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PAR
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ADVECTION DE PHYTOPLANCTON D'EAU DOUCE DANS LA ZONE DE TURBIDITÉ MAXIMALE DE L'ESTUAIRE DU FLEUVE SAINT-LAURENT

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AVANT-PROPOS

En vertu des articles 136 à 138 du règlement des études de cycles supérieurs, le présent document a été rédigé sous forme d'article scientifique. Il contient deux chapitres, le premier contenant un résumé substantiel, en français, des travaux et des résultats obtenus au cours de ma maîtrise. Le deuxième représente un article rédigé en anglais intitulé : «*Advection of freshwater phytoplankton in the St. Lawrence River estuarine turbidity maximum as revealed by sulfur stable isotopes* », qui sera soumis au journal « *Marine Ecology Progress Series* ». La littérature et les tableaux et figures cités dans ce chapitre sont les mêmes que lors du chapitre 1; le lecteur est invité à s'y référer.

Le manuscrit a été préparé selon le document intitulé « *Exigences et modalités liées à la présentation du mémoire ou de l'essai présenté sous forme d'article scientifique pour les programmes en Sciences de l'environnement (3403 et 3893)* ». L'article a été rédigé en suivant les directives aux auteurs pour la revue choisie, lesquelles se retrouvent en annexe.

RÉSUMÉ

La confrontation entre les eaux douces et salées dans l'estuaire du Saint-Laurent génère une zone de mélange intense occasionnant une remise en suspension des sédiments résultant en une forte turbidité qui limite la pénétration de la lumière dans la colonne d'eau. Cet endroit nommé la zone de turbidité maximale (ZTM), situé à des salinités entre 0.05 et 1 psu, est caractérisé par une colonne d'eau où la lumière ne pénètre que les premiers 10% tandis que les concentrations en Chl *a* dépassent $50\mu gL^{-1}$ en surface comme en profondeur, correspondant à une augmentation de 10 à 25 fois par rapport aux concentrations retrouvées en amont ou en aval de la ZTM. Les signatures isotopiques du Soufre du phytoplancton et du périphyton retrouvés dans le gradient de salinité indiquent que le phytoplancton retrouvé dans la ZTM a connu la majeure partie de sa croissance en eau douce. Les structures de communautés du phytoplancton sont similaires tout au long du gradient de salinité et la grande majorité des algues identifiées appartiennent à des genres communs aux sites en amont, à l'intérieur et en aval de la ZTM. Cette étude démontre que les apports d'algues depuis les eaux douces en amont, plutôt que la croissance locale, sont responsables des fortes abondances de phytoplancton à l'intérieur de la ZTM.

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Chl *a*	Chlorophyll *a*
ETM	Estuarine turbidity maximum
ETZ	Estuarine transition zone
K_d	Diffuse light attenuation coefficient
NO_2+NO_3	Nitrites+Nitrates
NTU	Nephelometric turbidity units
PAR	Photosynthetically active radiation
psu	Practical salinity units
SRP	Soluble reactive Phosphates
Z_{eu}	Euphotic depth
Z_m	Mixing depth

CHAPITRE 1

INTRODUCTION

Introduction générale

Puisqu'ils reçoivent de nombreux tributaires drainant des bassins versants diversifiés, qu'ils sont soumis à une hydrologie et une morphologie changeante et qu'ils servent à la fois d'habitat pour la faune aquatique et de route maritime pour la navigation, les écosystèmes fluviaux représentent un milieu complexe et hétérogène. La rencontre entre des milieux contrastés, que ce soit au niveau de la température, de la vitesse du courant ou de la densité de l'eau entraîne la formation de zones de front où on retrouve des phénomènes hydrodynamiques et biologiques particuliers. La zone de transition estuarienne (ZTE) du fleuve Saint-Laurent en est un bon exemple. La rencontre entre les eaux douces et chaudes continentales et les eaux salées et froides océaniques dans les environs de l'Ile d'Orléans crée une zone de brassage intense où les sédiments sont remis en suspension, augmentant de ce fait la turbidité et réduisant la pénétration de la lumière. C'est en cet endroit, au tout début de la ZTE, qu'on retrouve la zone de turbidité maximale (ZTM), laquelle est aussi caractérisée par une forte densité de matière organique particulaire (Martineau et al. 2004), de Chlorophylle *a* (Chl *a*) (Vincent et al. 1996), de zooplancton (Winkler et al. 2003) et de larves de poissons (Vincent et al. 1996, Vincent et Dodson 1999).

Problématique

Il est surprenant de retrouver de telles abondances de phytoplancton dans un milieu où les conditions de lumière semblent à première vue adverses et les tentatives d'élucider cette question sont nombreuses puisque la co-occurrence de pics de Chl *a* et de turbidité est commune à plusieurs autres estuaires, dans la portion amont de la ZTE, dont l'estuaire de San Francisco (É.-U., Kimmerer et al. 1998), la baie de Chesapeake (É.-U., Roman et al. 2001), le rivière Hudson (É.-U., Cole et al. 1992), l'estuaire de Schelde (Belgique, Muylaert et al. 2005) et l'estuaire de Colne (Royaume-Uni, Kocum et al. 2002). Les travaux de Cole et collaborateurs (1992) ont d'ailleurs démontré que la lumière est limitante pour la croissance algale dans la rivière Hudson lorsque la

profondeur dépasse 5 m alors que Alpine et Cloern (1988) ont démontré que la respiration à elle seule peut excéder la photosynthèse dans les environnements turbides et bien brassés.

Hypothèse

Ces observations ont amené les chercheurs à se questionner sur la cause de ces biomasses algales. Parmi d'autres hypothèses, Wofsy (1983) suggère qu'un apport externe continu d'algues pourrait contribuer à maintenir les fortes concentrations de Chl *a* dans la ZTM. Cette hypothèse est particulièrement pertinente pour la ZTM du Saint-Laurent étant donnée la présence d'une zone de rétention (Frenette et al. 1995), qui porte le temps de résidence des particules algales et détritiques dans la ZTM jusqu'à 15 jours sur une distance couvrant à peine 40 km (Simons et al. 2006). Ce temps de résidence combiné à un débit annuel moyen de 12 309 m^3s^{-1} d'eau comportant des concentrations élevées de phytoplancton (Vincent et al. 1996) au niveau de la ville de Québec (Environnement Canada, Service Météorologique), en amont, fait en sorte qu'il y a un potentiel d'accumulation d'algues ayant connu une croissance en amont à l'intérieur de la ZTM du Saint-Laurent. Le présent mémoire traite de la vérification de cette hypothèse.

MATÉRIEL ET MÉTHODES

Échantillonnage

L'échantillonnage s'est déroulé à bord du navire de recherche le *Lampsilis* à la mi-août (9-15) et à la mi-octobre (11-17) 2006 dans la zone illustrée à la figure 1, qui couvre un gradient de salinité allant des eaux douces (0.1 psu, site 45) jusqu'à des salinités d'environ 7 psu en surface (site 51). À chaque site, de l'eau a été récoltée en surface (0.5 m) et en profondeur (1.5 m au-dessus du fond) à l'aide d'une bouteille « Go-Flo » (General Oceanics) de huit litres pour fins d'analyses. Des profils verticaux de pénétration de la lumière, de turbidité et de salinité ont été réalisés à l'aide d'un Hyper-

spectroradiomètre (HyperPro, Satlantic Instruments) et d'une multi-sonde (6600 EDS-M, Yellow Spring Instruments), respectivement.

Analyses

Nous avons analysé l'eau échantillonnée pour déterminer les concentrations en nitrites et nitrates (NO_2+NO_3), en phosphore réactif soluble (SRP, en anglais) et en Chl *a*. Pour les analyses de phosphore et d'azote, un volume d'eau a été préfiltré sur des filtres GFF (Millipore) de diamètre de 47 mm avec des pores de 0.7 µm avant d'être analysé par spectrophotométrie selon les protocoles de l'APHA (1998). Un volume d'eau a été filtré sur des filtres GFF (Millipore) de diamètre de 25 mm avec des pores de 0.7 µm afin de concentrer le seston sur les filtres. Ces derniers ont ensuite été soniqués et la Chl *a* a été extraite dans de l'acétone (90%) froide pendant 24 heures dans le noir. Les concentrations ont été mesurées sur un fluorimètre « Turner Designs » (modèle 10-005R) tel que décrit par Parsons et ses collaborateurs (1984).

Isotopes stables de soufre

À chaque bouée de navigation illustrée à la figure 1, un volume d'eau de 20 L a été échantillonné afin d'isoler le phytoplancton du seston total par fractionnement densitaire (Hamilton et al. 2005) étant donné qu'il a été documenté que la fraction algale ne représente qu'un faible pourcentage du seston total dans la ZTM du Saint-Laurent (Martineau et al. 2004). Parallèlement, des bouteilles de polyéthylène de deux litres ont été attachées aux mêmes bouées et un poids de 5 kg y était attaché pour que les bouteilles flottent à environ 0.25 m de la surface, servant ainsi de substrat artificiel pour la colonisation par des algues périphytiques. Cette expérience a servi à déterminer le ratio isotopique du soufre pour des algues croissant à une salinité fixe et connue. Les substrats ont été installés lors de la première croisière et retirés lors de la seconde pour une période de croissance de 58 jours. Les ratios isotopiques du soufre pour le phytoplancton et le périphyton récoltés aux bouées de navigation ont été analysés par le laboratoire Iso-Analytical ltd. (Royaume-Uni) à l'aide d'un spectromètre de masse. Les

résultats sont exprimés sous la notation « δ » selon l'équation :

$$\delta X=\left[(R_{Sample}/R_{Standard})-1\right]\times 1000\,.$$

Structures de communautés

Des échantillons d'eau de 100 ml ont été conservés dans du gluteraldehyde-paraformaldehyde (concentration finale de 1 % v/v) dans le but de faire l'identification des algues phytoplanctoniques retrouvées aux sites 45, 46, 48 et 50. L'identification s'est faite au niveau du genre à l'aide d'un microscope inversé à épifluorescence. Nous avons étudié la structure de communauté phytoplanctonique en regroupant les genres en groupes dominants, i.-e. les cyanophytes, les diatomées, les chlorophytes, les cryptophytes et un groupe « autre » comprenant des algues mixotrophes (ayant la capacité d'assimiler du carbone sous forme inorganique et organique) qui n'entrent pas dans les précédentes catégories. Les résultats sont présentés sous forme de pourcentage d'occurrences pour un groupe, pourcentage qui représente le nombre d'algues identifiées appartenant à un groupe par rapport au nombre total d'algues identifiées pour un même échantillon.

RÉSULTATS

Environnement physique, chimique et biologique

La ZTM se situe au tout début de la ZTE entre les sites 46 et 49, où la salinité varie entre 0.05 et 1 psu (fig. 2). La turbidité atteint des valeurs allant jusqu'à 89.9 et 49.5 « Nephelometric turbidity units » (NTU) pour les croisières d'août et d'octobre respectivement, ce qui se manifeste par des coefficients d'atténuation de la lumière atteignant respectivement 7.3 m^{-1} et 3.8 m^{-1}. Tandis que l'azote démontre une diminution constante au travers de la région d'étude, le phosphore tend à augmenter pour atteindre des valeurs maximales en aval de la ZTM, aux sites 51 et 52 (fig. 3). Les concentrations de Chl *a* augmentent de pair avec la turbidité pour atteindre jusqu'à 54.8 et 22.9 μgL^{-1} dans le maximum de turbidité en août et octobre respectivement, à des sites où la zone photique (où on retrouve plus de 1% de la lumière de la surface de l'eau) représente à peine 5 % de la colonne d'eau (fig. 4). Ces concentrations représentent environ 25 et 12

fois, respectivement, la moyenne retrouvée aux sites hors ZTM pour les croisières d'août et d'octobre. La figure 5 illustre que les biomasses phytoplanctoniques sont reliées à la turbidité plutôt qu'aux variables associées à la croissance.

Isotopes de soufre des algues

Les ratios isotopiques du S pour le périphyton suivent fidèlement le patron de salinité (fig. 6), par opposition aux valeurs de phytoplancton qui sont similaires à celles du périphyton d'eau douce pour tous les sites, excepté une légère augmentation pour les bouées H89 et K65. Les ratios C/N indiquent un bon succès de la séparation puisqu'ils sont très inférieurs pour les algues isolées comparativement aux ratios du seston total (tableau 1), mais ils sont quand même nettement au-dessus des ratios théoriques de Redfield (1934) et d'une expérience de culture d'algues (Ho et al. 2003) qui ont évalué le ratio moyen d'une algue phytoplanctonique à environ 6.

Structures de communautés

Les structures de communautés démontrent peu de variation au fil du gradient de salinité et de turbidité, ne serait-ce qu'une augmentation de la proportion des diatomées au détriment des cyanophytes (fig. 7). Ceci est conséquent avec les données de co-occurrences des genres entre les zones (fig. 9) qui illustrent que près du tiers des genres retrouvés dans la zone d'étude sont communs aux portions amont, ZTM et aval ; ces genres sont aussi dominants au niveau des pourcentages d'occurrence, comparativement aux 7 et 9 genres indigènes à la ZTM pour les croisières d'août et d'octobre respectivement, qui ne représentent pas plus de 5 % des occurrences (fig. 10). La figure 8 illustre que la proportion de mixotrophes n'est pas plus élevée à l'intérieur de la ZTM par rapport aux zones en amont ou en aval ; elle tend plutôt à diminuer.

DISCUSSION

Les concentrations en Chl *a* ont augmenté de plus d'un facteur d'amplitude à l'intérieur de la ZTM malgré une forte augmentation de la turbidité et une diminution subséquente de la pénétration de la lumière. L'absence de lien avec les ressources liées à la croissance (nutriments, lumière) et la forte augmentation de biomasse aux sites où la turbidité est maximale suggèrent que l'hydrodynamique de la recirculation estuarienne est le facteur commun qui explique la remise en suspension des sédiments, l'accumulation de matière détritique et l'accumulation d'algues provenant de l'amont à l'intérieur de la ZTM. Ceci est bien supporté par les ratios isotopiques du soufre et par les structures de communautés qui reflètent une grande co-occurrence des genres tout au long de la zone d'étude sans qu'il n'y ait de changement majeur au niveau des structures de communautés précisément dans la ZTM. Des travaux antérieurs avaient évalué indirectement que la portion en amont de la ZTM pouvait contribuer jusqu'à 20-30 % de la biomasse algale retrouvée mais c'était pour des sites où la profondeur moyenne était d'environ 5 m et où les biomasses ne dépassaient pas les 10 μgL^{-1} (Vincent et al. 1996). Nos résultats démontrent directement une accumulation d'algues ayant connu une croissance en eaux douces et suggèrent une contribution beaucoup plus importante de l'amont comparativement aux calculs de Vincent et collaborateurs (1996) compte tenu des valeurs isotopiques, malgré le fait que l'absence de vraies valeurs limites (*end-members*) nous empêche de calculer un modèle de mélange pour évaluer quantitativement la contribution de l'amont étant donné que l'étude se situe dans un gradient de salinité. La présence de genres indigènes à la ZTM indique que la croissance y est possible mais ces genres ne représentent qu'un faible pourcentage des occurrences totales, et aucun signe au niveau des patrons de lumière ou des nutriments ne suggère que la croissance soit suffisante pour expliquer une telle augmentation de biomasse, bien au contraire.

La démonstration de l'advection d'algues depuis l'amont jusque dans la ZTM n'est pas sans soulever de questions. Simons et collaborateurs (2006) ont calculé un temps de résidence d'environ 15 jours ce qui correspond environ au facteur d'augmentation de la biomasse. Cependant si on accepte l'hypothèse que la majeure partie de la biomasse

phytoplanctonique provient de l'amont, cela signifie que le taux de renouvèlement de la biomasse et du signal isotopique des algues de la ZTM est relativement lent. Il est possible que les conditions de lumière et l'augmentation de la salinité ralentissent le métabolisme des algues et par conséquent le taux de renouvèlement, ou encore que la plupart des algues retrouvées à l'intérieur de la ZTM soient fraîchement advectées sauf aux sites où l'on retrouve les pics de biomasse. Frenette et collaborateurs (1995) ont justement observé une augmentation de la taille des cellules algales au maximum de turbidité, ce qui augmente leur temps de résidence à cause de taux supérieurs de précipitation et qui favorise un temps de survie supérieur grâce à l'accumulation de réserves (Sandgren 1991). Nos résultats isotopiques montrent que la contribution des algues d'eau douce semble même s'étendre au-delà de la ZTM; l'étendue limitée de la zone d'étude fait en sorte qu'il est impossible de déterminer à partir de quelle distance des eaux douces la croissance locale redevient dominante.

CONCLUSION

Il est tentant d'affirmer que l'advection de phytoplancton dans la ZTM explique la forte productivité secondaire qu'on y retrouve. Les pics de densités de zooplancton et d'ichtyoplancton se retrouvent à des salinités tout juste supérieures (Winkler et al. 2003) ce qui signifie qu'ils se retrouvent légèrement en aval, où les concentrations de Chl *a* commencent à diminuer. Nos données ne nous permettent cependant pas de vérifier directement cette hypothèse mais la démonstration d'un lien direct entre la salinité et les ratios isotopiques du soufre chez les algues rend possible la poursuite de recherches similaires chez les niveaux trophiques supérieurs. Il sera intéressant d'étudier les patrons de $\delta^{34}S$ chez les consommateurs en fonction de leur position dans l'estuaire, de leurs comportements alimentaires (planctonique ou benthique) et de leur sélectivité.

CHAPITRE 2

Title: Advection of freshwater phytoplankton in the St. Lawrence River estuarine turbidity maximum as revealed by sulfur stable isotopes

Running head: Advection of upstream algae

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Abstract:

The confrontation of fresh and marine waters in the St. Lawrence River generates an intense mixing zone responsible for sediments resuspension and a consequent high turbidity that strongly limits light penetration. This estuarine turbidity maximum (ETM) is situated in waters with salinities situated between 0.05 and 1 psu and is characterised by a water column where light penetrates below 10 % of the total depth while Chl *a* concentrations reach more than $50 \mu g L^{-1}$ in surface and depth samples, an increase of more than one order of magnitude compared to upstream and downstream sites. S stable isotopes from planktonic and periphytic algae in the salinity gradient showed that phytoplankton found in the ETM achieved most of its growth in freshwaters. Phytoplankton community structures are similar throughout the salinity gradient and the vast majority of identified algae correspond to genus common to the freshwater, ETM and downstream environments. This study provides evidences that high inputs of phytoplankton from upstream freshwaters, rather than local growth, are largely responsible for the observed peak in phytoplankton biomass within the ETM.

Key words: estuary, turbidity, light, phytoplankton, advection, sulfur stable isotopes

INTRODUCTION

The St. Lawrence River Estuarine turbidity maximum (ETM) is known for its high biological productivity. The first encounter between fresh and marine waters coincides with high levels of turbidity and peak concentrations of particulate organic matter (Martineau et al. 2004), Chl *a* (Vincent et al. 1996), zooplankton (Winkler et al. 2003) and fish larvae (Vincent et al. 1996, Vincent & Dodson 1999) which will eventually colonise downstream and upstream environments. Phytoplankton constitutes the major carbon source for secondary producers because of a strong selectivity for these algae which represent only 10% of the seston (Martineau et al. 2004). Such selectivity has also been observed in San Francisco estuary where phytoplankton represents about 5% of the total organic matter (Sobczak et al. 2002). The presence of peak algal biomasses in ETM is then questioned by the high loss rates of phytoplankton and the high turbidity which in turn reduces light penetration and limits high primary productivity. Attempts to explain this problematic are abundant since the occurrence of peak algal biomass with high turbidity is common in estuarine transition zones (ETZ) where salinities range from 0 to 3-4 psu. This has been observed in the San Francisco Estuary (US, Kimmerer et al. 1998), the Chesapeake Bay (US, Roman et al. 2001), the Hudson River (US, Cole et al. 1992), the Schelde Estuary (Belgium, Muylaert et al. 2005) and the Colne Estuary (UK, Kocum et al. 2002), among others. To explain this apparent paradox, Wofsy (1983) suggests the possibility of an exogenous input of algae, among other hypotheses. This is particularly relevant to the St. Lawrence ETM due to the presence of an entrapment zone that increases particles residence time (see Frenette et al. 1995 for a conceptual model). This retention zone has been documented in some estuaries enumerated above and

proposed to be responsible for the accumulation of zooplankton and phytoplankton in ETM (Kimmerer et al. 1998, Roman et al. 2001). In the St. Lawrence, Simons and collaborators (2006) calculated a residence time of 15 days in the 40 km long ETM for passive particles such as algae due to the combination of tides reaching 10 m and tidal currents up to 3 ms^{-1} (Mertz & Gratton 1990). This high residence time combined with a mean annual discharge of 12 309 m^3s^{-1} of water containing high concentrations of phytoplankton (Vincent et al. 1996) at Quebec City (Environnement Canada, Service Météorologique) may cause algae accumulation in the ETM. However, no conclusive evidence has been documented yet to demonstrate that mechanism. This study aims to directly test the hypothesis that high phytoplankton biomasses in the St. Lawrence ETM are due to an important input of algae imported from the upstream portion of the river using recent technical developments in stable isotopes analyses and separation of algae from the seston.

MATERIAL AND METHODS

Sampling

Two 8 days- sampling cruises were performed in mid August (9-15) and October (11-17) 2006 aboard the *Lampsilis* research vessel from the Université du Québec à Trois-Rivières. The study zone was situated between Quebec City and Ile-aux-Coudres (fig.1), where salinities range from 0 to ~8 practical salinity units (psu). At each site, water was collected at 0.5 m from the surface and at 1.5 m above the bottom of the river using a 8L Go-Flo bottle (General Oceanics). Salinity, temperature and turbidity vertical profiles were obtained using a multiprobe depth profiler (6600 EDS-M, Yellow Spring Instruments).

Underwater light

Photosynthetically available radiation (PAR) in the water column was measured using a spectroradiometer (HyperPro, Satlantic Instruments). The instrument was slowly lowered in the water column using a winch and a 40 kg weight was attached in order to achieve verticality in the strong currents. Diffuse vertical attenuation coefficients (K_d) were calculated by linear regression of natural logarithm of PAR irradiance versus depth. The photic depth (Z_{eu}), to which 1% of the subsurface irradiance penetrated, was calculated as $4.605/K_d$ (Kirk 1994). We then calculated the percentage of the water column situated in the photic depth as: $Z_{eu} / Z_m \cdot 100$ where Z_m is the mixing depth. The mixing depth has been determined as the upper limit of the thermocline according to the temperature profiles from the multiprobe profiler.

Nitrogen and Phosphorus analyses

At each site, water was collected from the Go-Flo bottle for nutrients analyses. Samples for soluble reactive phosphorus (SRP) and nitrites + nitrates (NO_2 + NO_3) were prefiltrated on 45 mm diameter, 0.7 µm pore-size GFF filters (Millipore). Concentrations of TP were obtained using the spectrophotometric determination of phosphates after digestion by persulfate. SRP was analysed using the acid molybdate technique. Nitrites and nitrates concentrations were determined as the sum of NO_2 + NO_3. Nitrates were first reduced into nitrites by agitation on cadmium then nitrites concentrations were determined by the sulphanilamide method. All nitrogen and phosphorus analyses were performed according to APHA (1998).

Chlorophyll *a*

Duplicate subsamples of water from surface and bottom water were filtrated on 25mm diameter, 0.7µm pore-size GFF filters (Millipore) immediately after collection and kept frozen until analysis. Filters were sonicated in cold 90% acetone and extraction continued for 24 hours in the dark at 4°C. After centrifugation, concentrations of Chl *a* were measured on a Turner Designs fluorometer (model 10-005R) before and after acidification (Parsons et al. 1984).

Periphyton growth experiment

Recent works demonstrated that S stable isotopes are useful to discriminate producers along a salinity gradient and proposed that S should be the element of choice in marine studies (Connolly et al. 2003). We installed capped 2L polyethylene containers on four navigation buoys (fig. 1) to allow colonisation by periphyton in order to obtain $\delta^{34}S$ from algae growing at a known salinity. A 5 kg weight was attached to the containers to obtain an incubation depth of approximately 0.25 m. Artificial substrates were installed during the first cruise and peryphyton was collected 58 days later during the second cruise. Algae were scraped off the container and transferred in a bottle containing filtrated water from the site using a tangential flow filtration apparatus (Millipore Pellicon, 0.45 µm pore-size). This volume of water containing periphyton was shaken then filtrated through 47 mm, 0.7µm pore-size GFF filters (Millipore) to collect algae. Filters were kept frozen at -80°C until analysis for stable isotopes signatures.

Separation of phytoplankton from the seston

Since algae in the St. Lawrence ETM represents about 10% of the total seston (Martineau et al. 2004), we followed the methodology of Hamilton and collaborators (2005) to isolate phytoplankton from detrital particles to obtain a "true" isotopic value of algal material. At each navigation buoy, 20 L of water was collected in acid-washed polyethylene containers. Particulate material was concentrated using a tangential flow filtration apparatus (Millipore Pellicon) equipped with 0.45µm pore-sized filters. Subsamples of the retentate were gently dispensed into 50ml centrifugal vials (Falcon) half-filled with silica gel (LUDOX TM50, Sigma-Aldrich) at a density of 1.27 $mg \cdot L^{-1}$. They were centrifuged for 10 minutes at 1000 rpm allowing heavy detrital material to precipitate. The light fraction containing the isolated algae was collected at the silica gel-water interface and dispensed into a graduated cylinder with filtrated water from the site as described above. Algae and the volume of water were then homogenised by gentle shaking before filtering on 47 mm, 0.7µm pore-sized GFF filters (Millipore) to collect isolated algae Filters were kept frozen at -80°C until analysis for stable isotopes signatures. Success of the separation was verified by comparing the C/N ratios of the light fraction and the total seston.

Isotopic analyses

Stable isotopes ratios for S were determined for total seston, isolated phytoplankton and periphytic algae. They were expressed in delta (δ) notation (‰) according to the equation :

$$\delta X = \left[(R_{Sample} / R_{Standard}) - 1 \right] \times 1000$$

Stable isotopes signatures were analysed at Iso-Analytical Limited laboratory (UK) using an Elemental Analysis system coupled with an Isotopic Ratio Mass-Spectrometre.

Phytoplankton community structure

Algal assemblages have been studied at surface and bottom of sites 45, 46, 48 and 50 for both cruises in order to cover the salinity and turbidity gradients of the ETM; site 45 corresponds to the freshwater, upstream site of the ETM and site 50 represents the estuarine site further downstream; sites 46 and 48 are situated in the ETM. Subsamples of site water were transferred into opaque 125 ml high density polyethylene bottles (Nalgene) containing gluteraldehyde-paraformaldehyde (1% v/v final concentration). Samples were settled in Utermöhl chambers. Two to four transects were counted at 400x magnification until al least 300 cells occurrence were observed (colonial cells account for 1 occurrence). Taxa were identified to the genus level. Cyanophytes were counted using green epifluorescence but were not identified to the genus level. We looked at the total diversity per site (number of genus, excluding cyanophytes) and at the relative importance, in terms of occurrence, of each major group, i-e chlorophytes, cryptophytes, diatoms, cyanophytes and an "other" group including mixotrophic algae that do not fit in the previously identified groups. Cells were subsequently separated in mixotrophic group, by summing cells counted into "cryptophytes" and "other", and autotrophic group, by summing cells counted into "chlorophytes", "diatoms" and "cyanophytes" to look for a potential increase in the proportion of mixotrophs in the ETM.

RESULTS

Physical and chemical environment

The Estuarine turbidity maximum (ETM) is situated at the freshwater most portion of the estuarine transition zone (ETZ), at sites 46-49 where salinities range between 0.05 and 1.10 practical salinity units (psu) (fig. 2). Water column in the ETM is relatively shallow with a mean value of 16.9 m but get deeper further downstream (58.7 m). The water column is well mixed for sites 45-50 while the mixing depth varies between 5.5 and 7.5 m for sites 51-52. Turbidity and diffuse light attenuation coefficient (K_d) respectively peak at 89.9 nephelometric turbidity units (NTU) and 7.3 m^{-1} in August compared to October cruise where peak values decrease at 49.5 ntu and 3.8 m^{-1} (fig. 2). The ETM moved slightly downstream in October compared to August and the turbidity gradient was also less pronounced. Nitrogen shows a continuous decrease with increasing salinity except for a peak found in surface sample of site 48, August (0.72 $mg \cdot L^{-1}$, see fig. 3). Soluble reactive phosphorus (SRP) shows an opposed pattern; it increases throughout the sampling region to peak a few kilometres downstream of the ETM at 31.3 $\mu g \cdot L^{-1}$ and 19.2 $\mu g \cdot L^{-1}$ in surface samples for August and October cruises, respectively.

Algal biomass and light available for photosynthesis

Concentrations of Chl *a* increased drastically in the ETM for surface and depth samples during both cruises with peak concentrations of 54.8 $\mu g \cdot L^{-1}$ and 22.9 $\mu g \cdot L^{-1}$ in surface samples at site 47 for August and October cruises, respectively (fig. 4). This represents approximately 25 times and 12 times the mean concentrations found at the sites

upstream and downstream of the ETM in the sampling region (means = 2.21 $\mu g \cdot L^{-1}$ and 1.97 $\mu g \cdot L^{-1}$ for August and October cruises, respectively). These peaks of algal biomass correspond to the turbidity peaks resulting in a water column where only 5.8 % and 7.5% of the mixing zone (corresponding to the whole water column for sites in the ETM and upstream) is situated in the euphotic zone; concentrations of Chl *a* were positively related to turbidity while they were not, or negatively related to growth proximate factors such as light and nutrients (fig. 5). The PAR irradiance is penetrating most of the mixing zone downstream of the ETM.

Algae stable isotopes signatures

Periphytic algae grown on the artificial substrates showed a $\delta^{34}S$ ranging from 5.97 to 20.00 ‰ that tracked closely the *in situ* salinity gradient (fig. 6). Comparisons of the C/N ratios from the isolated algae, the total seston, the Redfield ratio and phytoplankton species in cultures (Table 1) illustrate a good success of the density fractionation technique. However a small portion of detrital material was still present in the light fraction as its C/N ratio is situated halfway between the theoretical ratio for phytoplankton and that of the total seston at a same site. Moreover, considering that the seston includes a certain proportion of phytoplankton and is not a true end-member of the detrital, or terrestrial material, our results indicate that the major part of the light fraction is actually composed of phytoplankton. We will thus use the term "phytoplankton" to refer to the light fraction obtained from the fractionation (fig. 6). The S isotopic ratios from phytoplankton in the ETM (buoy k100) match the value from phytoplankton sampled upstream at the freshwater site (buoy k140); the phytoplankton

$\delta^{34}S$ during both cruises correspond to that of the periphyton growing in the freshwater portion (buoy k140). During August cruise, values for phytoplankton slightly increased going further in the ETZ but never got close to that of the periphyton for these sites.

Phytoplankton community structures

The distribution of cells occurrences in the different phytoplankton groups showed little variation moving from freshwaters to the downstream portion of the ETM (fig. 7). The proportion of diatoms increased with a concomitant decrease of the cyanophytes when moving downstream of site 45 during both cruises. No increase in the proportion of mixotrophs in the ETM could be observed during August or October (fig. 8). We rather observed a general tendency for mixotrophy to decrease with the salinity gradient except for site 46 during October cruise. No distinct dominance of a particular group was observed within the ETM (sites 46 and 48). This is well illustrated by the diagram showing the co-occurrence of species within the different regions (fig. 9). Eighteen of the 58 genus identified in August were common to all the portions of the sampling region while 31 genus are common to the upstream and ETM regions, compared to 21 between the ETM and the downstream sites. Seven genus were observed exclusively in the ETM during August cruise, compared to 9 during October cruise. However, these represented a low percentage of the total occurrences (<5%, fig. 10) and they corresponded mostly to benthic diatoms such as *Epithemia* , *Amphipleura* or *Cymatopleura*. The distribution of the diversity was well balanced between the different zones during October cruise compared to August where a higher rate of co-occurrence is observed between the upstream and the ETM region. During both cruises, the vast

majority of occurrences came from genus which are common to all the regions, such as *Rhodomonas* sp., *Cyclotella* sp. and *Navicula* sp.

DISCUSSION

The concentrations of Chl *a* increased by approximately an order of magnitude in both surface and bottom samples when entering in the ETM despite the sharp decrease in the underwater light availability in response to the prevailing high turbidity. Our results indicate that these abrupt increases could not be explained by local growth as illustrated by light or nutrients patterns in the ETM. It appears that the water recirculation pattern responsible for accumulation of detrital suspended matter and sediments resuspension in the ETM (Frenette et al. 1995, Martineau et al. 2004) is also causing an accumulation of phytoplankton advected from upstream. Cole and collaborators (1992) demonstrated that local growth appeared insufficient to sustain algal biomasses in the Hudson River ETM where phytoplanktonic primary production is strongly limited by light and mixing regime. They hypothesized that high algal biomasses could only be observed at shallow sites where depth was at most 4m. Studies conducted in the San Francisco Bay and the Orinoco River demonstrated that respiration alone can exceed photosynthesis in turbid and well mixed environments (Alpine and Cloern, 1988, Lewis, 1988). In the St. Lawrence, Vincent and collaborators (1996) calculated, based on a mass balance model, that 20 % to 30 % of the phytoplankton biomass found in the ETM is advected from upstream. However, we found Chl *a* concentrations peaking at much higher values (54.8 *vs* 9.9 $\mu g \cdot L^{-1}$) at sites where depth was about two fold higher while light penetration gradient was steeper. These differences are probably explained by a contrasting

distribution in the sampling sites. The observed patterns of light penetration and nutrients availability combined with documented high loss rates of phytoplankton (Winkler et al. 2003) agree with a high contribution from upstream to the ETM Chl *a*. The advected phytoplankton biomass appears even higher than previously described (Vincent et al. 1996).

The isotopic results provide direct evidence that freshwater algae constitute an important fraction of the ETM phytoplankton biomass. The relationship between periphyton S isotopic ratios and salinity demonstrates that increasing salinity results in a direct enrichment in the heavy isotope for algae in accordance with the results of Connolly and collaborators (2003) based on estuarine macrophytes. However, S ratios for phytoplankton from the whole sampling region were close to that of the periphyton growing in freshwaters, suggesting that algae communities from the ETM and further downstream spent a significant period of their growth in a freshwater site. While several studies denoted the importance of light, nutrients, growth rate and boundary layer effect on C and N isotopic ratios of algae (Hecky and Hesslein 1995, and references therein), none, to our knowledge, highlighted these mechanisms for S isotopic ratios that could limit the comparison of $\delta^{34}S$ between planktonic and periphytic algae. Similar results were obtained along a salinity gradient with the emergent macrophyte *Spartina* sp. (Connolly 2003) and indicate that such a relationship between salinity and $\delta^{34}S$ is not exclusive to periphyton. Nonetheless, if we assume conservatively a physiological mechanism causing a shift in $\delta^{34}S$ of phytoplankton (there is actually a ~2 ‰ shift between phytoplankton and periphyton growing in freshwaters), we should expect a

similar increase with salinity for both groups. These potential differences in fractionation and the lack of true end-members, since the study sites are situated in a salinity gradient, prevent the application of a mixing model to approximate the proportion of algal biomass found in the ETM that originates from upstream. As for the S isotopic ratios, the absence of true end-members for the C/N ratios prevents the application of a mixing model to evaluate quantitatively the success of the density fractionation to isolate the phytoplankton from the seston. On the higher end, the seston values include a certain portion of algae (~10% in the ETM according to Martineau et al. 2004) indicating that the real detrital, or terrestrial component of the seston would exhibit even higher ratios. On the lower end, Redfield (1934) and Ho and collaborators (2003) demonstrated that eukaryotic phytoplankton acquire a C/N ratio situated around 6. However, these values rather represent an average value of a ratio which undergoes high variability according to growth conditions and growth strategies (Arrigo 2005); resource-limited algae, as it appears to be the case with light in the ETM, would contain higher proportions of C rich pigments and proteins that would increase their C/N ratios. These considerations suggest that the light fraction identified as isolated phytoplankton is composed of a large majority of algal material.

The passive accumulation of phytoplankton in the St. Lawrence River ETM highlights further questioning. Simons and collaborators (2006) calculated a residence time for free floating particles such as algae of approximately 15 days in the ETM, which is in the same range as the increase in Chl *a* concentrations at peak values compared to upstream concentrations. This indicates that the phytoplankton in the ETM achieve a relatively slow turnover rate of $\delta^{34}S$ since the values for phytoplankton throughout the sampling

zone are very close to that of the freshwater periphyton. It is possible that adverse light conditions reduce the algal metabolic rates and biomass turnover time or that most of phytoplankton found in the ETM is recently advected except for sites where biomasses are very high due to the particular hydrodynamic at the salinity front; Frenette and collaborators (1995) noted an increase in cells size within the ETM which would promote a higher residence and survival time due to superior sinking rates and metabolic reserves in habitats when resources are limiting (Sandgren et al. 1991).This would limit the acquisition of a $\delta^{34}S$ representative of the ETM salinity for advected phytoplankton. Concentrations of Chl *a* sharply decreased just downstream of the salinity front with a concomitant increase in phytoplankton $\delta^{34}S$ indicating a higher contribution of local algal growth. However, the $\delta^{34}S$ values revealed that even downstream of the ETM, advection of freshwater phytoplankton contributes importantly to the total biomass. The low number of samples and the limited extent of the sampling region downstream in the ETZ do not allow determining where local growth becomes the main source of algal biomass. However, the isotopic data and taxonomy results suggest that freshwater algae are present up to sites where salinity reach 5.5 psu in surface waters. Roughly a third of the total genus are common to the upstream and downstream portion of the sampling region while the vast majority of occurrences is represented by these genus.

Diatoms dominated the phytoplankton community structure during both cruises at all sites with an increasing abundance with higher salinities (from sites 45 to 50), in agreement with previous studies in that zone (Lovejoy et al. 1993, Frenette et al. 1995, Vincent et al. 1996) who identified the ETM as a zone of diatoms growth. Moreover, in

this study most of the indigenous genus observed within the ETM are resuspended benthic diatoms. Given the extensive shallows surrounding the ETM along with tides reaching 10 m (Gratton and Mertz 1990), favourable growth conditions might indeed be met for benthic algae despite the turbidity conditions but our community structures and isotopes results suggest that these do not represent an important fraction of the standing planktonic biomass.

Since the peaks in zooplankton and ichtyoplankton biomasses are found at salinities slightly higher than the Chl *a* peaks (Winkler et al. 2003), it is tempting to conclude that phytoplankton advected from freshwaters supports the high ETM secondary productivity. The sharp decrease in algal biomass could be explained by an intense zooplankton grazing. However, further studies will be needed to confirm this hypothesis. Since we demonstrated the S isotopic ratios of algae are function of the salinities in the St. Lawrence River ETM, it will be interesting to observe the patterns in $\delta^{34}S$ found in consumers (planktonic and benthic) in order to determine if the advection of algae contributes to higher trophic levels, and at what extent different groups of estuarine consumers rely on phytoplankton advected from freshwaters.

REFERENCES

Alpine AE, Cloern JE (1988) Phytoplankton growth rates in a light-limited environment, San Francisco Bay. Mar Ecol Prog Ser 44: 167-173

American Public Health Association (1998) Standard Methods for the Examination of Water and Wastewater, 20th edn. APHA, Washington DC.

Arrigo KR (2005) Marine microorganisms and global nutrients cycling. Nature 437 : 349-355

Cole JJ, Caraco NF, Peierls BL (1992) Can phytoplankton maintain a positive carbon balance in a turbid, freshwater, tidal estuary ? Limnol. Ocenaogr. 37(8) : 1608-1617

Connolly RM, Guest MA, Melville AJ, Oakes JM (2003) Sulfur stable isotopes separate producers in marine food web analyses. Oecologia 138: 161-167

Frenette JJ, Vincent WF, Dodson JJ, Loveloy C (1995) Size-dependent variations in phytoplankton and protozoan community structures across the St. Lawrence River transition region. Mar Ecol Prog Ser 120: 99-110

Gingras D (1997) Le fleuve en bref, Environnement Canada, Centre Saint-Laurent, coll. « Bilan Saint-Laurent »

Gosselain V, Hamilton PB, Descy JP (2000) Estimating phytoplankton carbon from microscopic counts: an application for riverine systems. Hydrobiologia 438: 75:90

Hamilton SK, Sippel SJ, Bunn SE (2005) Separation of algae from detritus for stable isotope or ecological stoechiometry studies using density fractionation in colloidal silica. Limnol Oceanogr Methods 3:149-157

Hecky RE, Hesslein RH (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope.analysis. J N Am Benthol Soc 14(4): 631-653

Ho TY, Quigg A, Finkel ZV, Milligan AJ, Wyman K, Falkowski PG, Morel FMM (2003) The elemental compostion of some marine phytoplankton. J Phycol 39: 1145-1159

Kimmerer WJ, Burau JR, Bennett WA (2002) Persistence of tidally-oriented vertical migration by zooplankton in a temperate estuary. Estuaries 25(3) : 359-371

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems. 2nd ed. Cambridge University Press: United Kingdom

Kocum E, Underwood GJC, Nedwell DB (2002) Simultaneous measurement of phytoplanktonic primary production, nutrient and light availability along a turbid, eutrophic UK east coast estuary (the Colne Estuary). Mar Ecol Prog Ser 231: 1-12

Lewis J, Harris ASD, Jones KJ, Edmonds RL (1999) Long term survival of marine planktonic diatoms and dinoflagellates in stored sediment samples. J Plank Res 21(2): 343-354

Lovejoy C, Vincent WF, Frenette JJ, Dodson JJ (1993) Microbial gradients in a turbid estuary: Application of a new method for protozoan community analysis. Limnol Oceanogr 38(6): 1295-1303

Martineau C, Vincent WF, Frenette JJ, Dodson JJ (2004) Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. Limnol Oceanogr 49(5): 1679-1686

Mertz G, Gratton Y (1990) Topographic waves and topographically induced motions in the St. Lawrence Estuary p. 94–108. In M. I. El-Sabh and N. Silverberg [eds.], Oceanography of a large-scale estuarine system: The St.
Lawrence. Springer-Verlag.

Muylaert K, Tackx M, Vyverman W (2005) Phytoplankton growth rates in the freshwater tidal reaches of the Schelde estuary (Belgium) estimated using a simple light-limited primary production model. Hydrobiologia 540: 127-140

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Toronto

Roman MR, Holliday DV, Sanford LP (2001) Temporal and spatial patterns of zooplankton in the Chesapeake Bay turbidity maximum. Mar Ecol Prog Ser 213: 215-227

Redfield AC (1934) On the proportions of organic derivatives in sea water and their relation to the composition of plankton. James Johnstone Memorial Volume, Liverpool.

Sandgren CD (1991) Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press

Simons RD, Monismith SG, Johnson LE, Winkler G, Saucier FJ (2006) Zooplankton retention in the estuarine transition zone of the St. Lawrence Estuary. Limnol Oceanogr 51(6): 2621-2631

Sobczak WV, Cloern JE, Jassby AD, Muller-Solger AB (2002) Bioavailability of organic matter in a highly disturbed estuary: The role of detrital and algal resources. PNAS 99(12): 8101-8105

Vincent WF, Dodson JJ, Bertrand N, Frenette JJ (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 WF, Dodson JJ (1999) The St. Lawrence River,Canada-USA: the need for an ecosystem-level understanding of large rivers. Jpn J Limnol 60:29–50

Winkler G, Dodson JJ, Bertrand N, Thivierge D, Vincent WF (2003) Trophic coupling across the St. Lawrence River estuarine transition zone. Mar Ecol Prog Ser 251: 59-73

Wofsy SC (1983) A simple model to predict extinction coefficients and phytoplankton biomass in eutrophic wates. Limnol Oceanogr 28: 1144-1155

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Isolated algae	10.9
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Ho et al. 2003	6.6
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9. Co-occurrence of genus between the different portions of the sampling region. "Upstream" includes site 45, "ETM" includes sites 46 and 48 and "Downstream" includes site 50. The numbers indicate the diversity in number of species. Surface and bottom samples are included for each site

10. Co-occurrence of genus, in percent of the total occurrences within the ETM, between the ETM, the upstream and the downstream portion of the ETM

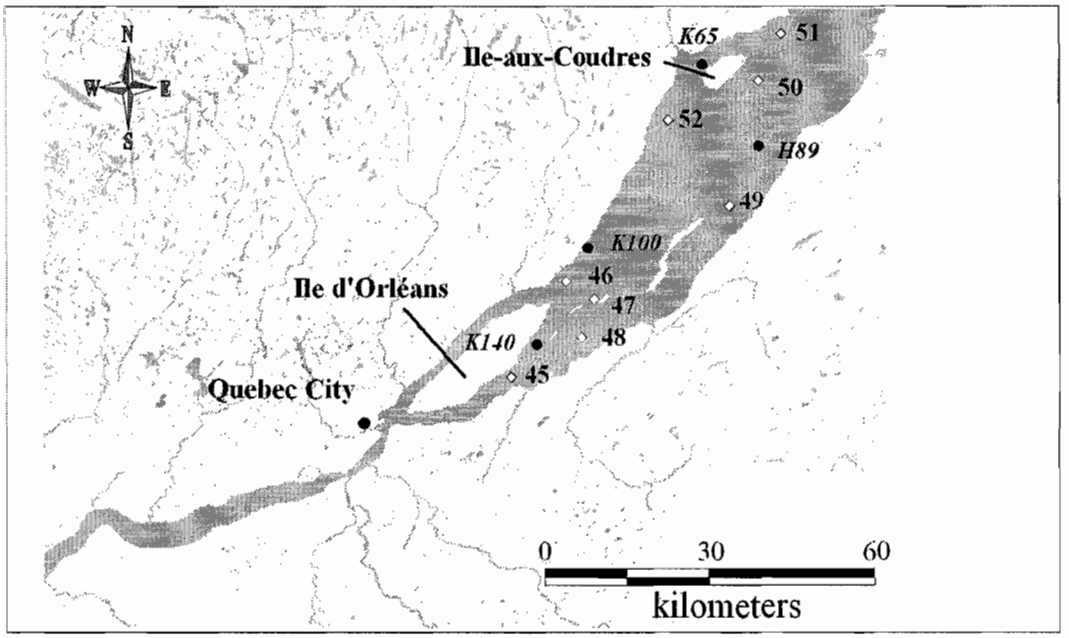
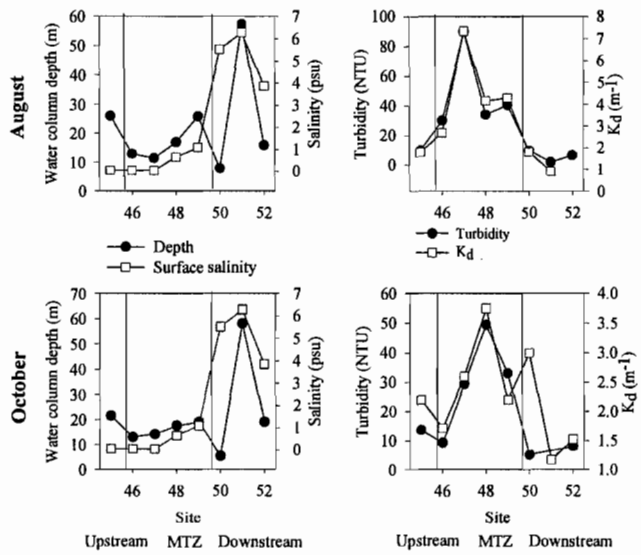


Figure 1



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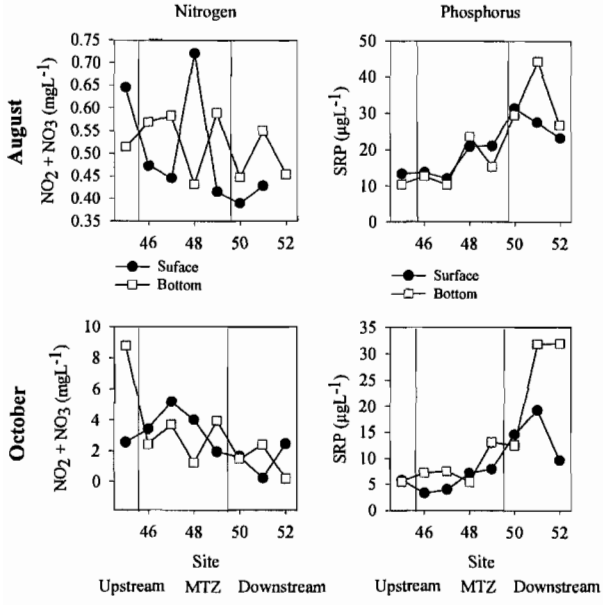

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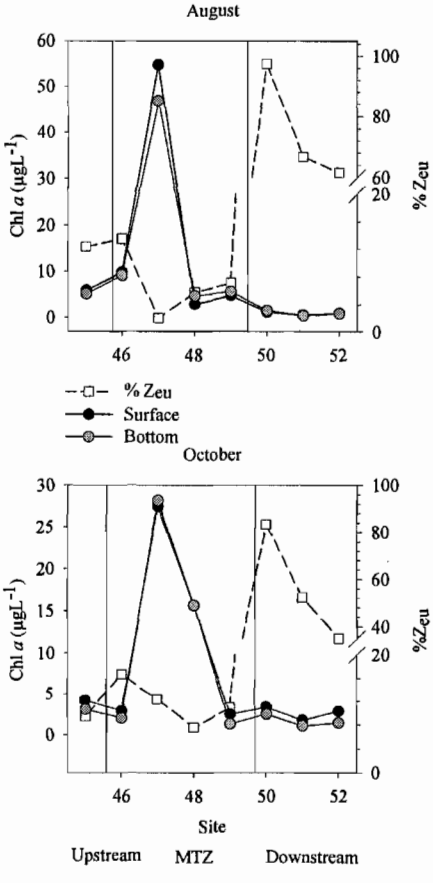

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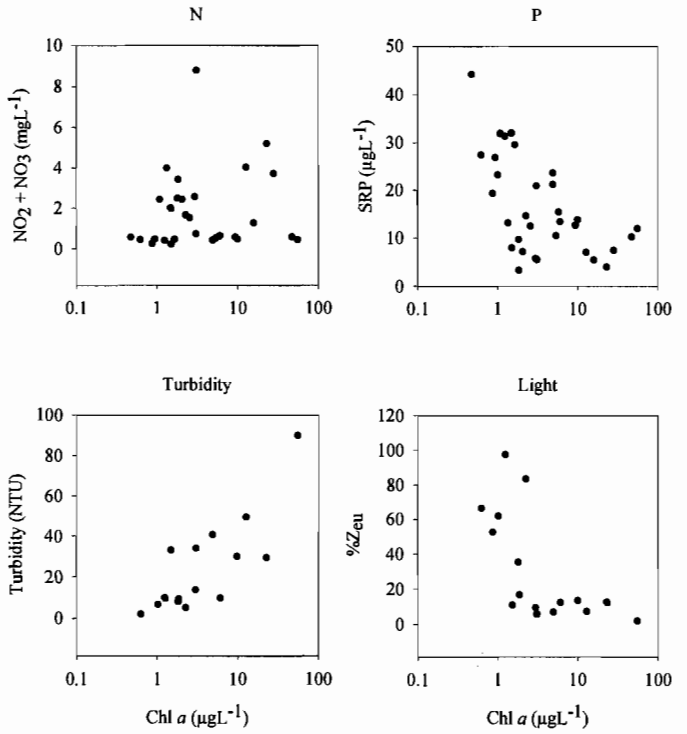

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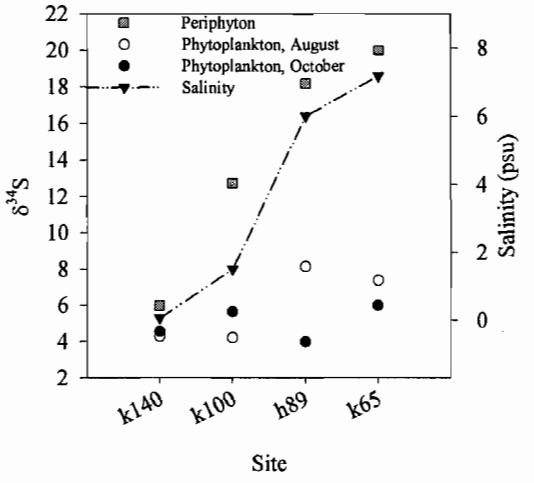

Figure 6

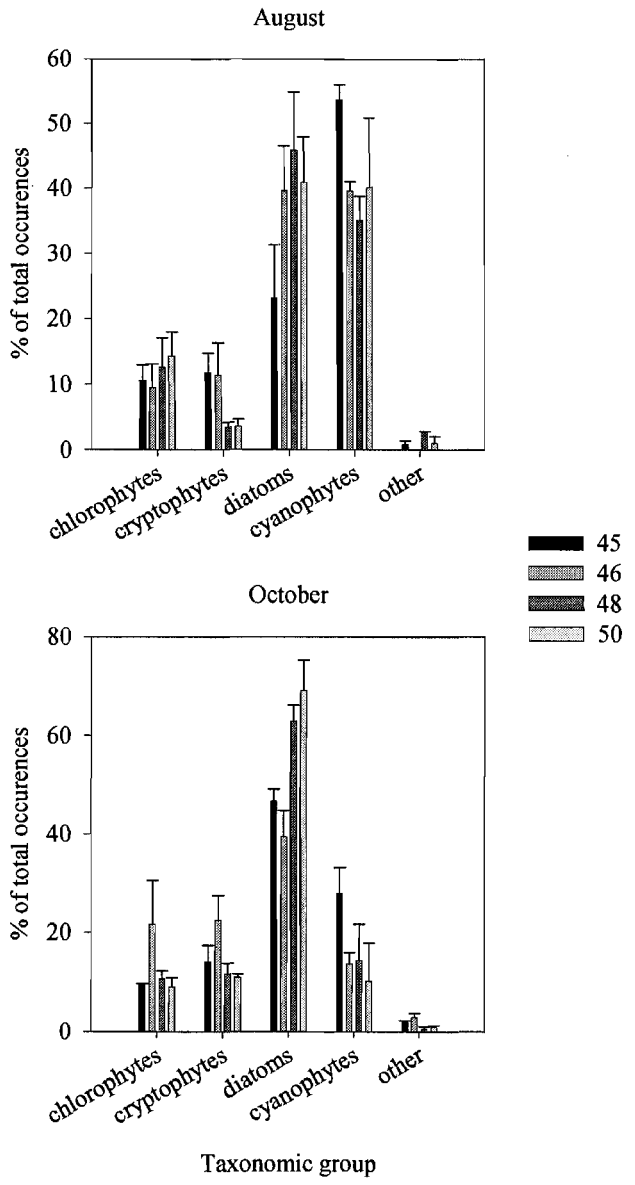
August
% of total occurences
60
50
40
30
20
10
0
chlorophytes
cryptophytes
diatoms
cyanophytes
other
45
46
48
50
October
% of total occurences
80
60
40
20
0
chlorophytes
cryptophytes
diatoms
cyanophytes
other
Taxonomic group

Figure 7

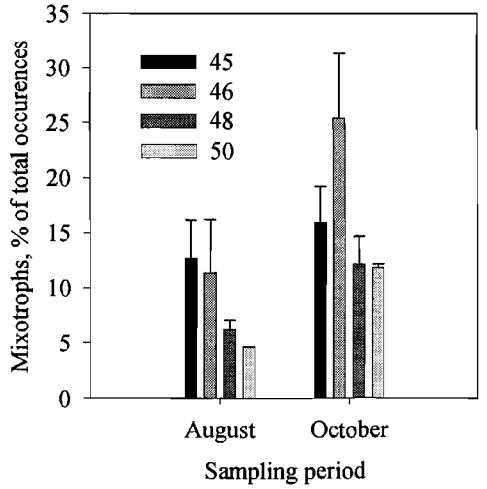

Figure 8

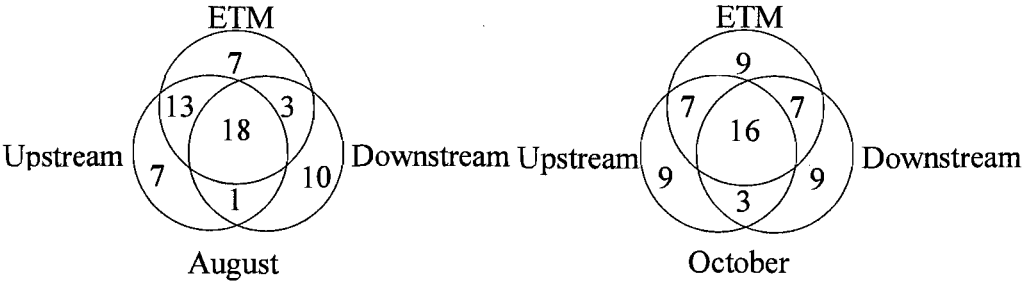

Figure 9

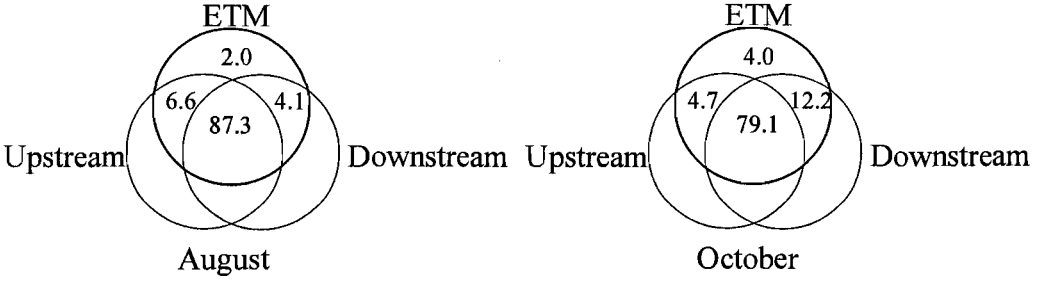

Figure 10

ANNEXE

GUIDELINES FOR MEPS AUTHORS

PREPARATION

New submissisions and revisions should if possible be uploaded or sent as one file (preferably pdf format) containing the complete text, tables, and figures. Once a ms has been accepted, please send it on CD (formatted as Mac/PC hybrid) or per email as a word-processing file (e.g. MS Word), together with separate figure files (if any). Large files (>1 MB) can be uploaded to our ftp site (ftp.int-res.com; the ftp site can be freely accessed, but please inform us if you upload anything).

To facilitate and accelerate the production process, please make sure that the ms conforms to the IR style. **For the appropriate format please refer to recent issues of MEPS.** Poor mss incur extra costs and delays; this applies particularly to figures and tables. If a ms requires excessive changes, we may have to return it, or charge you for the extra work involved in copy editing, typesetting and proofreading. To avoid this, please bring your ms in line with the following guidelines:

Manuscript length

The target length of Research Articles should be approximately 10 printed pages (about 6000 words, including references, plus Tables and Figures). Limit the number of citations to a maximum of about 1 page of citations for every 4 pages of text (i.e. Introduction, Methods, Results and Discussion).

Cover page

Title: Avoid the use of 'A', 'An', 'The', 'On', etc. at the beginning, eliminate unnecessary modifiers, and make the title as specific and concise as possible. It should preferably have up to 100 characters (ca. 15 words, 2 lines in print), and 150 characters at most. Compare

'A novel method for the production of monoclonal antibodies (MAbs) specific to an envelope protein (28kDa) of white spot syndrome virus (WSSV) of shrimp and detection of WSSV by MAb-based antigen-capture enzyme-linked immunosorbent assay'
(236 characters, 37 words)

vs.

'Detection of white spot syndrome virus (WSSV) of shrimp by means of monoclonal antibodies (MAbs) specific to an envelope protein (28 kDa)'
(137 characters, 22 words).

Provide a running head with 3 to 6 words; e.g. 'Detection of shrimp WSSV'.

Authors and addresses: If a ms has several authors from different institutions:

use superscript numerals for identification;

provide a full valid street address or PO Box for each institution, including present address(es) if applicable

use * to refer to a footnote that identifies the corresponding author and provide her/his e-mail.

Abstract: Limit the abstract (max. 250 words) to concise information on your work and its principal results. It should not contain literature cites, series of data, or meaningless clauses such as *'the results are discussed'*.

Key words: Supply 3 to 8 key words, listed in order of importance; these may be composites (e.g. 'environmental assessment', 'population dynamics'), but should not be phrases or sentences.

Text

Please use numbered pages and lines, 12 point font, and double spacing. Do your very best to use correct English grammar, spelling and punctuation; if you are not a native speaker, you should have the text edited by someone who is, before sending the ms to IR. You may also wish to consult a 'How to' book such as Day & Gastel (2006) *How to write and publish a scientific paper, 6th edn.* . (Greenwood Press, Westport, CT).

Headings: Our main headings are in capital letters. Subheadings are bold type lower case, usually centered. Further subheadings can be used and you need not worry about details as long as their order is clear; they should be kept short and in the same style as described under 'Title'. We do not accept solitary subheadings, i.e. any section must contain at least 2 subheadings, or none at all.

Verbosity: Please eliminate verbiage; examples (verbiage underlined) with improved versions:

'Numerous studies in recent years, such as those by Miller (1995) and Smith (1998), have shown that low salinities enhance oyster recruitment'.

'Low salinities enhance oyster recruitment (Miller 1995, Smith 1998)'.

'This speed was chosen because past studies have shown this to be slightly greater than the maximum sustained swimming speed.'

'This speed is slightly greater than the maximum sustained swimmng speed.'

Species names must be in italics, the genus is written in full at the first mention in each paragraph and abbreviated whenever mentioned again in the same paragraph. When referring to a species, do not use the genus name alone, unless you have previously defined it that way; be precise when using 'sp.' (singular) and 'spp.' (plural).

Abbreviations: Define unusual abbreviations and acronyms in the 'Abstract' (if used there) and at first mention in the main text, and thereafter use only the abbreviation / acronym.

Lists of items in the text should be run-on with numerals in parentheses; e.g. 'This study on mussels was conducted to: (1) assess their distributional range, (2) determine their population density, (3) collect specimens for culinary experiments'.

Literature cites in text: In cites with 2 authors, use '**&**' (e.g. '**Fesefeldt & Pritchard 2002**'); in cites with more than 2 authors use '**et al.**' but not in italics. Note there is no comma between authors and dates. When listing several cites in a row, these should be ordered by year (the earliest first), and if there are several with the same date, then these should go in alphabetical order. Cites are separated by a comma. Websites can be given in the main text (or as footnotes if referred to more than once), and they must be dated and still accessible when the article is published.

Equations and units: Use standard SI units. Relations or concentrations (e.g. mg per l) must be given as 'mg l^{-1}' (not mg/l); this applies to text, tables and graphs (e.g. axis labels). Variables are usually italicised (except for Greek letters). Italicisation should be consistent in text, figures and equations, and kept the same whether the symbols are in normal, superscript or subscripted text. Leave one blank space on either side of '=', '>', ± etc. where these denote equalities or inequalities.
Example: '$p < 0.05$, $r^2 = 0.879$' (*not* '$p<0.05$, $r^2=0.879$')
but: 'we studied organisms of size <0.5 µm'

Acknowledgements: Do not give first names in full, only initials (with period and space), e.g. 'We thank M. A. Smith and R. F. G. Miller'. Authors of the current ms should be given as initials only, e.g. 'We acknowledge a grant to M.A.S. from ...'.

Figures and tables

Figures: Please see Guidelines to Authors on Figure Preparation.

These should be self-explanatory; they must be referred to in correct numerical order in the text. Please prepare them very carefully; poor figures are a principal source of delay and additional work in the production process. High quality laser printouts, photographic prints (i.e. created by a camera), and electronic files in standard formats are acceptable.

Legends: Table legends should be given above each table; figure legends should be supplied as a list, and not placed with the individual figures. Captions should be brief and precise. If a figure or table provides data on biological species, its legend should begin with the full Latin name of that species. Example:

'Fig. 3. *Crassostrea gigas* and *Mytilus edulis*. Larval growth rates (mm d^{-1}; mean ± SD) at (a) 20°C and (b) 25°C'

Tables: Keep tables as simple and short as possible. Make sure the layout is clear. Preferably, write the rows as normal text lines and use tabs to indicate the columns (rather than using the 'Table' (cells) option in a word-processing program). For table footnotes, use superscripted lower case letters; asterisks can be used to indicate statistical significance. Tables too long to be printed in the journal can be published on our Website as an electronic supplement.

Literature cited

Limit the number of citations to a ratio of about 1 page of citations for every 5 pages of text. Use IR format (e.g. no periods or spaces with authors' initials, nor periods within journal names; examples below). All quoted literature must be listed, and all listed literature must be quoted. If in doubt with regard to abbreviations or how much information the cite should contain, provide all of it and let us shorten it.

Periodicals: Use standard abbreviations according to 'BIOSIS Serial Sources'. You may download a list of journal abbreviations from www.int-res.com/misc/journallist.txt or use the bibliographic database software 'EndNote' to import the list and obtain styles for IR journals at www.endnote.com/support/enstyles.asp. Example:

Blowden DA, Clarke A, Peck LS, Barnes DKA (2006) Antarctic sessile marine benthos: colonisation and growth on artificial substrata over three years. Mar Ecol Prog Ser 316:1-6

Books: Please write the title of the book in lower case, and give the publisher and place of publication. In the case of book series, give the series editor as well. Example:

Hanski I (2005) The shrinking world: ecological consequences of habitat loss. In: Kinne O (ed) Excellence in ecology, Book 14. International Ecology Institute, Oldendorf/Luhe

Papers from books, conference reports, symposium proceedings, etc.: Please give the title of the cited chapter, the editor(s) and title of the volume, the publisher and place of the publisher (not the location where the conference was held), and the pages of the chapter. The date of the cite must be the year of publication (not the year in which the conference was held). Examples:

Levin LA, Tolley D (2000) Influences of vegetation and abiotic enviromental factors on salt marsh invertebrates. In: Weinstein MP, Kreeger DA (eds) Concepts and controversies in tidal marsh ecology. Kluwer Acedemic Publishers, Dordrecht, p 661-707

West TL, Amrose WG (1992) Abiotic and biotic effects on population dynamics of oligohaline benthic invertebrates. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine eutrophication and population dynamics. Proc 25th Eur Mar Biol Symp. Olsen & Olsen, Fredensburg.

Certain conference proceedings/symposiums may be cited as a journal.

Bambach RK, Knoll AH, Sepkoski JJ Jr (2002) Anatomical and ecological constraints on Phanerozoic animal diversity in the marine realm. Proc Natl Acad Sci USA 99:6854-6859

Dissertations: Please write the title in lower case, 'MS / PhD thesis / dissertation' (no spaces or periods in 'MS' or 'PhD'), and give the university and its location. Example:

Eve TM (2001) Chemistry and chemical ecology of Indo-Pacific gorgonians. PhD dissertation, University of California, San Diego, CA

Websites: Permanent databases such as FishBase, GenBank, or those from climatological sources may be included in the Literature Cited list; the date accessed must be given. URLs for printed publications also available online may be included with their citations. Other website references should only be cited in the body text. Examples:

Froese F, Pauly D (2006) FishBase. Accessed 13 Dec. www.fishbase.org

IMGT/HLA sequence database (2006) European Bioinformatics Institute. Accessed 13 Dec. www.ebi.ac.uk/imgt/hla/

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