lucotte and d'Anglejan 1986).

Sampling was conducted during summer 2000 and summer 2001. The research goals of summer 2000 were to determine the factors controlling the veligers' horizontal distribution across the ETZ and to assess the physical, chemical and biological variables associated with veliger abundance. During summer 2001, the

veligers' vertical distribution was assessed in the strictly freshwater zone of the ETZ (Zone 1) as well as directly in the ETZ (Zone 2) during different tidal regimes. Veliger abundance was evaluated as a function of depth and water column stratification.

2.2.2. Summer 2000 sampling

Five cruises were undertaken during summer 2000: 4 and 14 June, 15 and 28 July and 8 August. During these cruises, 12 stations were sampled consisting of five in the north channel (N), two in the middle channel (M) and five in the south channel (S) (Figure 2.1). Each station was chosen according to salinity, implying that the geographical position of the station differed with every sampling period, depending on the tidal state. Five salinities were selected for the stations in the north and south channels: 0, 0 - 1, 1 - 3, 3 - 5 and finally 5 ‰ or more. The salinity of the stations in the middle channel varied between 1 - 3 and 3 - 5 ‰. Due to unfavourable meteorological conditions, certain stations had to be eliminated during the first and last cruise, specifically S - 13, N - 0, N - 01, N - 13 and N - 35 during the first cruise and M -13 and M – 35 during the last cruise.

Physical variables

The site of each station was chosen according to surface salinity, which was determined with a Yellow Springs Instrument salinometer. Water column salinity and temperature as a function of depth were recorded using a CTD (Conductivity-Temperature-Depth meter, Sea logger SBE-19) which was lowered from the surface to 2 m from the bottom. At each station, water was collected at the surface (0 - 2 m) and near the bottom (2 m from the bottom) with a 5 litre Go-Flo bottle. These water samples were stored cool and in the dark. Upon arrival at the laboratory, turbidity for each sample was recorded using an HF Scientific Inc. nephelometer, model DRT15-CE.

Veligers

To collect *D. polymorpha* veligers, a bottom to surface haul was made using a plankton net (63 μm mesh with a 50 cm diameter), and an additional haul was made

from 2 m to the surface. The contents were filtered through a 63 μm screen to eliminate smaller particles and then preserved in denatured ethanol (95%) at a final concentration of approximately 80%. Due to clogging of the net with sediments and subsequent loss of material during the haul, the veliger densities from the full water column hauls were equal to or lower than the veliger densities from surface hauls, hence only surface data were used in the statistical analyses for this year. An improved method of collection was used in summer 2001.

Veliger counts were conducted in the laboratory using cross-polarisation according to Johnson (1995). This method renders the veligers visible and easily distinguishable from other zooplankton and detritus. The volume of the counts had to correspond to a minimum of 10% of the total sample volume. If 300 individuals were counted before reaching this 10%, another sub-sample was counted in order to use the average of the two counts.

Chlorophyll a

Upon arrival at the laboratory, the water collected with the Go-Flo bottles was passed through GF/F Whatman 25 mm filters in duplicate. These filters were then frozen (-20 °C) and kept in the dark until extraction. They were subsequently ground in 90% acetone and extracted over 24 h. The extracts were then cleared by centrifugation and assayed by spectrofluorometry (Varian Cary-Eclipse) before and after acidification (1.0 N HCl). Chlorophyll *a* (Chl. *a*) from *Anacystis nidulans* (Sigma-Aldrich) was used for calibration.

Photosynthetic picoplankton

In a darkened room, immediately upon arrival in the laboratory, approximately 15 - 20 mL of water sample were filtered onto Anodisc Whatman filters which were mounted on slides using Aquapolymount (Polysciences). These slides were immediately stored in the dark at 4 °C for 24 h then stored frozen (–20 °C). Within days of the slide preparation, counting was undertaken with an Olympus epi-fluorescence microscope with exchangeable blue and green excitation filters and 1000 x oil immersion. When excited with a green light source, photosynthetic

picoplankton with phycocyanin and/or Chl. *a* fluoresce bright red (PC) while phycoerythrin-rich photosynthetic picoplankton (PE) fluoresce bright orange (Lovejoy et al. 1993). Three hundred individuals were counted with a minimum of 15 fields. A minimum of 50 fields was counted when counts were low.

Protist communities

Samples for protists (phytoplankton and protozoa) were examined and prepared using the procedures outlined by Lovejoy et al. (1993). These samples were fixed immediately with 1% gluteraldehyde and 0.1% paraformaldehyde (final concentrations), stored in the dark at 4 °C and later settled in Utermohl chambers. The auto- and fluorochrome (DAPI) fluorescence allowed us to differentiate organisms from abiotic particles and to locate and characterize cells. The organisms were grouped into two broad categories: autotrophs (including mixotrophs) and heterotrophs.

Chemical variables

Particulate organic carbon (POC), nitrogen (PON) and phosphorus (PP) were obtained by filtering 20 - 100 mL (depending on turbidity) of water through pre-combusted and acid-washed GF/F filters. These were then stored frozen in the dark (–20 °C). For the dissolved components (NO_3^-, total nitrogen (TN), and soluble reactive phosphorus (SRP)), the water was filtered through pre-combusted GF/F filters and stored cool in the dark until analysis. For total phosphorus (TP), unfiltered water was immediately acidified (1 mL 30% H_2SO_4 per 100 mL sample) upon arrival at the laboratory. All analyses were conducted at the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario according to the standard methods (NLET 1994).

2.2.3. Summer 2001 sampling

Two cruises were undertaken to assess the short-term dynamics of veliger vertical abundance at fixed stations during different tidal states. Both cruises occurred during the spring tides of June and July 2001. One fixed station was located in the

strictly freshwater zone (46° 52' 86" N, 70° 55' 60" W) and the other directly in the transition zone (47° 06' 71" N, 70° 42' 44" W) (Figure 2.1). Each site was sampled at three high, three low and four intermediate tides, thus a total of six high, six low and eight intermediate tides for each station for both cruises.

The veligers were collected with two 5 litre Go-Flo bottles. These bottles collected water at the surface (0 - 2 m), mid-column (depending on the column depth) and near the bottom (1 - 2 m from the bottom). The water was then filtered through a 63 μm sieve and preserved and counted as described above.

2.2.4. Data analysis

Spatial autocorrelation was tested in the summer 2000 veliger data by constructing a correlogram obtained using Moran's *I* (Legendre and Fortin 1989). This correlogram was globally significant at the alpha = 5% level since several individual values were significant at the Bonferroni corrected level (unpublished data) (Legendre and Fortin 1989). In order to account for the spatial structure in the data, spatial components were incorporated into the statistical analyses (Legendre 1993). For the summer 2001 data, the statistical model constructed took into account spatial and temporal autocorrelation (see below).

Water column stratification

A measure of water column stratification (Δs) was calculated as the difference between salinity at the surface and at the bottom of the water column for the stations in Zone 2 (summer 2001). If veliger larvae are passively transported, the vertical stratification of their distribution (abundance at the bottom – abundance at the surface = Δ_{vel}) should be correlated with Δs. Since these values (Δ_{vel} and Δs) did not have normal distributions, Spearman's nonparametric test was used to evaluate the correlation.

Spatial and temporal distribution of the veligers

Temporal variation in veliger abundance between the four cruises undertaken in summer 2000 data was tested using a repeated measure ANOVA (mixed-effect model) with SAS (version 8.2). The date was a fixed effect and the station was a

random effect. Temporal dependency was adjusted with heterogeneous compound symmetry based on the Akaike information criteria. Multivariate normality was verified using the Mardia Skewness and Kurtosis statistic. Multiple comparisons were made using the protected LSD method.

For the vertical distribution assessed during summer 2001, a split-split plot ANOVA (mixed-effects model) was used to fit the veliger abundance data. The effects of zone (1 and 2), depth (surface, mid-column and bottom), and tidal cycle (high, low and intermediate) on veliger abundance were verified. In the main plot, the date (cruise) was a random effect and zone was a fixed effect. The effect of depth was in the sub plot, while the effect of the tidal states was in the sub-sub plot. This model took into account the possible correlations between observations in the same zone.

Relationships between environmental, spatial and biological variables (RDA)

Redundancy analysis (RDA; CANOCO program 4.0, ter Braak 1998) was used to determine whether variation in species abundance was coordinated in response to environmental and spatial gradients. The variation in species abundance was partitioned into independent components: pure environmental, pure spatial, spatial component of environmental variation and unexplained (Borcard et al. 1992). This method was used to partial out the intrinsic spatial component of community structure and to establish the relative contribution of environmental factors in controlling the species distribution.

To choose between a linear and non-linear model, a detrended canonical correspondence analysis (DCCA) was employed and RDA was chosen over canonical correspondence analysis (CCA) because the range of the ordination sample scores in a detrended correspondence analysis (with detrending by segments and non-linear rescaling) was less than 1.5 standard deviation units (ter Braak 1998). This indicated that the application of a linear method was appropriate. This multivariate technique for direct gradient analysis graphically summarises the relationships between the species and the abiotic environmental and spatial variables.

Data from summer 2000 sampling were used whereby the species variables consisted of veliger and picophytoplankton (PE and PC categories) numerical densities and Chl. *a* biomass. The environmental variables were salinity, temperature, turbidity, TP, Cl^-, SO_4^{2-}, SRP, NO_3^-, TN and date (cruises 3, 4 and 5) to test for temporal variation in species abundance. Logarithmic and square root transformations were used to normalise the distribution of the data. In order to account for differences in units, the species data were centred and standardised (ter Braak 1998). The spatial component was a matrix of two-dimensional geographic coordinates including all the terms of a cubic trend surface polynomial, as suggested by Legendre (1990, cited from Borcard et al. 1992). This surface regression was of the form $Z = b_1X + b_2Y + b_3XY + b_4X^2 + b_5Y^2 + b_6X^2Y + b_7XY^2 + b_8X^3 + b_9Y^3$, where X was the longitude and Y was the latitude in Universal Transverse Mercator co-ordinates. This matrix represented the geographic surface over which the species were sampled and the model tested its relative influence on the variation in species abundance.

Forward selection of environmental variables and polynomial terms was applied to select the set of variables that significantly explained the variation in abundance of the species ($p < 0.05$). In order to account for collinearity, environmental variables with variance inflation factors greater than 15 were eliminated. Once the significant environmental and spatial variables identified, four analyses were conducted to partition the variance in the species data following Borcard et al. (1992): 1) RDA of the species matrix constrained by the environmental variables, 2) RDA of the species matrix constrained by the spatial variables, 3) like (1), after removing the effect of the spatial variables, 4) like (2) after removing the effect of the environmental variables. In RDAs calculated using CANOCO, the sum of all canonical eigenvalues can be interpreted as the fraction of explained variation (r^2). Monte Carlo permutation tests (999 unrestricted permutations) were used to assess the statistical significance of the relationship between the species and independent variables. The total explained variation (r^2) was the sum of the explained

variation in 1) and 4) or in 2) and 3). The "pure" environmental variation was defined by step 3), and the "pure" spatial variation was defined by step 4). The variation "shared" by the spatial and the environmental components was calculated by subtracting 3) from 1) or 2) from 4). The unexplained portion of the variation was obtained by subtracting the total variation explained from one.

2.3. Results

2.3.1. Horizontal and temporal distribution

Concentrations of veliger larvae dropped precipitously across the freshwater-saltwater gradient of the ETZ, and there was a negative exponential relationship with salinity (Figure 2.2). For summer 2000 and 2001, the maximum decrease in veliger abundance occurred approximately at 2 ‰ which may correspond to a physiological threshold. For summer 2000, the highest densities were obtained during the second and fourth cruises with densities reaching up to 260 individuals per litre at stations in the north channel with salinities between 0 and 1 ‰. For summer 2001, the highest densities were observed in the freshwater zone with a density of 240 individuals per litre. The greatest relative decrease in mean abundance occurred between salinity intervals 0 – 2 and 2 – 4 ‰ with decreases from 75.9 to 18.0 and from 75.2 to 13.2 individuals per litre for summers 2000 and 2001 respectively. The sampling undertaken during summer 2001 (spring tides of June and July) showed a sharp decline in veliger abundance with an increase in salinity from the freshwater zone 1 to the transition zone 2 (Figure 2.3). Densities were lowest during high and ebbing tides. The strong influence of salinity on veliger abundance, rather than geographical position, was evident when sampling was conducted from the fixed stations.

The presence of veligers in early summer appears to be determined by the water temperature as spawning occurs around 18 °C (Sprung 1993). During the first cruise (4 June 2000), their abundance was sparse, consistent with the low water temperatures, between 12.5 °C and 15 °C. For the second cruise (26 June 2000) onwards, water temperatures were > 18 °C and the veligers were abundant at low salinities (< 2 ‰). Date had a significant effect on the variation in veliger

concentrations (df = 3, 31; F = 5.58, p = 0.0035). Veliger densities from cruise 2 were significantly higher than those from cruises 3, 4 and 5. Densities from cruises 3, 4 and 5 were not significantly different. This was also evident in the RDA results with the near absence of correlation between veligers and cruises 3 and 5 (Figure 2.4).

2.3.2. Vertical distribution (summer 2001)

Veligers in Zones 1 and 2 were homogeneously distributed throughout the water column. No vertical stratification in distribution was observed (Table 2.1; Figure 2.3), even in stratified water columns. However, the zone and tidal state affected veliger abundance. There was a sharp decline in veliger densities when going from Zone 1 to 2 and tidal state played a significant role in influencing veliger densities in the transition zone only. Consistent with these results, there was no statistical relationship between Δ_{vel} and Δs when all the transition zone stations were considered (Spearman's rho = 0.02, $p > 0.05$, n = 19) nor when only stratified stations were considered (Spearman's rho = -0.02, $p > 0.05$, n = 9). These results underscore the homogeneous distribution of the veligers in the water column, regardless of stratification and mixing conditions.

2.3.3. Relationships between environmental, spatial and biological variables (RDA)

Six of the nine environmental variables were selected using forward selection ($p < 0.05$): temperature, salinity, turbidity, TP, cruise 3 and cruise 5. After environmental variable selection, the Monte Carlo tests of significance for the first canonical axis of step 1) yielded an eigenvalue of 0.399 (F-ratio = 17.926, p = 0.001) and of 0.630 for all canonical axes (Table 2.2).The first two axes explained 53.9% (39.9 + 14.0) of the total variance in the species data. The fraction of the species-environment variation that was jointly explained by the first two axes was high (0.855), thus little information on species-environment relationships was lost by discarding the additional axes. Since the eigenvalue of the third axis was small compared to the first two ($\lambda_1 = 0.399$, $\lambda_2 = 0.140$, $\lambda_3 = 0.074$), it can be ignored as can the higher numbered ordination axes. Veliger densities, Chl. *a* biomass and PC

picoplankton were positively cross-correlated, with temperature and turbidity strongly related to them (Figure 2.4). For interpretations of the biplot diagram, arrows pointing in the same direction indicate positively correlated variables, perpendicular arrows indicate lack of correlation and arrows pointing in opposite directions indicate negatively correlated variables. The angles between environmental and species arrows and the length of the arrows can be used jointly to infer the direction and intensity of species responses to environmental variables (ter Braak 1998). However, RDAs only reveal the shared variation among species variables explained by the environmental variables. Thus the relationship between veliger abundance and the species variables was verified separately from this analysis and revealed that veligers were indeed positively correlated with Chl. *a* (Pearson $r = 0.48$, $p = 0.004$) and PC picoplankton (Pearson $r = 0.45$, $p = 0.008$). Salinity and TP were negatively related to the veliger densities and Chl. *a* biomass, but positively correlated to PE picoplankton. Turbidity and temperature were negatively correlated with salinity and TP.

Among the nine terms in the surface polynomial, the following ones were retained in the forwards selection: $Z = b_1X + b_2Y + b_3XY + b_4X^2 + b_5Y^2 + b_6X^2Y + b_7XY^2$. After variable selection, the Monte Carlo tests of significance for step 2) for all canonical axes yielded an eigenvalue of 0.239 which was not significant (Table 2.2). Variation partitioning indicated that the total variation explained by environmental and spatial variables together accounted for 74.4% of the total variation while 25.6% was left unexplained. The environmental variables accounted for 63% of the total variation explained in the species matrix. Approximately 23% of this variation can also be predicted by the spatial matrix. Only 15% of the variation is attributable to spatial effects and cannot be related to the measured environmental variables (Table 2.2). This portion of variation acts partly as a descriptor of unmeasured underlying processes such as external causes or underlying biotic processes such as growth, reproduction, predation, input from various source populations and stress dependent mortality. The purely spatial effect was weak or insignificant. This statistical result is to be expected given that water masses are

advected up and down the estuary by as much as several tens of kilometres over each semidiurnal tidal cycle, and rapid changes take place at any fixed x-y locus. Biological and chemical properties are therefore more closely related to the shifting salinity field rather than geographic position. As for the environmental variable component, 66% of the total variation is accounted for by the purely environmental effects, indicative that the selected variables are effective descriptors of the species data in the ETZ. This underscores the influence of the position of the salinity front in the transition zone and its subsequent effects on community composition.

2.3.4. Food quality

According to the approximate limits set by Healey and Hendzel (1980) and Hecky et al. (1993), the stoichiometric ratios obtained of the ETZ seston showed no apparent nutrient deficiencies (Figure 2.5). The values for C: P, N: P and C: N (μmol: μmol) were consistently below the thresholds for limitation, with the exception of the C: N ratio where N seemed moderately deficient. The C: Chl. *a* ratios were exceptionally high, which may indicate general nutrient deficiency or high concentrations of heterotrophic organisms as well as detritus. Turbidity was highly positively correlated with particulate C, N and P (Figure 2.6). The C: N: P ratios (Table 2.3) lay well below the Redfield ratio of 106:16:1 which reveals the absence of nutrient limitation in the ETZ seston. In addition, SRP and nitrate were always at detectable levels (means = 0.011 ± 0.004 mg L^{-1} and 0.28 ± 0.076 mg L^{-1} respectively) implying adequate nutrient supply for the phytoplankton. Finally, veliger densities were weakly but positively correlated with the PP/TP ratio (r^2 = 0.18, p = 0.0001, n = 46) as well as with PP (r^2 = 0.14, $p < 0.05$, n = 46), indicative of their association with good quality seston.

2.3.5. Particle aggregation

Particle size appears to be one aspect that affects the food availability for zebra mussel veligers (Sprung 1993; S. Bernier, Département de biologie, Université Laval, Ste-Foy, Québec, G1K 7P4, unpublished data). We therefore examined the extent of cell aggregation across the ETZ given the known flocculation characteristics

of salinity gradients (Kranck 1979). We found a strong decrease in the bio-availability of the veligers' prey (Figure 2.7). This effect was greatest for the autotrophs with a 2.9 fold increase of organisms within aggregates (> 10 μm) from salinity interval 1 - 2 ‰ to 2 – 3 ‰. At this latter interval, up to 80% of the potential autotrophic prey were within aggregates and thus too large to be consumed by veligers.

2.4. Discussion

2.4.1. Spatial distribution of the veligers

The pattern of change in the veliger distribution across the ETZ showed several characteristics which are consistent with the joint effects of abiotic and biotic controlling factors. The changes in veliger density during the summer 2000 cruises can be explained by: (1) salinity, (2) stress due to advective transport, (3) retention and turbidity, (4) bio-availability of the veligers' prey, and (5) predation.

Salinity

The longitudinal distribution of zebra mussel veligers in the St. Lawrence ETZ was a strong function of salinity and tidal state. The highest densities were found at salinities below 2 ‰, but individuals were found up to 10 ‰. The maximum decrease in veliger abundance occurred around 2 ‰ which corresponds to a lower tolerance threshold than that reported in the literature. These results are consistent with the recent findings of S. Bernier (Département de biologie, Université Laval, Ste-Foy, Québec, G1K 7P4, unpublished data) who noted a sharp decrease in filtration rates of veligers at salinities greater or equal to 2 ‰. Past studies have indicated that their lethal incipient tolerance to salinity is at ~4.5 ‰ and maximum growth rates have been observed up to 2 ‰ (Strayer and Smith 1993; Kilgour et al. 1994; Wright et al. 1996). Their tolerance to salinities between 4 ‰ and 5 ‰ is for a short period of time, depending on the water temperature and acclimation period. Veliger abundance was positively correlated to temperature, but this is likely to reflect the close negative correlation between temperature and salinity. Temperature is not likely to have had a strong negative impact on the veligers as it never dropped

below 11.5 °C in the ETZ and veligers can tolerate temperatures between 0 and 29 °C (Sprung 1993 and references cited therein). At salinities exceeding 4 ‰, this freshwater species' physiological state deteriorates quickly and growth is inhibited (Sprung 1993). Zebra mussel veligers are extremely sensitive to sudden changes in salinity but their tolerance to such changes increases significantly if these changes are gradual (Strayer and Smith 1993; Kilgour et al. 1994). In the ETZ, the prevailing high mixing rates may not allow for an appropriate acclimation period. The veligers may thus be exposed to abrupt changes in salinity associated with this mixing and the different tidal regimes, which may explain the sharp decrease in abundance at 2 ‰.

Stress due to advective transport

Horvath and Lamberti (1999) found that veligers were highly susceptible to damage by physical forces (i.e., shear and turbulence) and that mortality in high current streams (> 1.0 m s^{-1}) could limit veliger survival during downstream transport. Veliger densities from summer 2001 cruises showed a significant decline from Zone 1 to 2, even during low tide (salinity < 0.5 ‰). This may imply that mortality due to advective transport could have come into play. As for the effect of turbulence on the veligers, those collected in the highly mixed waters of the ETZ were active, i.e., swimming and gut filled, at salinities varying between 1 ‰ and 3 ‰ (C. Barnard, unpublished data). The physiological state of the veligers was not thoroughly examined, yet their presence or absence appears to be representative of their abundance since the abundance dropped significantly from one fixed station to the other over a fairly short distance. The decline from Zone 1 to Zone 2 could be indicative of the negative impact of hydrodynamic forcing, salinity or other factors discussed below.

Retention and turbidity

Retention by hydrodynamic trapping can explain the high densities observed at stations N - 0 and N - 01. Frenette et al. (1995) documented a prolonged residence time for cells (2 - 200 μm) in the ETZ and pathways that favour retention of larger particles (greater than 20 μm). Veligers could then be subjected to this hydrodynamic

trapping, a consequence of opposing currents in stratified estuaries which position the organisms to be moved upstream on flooding tides and retained during ebbing tides (Frenette et al. 1995). This recirculation mechanism would tend to lower downstream advective losses in this stratified estuary.

The veligers were positively correlated with turbidity, implying that turbidity can be used as an approximate guide to their distribution in the ETZ. The turbidity maximum present in this part of the estuary is the result of the recirculation and retention mechanisms of suspended matter (Vincent and Dodson 1999). The stations where high veliger densities were found (N-0 and N-01) were situated directly in this maximum turbidity zone. Along with the potential accumulation of veligers due to hydrodynamic retention, these concentrations could be the result of the coriolis force in this section of the ETZ. These stations could also be receiving individuals originating from adult populations colonising the shores of Île d'Orléans.

Bio-availability of the veligers' prey

Kranck (1979) found that the particle size spectra of organisms in the maximum turbidity zone increases drastically when going from Zone 1 to Zone 2. According to this author, the majority of particles in the turbidity maxima (Zone 2) appear to be greater than 10 μm due to flocculation processes. Indeed, preliminary microscope results on the protist community of the ETZ revealed that the veligers' prey becomes unavailable to them due to the formation of aggregates, thus causing mechanical interference. This sharp decrease in prey bio-availability at salinity > 2 ‰ may be an important stressor to the veligers, in addition to salinity.

Predation

Previous studies conducted in the St. Lawrence ETZ observed sharp declines in Chl. *a*, photosynthetic picoplankton and protist concentrations between Zones 1 and 2 (Lovejoy et al. 1993; Frenette et al. 1995). The authors attributed this effect to zooplankton grazing, as there is a large standing stock of zooplankton in the ETZ (Vincent and Dodson 1999; Winkler et al. 2003). Predation pressures could then also be partially responsible for the decrease in veliger abundance. Wright et al. (1996)

observed that rotifer predation caused high mortality rates of their zebra mussel larvae culture. Mills et al. (1995) observed that alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) prey upon veligers but concluded that predation did not substantially reduce the number of veligers because they were either resistant to predation pressures and/or the predation rate was not high enough. In contrast, other authors have stressed the importance of predation on veligers by fish larvae (*Osmerus esperlanus, Lucioperca lucioperca, Acerina arnua,* and *Ruttilus rutilus*) (Sprung 1993).

2.4.2. Vertical distribution (summer 2001)

The veligers were evenly distributed throughout the water column, regardless of tidal state and zone. Even when the water column was stratified, no significant difference in abundance between surface, mid-column and bottom samples was observed. Kern et al. (1994) also observed homogeneous vertical distribution in the Rhine River which most likely had higher mixing rates than lakes where stratified distribution was observed under low wind conditions (Fraleigh et al. 1993; Garton and Haag 1993). With current speeds at approximately 2.5 m s^{-1} and horizontal and vertical mixing, the ETZ hydrodynamics are much more complex and intense than in lakes. The veligers are most likely undergoing passive transport under these conditions. The elevated current speed and mixing rates of the ETZ throughout the water column and the constant influence of the salinity front on the hydrodynamics most probably does not allow the veligers to actively maintain their position in a density gradient nor to passively settle. In this regard, veligers are also unlikely to have the capacity of actively moving to areas of high productivity to feed.

2.4.3. Temporal distribution

Certain authors have documented that a single massive explosion of individuals during the summer characterizes veliger larvae abundance in the plankton, while other authors have observed several pulses of high abundances over the summer (Stoeckel et al. 1997). These authors suggested that upriver source populations spawned in frequent distinct bursts throughout the summer rather than

just once or twice. Of the four cruises undertaken during summer 2000, our data revealed that veliger abundance was significantly higher during cruise 2 than during cruises 3, 4 and 5. Our data was not collected on a daily basis, but shows evidence of sporadic and distinct peaks in veliger abundance. This was consistent with the results obtained during the fixed station sampling of summer 2001 which showed large variation in veliger abundance between samples. Stoeckel et al. (1997) also observed the continual passage of pulses of individuals in a large river.

2.4.4. Food quality

Herbivores with high nutrient demands, such as the veligers, are frequently limited not by food quantity or available energy but by the quantity of mineral elements in their food, i.e., seston food quality (Sterner and Hessen 1994). It has been illustrated that food quality has a highly significant effect on the survival rates of *Dreissena* veligers (Wacker et al. 2002). Seston ratios have proven to be effective physiological indicators of the nutritional state of phytoplankton (Healey and Hendzel 1980). Veliger densities were weakly but positively correlated with the PP/TP ratio as well as with PP, indicating their association with good quality seston. The ratio PP/TP represents the amount of phosphorus within the particulate material (and potential prey items) relative to the total phosphorus. Under nutrient-poor or resource-limited conditions, organisms tend to have a high particulate carbon content relative to other nutrients (Sterner 1997). The seston throughout the ETZ has relatively low C: P, C: N and N: P ratios, indicative of its good overall nutritional quality and in this aspect is a favourable environment for veliger growth. According to pre-established criteria, the ratios suggest no severe limitation of single N or P of plankton in the water column (Healey and Hendzel 1980; Hecky et al. 1993) and SRP and nitrate were always at detectable levels suggesting adequate nutrient supply for the algae. The C: N: P ratios were generally in agreement, suggesting no simultaneous deficiencies. In general however, these did not correspond to the traditional Redfield ratio of 106:16:1. The concentrations of particulate matter in this dynamic estuary are presumably much higher and more variable than in typical oceanic environments. Redfield ratios tend to

be an exception rather than the rule in freshwater (Hecky et al. 1993) and studies have shown that although this ratio is found in the ocean and large lakes, it can vary greatly according to transient effects on cellular physiology (Falkowski 2000).

The high C: Chl. *a* ratios observed in the ETZ could potentially be interpreted as indicating a high general nutrient deficiency, or as a substantial detrital influence (Healey and Hendzel 1980; Hecky et al. 1993). The first scenario is highly unlikely considering the values of seston elemental ratios. As for the second scenario, some authors attempt to correct for the influence of detritus on particulate C, N and P, on the basis of a standard C: Chl. *a* ratio (Hecky et al. 1993). The latter assumption would not be appropriate for the St. Lawrence ETZ where physical gradients are pronounced. Even the C: Chl. *a* ratio of algae grown in culture varies significantly with varying degrees of nutrient stress and light conditions (Healey and Hendzel 1980). If detritus had an over-riding influence on the ratios, we would expect to find an increase of particulate C in the ratios with an increase in turbidity, but this was not the case. Moreover, there were no differences between the surface and bottom ratios, despite higher turbidities near the bottom (C. Barnard, unpublished data).

Järvinen et al. (1999) also observed high C: Chl. *a* ratios in large, turbid Lake Tanganyika which is characterized by low Chl. *a* concentrations. In the ETZ, Chl. *a* concentrations are tidally variable with lows occurring during high tide (Vincent and Dodson 1999). It has been shown that the phytoplankton in the ETZ are adapted to intermittent light rather than low light conditions (Vincent et al. 1994), which could result in lower cellular requirement for Chl. *a* and thus a higher C: Chl. *a* ratio.

Chl. *a* biomass, veliger abundance, particulate C, N and P were all positively correlated with turbidity. It is interesting to note that the turbidity does not seem to negatively affect Chl. *a* biomass nor seston quality. The positive relationship between turbidity and the biological variables is likely the result of the common influence of hydrodynamic retention on biotic as well as abiotic particles (Frenette et al. 1995). A similar peak in phytoplankton biomass, coincident with the turbidity maximum, has also been observed in the ETZ of the James River, USA (Moon and Dunstan 1990).

2.4.5. Impacts of veligers on the food web

The positive correlations between Chl. *a*, PC picoplankton and veliger concentrations implies that the veligers have no severe negative impacts on these food sources and that phytoplankton resources are not limiting for the veligers in the ETZ. These associations also suggest that veligers are subject to the same physical and chemical forces in the water column as the biological variables. These biological variables had a negative relationship with salinity and TP but a positive relationship with temperature and turbidity. As shown with variance partitioning, the spatial component did not have a strong influence on the biological community. On the other hand, the environmental variables were highly significant explanatory variables, underlying the pivotal role that the salinity front plays in structuring the biological community. The zone of high veliger abundance, the upstream portion of the ETZ, seems to be in a favourable environment for their growth with low salinity and abundant food resources. Bertrand and Vincent (1994) observed that picoplankton could contribute from 6% to 56% of the total Chl. *a* in the water column of the ETZ. This may imply that a high proportion of the Chl. *a* biomass is edible by the veligers (prey size spectrum 1 - 9 μm) (Sprung 1993; Wright et al. 1996). In the present study, the mean photosynthetic picoplankton densities at < 2 ‰ was 5.09 (standard deviation = 1.9) $x10^6$ individuals per litre. This is similar to the photosynthetic picoplankton densities previously documented by Bertrand and Vincent (1994) prior to the invasion. No decrease in the photosynthetic picoplankton densities of the ETZ has thus been observed since the arrival of this new species.

In summary, we found that the larval veligers of *Dreissena polymorpha* were abundant in the low salinity waters of the St. Lawrence ETZ. Their concentrations decreased exponentially with increasing salinity, with the maximum decrease observed at 2 ‰, suggesting salinity induced mortality, grazer or other loss processes. The veligers were homogeneously distributed throughout the water column, under all tidal regimes, even the water column was stratified. Despite their dominance in the ETZ plankton, the veligers have no severe effects on the lower food web since they

are positively correlated with Chl. *a* biomass, picoplankton densities and good quality seston. The high productivity of the ETZ may explain why the ecosystem does not appear to be negatively impacted by their invasion, i.e., the ETZ may have a sufficient surplus of food resources to support the high population of zebra mussel veligers in addition to the usual zooplankton residents. The high abundance of veligers may have an impact on higher trophic levels, but this was not assessed in our analysis. The low salinity waters (< 2 ‰) of the upper reaches of the St. Lawrence ETZ therefore provide a favourable environment for their survival. Future perspectives should investigate their impact on the biovolume size-spectrum of the microbial community and on higher trophic levels of this important larval fish nursery.

2.5. Acknowledgements

This research is a contribution to the programme of GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec) and CEN (Centre d'Études Nordiques). We thank Ladd Johnson, Connie Lovejoy and Paul Hamilton for assistance and support at various stages of the project. We also thank Hugues Boulanger, Julie Harnois, Kim Huggins, Brigitte Laberge, Marie-Audrey Livernoche, Carl Martin, Sébastien Roy, Gesche Winkler, the crew of the Willie DeRaspe and of the Marie-Karine D. for their help in the field and in the laboratory. The first cruise of summer 2001, conducted on the Willie DeRaspe, was possible thanks to the support of the Department of Fisheries and Oceans (Canada). Finally, we thank Dr. Christian Salesse for the use of his microscope and Andrea Bertolo for many helpful discussions on statistics. This study was funded by a strategic NSERC (Canada) grant led by Ladd Johnson, NSERC discovery grants to J.-J.F. and W.F.V., and a FCAR (Québec) post-graduate fellowship to C.B.

2.6. References

Bertrand, N., and Vincent, W.F. 1994. Structure and dynamics of photosynthetic picoplankton across the saltwater transition zone of the St. Lawrence River. Can. J. Fish. Aquat. Sci. **51**: 161-171.

Borcard, D., Legendre, P., and Drapeau, P. 1992. Partialling out the spatial component of ecological variation. Ecology **73**: 1045-1055.

Caraco, N.F., Cole, J.J., Raymond, P.A., Strayer, D.L., Pace, M.L., Findlay, S., and Fischer, D.T. 1997. Zebra mussel invasion in a large river, turbid river: phytoplankton response to increased grazing. Ecology **78**: 588-602.

d'Anglejan, B., and Smith, E. 1973. Distribution, transport, and composition of suspended matter in the St. Lawrence Estuary. Can. J. Earth Sci. **10**: 1380-1396.

de Kock, W.C. and Bowmer, C.T. 1993. Bioaccumulation, biological effects, and food chain transfer of contaminants in the zebra mussel (*Dreissena polymorpha*). *In* Zebra mussels: biology, impacts and control. *Edited by* T. Nalepa and D. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 503-533.

de Lafontaine, Y., Lapierre, L., Henry, M., and Grégoire, Y. 1995. Abondances des larves de moule zébrée (*Dreissena polymorpha*) et de Quagga (*Dreissena bugensis*) aux abords des centrales hydroélectriques de Beauharnois, Les Cèdres et Rivière-des-Prairies. Environnement Canada Région du Québec, Conservation de l'environnement, Centre Saint-Laurent. Rapport scientifique et technique St-14, Canada. pp. 52.

Falkowski, P.G. 2000. Rationalizing elemental ratios in unicellular algae. J. Phycol. **36**: 3-6.

Findlay, S., Pace, M.L., and Fischer, D.T. 1998. Response of heterotrophic planktonic bacteria to the zebra mussel invasion of the tidal freshwater Hudson River. Microb. Ecol. **36**: 131-140.

Fraleigh, P.C., Klerks, P.L., Gubanich, G., Matisoff, G., and Stevenson, R.C. 1993. Seasonal reproductive cycles and settlement patterns of *D. polymorpha* in

Western Lake Erie. *In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. pp. 129-143.

Frenette, J.-J., Vincent, W.F., Dodson, J.J., and Lovejoy, C. 1995. Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar. Ecol. Prog. Ser. **120**: 99-110.

Garton, D.W., and Haag, W.R. 1993. Seasonal reproductive cycles and settlement patterns of *D. polymorpha* in Western Lake Erie. *In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton, Fla. pp. 111-128.

Healey, F., and Hendzel, L. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Can. J. Fish. Aquat. Sci. **37**: 442-453.

Hebert, P.D.N., Muncaster, B.W., and Mackie, G.L. 1989. Ecological and genetic studies on *Dreissena polymorpha* (Pallas): a new mollusc in the Great Lakes. Can. J. Fish. Aquat. Sci. **46**: 1587-1591.

Hecky, R.E., Campbell, P., and Hendzel, L.L. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol. Oceanogr. **38**: 709-724.

Horvath, T.G., and Lamberti, G.A. 1999. Mortality of zebra mussel, *Dreissena polymorpha*, veligers during downstream transport. Freshwater Biol. **42**: 69-76.

Jarvinen, M., Salonen, J., Sarvala, J., Vuorio, K., and Virtanen, A. 1999. The stoichiometry of particulate nutrients in Lake Tanganyika - implications for nutrient limitation of phytoplankton. Hydrobiologia **407**: 81-88.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* sp.) veligers using cross-polarised light microscopy. Hydrobiologia **312**: 139-146.

Kern, R., Borcherding, J., and Neumann, D. 1994. Recruitment of a freshwater mussel with a planktonic life-stage in running waters: studies on *Dreissena polymorpha* in the Rhine River. Arch. Hydrobiol. **131**: 385-400.

Kilgour, B., Mackie, G., Baker, M., and Keppel, R. 1994. Effects of salinity on the condition and survival of zebra mussels (*Dreissena polymorpha*) Estuaries **17**: 385-393.

Kranck, K. 1979. Dynamics and distribution of suspended particulate matter in the St. Lawrence estuary. Naturaliste can. **106**: 163-173.

Legendre, P., and Fortin, M.-J. 1989. Spatial pattern and ecological analysis. Vegetatio **80**: 107-138.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? Ecology **74**: 1659-1673.

Lovejoy, C., Vincent, W.F., Frenette, J.-J., and Dodson, J.J. 1993. Microbial gradients in a turbid estuary: application of a new method for protozoan community analysis. Limnol. Oceanogr. **38**: 1295-1303.

Lucotte, M., and d'Anglejan, B. 1986. Seasonal control of the Saint-Lawrence maximum turbidity zone by tidal-flat sedimentation. Estuaries **9**: 84-94.

Mellina, E., and Rasmussen, JB. 1994. Occurrence of zebra mussel (*Dreissena polymorpha*) in the inter-tidal region of the St. Lawrence estuary. J. Freshwater Ecol. **9**: 81-84.

Mills, EL., O'Gorman, R., Roseman, EF., Adams, C., and Owens, RW. 1995. Planktivory by alewife (*Alosa pseudoharengus*) and rainbow smelt (*Osmerus mordax*) on microcrustacean zooplankton and dreissinid (Bivalvia: Dreissenidae) veligers in southern Lake Ontario. Can. J. Fish. Aquat. Sci. **52**: 925-935.

Moon, C., and Dunstan, WM. 1990. Hydrodynamic trapping in the formation of the chlorophyll *a* peak in turbid, very low salinity waters of estuaries. J. Plankton Res. **12**: 323-336.

National Laboratory for Environmental Testing. 1994. Manual of Analytical Methods, Volume 1, Major Ions and Nutrients. 84 p.

Pace, M., Findlay, S., and Fischer, D. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshwater Biol. **39**: 103-116.

Ricciardi, A., Neves, RJ., and Rasmussen, JB. 1998. Impending extinctions of North American freshwater mussels (Unionidae) following the zebra mussel (*Dreissena polymorpha*) invasion. J. Anim. Ecol. **67**: 613-619.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha. In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. pp. 39-53.

Sterner, R.W. 1997. Modelling interactions of food quality and quantity in homeostatic consumers. Freshwater Biol. **38**: 473-481.

Sterner, R.W., and Hessen, D.O. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annu. Rev. Ecol. Syst. **25**: 1-29.

Stoeckel, J.A., Schneider, D.W., Soeken, L.A., Blodgett, K.D., and Sparks, R.E. 1997. Larval dynamics of a riverine metapopulation : implications for zebra mussel recruitment, dispersal, and control in a large-river system. J. N. Amer. Benthol. Soc. **16**: 586-601.

Strayer, D.L., and Smith, L.C. 1993. Distribution of the zebra mussel (*D. polymorpha*) in estuaries and brackish waters. *In* Zebra mussels: biology, impacts and control. *Edited by* T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. Fla. pp. 715-725.

ter Braak, C.J.F. 1998. CANOCO a FORTRAN program for Canonical Community Ordination by [partial][detrended][canonical] correspondence analysis, principal components analysis and redundancy analysis (version 4.0). DLO-Agricultural Mathematics Group, Wageningen. The Netherlands.

Vanderploeg, HA., Liebig, JR., and Gluck, AA. 1996. Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena*

polymorpha): The importance of size and polyunsaturated fatty acid content. J. Great Lakes Res. **22**: 36-45.

Vincent, W.F., Bertrand, N., and Frenette, J.-J. 1994. Photoadaptation to intermittent light across the St. Lawrence estuary freshwater-saltwater transition zone. Mar. Ecol. Prog. Ser. **110**: 283-292.

Vincent, W.F., and Dodson, J.J. 1999. The St. Lawrence River, Canada-USA: the need for an ecosystem-level understanding of large rivers. Jpn. J. Limnol. **60**: 29-50.

Wacker, A., Becher, P., and Elert, E.V. 2002. Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha*. Limnol. Oceanogr. **47**: 1242-1248.

Winkler, G., Dodson, J.J., Bertrand, N., Thivierge, D., and Vincent, W.F. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. Mar. Ecol. Prog. Ser. **251**: 59-73.

Wright, DA., Setzler-Hamilton, EM., Magee, JA., Kennedy, V.S. and McIninch, S.P. 1996. Effect of Salinity and Temperature on Survival and Development of Young Zebra (*Dreissena polymorpha*) and Quagga (*D. bugensis*) Mussels. Estuaries **19**(3): 619-628.

Table 2.1. The results from a split-split plot analysis of variance for the effects of depth (surface, mid-column, bottom), tidal state (high, intermediate, low) and zone (freshwater and transition) on veliger abundance for summer 2001 sampling from the two fixed stations in the St. Lawrence estuarine transition zone. n = 114; results in bold are significant. DF = degrees of freedom, MS = mean square. (a) main plot, (b) subplot, (c) sub-subplot.

Source of variance	DF	MS	*F* value	*P*
(a) Cruise	1	620.71	0.72	0.5524
Zone	1	224609.99	259.88	**0.0394**
Error 1*	1	864.27		
(b) Depth	2	321.40	5.87	0.0645
Zone x Depth	2	202.14	3.69	0.1234
Error 2**	4	54.72		
(c) Tide	2	3924.25	4.07	**0.0204**
Zone x Tide	2	10271.29	10.64	**<0.0001**
Depth x Tide	4	309.96	0.32	0.8631
Zone x Depth x Tide	4	547.31	0.57	0.6871
Error 3***	90	965.00		
Total	113			

* Error 1 = cruise x zone

** Error 2 = (cruise x depth) + (cruise x zone x depth)

*** Error 3 = (cruise x tide) + (cruise x zone x tide) + (cruise x depth x tide) + (cruise x zone x depth x tide) + Tidal replicates (cruise x zone x tide x depth)

Table 2.2. The results of variation partitioning in redundancy analysis. The relative and shared contribution of the environmental and spatial components to the variation in the species data are indicated.

		r^2	*P*
Step	1	0.630	**0.001**
	2	0.239	0.057
	3	0.488	**0.001**
	4	0.114	0.233
Total explained		0.744	
Pure environmental		0.488	
Pure spatial		0.114	
Shared		0.142	
Unexplained		0.256	

Table 2.3. Molar ratios of particulate organic carbon to nitrogen to phosphorus (C: N: P) for the seston (0 - 2 m) at the 12 stations sampled during summer 2000. Stations are indicated by a letter representing the channel (N = north, S = south, M = middle) followed by numbers indicating the salinity (0, 0 - 1, 1 - 3, 3 - 5, 5 + ‰).

Station	Cruise 2	Cruise 3	Cruise 4	Cruise 5
S-0	43:3:1	87:91:1	48:5:1	77:10:1
S-01	46:4:1	70:6:1	58:6:1	64:6:1
S-13	74:5:1	58:7:1	54:6:1	62:6:1
S-35	23:2:1	53:5:1	56:6:1	45:5:1
S-5+	16:1:1	50:6:1	34:5:1	68:6:1
M-13	28:2:1	61:6:1	54:6:1	n/a
M-35	40:3:1	30:3:1	54:7:1	n/a
N-5+	44:4:1	34:3:1	63:7:1	92:11:1
N-35	37:3:1	45:4:1	69:9:1	82:8:1
N-13	23:2:1	66:5:1	61:6:1	63:7:1
N-01	24:2:1	40:3:1	56:6:1	70:8:1
N-0	24:2:1	50:3:1	66:7:1	49:4:1

n/a= not available

2.7. List of figures

Figure 2.1. Estuarine transition zone (ETZ) of the St. Lawrence River, Québec, Canada. Summer 2000: stations are indicated by a letter representing the channel (N = north, S = south, M = middle) followed by numbers indicating the salinity (0, 0 - 1, 1 - 3, 3 - 5, 5 + ‰). Summer 2001: Triangles mark the fixed stations

Figure 2.2. Veliger larvae densities in the St. Lawrence estuarine transition zone (ETZ) as a function of salinity for (a) summer 2000 cruises where closed circle = 14 June, open circle = 15 July, closed square = 28 July, open square = 8 August and (b) summer 2001 fixed stations in zones 1 and 2 where closed circle = 25-29 June and open circle = 23-27 July . For both graphs, the greatest relative decrease in veliger occurs at ~ 2 ‰.

Figure 2.3. Veliger larvae densities as a function of tidal state and salinity in the estuarine transition zone (ETZ) for (a) cruise one (25-29 June 2001) and (b) cruise two (23-27 July 2001). For the transition zone, tidal cycles were further divided into flood and ebb tides, as veliger densities varied significantly between the two tidal regimes. Values are the means ± standard deviation (when available). Black = surface densities, white = mid-column densities, grey = bottom densities, triangles = salinity.

Figure 2.4. (a) Redundancy analysis biplot of the species matrix constrained by the environmental variables. Percent variance in species data for each axis is specified in brackets. Species arrows are solid lines. Significance of axis 1 is P = 0.001, significance of overall test is P = 0.001. Environmental variables are dotted lines with TP = total phosphorus. PC = photosynthetic picoplankton (phycocyanin-rich and/or Chl.*a*); PE = photosynthetic picoplankton (phycoerythrin-rich). (b) Chl. *a* biomass and picoplankton (PC and PE) densities are stacked as a function of the station for

each cruise. Note: picoplankton densities were available solely for cruises 3, 4 and 5. Cruises are indicated by colours: cross-hatched = 14 June (cruise 2), black = 15 July (cruise 3), white = 28 July (cruise 4), grey = 8 August 2000 (cruise 5).

Figure 2.5. Particulate nutrient stoichiometry and the C: Chl. *a* ratio of the seston in the St. Lawrence estuarine transition zone (ETZ) during summer 2000. Samples were taken from the surface (0 – 2 m). Indication of nutrient deficiency according to Healey and Hendzel (1980) and Hecky et al. (1993): P = P deficiency; N = N deficiency; G = general nutrient deficiency; single symbol = moderate deficiency; double symbols = severe deficiency. Stations are indicated by a letter representing the channel (N = north, S = south, M = middle) followed by numbers indicating the salinity (0, 0 - 1, 1 - 3, 3 - 5, 5 + ‰).

Figure 2.6. Relationship between the log of (a) particulate organic carbon (POC), (b) nitrogen (PON) and (c) phosphorus (PP) as a function of turbidity (Nephelometric Turbidity Units (NTU)) in the St. Lawrence estuarine transition zone (ETZ).

Figure 2.7. Percentage of organisms within aggregates (> 10 μm) as a function of salinity in the St. Lawrence estuarine transition zone (ETZ). The protist community was grouped into two functional groups: autotrophs = circles, solid line and heterotrophs = open triangles, dotted line. Organisms within these aggregates are not available to veligers due to their large size.

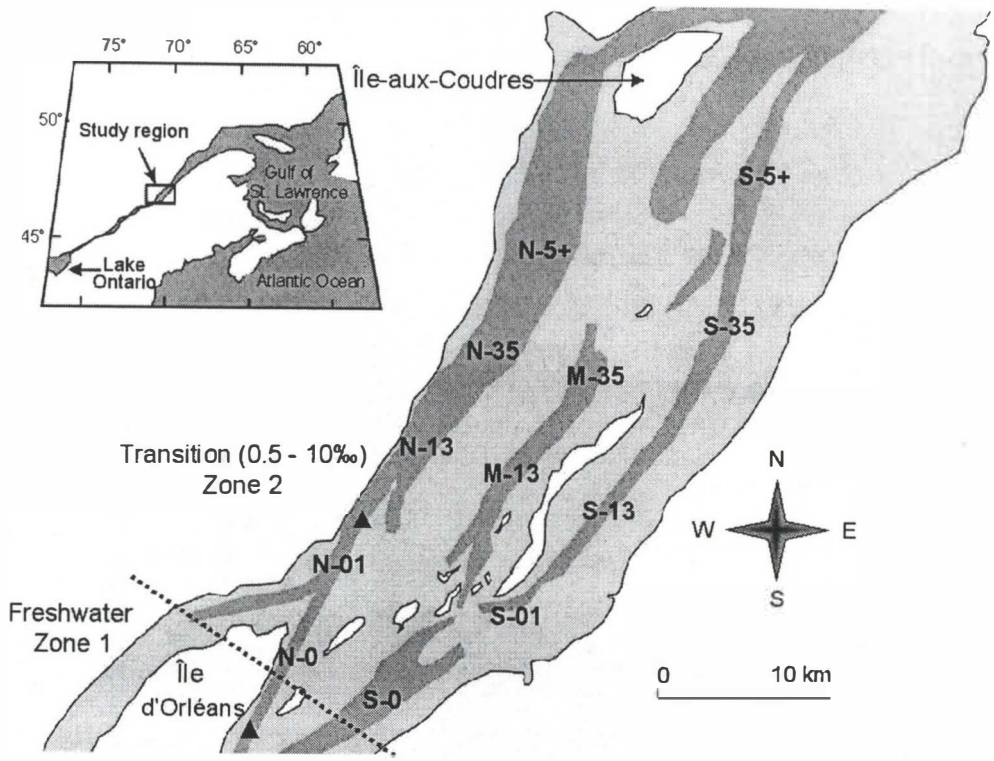

75°
70°
65°
60°
50°
45°
Study region
Gulf of St. Lawrence
Lake Ontario
Atlantic Ocean
Île-aux-Coudres
S-5+
N-5+
S-35
N-35
M-35
Transition (0.5 - 10‰)
Zone 2
N-13
M-13
S-13
N
W
E
S
N-01
S-01
Freshwater
Zone 1
N-0
Île
d'Orléans
S-0
0
10 km

Figure 2.1.

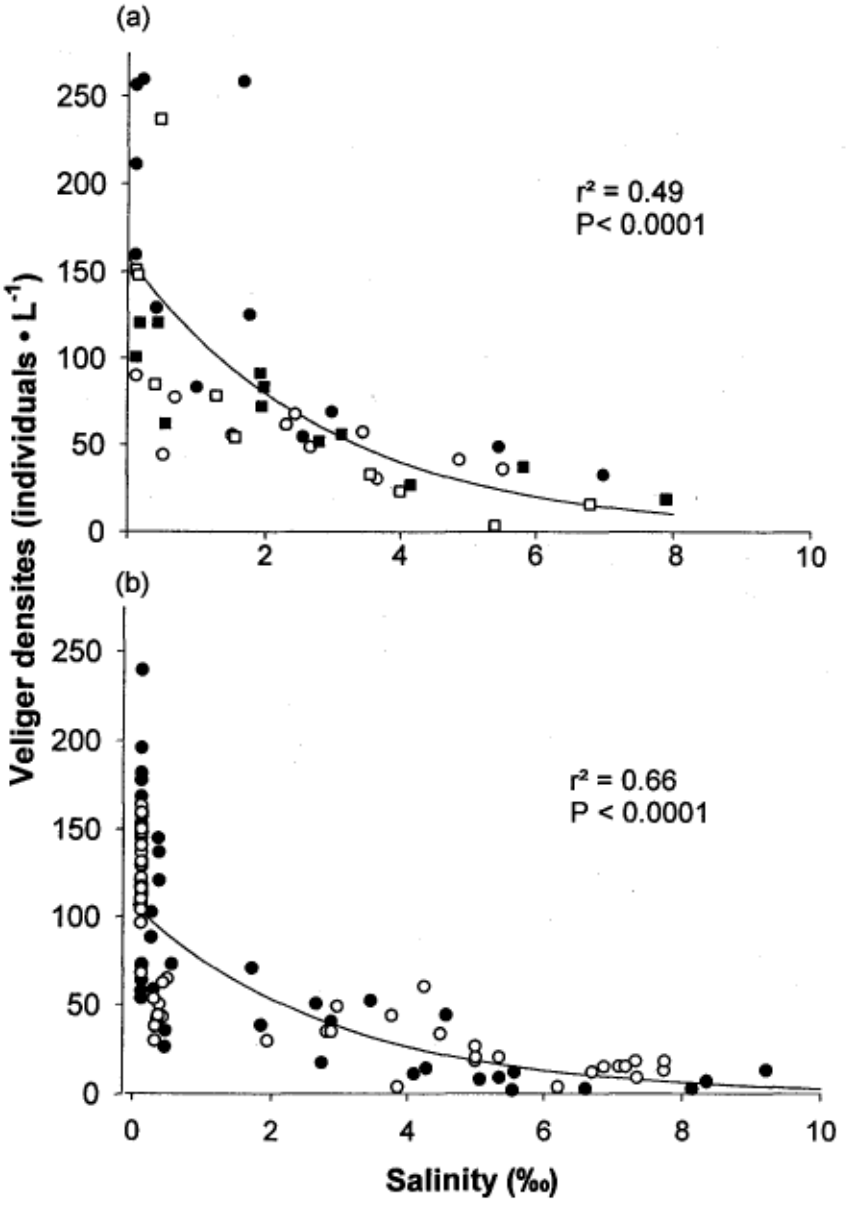

(a)
r² = 0.49
P< 0.0001
(b)
r² = 0.66
P < 0.0001
Veliger densites (individuals • L-1)
Salinity (‰)
0
50
100
150
200
250
2
4
6
8
10

Figure 2.2.

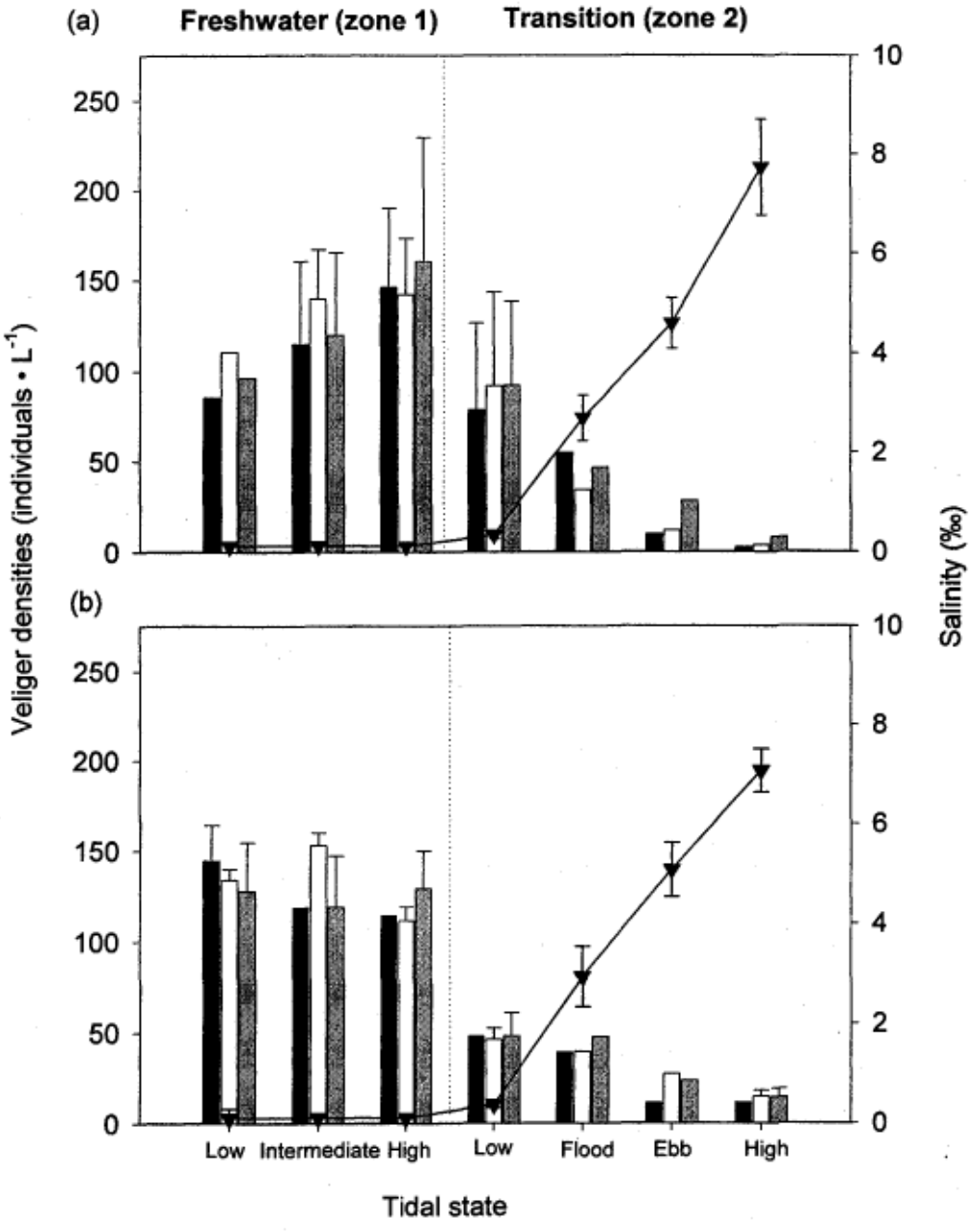

(a)
Freshwater (zone 1)
Transition (zone 2)
(b)
Veliger densities (individuals • L-1)
Salinity (‰)
0
50
100
150
200
250
0
2
4
6
8
10
Low
Intermediate
High
Low
Flood
Ebb
High
Tidal state

Figure 2.3.

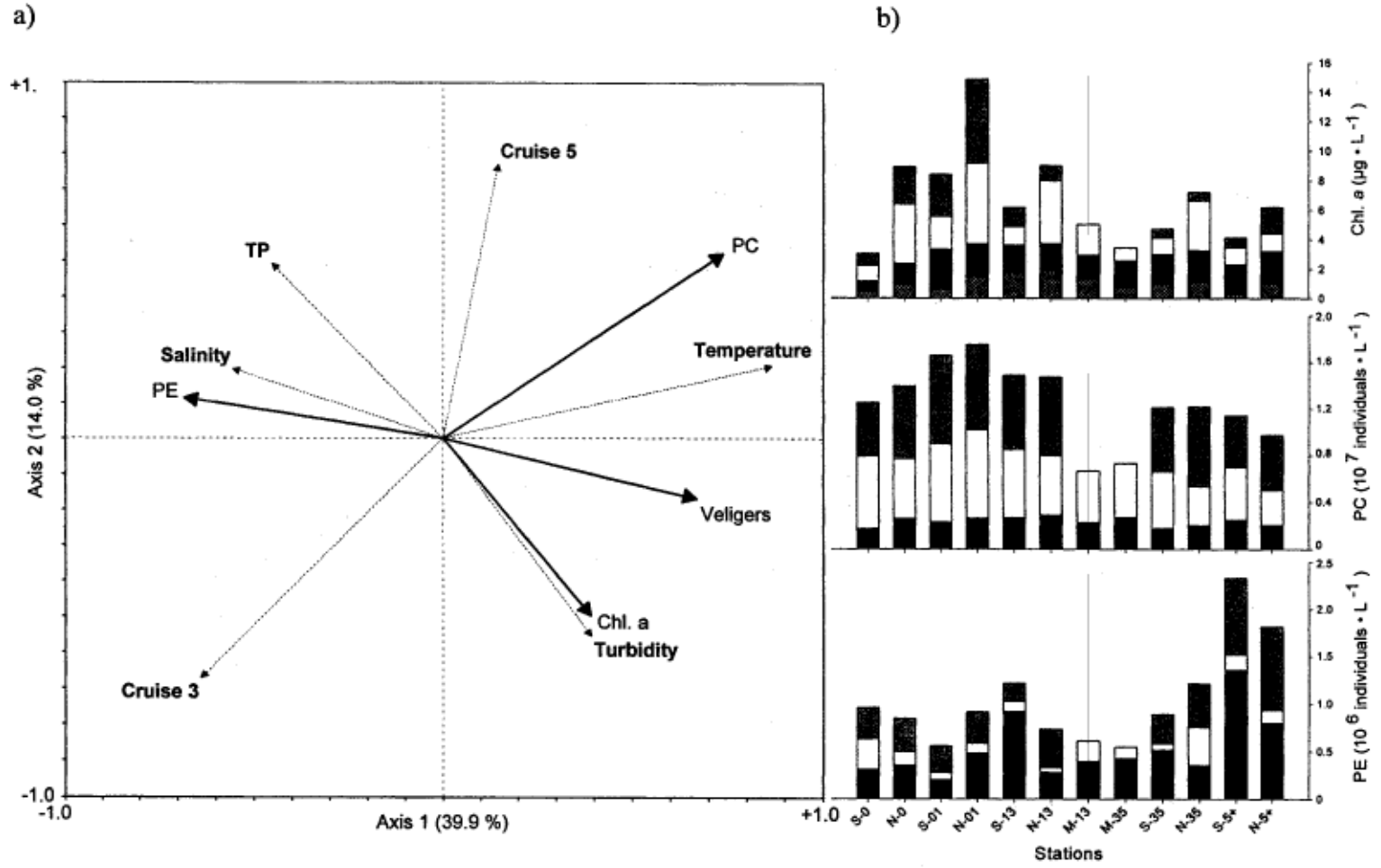

a)
b)
+1.
-1.0
-1.0
+1.0
Axis 2 (14.0 %)
Axis 1 (39.9 %)
Cruise 5
TP
PC
Salinity
Temperature
PE
Veligers
Chl. a
Turbidity
Cruise 3
Chl. a (µg • L-1)
PC (10 7 individuals • L-1)
PE (10 6 individuals • L-1)
S-0
N-0
S-01
N-01
S-13
N-13
M-13
M-35
S-35
N-35
S-5+
N-5+
Stations

Figure 2.4.

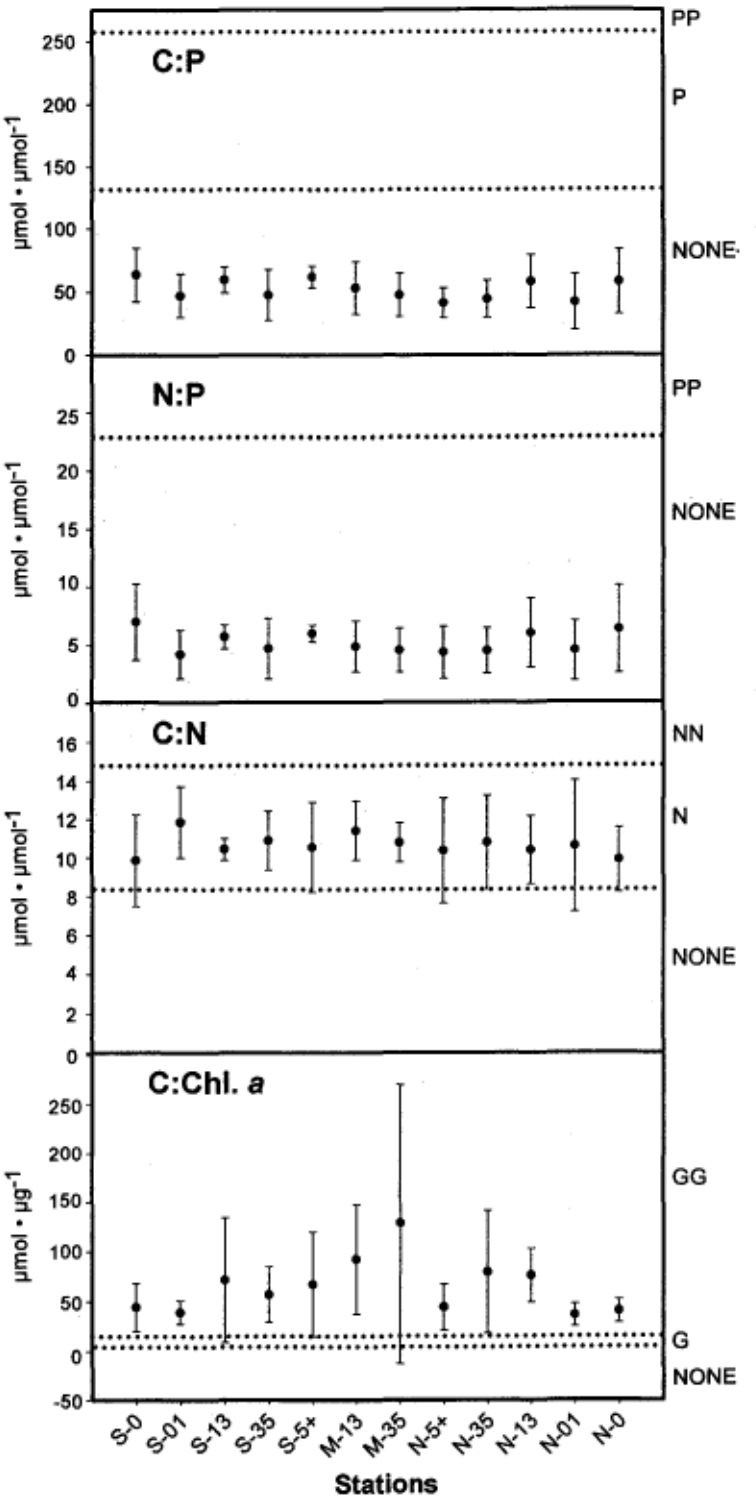

C:P
µmol • µmol-1
PP
P
NONE
N:P
µmol • µmol-1
PP
NONE
C:N
µmol • µmol-1
NN
N
NONE
C:Chl. a
µmol • µg-1
GG
G
NONE
S-0
S-01
S-13
S-35
S-5+
M-13
M-35
N-5+
N-35
N-13
N-01
N-0
Stations

Figure 2.5.

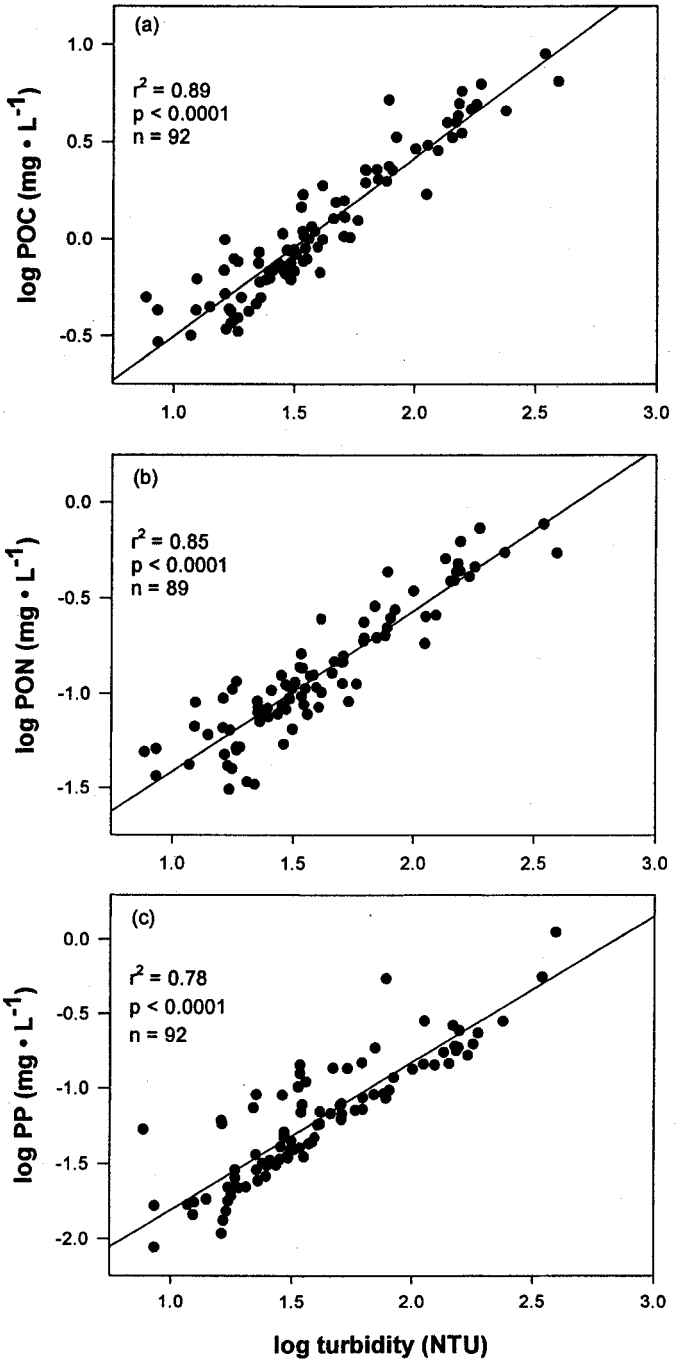

(a)
r^2 = 0.89
p < 0.0001
n = 92
log POC (mg • L^{-1})
(b)
r^2 = 0.85
p < 0.0001
n = 89
log PON (mg • L^{-1})
(c)
r^2 = 0.78
p < 0.0001
n = 92
log PP (mg • L^{-1})
log turbidity (NTU)

Figure 2.6.

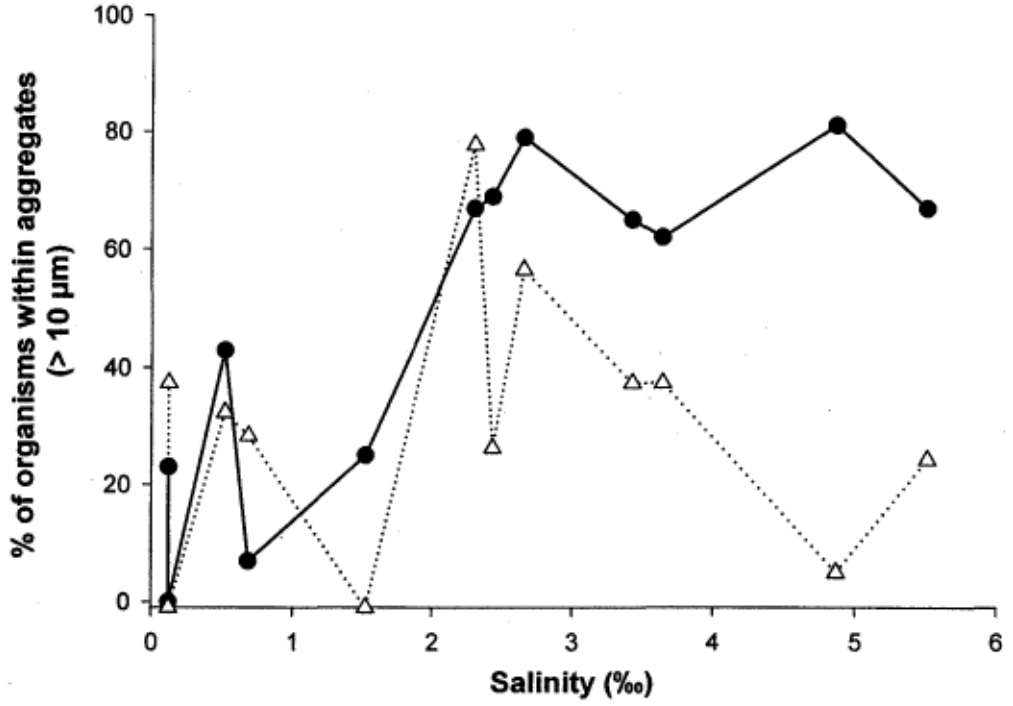

% of organisms within aggregates (> 10 µm)
100
80
60
40
20
0
0
1
2
3
4
5
6
Salinity (‰)

Figure 2.7.

CHAPITRE 3

RÔLE TROPHIQUE DES LARVES VÉLIGÈRES DE LA MOULE ZÉBRÉE ET LEUR ASSIMILATION DE CARBONE ORGANIQUE DISSOUT

TROPHIC POSITION OF ZEBRA MUSSEL VELIGERS AND THEIR USE OF DISSOLVED ORGANIC CARBON

Résumé

La structure du réseau trophique de la zone de transition estuarienne (ZTE) du fleuve Saint-Laurent a été analysée ainsi que le rôle trophique exercé par les larves véligères de moule zébrée, *Dreissena polymorpha*, une espèce envahissante. Les sources de carbone qu'assimilent les organismes de la ZTE et leurs rôles trophiques ont été établis à l'aide d'isotopes stables de carbone et d'azote ($\delta^{13}C$ et $\delta^{15}N$). Les valeurs de $\delta^{13}C$ variaient de -31.2 ‰ (seston) à -16.1 ‰ (poisson adulte) et celles de $\delta^{15}N$ variaient de 2.6 ‰ à 17.4 ‰. Le réseau trophique semble être largement supporté par la matière autochtone (production primaire in situ) plutôt que par la matière allochtone (d'origine terrestre), malgré les importantes quantités de subsides terrestres reçues par ce système. Les relations trophiques démontrent que les véligères utilisent des sources de carbone semblables à celles ingérées par d'autres consommateurs primaires, mais dans des proportions différentes. Les valeurs isotopiques du poisson n'indiquent pas une assimilation significative de véligères. Les valeurs de $\delta^{13}C$ indiquent que les véligères s'alimentent de bactéries libres, de carbone organique dissous (COD) et d'algues d'eau douce. Afin de tester la possibilité d'une assimilation de COD par les véligères, nous avons exposé les véligères aux lysats d'algues radio marqués. Nous avons démontré qu'elles peuvent assimiler rapidement le COD et incorporer ce carbone dans leur biomasse à un taux équivalent à 6% de leur poids sec en tissus mou par heure. Les véligères semblent avoir la capacité d'utiliser directement le COD autochtone, sans l'intermédiaire du réseau microbien, et occupent ainsi une position trophique unique dans le réseau alimentaire de la ZTE, sans entrer en interaction direct avec d'autres composantes du réseau trophique. Cette position trophique unique pourrait expliquer l'absence d'impacts sévères des véligères sur la communauté planctonique.

Abstract

We evaluated by stable isotope analysis the trophic structure of an estuarine transition zone (ETZ) food web and the role of an invasive species, the veliger stage of the zebra mussel *Dreissena polymorpha.* In the St. Lawrence ETZ, where zebra mussel veligers are now the dominant zooplankton in summer, $\delta^{13}C$ ranged from -31.2 ‰ (seston) to -16.1 ‰ (adult fish) and $\delta^{15}N$ ranged from 2.6 ‰ to 17.4 ‰. Isotopic analysis of samples indicated that the overall food web was largely supported by autochthonous phytoplankton rather than by allochthonous terrestrial carbon. Large differences among the isotopic signals of veligers, cladocerans and copepods suggested the use of different proportions of food items, and the isotopic values of fish larvae indicated no significant assimilation of veligers. The $\delta^{13}C$ signature of the veligers was in a range consistent with feeding on free-living bacteria and DOC or both, and freshwater algae incubated in situ. To investigate the possibility of DOC uptake by the veligers, we incubated veligers on ^{14}C-labelled algal lysates. There was rapid uptake of DOC and incorporation into biomass, equivalent to 6% of the soft tissue dry weight per hour. Zebra mussel veligers are likely using autochthonous DOC as an alternate food source, and they occupy an exotic trophic position in which there is little direct interaction with other major components of the ETZ food web.

Key words: veligers, stable isotopes, estuary, DOC uptake, zebra mussel, St. Lawrence

3.1. Introduction

Estuarine transition zones (ETZ), where riverine waters first mix with seawater, are typically rich in both allochthonous (terrestrial) and autochthonous (in situ photosynthetic) organic matter. They also contain abundant consumer populations, but little attention has been given to the sources and pathways of carbon flow. Some studies have argued for a dominant role of allochthonous carbon in sustaining food webs in rivers (McCallister et al. 2004), while other authors have stressed the greater importance of autochthonous sources (Martineau et al. 2004 and references therein). The St. Lawrence River ETZ has long been recognized as supporting high biological productivity and an important larval fish nursery, with large standing stocks of phytoplankton and zooplankton (Frenette et al. 1995; Winkler et al. 2003). In spite of the ecological importance of this ETZ, there has been no analysis of its overall food web structure.

Like many waterways in North America and elsewhere, the St. Lawrence River has recently been invaded by the zebra mussel, *Dreissena polymorpha,* and since July 1994 large concentrations of zebra mussel larvae (veligers) have been registered in the river and ETZ. These veligers are now recognized as the dominant zooplankton component, constituting between 52 and 90% of the total zooplankton counts in summer (Winkler et al. 2005). The larvae are likely retained and concentrated in the ETZ by the same physical mechanisms that cause the high turbidity (Frenette et al. 1995). There is now an extensive literature on the effects of adult zebra mussels in aquatic ecosystems; however the ecological role of their abundant veliger stage has rarely been addressed.

Our previous distributional analysis of veligers and microbial food web components in the St. Lawrence ETZ indicated that there was little effect of this invasion on the pelagic microbial community, but we also drew attention to the need to analyze potential impacts on both primary consumers that may utilize similar resources and on higher trophic levels that could potentially feed on veligers (Barnard

et al. 2003). Laboratory experiments have shown that veligers feed on particles ≤ 10 μm, including bacteria, protists and detritus (Sprung 1993; Wacker et al. 2002); however, there is increasing evidence that adult zebra mussels are able to use dissolved organic carbon (DOC) as an alternate carbon source (Roditi et al. 2000). Recent measurements of DOC uptake in combination with chemical analysis of monomers such as acetate and amino acids in natural DOC showed that adult zebra mussels could potentially obtain 10-25% of their metabolic needs for carbon from DOC in river water (Baines et al. 2005). To our knowledge, similar experiments have not been conducted on zebra mussel veligers, although active DOC uptake is known for the larval stage of some marine invertebrates, including bivalves (Manahan et al. 1982).

The aims of the present study were to determine: 1) the trophic position of zebra mussel veligers in an ETZ ecosystem, the St. Lawrence River and upper estuary; 2) the potential interactions between these organisms and other food web components; 3) the primary carbon source for the overall ETZ food web and the carbon sources used by veligers; and 4) the capacity for DOC uptake by veligers. We applied stable isotope techniques ($\delta^{13}C$ and $\delta^{15}N$) to the ETZ and the upstream freshwater community during the summer period of maximum veliger abundance, and combined these measurements with experimental analysis of DOC uptake.

3.2. Materials and methods

3.2.1. Study area

The St. Lawrence River is the third largest river on the North American continent, extending 500 km from the Great Lakes to the sea. The turbid waters of the estuary extend 150 km from eastern tip of Île d'Orléans to the Saguenay River mouth, with a maximum turbidity zone located 30-45 km downstream of Québec City. Salinity in this freshwater-saltwater transition zone ranges from 0.2 to 10 and turbidity reaches several hundred NTU. There is a shift in the bacterial community

across the ETZ, from free-living to attached cells (Painchaud et al. 1995), and the food web structure changes substantially. Amphipods (*Gammarus* spp.) and cladocera (*Bosmina longirostris)* are the predominant zooplankton in the freshwater part of the estuary and in the South channel of the ETZ, shifting further into the ETZ to estuarine mysids (*Neomysis americana* and *Mysis stenolepis*), copepods (notably *Eurytemora affinis)* and the sand shrimp *Crangon septemspinosa.* The high primary production and zooplankton biomass sustain large stocks of fish larvae, with concentrations of 85 *Osmerus mordax* (rainbow smelt) and 153 *Microgadus tomcod* (Atlantic tomcod) per 100 m^3 (Winkler et al. 2003 and references therein).

3.2.2. Sampling sites

Zooplankton and ichthyoplankton were sampled in each of the three main channels of the ETZ (Fig. 3.1) in summer 2000 (*Stizostedium canadense*; Sauger) and 2001 (all taxa). *Dreissena bugensis* (quagga mussels) are also present in the St. Lawrence River, but are much less abundant than *Dreissena polymorpha* and represent less than 2% of the *Dreissena* population near Québec City (De LaFontaine, Y., pers. comm.). Quagga and zebra mussel veliger larvae can be readily distinguished since quagga veligers are kidney-shaped while zebra mussel veligers are D-shaped (Johnson 1995). The weak proportion of quagga veligers is consistent with the known preference of their adult stage for deep waters in the Great Lakes (> 20 m), and the paucity of such deep habitats in the St. Lawrence River upstream of Québec City. Adult zebra mussels were sampled 30 km upstream of the ETZ (Québec City) at U1, and at two sites within the ETZ (Berthier-sur-Mer and St. François). Fish samples were collected near Île Madame (*S. canadense* from 2000 sampling) and from a trap on the southern bank of Île-aux-Coudres (*O. mordax* and *M. tomcod*) at the downstream end of the ETZ. Larval zebra mussels (D-stage veligers) were collected within the ETZ and at U1. Dissolved inorganic carbon (DIC), DOC, and microbial components (seston fractions and bacteria) were sampled from 60 L of water collected during July and August 2003 at U2 and at the North Channel station

in the ETZ. Algal cultures were incubated in situ at the latter stations to estimate the isotopic signature of the phytoplankton.

3.2.3. DIC and fractionation estimates

To measure the $\delta^{13}C$ and concentration of DIC, amber bottles fitted with gas-tight septa (VWR cat # 15900-024) were filled with water obtained from tangential flow filtration with fluorocarbon polymer filters of 0.45 μm pore size (Millipore Pellicon system). For the purpose of comparing with the $\delta^{13}C$ of algae incubated in situ, we used DIC samples obtained during the incubation in August 2003. One sample at U2 and two samples at North channel (low and high tide) were kept cool and immediately sent to the G.G. Hatch Isotope Laboratories (Ottawa) for analysis using a TOC-Analyser (OI Analytical Model 1010) interfaced with a Delta Plus Isotope Ratio Mass Spectrometer (IRMS; Thermo Finnigan).

The $\delta^{13}C$ of algae was predicted from the $\delta^{13}C$ of dissolved CO_2, ($CO_{2(d)}$), the DIC species that is mainly fixed enzymatically by phytoplankton (Rau et al. 1996), and the expected fractionation by phytoplankton. In the St. Lawrence estuary during the summer, the average pH is 7.84, ranging between 7.6 and 8.2 (Hélie et al. 2002). The principal species of DIC is therefore bicarbonate, by several orders of magnitude. Equilibrium isotopic fractionation (ε_d) between bicarbonate and $CO_{2(d)}$ was calculated as (Mook et al. 1974):

$$\varepsilon_d = -(9.866 \pm 0.23) \times 10^3 / T + (24.12 \pm 0.78)\ ‰ \qquad (1)$$

where T = temperature in degrees Kelvin. For the North channel station, the average temperature between high and low tide was used. Algal fractionation (ε_a) was calculated using $CO_{2(d)}$ with the following equation from Laws et al. (1998):

$$\varepsilon_a = (\delta^{13}C_{DIC} - \delta^{13}C_a) / (1 + (\delta^{13}C_a / 1000)) \qquad (2)$$

where $\delta^{13}C_{DIC} = \delta^{13}C$ value of $CO_{2(d)}$ and $\delta^{13}C_a = \delta^{13}C$ value of the algae.

3.2.4. Seston

At 0.5 to1 m from the surface, 10 L water samples were collected in acid-washed polyethylene containers and kept cool in the dark. For seston isotopic ratios, the samples were passed through Nitex filters to obtain the following size fractions : < 10 μm (n = 11), 10-64 μm (n = 14) and > 64 μm (n = 14), which were filtered onto precombusted (4 h at 450 °C) GF/F filters and stored at -80 °C prior to stable isotope analysis.

3.2.5. Bacteria

Upon arrival at the laboratory, particle-containing water > 0.45 μm in 40 L of water was concentrated to 500 mL by tangential flow filtration with fluorocarbon polymer filters (Millipore Pellicon). The remaining 500 mL were passed through a 1.0 μm filter (n = 8) and this 0.45-1.0 μm fraction containing free bacteria was frozen at -80 °C. For bacteria in aggregates (n = 7), which constitute a substantial fraction of total bacterial biomass in the maximum turbidity zone (Painchaud et al. 1995), the > 10 μm fraction of the 500 mL concentrate was sonicated (100 W for 30 to 60 s) and then passed through a 1 μm filter and stored frozen at -80 °C.

3.2.6. Algae

To complement the prediction of algal $\delta^{13}C$ using ε, algae were incubated in situ to obtain their isotopic signatures. The use of total seston for estimation of the phytoplankton isotopic signature is inappropriate in the ETZ as terrestrial-derived detritus and aggregates are dominant components of the seston and thus obscure the signal of autochthonous carbon (Martineau et al. 2004). Three protist species representing genera that are abundant in the St. Lawrence ETZ were obtained from University of Toronto Culture Collection (UTCC): estuarine diatom UTCC 624 *Thalassiosira pseudonana*, freshwater mixotroph UTCC 336 *Cryptomonas* sp. and freshwater diatom UTCC 520 *Cyclotella* sp. (for detailed methods see Web Appendix, section 3a, page 157). The protists were incubated in situ at sites U2 and North channel in Fisherbrand™ regenerated cellulose dialysis tubing (12,000-14,000

Dalton MWCO). After a growth period of 7 days, the algae were filtered onto GF/F filters for isotopic analysis and stored frozen at -80 °C. Several tests were conducted to evaluate any boundary layer effects, DOC release from the tubes, and the isotopic equilibration time of the algae (Web Appendix 1).

3.2.7. Zooplankton and ichthyoplankton

Trawl sampling in the ETZ was carried out using two mesh-sizes of net, 64 μm for zooplankton and 500 μm for fish larvae. A centrifugal pump was used to obtain the veliger larvae (flow of 20 L min^{-1}) between 2 and 8 m depth at all stations. Five to ten adult zebra mussels were collected by hand at low tide at St. Francois, Berthier-sur-Mer and Québec City on rocky substrata near the shore. All organisms were preserved on ice until laboratory processing and then sorted by hand, washed with deionized water, freeze-dried and kept in a desiccator. The larger samples were ground using an agate mortar and pestle.

3.2.8. Laboratory preparation of veligers for isotopic analysis

The presence of high concentrations of sediment, detritus and microalgae created major difficulties for the sorting of small organisms, especially the veligers. We developed and applied an improved concentration protocol that yielded 20 composite samples with enough material (50-100 animals per sample) to give excellent resolution of the mass spectrometry peaks. For this revised protocol we used the colloidal silica matrix Ludox® AM (specific gravity of 1.2) to separate veligers from heavier seston, as previously employed for studies of other bivalve larvae (Tremblay et al. 1987), and centrifugation in water to eliminate lighter detritus and microalgae from the veliger samples. An acid treatment was used to remove carbonate shell material (Pennington and Hadfield 1989) which could have masked the dietary carbon signal.

3.2.9 Isotopic analysis

Samples were freeze-dried (zooplankton and bacteria) or air-dried at 60 °C (algae and seston) for 24 h and any carbonate was removed by exposure to HCl vapor. All isotopic analyses, except for bacteria and DOC, were carried out with an

elemental analyzer NC 2500 (CE instruments) coupled with an IRMS (VG Prism III, Fisons Instruments) at Commission Géologique du Canada, QC. Bacteria were analyzed at the G.G. Hatch Laboratory (Ottawa) using a Finnigan MAT DeltaPlus IRMS with an online elemental analyzer. DOC samples were analyzed at the same laboratory using a TOC-Analyser (OI Analytical Model 1010) interfaced with a Delta Plus IRMS (Thermo Finnigan). Stable isotope ratios were expressed in delta (δ) notation (‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (3)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio ^{13}C : ^{12}C or ^{15}N : ^{14}N. The reference standards for ^{13}C and ^{15}N were PeeDee Belemnite (PDB) and atmospheric N_2 respectively. The analytical error (SD) was 0.23 ‰ for carbon and 0.28 ‰ for nitrogen.

Trophic level (TL) for each sample was estimated by assuming a $\delta^{15}N$ trophic enrichment factor of 3.4 ‰. This value when applied to an entire food web with multiple trophic pathways and many species is a widely used average (reviewed in Post 2002). The natural variability in source N was corrected for by subtracting the value of $\delta^{15}N$ in local zebra mussels, assumed to occupy TL position 2 (Cabana and Rasmussen 1996):

$$TL = [(\text{mean organism } \delta^{15}N - \text{mean mussel } \delta^{15}N) / 3.4] + 2 \quad (4)$$

3.2.10. Radiolabelled DOC uptake

We obtained radiolabelled DOC by incubating an algal culture with ^{14}C-bicarbonate (Roditi et al. 2000). In brief, 370 kBq of $NaH^{14}CO_3$ was added to 500 mL of an exponentially growing culture of the euryhaline diatom *Thalassiosira pseudonana* (UTCC 624). After five days and an increase in algal biomass from 8.6 x 10^3 to 7.9 x 10^4 mg m^{-3}, the cells were filtered onto 1.0 μm filters and lysed in 500 mL of 0.2 μm filtered freshwater from Lake St. Pierre (Canada) in which naturally occurring DOC had been photo-oxidized (< 0.10 mg DOC L^{-1}) using a 450 W Hanovia UV lamp (Armstrong et al. 1966). The final concentration of the radioactive

DOC (filtered through a 0.2 μm membrane) used in the uptake experiments was 4.15 mg DOC L^{-1} with an associated radioactivity of 1.5 x 10^5 kBq, after dilution with DOC-free water. This concentration was similar to naturally occurring concentrations at the site where the veligers were collected (2.8 ± 0.5 mg DOC L^{-1}), downstream of Lake St. Pierre where concentrations averaged 5.0 ± 1.5 mg DOC L^{-1}.

Veligers were collected on the south shore of the St. Lawrence River at the outlet of fluvial Lake St. Pierre using slow moving horizontal 63 μm zooplankton net hauls. The veligers were gently transferred to 2 L polyethylene bottles which were filled to the rim with water (< 63 μm) to minimize sloshing and mechanical damage of the animals during their immediate transport to the laboratory. The veligers were kept at a temperature of 22 ± 2 °C at all times, similar to natural conditions. Upon arrival, they were left undisturbed for 45 to 60 min and then filtered between 150 and 63 μm to ensure uniformity in size (length = 102 ± 5 μm, width 78 ± 4 μm; n = 50). Veligers were then placed under a dissecting scope equipped with cross-polarization (Johnson 1995). Actively swimming veligers were isolated with a micropipette (10 μL) to minimize uptake of other particles and were transferred to DOC-free water a minimum of three times to minimize carry-over effects. One hundred veligers were placed into 2 mL of DOC-free water in modified glass scintillation vials which had 63 μm screens across the top and screw caps with holes (diameter 1.4 cm). Veligers were left undisturbed for 45 min to decrease stress and to standardize their nutritional status. Veliger activity was then evaluated by inspection under the dissecting scope. Blanks were prepared with veligers that were killed by being kept in water at 60 °C for at least 1 h.

Fifteen ml of freshly filtered (0.2 μm) radioactive DOC was added to each vial for the following time intervals: 5, 10, 20, 30, 40, 50, 60, and 120 min. Three trials were conducted with 100 live and dead veligers at each time interval. For blanks, the time intervals were 0, 10, 30, 50, and 120 min. When time was up, solution was sucked out of the vial through the 63 μm mesh. The veligers were thoroughly rinsed at least 3 times with DOC-free water. To dissolve the shell, 5 mol

L^{-1} HCl (40 µL) was added for 2 min. The pH was then neutralized with 5 N NaOH (45 µL). Veliger soft tissues were solubilized with 1 ml Hyamine Hydroxide (ICN Biomedicals) for 6 h at 60 °C. The vials were allowed to cool, filled with the scintillation cocktail CytoScint ES™ (ICN Biomedicals), shaken and placed in the dark for a minimum period of 7 days before reading by scintillation counter. All samples were counted in a Wallac 1409 liquid scintillation counter, with the counts corrected for quenching by external standards. To test for the effect of DOC on veliger behavior, 9 veligers were isolated and kept in each DOC-containing and DOC-free water for a time series of 0, 44, 103, 223, and 403 min. Swimming activity, ciliate movement, and tissue contraction were noted from microscopic observations at each time interval.

3.2.11. Uptake calculations

Clearance rate (CR) and net DOC uptake rate (U) were calculated as follows, modified from Baines et al. (2005):

$$CR = \ln(((^{14}C_{initial} - {}^{14}C_{veligers} \text{ at time } t) \times [DOC]) / {}^{14}C_{initial}) \times \text{mL solution / number of veligers} \quad (5)$$

$$U = CR \times \text{ng DOC mL}^{-1} \text{ in solution} \quad (6)$$

Similar rates were also obtained using a linear net-uptake model.

3.2.12. Autochthonous to allochthonous ratios of DOC

The DOC used in our uptake experiment was composed of autochthonous carbon derived from algal lysis. This type of DOC is considered labile, while allochthonous matter, originating from terrestrial sources, tends to be more recalcitrant and less suitable as a biological carbon source. In the natural river environment, both sources are likely to be present, and the extent of DOC utilization by veligers may depend on their relative concentrations. As one approach toward assessing the proportions of each type of DOC, we applied a synchronous fluorescence index (Belzile et al. 2002 and references therein) to 57 and 56 samples collected in 2001 at U2 and North channel respectively. Synchronous fluorescence scans (SFS) were recorded with a Cary Eclipse spectrofluorometer. The instrument

was used in synchronous mode with a slit width of 5 nm on both the excitation and emission side and a difference of 14 nm ($\Delta\lambda$) between both light beams, and the results were corrected for the inner-filter effect based on spectrophotometric measurements of the same water. The SFS waveband ratio 265-335 nm to 336-500 nm was used as a guide to the autochthonous: allochthonous DOC ratio, modified from Belzile et al. (2002).

3.3. Results

3.3.1. Overall trophic structure

The ETZ food web components were ranked according to their carbon and nitrogen isotopic ratios (Figs. 3.2, 3.3; Table 3.1). The $\delta^{13}C$ values ranged from -32.5 ‰ (seston < 10 μm) to -15.9 ‰ (adult *M. tomcod*) while $\delta^{15}N$ ranged from 2.6 ‰ (seston < 10 μm) to 18.1 ‰ (adult *S. canadense*). There was no obvious clustering of species into functional trophic groups (primary consumers versus predators, for example).

3.3.2. DOC and microbial food web

DOC, free bacteria and freshwater diatoms (*Cyclotella* sp.) and mixotrophs (*Cryptomonas* sp.) had overlapping $\delta^{13}C$ values, ranging from -30.2 to -18.6 ‰ (Fig. 3.3). The lowest values were for aggregate bacteria and the highest for the estuarine alga *Thalassiosira pseudonana*. Free bacteria had very similar $\delta^{13}C$ to that of DOC (Table 3.1) while the $\delta^{13}C$ of aggregate bacteria was about 4 ‰ lower and very close to that of the < 10 and 10-64 μm seston fractions (Fig. 3.2). Large differences in $\delta^{13}C$ were observed between the freshwater and estuarine algae (Fig. 3.3). There was much overlap in the $\delta^{15}N$ at this lowest trophic level, ranging between 3.4 and 9.7 ‰, with the highest values obtained for the mixotrophs and the lowest for the free bacteria (Fig. 3.3).

3.3.3. DIC and fractionation estimates

Measured $\delta^{13}C$ of DIC and calculated $\delta^{13}C$ of $CO_{2(d)}$ are shown in Table 3.2. These were within the range of previously obtained values in the St. Lawrence upper estuary (Hélie et al. 2002).

3.3.4. Seston and primary consumers

For seston, means for July and August and high and low tides were calculated. Seston $\delta^{13}C$ for the < 10 μm fraction was well below the values of all components of the food web, including the primary consumers *Dreissena polymorpha*, *Keratella* sp., *Bosmina longirostris* and *Eurytemora affinis*. The $\delta^{13}C$ increased with increasing size fraction, averaging -31.21 ‰, -28.1 ‰, and –25.5‰ for the fractions < 10, 10-64 and > 64 μm respectively (Table 3.1). The four primary consumer species in the ETZ ranged in $\delta^{13}C$ from –22.5 to –20.5 ‰ with a mean of –21.0 ± 0.9 ‰, well above the seston values. There was no overlap in $\delta^{13}C$ of these four consumers. The disparity between the seston and the primary consumers was even more striking for the < 10 μm fraction which contributes 55-80% of total seston carbon, with $\delta^{13}C$ averaging about 11 ‰ below the primary consumers. The $\delta^{15}N$ for the seston fractions averaged 6.6 ± 2.3, 6.6 ± 1.6 and 7.1 ±1.2 ‰ respectively for the fractions < 10, 10-64 and > 64 μm, while primary consumers averaged 8.5 ± 1.9 ‰, with significantly higher values for the copepod *E. affinis*. The consumers of lowermost trophic rank, the zebra mussel veligers, had $\delta^{13}C$ intermediate between freshwater and estuarine diatoms incubated in situ in the river and were not significantly different from freshwater *Cyclotella* sp., DOC and free bacteria (Fig. 3.3). Much $\delta^{15}N$ variability was observed with the veligers, and these values overlapped with those of bacteria (free-living and aggregated), seston fractions, adult zebra mussels, and the rotifer *Keratella* sp.

Application of a 3-end-member mixing model (the MIXMODEL of Phillips and Gregg (2003)) to these isotopic data indicated that DOC could provide 26 – 33 % of food sources for the veligers with the remainder contributed by algae and free-

living bacteria. For this analysis we assumed fractionation by the veligers of 1 ‰ for ^{13}C and 3.4 ‰ for ^{15}N.

Zebra mussel veligers and adults showed similar average isotopic values and trophic ranking, implying a similar diet and carbon source (Table 3.1). There was no difference in the isotopic composition of *D. polymorpha* veligers between the upstream and ETZ areas, nor was there any difference between adult populations from the two regions. The veligers showed a gradual, linear enrichment in their ^{13}C content throughout the summer ($y = 0.0961x - 42$; $n = 14$; $r^2 = 0.59$). The $\delta^{13}C$ ranged from -26.8 to -19.3 ‰ with no significant difference between upstream U1 and ETZ samples ($t = -0.0456$; $n = 14$; df = 12; $p = 0.96$). Upstream veligers averaged a lower $\delta^{15}N$ (1.6 ‰) relative to those in the ETZ (6.3 ‰), but given the large variability within each population this difference was not significant ($t = 3.0$; $n = 6$; df = 4; $p = 0.13$). For the adults, there was no significant difference in carbon isotopic ratios between ETZ and upstream individuals ($t = 0.573$; $n = 8$; df = 6; $p = 0.59$). However the average $\delta^{15}N$ was higher (7.3 ‰) in the ETZ mussel tissues relative to upstream mussels (6.6 ‰; $t = 2.54$; $n = 8$; df = 6; $p = 0.04$).

Bosmina longirostris had a higher trophic position (2.4) relative to adult *D. polymorpha* (2.0) and veligers (1.7). Adult *D. polymorpha* had isotope ratios that were closer to those of *B. longirostris* for both carbon (depleted by 1.97 ‰) and nitrogen (depleted by 1.20 ‰) than veliger larvae, which were depleted by 2.71 ‰ and 2.17 ‰ respectively for carbon and nitrogen relative to *B. longirostris*. Statistical analysis of these results for adult *D. polymorpha* and *B. longirostris* confirm that enrichment in $\delta^{13}C$ was greater than 1 ‰ ($t = 5.05$; $n = 30$; df = 28; $p < 0.0001$) but the $\delta^{15}N$ enrichment was smaller than 3.4 ‰ ($t = -4.6097$; $n = 18$; df = 16; $p = 0.0002$), indicating an indistinguishable trophic level for these organisms according to the criteria of Post (2002). The differences in isotopic ratio between *B. longirostris* and veliger larvae were statistically greater than 1 ‰ for carbon

(t = 3.978; n = 20; df = 18; p = 0.0008) and equal to 3.4 ‰ for nitrogen (t = -0.994; n =12; df = 10; p = 0.34).

3.3.5. Secondary consumers

Gammarus sp. averaged –18.7 ‰ for $\delta^{13}C$ and 9.4 ‰ for $\delta^{15}N$ (Table 3.1). *Eurytemora affinis* and its larval nauplii had similar isotopic values (t-test: t = -0.115; df = 17; p = 0.91 for carbon; Mann-Whitney Rank Sum Test; T = 60.5; $n_{nauplii}$ = 6; $n_{E. affinis}$= 14; p = 0.87 for nitrogen) and thus occupied the same trophic level (Table 3.1). The sand shrimp *Crangon septemspinosa* averaged a relatively high trophic position (3.4) with a wide range of $\delta^{15}N$ suggesting large variations in diet among individuals. The mysids (*Mysis stenolepis* and *Neomysis americana*) averaged a trophic position of 3.6, just below that of the fish larvae *O. mordax* and *M. tomcod*, with $\delta^{13}C$ ranging between -21.1 and -18.9 ‰ (Table 3.1).

3.3.6. Fish populations

The abundant larval fish species of the ETZ, *O. mordax* and *M. tomcod,* had very similar isotopic ratios, and consequently trophic rankings (Table 3.1). For the adults, *O. mordax*, *M. tomcod* and *S. canadense* were statistically different in their isotopic ratios (Kruskal-Wallis One-way ANOVA; H = 9.24; df = 2; $p < 0.001$ for carbon and H = 9.791; df = 2; $p < 0.001$ for nitrogen). *S. canadense* occupied the highest trophic position (TL = 5).

3.3.7. DOC uptake and in situ composition

A mean net uptake rate of 0.031 ng (DOC) veliger^{-1} min^{-1} was calculated for the 2 h incubation of veligers on algal lysates, equivalent to 1.5 % of the total dry weight of veligers and 6 % of soft tissue dry weight per hour. The 2-h incubation gave a mean net uptake rate by veligers of 14.2 mg (DOC) g dry weight^{-1} h^{-1}. In the absence of DOC, the veligers contracted their tissues over time and were significantly smaller at 403 than at 0 min (t = 3.93; n = 9; df= 15; p = 0.001). Also, swimming activity eventually stopped and the cilia were completely contracted. However, in the presence of DOC there was some initial contraction then full recovery; swimming

activity was maintained throughout the experiment, and there were no significant differences in size between the beginning and end of the experiment (t = 0.40; n = 9; df= 15; p = 0.69). The SFS analysis of DOC in during July-August 2001 showed that the mean (± SE) autochthonous: allochthonous ratio was 1.65 ± 0.13 for freshwater station U2, and 1.26 ± 0.06 for the ETZ North channel station.

3.4. Discussion

3.4.1. Overall food web

The isotopic ranges observed in the St. Lawrence ETZ are similar to those in other estuarine ecosystems, with broad omnivory and an absence of distinct trophic levels (France et al. 1998). The pronounced $\delta^{13}C$ offset between consumers and seston and the relatively small $\delta^{15}N$ difference relative to primary consumers of lowest trophic rank, were previously reported by Martineau et al. (2004). This disparity was interpreted as evidence both of weak trophic coupling between consumers and the bulk seston and of strong selectivity by the consumers for prey items that constitute only a minor fraction of the total seston. The more negative seston values for the transition zone likely reflect the high detrital and terrestrial influence in this maximum turbidity zone and are consistent with the values obtained by Martineau et al. (2004). The present study extends these observations to other animals including veligers and higher trophic levels and shows that the entire ETZ food web is trophically decoupled from most of the particulate organic matter. This unusual feature sets the ETZ apart from many other types of aquatic ecosystems (see the intersystem comparison in Martineau et al. 2004). This was also observed in Bothnian Bay (Baltic Sea) where two separate trophic structures were noted. These included the smallest seston fractions (microbial components and terrigenous matter) with very negative $\delta^{13}C$ values and larger seston fractions (mesozooplankton) with higher $\delta^{13}C$ values, perhaps representative of the microbial-decomposer and the algal-grazer pathways (Rolff and Elmgren 2000) and consistent with our observations. Our

data lend support to the contention of high prey selectivity. The $\delta^{13}C$ values of the ETZ consumers are intermediate between that of the freshwater protists, > 10 μm seston, and estuarine algae, and consequently these consumers may rely on a combination of these sources. The observations are also consistent with a previous study which found that the maximum turbidity zone of the ETZ acted as a retention zone where diatoms and larger particles advected from upstream and produced in situ were concentrated, resulting in a rich feeding zone characterized by high diversity and productivity (Frenette et al. 1995). Aggregates and their associated organisms have been documented as an important food source for *Eurytemora* sp. (Zimmermann-Timm 2002). Due to the high $\delta^{13}C$ of the primary and secondary consumers of the ETZ relative to the freshwater algae, estuarine phytoplankton is likely to be a more important carbon source for the food web.

3.4.2. Zebra mussel carbon sources

The difference between veliger and < 10 μm seston isotopic characteristics implies that the veligers selected specific components of the total seston or that they used different carbon sources which were not well represented in our < 10 μm seston samples. The carbon signature of bacteria in aggregates resembled that of the total seston, which is dominated by detrital material of largely terrigenous origin (Martineau et al. 2004). The isotopic data imply that bacteria in aggregates are not an important food source, but they do not exclude direct feeding by the veligers on DOC, free-living bacteria or phytoplankton. There was much overlap between the $\delta^{15}N$ of the veligers and freshwater diatoms. Trophic $\delta^{15}N$ offsets are typically in the range 2-4 ‰ (but with large variability between systems; Martineau et al. 2004) and the results imply that upstream phytoplankton populations were not a major nutritional source for the veligers. The large variability in $\delta^{15}N$ of the veligers, however, implies nitrogen uptake from diverse sources.

The isotopic characteristics of both zebra mussel life stages deviated greatly from the seston, especially the smaller seston size class containing particles they are

likely to filter most efficiently (the size range 1 to 10 μm; Bernier 2003, Sprung 1993). As noted above, this disparity is representative of all ETZ animals and suggests a high selectivity for prey items. This feature is unusual relative to several other studies on mussels that have shown that the isotopic signature of the animals closely reflects that of the locally produced particulate organic matter (Thorp et al. 1998 and references therein). However, bivalve selectivity is known from other environments. For example, Raikow and Hamilton (2001) used both natural abundance and enrichment of ^{15}N to show that the freshwater bivalve *Sphaerium striatinum* preferentially assimilated the highly enriched living component of suspended and/or benthic organic matter, rather than assimilating the bulk material. Our results contrast markedly with those reported by Mitchell et al. (1996) who examined adult zebra mussels in Oneida Lake (New York). They observed highly depleted carbon ratios (-33.6 to -31.3 ‰) and concluded that the animals were using the entire seston resource rather than only one component, and that they were not feeding on sediment material ($\delta^{13}C$ = -26.2 to -25.6 ‰). The large variability in the $\delta^{15}N$ of veligers in this study may reflect the small number of samples, although each sample represented more than 50 individuals. Contamination of the samples is possible given the complex manipulations required to obtain a sufficient sample mass. However, it is likely that much of this variability is real and reflects broad omnivory by the larvae and variations in food composition.

3.4.3. Autochthonous $\delta^{13}C$

There were large differences in $\delta^{13}C$ among the three protist species incubated in situ. Given this interspecific variability, no additional precision was gained relative to using only DIC $\delta^{13}C$ and assuming a range of algal fractionation. However, the in situ incubation yielded algal biomass $\delta^{13}C$ that was substantially heavier than the seston (notably for the estuarine species), thereby corroborating the estimates based on fractionation. The in situ incubation data provided additional support for the contention that the isotope ratio of total seston is a poor indication of autochthonous

sources, particularly in ecosystems that receive large inputs of allochthonous materials and detritus. The incubation data point to the differences in $\delta^{13}C$ that can occur among phytoplankton species even within the same physical and chemical environment, as well as the variability between sites for the same species. Such variability could not have been discerned from the fractionation estimates.

The values in Table 3.3 for fractionation relative to $CO_{2(d)}$ compare well with those of Fogel et al. (1992) for phytoplankton-dominated seston in the Delaware Estuary (pH from 7.0 to 8.4). Marine diatoms have been shown to directly take up bicarbonate without the energy requiring steps of the enzyme carbonic anhydrase which converts accumulated bicarbonate to CO_2, thereby reducing the overall carbon isotope fractionation (Korb et al. 1997; Tortell et al. 1997). This direct uptake mechanism is known for *T. pseudonana* (Laws et al. 1998) which may explain the lower carbon isotope fractionation observed in our study for this species relative to the freshwater taxa.

3.4.4. Veligers as competitors with indigenous species

Veligers and indigenous zooplankton species do not appear to be sharing the same carbon sources. The trophic position of veligers was well below that of *B. longirostris*, implying that dietary overlap is unlikely between these taxa. The nitrogen and carbon isotopic ratios of the adult mussels were more similar to those of *B. longirostris* than to the veligers, and therefore interspecific competition is likely to be greater between *B. longirostris* and the adults. However, no adults have been observed on the fluvial bed of the ETZ (A. Casper, unpublished data, Biology Dept, Laval University, Quebec, Canada), hence any impacts on potential competitors should not be due to adult populations. Veligers had a trophic position (1.7) that was very close to that of the rotifer *Keratella* sp. (1.8), implying potential dietary overlap. Yet their $\delta^{13}C$ differed, indicating some dietary difference. Veligers may be feeding less efficiently in the ETZ than upstream due to the stress induced by high turbidity, high turbulence and changing salinity. This reduced feeding would be consistent with

the lack of significant difference between their upstream and downstream carbon signatures.

3.4.5. Veligers as prey for indigenous species

The $\delta^{15}N$ values of the adult fishes occupying high trophic positions (*S. canadense, M. tomcod* and *O. mordax*) were well above that of the veligers (more than one trophic position), indicating that the latter was not a major source of food for top predators. Consistent with our results, the stomach contents of *O. mordax* and *M. tomcod* larvae showed no evidence of predation on veligers by these species (H. Yoneyama, unpublished data; Biology Dept, Laval University, Quebec, Canada). Based on this same isotopic argument, similar conclusions pertain to *M. villosus* larvae because their isotopic ratios were similar to those of other larval fish. Furthermore, the veliger nitrogen isotopic ratios were about two trophic levels below those for the fish larvae, indicating that they were not a major component of the larval fish diet.

Consumers dependent on veliger larvae should be found one trophic level higher in terms of $\delta^{15}N$, and with a $\delta^{13}C$ value close to that of veligers. Three fish species meet these criteria (Fig. 3.2): *A. sapidissima* (TL = 2.9), *C. commersoni* (TL = 2.9) and *A. pseudoharengus* (TL = 3.0). These species fed 1 TL higher than the veligers and showed similar $\delta^{13}C$. However, *C. commersoni* is likely to be a benthic feeder, with high $\delta^{13}C$ values. *E. affinis* and nauplii larvae were also one trophic level higher than the veligers, had similar carbon isotopic ratios, and thus could be potential consumers. Amphipods (*Gammarus* spp.) were also one trophic level higher than the veligers, but had high $\delta^{13}C$ values consistent with their largely benthic habit. The isotope ratios of the mysids suggest little potential for veligers to serve as prey.

3.4.6. Prey of fish larvae

Consistent with previous studies (Winkler et al. 2005 and references therein), the isotopic data indicate that the zooplankton *B. longirostris* and *E. affinis* are important food sources for fish larvae. All the fish larvae analyzed in the present

study (*M. villosus*, *M. tomcod* and *O. mordax*) had very similar carbon and nitrogen isotopic signatures, suggesting their dependence on the same combination of prey items. Their $\delta^{13}C$ values suggest that *N. americana*, the copepods *E. affinis* and *E. curticorne*, nauplii larvae and *B. longirostris* are likely to be the major prey items for fish larvae.

3.4.7. Secondary consumers

Gammarus sp. showed a large range of carbon and nitrogen isotopic ratios implying an omnivorous behavior, and their relatively high $\delta^{15}N$ likely reflects foraging on benthic invertebrates. Sand shrimp had a wide range of $\delta^{15}N$ suggesting an omnivorous diet, consistent with studies on feeding habits by Wilcox and Jeffries (1974) which showed that this species fed on many crustacean taxa and other invertebrates as well as detritus.

3.4.8. Adult fish

The two planktivorous fishes sampled, *A. pseudoharengus* and *A. sapidissima*, are known to have very similar diets of cladocera, copepods and insect larvae (Scott and Crossman 1974). Consistent with these previous observations, these two species had similar carbon and nitrogen isotopic ratios in the ETZ and occupied a similar trophic position (3.0). The adults of *C. commersoni* had $\delta^{13}C$ higher than either *Alosa* species, consistent with the known importance of benthic organisms in its diet, but $\delta^{15}N$ was similar (Scott and Crossman 1974). The highest trophic rankings in our analysis were for the three most abundant adult fishes in the ETZ: *O. mordax*, *M. tomcod* and *S. canadense*. *S. canadense* occupied the highest trophic position. In the St. Lawrence estuary, they consume principally amphipods as juveniles and tomcod as adults (J. Dodson, unpublished data). *M. tomcod* typically preys on smaller fish (i.e., *Osmerus* sp., *Gasterosteus* sp.) and many small crustacea such as shrimp and amphipods (Scott and Crossman 1974). Consistent with the partial feeding on small fish, the isotopic ratios of adult *M. tomcod* in the ETZ place it just above that of *O.*

mordax. In the St. Lawrence estuary, smelt feed primarily on mysids, amphipods, *C. septemspinosa* and nereids (Lecomte and Dodson 2005).

3.4.9. DOC uptake

Radiolabelled uptake experiments in the laboratory confirmed that veligers from the St. Lawrence River have the capacity to directly assimilate DOC from the water (Fig. 3.4). The 2-h incubation gave a mean net uptake rate by veligers of 14.2 mg (DOC) g dry weight^{-1} h^{-1}, while that for adults has been estimated as 0.03 mg (DOC) g dry weight^{-1} h^{-1} (Roditi et al. 2000). This difference may reflect the allometric relationship between specific growth rate and body size. This high rate for veligers is unlikely to be sustained for long periods of time, and although the algal lysates were supplied at concentrations similar to the DOC of the St. Lawrence River, they are likely to have been of much higher nutritional quality relative to a large fraction of DOC occurring naturally in the river. Furthermore, the veligers were preincubated in food-free water for 1 h prior to the start of the experiment, and this may have stimulated uptake rates. However, the mixing model supports our high uptake rates since it showed that DOC could represent an important carbon source for the veligers. The model's results may underestimate the importance of DOC for veligers if they discriminate against some phytoplankton cells, for example based on size, or differentially take up algal exudates in the dissolved organic matter pool that have a similar isotopic signature to algal carbon and nitrogen. The large DOC uptake capacity shown here points to algal exudates as an important carbon source that could meet much of the total growth and maintenance requirements of zebra mussel veligers. Our behavioral observations of the veligers with and without the algal lysates also implied a beneficial maintenance effect of DOC.

Previous work on bivalves in marine systems has similarly shown a large difference in DOC uptake capacity between life history stages. Specifically, *Mytilus edulis* veligers and pediveligers have up to an order-of-magnitude faster uptake rate of DOC relative to the adults, and this may confer an advantage on the larval stages given that the energy reserves provided by the adults are minimal (Manahan et al.

1982). Furthermore, the reorganization of tissues during metamorphosis from veliger to adult may interrupt particulate feeding for a period of several days, and DOC uptake may at that time provide the sole mechanism of carbon acquisition; it has been noted for example that during metamorphosis, *Mytilus edulis* veligers can absorb [^{3}H] glycine through developing gill buds (Manahan et al. 1982). Recent evidence has shown that the larvae of *Crassostrea gigas* can survive long feeding delays while maintaining a constant rate of metabolism, and the authors suggested that the larvae feed on detrital matter or DOC which would fuel their maintenance metabolism for extended periods equivalent to four times the predicted lifespan (Moran and Manahan 2004). Previous studies have drawn attention to the high metabolic demands of zebra mussel veligers that seem incompatible with their low particle uptake rates (Sprung 1993). The direct uptake of DOC as observed here could satisfy this apparent imbalance in energy supply and demand. Polis et al. (1997) have suggested that the use of terrestrial subsidies by organisms could stabilize food webs. This could be the case with the veligers, the dominant zooplankton component, using an alternative subsidy, thereby not directly disturbing food web structure.

3.4.10. DOC: in situ composition

The autochthonous: allochthonous ratios of the natural DOC as determined by SFS analysis were >1, implying that labile carbon, perhaps algal-derived, makes a large contribution to the total DOC pool of the river and transition zone during the summer, consistent with previous biogeochemical analyses (Hélie 2004). Similarly, in large northeastern United States rivers, the composition of DOC was similar to that of algal exudates (Roditi et al. 2000). In the Mississippi River plume, elevated DOC concentrations were probably from direct release by the high phytoplankton biomass or from grazing-mediated processes during spring and summer (Dagg et al. 2004). High concentrations of combined dissolved carbohydrates provided molecular evidence for the production of DOC at mid-salinities in that plume (Benner and Opsahl 2001). In the present study, the $\delta^{13}C$ for DOC and free bacteria was closer to

values inferred for phytoplankton rather than the total seston, the latter more depleted and more strongly influenced by terrestrial organic matter and detritus (Martineau et al. 2004). This is consistent with of the findings of Chin-Leo and Benner (1992) who found that plankton-derived organic matter supported 68% of bacterial production in the Mississippi River during the summer. Nevertheless, the uptake of some allochthonous materials by veligers cannot be ruled out. Adult zebra mussels are known to take up humic acids in addition to algal lysates (Voets et al. 2004), but the $\delta^{13}C$ of humics is likely to reflect their terrestrial origins and the non-terrestrial signature of zebra mussel veligers in the St. Lawrence River implies that this is not a primary source in their diet.

3.4.11. DOC uptake mechanisms

Apart from the observational evidence presented here, there are also mechanistic reasons for considering DOC as a food source for zebra mussel veligers. Bivalves are known to take up amino acids by substrate-specific transporters located across the membranes of epithelial cells in their gills (Wright and Manahan 1989). No bacterial associations have been identified in conjunction with the uptake of DOM in adult and veliger bivalves (Manahan et al. 1982). Rapid, intense labeling of the gill epithelium of *Mytilus edulis* was revealed by autoradiograms of adults exposed for brief periods to radiolabeled amino acids (Manahan et al. 1982), indicative of direct assimilation rather than via any intermediate steps associated with symbiotic bacteria.

Although veligers have distinctive feeding mechanisms by comparison with other invertebrates, it may be that their rapid rates of DOC uptake are also to be found in other groups of freshwater taxa. Early studies gave conflicting results on the capacity of marine crustaceans to assimilate DOC (McWhinnie and Johanneck 1966; Anderson and Stephens 1969) yet they nonetheless resulted in the premature generalization that crustaceans cannot take up DOC. Studies on larval insects have shown the capacity for DOC uptake (Ciborowski et al. 1997), and this requires further investigation in other arthropod taxa. In some strongly heterotrophic systems,

terrestrial matter seems to be the principal carbon source via bacterioplankton and their subsequent consumption, yet this bacterial carbon is inefficiently transferred to consumers, as well as insufficient in biomass to meet the carbon requirements for higher consumers. A recent study indicated that terrestrial matter was an important source of carbon for a lentic food web (Pace et al. 2004), yet imbalances in the carbon budget were noted since the sum of particulate organic carbon (POC) from both terrestrial sources and from in-lake production could not meet the carbon requirements of the food web. It was speculated that the aggregation of DOC into microparticles and their subsequent ingestion of POC could possibly account for some of this carbon. Given that DOC is generally ten times more abundant than POC (Wetzel 2001), the direct transfer of DOC to primary consumers and subsequently higher trophic levels deserves closer attention.

These results define the ETZ food web as a complex network in which omnivory dominates. Although this ecosystem is likely to be net heterotrophic (Painchaud et al. 1995), autochthonous sources appear to dominate the trophic supply of carbon to ETZ animals, and the productive food web seems largely decoupled from decomposer pathways based on allochthonous organic matter. Despite their high abundance in the ETZ, zebra mussel veligers were not sharing resources with native zooplankton and did not appear to serve as important carbon sources for higher trophic levels. Veliger carbon sources likely include free bacteria, phytoplankton and DOC. Uptake experiments resulted in relatively high uptake rates of algal lysates, implying that autochthonous DOC represents an alternate food source for this invasive zooplankton. Exotic invaders including adult zebra mussels often occupy unusual niches that have no parallel in the native community (Ricciardi and Rasmussen 1998). The larval stage of zebra mussels appears to follow this pattern and to neither compete with nor act as a major food source for other major food web components in the ETZ ecosystem.

3.5. Acknowledgments

This work was funded by strategic and discovery research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and a post-graduate NSERC fellowship to CB. This is a contribution to the programs of Québec-Océan, Centre d'Études Nordiques, Groupe de Recherche sur les Écosystèmes Aquatiques (GRÉA) and the Canada Research Chair in Aquatic Ecosystem Studies. We thank Hugues Boulanger, Andy Casper, Marie-Audrey Livernoche, Marie-Noël M'Boutchou, Didier M'Radamy, Carl Martin, Leira Retamal, Sébastien Roy, Geneviève Trudel, and Gesche Winkler for field and laboratory assistance, Martine Savard and Anna Smirnoff for their expert advice and assistance with mass spectrometry at the Commission Géologique du Canada, Québec, Nicholas S. Fisher for helpful advice on protocols, Judy Acreman at University of Toronto Culture Collection, Lise Rancourt and Sébastien Duval at the Institut National de la Recherche Scientifique, Paul Middlestead at the G.G. Hatch Laboratory and Ladd Johnson for his leadership of the NSERC strategic research program on the St. Lawrence estuary.

3.6. References

Anderson, J.W., and G.C. Stephens. 1969. Uptake of organic material by aquatic invertebrates, VI. Role of epiflora in apparent uptake of glycine by marine crustaceans. Mar. Biol. **4**: 243-249.

Armstrong, F.A.J., P.M. Williams, and J.D.H. Strickland. 1966. Photo-oxidation of organic matter in sea water by ultra-violet radiation, analytical and other applications. Nature **211**: 481-484.

Baines, S.B., N.S. Fisher, and J.J. Cole. 2005. Uptake of dissolved organic matter (DOM) and its importance to metabolic requirements of the zebra mussel, *Dreissena polymorpha*. Limnol. Oceanogr. **50**: 36-47.

Barnard, C., J.-J. Frenette, and W.F. Vincent. 2003. Planktonic invaders of the St. Lawrence estuarine transition zone: environmental factors controlling the distribution of zebra mussel veligers. Can. J. Fish. Aquat. Sci. **60**: 1245–1257.

Belzile, C., J.A.E. Gibson, and W.F. Vincent. 2002. Colored dissolved organic matter and dissolved organic carbon exclusion from lake ice: implications for irradiance transmission and carbon cycling. Limnol. Oceanogr. **47**: 1283-1293.

Benner, R., and S. Opsahl. 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi River Plume. Org. Geochem. **32**: 597-611.

Bernier, S. 2003. Efficacité et sélectivité alimentaire des larves de la Moule Zébrée : effets potentiels sur le réseau alimentaire de la zone de transition estuarienne du Saint-Laurent. MSc thesis. Université Laval.

Cabana, G., and J. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Nat. Acad. Sci. **93**: 10844-10847.

Chin-Leo, G., and R. Benner. 1992. Enhanced bacterioplankton production and respiration at intermediate salinities in the Mississippi River plume. Mar. Ecol. Prog. Ser. **87**: 87-103.

Ciborowski, J.J.H., D.A.Craig, and K.M. Fry. 1997. Dissolved organic matter as food for black fly larvae (Diptera: Simuliidae). J. N. Am. Benthol. Soc. **16**: 771-780.

Dagg, M.J., R. Benner, S. Lohrenz, J. O'Donnell, and D. Lawrence. 2004. Transport and transformation of dissolved and particulate materials on continental shelves influenced by large rivers: plume processes. Cont. Shelf Res. **24**: 833-858.

Fogel, M.L., L.A. Cifuentes, D.L. Veliinsky, and J.H. Sharp. 1992. Relationship of carbon availability in estuarine phytoplankton to isotopic composition. Mar. Ecol. Prog. Ser. **82**: 291-300.

France, R., M. Chandler, and R. Peters. 1998. Mapping trophic continua of benthic foodwebs: body size - $\delta^{15}N$ relationships. Mar. Ecol. Prog. Ser. **174**: 301-306.

Frenette, J.-J., W.F. Vincent, J.J. Dodson, and C. Lovejoy. 1995. Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar. Ecol. Prog. Ser. **120**: 99-110.

Hélie, J.-F., C. Hillaire-Marcel, and B. Rondeau. 2002. Seasonal changes in the sources and fluxes of dissolved inorganic carbon through the St. Lawrence River - isotopic and chemical constraint. Chem. Geol. **186**: 117-138.

Hélie, J-F. 2004. Géochimie de carbone organique et inorganique dans les milieux aquatiques de l'est du Canada: exemples du Saint-Laurent et du réservoir Robert-Bourassa - Approche isotopique. PhD Thesis. Université du Québec a Montréal.

Johnson, L.E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* sp.) veligers using cross-polarized light microscopy. Hydrobiologia **312**: 139-146.

Korb, R.E., P.J. Saville, A.M. Johnston, and J.A. Raven. 1997. Sources of inorganic carbon for photosynthesis by three species of marine diatom. J. Phycol. **33**: 433-440.

Laws, E.A., P.A. Thompson, B.N. Popp, and R.R. Bidigare. 1998. Sources of inorganic carbon for marine microalgal photosynthesis: a reassessment of $\delta^{13}C$ data from batch culture studies of *Thalassiosira pseudonana* and *Emiliana huxleyi*. Limnol. Oceanogr. **43**: 136-142.

Lecomte, F., and J.J. Dodson. 2005. Distinguishing trophic and habitat partitioning among sympatric populations of an estuarine fish (*Osmerus mordax* Mitchell). J. Fish Biol. **66**: 1601-1623.

Manahan, D.T., S.H. Wright, G.C. Stephens, and M.A. Rice. 1982. Transport of dissolved amino acids by the mussel, *Mytilus edulis*: Demonstration of net uptake from natural seawater. Science **215**: 1253-1255.

Martineau, C., W.F. Vincent, J.-J. Frenette, and J.J. Dodson. 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnol. Oceanogr. **49**: 1679-1686.

McCallister, S.L., J.E. Bauer, J.E. Cherrier, and H.W. Ducklow. 2004. Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) approach. Limnol. Oceanogr. **49**: 1687-1702.

McWhinnie, M.A., and R. Johanneck. 1966. Utilization of inorganic and organic carbon compounds by Antarctic zooplankton. Antarct. J. U.S. **1**: 210.

Mitchell, M.J., E.L. Mills, N. Idrisi, and R. Michener. 1996. Stable isotopes of nitrogen and carbon in an aquatic food web recently invaded by *Dreissena polymorpha* (Pallas). Can. J. Fish. Aquat. Sci. **53**: 1445-1450.

Mook, W.G., J.C. Bommerson, and W.H. Staverman. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet. Sci. Lett. **22**: 169-176.

Moran, A.L., and D.T. Manahan. 2004. Physiological recovery from prolonged 'starvation' in larvae of the Pacific oyster *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. **306**: 17– 36.

Pace, M.L., J.J. Cole, S.R. Carpenter, J.F. Kitchell, J.R. Hodgson, M.C. Van de Bogert, D.L. Bade, E.S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**: 240-243.

Painchaud, J., J.-C. Therriault, and L. Legendre. 1995. Assessment of salinity-related mortality of freshwater bacteria in the St. Lawrence Estuary. Appl. Environ. Microbiol. **61**: 205-208.

Pennington, J. T., and M.G. Hadfield. 1989. A simple nontoxic method for the decalcification of living invertebrate larvae. J. Exp. Mar. Biol. Ecol. **130**: 1-7.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia **136**: 261-269.

Polis, G.A., W.B. Anderson, and R.D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu. Rev. Ecol. Syst. **28**: 289-316.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**: 703-718.

Raikow, D. F., and S.K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: a stable isotope enrichment study. Limnol. Oceanogr. **46**: 514-522.

Rau, G.H., U. Riebesell, and D. Wolf-Gladrow. 1996. A model of photosynthetic ^{13}C fractionation by marine phytoplankton based on diffusive molecular CO_2 uptake. Mar. Ecol. Prog. Ser. **133**: 275-285.

Ricciardi, A. and J.B. Rasmussen. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can. J. Fish. Aquat. Sci. **55**: 1759-1765.

Roditi, H.A., N.S. Fisher, and S.A. Sanudo-Wilhelmy. 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407**: 78-80.

Rolff, C., and R. Elmgren. 2000. Use of riverine organic matter in plankton food webs of the Baltic Sea. Mar. Ecol. Prog. Ser. **197**: 81-101.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada (Bulletin of the

Fisheries Research Board of Canada, No. 184). Fisheries Research Board of Canada, Ottawa.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*, p. 39-53. T.F. Nalepa and D.W. Schloesser [eds.], *In* Zebra mussels: biology, impacts and control. Lewis Publishers.

Thorp, J.H., M.D. Delong, K.S. Greenwood, and A.F. Casper. 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. Oecologia **117**: 551-563.

Tortell, P.D., J.R. Reinfelder, and F.M.M. Morel. 1997. Active uptake of bicarbonate by diatoms. Nature **390**: 243-244.

Tremblay, M .J., L.D. Meade, and G.V. Hurley. 1987. Identification of planktonic sea scallop larvae (*Placopecten magellanicus*)(Gmelin). Can. J. Fish. Aquat. Sci. **44**: 1361-1366.

Voets, J., L. Bervoets, and R. Blust. 2004. Cadmium bioavailability and accumulation in the presence of humic acid to the zebra mussel, *Dreissena polymorpha*. Environ. Sci. Technol. **38**: 1003-1008.

Wacker, A., P. Becher, and E.V. Elert. 2002. Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha*. Limnol. Oceanogr. **47**: 1242-1248.

Wetzel, R.G. 2001. Limnology: Lake and River Ecosystems. Academic Press.

Wilcox, J. R., and H. P. Jeffries. 1974. Feeding habits of the shrimp *Crangon septemspinosa*. Biol. Bull. **146**: 424-434.

Winkler, G., J.J. Dodson, N. Bertrand, D. Thivierge, and W.F. Vincent. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. Mar. Ecol. Prog. Ser. **251**: 59-73.

Winkler, G., P. Sirois, L.E. Johnson, and J.J. Dodson. 2005. Invasion of an estuarine transition zone by *Dreissena polymorpha* veligers had no detectable effect on zooplankton community structure. Can. J. Fish. Aquat. Sci. **62**: 578-592.

Wright, S., and D. Manahan. 1989. Integumental nutrient uptake by aquatic

organisms. Annu. Rev. Physiol. **51**: 585-600.

Zimmermann-Timm, H. 2002. Characteristics, dynamics and importance of aggregates in rivers - an invited review. Int. Rev. Hydrobiol. **87**: 197-240.

Table 3.1. Trophic position and stable isotope data for each food web component sampled in the St. Lawrence estuarine transition zone. For the algae, values are means from U2 and North channel.

Species	Trophic position	$\delta^{13}C$ (‰)	Range	$\delta^{15}N$ (‰)	Range
Stizostedion canadense	5.0	-17.3	-16.9 to -17.8	17.4	16.7 to 18.1
Microgadus tomcod	4.6	-16.1	-16.3 to - 15.9	16.1	16.0 to 16.3
Osmerus mordax	4.0	-19.4	-19.9 to -17.7	14.2	13.7 to 14.6
Mallotus villosus larvae	3.8	-20.4	-21.5 to -19.9	13.4	13.2 to 13.5
O. mordax larvae	3.7	-21.0	-22.3 to -19.7	13.0	11.8 to 14.4
M. tomcod larvae	3.7	-19.9	-21.6 to -18.7	12.9	12.0 to 13.6
Mysids	3.6	-20.2	-21.1 to -18.9	12.6	10.6 to 18.4
Crangon septemspinosa	3.4	-18.5	-20.4 to -15.7	12.0	11.3 to 12.8
Ectinosoma curticorne	3.3	-21.1	-22.2 to -21.1	11.7	11.4 to 12.0
Nauplii larvae	3.1	-20.7	-21.0 to -20.6	11.0	10.2 to 11.8
Eurytemora affinis	3.0	-21.0	-21.8 to -20.3	10.6	9.2 to 12.5
Gammarus sp.	2.6	-18.7	-20.4 to -17.6	9.4	8.5 to 11.0
Bosmina longirostris	2.4	-20.5	-20.9 to -20.1	8.5	8.2 to 8.8
Cryptomonas sp.	2.4	-26.6	-28.8 to -25.4	8.6	7.8 to 9.7
Dreissena polymorpha	2.0	-22.5	-23.9 to -20.5	7.3	6.1 to 7.7
Keratella sp.	1.8	-20.7	-20.9 to -20.2	6.8	6.5 to 6.9
D. polymorpha veliger	1.7	-23.2	-24.6 to -19.9	6.3	3.7 to 8.6
Cyclotella sp.	1.6	-25.6	-28.3 to -24.2	6.0	4.3 to 8.2
Thalassiosira sp.	1.5	-19.6	-20.9 to -18.6	5.7	4.3 to 6.9
Bacteria (aggregates)	1.4	-29.1	-30.2 to -27.8	5.1	4.8 to 5.5
Bacteria (free cells)	1.2	-25.0	-27.2 to -22.1	4.6	3.4 to 5.8
> 64 µm seston	-	-25.5	-27.7 to -24.3	7.1	5.6 to 9.5
10-64 µm seston	-	-28.1	-31.2 to -23.8	6.6	5.0 to 7.9
0-10 µm seston	-	-31.21	-32.5 to -29.7	6.6	2.6 to 10.5
DOC	-	-25.5	-26.7 to -24.7	-	-

Table 3.2. Physical variables in the St. Lawrence estuarine transition zone. Station locations are shown in Fig. 3.1.

Station	$\delta^{13}C$ DIC (‰)	$\delta^{13}C$ $CO_{2(d)}$ (‰)*	[DIC] mg L^{-1}	Temperature (°C)†	Salinity†
U2	-2.95	-9.33	16.55	21.78	0.12
North channel					
High tide	-2.02	-9.62††	17.25	17.73	7.35
Low tide	-5.05		15.00	20.00	1.12

*Calculated value (see Materials and methods)

†Average from surface to 2 m

††Average for high and low tide in the transition zone

Table 3.3. Carbon isotope fractionation of algae (ε_a) based on uptake of $CO_{2(d)}$ in the freshwater zone (U2) and North channel in the transition zone. Values are means ± SD, *n* indicated in parentheses.

Algae	U2	North channel
Cryptomonas sp.	18.5± 2.3	16.1
	(2)	(1)
Cyclotella sp.	17.3 ± 2.0	15.4 ± 0.7
	(3)	(2)
Thalassiosira pseudonana	11.1 ± 0.7	9.7 ± 0.6
	(3)	(4)

3.7. List of figures

Figure 3.1. Sampling sites in the estuarine transition zone (ETZ) of the St. Lawrence River. Pelagic organisms and microbial components were collected in the three main stations (North, Middle, and South). Berthier-sur-Mer and St-François were the sites of adult mussel collection and Île-aux-Coudres was the location where the adult fishes were caught. Microbial components were also collected at the freshwater (U2) station. The upstream site (U1) was located in front of Québec City. Dotted lines indicate isohalines. Algal incubations were at U2 and North channel.

Figure 3.2. Dual isotopic plot for all food web components in the St. Lawrence River ETZ. Sampling was conducted in summer 2001, with the exception of *S. canadense* (summer 2000) and microbial components (summer 2003). All data are averages ± SD. $\delta^{13}C$ of DOC is represented by the gray rectangle. Dotted lines delimit trophic levels 1-5. 1) free bacteria, 2) aggregate bacteria, 3) estuarine algae, 4) *D. polymorpha* veligers, 5) seston 10-64 μm, 6) seston 0-10 μm, 7) *Keratella* sp., 8) freshwater algae, 9) seston > 64 μm, 10) *D. polymorpha* adults, 11) *B. longirostris*, 12) *Gammarus* sp., 13) *C. commersoni*, 14) *A. sapidissima*, 15) *E. affinis*, 16) *A. pseudoharengus*, 17) Nauplii larvae, 18) *E. curticorne*, 19) *C. septemspinosa*, 20) Mysids, 21) *M. tomcod* juvenile, 22) *M. tomcod* larvae, 23) *O. mordax* larvae, 24) *G. aculateus*, 25) *M. villosus* larvae, 26) *O. mordax* adults, 27) *S. canadense* juvenile, 28) *M. tomcod*, 29) *S. canadense* adults.

Figure 3.3. Dual isotope plot for microbial components and primary consumers in the St. Lawrence River system. Values are means ± SD. $\delta^{13}C$ of DOC is represented by the gray rectangle. Black dots (North channel): 1) Free bacteria (mean for U2 and North channel), 2) *Cyclotella* sp., 3) *Thalassiosira* sp.,4) aggregate bacteria (mean for U2 and North channel), 5) *D. polymorpha* veligers (mean for U2 and North channel),

8) *D. polymorpha* adults, 9) *Cryptomonas* sp., 10) *Bosmina* sp., 12) *Eurytemora* sp.; White dots (U2): 6) *Thalassiosira* sp., 7) *Cyclotella* sp., 11) *Cryptomonas* sp. Values in parenthesis below are the results from paired *t*-tests between the veligers' carbon signature (mean of ETZ) and individual potential carbon sources, for U2 and ETZ (North channel) respectively. *Cyclotella* sp. ($t = 1.96$; df = 19; $p = 0.065$ and $t = 0.67$; df = 18; $p = 0.511$); *Cryptomonas* sp. ($t = 2.28$; df = 18; $p = 0.035$ and NA ($n = 1$)); *Thalassiosira pseudonana* ($t = -2.64$; df = 19; $p = 0.016$ and $t = -3.975$; df = 20; $p < 0.001$); free bacteria ($t = 1.19$; df = 20; $p = 0.25$); aggregate bacteria ($t = 5.86$; df = 22; $p < 0.001$); DOC ($t = 0.79$; df = 18; $p = 0.439$ and $t = 2.04$; df = 20; $p = 0.055$).

Figure 3.4. Uptake of DOC by zebra mussel veligers. Each value is the mean of triplicates ± SD, with 100 veligers per replicate. Uptake from live veligers was significantly different from that of blanks (ANCOVA, $F_{1,36} = 131.56$; $n = 39$; $p < 0.0001$). The linear regression lines are for untransformed data ($r^2 = 0.84$; $n = 8$; $p = 0.001$; blank regression: $r^2 = 0.88$; $n = 5$; $p = 0.02$).

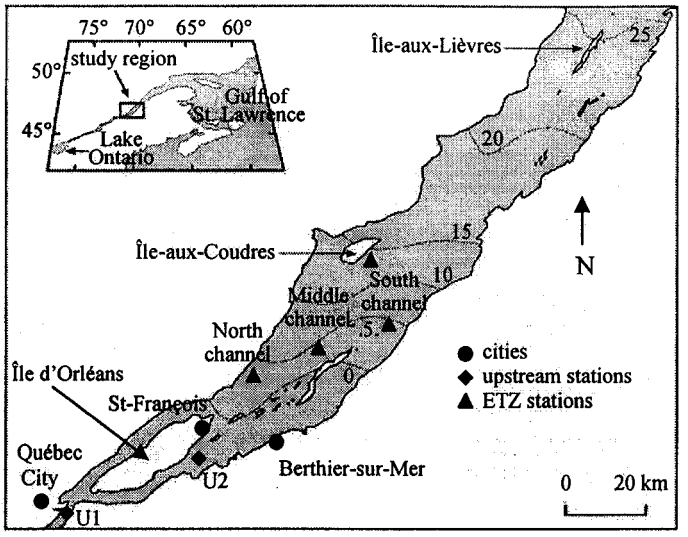

75° 70° 65° 60°
50°
45°
study region
Gulf of St. Lawrence
Lake Ontario
Île-aux-Lièvres
25
20
15
10
5
0
N
Île-aux-Coudres
South channel
Middle channel
North channel
Île d'Orléans
St-François
Québec City
U1
U2
Berthier-sur-Mer
cities
upstream stations
ETZ stations
0 20 km

Figure 3.1.

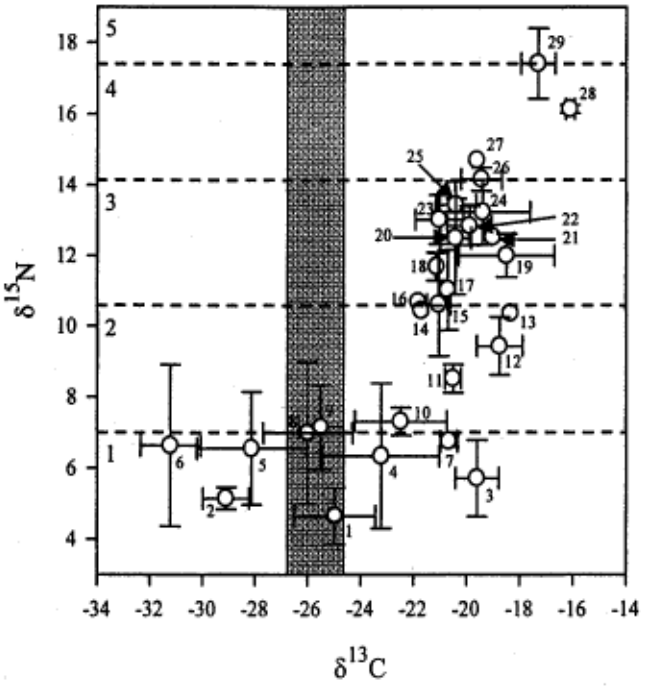


Figure 3.2.

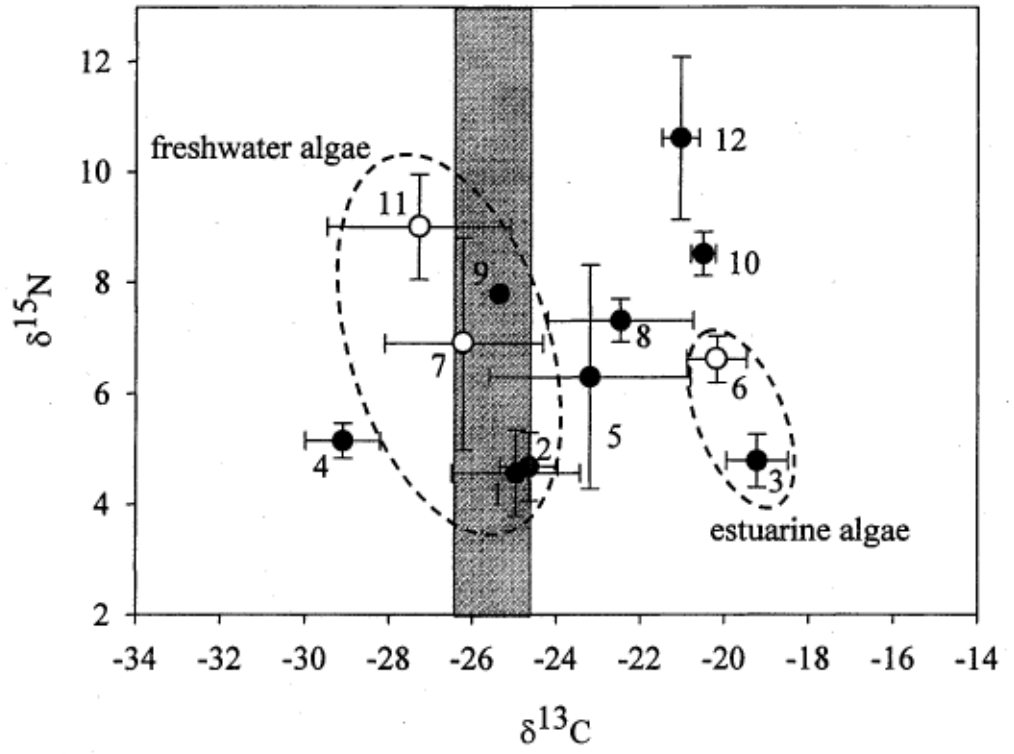

freshwater algae
estuarine algae
12
10
8
6
4
2
-34
-32
-30
-28
-26
-24
-22
-20
-18
-16
-14
$\delta^{15}N$
$\delta^{13}C$
1
2
3
4
5
6
7
8
9
10
11
12

Figure 3.3.

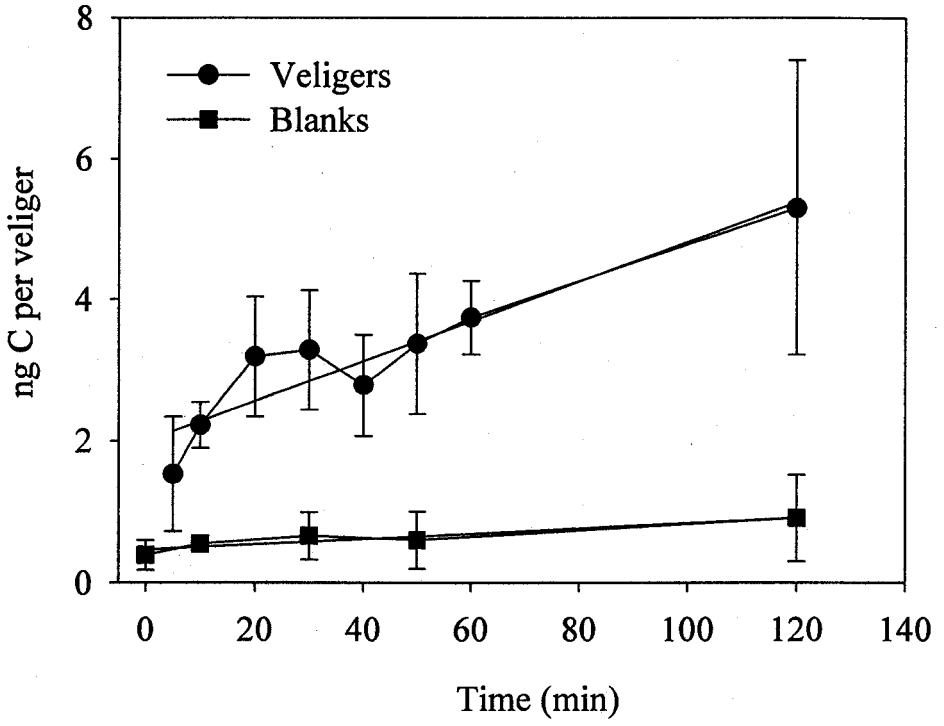

Veligers
Blanks
ng C per veliger
Time (min)
0
2
4
6
8
0
20
40
60
80
100
120
140

Figure 3.4.

3a. Web Appendix

Barnard, Christine, Martineau, Christine, Frenette, Jean-Jacques, Dodson, Julian J. and Vincent, Warwick F. 2006. Trophic position of zebra mussel veligers and their use of dissolved organic carbon. Limnology and Oceanography.

3a.1. Method to determine $\delta^{13}C$ of phytoplankton from in situ incubations

Three protist species representing naturally abundant genera in the St. Lawrence ETZ were obtained from University of Toronto Culture Collection (UTCC): estuarine UTCC 624 *Thalassiosira pseudonana*, freshwater UTCC 336 *Cryptomonas* sp. and freshwater UTCC 520 *Cyclotella* sp. The estuarine species were kept in ESAW (Harrison et al. 1980) and the freshwater species in CHU-10 culture medium (Stein 1973). The cells were incubated in a PsycroTherm™ Controlled Environment Incubator Shaker (New Brunswick Scientific Co. Inc.) at 18.5 ± 1 °C with a light: dark cycle of 12:12 and irradiance (photosynthetically available radiation) provided by neon cool white fluorescent lamps with an irradiance of 149 and 224 μmol m^{-2} s^{-1}, for the agitated and non-agitated cultures respectively. *Cyclotella* sp. and *T. pseudonana* were constantly agitated at a speed of 50 rpm while *Cryptomonas* sp. cultures were not agitated.

For the in situ incubations, the cultures were transferred to Fisherbrand® regenerated cellulose dialysis tubes (12,000-14,000 Dalton MWCO). The autoclaved tubing (10 min at 121°C) was filled with approximately 20 mL of the individual cultures (mid-exponential growth phase) and both ends were tied with double knots. The sealed tubes were submerged in containers of culture medium for up to 48 h prior to and during transport to the incubation site. For deployment, the dialysis tubes were placed in polystyrene bottles perforated with large holes to allow the river water to circulate freely inside. The bottles were then placed in stainless steel protective cages and attached to navigation buoys (sites U2 and North channel) at a depth of

approximately 0.5 m. The underwater light climate varies markedly between the freshwater and transition zones and was measured in summer 2001 with a spectroradiometer (Model PUV-500, Biospherical Instruments, San Diego, USA). Two cages, each containing one bottle, were placed on each buoy. At least two dialysis tubes of each species were placed in each bottle. On day 7, the dialysis tubes were collected, rinsed thoroughly with 0.22 μm membrane filtered MilliQ-water, cut open and their contents then filtered onto precombusted (4 h at 450 °C) GF/F 47 mm filters. The filters were stored frozen at -80 °C until isotopic analysis (see article).

A large increase in algal cell concentrations was visually noted within the tubes implying sufficient incubation for the cells to come to isotopic equilibrium with the river water. There was a large shift in the nitrogen but not carbon isotopic signatures of the algae from the culture media to the field (Fig. 3a.1). Within each environment, the three algal species differed in their $\delta^{13}C$ signatures most likely reflecting differences in photosynthetic pathways and in the extent of carbon fractionation during CO_2 or bicarbonate utilization. Taxon-specific differences may also lead to much of this variability in carbon signatures. Nitrogen values from the South channel (U2) were consistently higher than transition zone values. This may be the result of nutrient enrichment due to the intense agricultural activities on the south shore (Lavoie et al. 2003) and the human and industrial effluents discharged from the upstream Québec City area, given that urban wastes can also lead to enriched ^{15}N values (Cabana and Rasmussen 1996). Wada and Hattori (1978) found that decreased irradiance values could reduce nitrogen fractionation in diatoms which could explain the consistently lower $\delta^{15}N$ values for the algae in the TZ station where light penetration during low and ebbing tides is greatly inhibited by high turbidity. The K_d values as well as the depth to which 1% of the surface irradiance penetrated ($Z_{1\%}$) differed substantially between the zones, consistent with the observed turbidity in the transition zone. The euphotic depth (1% PAR) extended to 4.5 m (range 3.3 to 4.5 m) in the freshwater zone and decreased to 0.6-2.6 m in the transition zone.

3a.2. Boundary layer tests

In order to evaluate potential boundary layer effects on fractionation (cf. Turner et al. 1994), we filled autoclaved dialysis tubes (see above) with 30 mL of UTCC 624 *Thalassiosira pseudonana* at mid-exponential growth. The tubes were placed in ESAW media at a ratio of ESAW to algal solution of 10:1 to ensure optimum growth. Three separate glass flasks, with three tubes in each, were placed in the incubator with the same growing conditions as above with one flask constantly agitated at 55 rpm, one incubated without agitation, and the tubes in the third flask inverted by hand three times daily at mid-day. Stable isotope analysis as above was conducted at day 0 and day 14 of the experiment. The measurements showed that the $\delta^{13}C$ values of the algae (all treatments) on day 14 were significantly different from the $\delta^{13}C$ of the time zero algae (one-way ANOVA, $F_{3,10} = 16.4$; $p = 0.002$) however there were no significant differences between the static cultures and those with agitation (Bonferroni *t*-test, $t = 0.112$, $p = 1$), implying no boundary layer effects on fractionation. For the in situ incubations, DIC limitation is additionally unlikely as water movements in this region of the St. Lawrence River are strong and the tubes were constantly under agitation, and furthermore the ETZ and river contain high concentrations of DIC (Table 3.2 in article) relative to systems in which carbon limitation has been observed.

3a.3. Isotopic equilibration tests

Dialysis membranes allow diffusion of molecules smaller than proteins, and equilibration with ambient sea water is known to occur in less than 4 h (Wood et al. 1992; Mura et al. 1996). The time required for the media and algae in the tubes to reach isotopic equilibrium with natural waters in the St. Lawrence River was tested during a seven day incubation. Thirty mL of algal culture were placed in each tube in order to have enough water for DIC analysis. Samples for stable isotope analysis were taken on days 1, 4, and 7 as well as the DIC in the surrounding water and within the tubes using the same methods described above. The $\delta^{13}C$ of DIC in the river water

and in the tubes was approximately the same by day 4 of the incubation (Fig. 3a.2), indicating rapid equilibration between the internal and external dissolved pools.

3a.4. DOC release from tubes

Evidence suggests that dialysis tubes do not generate transparent exopolymers (Passow 2000) or DOC (Kritzberg et al. 2004), and we verified this for our own tubes during a 7 day incubation period. Any DOC released from the tubes could serve as a substrate for bacteria and the subsequent respired CO_2 could then be used by the algae, instead of the naturally occurring CO_2, thereby influencing the algal carbon signature. To test this, the concentration of DOC was analyzed in 3 control beakers containing MilliQ-water on day 0 and day 7 by filtering the water through 0.2 µm Millipore Isopore membranes. In 6 beakers, autoclaved dialysis tubes (see above) were placed in Milli-Q water and the volume to length of tubing ratio of 6.45 mL cm^{-1} was preserved to test the amount of DOC produced within the tubes once closed. These beakers were kept in the incubator with the same light and temperature conditions as above. Three beakers were agitated at a speed of 55 rpm while 3 others were without agitation. After 7 days, 20 mL of water was obtained from each beaker and filtered through 0.2 µm Millipore Isopore membranes for DOC analysis at the Institut National de la Recherche Scientifique. Samples were kept cool in amber bottles and subsequently analyzed for DOC using a TOC-analyzer (Shimadzu Model TOC-5000A).

Over the 7 day trial period, an average of 1.49 ± 0.07 and 1.76 ± 0.32 mg DOC L^{-1} (DOC in MilliQ-water subtracted) was generated in beakers without and with agitation respectively (Fig. 3a.3). This represents approximately 0.23 mg DOC L^{-1} released from the tubes daily, providing an upper estimate of the amount of DIC released from this source if it was all converted to CO_2 by bacterial respiration. This amount of DIC is negligible compared to the ambient concentrations of DIC in the surrounding waters (Table 3.2 in article), representing 1.4 % of the total DIC pool per day, and hence should not influence the algal carbon signatures.

3a.5. References

Cabana, G., and J. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Nat. Acad. Sci. **93**: 10844-10847.

Fogel, M.L., L.A. Cifuentes, D.L. Veliinsky, and J.H. Sharp. 1992. Relationship of carbon availability in estuarine phytoplankton to isotopic composition. Mar. Ecol. Prog. Ser. **82**: 291-300.

Harrison, P. J., R. Waters, and F.J.R. Taylor. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. **16**: 28-35.

Kritzberg, E.S., J.J. Cole, M.L. Pace, W. Granéli, and D.L. Bade. 2004. Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake ^{13}C addition experiments. Limnol. Oceanogr. **49**: 588-596.

Lavoie, I., W.F. Vincent, P. Reinhard, and J. Painchaud. 2003. Effet du débit sur la dynamique temporelle des algues périphytiques dans une rivière influencée par les activités agricoles. Rev. Sci. Eau **16**: 55-77.

Mura, M.P., S. Agusti, P.A. del Giorgio, J.M. Gasol, D. Vaqué, and C.M. Duarte. 1996. Loss-controlled phytoplankton production in nutrient-poor littoral waters of the NW Mediterranean: in situ experimental evidence. Mar. Ecol. Prog. Ser. **130**: 213-219.

Passow, U. 2000. Formation of transparent exopolymer particles, TEP, from dissolved percursor material. Mar. Ecol. Prog. Ser. **192**: 1-11.

Stein, J. 1973. Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press. 448 pp.

Turner, M.A., E.T. Howell, G.G.C. Robinson, P. Campbell, R.E. Hecky, and E.U. Schindler. 1994. Roles of nutrients in controlling growth of epilithon in oligotrophic lakes of low alkalinity. Can. J. Fish. Aquat. Sci. **51**: 2784-2793.

Wada, E., and A. Hattori. 1978. Nitrogen isotope effects in the assimilation of inorganic nitrogenous compounds by marine diatoms. Geomicrobiol. J. **1**: 85-

101.

Wood, A.M., H. Rai, J. Garnier, T. Kairesalo, S. Gresens, E. Orive, and B. Ravail. 1992. Practical approaches to algal excretion. Mar. Microb. Food Webs **6**: 21-38.

Table 3a.1. Results from paired *t*-tests to assess the differences between in vivo and in situ carbon and nitrogen isotope ratios of the algae. Results in brackets are significant.

In vivo versus in situ	*n*	df	*t*	*p*
$\delta^{13}C$				
Cyclotella sp. U2	3	4	-1.949	0.123
Cyclotella sp. TZ	2	3	0.998	0.392
Cryptomonas sp. U2	2	3	-1.318	0.279
Cryptomonas sp. TZ	1	-	-	-
Thalassiosira pseudonana U2	3	4	-0.684	0.532
Thalassiosira pseudonana TZ	4	5	-1.688	0.152
$\delta^{15}N$				
Cyclotella sp. U2	3	4	-9.955	(<0.001)
Cyclotella sp. TZ	2	3	-20.797	(<0.001)
Cryptomonas sp. U2	2	3	-6.952	(0.006)
Cryptomonas sp. TZ	1	-	-	-
Thalassiosira pseudonana U2	3	4	-6.652	(0.003)
Thalassiosira pseudonana TZ	4	5	-4.689	(0.005)

3a.6. List of figures

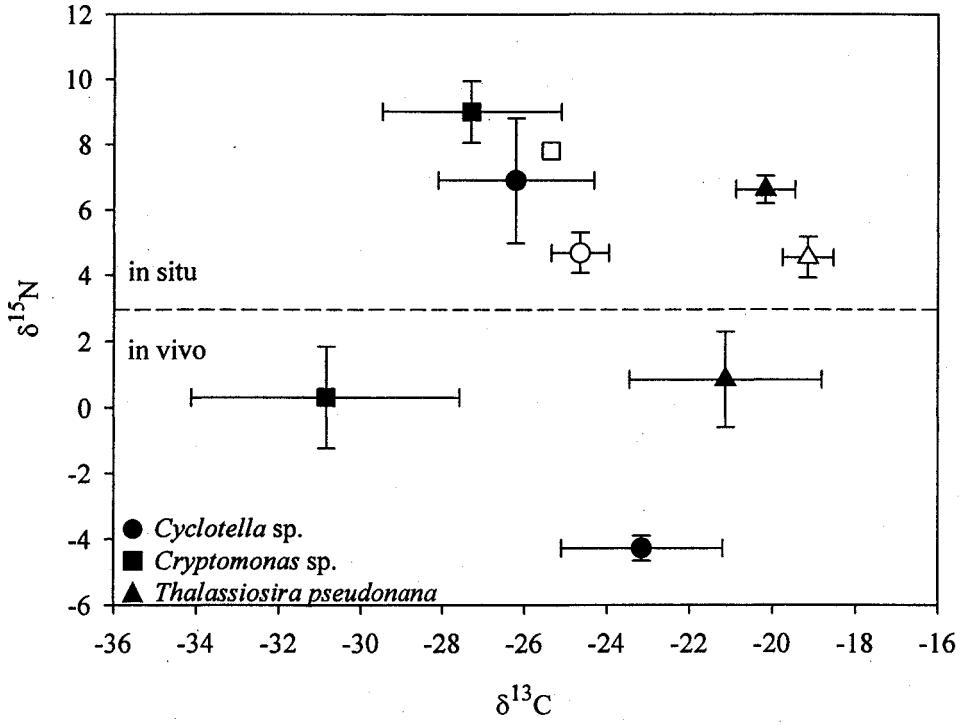

12
10
8
6
4
2
0
-2
-4
-6
$\delta^{15}N$
in situ
in vivo
● *Cyclotella* sp.
■ *Cryptomonas* sp.
▲ *Thalassiosira pseudonana*
-36
-34
-32
-30
-28
-26
-24
-22
-20
-18
-16
$\delta^{13}C$

Figure 3a.1.

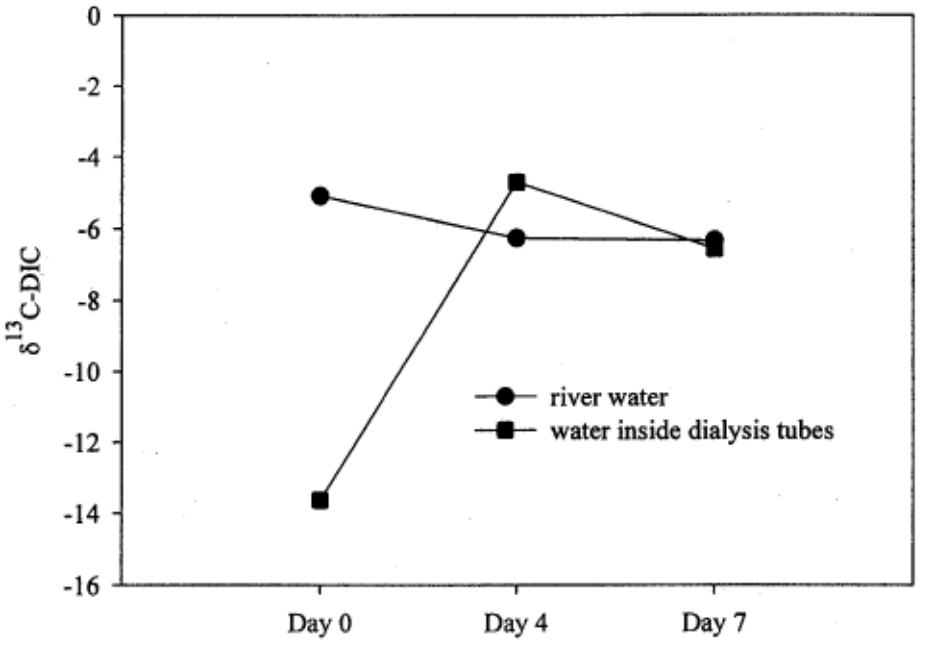

0
-2
-4
-6
-8
-10
-12
-14
-16
δ13C-DIC
river water
water inside dialysis tubes
Day 0
Day 4
Day 7

Figure 3a.2.

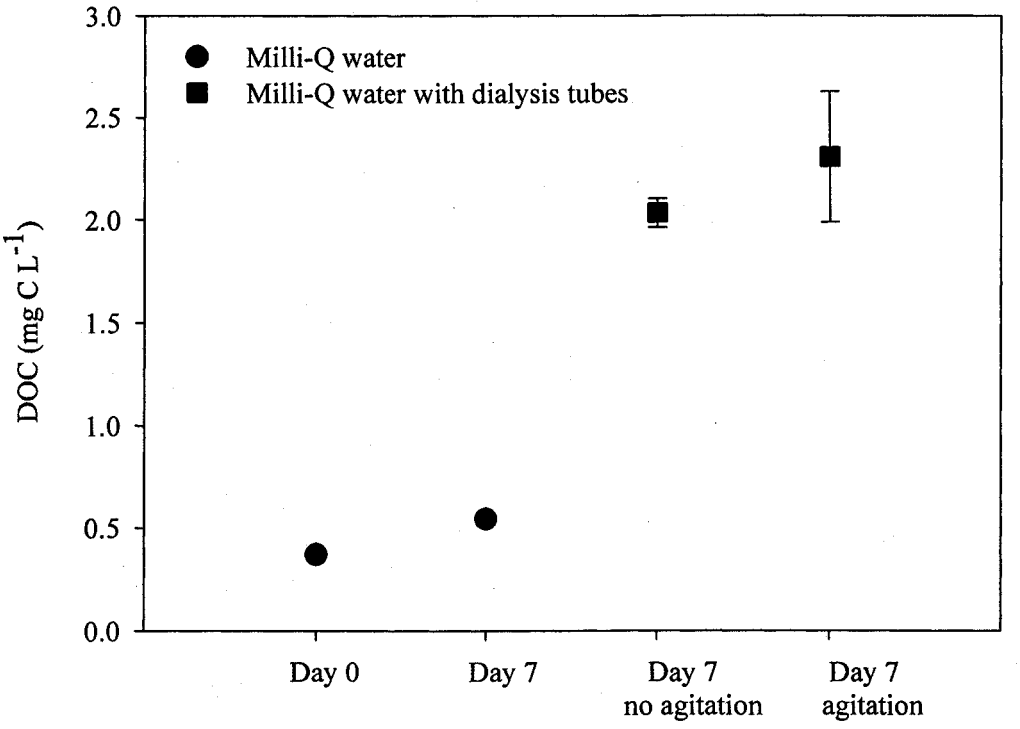

3.0
2.5
2.0
1.5
1.0
0.5
0.0
DOC (mg C L-1)
Milli-Q water
Milli-Q water with dialysis tubes
Day 0
Day 7
Day 7
no agitation
Day 7
agitation

Figure 3a.3.

Conclusion générale

La ZTE et la communauté microbienne depuis l'invasion

Depuis l'invasion des véligères de la ZTE, la structure de la communauté microbienne n'a pas changé de façon significative. Cette communauté demeure dominée par les autotrophes depuis l'eau douce jusqu'en eau saumâtre dans la zone maximale de turbidité. La classe de taille représentée par le nanophytoplancton (2-20 μm) était aussi très abondante en 1991 (Vincent et al. 1994) et ce patron a été observé de nouveau en 2001, malgré leur potentiel d'exploitation par les véligères omniprésentes dans le microzooplancton. Les analyses de corrélation démontrent que les véligères sont positivement corrélées à leurs proies, indiquant qu'elles ne sont pas limitées par la quantité de proies et qu'elles ne causent pas de diminution importante de leur densité de proies dans cette communauté.

Les véligères ne peuvent expliquer à elles seules les variations observées dans la structure de la communauté microbienne. La salinité, le broutage par le zooplancton et la rétention hydrodynamique sont les facteurs contrôlant l'abondance et la distribution de cette communauté. Selon plusieurs études, la salinité semble expliquer en partie la diminution de cellules autotrophes et conséquemment la biomasse de la Chl *a* en milieu estuarien (Bernat et al. 1994; Hollibaugh et Wong 1999; Winkler et al. 2003). En plus de l'effet de salinité, le broutage semble être un facteur de contrôle important dans la ZTE. Winkler et al. (2003) avait noté une relation exponentielle négative entre la salinité et la Chl *a*, ce qui permettait d'illustrer la pression que le broutage peut exercer sur le phytoplancton. Ces auteurs ont aussi noté une augmentation importante de la biomasse zooplanctonique qui concordait avec la diminution d'autotrophes. Nos résultats appuient ces recherches, car nous avons observé une diminution d'autotrophes avec une augmentation d'hétérotrophes >25 μm.

La rétention hydrodynamique joue un rôle primordial dans la définition de la structure de la communauté microbienne de la ZTE. De l'eau douce à la ZTE, nous avons observé une augmentation abrupte de deux ordres de grandeur au niveau de la biomasse de Chl *a* et de la densité d'agrégats; les valeurs maximales d'abondance chez les organismes de taille > 25 µm et de 3-12 µm ont été observées dans la ZTE. Ces résultats sont cohérents avec ceux de Franks (1992) et Jochem (2003) qui ont aussi noté une accumulation de cellules de petite taille au front d'eau salée estuarien. Par ailleurs, Frenette et al. (1995) ont observé une diminution de petites cellules et plutôt une augmentation de cellules de grande taille dans la ZTE. Cette divergence peut s'expliquer par des périodes d'échantillonnage différentes. En effet, Frenette et al. (1995) ont échantillonné une colonne d'eau stratifiée alors que dans notre étude, la colonne d'eau était non stratifiée et fortement mélangée sous l'effet des grandes marées, ce qui rendait la sédimentation et l'accumulation de cellules de grandes tailles peu probable.

Le premier chapitre a défini la structure de la communauté microbienne de la zone d'eau douce et de la zone de transition depuis l'invasion par les véligères de moule zébrée et ensuite identifie les caractéristiques physiques et chimiques pouvant expliquer la structure observée. Nous avons également évalué comment une zone de transition estuarienne s'insère dans le contexte de concepts lotiques. Les zones de transition estuariennes sont reconnues pour leur dynamisme et leur hétérogénéité longitudinale aux niveaux chimique, physique et biologique. Cependant, les concepts lotiques n'ont jamais été utilisés pour l'analyse des ZTE, comme si les grandes rivières devenaient soudainement l'affaire des océanographes aussitôt qu'un changement de salinités se produisait et l'inverse est vrai en ce qui concerne la perspective des océanographes pour les eaux saumâtres. Nous avons noté une discontinuité accrue dans la structure de la communauté microbienne tout au long du gradient de l'eau douce vers la zone de transition. La structure de la communauté a changé en terme de classes de tailles des organismes et des variations subtiles ont été observées au niveau des proportions de groupes fonctionnels. Cette discontinuité

s'insère dans le contexte des récentes études qui critiquent le Rivier Continuum Concept (RCC; Vannote et al. 1980) en soulignant des discontinuités longitudinales et latérales causées par l'apport de matières organiques des berges (Junk et al. 1989), par la présence de tributaires (Rice et al. 2001), de barrages (Ward et Stanford 1983) et finalement par des séquences de *zones de processus écologiques fonctionnelles* (ecological functional process zones) qui introduisent des discontinuités hydro-géomorphiques et écologiques tout au long de ce continuum longitudinal des rivières (Thorp et al. 2006). Les ZTEs, telle la ZTE du fleuve Saint-Laurent, représentent donc aussi une forte discontinuité longitudinale par leurs processus hydrodynamiques et gradients physiques et chimiques qui imposent des changements dans la communauté biotique.

De tels gradients physiques, chimiques et biologiques imposent un contrôle redoutable sur les communautés zooplanctoniques (Vincent et Dodson 1999; Winkler et al. 2003) et microbienne (Frenette et al. 1995; Chapitre 1) de la ZTE. Il semble que, par son dynamisme et sa productivité, la ZTE est en mesure de supporter les besoins des véligères sans que cela n'entraîne d'impact sévère sur le réseau microbien.

Les véligères et les variables environnementales contrôlant leur distribution spatiale et temporelle dans la ZTE

Les variations observées dans la distribution des véligères à travers la ZTE peuvent être expliquées par une combinaison de variables environnementales. Les changements dans l'abondance des véligères au cours de l'été 2000 s'expliquent par l'effet de 1) la salinité; 2) la chute de biodisponibilité de leurs proies; 3) le stress engendré par le transport; 4) la rétention et la turbidité; et 5) la prédation.

Les densités les plus élevées de véligères se trouvèrent dans les zones de salinité < 2 ‰ avec une diminution abrupte à 2 ‰. Ces résultats corroborent ceux de Bernier (2003), qui avait noté une diminution accrue du taux de filtration des

véligères à la salinité 2 ‰. Un déclin simultané de la biodisponibilité de leurs proies à >2 ‰ fut observé, impliquant que cette chute de biodisponibilité pourrait constituer un stress additionnel à l'effet de la salinité.

Les mouvements de l'eau peuvent entraîner une diminution ou une augmentation de l'abondance des véligères. Horvath et Lamberti (1999) ont observé que les véligères sont sensibles à la turbulence et aux forces de cisaillement causées par les courants fluviaux. Le déclin que nous avons noté dans la densité des véligères entre l'eau douce et la ZTE que nous avons noté pourrait aussi être expliqué par ce phénomène. Les courants atteignant des vitesses de plus de 2.0 m s^{-1} et un taux de mélange vertical élevé dans la colonne d'eau pourraient affecter négativement l'état physiologique des véligères. Les courants pourraient également expliquer leur forte abondance à certaines stations de la ZTE. L'advection amène une recirculation cyclonique responsable de la rétention du plancton dans cette zone où vont s'accumuler des cellules de grande taille et une communauté plus diversifiée (Frenette et al. 1995). Un facteur important de contrôle de la communauté planctonique de la ZTE est la prédation (Vincent et Dodson 1999; Winkler et al. 2003). La chute de véligères entre l'eau douce et la ZTE pourrait être causée par la prédation puisqu'on observe une augmentation concomitante de la communauté zooplanctonique. Wright et al. (1996) avaient observé une prédation accrue de véligères par les rotifères et d'autres auteurs avaient documenté l'importance de la prédation de véligères par les larves de poisson *Osmerus esperlans*, *Lucioperca lucioperca*, *Acerina arnua* et *Ruttilus rutilus* (révisé dans Sprung 1993). Cependant, nous avons observé (Barnard et al. 2006) que les véligères pourraient servir de proie pour les espèces *Alosa sapidissima* et *Alosa pseudoharengus* mais qu'elles ne représentent pas une source de carbone importante pour les consommateurs de la ZTE.

Les véligères auraient pu être affectées par la qualité du seston de la ZTE, vu leur sélectivité pour les algues de haute qualité alimentaire (Wacker et al. 2002). Nos analyses stoechiométriques ont démontré que le seston de la ZTE est de bonne

qualité. Les véligères sont positivement corrélées à leurs proies, i.e., à la biomasse de chlorophylle *a*, au picophytoplancton et au nanoplancton, impliquant qu'elles ne semblent pas avoir d'impacts sévères sur leurs proies phytoplanctoniques. Dans les eaux de faible salinité, la ZTE semble être un environnement favorable pour la croissance des véligères avec une communauté microbienne abondante et de bonne qualité.

Le rôle des véligères dans le réseau trophique de la ZTE : sources de carbone et assimilation de carbone organique dissout (COD)

En tant que consommateurs primaires, les véligères se retrouvent à la base de la chaîne trophique et ne jouent pas un rôle interactif avec les autres consommateurs. Selon les analyses isotopiques, les véligères se nourrissent d'algues d'eau douce, de bactéries libres et de COD. La différence notée entre la signature isotopique $\delta^{13}C$ des véligères et celle du seston <10 µm révèle que les véligères sélectionnent certaines composantes du seston total ou qu'elles utilisent des composantes qui ne sont pas bien représentées dans nos échantillons de seston. Tel que noté par Martineau et al. (2004), le seston total est majoritairement constitué de matière allochtone et n'est pas représentatif de la matière autochtone. Nos expériences d'incubation d'algues *in situ* ont démontré que la signature autochtone varie significativement de la signature allochtone dans la ZTE. De plus, nos données démontrent que les véligères et le réseau trophique semblent être supportés par le carbone autochtone plutôt que le carbone allochtone, et ce, malgré les subsides importants de matière allochtone que reçoit la ZTE. Au niveau des interactions trophiques, les véligères ne semblent pas servir de proie principale ni utiliser les mêmes proportions de proies que d'autres consommateurs primaires. Par contre, quelques espèces de poissons, telles *Alosa sapidissima* et *Alosa pseudoharengus*, peuvent s'en alimenter.

L'assimilation de COD a été évaluée en laboratoire. Un taux d'assimilation de 0.031 ng (COD) véligère^{-1} min^{-1} fut calculé, démontrant une assimilation rapide et efficace de lysats d'algues. En milieu naturel, une proportion importante du COD est composée de matière dissoute récalcitrante, ce qui pourrait diminuer significativement le taux d'assimilation en milieu naturel. Cependant, nos observations nous permettent d'affirmer que le COD représente une source de carbone importante pour les véligères et cette utilisation pourrait expliquer leur rôle passif ou non perturbateur dans le réseau trophique de la ZTE. Les espèces exotiques occupent souvent des niches distinctes qui n'ont pas d'équivalent dans la communauté indigène (Ricciardi et Rasmussen 1998). Les études à venir devront de façon générale quantifier les différents flux de carbone faisant intervenir les véligères dans un réseau trophique. Il faudrait plus particulièrement déterminer l'importance du cheminement direct du COD vers le zooplancton et les niveaux supérieurs. En effet, plusieurs études ayant évalué les budgets de carbone en milieu aquatique ont démontré que la matière particulaire n'est pas suffisante pour soutenir les niveaux trophiques supérieurs (e.g. Pace et al. 2004). Il serait donc pertinent d'examiner plus en profondeur le rôle joué par la matière dissoute dans le transfert de carbone au sein d'autres organismes zooplanctoniques de façon à mieux cerner son impact parmi les réseaux trophiques en général.

En résumé, la ZTE est une interface critique entre la section fluviale et la section estuarienne du fleuve Saint-Laurent. La ZTE est une zone de discontinuité fluviale où l'on retrouve des gradients abrupts dans les caractéristiques physiques et chimiques qui engendrent un changement dans la structure de communauté microbienne et zooplanctonique. La présence et l'abondance des véligères dans la ZTE sont affectées majoritairement par la salinité, la chute de biodisponibilité de leurs proies, le stress engendré par le transport et la rétention hydrodynamique. Les véligères ne semblent pas avoir d'impacts majeurs sur la communauté microbienne, ni sur le réseau trophique supérieur et jouent donc un rôle non perturbateur dans cette

communauté. La turbidité et l'agrégation d'organismes pourraient nuire à leur alimentation sélective, en conséquence, l'assimilation de COD semble une alternative efficace pour leur survie. Dans ce sens, les véligères jouent un rôle trophique unique dans la ZTE par cette assimilation de COD.

Références

Barnard, C., Martineau, C., Frenette, J.-J., Dodson, J.J., et Vincent, W.F. 2006. Trophic position of zebra mussel veligers and their use of dissolved organic carbon. Limnol. Oceanogr. **51**: 1473-1484.

Bernat, N., Köpcke, B., Yasseri, S., Thiel, R., et Wolfstein, K. 1994. Tidal variation in bacteria, phytoplankton, zooplankton, mysids, fish and suspended particulate matter in the turbidity zone of the Elbe estuary; interrelationships and causes. Netherlands J. Aquat. Ecol. **28**: 467-476.

Bernier, S. 2003. Efficacité et sélectivité alimentaire des larves de la Moule Zébrée : effets potentiels sur le réseau alimentaire de la zone de transition estuarienne du Saint-Laurent. Mémoire MSc, Université Laval, 43 pp.

Franks, P.J.S. 1992. Sink or swim: accumulation of biomass at fronts. Mar. Ecol. Prog. Ser. **82**: 1-12.

Frenette, J.-J., Vincent, W.F., Dodson, J.J., et Lovejoy, C. 1995. Size-dependent variations in phytoplankton and protozoan community structure across the St. Lawrence River transition region. Mar. Ecol. Prog. Ser. **120**: 99-110.

Hollibaugh, J.T. et Wong, P.S. 1999. Microbial processes in the San Francisco Bay estuarine turbidity maximum. Estuaries 22: 848-862.

Horvath, T.G., et Lamberti, G.A. 1999. Mortality of zebra mussel, *Dreissena polymorpha*, veligers during downstream transport. Freshwater Biol. **42**: 69-76.

Jochem, F.J. 2003. Photo- and heterotrophic pico- and nanoplankton in the Mississippi River plume: distribution and grazing activity. J. Plankton Res. **25**: 1201-1214.

Junk, W., Bayley, P., et Sparks, R. 1989. The flood pulse concept in river-floodplain systems. In Proceedings of the international large river symposium. Edited by D. Dodge. Can. Spec. Publ. Fish. Aquat. Sci., pp. 110-127.

Martineau, C., Vincent, W.F., Frenette, J.-J., et Dodson, J.J. 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnol. Oceanogr. **49**: 1679-1686.

Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van de Bogert, M.C., Bade, D.L., Kritzberg, E.S., et Bastviken, D. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**: 240-243.

Ricciardi, A. et J.B. Rasmussen. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can. J. Fish. Aquat. Sci. **55**: 1759-1765.

Rice, S., Greenwood, M., et Joyce, C. 2001. Tributaries, sediment sources, and the longitudinal organization of macroinvertebrate fauna along river systems. Can. J. Fish. Aquat. Sci. **58**: 824-840.

Sprung, M. 1993. The other life: an account of present knowledge of the larval phase of *Dreissena polymorpha*. *In* Zebra mussels: biology, impacts and control. Edited by T.F. Nalepa and D.W. Schloesser. Lewis Publishers, Boca Raton. Fla.

Thorp, J.H., Thoms, M.C., et Delong, M.D. 2006. The riverine ecosystem synthesis: biocomplexity in river networks across space and time. River Res. Appl. **22**: 123-147.

Vannote, R., Minshall, G., Cummins, K., Sedell, J., et Cushing, C. 1980. The River Continuum Concept. Can. J. Fish. Aquat. Sci. **37**: 130-137.

Vincent, W.F., Bertrand, N., et Frenette, J.-J. 1994. Photoadaptation to intermittent light across the St. Lawrence estuary freshwater-saltwater transition zone. Mar. Ecol. Prog. Ser. **110**: 283-292.

Vincent, W.F., et Dodson, J.J. 1999. The St. Lawrence River, Canada-USA: the need for an ecosystem-level understanding of large rivers. Jpn. J. Limnol. **60**: 29-50.

Wacker, A., Becher, P., et Elert, E.V. 2002. Food quality effects of unsaturated fatty acids on larvae of the zebra mussel *Dreissena polymorpha*. Limnol. Oceanogr. **47**: 1242-1248.

Ward, J. et Stanford, J. 1983. The serial discontinuity concept of lotic ecosystems. *In* Dynamics of lotic ecosystems. Edited by T. Fontaine and S. Bartell. Ann Arbor Science Publishers, Ann Arbor pp. 29-42.

Winkler, G., Dodson, J.J., Bertrand, N., Thivierge, D., et Vincent, W.F. 2003. Trophic coupling across the St. Lawrence River estuarine transition zone. Mar. Ecol. Prog. Ser. **251**: 59-73.

Wright, DA., Setzler-Hamilton, EM., Magee, JA., Kennedy, V.S. et McIninch, S.P. 1996. Effect of salinity and temperature on survival and development of young Zebra (*Dreissena polymorpha*) and Quagga (*D. bugensis*) Mussels. Estuaries **19**(3): 619-628.