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ÉVALUATION ISOTOPIQUE DE L'IMPORTANCE RELATIVE DES PRODUCTIONS PHYTOPLANCTONIQUE VS PÉRIPHYTONIQUE DES ZONES DE FAIBLES VÉLOCITÉS POUR LES CONSOMMATEURS PRIMAIRES D'UN ÉCOSYSTÈME FLUVIAL

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AVANT-PROPOS

En accord avec les articles 136 et 138 du *Règlement des études de cycles supérieurs* de l'UQTR, le présent document est présenté sous la forme d'un article scientifique. Il est divisé en deux chapitres, un premier présentant un résumé substantiel (en français) du mémoire et un second présentant l'article (en anglais) qui sera soumis à la revue *Limnology and Oceanography* et intitulé *Isotopic evaluation of the relative importance of planktonic vs periphytic production in a large river's slackwater for primary consumers*.

RÉSUMÉ

Les isotopes stables du carbone ($\delta^{13}C$) sont des traceurs naturels de plus en plus utilisés pour estimer la contribution relative des algues périphytiques et planctoniques à la production secondaire des rivières (invertébrés, poissons). Cette approche, essentiellement développée dans des études de lacs profonds et stratifiés, reste à être vérifiée dans le contexte des zones de faibles vélocités des écosystèmes fluviaux, où le temps de résidence est court, la stratification est faible, la turbidité variable et les patrons de mélange complexes, dû à l'apport des tributaires. Nous avons analysé des filtreurs et des brouteurs, à 12 stations dans un lac fluvial du fleuve Saint-Laurent, Canada. Nous avons observé une forte variation du $\Delta\delta^{13}C$ ($\delta^{13}C$ des brouteurs – $\delta^{13}C$ des filtreurs), allant de 1 à 7‰. Lorsque cette différentiation benthique-pélagique était non détectable, le phytoplancton (chlorophylle *a*) dominait. À ces endroits, les ratios isotopiques des brouteurs étaient similaires à ceux des filtreurs, suggérant que du phytoplancton déposé était assimilé par la communauté benthique. Nos résultats isotopiques suggèrent que les lacs fluviaux peu profonds sont constitués d'une mosaïque de secteurs allant d'une dominance complète du phytoplancton à une contribution importante du périphyton.

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C	Carbone
^{12}C	Carbone 12
^{13}C	Carbone 13
chl *a*	Chlorophylle *a*
DIC	Carbone inorganique dissous
K_d	Coefficient d'atténuation des rayons photosynthétiques actifs
TN	Nitrates et nitrites
TP	Phosphore total
Turb	Turbidité
LSP	lac Saint-Pierre
PPR	Ratio Phytoplancton Périphyton
Phyto	Phytoplancton
Peri	Périphyton
$\delta^{13}C$	Ratio des isotopes stables du carbone
$\Delta\delta^{13}C$	$\delta^{13}C$ des brouteurs – $\delta^{13}C$ des filtreurs

CHAPITRE I

INTRODUCTION

À l'état naturel, les écosystèmes fluviaux sont composés d'une variété d'habitats passant de conditions lotiques, au niveau du chenal principal, à des conditions lentiques, en bordure des rives. Selon Thorp et Delong (1994), la principale source de carbone de ces écosystèmes serait de type autochtone et proviendrait des zones littorales. Plus particulièrement, les zones de faibles vélocités inondées en permanence, possèdent un potentiel de production primaire élevé et constant (Thorp et Delong, 2002). Cependant, cette production primaire, plus particulièrement la biomasse de phytoplancton (algues en suspension dans la colonne d'eau), de macrophytes (plantes aquatiques) et d'épiphytes (algues attachées à des macrophytes) peut varier de façon importante spatialement et temporellement à l'intérieur de ces zones (Vis et al., 2007). Ces variations peuvent provoquer des modifications dans la contribution relative des différents producteurs primaires au réseau alimentaire et ainsi influencer le fonctionnement de tout l'écosystème : transfert d'énergie, recyclage des nutriments, structure de la chaîne alimentaire et des habitats, etc. (Wetzel, 2001).

Les isotopes stables du carbone ($\delta^{13}C$) constituent un traceur naturel de plus en plus utilisé pour évaluer l'importance relative du phytoplancton et du périphyton (algues attachées à un substrat) pour les consommateurs (invertébrés, poissons, etc.) (Forsberg et al., 1993, Lewis et al., 2001, Delong et Thorp, 2006). Les différences d'habitats entre ces deux producteurs primaires mènent à une différenciation isotopique du C d'environ 7‰ en lac profond (France, 1995b). Cependant, dans les zones de faibles vélocités des rivières, cette différenciation isotopique semble plus incertaine (Delong et Thorp, 2006). En comparaison avec les lacs profonds, ces écosystèmes possèdent généralement une colonne d'eau non stratifiée, une turbidité élevée et des patrons de mélange complexes occasionnés par l'importance des macrophytes et l'apport des nombreux tributaires. Ces caractéristiques peuvent influencer la distribution spatiale du périphyton et du phytoplancton ainsi que leur signature isotopique.

L'objectif de cette étude est de vérifier si les isotopes stables du carbone peuvent être utilisés afin de distinguer les chaînes alimentaires phytoplanctonique et périphytique à l'intérieur des zones de faibles vélocités des rivières. Dans le cas où il y aurait chevauchement isotopique entre ces deux chaînes alimentaires, nous tenterons de déterminer quel est le maillon de la chaîne (producteur primaire ou consommateur primaire) qui est à l'origine de ce chevauchement. Chez les producteurs primaires, c'est principalement la demande en C et la limitation de ce dernier qui permettent de différencier isotopiquement le phytoplancton et le périphyton. Une variation de la demande en carbone, occasionnée par exemple par une diminution de l'intensité lumineuse chez les algues périphytiques, pourrait donc augmenter le fractionnement isotopique et ainsi mener à un chevauchement du δ^{13}C des algues planctoniques et benthiques. Puisque le δ^{13}C est un isotope de type conservateur, la signature isotopique des consommateurs est le reflet du δ^{13}C de la nourriture qu'ils ont assimilée. L'ingestion simultanée d'algues planctoniques et périphytiques par les consommateurs primaires, pourrait, elle aussi, mener à un chevauchement isotopique des chaînes alimentaires phytoplanctonique et périphytique.

RÉSULTATS

Il existe un important chevauchement entre les signatures isotopiques des consommateurs primaires filtreurs (se nourrissant de phytoplancton en suspension) et brouteurs (se nourrissant de périphyton) des zones de faibles vélocités du lac Saint-Pierre (LSP) (dernier lac fluvial du fleuve Saint-Laurent) comparativement aux résultats de France (1995b) obtenus en lacs profonds. Les résultats pairés, par station et par date d'échantillonnage des brouteurs et des filtreurs, montrent que les brouteurs sont généralement enrichis en ^{13}C par rapport aux filtreurs, mais que cet enrichissement varie de 1 à 7‰.

Une expérience effectuée en milieu semi-contrôlé montre que le périphyton croissant sous de fortes intensités lumineuses (31% de la lumière incidente) possède des concentrations plus élevées en Chlorophylle *a* (Chl *a*) que celui croissant sous de plus faibles intensités lumineuses (4, 6 et 15% de la lumière incidente) (ANOVA : F = 34,9 ; $p < 0,001$). Ces résultats suggèrent que les taux photosynthétiques des algues benthiques diminuent lorsqu'elles sont soumises à de faibles intensités lumineuses. Les résultats du $\delta^{13}C$ montrent que le fractionnement isotopique du C augmente significativement sous de faibles intensités lumineuses (ANOVA : F = 16,6 ; $p = 0,001$). La différence entre le $\delta^{13}C$ du périphyton soumis à de faibles ou de fortes intensités lumineuses est d'environ 1‰.

La variation du $\Delta\delta^{13}C$ ($\delta^{13}C$ des brouteurs – $\delta^{13}C$ des filtreurs) est expliquée par le Ratio Phytoplancton Périphyton (PPR) (PPR = log ([phytoplancton mg/m^2] / [périphyton mg/m^2]) ($r^2 = 0.80$; $p < 0.001$). La relation inverse entre le $\Delta\delta^{13}C$ et le PPR suggère que lorsque le périphyton est abondant, les brouteurs et les filtreurs possèdent des signatures isotopiques similaires, tandis que lorsque le phytoplancton est abondant, les brouteurs et les filtreurs possèdent des $\delta^{13}C$ similaire.

DISCUSSION

Nos résultats isotopiques montrent un chevauchement du $\delta^{13}C$ des filtreurs et des brouteurs et d'importantes variations du $\Delta\delta^{13}C$. Ce chevauchement n'est pas consistant avec la revue de littérature de France (1995b), qui montre une différenciation isotopique de 7‰ entre des organismes brouteurs et filtreurs de lacs oligotrophes profonds.

Le pourcentage de lumière incidente atteignant le fond varie entre 0,2 et 37,4% à l'intérieur de nos 12 stations au LSP. Or, les résultats de notre expérience en milieu semi-contrôlé, montrent que le périphyton croissant sous de fortes intensités lumineuses (31% de la lumière incidente) est enrichi en ^{13}C comparativement au périphyton croissant sous de plus faibles intensités lumineuses (4, 6 et 15%). Ceci s'explique par l'importance de la couche limite chez les algues benthiques qui limite la diffusion du C et, puisque le ^{12}C est préférentiellement assimilé par les algues (Smith et Walker, 1980), plus la demande en C du périphyton est élevée, plus il s'enrichi en ^{13}C. Cependant, une variabilité isotopique de 1‰ du périphyton, liée à l'intensité lumineuse, est insuffisante pour expliquer un chevauchement du $\delta^{13}C$ des consommateurs primaires.

L'utilisation du $\delta^{13}C$ des consommateurs primaires en tant qu'indicateurs des algues planctoniques et benthiques s'appuie sur le fait que ces organismes consomment uniquement des algues planctoniques dans le cas des filtreurs, et du périphyton, dans le cas des brouteurs. Or, la relation inverse observée entre le $\Delta\delta^{13}C$ et le PPR suggère que dans un environnement dominé par le périphyton, les consommateurs primaires se nourrissent sur leur source respective de C, tandis que dans un environnement dominé par le phytoplancton, les brouteurs et les filtreurs se nourrissent de phytoplancton. Ce changement d'alimentation des brouteurs allant du périphyton au phytoplancton, selon l'abondance des sources peut être le résultat de la sédimentation du phytoplancton, lorsqu'il abonde, le rendant ainsi disponible au mode d'alimentation des brouteurs. On estime qu'au lac Saint-Pierre les algues benthiques représentent entre 96 et 27% du carbone des organismes brouteurs.

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CHAPITRE II

Isotopic evaluation of the relative importance of planktonic and periphytic production for primary consumers in a large river's slackwater

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ABSTRACT

Stable carbon isotopes are a natural tracer which is increasingly used to estimate the relative contribution of phytoplankton and periphyton to secondary production. This approach, developed mostly through studies of deep stratified lakes, remains to be evaluated in the context of slackwaters in large rivers. These slackwaters have a short residence time, little stratification, variable turbidity, and complex patterns of mixing due to inputs from tributaries. We used filterers and grazers as integrators of isotopic signals of phytoplankton and periphyton, respectively, at 12 stations in a fluvial lake of the Saint-Lawrence River, Canada. We found strong differences in $\Delta\delta^{13}C$ between grazers and filterers ranging from 1 to 7‰. Areas of the lake where benthic-pelagic isotopic differentiation was not detected were dominated by phytoplankton (chl *a*). In these areas, isotopic ratios of grazers were similar to those of filterers, suggesting that deposited phytoplankton were consumed by the benthic community. Our isotopic data suggest that large river slackwaters comprise a mosaic of areas in which trophic states range from complete dominance of phytoplankton to strong reliance on periphytic production.

INTRODUCTION

The floodplains of large rivers comprise a variety of habitats ranging from lotic to lentic conditions. The riverine productivity model, postulates that the main source of carbon in large rivers is autochthonous primary production in the littoral zone (Thorp et Delong, 1994). Permanently inundated slackwater zones, such as shorelines, embayments and other areas outside the main channel where current velocities are substantially reduced (Thorp et Mantovani, 2005), are potentially one of the most persistent sources of autochthonous carbon for large rivers food webs (Thorp et Delong, 2002). In these ecosystems, phytoplankton, periphyton and macrophytes compete for light and nutrients and their relative contribution to biomass will depend on river morphometry, depth, and nutrients (Sand-Jensen et Borum, 1991).

In a study on lake Saint-Pierre (LSP), a large fluvial lake with an important slackwater zone, Vis et al. (2007) used an empirical model to estimate the relative contribution of phytoplankton, epiphyton and macrophytes to total primary production. They reported important temporal and spatial variations in the relative biomasses of primary producers. These variations may lead to changes in the relative abundance and in the community structure of planktonic and benthic primary consumers. This, in turn, may influence the entire ecosystem's metabolism, the energy flow, the recycling of nutrients and, the food web and habitat structure etc. (Wetzel, 2001). For example, in a long term study of a coastal area, Josefon et al. (1993) showed that changes in the benthic community structure were influenced by increased sedimentation of phytoplankton induced by the eutrophication of the environment.

Stable carbon isotopes (δ^{13}C) are a natural tracer, increasingly used to estimate the relative contribution of periphyton (attached algae) *vs* phytoplankton (suspended algae) to secondary production (invertebrates, fish) in rivers (Forsberg, et al., 1993, Lewis, et al., 2001, Delong et Thorp, 2006). In other ecosystems, such as deep lakes and marine coastal areas, the $\delta^{13}C$ of periphyton is enriched by approximately 7‰ compared to that of phytoplankton. This isotopic enrichment is generally ascribed to differentiation in the turbulence of their respective environments (France, 1995a). Periphytic algae grow in environments that have relatively low turbulence, which favour a thicker boundary layer. This limits the diffusion of C, increases limitation by ^{12}C, which is preferentially used for photosynthetic processes, and decreases isotopic discrimination (Smith et Walker, 1980).

Also, due to respiration processes, $\delta^{13}C$ of dissolved inorganic carbon (DIC) decreases with depth in stratified lakes. This depleted carbon can be assimilated by phytoplankton and increases the isotopic differentiation between planktonic and periphytic algae (Rau, 1978). After being fixed by photosynthesis, organic carbon keeps its isotopic properties which are transferred to higher trophic levels with an enrichment lower than 1‰ per trophic transfer (Peterson et Fry, 1987).

Although this isotopic discrimination between periphytic and planktonic algae is commonly observed in stratified lakes (France, 1995b, Vander Zanden et Vadeboncoeur, 2002, Sierszen et al., 2006) it appears to be more uncertain in slackwaters of large rivers (Delong et Thorp, 2006), for several reasons: 1) compared to deep lakes, large rivers typically have a well-mixed water column, which induces vertical homogenisation of $\delta^{13}C$ of DIC; 2) large rivers have short residence times, high turbidity, and complex patterns of mixing due to their well-eveloped macrophyte beds and inputs from tributaries. Those characteristics affect the spatial distribution of periphyton and phytoplankton and their carbon isotopic signature. For example, a study of the upper

Mississippi River, showed that transported algal matter was the major carbon source assimilated by primary consumers (Delong et Thorp, 2006). In that study, even collector-gatherers/detritivores or scrapers had isotopic signatures corresponding to that of planktonic algae. This results, which runs counter to their expectation that isotopic ratios of scrapers should be closer to those of benthic algae, could be due to benthic-pelagic coupling induced by sedimentation (Delong et Thorp, 2006).

Overlap in δ^{13}C of food sources of filterers and grazers could also be explained by variable fractionation in periphyton as a function of light levels. High densities of phytoplankton decrease the quantity of light available for photosynthesis by periphytic algae. This causes a reduction in the C demand and, results in an increase in isotopic fractionation from DIC (Laws et al., 1995). Under light stress, periphyton would acquire an isotopic ratio similar to that of phytoplankton, and a convergence in δ^{13}C would be observed for filterers and grazers.

The aim of this study is to examine the sources of carbon for primary consumers in the slackwater zone of a large fluvial lake. To achieve this goal, we used the carbon isotopic ratios of grazers and filterers as integrators of the signal for periphyton and phytoplankton. We show that the isotopic differentiation between consumers is highly variable within the lake. This result could be brought about by at least two mechanisms: 1) variable fractionation by primary producers from their carbon source (s) and transfer of this signal to their consumers and 2) pelagic-benthic coupling leading to the mixing of food sources. Following an experimental approach, we first showed that the light regime, known for influencing isotopic fractionation from DIC (MacLeod et Barton, 1998), accounted for very little variability in the isotopic ratio of periphyton. On the other hand, field data showed that the isotopic difference between filterers and grazers was strongly related to spatial variation in the relative abundance of phytoplankton and periphyton within the lake. This study demonstrates that food web structure in the slackwater zone of a fluvial lake is spatially highly variable, ranging from reliance on distinct carbon sources to being almost solely dependent on phytoplankton.

MATERIALS AND METHODS

Study area

Lake Saint-Pierre (LSP) (mean surface area:480km^2; mean depth: 3m), has a large width/depth ratio, which reduces the horizontal mixing of water and, leads to persistence of three main water masses (Frenette et al., 2003). Its large littoral zone offers a variety of colonisable substrates for benthic algae and the distinctness of the water masses generates spatial heterogeneity in environmental conditions. In July and August 2006, we sampled 12 stations; 10 in slackwater area and 2 near the central water mass (Fig. 1). Each station included five sampling sites located at the four vertices and the center of a square measuring 300m along the diagonal.

Water characteristics

At each station, we measured vertical profiles of turbidity (multiprobe: Yellow Spring Instruments, 650) and light (spectroradiometer: PUV2545, Biospherical Instruments). A sample of surface water for nutrient analyses (total nitrogen (TN) and total phosphorus (TP)) was collected (acid-washed polyethylene containers) at each station. Analyses of TN were accomplished by reduction of nitrates to nitrites by cadmium followed by spectrophotometry (APHA, 1998). Analyses of TP were accomplished by hydrolytic transformation of organic phosphorus, by persulfate and boric acid into ortho-phosphates followed by spectrophotometry (APHA, 1998).

We sampled each station for dissolved inorganic carbon (DIC) in 2007. Water samples collected near the surface with a syringe were immediately filtered on a 0.22μm syringe filter in an amber glass bottle to limit exchanges with the atmosphere. The bottle was completely filled and capped with a double septum, kept cold (4°C), and sent to GG Hatch Isotope Lab (University of Ottawa, Canada) the following day for C isotope analyses.

Primary producers

Phytoplankton and periphyton biomasses at each station were determined, respectively, by the concentration of chlorophyll *a* (chl *a*) in water samples and on artificial substrates. Four litres of water from the first 1.5 m were sampled at each station for phytoplankton analyses. We introduced 1.5 m of an open PVC tube below the water and closed the top extremity in order to sample, by suction, the entire water column. Artificial substrates (10 by 10 cm porous ceramics plates) were set on June 12 and were collected between the 4 and 12 July (first sampling period), and between the 17 and 28 August (second sampling period). Matter covering the artificial substrates was collected using toothbrushes.

In the laboratory, a homogenized fraction of each sample was collected on Millipore APFF filters (0.7μm) until the filters were visibly clogged. The filters were then frozen at -20 °C until chl *a* analyses. Filters were sonicated in cold acetone (90 %) and extraction continued in the dark for 24 hours at 4 °C. After centrifugation (5 000 rpm, 5 min), we used a Turner Design fluorometer (model 10-005R) to measure chl *a* (Parsons, 1984). We averaged phytoplankton and periphyton biomasses for the 5 sites at each station/date.

As suggested by Vadeboncoeur et al. (2002), values of chl *a* were transformed into mg m^{-2} by multiplying volumetric concentration (mg chl *a* m^{-3}) by depth (m). A Phytoplankton to Periphyton Ratio (PPR) was calculated as follows:

$$PPR = \log([phyto]/[peri])$$

where [phyto] and [peri] correspond to the concentration of chl *a* in mg m^{-2} for phytoplankton and periphyton, respectively.

Primary consumers

Primary consumers are frequently used as an indicator of the $\delta^{13}C$ of primary producers in order to obtain values of $\delta^{13}C$ integrated over longer time periods (Post, 2002). The difference between the $\delta^{13}C$ of the indicator organisms of the pelagic and benthic food web ($\Delta = \delta^{13}C_{grazers} - \delta^{13}C_{filterers}$) determines if the ultimate C sources of these two food webs are similar or not. Zoobenthos was collected at each station using a biological dredge (mesh size of 1cm) and the contents of the dredge were kept cold in the field until frozen in the laboratory. Grazing organisms selected as indicators of the benthic food web were the gammarid (*Gammarus fasciatus*) and the gastropods (*Goniobasis livescens* and *Planorbella trivolvis*). For the pelagic food web, three bivalves (filterer organisms) were selected: *Elliptio complanata, Lampsilis radiate,* and *Dressenia polymorpha*. We also selected *Bithynia tentacula* and *Viviparus georgianus*. These gastropods are able to feed simultaneously on benthic and planktonic algae (Brendelberger et Jurgens, 1993, Declerck, 1995).

For *G. Gammarus,* whole individuals were used, while only the soft body of the gastropods and the posterior delivery muscle of the bivalves were used. Thereafter, the samples were dried (3 days at 60 °C), crushed, then acidified drop by drop (HCl 1mol L^{-}

^{1}) to remove carbonates (Jacob et al., 2005). The samples were dried again (three days at 60 °C) and 0.20 ±0.02mg were weighed in a tin cup for isotopes analyses. Stable isotopes of C were analyzed with a Finnigan Delta mass spectrometer at the Stable Isotopes in Nature Laboratory (SINLAB) (New Brunswick, Canada). Isotopic ratios are expressed in the usual δ notation, the deviation in ‰ being compared to a reference standard, Pee Dee Belemnite:

$$\delta^{13}C = \left[\left(R_{sample}/R_{s\tan dard}\right)-1\right]\cdot 1000;$$
$$R = {}^{13}C/{}^{12}C$$

Isotopic fractionation of benthic algae

An experiment in a semi-controlled environment was carried out to examine the relationship between periphyton δ^{13}C fractionation and light intensity. During summer 2007, we installed four containers, each containing four artificial substrates (porous ceramics plates ; 10 by 10 cm) on a floating platform on Lake Joseph (Canada), an eutrophic lake (Simoneau et al., 2004). A pump constantly supplied lake water to the containers, maintaining the substrates always covered by 5cm of water. Above each substrate, the container lids were perforated and covered with screen filters letting light pass at 4,6,15 and 31% of the incidental light. The choice of filters corresponded to incident light levels at the bottom of the 12 stations in LSP.

Periphyton was collected as described above after 1 month of growth. A portion of each sample was filtered and immediately analysed for chl *a*. The other portion was reserved for density fractionation in order to separate the algal and detritus components (Hamilton et al., 2005). The algal fraction was then collected on filters (Millipore APFF, 0.7μm) that had been rinsed beforehand with acid (HCl 0.1N) and pre-dried (230 °C, during six hours). The filters were then frozen (-20 °C). Before isotopic analyses, the filters were rinsed with acid (HCl 1mol L^{-1}) in order to eliminate carbonates and then dried following Jacob et al. (2005).

Statistical analyses

$\delta^{13}C$ of filterers and grazers species were analyzed using a paired *t*-test procedure with a Bonferonni adjustment in order to evaluate differences between isotopic values of species. The semi-controlled experiment results (chl *a* and $\delta^{13}C$) were analyzed using the ANOVA procedure with a Tukey post-hoc test. An ANCOVA was used to evaluate the homogeneity of slope and y-intercept between the two regressions linking the $\Delta\delta^{13}C$ (difference between $\delta^{13}C$ of filterers and grazers) and PPR. We use $p < 0.05$ as the significance criterion for all our statistical analyses.

RESULTS

Limnological characteristics of stations

The 12 stations were highly variable with respect to physical and chemical characteristics (Table 1). The turbidity for the two sampling periods varied between 0.3 at station 4 and 121.3 NTU at station 8 and the percentage of the incident light reaching the bottom at each station varied accordingly between 0.2 to 37.4%. The concentration of nutrients also varied between the 12 stations (TN ranged from 0.05 to 1.49mg/L and TP ranged from 7.4 to 165.4 µgP/L). In 2007, $\delta^{13}C$-DIC varied between -1.6 (11) to -12.9‰ (8) among stations (Table 1).

Selection of isotopic integrators

There were no differences between the $\delta^{13}C$ of the filterers (mean pairwise differences ranging from 0.16 to 0.95; $p > 0.7$ in all cases), indicating that these three species are consistent integrators of sestonic sources of C. Here, we report average δ^{13}C values of filterers present at each station/date.

δ^{13}C of *B. tentacula* and *V. georgianus* were significantly lower than the other grazer species collected at the same station (mean paired differences ranging from -1.8 to -7.1; $p = 0.016$ and 0.014 respectively). This isotopic shift confirms the capacity of these organisms to feed on suspended algae (Brendelberger et Jurgens, 1993, Declerck, 1995). We therefore excluded *B. tentacula* and *V. georgianus* as indicators of the periphytonic carbon source.

Mean differences in δ^{13}C among *G. fasciatus*, *G. livescens,* and *P. trivolvis* ranged from 0.6 to 2.7 ($p > 0.8$). Thus, δ^{13}C values presented here, as indicators of the benthic food web, are the average of δ^{13}C of *G. fasciatus*, *G. livescens,* and *P. trivolvis* found at each station/date.

Isotopic differentiation between pelagic and benthic primary consumers

In LSP, δ^{13}C was extremely variable for grazers and filterers, ranging respectively between -28 to -16‰ and -32 to -19‰, and therefore resulting in an substantial overlap between the δ^{13}C of filterers and grazers (Fig. 2 A) compared to the values reported by France (1995b) (Fig. 2 B). However, averages by station/date revealed a significant correlation between δ^{13}C of grazers and filterers ($p < 0.001$). The slope of that relationship was not significantly different from one ($t = 1.88$, df= 17, $p > 0.05$). Grazers were generally enriched in ^{13}C compared to filterers. However, this enrichment was variable ranging from 1 to 7‰ (Fig. 3).

Isotopic fractionation of benthic algae

Periphyton grown under high light intensity (31% of incident light) showed the highest concentration biomass ($F = 34.94$; $p < 0.001$) (Fig. 4 A) and isotopic fractionation significantly increased by about one 1‰ under low light intensity (4, 6 and 15%) ($F = 16.75$; $p = 0.001$), resulting in more negative δ^{13}C periphyton (Fig. 4 B).

Contributions of phytoplankton and periphyton to primary consumers

Phytoplankton biomasses varied by approximately an order of magnitude (1.7 to 12.4 µg L^{-1}) while periphyton varied by more than two orders of magnitude (0.03 to 8.2mg m^{-2}) (Table 1). The Phytoplankton to Periphyton Ratio (PPR) varied from -1.1 to 2.4 (Fig. 5). Therefore, the biomass of phytoplankton relative to that of periphyton also varied by more than two orders of magnitude among our stations. Variation in $\Delta\delta^{13}$C (difference between δ^{13}C of grazers and filterers) was related to PPR ($r^2 = 0.80$; $p <$ 0.001) (Fig. 5). Separate analyses of the two species known to be facultative grazers/filtereres (*B. tentacula* and *V. georgianus*) resulted in a similar negative slopes between $\Delta\delta^{13}$C and PPR (ANCOVA test for homogeneity of slope; $F = 0.655$; $p > 0.05$), but with a significantly lower intercept (ANCOVA; $F = 43.278$; $p < 0.001$).

DISCUSSION

Chlorophyll *a* results demonstrate a strong gradient in the concentration of primary producers within LSP. Phytoplankton values varied from 1.7 to 12.5 µg L^{-1} (stations 4 and 9 respectively; Table 1) indicating that, in terms of chl *a*, some areas would be considered as oligotrophic and others as eutrophic (Wetzel, 2001). However, those variations are small relative to the large range of phytoplankton biomass reported for rivers (<1 to >400 µg chl a L^{-1}; Wehr et Descy, 1998). Even if periphyton concentration ranged over two orders of magnitude, our values were relatively low in comparison with stream with high nutrient loadings, maximum periphyton biomass can range from 300 to 400 mg m^{-2} (Stevenson et al., 1996). The variations in density of primary producers induced a large PPR gradient, ranging from -1.1 to 2.4, within a single ecosystem. This result indicates that in some areas, periphyton is approximately three times more abundant than phytoplankton whereas others, phytoplankton is approximately eleven times more abundant than periphyton. Even if biomass and productivity are different measure, the variations in the relative abundance of this study are comparable to those observed between phytoplankton and periphyton productivity compiled by Vadeboncoeur et al. (2002), ranging from periphyton which is about five times more productive than phytoplankton to phytoplankton which is about forty times more productive than periphyton.

A study conducted by Fry (2002), in a δ^{13}C-DIC gradient ranging from -2 to -10‰, showed a relationship between the δ^{13}C of the DIC and that of bivalves. Thus, the great range of primary consumers δ^{13}C reported in LSP could be related to spatial variability in the δ^{13}C-DIC available to primary producers. The variation in δ^{13}C-DIC observed in LSP (-1.6 to -12.9‰; Table 1) could be explained by the inputs of low δ^{13}C-DIC tributaries along the north and south shores of LSP, in contrast with the high carbonate, high δ^{13}C-DIC waters arriving from the Great Lakes (Yang et al., 1996, Barth et Veizer, 1999).

Isotopic values of primary consumers showed a substantial overlap between carbon signatures of invertebrates feeding on phytoplankton and periphyton (Fig. 2) and substantial variability in $\Delta\delta^{13}$C (Fig. 5). This variation, in the scope of isotopic differences between grazers and filterers, is in contrast with the comparative data summarized by France (1995a) (Fig. 2 B). In that study, a very small overlap in δ^{13}C was observed between filterers and grazers and the range of differences between these two functional groups averaged 7‰. However, these results were based on isotopic ratios obtained from deep oligotrophic lakes which are subject to vertical variation in the δ^{13}C-DIC (Rau, 1978), thus increasing the likelihood of isotopic differentiation between phytoplankton and periphyton. In the absence of strong stratification in shallow lakes and rivers, both phytoplankton and periphyton should be using the same pool of DIC. Therefore, vertical variation in δ^{13}C-DIC in slackwaters should not be a driving factor controlling the isotope differentiation between primary producers. However, the fractionation effects by boundary layer phenomena observed in periphyton should be present in shallow water bodies as well as in deeper one, potentially explaining the site specific shift between δ^{13}C of grazers and filterers observed in the present study (Fig. 3).

The variation in light intensity reaching the bottom in our study (0.2 to 37.4%; Table 1) caused a significant but modest fractionation of periphyton from DIC. The potential fractionation linked to light levels (1‰, Fig. 4B) is insufficient to explain the among-station variation in $\Delta\delta^{13}$C. In a field experiment under different light regime (100 and 10% of incident light), the δ^{13}C of periphyton varied about 3‰ during the growth season (MacLeod et Barton, 1998). However, that experimental light regime varied more widely than the light conditions observed at the bottom of LSP in our experiment. Therefore, fractionation by periphyton related to variable light regime is not likely to be an important factor explaining variation in the isotopic ratios of grazers in LSP.

Another possible mechanism explaining the variability of $\Delta\delta^{13}$C between primary consumers is the mixing of C sources available to filterers and grazers. The negative relationship between $\Delta\delta^{13}$C and the PPR indicated that when periphyton was dominant, grazers and filterers had distinct δ^{13}C values and conversely, when phytoplankton was dominant, grazers and filterers had similar isotopic signatures (Fig. 5). This suggests that in an environment dominated by periphyton, grazers and filterers fed on their respective

C source. However, in an environment dominated by phytoplankton, both grazers and filterers depended almost entirely on phytoplankton, result possibly explained by a pelagic-benthic coupling, in which phytoplankton deposited at the bottom becomes readily available to grazers.

Vadeboncoeur et al. (2003) obtained similar results in shallow productive lakes where benthic primary consumers had δ^{13}C close to phytoplankton primary producers. They suggested that dominatnce of planktonic algae by biomass caused a diet change in grazers, which shifted from periphyton to phytoplankton. Such changes in trophic relationships could be related to grazing on deposited phytoplankton or to modifications of grazer feeding systems. Our results suggest that both mechanisms could be at work in slackwaters of fluvial lakes. Indeed, the relationship between $\Delta\delta^{13}$C of grazers that can modify their feeding system to consume suspended planktonic algae (i.e., facultative grazers such as *B. tentacula* and *V. georgianus*) and PPR is similar to that of obligate grazers (Fig. 5). However, for a same PPR, values of $\Delta\delta^{13}$C of grazers/filterers are always smaller than those of grazers. This indicates that even in an environment dominated by periphyton, this facultative grazer use filter feeding to collect suspended phytoplankton. This result agrees with the study of Tashiro and Colman (1982) which showed that *Bithynia tentacula* had greater net energy gain when they fed on suspended phytoplankton; this adaptation may provide a competitive advantage relative to other benthic invertebrates. Therefore, facultative grazers should not be used as indicators of benthic food webs.

To calculate the relative importance of phytoplankton and periphyton to primary consumers we assumed that: 1) filterers fed exclusively on phytoplankton, and 2) $\delta^{13}C_{periphyton} - \delta^{13}C_{phytoplankton}$ was equal to 7‰. The first assumption is based on the observation that filter feeders can only rely on planktonic algae and, therefore, periphyton should not be an available carbon source. The second assumption seems feasible at the light levels measured from our experimental and field results, which suggest that there is negligible variation in the isotopic fractionation of primary producers and that the greatest $\Delta\delta^{13}$C between filteres and grazers was of 7‰. Thus, the grazers' reliance on periphytic carbon is calculated as follows:

$$\%\ dependence\ on\ periphyton\ for\ grazers = \frac{\Delta\delta^{13}C}{7\text{‰}} \cdot 100$$

The percent reliance of grazers on periphyton is also variable, ranging from 27% (station 9) to 96% (station 4; Table 1). The mean for the 12 station/dates shows that grazers obtain approximately 65% of their carbon from periphyton and 35% from phytoplankton.

Despite the marked variability of $\Delta\delta^{13}$C in fluvial slackwaters, our results suggest that stable isotopic data may be used to trace the relative contribution of phytoplankton and periphyton to consumers in these systems. Large river slackwaters comprise mosaic of areas in which carbon flows to consumers range from dominance by phytoplanktonic sources to strong reliance on periphytic production. Grazers can vary drastically in their use of carbon sources, from almost sole reliance on perphyton (96%) to strong dependence on phytoplankton (73%). Therefore, slackwater environments, because of their shallow depth, low current velocities, and abundant macrophyte beds, are subject to benthic-pelagic coupling, as illustrated in the present study by the deposition and consumption of phytoplankton by benthic primary consumers.

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TABLE

Table 1 Averages of July and August limnological characteristics for 12 stations in LSP: depth, turbidity (Turb), coefficient of light attenuation of photosynthetic available radiation (K_d), sum of nitrates and nitrites (TN), total phosphorus (TP), concentration of chl *a* in seston (Phyto), concentration of chl *a* collected on artificial substrates (Peri), C isotope values of the total dissolved inorganic carbon (δ^{13}C-DIC) and the percentage of grazer carbon provided by periphytic algae (graze reliance on periphyton).

Stn	Depth m	Turb NTU	K_d	TN mg/L	TP µgP/L	Phyto µg L^{-1}	Peri mg m^{-2}	δ^{13}C-DIC (‰)*	Grazer reliance on periphyton (%)
1	1.3	37.6	4.17	1.49	18.6	4.6	2.0	-5.6	65.8
2	0.8	4.0	2.87	0.39	30.4	2.9	8.2	-8.5	94.9
3	0.9	1.1	1.78	0.21	20.7	3.6	4.9	-8.7	93.9
4	0.8	0.3	2.25	0.21	99.7	1.7	3.4	-8.6	95.9
5	0.9	1.2	2.34	0.18	11.3	1.7	6.9	.	73.5
6	0.6	30.4	1.56	0.05	26.3	3.7	1.6	-9.0	71.8
7	1.1	1.6	1.98	0.20	26.6	6.3	0.04	-7.4	38.9
8	0.6	121.3	9.76	0.88	165.4	4.2	0.5	-12.9	48.3
9	0.8	27.6	3.34	0.40	44.2	12.5	0.03	-10.4	27.0
10	0.6	61.6	2.31	0.34	42.8	3.0	1.6	-10.8	83.0
11	1.9	15.9	1.52	0.50	7.4	4.0	.	-1.6	29.7
12	1.7	5.7	2.52	0.37	34.7	6.9	4.6	-7.9	57.5

*** Samples for DIC were collected in August 2007.**

FIGURE LEGENDS

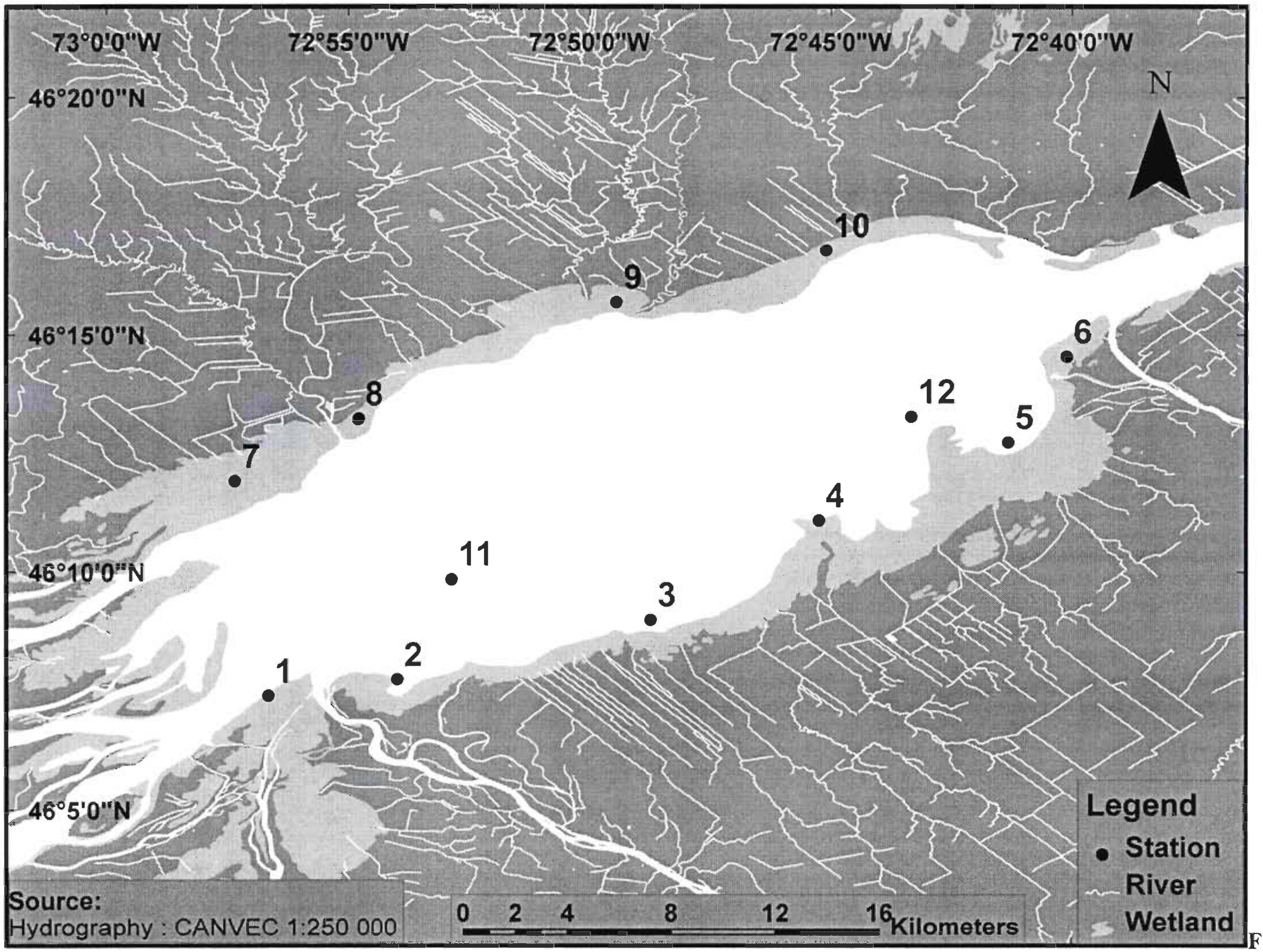
Figure 1 Location of the 12 sampling sites in Lake Saint-Pierre.

Figure 2 Percentage frequency distribution of δ^{13}C (‰) for filterers and grazers in (A) the present study and (B) the study of France (1995b).

Figure 3 δ^{13}C (‰; mean and standard deviation) of grazers and filterers for each station in July and August 2006. $\delta^{13}C_{\mathrm{grazers}} = -2.7 + 0.7 \cdot \delta^{13}C_{\mathrm{filterers}}$ ($p < 0.001$). The 1:1 line is shown.

Figure 4 Box plots of Chl *a* concentration (mg m^{-2}) (A) and δ^{13}C (‰) of benthic algae (B) in relation to the percentage of incident light reaching the artificial substrate during the experiment in a semi-controlled environment, letters represent the results of Tukey post-hoc test of an ANOVA analyse.

Figure 5: $\Delta\delta^{13}$C (‰); the difference between the δ^{13}C of grazers (dark circle) and of grazers known to be able to filter (*B. tentacula and V. georgianus*) (open circle) and the δ^{13}C of filterers, in relation with the index of abundance of phytoplankton (PPR) for the 12 stations at the two sampling dates.



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Hydrography : CANVEC 1:250 000

Fig. 1

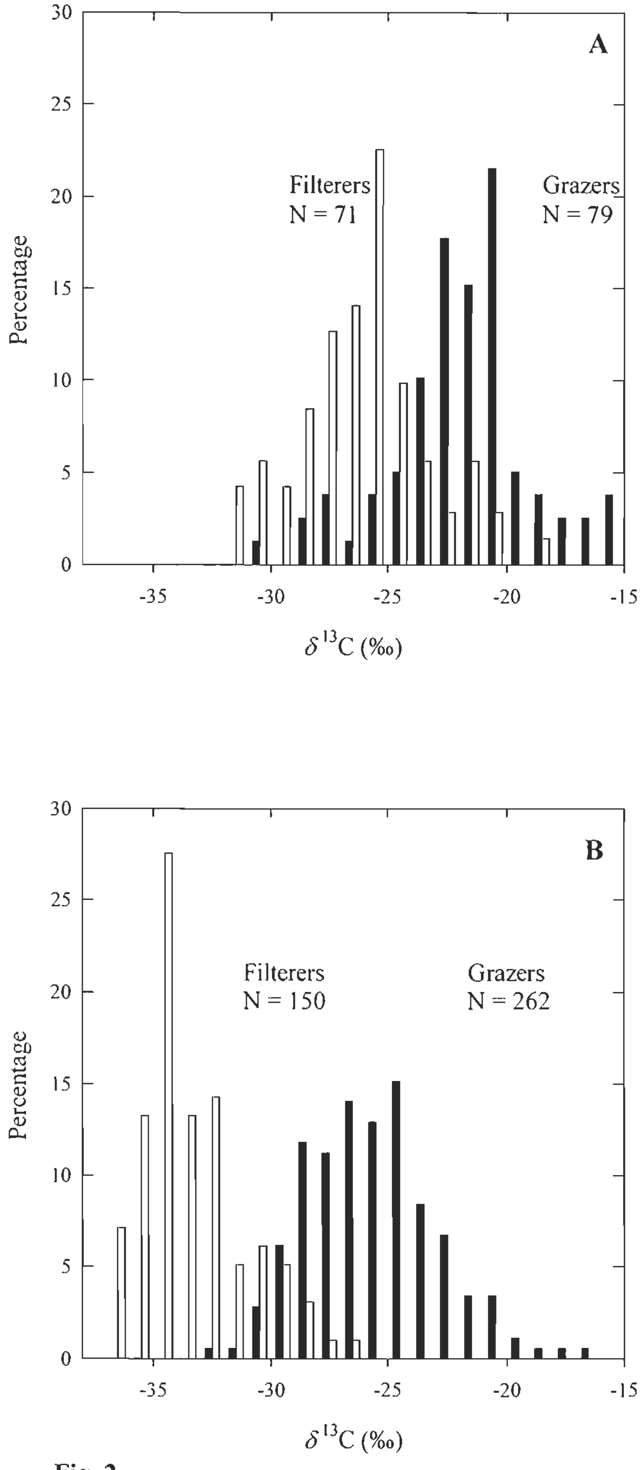
A
Filterers
N = 71
Grazers
N = 79
Percentage
0
5
10
15
20
25
30
-35
-30
-25
-20
-15
δ^{13}C (‰)
B
Filterers
N = 150
Grazers
N = 262
Percentage
0
5
10
15
20
25
30
-35
-30
-25
-20
-15
δ^{13}C (‰)

Fig. 2

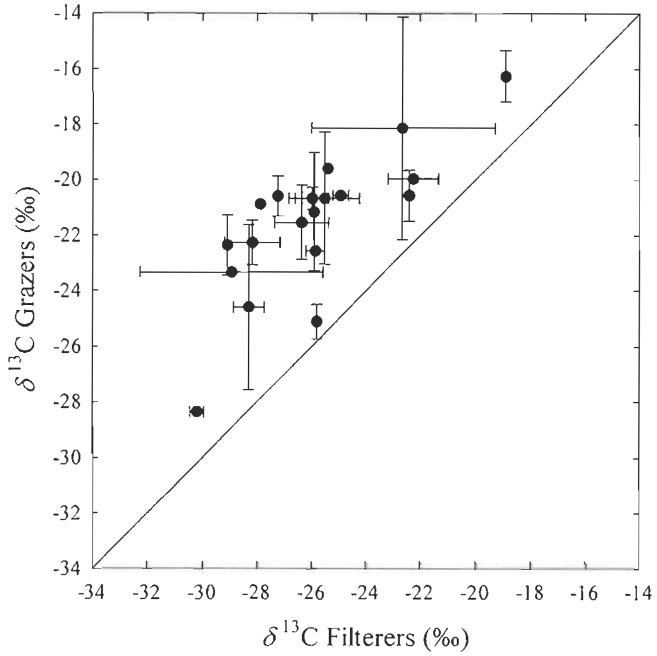
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
-34
δ^{13}C Grazers (‰)
-34
-32
-30
-28
-26
-24
-22
-20
-18
-16
-14
δ^{13}C Filterers (‰)

Fig. 3

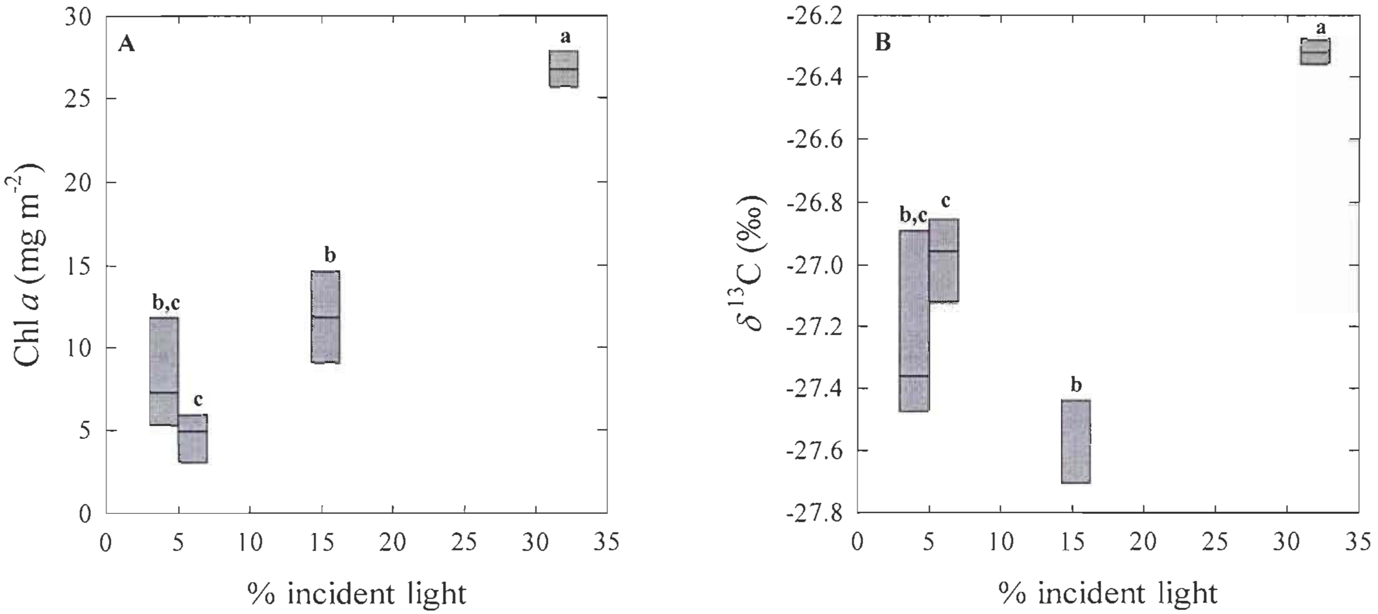
A
Chl *a* (mg m^{-2})
30
25
20
15
10
5
0
a
b
b,c
c
0
5
10
15
20
25
30
35
% incident light
B
δ^{13}C (‰)
-26.2
-26.4
-26.6
-26.8
-27.0
-27.2
-27.4
-27.6
-27.8
a
b,c
c
b
0
5
10
15
20
25
30
35
% incident light

Fig. 4

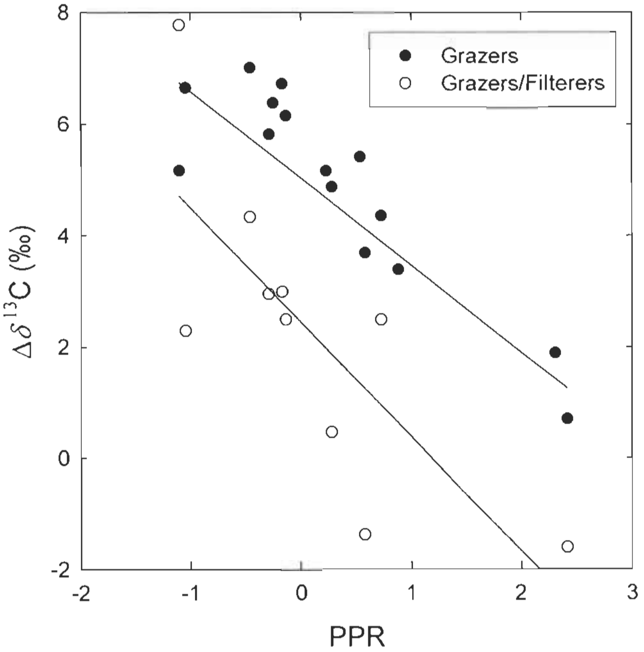
8
6
4
2
0
-2
-2
-1
0
1
2
3
$\Delta\delta^{13}C$ (‰)
PPR
● Grazers
○ Grazers/Filterers

Fig. 5

ANNEXE

Author Instructions

General points

The American Society of Limnology and Oceanography (ASLO) publishes six regular issues of Limnology and Oceanography (L&O) (ISSN 0024-3590). In addition, Special Issues that deal with a topic that is both timely and of general interest to the ASLO membership are published occasionally. For further information regarding Special Issues, and the requirements for publishing a Special Issue, click here.

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$50 per typeset page (including web appendices), if either the lead author or the corresponding author is an ASLO member. If neither lead or corresponding author is an ASLO member, the charge is $75 per page.

Tips to successful publication in L&O

The most common reasons for manuscript rejection are flawed study design or lack of detail in methods. Rejection is also likely if the writing is unclear, the manuscript is poorly organized, incomplete, or deviates significantly from the L&O style. Authors should also be aware that L&O permits only one major revision of any submission. That is, if the revision of a paper is still not scientifically acceptable the manuscript will be rejected and resubmission will not be allowed. To prevent reviewers from dwelling on issues of style in the first round of review and overlooking substantive issues that subsequently result in the rejection of the revision, authors are advised to submit only fully polished manuscripts. In rare instances, the editor may invite an author to resubmit a paper for consideration as a new manuscript after, e.g., further research has been done. Such a revision is expected to be so profound that the revision will truly be a new manuscript. Under no circumstances will more than one such resubmission be allowed.

How to submit a manuscript

Proposals (for Reviews only)

Because space is limited and because Reviews in Limnology and Oceanography are intended to serve multiple purposes, including education and outreach, we strongly encourage submissions of proposals for reviews. These proposals will be formally reviewed by experts in the field, with the explicit function of providing recommendations for improvement of the eventual review. Proposals should be limited to no more than five double-spaced pages. Each should include the following:

- a provisional title, along with a fuller explanation of material to be covered and excluded;
- a listof authors and roles, including all institutional affiliations (We particularly encourage mentee-mentor collaborations in which a junior researcher who would find a review most useful engages a senior researcher with recognized perspective on a field.);
- a statement indicating why the review is both timely and needed (e.g., the citation for the most recent review on the same or a closely related topic and a summary of the significant advances after its publication);
- an explanation of the approach to be taken (e.g., a focus on a new piece of theory or a novel interpretation of past results);
- an explanation of the overall novelty of the approach and its likely impact on practice or thought; and,
- a description of the companion materials planned for the ASLO Teaching Tools web page (e.g., PowerPoint lectures on the review topic or editable vector graphics files of figures for educational use). Such materials are not required but are strongly encouraged as means to enhance the broader impact of the review.

All submissions

Do not submit a revision of a manuscript that was rejected by L&O unless you were specifically invited to do so! Uninvited resubmissions of rejected manuscripts will be turned away without review.

Send all submissions, including revisions, to the L&O Editorial Office, i.e., never send a manuscript directly to an L&O Associate Editor because doing so defeats our tracking system and will delay processing.

Be sure that the cover letter contains the corresponding author's surface and E-mail addresses, and telephone and fax numbers.

Manuscripts must be double-spaced throughout (i.e., including references and figure legends) and must be printed on only one side of each page (i.e., single sided). Table captions must be double-spaced, but not the tables themselves. Start each section of the manuscript on a new page, and put these in the following order: title page, acknowledgments, abstract (omitted if the submission is a Comment), text, references, tables (each on a separate page), figure legends, and figures (each on a separate page). If your submission is a Note or Comment, do not attempt to mimic the format of these types of papers in the printed journal.

Hard submissions

Include one copy of the cover letter.
Include one copy of the manuscript.
If a customs declaration is required, declare the contents to be "Educational materials, no commercial value" (otherwise the L&O office will have to pay a customs brokerage fee, which will be charged back to the author).

Electronic submissions

We encourage authors to submit new manuscripts and revisions electronically. Acceptable electronic formats are Adobe PDF and MS-Word. The manuscript must be in a single file that contains all text, tables, and figures.
To ensure that reviewers and editors can print your manuscript, all fonts must be embedded in PDF files, and PDF files must NOT contain security settings. If you are unsure how to create an acceptable PDF file, submit your manuscript as an MS-Word document and let us make the PDF file for you.
To submit electronically, attach two files (1: the cover letter, which must include the response to reviews if the submission is a revision; and 2: the manuscript) to an e-mail message addressed to the Editor-in-chief

Do not send hard copy unless you are specifically instructed to do so by the L&O office.

Original submissions

The cover letter must contain the names and complete addresses (including E-mail) of four people who the authors believe to be qualified reviewers for the paper. Suggested reviewers must be free of any potential conflict of interest. Any of the following situations may constitute a conflict of interest, so persons with these potential conflicts should be omitted from your list:

- someone with whom you or a co-author have had a significant and acrimonious disagreement with at any time in the past;
- a co-investigator with either you or a co-author on a current research project;
- a co-author with your or with one of your co-authors on the current manuscript in an article published within the past 5 years;
- a close friend of yours or of a co-author's;
- someone who works at your institution (or that of a co-author); or,
- someone who has seen and commented on the manuscript prior to its submission to L&O.

To provide balance and avoid overworking particular reviewers, L&O will probably go outside the list of reviewers you provide. Thus you may wish to make other potential conflicts known to us.

Clearly indicate in the cover letter whether the submission is intended as an Article/Note, Review, or Comment (the Editorial Comments website describes how these manuscript types differ). Include a statement that the manuscript contains only original data (i.e., no data in it are already published or currently submitted for review to another journal), and a statement that publication charges will be paid if the paper is accepted for publication in L&O. Finally, briefly identify the novel contribution of this work and how it will affect interpretation and
practice in aquatic sciences.

If any data in the manuscript were previously published or are used in another manuscript presently under consideration elsewhere, describe the extent of the overlap in the cover letter and include copies of the relevant papers. Similarly, it will speed review if you include copies of related manuscripts that are in press, submitted to another journal, or that reviewers are likely to have difficulty locating. We prefer to receive copies of all such manuscripts electronically (as PDF files).

Revisions

The cover letter must contain detailed responses to the Reviewers' and Editor's comments. Describe how you modified the manuscript in response to each comment or outline your reasoning carefully if you disagree with the comment.

Final Submissions

Send one complete hard copy of the final manuscript, including figures. You must include a cover letter where you state in detail how the manuscript was changed in response to the editor's letter and reviews (if any).

We also need an electronic copy. The preferred format for the text and references is Microsoft Word. (PDF files are not accepted at this stage.) Tables can be embedded in the file as MS-Word tables, but not as pictures (e.g., .pic, .gif, or .tif formats). If you cannot insert them as Word tables, then send all tables in one MS-Excel file. Send the figures in one PDF file created using the "Press Quality" Acrobat setting unless otherwise instructed.

The cover letter and any extra material (e.g., web appendices) should be in separate files.

The L&O Style

As you prepare your paper, refer to a recent issue of L&O for examples of the journal's style. The ASLO Journals Manager (lo-manager@aslo.org) will be happy to answer any questions that you cannot resolve in this way or by referring to the detailed L&O style specifications below.

The order of the different parts of a submission should be:

- Title page
- Acknowledgments page
- Abstract page
- Text
- References
- Tables
- Figure Legends
- Figures

General style:

Use a 12-point font (Times Roman preferred), double-spaced on one side of non-glossy A4 or "letter" (8-1/2x11 inch; 21.6x28 cm) paper throughout the manuscript. Use 1-inch (2.5-cm) margins on all sides.

Number all pages, starting with 1 on the title page. If the software used to prepare the manuscript can do so, number all lines of text (making it easier for reviewers to comment on the manuscript).

Do not justify (i.e., align text) on the right-hand margin.

Do not break (hyphenate) words over lines.

Indent the first line of each paragraph. Do not put a blank line between paragraphs.

The only allowable footnotes are for author addresses on the title page or when they are unavoidable in tables.

L&O does not publish printed appendices. We do, however, publish electronic appendices on the L&O website. Such appendices may contain materials that cannot be printed in L&O (e.g., video clips) or tables that would take up too much space in the printed journal. The reviewers and editor must agree that this material is essential to understanding the associated L&O paper; i.e., L&O Web Appendices are not intended to be used to archive raw data. Submit material intended for publication on the L&O website as separate, numbered, electronic files and refer to each appendix in the manuscript as "Web Appendix n" where n is the number of the corresponding electronic file. Submit the material in an MS-Word or pdf file. The first reference to each such appendix must include the URL; e.g., see Web Appendix 1: www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa1.pdf (or appropriate file extension).

Do not number or letter sections of the manuscript.

Use an italic font for lower case Greek letters; but use a regular font (i.e., not italic) for upper case Greek letters.

Thoroughly proofread and spell-check the manuscript with a computer program.

Use a single serifed font (Times New Roman preferred); if special mathematical or Greek symbols not available in that font are needed, use the Symbol font. Note: superscripts, subscripts, italic, boldface, underline, and changes of font size are not considered to be different fonts.

Cite all figures and tables in the text and number them in the order that they appear in the text.

Do not use punctuation (commas or periods) in numbered equations.

Cite literature in the text in chronological, followed by alphabetical, order and formatted like these examples: "Campbell (1983, 1987b)," "(Smith et al. 1984; Karl and Craven 1988; Korobi 1997, 1998)." In the References section, list citations in alphabetical, followed by chronological, order.

Manuscripts must be written in English. Before submission, the manuscript should be proofread by a person fluent in English.

Order the manuscript as: title page, acknowledgments page, abstract page (not required for a Comment), manuscript body, references, tables, figure legends, and figures. All papers should be formatted in this way, i.e., do NOT place author names and acknowledgments at the end of the manuscript, which is how Notes and Comments are formatted in the journal.

Use only SI units (metric and Celsius; for detailed SI specifications, click here). The following are required formats for situations that are commonly formatted incorrectly:

- Use exponents to indicate multiplication or division in units (slashes are not allowed).

- Use mol $L^{-1}$for molar concentrations ('M' is not acceptable).
- Use mol quanta for photosynthetically available radiation (PAR) (Einsteins is not acceptable).
- Use × for multiplication (* is not acceptable).
- To indicate a power of 10, write, e.g., 5×10-8(5E-8 is not acceptable).

Do not italicize common Latin terms and abbreviations such as i.e., e.g., in situ, in vivo, and et al.

The Title page:

Capitalize only the first word, proper nouns, and acronyms in the title. I.e., Do not capitalize all words nor use all capitals for the entire title.

Do not use abbreviations in the title (e.g., use 'iron', not 'Fe'; and 'southeast', not 'SE').
List the names of all authors in a single continuous character string below the title. Use footnotes to indicate the corresponding author (if different than the first author listed) and author addresses; these addresses should be those where the authors resided at the time that the work presented in the paper was done (use separate footnotes for current addresses, if different). Spell out state or province names in full and include postal codes. Double-space all footnotes on the title page.

For Articles, provide a condensed running head of no more than 40 characters (including spaces) at the bottom of the page.

The Acknowledgments page:

Include brief statements about granting agencies, important aid received from institutions, and any potential conflicts of interest (as detailed in the L&O Ethics statement section 3.4 and 3.4.1).

Thank anyone who made a substantial contribution to the work (e.g., data collection, analysis, or writing or editing assistance) but who did not fulfill the authorship criteria, along with their specific contributions.

You are responsible for ensuring that all persons named in the Acknowledgments section know and agree to being identified there (since it may be interpreted as endorsement of the data or conclusions).

The Abstract:

A single paragraph of no more than 250 words (15 to 17 lines of text in a 12-point, Times New Roman font, where the line width is 17 cm [=6.5 in]). State what you did and what you found; omit 'introductory' statements that summarize previous work and avoid statements that do not identify actual findings (e.g., "The implications of these results are investigated with a dynamic model.") Summarize rather than advertise important findings and their significance. (In the jargon of scientific writing, L&O abstracts must be informative rather than indicative. See http://www.southernct.edu/~brownm/inform_ab.html for further explanation of these terms.) Because the abstract must stand on its own, it cannot include references. Comments have no abstracts.

Text:

Follow all directions given in the General style section above.

Describe statistical methods in enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. Give degrees of freedom for F-tests as subscripts (e.g., F3,4); for other statistics, report degrees of freedom as "df=n" following the test result (e.g., t=3.4, df=20). Use italics for symbols representing a statistic: p for probability level, n for the sample size, r for the correlation coefficient, R^2 to denote the coefficient of determination. ($r^2 = R^2$ only for a linear regression.)

Use the same font for the same mathematical symbol regardless of where it appears in the manuscript (text, displayed equations, tables, figures, or figure legends).

Use periods after all abbreviations except for metric measures, compass directions, and time (s, min, h, d, yr; do not abbreviate 'week' or 'month'). Use hh:mm h or hh:mm:ss h for time of day. Do not use a.m. or p.m. E.g., 09:30 h, 18:24:44 h.

Provide the full expansion of all acronyms on first use (even common ones like DNA).

Format dates like "15 June 1999" throughout the text, figures, and tables. If it is necessary to conserve space, abbreviate month names to the first 3 letters of the month name (no period) and the year to the last two digits.

Do not abbreviate names of states, provinces, or cities. Abbreviate names of countries only after defining on first use, e.g., United Kingdom (U.K.), United States of America (U.S.A.)

References:

The ratio of pages of references to pages of text must be less than 1:4.unpubl. (See the editorial commentary Web page for reasons.) For Reviews only, the ratio of references to text may be relaxed at the discretion of the editor. Nevertheless, Reviews should limit citations to prior reviews and key papers published since the last review or omitted from prior reviews. Exhaustive bibliographies (annotated or not) may be useful and can be submitted to the ASLO Teaching Tools web page.

All references cited in the text must appear in the References, and vice versa.

No more than 3 references can be cited to support any statement. (See the editorial commentary Web page for reasons.)

Double check the spelling of author names and years of publication. All author names must be given--even if there are more than eight (the copyeditor will abbreviate the list to 'and others' if appropriate).

Manuscripts in preparation, submitted, unpublished theses, or other inaccessible sources should be cited in text by giving the author(s) initial(s), last name(s), and 'pers. comm.' or 'unpubl.' For example, (A. B. Jones unpubl.) Such materials must NOT appear in the References.

Verify all references against original sources; check especially journal titles, accents, diacritical marks, and spelling in languages other than English. Make sure that each citation is complete, according to the following examples:

Article:
Fenchel, T. 1986. Protozoan filter feeding. Prog. Protistol. 1: 65-113.
Articles with a Digital Object Identifier (DOI): Many older papers that were originally published with page numbers have been retroactively assigned DOI's while some newer electronic journals assign article identifiers instead of page numbers (HTML being the primary form of publication). Thus, a paper with a DOI may contain page numbers, an article identifier, or both, and at least one of these is needed to complete the reference.
De Pol-Holz, R., O. Ulloa, L. Dezileau, J. Kaiser, F. Lamy, and D. Hebbeln. 2006. Melting of the patagonian ice sheet and deglacial perturbations of the nitrogen cycle in the eastern South Pacific. Geophys. Res. Lett. 33: L04704, doi:10.1029/2005GL024477. If there are page numbers, the last part would be 33: 15-32, doi:10.1029/2005GL024477.
If there are both page numbers and an article identifier, the last part would be 33: 15-32, L04704, doi:10.1029/2005GL024477.

Book:
Stumm, W., and J. Morgan. 1981. Aquatic chemistry, 2nd ed. Wiley.
Chapter:
Codispoti, L. A. 1983. Nitrogen in upwelling systems, p. 513-564. In E. J. Carpenter and D. G.
Capone [eds.], Nitrogen in the marine environment. Academic.
Thesis:
Kimmance, S. A. 2001. The interactive effect of temperature and food concentration on plankton grazing and growth rates. Ph.D. thesis. Univ. of Liverpool.
Papers which are unconditionally accepted for publication but for which exact publication data are not yet available should be formatted according to the above examples but with the phrase "In press" appearing instead of the year of publication.

Use mixed case (upper and lower case OR caps and small caps) for all text in the References section. In particular, do not use all capital letters for author names because doing so makes it impossible to for the copyeditor to properly typeset names like "MacKenzie".

For abbreviations of journal names refer to Chemical Abstracts Service Source Index (CASSI) or Biosis.

Do not include part (issue) numbers after volume numbers unless each part of the volume is paginated separately.

Websites. A websites may be referred to only if it is sponsored by an organization that is committed to maintaining it in perpetuity. Personal or university-based websites are not allowed in L&O because such websites are prone to disappear when the scientist who created them moves or loses interest in material. Websites are referred to only in the text and are not included in the list of references.

Tables:

Start each table on a new page.

Format tables so that they will fit on the printed page: A 1-column table can be up to 60 characters wide, and a 2-column table up to 130.

Type table legends as double-spaced paragraphs at the top of each table.

Figure Legends:

Group figure legends together on the page(s) preceding the figures; one paragraph per figure.

Explain all panels in each figure (A), (B), ...

Symbols used in the figure (e.g., circles, squares, ...) must be explained on the figure itself (i.e., not in the figure legend). No special symbols are allowed in the figure legend.

Figures:

Do not put figure legends on the figures. Put only "Fig. #." on the figure.

Figures must be camera-ready (no modifications will be made by the L&O editorial staff or printer). They must be printed at high resolution (minimum of 600 dpi).

Number all figures serially (In figure numbering, L&O does not distinguish color "plates" from black-and-white figures).

Number figures with Arabic numerals in the order of their citation in the text. If panels of a figure are labeled (A, B, ...) use the same case when referring to these panels in the text (A, B, ..., not a, b,...).

If a figure consists of multiple panels, put all panels on one page and repeat axes titles on each panel only if they are different.

Put scale bars on the figure, NOT in the figure legend.

Use the Times New Roman font for all text and numerals on figures. Font sizes size should be from 9 to 11 points. If mathematical or Greek symbols are not available in Times New Roman, use the Symbol font.

Page layout: See page layout diagram.

Submit figures at the intended print size. The L&O column width is 8.9 cm (3.5 in) and full page width is 18.4 cm (7.25 in). The maximum size for a figure is 18.4 x 23.2 cm (7.25 x 9.125 in).

Make figures as simple as possible. For example, avoid grid lines and boxes around symbol definitions.

Maps must include latitude and longitude, an indication of compass direction, and a thin line as a border. All markings must be legible.

If a figure is submitted as mounted artwork, mount it on flexible paper because it will be scanned on a drum scanner; use glue stick to attach just the top edge of each panel to the paper, making sure that the plate is flat (i.e., there are no bumps or bubbles); any unevenness will cause distortion of the final image.

Color figures:

See detailed instructions.