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Nutritional quality of biofilms with respect to light regime in Lake Saint-Pierre (Québec, Canada)

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SUMMARY

1. *In situ* experiments were conducted using specialised incubation devices to grow biofilms under varying light regimes and grazing intensities (by excluding fish and large-sized zooplankton, >2 mm) both within and between two sites in Lake Saint-Pierre.
2. Biofilms growing under greater *in situ* UVR and light exposures found in the south water mass were characterised by a greater biomass and nutrient content, but their total fatty acid (FA) contents and ratios of elemental nutrients were not significantly different from the north. There was a relatively greater abundance of chlorophytes and cyanobacteria in the south water mass, along with a greater proportion of low nutritional quality saturated fatty acids (SAFA). Conversely, biofilms growing in the north had a greater relative abundance of diatoms, as well as greater eicosapentaneoic acid (20:5ω3) and docosahexaneoic acid (22:6ω3) concentrations (two FAs implicated in the physiological competency of grazers).
3. The prevailing community structures created differences in terms of nutritional status of the biofilms for benthic grazers and their predators at the two sites. The biofilms from the southern site were characterised by greater food quantity at the expense of quality, while biofilms from the northern site contained less food of a better quality. Despite this, the nutritional regime in the south supported a greater productivity at higher trophic levels. The secondary treatments (light and grazing by fish and macro-invertebrates) had lesser effects on food quality.

Keywords: biofilms, community structure, fatty acids, fluvial lake, lipids, UV radiation

Introduction

The importance of algal food quality for zooplankton and fish has become the focus of extensive research. Studies have mainly focussed on two indicators of algal food quality, namely phosphorus (Urabe, Clasen & Sterner, 1997) and long-chain poly-unsaturated fatty acids (PUFA) (Brett & Müller-Navarra, 1997; Weers & Gulati, 1997). The majority of studies have been conducted directly on cultured algae fed to zooplankton, or indirectly by implying effects on consumers based on field observations, but comparatively few studies have been conducted *in situ* using direct experimental approaches and natural algal communities. Although phosphorus and PUFA may act separately or in conjunction to constrain the growth and physiological competencies of grazers there is general consensus that when phosphorus is not limiting PUFA becomes the main factor limiting growth (Gulati & Demott, 1997).

Light is crucial for photosynthesis and is thus the most critical environmental factor regulating biofilm growth, community structure and productivity (Hill, Ryon & Schilling, 1995). High light, high ultraviolet radiation (UVR) conditions have been shown to increase chlorophyte abundance (Wellnitz & Ward, 1998), which are generally of a lesser nutritional

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quality on the basis of fatty acid composition compared with most diatom species. The latter have been shown to decrease under these light conditions, being particularly sensitive to UVR (Watkins *et al.*, 2001). Further, light quantity (intensity) and quality (wavelength dependent energy) are, in part, responsible for the biochemical composition of algae (e.g. McNamara & Hill, 2000; Hessen, Faerovig & Andersen, 2002). For example, there is evidence to suggest that light quality and/or quantity influences lipid content and composition (Bigogno, Khozin-Goldberg & Cohen, 2002; Khozin-Goldberg *et al.*, 2002).

Light quantity may affect algal food quality by altering the cellular carbon to phosphorus (C : P) ratio (e.g. Sterner & Schulz, 1998). However, the utility of the C : P ratio as a food quality indicator is of limited value when phosphorus is in sufficient supply and/or is similar amongst sites (von Elert & Stampfl, 2000). Another useful indicator of food quality is the FA content of the biofilms. Although the biosynthesis of FA is just beginning to be understood, it is well known that saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) are the major components of neutral lipids. These lipids function mainly as energy storage reserves, which, in algae, generally increase as a result of exposures to stressful environmental conditions, such as high temperature, nutrient extremes and harsh light conditions. In contrast, PUFA affect many physiological processes and are major constituents of polar lipids, which are present in cell and chloroplast membranes.

Although recent studies have shown that some organisms, such as the nematode *Caerhabditis elegans*, can synthesize PUFA containing more than 20 carbon atoms directly from SAFA and MUFA (Wallis, Watts & Browse, 2002), most animals cannot synthesize essential fatty acids (EFA) de novo from linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3) in sufficient amounts to achieve optimal physiological performance (Cunnane, 1996; Arts, Ackman & Holub, 2001). They must therefore be obtained from dietary sources (e.g. algae). Eicosapentaneoic acid (20:5ω3) and docosahexaneoic acid (22:6ω3) are two EFA that have been shown to limit the growth and development of aquatic invertebrates. For example, EPA has been shown to increase growth rates and fecundity in *Daphnia* (DeMott & Müller-Navarra, 1997) and copepods (Jünasdüttir, Fields & Pantoja, 1995).

Grazing also plays a major role, in addition to nutrients and light, in regulating biofilm nutrient content and stoichiometry (Wellnitz & Ward, 1998). Numerous authors (e.g. Müller-Navarra, 1995) have described how algal PUFA content increases grazer growth and reproduction rates, but the full effects of grazing on FA biosynthesis in algae remain unclear. One possibility is that biofilms exposed to grazers would undergo a selective removal of higher quality cells decreasing their biomass and the overall EFA content of the biofilms, whereas biofilms which experience reduced grazing pressures should be characterised by higher EFA contents and biomass.

Lake Saint-Pierre is characterised by three main water masses that differ in their optical properties (Frenette, Arts & Morin, 2003), thus constituting a natural experimental site for intralake comparisons of light effects on algal food quality. The objectives of this study were to determine, *in situ*, how differing light regimes (PAR = photosyntically active radiation and UVR), as well as the indirect effects of macrograzers, influence algal food quality (composition and quantity of individual PUFA, and elemental stoichiometry).

Methods

The experiments were conducted to study the effects of variable light regime between sites, amongst sites, and grazing by macro-invertebrates (and perhaps fish) on biofilms. Six incubation rafts were placed in the north water mass and six in the south to study the effect of site, whereas six different light filters (later combined to form four light treatments; see below) on each incubation raft allowed for the study of fluctuating light regimes within a single water mass. The macrograzer and fish exclusion treatment was achieved by placing nets with a mesh size of 4 mm^2 on three of the six rafts within each water mass. All combinations of treatments (2 sites × 4 light regimes × 2 grazing levels) were replicated threefold, resulting in a split-plot design with 48 sampling units.

Study area

Incubation devices were deployed in the north (lat: 46°12′, long: 72°55′) and south (lat: 46°8′, long: 72°51′) water masses of Lake Saint-Pierre for 47–49 days,

from 24–25 July 2001 until 9–11 September 2001. This fluvial lake is situated within the St Lawrence River system and occupies a mean annual area of 480 km^2 (Langlois *et al.*, 1992), and extends from the Berthier-Sorel Islands to the city of Trois-Rivières, Québec, Canada. Hydrodynamic conditions of these two sites were comparable as both were situated in shallow embayments east of the Chenal Tardif on the south shore and in the Baie de Yamachiche on the north shore, which ranged in depth from 1 to 3 m from the beginning to the end of the experiment. Distinct light climates have been defined at each site. Underwater UVR intensity is roughly threefold greater in the south water mass.

Experimental setup

Aluminium rafts consisting of two stories were built: a lower story supporting a series of unglazed ceramic tiles (Céramique Des Rochers, Trois-Rivières, Canada) of two sizes (232.3 cm^2 and 25.8 cm^2) and an upper story on which up to six light filters could be affixed (Fig. 1). Three of the six filters consisted of different combinations of acrylic sheeting and/or polyester film (Mylar). This arrangement produced the following light treatments: UVR + 100% PAR, no UVR + 93% PAR, and no UVB + 100% PAR. The three other filters consisted of neutral density filters, made of opaque plastic window screen material varying in mesh size. These filters were used to reduce ambient light by 50, 70 or 90%. These materials, along with their transmission properties, are described in Table 1.

Because of the loss of two of our sampling rafts the samples from the control treatment, which allowed 93% PAR penetration + full UVR exposure, were combined with those from the 90% PAR, to form the high light treatments (HL), and the samples under the two neutral density filters allowing 70 and 50% PAR were combined together to form the low light treatments (LL) (Table 1). Consequently, the main effects were: site with two levels [north (N) and south (S)], light with four levels (no UVB, no UVR, HL, LL), and grazing with two levels [complete grazing (PRES) and partial grazing (PART)], resulting in a sampling unit of 42 instead of 48.

The upper story was fixed at a height of 2.5 cm above the lower one. This reduced stray light contamination and shading, without drastically modifying water flow. This assemblage of tiles and filters produced a platform of 1.52 × 1.80 m that was suspended from a rectangular floatation system designed to limit shading (Fig. 1). Stakes placed at the corners of the floatation device secured the rafts in position. The rafts were oriented at 45° with respect to water flow in order to minimise water turbulence and drag.

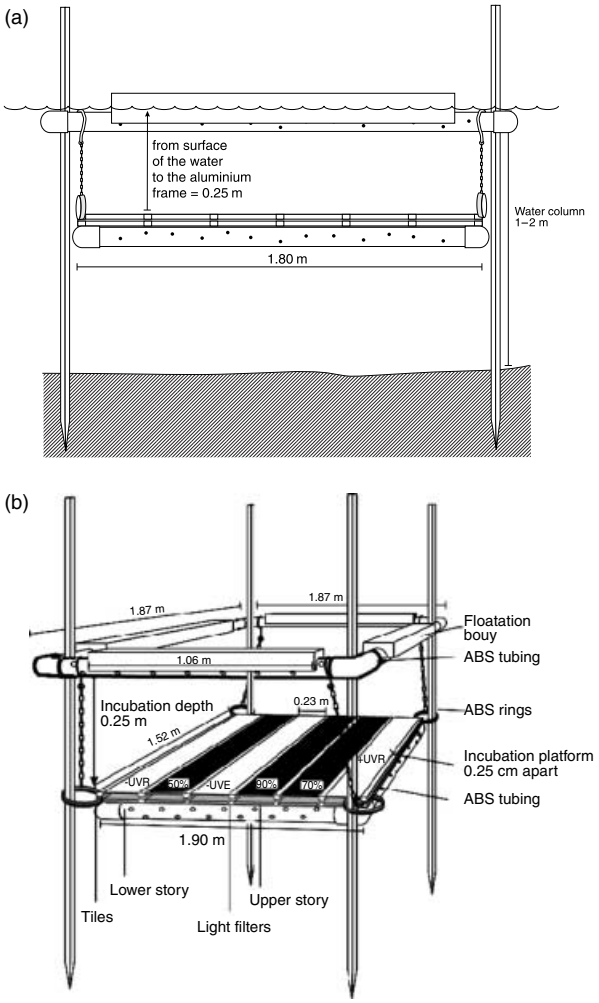

Fig. 1 (a) Side view of the incubation platform within the water. A square perforated ABS tubing maintained the rigidity of the incubation platform and supported the floatation buoys. The incubation raft maintained a constant distance from the water surface for the entirety of the incubation period. (b) Diagram of an incubation raft with the two main decks: a lower story supporting the substrates and an upper story with the light filters. The platform was attached to a square float and was anchored to the bottom sediments with wooden stakes. The rings at the corners allowed it to move vertically with changes in water depth.

Table 1 Average UVB (W m^{-2}), UVA (W m^{-2}), and PAR (μmol m^{-2} s^{-1}) intensities received by the biofilms at the incubation depth during the experiment with respect to the four different light treatments (no UV-B, no UVR, HL, and LL)

			High light		Low light	
	no UVB	no UVR	100% PAR	90% PAR	70% PAR	50% PAR
Materials used	Mylar®D	Acrylite® OP3	Acrylite® OP4	Neutral density filters	Neutral Density	Neutral Density
Transmission properties	Removes >94% ambient UVB with a 50% transmission at 318 nm	93% PAR with 0% transmission <375 nm	Allows 93% PAR and most of the UV-A and UV-B (65% transmission at 280 nm)	Opaque screening material which allows 90% transmission of PAR	Opaque screening material which allows 70% transmission of PAR	Opaque screening material which allows 50% transmission of PAR
North						
Average UVR (μW m^{-2})	UVB:0–8.33	–	UV-B: 13.32–16.65	UV-B: 14.99	UV-B: 11.66	UV-B: 8.33
	UVA: 131.69		UV-A: 131.69	UV-A: 118.52	UV-A: 92.18	UV-A: 65.85
Average PAR (μmol m^{-2} s^{-1})	600.53	558.49	558.49	540.48	420.37	300.27
South						
Average UVR (μW m^{-2})	UVB: 0–28.54	–	UV-B: 37.10–57.07	UV-B: 51.36	UV-B: 39.95	UV-B: 28.54
	UVA: 413.37		UV-A: 413.37	UV-A: 372.03	UV-A: 289.36	UV-A: 206.69
Average PAR (μmol m^{-2} s^{-1})	795.60	739.91	739.91	716.04	556.92	397.80

Substrate testing

Algal community structure was examined for periphyton growing on natural rocks, plants and our artificial tiles (Fig. 2). Polyethylene sheets fixed onto 'L-shaped' galvanised angles were fastened horizontally at different heights on wooden stakes to support rocks and tiles. Three stakes were deployed at both the north and the south incubation sites. The stakes were removed after 28 days (30 July to 26 August). The substrates (rocks and tiles) were cut loose from the polyethylene sheets and immediately placed in individual plastic bags filled with lake water, which were then put directly in coolers. Macrophytes (*Vallisneria* sp.) were gently uprooted, removed from the water, and placed in plastic bags in the same coolers. In the laboratory, the tiles and rocks were scraped with razor blades and the live material was placed in 1% Lugol's solution for later identification. Water was added to the plastic bags containing the plants and they were manually shaken for 1 min after which the slurry was filtered through a 0.1 mm sieve before being placed in Lugol's solution. The plants were placed in preweighed aluminium trays and ovendried at 60°C for 24 h. Identification of algae was carried out to the level of class. After 12 h of sedimentation in 50 mL Utermöhl chambers, counts of 600 or more cells and biovolume estimates were done under an inverted microscope at 100×, 200× and 400× magnification. The counts and biovolumes were entered into ALGAMICA plankton counting package (program version 4.1; developed by Hamilton and Gosselain 2001 (http://ibelgique.ifrance.com/algamica) for appropriate biovolume calculations of periphytic cells according to their shape (Hillebrand *et al.*, 1999).

Sampling and analysis

Physicochemical variables of lake water. During the incubation period, 2 L water samples were collected every 2–3 days from the north and south shores. Analyses were carried out at the National Laboratory for Environmental Testing (NLET, Burlington, Ontario). Total phosphorus (TP) and soluble reactive phosphorus (SRP) were obtained spectrophotometrically at 660 nm after the addition of ammonium molybdate (NLET, 2000–2001). Total nitrogen (TN) concentrations were measured spectrophotometrically at 520 nm after oxidation of organic N into nitrates

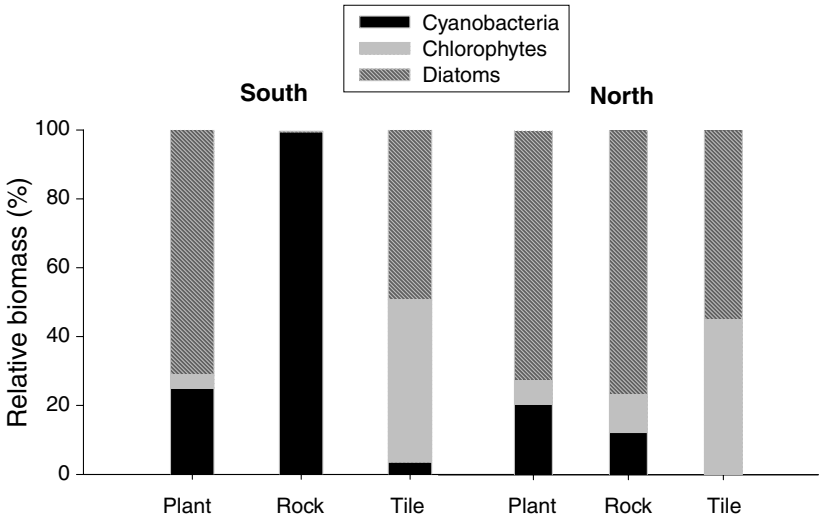

Fig. 2 Results of the simple substrate testing experiment comparing the community structure present on plants, rocks and tiles. Relative biomass (%) of cyanophytes, chlorophytes, and diatoms are shown for each substrate in the north and south. Note: polyethylene sheets attached horizontally onto wooden stakes were used in this experiment, contrary to the incubation rafts detailed in Fig. 1.

during their digestion in an autoclave. Nitrite-nitrate (NO_3-NO_2) was also measured spectrophotometrically at 520 nm after reacting with sulphanilamide and N-(1-naphtyl) ethylenediamine dichlorohydrate. Ammonia (NH_3) was measured spectrophotometrically at 630 nm after addition of ammonium salt or sodium phenate, and sodium hypochlorite. Silicates (SiO_2) were measured with a colorimeter set at 660 nm after addition of oxalic and ascorbic acids. Sulphates (SO_4) were measured spectrophotometrically at 460 nm after reacting with dichloride beryllium and blue methyl-thymol. Chlorine (Cl) was measured with a colorimeter set at 480 nm after addition of mercuric thiocyanate.

Concentrations of the portion of DOC which absorbs more strongly at lower wavelengths [i.e. chromophoric dissolved organic carbon (CDOM)] were obtained by measuring the absorption spectra on a spectrophotometer (Cary 100Bio, Varian Co., Palo Alto, CA, U.S.A.) of GF/F-filtered water from 290 to 750 nm using a 1 cm pathlength quartz cuvette. The absorption at 340 nm was used as the value for CDOM concentration, as in Frenette *et al.* (2003).

Irradiance values were measured at approximately 0.05 m intervals using a spectroradiometer (Model PUV-2545, Biospherical Instruments, San Diego, CA, U.S.A.), which was slowly lowered through the water column and which measured the energy at 313, 320, 340, 443, 550 nm wavelengths, and for PAR (400–700 nm). Underwater cosine-corrected downwelling irradiance at different depths (Edz) and vertical attenuation coefficients (K_d) were calculated as in Frenette *et al.* (2003) and 1% penetration depths were calculated using the equation $4.605/K_d$ (Kirk, 1994).

Biofilm samples. At the end of the incubation period, the rafts were removed from the water and the tiles were placed in plastic bags in a container filled with lake water. In the laboratory, tiles from the same replicate were scraped with razor blades and the biofilms were placed in cryo-vials that were immediately stored at −80°C for subsequent lipid analysis. Additional tiles were scraped and placed in amber glass bottles containing 1% Lugol's solution for community structure analyses. A slurry was formed by adding distilled water to the scraped biofilm material and gently blending the mixture to obtain a more homogeneous solution. This slurry was divided into three different subsamples for each replicate and filtered on, (i) 25 mm Whatman GF/F filters for chlorophyll *a* (Chl *a*), (ii) precombusted 25 mm Millipore glass fibre filters for ash free dry weight (AFDW), particulate carbon (PC) and nitrogen (PN), and (iii) precombusted and acid prewashed 47 mm Millipore GF/F filters for particulate phosphorus (PP) contents. PC, PN, PP, and Chl *a* were filtered in duplicate and averaged to obtain one value per experimental unit. The filters were stored at −20°C for subsequent analyses.

Community structure, biomass and stoichiometry. Identification of algae was carried out following the procedures described earlier. Chl *a* concentrations

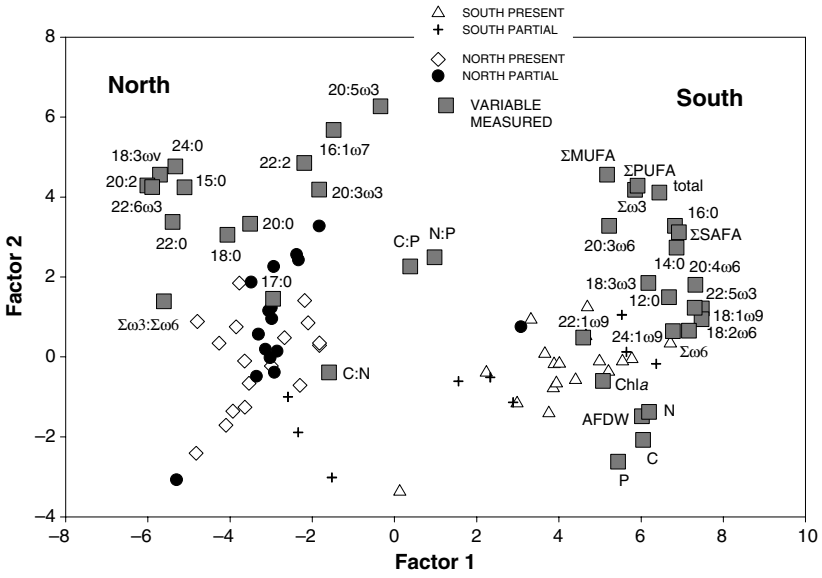

Fig. 3 Principle components analysis illustrating the association between biomass, nutrient content, stoichiometric variables and fatty acids for the two sites (north and south) and for the grazer treatments (presence and partial).

were measured by extraction of the filters in the dark in 8 mL 90% ethanol at 70°C for 5 min. Extractions continued in the dark at 4°C for 1 h, after which the samples were analysed in a spectrophotometer (Shimadzu, UV-Probe, Columbia, MD, U.S.A.) (A. Cattaneo, unpublished). Absorption measurements were taken at 665 and 750 nm before and after acidification to correct for phaeopigments, according to Wetzel & Likens (2000). Analyses of PC, PN and PP, were carried out at the NLET. Particulate carbon and nitrogen were measured by combusting the filters using pure oxygen in the presence of either helium or argon. For PP, acid digestion was followed by the addition of ammonium molybdate, which is reduced with stannous chloride to form a molybdenum blue complex measured spectro-photometrically at 660 nm.

Fatty acids. Fatty acid methyl esters (FAME) of the samples were obtained by a three-step process: gravimetric extraction, derivatisation, and quantification on a gas chromatograph (GC). Samples were extracted three times by grinding freeze-dried tissue in the presence of a chloroform : methanol (2 : 1 vol : vol) solution (Bligh & Dyer, 1959). After centrifugation at 4000 rpm (800 *g*) the supernatant was transferred to acid-washed, 15-mL centrifuge tubes and rinsed with chloroform : methanol. This procedure was followed by a salt wash (0.9% aqueous NaCl solution) before the samples were evaporated to 2 mL. From this volume, a 200 μL of sample extract was removed, dried and then weighed on a Cahn Model C-30 electron balance to provide a measure of total lipid content. The remaining 1.8 mL of each extract was transferred into 5-mL Shimadzu vials, evaporated to dryness using extra-dry nitrogen gas, and stored at −80°C until the derivatisation step could be performed. For the derivatisation process, hexane and BF3-methanol (10% w/w) were added and the headspace of the vials purged with nitrogen. They were then heated (70°C) for 2 h, after which, 1 mL of water and 1 mL of hexane were added and the vials were shaken. The upper hexane-layer containing the FAME was then removed and dried down to 2.0 mL using nitrogen gas. FAME concentrations were quantified using a gas chromatograph (Hewlett Packard 6890; Agilent Technologies, Wilmington, DE, U.S.A.) with a splitless injection on a Supelco (SP-2560) column (100 m × 0.25 mm ID × 0.20 μm thick film). Hydrogen was used as the carrier gas and the temperature programming was: 100 (hold 1 min) to 240°C at 5°C/min (hold for 38 min). Three individual pure FA internal standards (C20:2, cis-11,14-eicosadienoic acid; 20:5ω3 and 22:6ω3) were used to estimate the derivatisation efficiency. A 37-component FAME standard (Supelco No. 47885-U) was used to produce four-point calibration curves and establish the identity of unknown sample peaks by comparing their retention times to those of the FAME standard.

Statistical analysis

Community structure. The differences in community structure between the north and south sites, grazer and partial-grazer treatments were tested using a two-way ANOVA with site and grazing as the independent variables and the log-transformed relative biomasses of the three main algal groups (cyanophytes, chlorophytes and diatoms) as the dependant variables. The differences in taxonomic composition between tile, rock and plant substrates was tested using a one-way ANOVA, with substrate as the independent variable and the relative biomass of the three groups used as dependent variables. In order to achieve normal distributions log transformations were applied on the relative biomass data prior to the statistical analysis.

Stoichiometry and fatty acid content. The effects of site, light, and grazing by fish and macro-invertebrates on stoichiometry and FA content were tested using SPLIT-PLOT analyses for each variable, with the error term adjusted for the grouping effect of light levels within rafts. When the presence of outliers persisted, our results were treated conservatively and only the treatments that were significant before and after removal were kept for interpretation. For the FA data, the split-plot analyses were carried out on the microgram FAME per milligram dry weight of tissue data. Residuals from all analyses were verified for normality and homoscedasticity, and if these conditions were not respected, appropriate transformations (e.g. log, root, square and inverse) were applied to meet the required assumptions. We used principle components analysis to further illustrate the associations between nutrients, biomass, stoichiometry, and lipids as a function of location (north and south).

Results

Experimental setup

The tiles were abundantly colonised by biofilms. Ceramic tiles are widely used as substrates for studying biofilm growth and community structure. However, the most widely available natural substrates in Lake Saint-Pierre are the submerged macrophytes. Therefore, we tested the suitability of tiles as substrates by comparing the community structure of biofilms growing on tiles, macrophytes (*Vallisneria* sp.), and rocks (Fig. 2). There were significant differences (ANOVA; $P < 0.05$) in the community structures of the algal mats that colonised these substrates in the vicinity of our incubation sites. We recorded a greater abundance of chlorophytes growing on tiles compared with rock and plant substrates; however, differences in relative diatom biomass in the north and south were not significantly different between tiles and plants. The diatom relative biomass was significantly lower on the rocks in the south, while it was similar to the other substrates in the north. In general, the diatom relative biomass was greater in the north than in the south for all substrates used.

Biofilm is a term that refers to a matrix composed of algae, bacteria, fungi, micro- and meiofauna, and detritus. However, for testing the effects of light on the food quality, we focused our analyses on the periphyton component of the biofilms and did not attempt to estimate the biomass of other faunal components (e.g. flagellates and ciliates). Microfauna (e.g. nematodes, ostracods) and macrofauna (e.g amphipods; notably *Gammarus lacustris* Sars), were purposefully removed during the scraping process.

Physicochemical variables of lake water

The north and south water masses varied markedly in their spectral regime with respect to the 1% penetration depths of UVR and PAR owing to differences in their chromophoric dissolved organic matter (Fig. 4) and total suspended particle concentrations, respectively. Measurements of downward irradiance at the incubation depths (30 cm from the surface) were averaged over the incubation period along with the light intensities corresponding to the neutral density filters (Table 1). Calculations of UVB ($\mu W\ m^{-2}$), UVR (m^{-2}) and PAR (μmol photons m^{-2} s) intensities at the incubation depth indicated that the south biofilms were, on average, exposed to 3.4-fold greater UVB, threefold greater UVR (UVB + UVA), and 1.3-fold greater PAR throughout the incubation period.

Total N, NO_3-NO_2, NH_3, SRP, and P did not vary significantly between the two sites (north and south) (Table 2). On the basis of total N and total P, the two sites reflected a trophic state situated between mesotrophic and eutrophic according to the general trophic classification of lakes (Vollenweider in Wetzel, 2001),

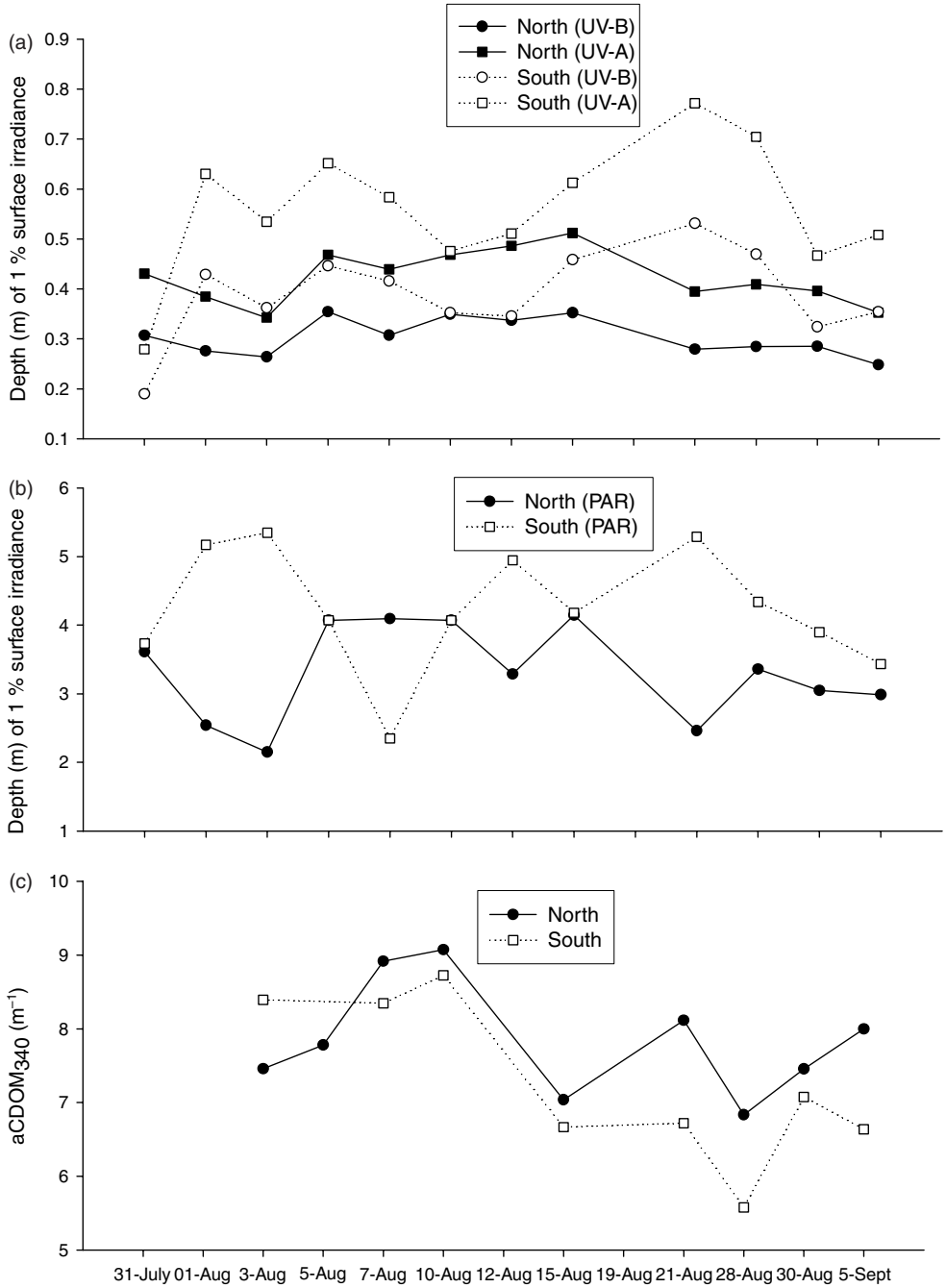

Fig. 4 (a) One per cent UVB, UVA penetration depths; (b) 1% PAR penetration depths (m), and (c) CDOM concentrations (Absorption at 340 nm) for the north and south water masses.

indicating that nutrients were probably not limiting at either study site.

Biofilm samples

Effect of site. Amongst the three taxonomic groups examined (diatoms, cyanobacteria, chlorophytes), chlorophytes had the greatest relative biomass in the north and south (ANOVA, $P < 0.05$) (Fig. 5). In the north, the relative abundance of diatoms equalled that of chlorophytes. The chlorophytes found in the south were dominated by *Cladophora* sp. and *Cloeochaete* sp., while *Cladophora* sp., *Oedogonium* sp. and *Stigeoclonium* sp. were greater in the north. Diatom species

Table 2 Average (±SD) for the water chemical variables (mg L^{-1}) analysed every 2–3 days. The variables, which differed significantly between the north and south water masses are in bold

	North ($n = 13$)	South ($n = 13$)	P-value (t-test)
SRP	0.006 (0.001)	0.007 (0.001)	0.3177
NO_2-NO_3	0.033 (0.01)	0.063 0.01)	0.1343
NH_3	0.0138 (0.002)	0.016 (0.002)	0.4284
TN	0.46 (0.03)	0.39 (0.01)	0.3417
TP	0.028 (0.001)	0.026 (0.001)	0.1407
Cl	**15.42 (0.58)**	**20.66 (0.58)**	**<0.0001**
SO_4	**18.13 (0.72)**	**23.85 (0.72)**	**<0.0001**
SiO_2	**1.47 (0.11)**	**0.74 (0.12)**	**0.0002**
DOC	**3.97 (0.15)**	**3.49 (0.15)**	**0.0339**

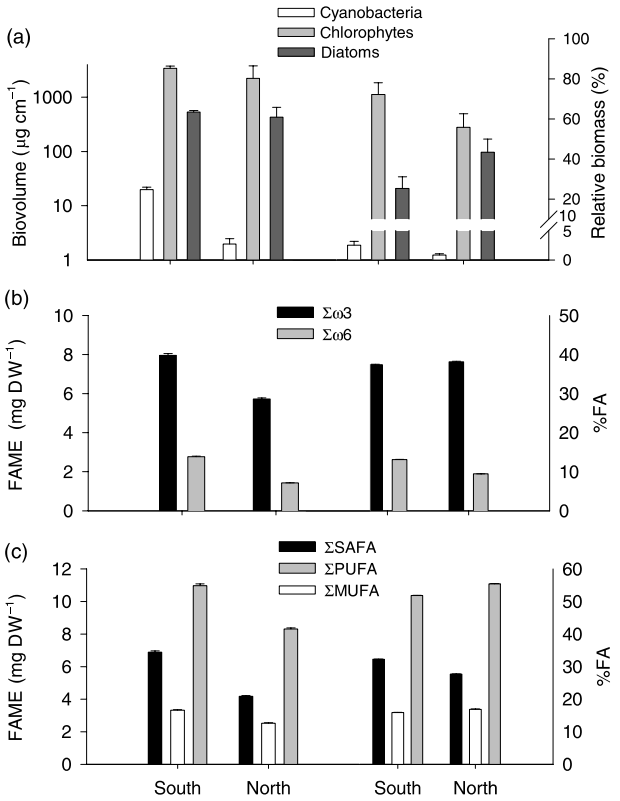

Fig. 5 Gross taxonomic composition and summary fatty acid indices for the biofilms in the southern and northern biofilms. The left y-axis shows absolute data, while the right y-axis presents relative information according to the north and south water masses. (a) Absolute (log-transformed) and relative (%) biomass of cyanobacteria, chlorophytes, and diatoms (b) absolute and relative (%) ΣSAFA, ΣMUFA, ΣPUFA, and (c) absolute and relative (%) ω3 and ω6.

richness and relative abundance were greater in the north, with *Melosira* sp. and *Amphora* sp. as the dominant species, while *Cocconeis* sp. was the most abundant diatom in the south.

The principle components analysis (Fig. 3) clearly illustrates the separation between the north and south biofilms (along factor 1) and the stoichiometric patterns and individual FA which were associated with each of the sites. The three factor SPLIT-PLOT analysis on log-transformed periphyton variables confirmed these results, where the south biofilms contained a greater biomass ($P < 0.001$), with respect to Chl *a*, particulate C, N and P and AFDW (Table 3, Fig. 3). However, C : P, C : N, N : P ratios did not differ significantly between the two sites.

The average ratios of SAFA : MUFA : PUFA in the south was 32 : 16 : 52, compared with 28 : 17 : 55 in the north, showing an increased proportion of SAFA at the expense of PUFA in the south, while MUFA did not vary significantly (Table 3; Fig. 5). PUFA can be separated into two groups, either the linoleate (ω6s) family, comprising the sum of all the ω6 FA (18:2ω6, 18:3ω6, 20:4ω6, and 20:3ω6), and the linolenate family, corresponding to the sum of all the ω3 FA (18:3ω3, 20:5ω3, 22:5ω3, 22:6ω3, and 20:3ω3). As shown by the principle components analysis (Fig. 3), the south was associated with greater total linoleates owing to the twofold greater 18:2ω6 ($P < 0{,}001$) and 20:4ω6 ($P < 0{,}001$) content compared with the north (Table 3; Fig. 5). Amongst the linolenates, 22:5ω3 ($P < 0.001$) and 18:3ω3 ($P = 0.016$) were greater in the south, while greater contents of 20:5ω3 and 22:6ω3 ($P = 0.046$ and <0.001, respectively) were found in the north. The following SAFA: 12:0, 14:0 and 16:0, and MUFA: 18:1ω9, 22:1ω9 and 24:1ω9 characterised the south biofilms, whereas the north biofilms were associated with the following SAFA: 15:0, 18:0, 20:0, 22:0 and 24:0, and MUFA: 16:1ω7. Some authors further suggest using the ratios of the sum of saturated to the sum of unsaturated and the sum of omega-3 (ω3) to the sum of omega-6 (ω6) FA as an indicator of lipid quality (Ahlgren *et al.*, 1994). The Σω3:Σω6 ratio was 1.5-fold greater in the north ($P < 0.001$), and the saturated : unsaturated FA ratio was 25-fold greater in the south ($P = 0.002$) (Table 3; Fig. 5).

Effect of light. The differing light treatments within each site did not account for any differences in terms of stoichiometry and biomass of the biofilms. Only a few of the FA were significantly affected by altering light regimes within each site; namely, 16:1ω7 ($P = 0.003$) and ΣMUFA ($P = 0.034$) (Table 3). For both of these, the high light treatments were, on average

Table 3 Averages (±SD) of nutrients (mg L^{-1}), stoichiometric ratios (mol : mol) and fatty acids (μg FAME mg dry $weight^{-1}$) analysed for the main treatments. The variables that are significantly different across main treatments are in bold. The significant interaction effects are not represented here

	Site		Grazing		Light			
	South	North	Presence	Partial	no-UVB	no-UVR	HL	LL
C	**1.18 (0.47)**	**0.34 (0.08)**	0.91 (0.61)	0.66 (0.48)	0.79 (0.57)	0.81 (0.68)	0.71 (0.70)	0.56 (0.50)
N	**0.14 (0.06)**	**0.04 (0.01)**	**0.12 (0.08)**	**0.07 (0.05)**	0.10 (0.08)	0.04 (0.01)	0.07 (0.05)	0.045 (0.10)
P	**0.017 (0.008)**	**0.005 (0.002)**	**0.013 (0.010)**	**0.009 (0.006)**	0.01 (0.01)	0.006 (0.004)	0.008 (0.006)	0.006 (0.003)
C : P	236.96 (67.21)	204.37 (37.19)	217.91 (63.26)	218.74 (51.93)	227.82 (48.59)	211.21 (26.07)	223.42 (49.87)	196.34 (30.83)
C : N	10.12 (1.20)	9.69 (0.47)	9.25 (0.50)	10.61 (0.76)	10.06 (1.07)	9.89 (0.56)	10.57 (0.72)	9.69 (0.52)
N : P	23.48 (6.07)	21.36 (4.03)	23.39 (6.25)	20.81 (3.62)	22.73 (3.52)	21.91 (3.28)	21.46 (3.8)	20.56 (3.64)
AFDW	**4.28 (1.57)**	**1.49 (0.59)**	2.97 (1.44)	2.95 (2.41)	2.91 (2.04)	1.49 (0.25)	2.80 (2.27)	1.62 (0.71)
Chl *a*	**27.67 (10.27)**	**7.87 (2.29)**	19.77 (11.99)	17.05 (14.03)	18.83 (14.87)	8.17 (2.12)	15.76 (13.49)	9.42 (3.39)
12:0	**0.22 (0.08)**	**0.12 (0.04)**	0.19 (0.09)	0.12 (0.04)	0.19 (0.05)	0.18 (0.10)	0.17 (0.10)	0.13 (0.05)
14:0	**1.70 (0.63)**	**0.85 (0.37)**	1.53 (0.68)	0.87 (0.39)	1.69 (0.40)	1.32 (0.83)	1.21 (0.75)	0.97 (0.42)
15:0	**0.02 (0.00)**	**0.03 (0.01)**	0.03 (0.01)	0.03 (0.01)	0.02 (0.00)	0.03 (0.02)	0.03 (0.01)	0.03 (0.01)
16:0	**4.83 (1.43)**	**2.79 (0.94)**	4.41 (1.60)	2.87 (0.38)	4.85 (1.22)	4.02 (1.89)	3.68 (1.63)	2.99 (1.05)
16:1ω7	**1.29 (0.29)**	**1.72 (0.79)**	**1.37 (0.42)**	**1.71 (0.83)**	**1.95 (0.96)**	**1.25 (0.41)**	**1.66 (0.58)**	**1.27 (0.48)**
17:0	**0.02 (0.01)**	**0.03 (0.01)**	**0.02 (0.01)**	**0.03 (0.01)**	0.02 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)
18:0	**0.10 (0.04)**	**0.21 (0.13)**	0.13 (0.10)	0.20 (0.12)	0.10 (0.04)	0.15 (0.13)	0.18 (0.14)	0.18 (0.09)
18:1ω9	**1.98 (0.65)**	**0.77 (0.35)**	1.75 (0.77)	0.79 (0.37)	2.02 (0.54)	1.54 (0.94)	1.32 (0.80)	0.84 (0.43)
18:ω6	**2.25 (0.70)**	**0.90 (0.36)**	1.99 (0.86)	0.94 (0.38)	2.21 (0.46)	1.64 (0.93)	1.62 (1.02)	0.97 (0.40)
20:0	**0.00**	**0.04 (0.01)**	0.05 (0.00)	0.04 (0.01)	0.00	0.05 (0.00)	0.04 (0.02)	0.03 (0.01)
18:3ω6	**0.09 (0.04)**	**0.31 (0.11)**	0.13 (0.09)	0.32 (0.12)	0.08 (0.03)	0.15 (0.10)	0.21 (0.10)	0.34 (0.13)
18:3ω3	**5.34 (1.88)**	**2.98 (1.59)**	4.77 (2.11)	3.16 (1.68)	5.03 (1.66)	4.38 (2.08)	4.36 (2.35)	3.01 (1.64)
20:2	**0.19 (0.13)**	**1.02 (0.40)**	0.31 (0.30)	1.05 (0.42)	0.17 (0.11)	0.41 (0.33)	0.52 (0.35)	1.17 (0.45)
22:0	0.048 (0.024)	0.048 (0.16)	0.05 (0.02)	0.05 (0.02)	0.035 (0.00)	0.05 (0.01)	0.05 (0.02)	0.05 (0.02)
20:3ω6	0.04 (0.01)	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)	0.03 (0.02)	0.03 (0.01)	0.03 (0.01)
22:1ω9	**0.03 (0.01)**	**0.02 (0.01)**	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.04 (0.02)	0.02 (0.01)	0.02 (0.01)
20:3ω3	0.05 (0.01)	0.08 (0.05)	0.06 (0.02)	0.08 (0.05)	0.05 (0.02)	0.07 (0.02)	0.07 (0.03)	0.08 (0.05)
20:4ω6	**0.42 (0.13)**	**0.19 (0.08)**	0.38 (0.15)	0.20 (0.08)	0.41 (0.08)	0.34 (0.18)	0.31 (0.17)	0.21 (0.09)
22:2	**0.07 (0.03)**	**0.14 (0.06)**	0.08 (0.03)	0.14 (0.07)	0.08 (0.02)	0.09 (0.05)	0.10 (0.04)	0.15 (0.07)
24:0	**0.04 (0.01)**	**0.10 (0.04)**	0.06 (0.03)	0.10 (0.04)	0.04 (0.01)	0.06 (0.03)	0.08 (0.04)	0.11 (0.04)
20:5ω3	**1.98 (0.61)**	**2.46 (0.80)**	2.02 (0.61)	2.51 (0.84)	2.15 (0.55)	1.90 (0.66)	1.20 (0.54)	2.73 (0.90)
24:1ω9	**0.03 (0.01)**	**0.00**	0.03 (0.01)	0.00	0.03 (0.01)	0.04 (0.02)	0.03 (0.01)	0.00
22:5ω3	**0.59 (0.30)**	**0.11 (0.09)**	0.50 (0.33)	0.11 (0.10)	0.50 (0.14)	0.49 (0.47)	0.35 (0.33)	0.11 (0.12)
22:6ω3	**0.02 (0.01)**	**0.11 (0.04)**	0.07 (0.05)	0.11 (0.05)	0.02 (0.01)	0.09 (0.05)	0.08 (0.03)	0.12 (0.05)
Σω3	7.96 (2.46)	5.73 (1.85)	7.36 (2.59)	5.96 (1.91)	7.74 (2.10)	6.87 (2.85)	6.83 (2.62)	6.03 (1.96)
Σω6	**2.77 (0.80)**	**1.43 (0.47)**	2.50 (0.95)	1.48 (0.49)	2.73 (0.56)	2.13 (1.04)	2.12 (1.08)	1.55 (0.53)
ΣSAFA	**6.89 (2.10)**	**4.18 (1.34)**	6.31 (2.30)	4.29 (1.38)	6.88 (1.70)	5.70 (2.77)	5.37 (2.31)	4.51 (1.47)
ΣMUFA	3.32 (0.87)	2.52 (0.96)	3.13 (0.92)	2.59 (1.01)	**32.3 (1.00)**	**2.47 (0.81)**	**3.11 (0.88)**	**2.70 (1.14)**
ΣPUFA	10.97 (3.18)	8.31 (2.53)	10.23 (3.35)	8.63 (2.60)	10.72 (2.63)	9.46 (3.74)	9.54 (3.39)	8.89 (2.71)
Total	21.19 (6.02)	15.01 (4.40)	19.67 (6.44)	15.51 (4.53)	20.97 (4.95)	17.94 (7.63)	17.66 (6.35)	16.24 (4.81)

1.2-fold greater than under low light, and for 16:1ω7 only the no UVB was 1.6-fold greater than the no UVR and 1.2-fold greater than the low light. The ratio of saturated : unsaturated FA responded differently to light in the north and south ($P = 0.002$). The ratio in the no UVB treatment in the south was the lowest, being equivalent to all the light treatments in the north (average = 0.38), whereas the no UVR, HL, and LL treatments maintained greater ratios (average = 0.48). The saturated : unsaturated FA were not significantly different between the light treatments in the north.

Effect of grazing. Grazing by fish and macrozooplankton resulted in an increase in the relative biomass of diatom (ANOVA; $P = 0.049$) compared with the macrograzer exclusion treatments. Particulate N ($P = 0.025$) and particulate P were also greater in the presence of large-bodied grazers. The triple interaction (site × light × grazer) was significant for particulate P ($P =$

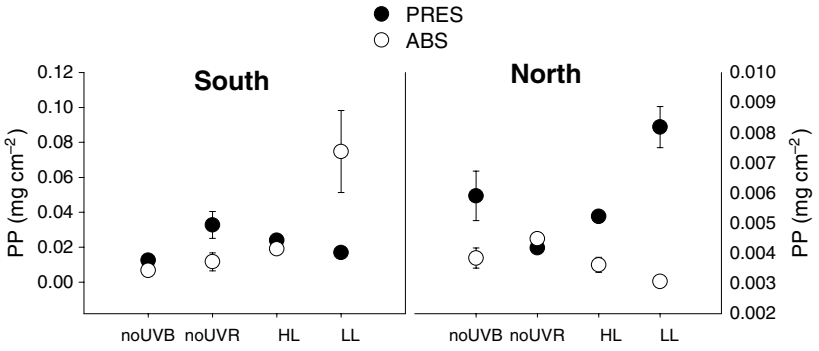

Fig. 6 Variations in particulate P with respect to site (north, south), light (no UVB, no UVR, HL, LL), and grazing (pres, part) for PP ($P = 0.027$).

0.027) (Fig. 6), indicating that P was influenced simultaneously by site, light regime and grazing.

The presence of large-sized grazers had variable effects on FA content. Split-plot analyses revealed that biofilms, which were grazed by large-bodied organisms had a greater per cent FA content ($P = 0.013$) as well as 17:0 ($P = 0.019$). For other FA, variable effects because of grazing were observed in the north and south, revealing significant interaction terms for grazing × water mass. Removal of macrograzers in the north had a positive effect on: 14:0 ($P = 0.002$), 16:1ω7 ($P = 0.013$), 17:0 ($P = 0.041$), 18:1ω9 ($P = 0.033$), 18:2ω6 ($P = 0.022$), 20:4ω6 ($P = 0.047$), 20:3ω6 ($P = 0.14$), 20:5ω3 ($P = 0.027$), the sum of SAFA ($P = 0.034$), and the sum of MUFA ($P = 0.017$). Contrastingly, in the south, removing macrograzers decreased 14:0, 18:1ω9, 18:2ω6, 20:4ω6, 20:3ω6, 20:5ω3, the sum of SAFA, and the sum of MUFA, while a non-significant effect was observed for 16:1w7 and 17:0.

Triple interaction. The triple interaction (water mass × light × grazing) was significant for particulate P ($P = 0.027$) (Fig. 6), which was, on average, threefold greater in the south than in the north, except under the no UVB and no UVR treatments. The greatest P concentrations were found in the south low light, macrograzer removal treatment, which was, on average, fourfold greater than under all the light treatments in the north. In fact, particulate P content was depleted under all light and grazer treatments in the north, excluding the biofilms found in the no UVB (presence of grazers) and low light (presence of grazers).

Discussion

The different light regimes in the north versus the south strongly influenced biofilm species composition and food quality while our intrasite manipulations of light regimes and grazers had less impact. When studying naturally occurring communities, large variations within treatments often mask the effects caused by more subtle treatments (Rae & Vincent, 1998), such as the light and grazing treatments in our experiment. Therefore, the effect of site was the predominant treatment, which created a first-order selection on algal species composition, while the light filters and grazer treatments within each water mass created secondary selective pressures. The habitats at each site constitute important retention zones, where water can remain from 2 to 13 or more days, depending on water levels (Hudon, Paquet & Jarry, 1996).

The study of light effects on lipid composition of the algae within biofilms is difficult because other organisms (e.g. bacteria, ciliates, flagellates and fungi) embedded in the biofilm matrix also contribute to any effects that may be observed. A more thorough microscopic survey of the organisms inhabiting the biofilms would have allowed us to more completely describe the variations in food quality with respect to changes in species composition. We recognise these limitations, but chose to limit our focus to algae because (i) they make up the greatest proportion of biomass in these biofilms and (ii) algae generally contain a greater quantity and quality FA than bacteria and fungi.

First-order level of selection – effect of site

At the northern site, the greater CDOM concentrations were responsible for absorbing high-energy wavelengths (313 and 320 nm) in the UVR-spectrum (Fig. 4), as shown in Frenette *et al.* (2003), while the greater turbidity caused by suspended particulate

matter could have contributed to the absorption of the longer wavelengths in the UVA (340 nm) and PAR regions (Rae & Vincent, 1998). These differences in light intensities and UVR between the two sampling sites had a significant effect on community structure, biomass, nutrient (PC, PN, PP) and biochemical composition (FA content) of the biofilms, but had no effect on the total lipids or stoichiometry of the biofilms (C : N : P ratios).

Under greater UVR and PAR exposures in the south water mass (Table 1; Fig. 4), the community structure of the biofilms reflected a greater proportion of larger, filamentous chlorophytes and cyanobacteria and exhibited a decrease in the more sensitive diatoms (Fig. 5). These results demonstrate that the prevailing light conditions contributed directly to the food quality of the biofilms primarily by controlling the species composition of the biofilms.

The greater biomass (AFDW and Chl *a*) of biofilms in the south resulted in a greater cellular nutrient content (C, N, and P). Average C : P ratios of the biofilms in the north and south (204 ± 37 and 237 ± 67, respectively) were both <300, which is the threshold value used as an indicator for zooplankton phosphorus limitation in numerous studies (e.g. Urabe *et al.*, 1997). Therefore, on the basis of this indicator alone, differences in food quality could not be detected. There is evidence that, in phosphorus sufficient environments, where algal C : P <300, food quality for consumers is mainly dependant on the EFA content of algae.

During our experiment, the average irradiance received by the biofilms in the 100% PAR treatment (Table 1) were 601 μmol m^{-2} s^{-1} and 796 μmol m^{-2} s^{-1} in the north and south, respectively, while average UVB (μW cm^{-2}) and UVA (μW cm^{-2}) intensities of 16.7 and 131.7 were recorded in the north and 57.1 and 413.4 in the south. Previous experiments have demonstrated that limiting or inhibiting light intensities can play a detrimental role (Klyachko-Gurvich *et al.*, 1999) in the desaturation and elongation of FA, resulting in an accumulation of the shorter chain precursors of highly unsaturated FA (HUFA = PUFA with 20+ carbons). It is possible that the greater PAR and UVR intensities in the south (Fig. 4) contributed to the accumulation of carbon within the cells in the form of FA that are more abundant in neutral, energy reserve lipids (SAFA, MUFA, such as 12:0, 14:0, 17:0, and 18:1ω9) at the expense of other FA that are abundant in polar lipids (PUFA such as; 18:3ω3, 18:3ω6, 20:4ω6, 20:5ω3, 22:6ω3) (Table 3; Fig. 3). Similar results have been obtained by other researchers who have examined FA profiles in relation to light quantity and/or quality (e.g. Bigogno *et al.*, 2002; Zhekisheva *et al.*, 2002). The peroxidative capacities of UVB and their negative effects on long chain unsaturated FA (Girotti, 2001) could also have led to the observed decrease in PUFA in the south biofilms.

Both 20:5ω3 and especially16:1ω7 are indicative of the presence of diatoms (Goedkoop *et al.*, 2000). The greater concentrations of these two FA in biofilms from the north (Table 3) agree with our community structure analysis (Fig. 2) demonstrating a greater relative abundance of diatoms in the north. This group of algae is recognised as a rich 20:5ω3 source, constituting a key food source for consumers. We hypothesise that the light climates characterising each site were primarily responsible for the observed differences in algal community structure.

Secondary levels of selection

Effect of light and grazing. Manipulating the light regimes within each site produced very few effects on the FA composition of the biofilms. We did observe effects on ΣMUFA, 16:1ω7, and on the saturated : unsaturated ratio. These fine alterations in cell FA content led us to hypothesise that under persistent unfavourable light conditions, there may be subtle changes at basic biochemical levels. Although the light filters reduced ambient light exposures, they were apparently less effective, at least over these timescales, in bringing about gross changes in community structure and physiology (e.g. stoichiometry and FA content) than were the long-term light climate differences between the northern and southern regions of the lake.

Removal of macrograzers (>2 mm) increased the relative biomass of diatoms in the north and south; however, this result was not highly significant ($P = 0.049$). The decrease in 16:1ω7, a diatom biomarker, in the north in the presence of grazers further questions this result. Although we did not measure this, it may be that the removal of macrograzers favoured the presence of microsized grazers on those biofilms. Previous studies have shown that grazing promotes an increased rate of periphyton succession, allowing

for continual replacement of older senescent cells by the colonisation and formation of new cells. This allows the shorter cells situated lower down in the biofilm mat, such as diatoms, to receive more light and nutrients for optimal growth (Lamberti *et al.*, 1987).

The exclusion of macrograzers had opposing effects on 14:0, 16:1ω7, 17:0, 18:0, 18:3ω3, 20:3ω6, 20:4ω3, 20:5ω3, ΣSAFA and ΣMUFA in the north and south. Removal of grazers in the north resulted in an accumulation of these FA, while in the south there was either a decrease or a negligible effect. The contrasting effects on FA caused by our macrograzer exclusion treatments at the two sites suggest that, depending on habitat characteristics (i.e. algal and grazer community structure), grazing pressures will differentially affect algal biochemical composition.

Food quantity versus quality

Our incubation rafts served as ideal structures for studying the impact of varying light climates on the food quality of biofilms. They allowed us the unprecedented ability to measure these effects *in situ* in a fluvial lake typified by harsh hydrodynamic conditions. Future studies on the determinants of food quality in aquatic systems should emphasise natural communities of algae in conjunction with detailed analyses of grazer feeding behaviours.

The importance of FA composition in determining the nutritional quality of algae has been extensively studied because of their importance to freshwater and marine organisms (Sargent *et al.*, 1995; Arts & Wainman, 1999). However, little is known about how tradeoffs between food quantity and quality will be reflected in the foodweb in terms of productivity and/or fitness of organisms at different trophic levels.

Food quality involves many dimensions, including the inherent properties of the food itself (e.g. stoichiometry, concentration of essential compounds, energetic content, presence of toxins etc) and its availability and suitability (e.g. digestibility) for consumers. We demonstrate that, on the basis of FA content alone, the quality of biofilms varied greatly between the north and south water masses and that the observed patterns in FA composition probably reflected the differing community structures in the two water masses. The biofilms in the north contained greater per cent PUFA, ω3/ω6, 20:5ω3 and 22:6ω3 but had lower ΣSAFA concentrations and saturated : unsaturated FA ratios. Benthic macrograzers, such as amphipods, feed on a variety of foods but benefit, in terms of reproduction, survivorship, and growth, by selecting richer quality foods (Cruz-Rivera & Hay, 2000). Given the importance of amphipods as an important dietary EFA source (Arts *et al.*, 2001) for fish, the quality of their food could have important repercussions for the productivity of this lake.

Our findings suggest that light intensity and UVR play a major role in regulating algal community structure, and hence food quality as exemplified by the FA profiles of the biofilms at the two sites. The intensity of light and UVR reaching the biofilms is directly influenced by fluctuating water levels and land use practices, which alter the amount of CDOM draining into this fluvial lake through its incoming tributaries. This could have serious implications for the overall productivity of Lake Saint-Pierre, as hypothesised by Frenette *et al.* (2003). Further studies detailing temporal and spatial patterns in FA signatures amongst the various water masses in Lake Saint-Pierre may provide additional insights into the foodweb structure (e.g. fish distributions) of this UNESCO world heritage site.

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