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By:

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Kim Huggins, Jean-Jacques Frenette and Michael T. Arts

Summary

1. *In situ* experiments were conducted using specialized incubation devices for the growth of aquatic biofilms (algae) to compare the effects of varying light regimes between sites, within each site, and of grazer exclusion on food quality in Lake Saint-Pierre.
2. Differing *in situ* UVR and visible light exposures in the north and south water masses had significant effects on biomass, nutrient, and relative fatty acid (FA) content of biofilms, owing to changes in community structure, but had no effect on the ratios of elemental nutrients (nitrogen and phosphorus).
3. Distinct community structures and fatty acid groups characterized each site; the biofilms in the south contained a greater relative abundance of chlorophytes and cyanophytes, along with greater amounts of low nutritional quality saturated fatty acids (SAFA) at the expense of greater quality poly-unsaturated fatty acids (PUFA). Conversely, the north biofilms had a greater relative abundance of diatoms, along with, greater PUFA, including two FA implicated in the physiological competency of grazers, i.e., eicosapentaneoic acid (EPA) and docosahexaneoic acid (DHA). This effect of site represented a primary level of natural selection on community structure.
4. The secondary level treatments (light and grazing treatments) had little to no detectable effects on food quality on the already existing community structures.

Valeur nutritive des biofilms en fonction des conditions d'éclairement au lac Saint-Pierre (Québec, Canada)

Kim Huggins, Jean-Jacques Frenette et Michael T. Arts

Résumé

1. Nous avons mené des expériences *in situ* à l'aide de dispositifs d'incubation spécialisés permettant la croissance de biofilms aquatiques (algues) dans le but de comparer les effets de différences d'éclairement, entre les stations et à l'intérieur de chacune, ainsi que ceux de l'absence de broutage sur la valeur nutritive des biofilms présents dans le lac Saint-Pierre.
2. Les différences d'exposition *in situ* aux rayons U.V. et à la lumière visible, chez les masses d'eau du sud et du nord, influaient de façon significative sur la biomasse, la teneur en nutriments et le profil d'acides gras (AG) des biofilms, en raison d'une restructuration des communautés. Par contre, ces différences n'influaient pas sur la concentration des nutriments élémentaires (azote et phosphore).
3. Des structures de communauté distinctes ainsi que des profils particuliers d'acides gras caractérisaient chaque station; dans le sud, les biofilms possédaient une plus grande abondance relative de chlorophytes et de cyanophytes et une plus grande proportion d'acides gras saturés (AGS), de faible valeur nutritive, au dépens des acides gras polyinsaturés (AGPI), de meilleure valeur nutritive. Inversement, les biofilms du nord avaient une plus grande abondance relative de diatomées et une plus grande proportion d'AGPI, dont deux AG jouant un rôle dans la compétence physiologique des brouteurs, l'acide eicosapentanoïque (EPA) et l'acide docosahexanoïque (DHA). Cet effet stationnel constitue un niveau primaire de sélection naturelle agissant sur la structure des communautés.
4. Les traitements de niveau secondaire (éclairement et broutage) n'ont eu essentiellement aucun effet notable, en matière de valeur nutritive, sur les structures de communauté déjà existantes.

NWRI RESEARCH SUMMARY

Plain language title
Effect of light climate on nutritional quality of aquatic biofilms

What is the problem and what do sicentists already know about it?
Large fluvial lakes are highly dynamic environments. We do not yet understand how the light climate in such systems affects food quantity and quality at the base of the food chain (algae). Scientists are becoming increasingly aware that both visible light and UV radiation exert strong effects on the biochemical composition of both attached (biofilm) and free-floating (sestonic) algae.

Why did NWRI do this study?
Lake Saint-Pierre has recently been classified by UNESCO as an "ecological reserve of the biosphere" and is now officially recognized as a world heritage site. This recognition arose, in part, because of its high biodiversity; the lake supports 83 fish species and harbors 288 bird species. In Lake Saint-Pierre, the areas adjacent to the south shore are known to be the most productive. For example, there are higher densities of zebra mussel larvae (Dreissena polymorpha) in the south compared with the north water mass. Perch (Perca flavescens) and American eel (Anguilla rostrata) growth rates are highest in the south water mass as are densities of perch and other fish species. Finally, most of the commercial fisheries are preferentially located along the south shore. This study was conducted, in part, to determine the underlyng reasons for the high biodiversity in this lake and also to provide an explanation for the higher productivity in the southern areas of the lake.

What were the results?
We designed, tested and proved a new incubation device that permits biofilms to be grown in highly hydrodynamic environments such as the shallow waters of rivers and fluvial lakes. The device maintains the artificial substrates at a fixed depth with respect to the surface despite rapid, and often unpredictable, changes in water level. It ably resists waves and strong currents. This design permits the operator the option of using optical and neutral density cut-off filters for precise control of the light climate impinging on the artificial substrates. Distinct algal communities and fatty acid groups characterized each site; the biofilms that grew in the south contained a greater relative abundance of green and blue-green algae, along with greater amounts of low nutritional quality saturated fatty acids (SAFA) at the expense of greater quality poly-unsaturated fatty acids (PUFA). Conversely, the northern biofilms had a greater relative abundance of diatoms, along with, greater PUFA, including two fatty acids implicated in the physiological competency of grazers, i.e., eicosapentaneoic acid (EPA) and docosahexaneoic acid (DHA). However, the low quality food effect in the south was compensated for by a much higher biomass of biofilms. This explains why the southern areas of the lake have higher overall productivity.

How will these results be used?
The results will be published in an International, peer-reviewed journal. The incubation

device should prove broadly useful to regulators/researchers interested in the effects of toxicants, nutrients, and/or light climate on the growth of aquatic biofilms in harsh environments. These results will be used by the Lac Saint-Pierre Fishermen's Association to help establish harvests limits for commercially important fish species in the lake. They will also be used by GRIL (compendium of Quebec universities conducting research on aquatic systems) to plan future studies on Lake Saint-Pierre.

Who were our main partners in the study?
University of Quebec, NSERC, Lac Saint-Pierre Fishermen's Association

Sommaire des recherches de l'INRE

Titre en langage clair
Effet du climat lumineux sur la valeur nutritive de biofilms aquatiques.

Quel est le problème et que savent les chercheurs à ce sujet?
Les grands lacs fluviaux sont des milieux très dynamiques. Nous ne comprenons pas encore comment le climat lumineux de ces milieux influe sur la quantité et la qualité de la nourriture constituant la base de la chaîne alimentaire (algues). Les chercheurs sont de plus en plus conscients du rôle très important joué par la lumière visible et les rayons U.V. dans la composition biochimique des algues fixées (biofilm) et des algues flottant librement (seston).

Quels étaient nos principaux partenaires dans cette étude?
Le lac Saint-Pierre a récemment été classé par l'UNESCO comme « réserve écologique de la biosphère » et est maintenant reconnu officiellement comme site du patrimoine mondial. Cette reconnaissance a été attribuée, en partie, à cause de sa grande biodiversité : le lac héberge 83 espèces de poissons et 288 espèces d'oiseaux. On sait que les zones adjacentes à la rive sud sont les plus productives du lac. Par exemple, on observe une plus grande densité de larves de moule zébrée *(Dreissena polymorpha)* dans la masse d'eau du sud que dans celle du nord. Les taux de croissance de la perchaude *(Perca flavescens)* et de l'anguille *(Anguilla rostrata)* sont plus élévés dans la masse d'eau du sud. Les densités de perchaude ainsi que d'autres espèces de poissons sont également plus élevées dans la masse d'eau du sud. Finalement, la plupart des pêches commerciales se situent de préférence le long de la rive sud. La présente étude visait, en partie, à déterminer quels facteurs expliquent la grande biodiversité du lac et pourquoi la partie sud du lac est la plus productive.

Pourquoi l'INRE a-t-il effectué cette étude?
Nous avons conçu et testé avec succès un nouveau dispositif d'incubation qui permet la culture de biofilms dans des milieux très hydrodynamiques tels que les eaux peu profondes des rivières et des lacs fluviaux. Le dispositif maintient des substrats artificiels à une profondeur fixe par rapport à la surface malgré les changements rapides et souvent imprévisibles du niveau de l'eau. De plus, ce dispositif résiste bien à l'action des vagues et des courants forts. Il est possible d'utiliser des filtres optiques ou neutres pour régler de façon précise le climat lumineux auquel sont exposés les substrats artificiels. Des communautés d'algues ainsi que des groupes d'acides gras distincts caractérisent chaque station; les biofilms qui croissent dans la partie sud du lac contiennent une plus grande abondance relative d'algues vertes et bleues et une plus grande proportion d'acides gras saturés (AGS), de faible valeur nutritive, au dépens des acides gras polyinsaturés (AGPI), de meilleure valeur nutritive. Inversement, les biofilms du nord ont une plus grande abondance relative de diatomées et une plus grande proportion d'AGPI, dont deux acides gras jouant un rôle dans la compétence physiologique des brouteurs, l'acide eicosapentanoïque (EPA) et l'acide docosahexanoïque (DHA). Cependant, l'effet de la faible valeur nutritive observée dans la partie sud du lac est compensé par une biomasse nettement plus élevée des biofilms. Ce phénomène explique pourquoi la productivité globale de la partie sud du lac est la plus élevée.

Quels sont les résultats?
Les résultats seront publiés dans une revue internationale possédant un comité de lecture. Le dispositif d'incubation devrait s'avérer utile de façon générale pour les organismes de réglementation et les chercheurs voulant étudier les effets des substances toxiques, des nutriments ou du climat lumineux sur la croissance des biofilms aquatiques dans les milieux difficiles. Ces informations aideront l'Association des pêcheurs commerciaux du lac Saint-Pierre à établir des limites de capture pour les espèces de poissons ayant une valeur commerciale. Le GRIL (Groupe de recherche interuniversitaire en limnologie) utilisera également ces résultats pour planifier de nouvelles études sur le lac Saint-Pierre.

Comment ces résultats seront-ils utilisés?
Université du Québec, CRSNG, Association des pêcheurs commerciaux du lac Saint-Pierre.

Introduction

The importance of algal food quality for zooplankton and fish has become the focus of extensive research over the last few years. These studies have mainly focussed on two indicators of high algal food quality, i.e. phosphorus (P) (Urabe et al. 1997; De Mott et al. 1998) and long-chained poly-unsaturated fatty acid (PUFA) content (Brett and Müller-Navarra 1997; Weers and Gulati 1997). The majority of such studies were conducted in the laboratory, directly on algal cultures (e.g. Boersma and Schöps 2001), or indirectly, by studying effects on consumers (e.g. Wacker and von Elert 2001), but there are comparatively few studies that have been conducted *in situ* on natural algal communities. Although these two factors may act separately or in conjunction as major control factors for the growth and physiological competency of grazers in freshwater ecosystems there is general consensus that when P is not limiting, PUFA becomes the main limiting factor (Gulati and Demott 1997; Sterner and Schulz, 1998).

Light is crucial for photosynthesis and is thus the most critical environmental factor for regulating biofilm growth, community structure, and productivity (Wellnitz and Ward 1998, Hill and al. 1995, Watkins et al. 2001). High light and UVR exposures have been shown to increase chlorophyte abundance (Wellnitz and Ward 1998, Donahue 2000 in Watkins and al. 2001), while decreasing that of most diatom species which are usually considered to be particularly sensitive to UVR (Watkins et al. 2001, Vinebrooke and Leavitt 1996, Francoeur and Lowe 1998). Furthermore, light quantity (intensity) and quality (wavelength dependent energy) are in part responsible for the biochemical composition of algae (e.g. Hessen et al. 2002, McNamara and Hill 2000), but it is still

unclear how light conditions affect food quality through P assimilation and PUFA biosynthesis.

Light quantity may affect algal food quality by altering the cellular carbon to phosphorus (C:P) ratio (e.g. Urabe and Sterner 1996; Sterner et al. 1997; Sterner and Schulz 1998). Although carbon:nitrogen (C:N) ratios have also been shown to vary under the influence of light intensities (Frenette et al. 1998), most measures of food quality incorporate the C:P ratio owing to its predictive power for zooplankton growth over a broader range of lake types (Sterner and Hessen 1994). This ratio is used because P is generally the most limiting nutrient in freshwater systems and its presence within the cells is necessary for many metabolic processes (Wetzel 2001). However, the power of C:P as a food quality indicator is of limited value when the P is in sufficient supply and/or is similar amongst sites (von Elert and Stampfl 2000).

Another useful indicator of food quality is the FA content of the biofilms. FA belong to one of three groups (SAFA, MUFA, and PUFA) depending on their degree of desaturation.. Although the biosynthesis of these groups of FA is just beginning to be understood, it is well known that SAFA and MUFA are the major components of neutral lipids. These lipids function mainly as energy storage reserves, which generally increase as a result of exposures to stressful environmental conditions, such as variations in temperature, nutrients, and light climate. In contrast, PUFA are major constituents of polar lipids, which are present in cellular and chloroplast membranes in algae. In organisms, PUFA function as neural transmitters, they are necessary for proper vision, and ensure thermal protection in cold environments (Arts et al. 2000). Certain PUFA can be defined as essential (EFA) meaning that they cannot be synthesized de novo from LA

and ALA in sufficient amounts to optimize physiological performance (Arts et al. 2001, Cunnane 1996). They must therefore be obtained from dietary sources, such as algae. In most studies the two main EFA considered to be most likely limiting for the growth and development of aquatic invertebrates and vertebrates are 20:5ω3 (EPA) and 22:6ω3 (DHA) (Arts and al. 1997, Brett and Müller-Navarra 1997, DeMott and Müller-Navarra 1997). A more recent study however suggests that the situation is more complex and that, in *Daphnia*, ALA may be converted to EPA but not the reverse (von Elert, 2002). The biosynthetic pathways (Fig. 1) leading to the synthesis of these two EFA include a series of pre-cursors, which are, from the linolenic series (ω3): 18:3ω3 (α-linolenic acid or ALA), and from the linoleic series (ω6): 18:2ω6 (linoleic acid or LA), 18:3ω6 (γ-linoleic acid or GLA) and 20:4ω6 (arachidonic acid or ARA). The biosynthesis pathways for PUFA in algae are less understood than those of higher plants and even less is known about the ancillary factors influencing their biosynthesis. There is good evidence to suggest that light quality and/or quantity influences lipid content and composition (Bigogno et al. 2002; Khozin-Goldberg et al. 2002). In fact, lipid content of algae should be greatest when lipid synthesis is light saturated, i.e. between 300-800 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (Wainman et al. 1999).

Grazing also plays a major role, in addition to nutrients and light, in regulating biofilm nutrient content and stoichiometry (Wellnitz and Ward 1998, Steinman and al. 1987). With respect to FA, numerous publications (e.g. Ahlgren 1990; Müller-Navarra 1995) have described how algal PUFA content increases grazer growth and reproduction rates, but the effects of grazing on algal FA biosynthesis are still unclear. We hypothesize

that grazers will selectively remove cells of richer food quality, thus decreasing biofilm EFA content.

Lake Saint-Pierre is characterized by three main water masses that differ in their optical properties (Frenette et al. 2003), thus constituting a natural experimental site for intra-lake comparisons of light effects on algal food quality. The objectives of this study were to determine, *in situ*, how differing PAR and UVR exposures, as well as the indirect effects of grazing, influence algal food quality (stoichiometry and PUFA synthesis). No evidence presently exists on potential differences in biofilm community structure amongst the north and south water masses of Lake Saint-Pierre. These two water masses are separated by a fast-flowing maritime channel and are influenced by their incoming tributaries. The tributaries in the south water mass in particular come from irrigated lands and thus are highly influenced by agricultural activities. The three main water masses provide important retention zones in which organisms have various amounts of time to adapt and develop to local conditions. The algal communities which develop in these habitats are likely to have experienced a natural selection process influenced in part by the ambient light environments to which they are exposed. Furthermore, present environmental threats, such as, ozone deterioration and global warming may alter existing light regimes and potentially modify the present benthic and pelagic algal communities. Thus part of our motivation to conduct this study was to more fully understand how aquatic biofilms are affected by light climate in this system.

Materials and Methods

Study area

Incubation devices (Fig. 2) 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 a period of 47 d to 49 d: from July 24 & 25, 2001 until Sept. 9, 10, & 11, 2001. This fluvial lake is situated within the St. Lawrence River system and occupies a mean annual area of 472 km² (Environment Canada, 2000), which extends from the Berthier-Sorel Islands to the city of Trois-Rivières, Québec, Canada. Hydrodynamic conditions of these two sites were comparable since 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 between 3 m at the beginning of the experiment to ~1 m by the end.

Experimental setup

Aluminum rafts consisting of 2 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 & 25.8 cm^2) and an upper story on which up to six light filters could be affixed. Three of the six filters consisted of different combinations of acrylic sheeting and/or polyester film (Mylar). This arrangement produced the following light treatments: 1) +UVR treatment: Acrylite® OP4, which transmits 93% of PAR and most of the UV-A and UV-B (65% transmission at 280 nm), 2) no UVR treatment: Acrylite® OP3, which transmits 93% of PAR with 0% transmission <375 nm, and, 3) no UVB: a film of Mylar®D, which allows 50% transmission at 318 nm was fixed to a sheet of Acrylite® OP4. 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%. The upper story was fixed at a height of 2.5 cm above the lower one, which reduced stray light contamination and shading, without drastically modifying water flow. This assemblage of tiles and filters produced a platform of 1.52 m by 1.80 m that was suspended from a rectangular floatation system designed to limit shading. Stakes placed within the corners of the floatation device secured the rafts in their attributed positions at 45° with respect to water flow in order to minimize water turbulence and drag.

The experiments were conducted to study the main and interactive effects of site, light regime, and grazing on biofilms. Six incubation rafts were placed in the north water mass and six in the south to study the effect of site, whereas, the light filters on the incubation rafts allowed for the study of fluctuating light regimes within a same mass with identical chemical properties. The macro-grazer 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 combination of treatments (2 sites X 6 light regimes X 2 grazing levels) was replicated threefold, resulting in a split-plot design with 72 sampling units.

Substrate testing

The similarities between the communities growing on the tiles with the natural benthic community structure was verified by examining the community structure of the periphyton growing on rocks, plants and tiles. Platforms supporting rocks and tiles were fixed on wooden stakes at different heights. Three stakes were deployed at both the north and the south incubation sites.

The stakes were removed after 28 d (July 30 to August 26). The substrates (rocks and tiles) were cut loose from the platforms and immediately placed in individual plastic bags in coolers on the field. Macrophytes (*Vallisneria* sp.) were gently uprooted, removed from the water, and placed in plastic bags. In the laboratory, the tiles and rocks were scraped with razor blades and the live material was placed in 1% Lugol's solution for future 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 pre-weighed aluminium trays dried them and oven-dried at 60° C for 24 h. Identification of algae was done to the level of class except in the case of diatoms. Diatom identification was performed at high magnification on specimens mounted on permanent slides, after the frustules were acid-cleared and mounted using Naphrax®. 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 100X, 200X and 400X magnification. The counts and biovolumes were entered into ALGAMICA 6, program version 4.1 (Hamilton and Gosselain 2001, available on internet) for appropriate calculations.

Sampling and analysis

Chemical and physical variables of lake water

During the incubation period, 2 L water samples were collected every 2-3 d from the north and south shores. Analyses were carried out at the National Laboratory for Environmental Testing (NLET, Burlington, Ontario). Total P was obtained after acid digestion, followed by the addition of ammonium molybdate, which is reduced with

stannous chloride to form a molybdenum bleu complex measured spectrophotometrically at 660 nm Total N concentrations were measured spectrophotometrically at 520 nm after oxidation of organic N into nitrates during their digestion in an autoclave (NLET, 2001).

The portion of DOC which absorbs more strongly at lower wavelengths, i.e. chromophoric dissolved organic carbon (CDOM) concentrations were obtained by measuring the absorption spectra on a spectrophotometer (Cary 100Bio, Varian Co., Palo Alto, CA, USA) 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).

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). The irradiance values were obtained using a spectroradiometer (Model PUV-2545, Biospherical Instruments, San Diego, USA), which measured the energy at 313, 320, 340, 443, 550 nm wavelengths, and for PAR (400-700 nm).

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 future community structure analyses. A slurry was formed with the remaining tiles by adding distilled water to the biofilms, which were then gently blended

to obtain a homogeneous solution. This slurry was divided into 3 different subsamples for each replicate and filtered on, 1) 25 mm Whatman GF/F filters for chlorophyll *a* (Chl *a*), 2) pre-combusted 25 mm Millipore glass fiber filters for ash free dry weight (AFDW), particulate organic carbon (POC) and nitrogen (PON), and 3) pre-combusted and acid pre-washed 47 mm Millipore GF/F filters for particulate organic phosphorus (PP) contents. POC, PON, PP, and Chl *a* were filtered in duplicates 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 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 analyzed in a spectrophotometer (Shimadzu, UV-Probe, Columbia, MD, USA) (Cattaneo unpublished). Absorption measurements were taken at 665 and 750 nm before and after acidification to correct for phaeopigments, according to Wetzel and Likens (2002). Analyses of POC, PON, and PP, were carried out at the National Laboratory for Environmental Testing (NLET, Burlington, Ontario). POC and PON 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 spectrophotometrically at 660 nm.

Fatty acids - extraction and fractionation

Fatty acid methyl esters (FAME) of the samples were obtained by a three-step process: gravimetric extraction, derivitization, 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 and Dyer, 1959). Following centrifugation, removal of the supernatant, and salt-wash, the samples were evaporated using gaseous nitrogen and a fixed volume was weighed to provide a quantitative measure of total lipid content. The remaining portion of each extract was evaporated to dryness using extra-dry nitrogen gas and stored at -80°C until the derivitization step.

For the derivitization process, hexane and BF3-methanol (10% w/w) were added and the head space of the vials were 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) using a splitless injection on a Supelco (SP-2560) column (100 m x 0.25 mm ID x 0.20 μm thick film). Hydrogen was used as the carrier gas and the temperature programming was: 100°C (hold 1 min) to 240°C at 5°C/min (hold for 38 min). Three individual pure FA standards (C20:2, cis-11,14-eicosadienoic acid; C20:5n3, eicosapentaenoic acid [EPA] and, C22:6n3, docosahexaenoic acid [DHA]) were used to estimate the derivitization efficiency. A 37-component FAME standard (Supelco #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. All FA results are reported either as μg FAME/mg dry weight tissue extracted

Statistical analysis

Community structure

The differences in community structure between the north and south sites, grazer and non-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 log-transformed relative biomass of the same three groups used as dependent variables.

Stoichiometry and FA content

The effects of site, light, and grazing 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. Because one of the rafts in the south water mass drifted away during the experiment, our analyses were conducted on n = 66 instead of 72 for the main treatments; the number of samples remaining for testing the two- and three-way interaction terms were consequently reduced. Because of this, and because of high standard deviations caused by a few unexplainably extreme values, the four neutral density treatments were combined to form two groups, for a total of four light treatments instead of six. Thus, the samples from the control treatment, which allowed 93% PAR

penetration + full UVR exposure, were combined to those from the 90% cut-off, to form the high light treatments (HL), and the samples under the 70 and 50% cut-offs were combined together to form the low light treatments (LL); the no UVB and no UVR treatments could not be combined.. Consequently, the main effects were: site with two levels; north (N) and south (S), light (L) with four levels (no UVB, no UVR, HL, LL), and grazing (G), with two levels; grazer presence (PRES) and grazer absence (ABS).

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 relative values (% of each FA/total of FA^{-1}). Residuals from all analyses were verified for normality and homoscedasticity; appropriate transformations (log, root, square, inverse) were applied when necessary to meet the required assumptions. To further illustrate the associations between nutrients, biomass, stoichiometry, and lipids, we used an ordination biplot (Fig. 3).

Results

Experimental setup

The clay tiles were abundantly colonized by biofilms. The regulated incubation depth with respect to the surface allowed us to thoroughly document of the light climate reaching the algal cells for photosynthesis. The use of tiles as substrates for studying biofilm growth is widely used in research. However, amongst our sites, the most available natural substrates mainly consist of submerged macrophytes. We tested the suitability of our tiles as substrates by comparing the community structure of the biofilms

growing on tiles, on macrophytes (*Vallisneria* sp.), and on rocks (Fig. 4). The one-way ANOVA revealed significant differences in the community structures of the algal mats that colonized these substrates at our incubation sites in the Lake Saint-Pierre. These reflected a greater abundance of chlorophytes growing on the tiles compared to the rock and plant substrates; however, the differences in relative diatom biomass in the north and south was not significantly different on the tiles from that on the plants, while the rocks supported the least abundant diatom relative biomass. The diatom relative biomass was greater in the north than in the south for all substrates used.

Physico-chemical variables of lake water

The north and south water masses varied markedly in their spectral regime with respect to UVR and PAR 1% penetration depths owing to their CDOM (Fig. 5) and total suspended particles concentrations. The measurements of downward irradiance at the incubation depths (0.03 m 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, UVR and PAR intensities at the incubation depth indicate that the south biofilms were, on average, exposed to 3.4 X greater UVB, 3X greater UVR (UVB + UVA), and 1.3X greater PAR throughout the incubation period. Total N (average = 0.40 $mg \cdot L^{-1}$) and P (average = 0.03 $mg \cdot L^{-1}$) did not vary significantly between the two sites (north and south) (t-test, $p = 0.34$ and 0.14 respectively). The values for each corresponded to an trophic state situated between mesotrophic to eutrophic according to the general trophic classification of lakes (Vollenweider in Wetzel 2001), indicating that nutrients were likely not limiting at both study sites.

Biofilm samples

Effect of site

Amongst the three taxonomic groups examined (diatoms, cyanophytes, chlorophytes), relative abundance of chlorophytes was greatest in the north and south (Fig. 6), with *Cladophora* sp. and *Coleochaete* sp. dominating in the south, and *Cladophora* sp., *Oedogonium* sp. and *Stigeoclonium* sp. dominating in the north. In the north, relative abundance of diatoms equaled that of chlorophytes, with *Melosira* sp. and *Amphora* sp. as the dominant species, while *Cocconeis* sp. was the most abundant diatom in the south.

The three factor SPLIT-PLOT on log-transformed periphyton variables (Table 2) revealed greater biomass ($p < 0.001$) in the south biofilms, with respect to Chl *a*, POC, PON, PP, and AFDW. However, the stoichiometric ratios (C:P, C:N, N:P) did not differ significantly between the two sites.

The average ratios of SAFA:MUFA:PUFA in the south was 32:16:52, compared to 28:17:55 in the north, showing an increased proportion of SAFA at the expense of PUFA in the south when compared to the north, while MUFA did not vary significantly (Table 2, Fig. 6). PUFA can be separated in 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). Total linoleates were greater in the south owing to two-fold greater 18:2ω6 and 20:4ω6 content compared to the north (Table 2). Amongst the linolenates, DPA ($p < 0.001$) and ALA ($p = 0.019$) were greater in the south, while greater contents

of EPA, DHA, and 20:3ω8 ($p < 0.001$) were found in the north. The ω8:ω6 ratio revealed that linolenates were 4X and 3X greater than linoleates in the north and south, respectively ($p < 0.001$) (Table 2, Fig. 6).

Individual FA all varied significantly with respect to site, excluding 20:3ω6 and 22:1ω9 (Table 2; Fig. 3). The southern biofilms were associated with greater biomass (Chl *a*, and AFDW) and nutrients (POC, PON, PP), along with the following PUFA: ALA, LA, ARA, DPA, and 20:3ω6, whereas the northern biofilms were associated with the following PUFA: EPA, DHA, 18:3ω6, 20:3ω8, 20:2, and 22:2, the following MUFA: 16:1ω7, and the following SAFA: 20:0, 24:0, 22:0, 18:0, and 15:0.

Effect of light

The differing light regimes within each site did not account for any variation in stoichiometry and biomass. Therefore, the taxonomic composition of the samples under the four differing light regimes was not compared. Amongst the FA analyzed, only a few were significantly affected by altering light regimes within each site; i.e., 12:0, ($p = 0.007$), 16:1ω7 ($p= 0.045$), 20:3ω6 ($p = 0.035$), ω8 ($p = 0.004$), ω8/ω6 ($p = 0.007$), and MUFA ($p = 0.015$). The LL treatment was significantly greater than HL in the case of 12:0, greater than no UVB for ω8/ω6 and greater than HL and no UVB for ω8. However, LL was lower than HL for 20:3ω6 and lower than no UVB in the case of 16:1ω7 and MUFA (Table 2). Linoleates responded differently to light in the north and south. The removal of UVB in the south increased linoleates, while the removal of entire UVR (UVB + UVA) was associated with an increased linoleate content in the north.

Effect of grazing

The two-way ANOVA revealed that grazers slightly increased diatom relative biomass ($p = 0.049$). POC ($p = 0.057$), PON ($p = 0.025$), and PP were greater in the presence of grazers whereas C:N decreased ($p = 0.051$), but these observations were not highly significant. The triple interaction (site X light X grazer) was significant for PP ($p = 0.011$), indicating that it was influenced simultaneously by site, light regime, and grazing, which does not allow for main treatment interpretation; this effect is described further in triple interactions.

The presence of grazers had variable effects on FA content. The split-plot analysis revealed that the presence of grazing contributed to increasing DPA and 22:2, while decreasing MUFA. Whereas for other FA, variable effects due to grazing were observed in the north and south as well as under differing light regimes for certain FA, revealing significant interaction terms for grazing X mass and grazing X light. In the south, the presence of grazing stimulated total FA content ($p = 0.062$) and 14:0 ($p = 0.002$), whereas in the north, presence of grazers had no or less effect. In the north, however, grazing increased the presence of 12:0, 17:0, and linolenates. Grazers had little or no effect on 20:3ωβ and 17:0 in the south, while they increased the contents of these FA in the north (Fig. 7). The linoleates were affected by light and grazing simultaneously, where grazers increased ω6, notably 18:2ω6, under the no-UVB and under the LL treatments.

Triple interaction (PP, PUFA)

The triple interaction (mass X light X grazing) was significant for PP ($p = 0.027$) and PUFA ($p = 0.038$) content (Fig. 8, Table 3). PP was on average significantly greater in the south than in the north, except under the no-UVB and no-UVR (ABS). PP found in the LL (south, ABS) was, on average, 4X greater than in the south. In the north, PP content was low under all light and grazer treatments, excluding the biofilms found in no-UVB (PRES) and LL (PRES). Conversely, PUFA was on average greater in the north, reaching its greatest values under LL (PRES and ABS), no-UVR (PRES) and no-UVB (PRES). There were no differences amongst the south biofilms with respect to PUFA content, but the lowest values were found under the HL (ABS and PRES) (Table 3).

Discussion

Location most strongly influenced biofilm food quality while the effects caused by light regime and grazers had less impact. When studying natural occurring communities, large variations within the treatments can reduce the effects caused by more subtle treatments (Rae and Vincent 1998), such as light filters and grazing in our experiment. Therefore, the effect of site was the predominant treatment, which created a first-order selection on algal communities, while the treatments of light and grazing within each water mass created secondary, less efficient levels of selection. The habitats at each site correspond to important retention zones that can last from 2 to over 13 d, depending on water levels (Hudon et al. 1996). Furthermore, these waters are fed with diverse incoming tributaries probably characterized by diverging communities of algal propagules that have been pre-selected on the basis of natural processes. Although the

light filters and grazing treatments had weak and inconclusive effects on FA content, they did aid in discriminating the FA which could be particularly vulnerable to these treatments.

First-order level of selection - effect of site

At the northern sites, the greater CDOM concentrations were responsible for absorbing high-energy wavelengths (313 and 320 nm) in the UVR-spectrum (Fig. 5), as shown in Frenette et al. (2003), while the greater turbidity observed caused by suspended particulate matter, could have contributed to the absorption of the shorter wavelengths in the UVA (340 nm) and in the PAR region (Rae and Vincent 1998). These differences in light intensities and UVR between the two sampling sites during the incubation period had a significant effect on community structure, biomass, nutrient (POC, PON, PP) and biochemical composition (FA content) of the biofilms, but none on the stoichiometric content of the cells in terms of C:N:P.

The optimal light regime for maximum photosynthetic rates and algal growth varies according to species. However, biofilms comprise a diverse community structure that is likely to react to high light coupled to high UVR intensities by producing a shift towards a more UV-resilient community (Bothwell et al. 1993). Studies have shown that increased HL and UVR, have a positive effect on chlorophytes, whereas diatom abundance generally decreases in the presence UVR. Our results show that differences in the underwater light environment between sites were responsible for naturally selecting the most competitive and adapted algal communities for each environment. The greater UVR (and perhaps HL) exposure in the south was the most probable cause for the

decrease in diatom relative abundance and the increase in larger, filamentous chlorophytes and cyanophytes (Fig. 6). This supports recent findings that diatoms are particularly sensitive to UVR exposure (e.g. Vinebrooke and Leavitt 1996, Rae and Vincent 1998, Francoeur and Lowe 1998, Watkins et al. 2001).

The greater nutrient contents (C, N, and P) found in the south can be accounted for by the differences in community structure coupled with greater biomass values (AFDW and Chl *a*). In the north and south, average C:P ratios of 204 +/- 37 and 237 +/- 67 respectively, corresponded to values situated below the threshold of 300 used as an indicator for zooplankton P-limitation in various studies, suggesting that, on the basis of this indicator alone, differences in food quality could not be detected. Furthermore, studies have provided evidence that in P sufficient environments (C:P < 300), food quality is mainly dependant on biochemical composition (Urabe et al. 1997).

Previous experiments have demonstrated that limiting or inhibiting light intensities can play a detrimental role in the desaturation and elongation processes involved in FA biosynthesis (Fig. 1), resulting in an accumulation of pre-cursors to the detriment of the end products of PUFA synthesis (Klyachko-Gurvich et al. 1999). This generally results in carbon accumulation within the cells in the form of neutral lipids (SAFA and MUFA, such as 16:0, 16:2, 18:0, 18:1, 18:2) at the expense of other FA that are abundant in polar lipids (e.g. PUFA such as; ALA, GLA, ARA, EPA, DHA) (Bigogno et al. 2002; Zhekisheva et al. 2002).

With respect to light quality, UVB has been shown to alter the allocation of carbon to the various biochemical pools (lipid, protein, polysaccharide and low molecular weight compounds), thus altering the lipid content of algae (Arts and Rai 1997). Others

have demonstrated that the detrimental effects of UVR on fatty acid synthesis cause reductions in chain elongation and desaturation processes (Goes and al., 1994), due to its peroxidation capacities (Girotti 2001) , thus bringing about subsequent decreases in %PUFA, notably EPA and DHA (Wang and Chai 1994).

The average irradiance received by the biofilms during our experiment was located between 300-800 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ for the north and south (Table 1), indicating that lipid content of the biofilms should be near maximal (Wainman 1998). However, the composition in FA of these lipids varied largely amongst the sites. On the basis of the studies described previously, the greater SAFA content (12:0, 14:0, 16:0, and 17:0) in the biofilms in the south compared to the north at the expense of PUFA, were probably a result of greater UVR (and PAR) intensities. Furthermore, the southern biofilms contained greater contents of 18:1ω9, ALA, LA, ARA, and 20:3n6, which are precursors in the synthesis of EPA and DHA. This inhibition of desaturation processes just previous to the formation of EPA and DHA remains to be explored further and, to our knowledge, has not been observed before. It is well known that 16:1ω7 and EPA indicate a predominance by diatoms (Léveillé et al. 1997), supporting our community structure analysis of greater diatoms in the north. This group of algae is recognized as a rich lipid source, thus constitutes a key food source for consumers. We hypothesize that the effect the light climate present at each site were primarily responsible for the differing algal community structures. Physiological mechanisms probably allowed them to adjust their FA biosynthetic pathways in order to attain FA profiles to optimize their survival in the northern and southern areas of the lake, although this remains to be explored further.

Secondary levels of selection

Effect of light

In this study, the deleterious effects caused by UVB and/or UVR exposure and/or high light were only detectable on a few variables within each water mass, perhaps because the differences between UVB, UVA, and PAR were not large enough to cause irreversible damage to the cells. UVA along with shorter wavelengths of PAR and/or high light intensities can contribute to repairing the damaging effects of UVB through photoreactivation (Quesada et al. 1995), stimulating the synthesis of photo-protective pigments, such as mycosporine-like amino acids (MAAs) in many algal species (Neale and al. 1998). Decreasing ozone levels increase the proportion of shorter-wavelength UVB radiationreaching the earth's surface. Algal communities will adapt to such changes but perhaps in ways that would reduce their nutritional quality and quantity.

The few effects of light that we did observe were on; Γlinolenates, 20:3ω6, 12:0, ΓMUFA, and 16:1w7. These fine alterations in cell FA lead us to hypothesize that the changes occur gradually at the physiological level, which, if conditions persist, could have repercussions on nutrient assimilation processes, growth, and stoichiometry.

Effect of grazing

Grazing had a significant effect on community structure. There was an increase in the relative biomass of diatoms in the PRES treatments. One hypothesis to explain this is that the grazing of larger cells promotes an increased rate of periphyton succession, allowing for continual replacement of older senescent cells by the colonization and formation of new cells. This allows the shorter cells situated lower down in the biofilm

mat, such as diatoms, to receive sufficient light and nutrients for optimal growth (Lamberti et al. 1987).

The presence of large grazer increased nutrient content of the cells (C, N, and usually P) and biomass (Chl *a*, AFDW). Similar increases in POC and PON have previously been reported in the presence of grazers (Lamberti et al. 1987), as well as a decrease in C:N (Hillebrand and Kahlert 2001), but these effects remain unclear.

Contrary to our expectation, grazing did not significantly decrease PUFA content. No other studies that we know of have examined, in detail, how grazing affects FA content of algae, therefore the reasons for the opposing effects of grazing on linolenates, 20:3n3, 14:0, 17:0, 18:0, and 22:0 in the north and south are unknown. These contrasting effects of grazing at the different sites suggest that grazing pressures differentially affect algal communities depending on the habitat, where the community structure of grazers and algae are likely to differ.

Food quantity vs quality

The existing studies on the impacts of food quality vs. food quantity have examined quality on the basis of nutrient content (Sterner and Hessen 1994), stoichiometry (Sterner 1997), or protein content (Cruz-Rivera and Hay 2000). None amongst these have considered FA content even though the lipid production by algae can be particularly important at certain periods (Arts et al. 1997), and essential requirements for PUFA in nutrition has been shown in all vertebrates studied to date (Sergant et al. 1995 in Gulati and Demott 1997). Furthermore, food quality includes many dimensions (stoichiometry, P-content, and essential biochemical content), and also depends on the

inherent demands of the consumers. Further exploration of other biochemical aspects (proteins and vitamin content) are planned in near future studies; however, our observations based on stoichiometry, P-content and FA content have shown that the food quality differs greatly between the north and south water masses on the basis of FA content. Studies have shown that ALA, EPA, and DHA supplemented diets, increase growth rates and fecundity in *Daphnia* (Demott and Muller-Navarra 1997; Weers and Gulati 1997; von Elert 2002). Although some animals are capable of desaturation and elongation of shorter-chain, dispensable FA, de novo synthesis of lipids most probably occur at slow rates and are sometimes inhibited (Weer and al. 1997; Ahlgren 1992; Goulden and Place 1993). Our results show that the southern biofilms contained a greater quantity of food of a lesser quality, whereas the northern biofilms contained less food of greater quality.

In the presence of lower quality food, certain grazers have developed feeding compensation behaviors by increasing feeding rates (Cruz-Rivera 2000), while others increase their search period resulting in lowered consumption rates of better quality foods and increased risks of predation (Begon et al. 1990 in Frost and Elser 2002). Although no quantification of grazer densities were performed in this study we observed that *Gammarus lacustris* was the dominant grazer on our tiles. Cruz-Rivera and Hay (2000) examined feeding behaviours of amphipods with respect to food quality and quantity. They demonstrated that these grazers feed on a high variety of foods, but benefit in terms of reproduction, survivorship, and growth, by selecting richer quality foods (Cruz-Rivera and Hay 2000). Given the importance of amphipods as an important dietary EFA source

(Arts and al. 2001) for fish, the quality of their ingested food could have important repercussions on the productivity of this lake.

Our findings clearly suggest that light intensity and UVR, which are directly affected by fluctuating water levels, play major roles in regulating food quality of algae in nature. This could have serious implications for the productivity of this ecosystem, as hypothesized by Frenette et al. (2003). Our incubation rafts served as ideal structures for studying the impact of varying light climates on the food quality of biofilms. They allowed us to measure these effects *in situ*, and, in a fluvial lake where harsh hydrodynamic conditions persisted, which is unprecedented to our knowledge. Future studies on the determinants of food quality in aquatic systems should emphasize natural communities of algae in conjunction with detailed analyses of grazer feeding behaviors.

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Figure 1: Main poly-unsaturated fatty acid biosynthetic pathways in algae. SAFA are desaturated into MUFA, which are further desaturated and elongated into PUFA. The EFA, EPA and DHA, are amongst the final products of the biosynthetic pathways formed from more saturated, shorter chain length pre-cursors.

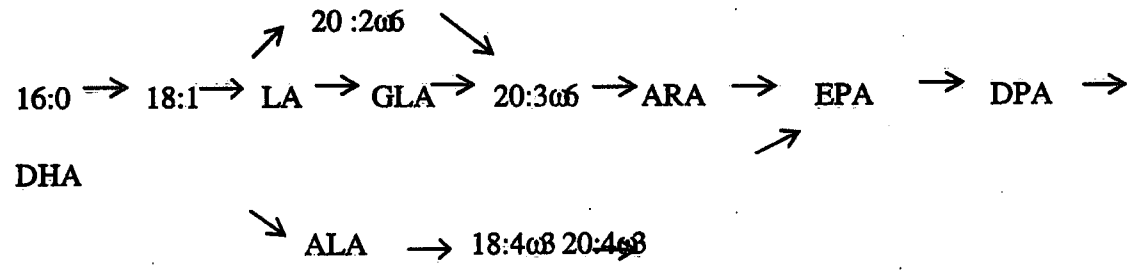


Figure 2: Diagram of an incubation raft with the two main decks: a lower story supporting the substrates (tiles) and an upper story with the light filters (no-UVB, no-UVR, HL (100% and 90% cut-off), LL (70 and 50% cut-offs)). The platform was attached to a square float and was anchored to the bottom sediments with wooden stakes. The rings at the corners allowed it to move vertically with the water movements so that the incubation depth could be precisely maintained.

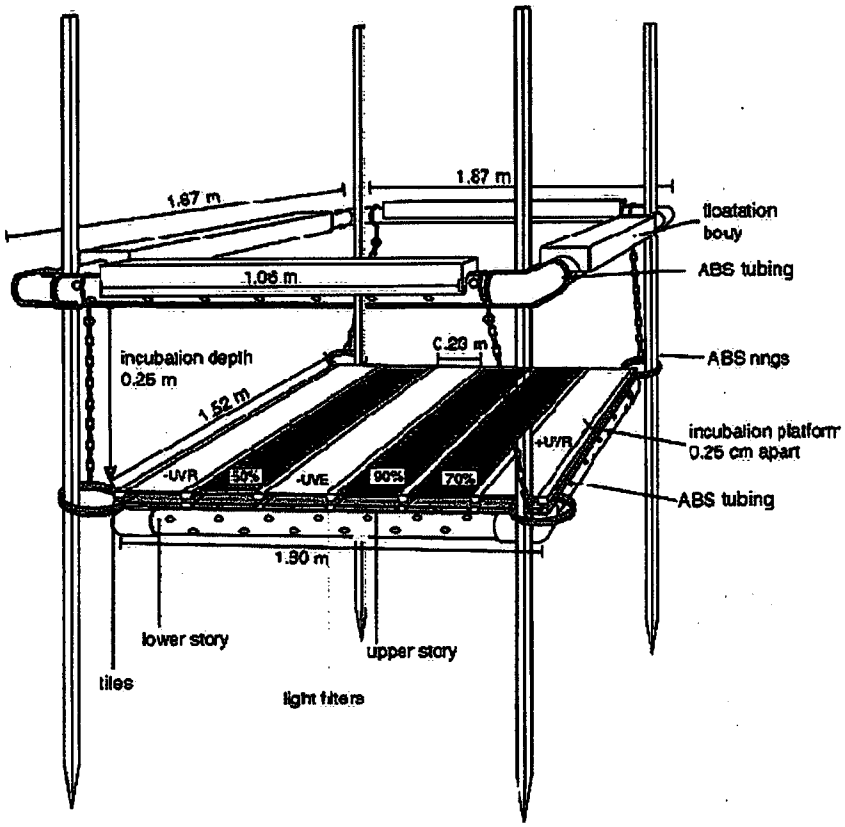

1.87 m
1.87 m
floatation bouy
ABS tubing
1.06 m
incubation depth 0.25 m
0.23 m
ABS rings
1.52 m
incubation platform 0.25 cm apart
+UVR
-UVR
50%
-UVB
90%
70%
ABS tubing
1.90 m
lower story
upper story
tiles
light filters

Figure 3: Ordination biplot illustrating the association between biomass, nutrient content, stoichiometric variables and fatty acids for the two sites (north and south) and for the grazer treatments (PRES and ABS). The fatty acids are mainly separated on the basis of site. The south biofilms were characterized by a greater biomass (chl *a*, AFDW), nutrients (C, N, and P) and more SAFA, while the north contained more PUFA, notably the EFA, EPA and DHA.

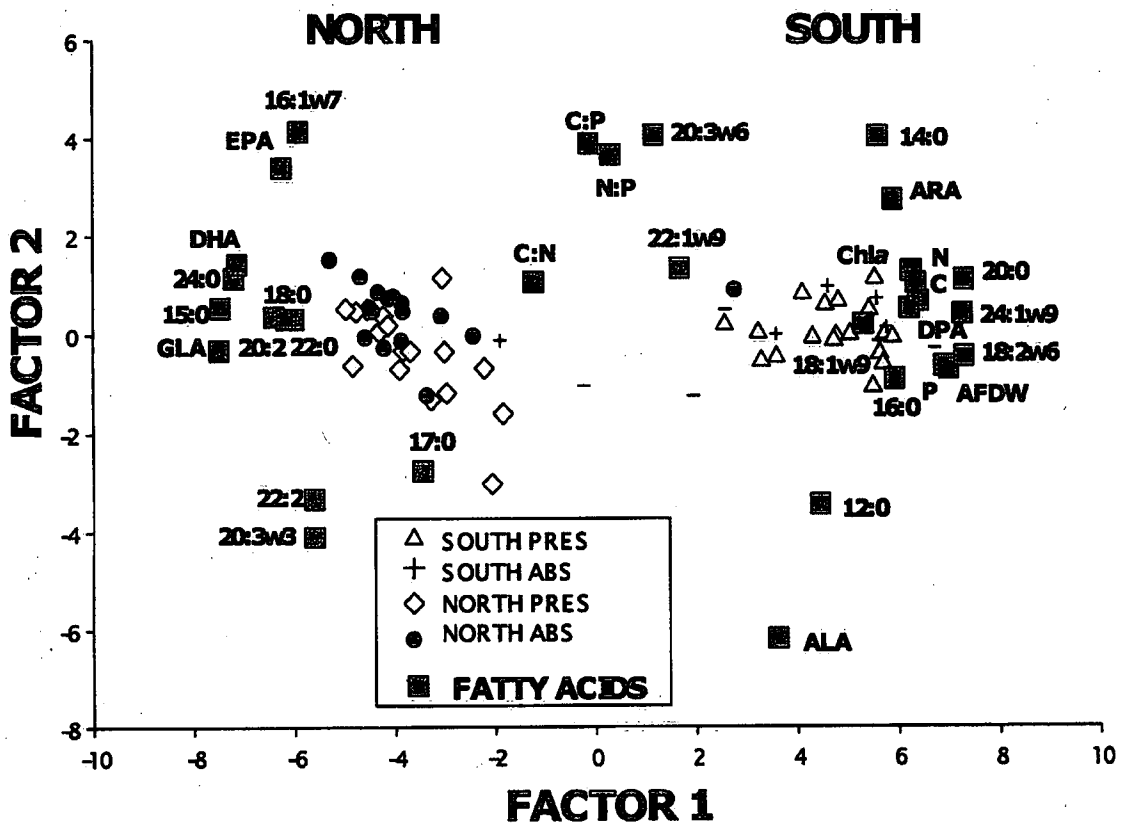


Figure 4: Results of the substrate testing experiment comparing the community structure present on plants, rocks and tiles. Relative biomass (%) of cyanophytes, chlorophytes, and diatoms are shown for each substrate in the north and south.

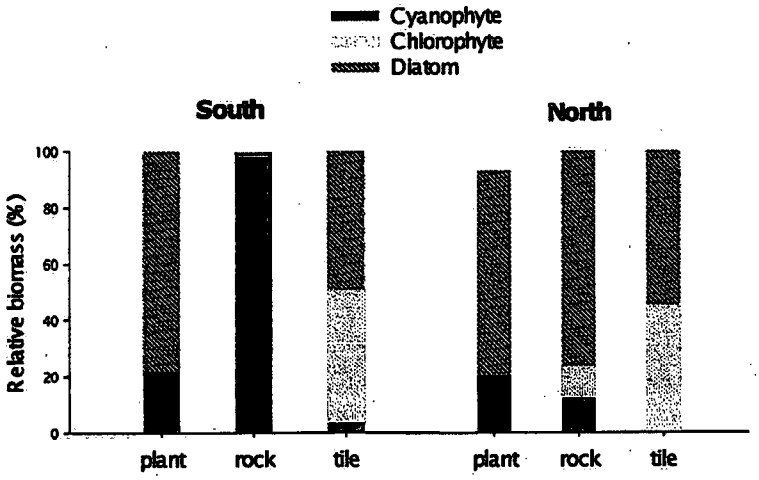


Figure 5: 1% UVB, UVA, and PAR penetration depths and CDOM concentrations (Absorption at 340nm) (on the right y-axis). CDOM concentrations were missing for

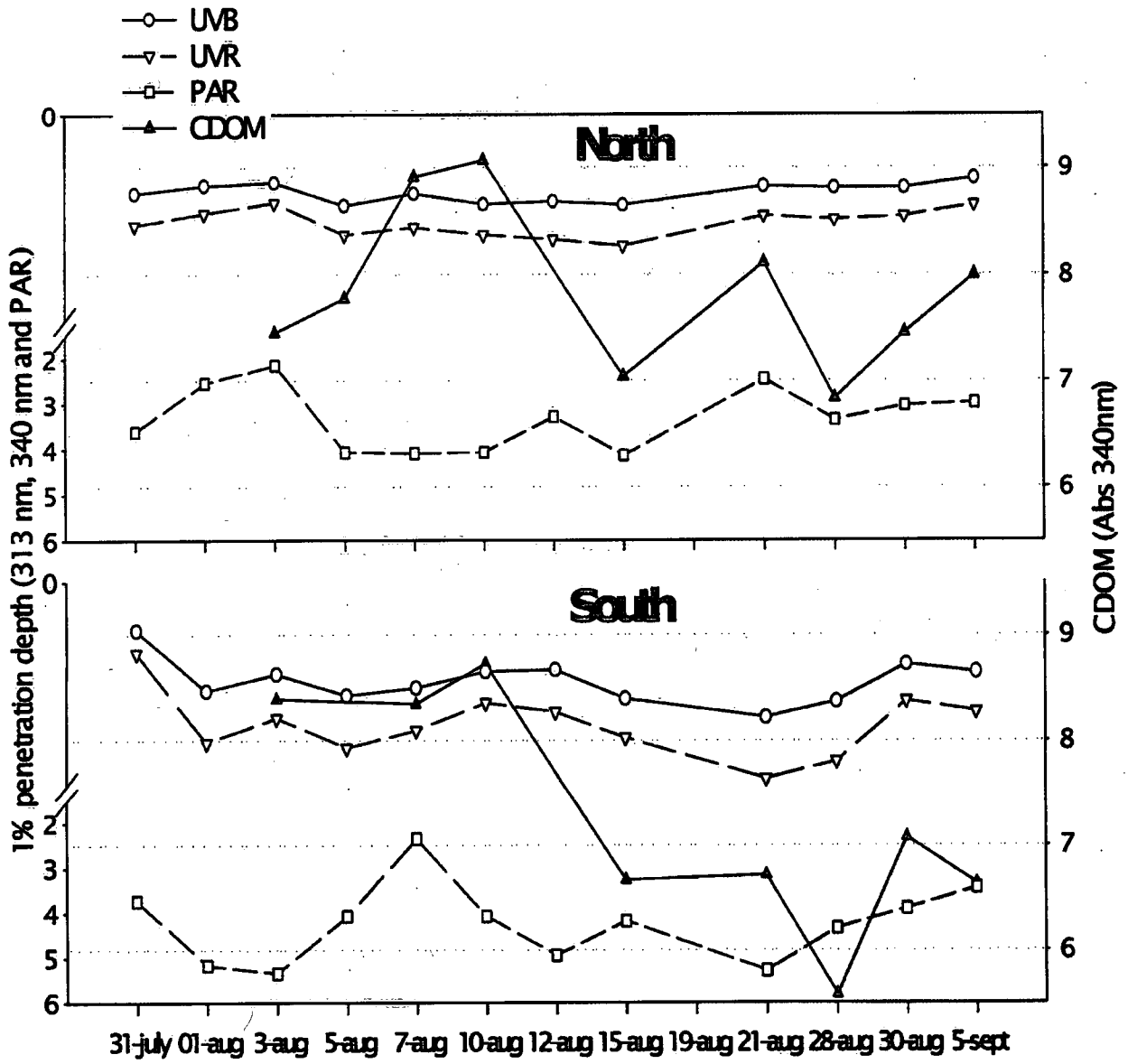
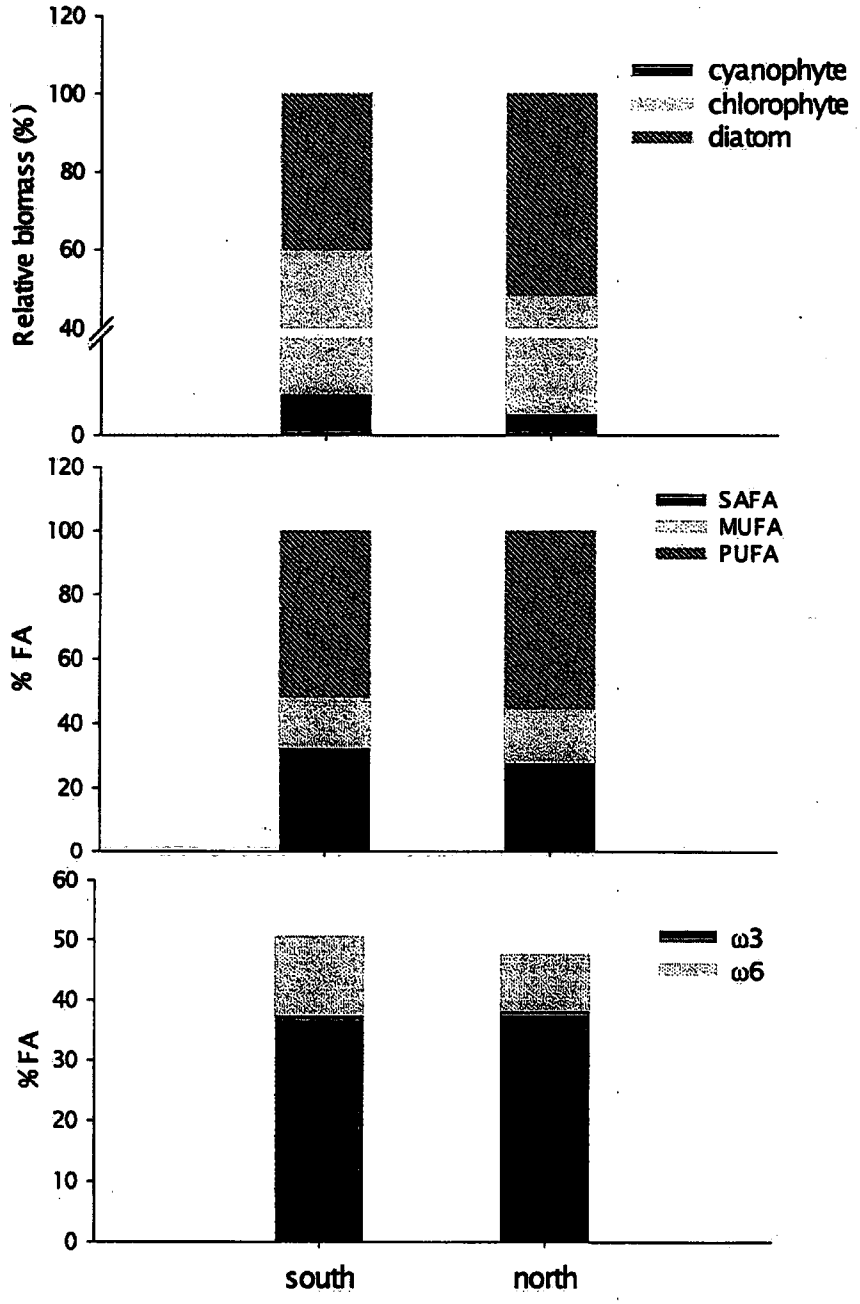


Figure 6: Relative biomass (%) of cyanophytes, chlorophytes, and diatoms and %FA



content of SAFA, MUFA, PUFA, ω3 and ω6 at the two study sites (south and north).

Figure 7: % FA (14:0, 20:3•3, •3, and total (left y-axis); 17:0 and 12:0 (right y-axis)) that differed significantly with respect to the grazing treatment (PRES vs. ABS).

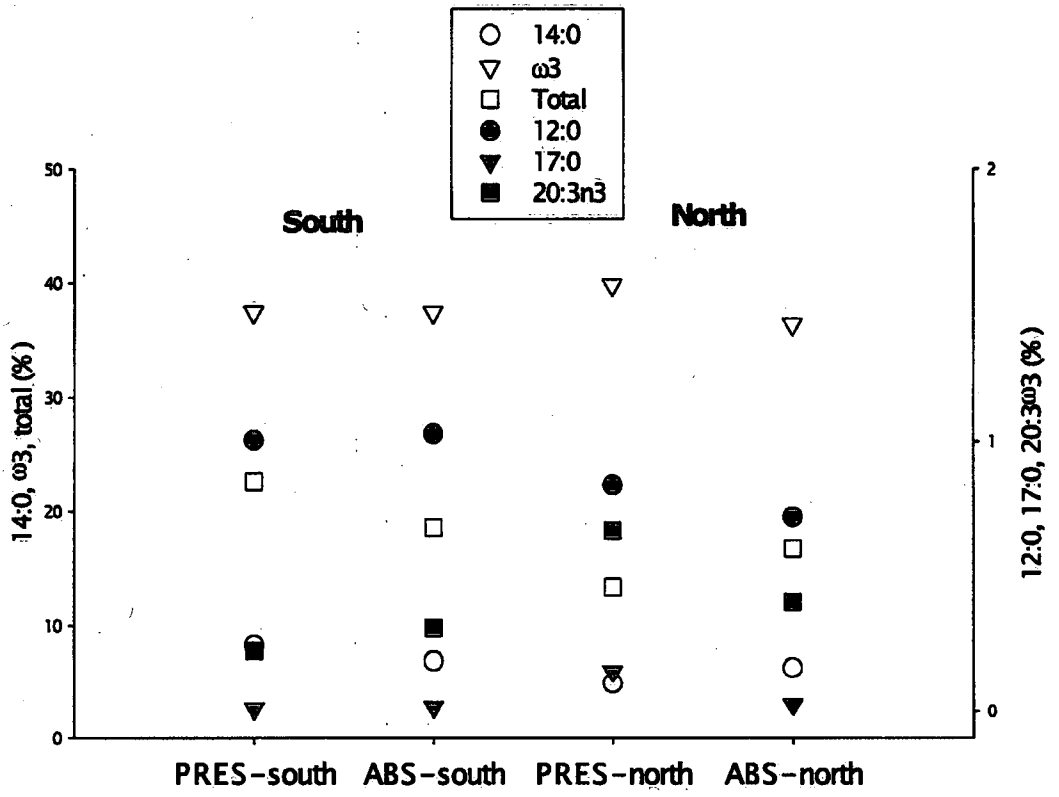


Figure 8: Significant triple interaction (site X light X grazing) for PUFAs (p = 0.038) and PP (p = 0.027).

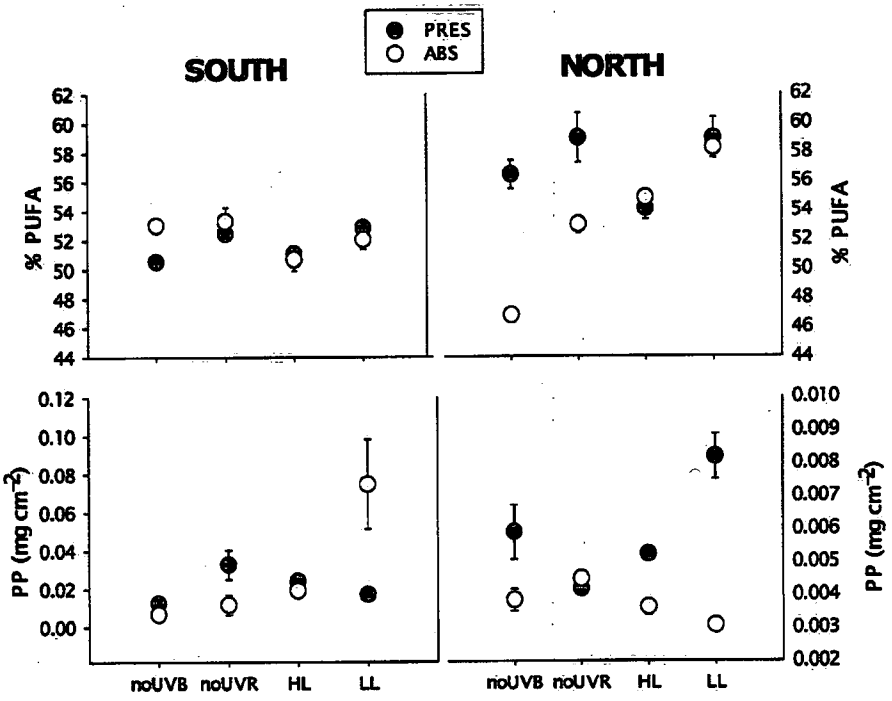


Table 1: Average UVB ($W \cdot m^{-2}$), UVA ($W \cdot m^{-2}$), and PAR ($\mu mol \cdot m^{-2} sec^{-1}$) intensities received by the biofilms at the incubation depth during the experiment.

		north	south
	UVB	0.02	0.06
	UVA	0.13	0.41
HL treatment ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	**100% PAR**	600.53	795.60
	90% PAR	540.48	716.04
LL treatments ($\mu mol \cdot m^{-2} \cdot sec^{-1}$)	**70% PAR**	420.37	556.92
	50% PAR	300.27	397.80

Table 2: Average (+/- s.d.) of relative fatty acids analyzed for the main treatments. The variables that are significantly different across main treatments are in bold. The

	SITE		GRAZING		LIGHT			
	south	north	PRESENCE	ABSENCE	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)	0.71 (0.70)	0.81 (0.68)	0.56 (0.50)
N	**0.14 (0.06)**	**0.04 (0.01)**	**0.12 (0.08)**	**0.07 (0.05)**	0.098 (0.08)	0.07 (0.05)	0.04 (0.01)	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)	0.008 (0.006)	0.006 (0.004)	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)	223.42 (49.87)	211.21 (26.07)	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)	10.57 (0.72)	9.89 (0.56)	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)	21.46 (3.8)	21.91 (3.28)	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)	2.80 (2.27)	1.49 (0.25)	1.62 (0.71)
chla	**27.87 (10.27)**	**7.87 (2.29)**	19.77 (11.99)	17.05 (14.03)	18.83 (14.87)	15.76 (13.49)	8.17 (2.12)	9.42 (3.39)
12:0	**1.00 (0.12)**	**0.77 (0.16)**	0.90 (0.16)	0.89 (0.23)	0.90 (0.21)	0.87 (0.21)	**0.95 (0.16)**	**0.84 (0.14)**
14:0	**7.50 (0.75)**	**5.63 (1.06)**	6.49 (1.81)	6.60 (0.70)	6.58 (1.71)	6.74 (0.97)	6.52 (0.78)	6.17 (0.92)
15:0	**0.03 (0.03)**	**0.21 (0.06)**	0.14 (0.11)	0.10 (0.10)	0.14 (0.12)	0.12 (0.11)	0.08 (0.07)	0.15 (0.09)
16:0	**22.59 (0.69)**	**18.33 (0.81)**	20.52 (2.48)	20.45 (2.49)	20.28 (2.24)	20.50 (2.15)	20.89 (2.33)	19.87 (1.94)
16:1ω7	**6.83 (0.85)**	**11.74 (3.19)**	8.26 (2.30)	10.27 (4.45)	**9.06 (4.04)**	10.18 (4.17)	8.60 (2.30)	**10.26 (2.55)**
17:0	**0.01 (0.01)**	**0.09 (0.08)**	0.08 (0.09)	0.02 (0.04)	0.07 (0.07)	0.03 (0.06)	0.03 (0.03)	0.07 (0.08)
18:0	**0.55 (0.17)**	**1.48 (0.48)**	1.14 (0.76)	0.90 (0.42)	1.07 (0.72)	0.96 (0.55)	0.83 (0.32)	1.23 (0.59)
18:1ω9	**9.26 (0.62)**	**5.24 (0.81)**	7.24 (2.44)	7.30 (2.24)	6.94 (2.42)	7.32 (2.16)	7.58 (1.82)	6.70 (1.83)
LA	**10.64 (0.89)**	**5.91 (0.62)**	8.59 (2.75)	8.10 (2.64)	8.37 (2.75)	8.25 (2.67)	8.43 (2.18)	7.47 (2.17)
20:0	**0.00**	**0.11 (0.08)**	0.06 (0.08)	0.06 (0.09)	0.07 (0.09)	0.06 (0.09)	0.02 (0.04)	0.08 (0.07)
GLA	**0.41 (0.22)**	**2.06 (0.25)**	1.14 (0.99)	1.30 (0.88)	1.22 (1.00)	1.26 (0.90)	1.13 (0.76)	1.51 (0.74)
ALA	**24.07 (2.00)**	**18.93 (5.00)**	22.96 (2.72)	20.27 (6.05)	22.25 (5.26)	19.99 (5.44)	22.57 (3.23)	20.35 (3.02)
20:2	**1.09 (0.45)**	**6.77 (0.86)**	3.57 (3.07)	4.14 (3.33)	3.88 (3.22)	4.07 (3.04)	3.578 (2.88)	4.77 (2.57)
22:0	**0.07 (0.08)**	**0.30 (0.07)**	0.18 (0.17)	0.20 (0.12)	0.17 (0.17)	0.20 (0.14)	0.18 (0.07)	0.244 (0.12)
20:3ω6	0.17 (0.02)	0.16 (0.05)	0.16 (0.04)	0.16 (0.03)	0.16 (0.05)	0.70 (0.03)	**0.15 (0.03)**	**0.16 (0.01)**
22:1ω9	0.13 (0.04)	0.08 (0.05)	0.08 (0.04)	0.14 (0.04)	0.08 (0.05)	0.12 (0.05)	0.14 (0.04)	0.12 (0.03)
20:3ω8	**0.27 (0.05)**	**0.53 (0.20)**	0.46 (0.26)	0.35 (0.10)	0.43 (0.25)	0.35 (0.15)	0.38 (0.11)	0.44 (0.16)
ARA	**2.09 (0.26)**	**1.33 (0.14)**	1.69 (0.40)	1.75 (0.54)	1.72 (0.53)	1.76 (0.51)	1.74 (0.36)	1.64 (0.36)
22:2	**0.34 (0.05)**	**0.92 (0.17)**	**0.69 (0.36)**	**0.54 (0.30)**	0.65 (0.36)	0.57 (0.30)	0.56 (0.30)	0.69 (0.29)
24:0	**0.12 (0.09)**	**0.72 (0.12)**	0.40 (0.35)	0.43 (0.34)	0.39 (0.38)	0.41 (0.32)	0.38 (0.28)	0.52 (0.29)
EPA	**10.26 (1.60)**	**16.95 (1.76)**	12.95 (3.39)	13.95 (4.58)	13.28 (3.32)	13.94 (4.06)	13.17 (3.88)	14.72 (3.25)
24:1ω9	**0.08 (0.04)**	**0.01 (0.02)**	0.05 (0.05)	0.03 (0.04)	0.04 (0.05)	0.04 (0.04)	0.05 (0.03)	0.03 (0.03)
DPA	**2.45 (0.33)**	**0.71 (0.27)**	**1.66 (0.99)**	**1.55 (1.03)**	1.60 (1.03)	1.56 (0.90)	1.70 (0.92)	1.33 (0.80)
DHA	**0.04 (0.04)**	**0.71 (0.14)**	0.37 (0.38)	0.37 (0.40)	0.39 (0.38)	0.39 (0.37)	0.28 (0.32)	0.49 (0.33)
ω8	37.10 (1.39)	37.84 (4.02)	38.40 (2.55)	36.49 (3.50)	**34.84 (4.30)***	38.87 (3.47)	**36.95 (3.26)***	**39.74 (3.90)****
ω6	**13.30 (0.93)**	**9.45 (0.48)**	11.58 (2.19)	11.30 (2.30)	11.47 (2.27)	11.45 (2.29)	11.46 (1.79)	10.77 (1.78)
ΣSAFA	**31.87 (1.10)**	**27.73 (1.50)**	29.96 (2.83)	29.69 (2.46)	29.70 (2.55)	29.91 (2.03)	29.91 (2.42)	29.25 (1.71)
ΣMUFA	16.31 (1.16)	17.30 (3.05)	**15.79 (1.50)**	**17.83 (2.78)**	**16.35 (2.95)**	17.77 (2.62)	16.41 (0.90)	**17.19 (1.23)**
ΣPUFA	**51.82 (1.36)**	**54.97 (4.13)**	54.25 (3.58)	52.47 (3.42)	53.95 (4.17)	52.31 (2.91)	53.68 (2.29)	53.56 (1.69)
ARA:DHA	16.66 (8.28)	1.81 (0.47)	54.24 (0.13)	53.06 (0.15)	**20.18 (0.46)***	**7.12 (10.56)***	**3.66 (0.53)****	**1.75 (0.45)****
ω8:ω6	**2.89 (0.43)**	**4.04 (0.46)**	3.47(0.02)	3.54 (0.03)	**3.23 (0.08)**	3.53 (0.06)	3.49 (0.03)	**3.65 (0.04)**
Total	19.46 (3.04)	15.15 (2.33)	17.09 (4.57)	17.57 (2.23)	17.33 (4.22)	17.74 (2.68)	16.76 (1.84)	16.28 (2.36)

significant interaction effects are not represented here.

Table 3: Significant triple interaction for P and PUFA; averages (s.d.) are written. The averages (s.d.) are in grey to illustrate the first set of differences and the second set of differences are in bold.

SITE	GRAZER	LIGHT	PP			Σ PUFA		
SOUTH	PRES	no-UVB	0.01253 (0.0013)			50.54 (0.49)	*	
		no-UVR	0.0328 (0.0077)			52.46 (0.47)		
		LL	**0.01670 (0.0014)**		**	52.86 (0.23)		
		HL	0.0240 (0.0025)			51.07 (0.32)	*	
	ABS	no-UVB	0.0068 (0.0025)			53.04 (n.a.)		
		no-UVR	**0.0117 (0.0051)**		**	53.32 (0.94)		
		LL	**0.0748 (0.0234)**	**	**	52.05 (0.65)		
		HL	**0.01901 (0.0024)**		**	50.70 (0.78)	*	
NORTH	PRES	no-UVB	0.0060 (0.0008)	*		**56.48 (0.97)**		*
		no-UVR	**0.0042 (0.0001)**	*	*	**58.98 (1.69)**		*
		LL	0.0082 (0.0007)	*		**58.94 (1.40)**	**	*
		HL	**0.0052 (0.0002)**	*	*	54.11 (0.73)		
	ABS	no-UVB	**0.0038 (0.0003)**	*	*	**46.84 (0.30)**		**
		no-UVR	**0.0045 (0.0002)**	*	*	53.04 (0.57)		
		LL	**0.0031 (0.0002)**	*	*	**58.29 (0.66)**		*
		HL	**0.0036 (0.0003)**	*	*	**54.86 (0.44)**		*

NWRI Research Summary

Plain language title

Effect of light climate on nutritional quality of aquatic biofilms

What is the problem and what do scientists already know about it?

Large fluvial lakes are highly dynamic environments. We do not yet understand how the light climate in such systems affects food quantity and quality at the base of the food chain (algae). Scientists are becoming increasingly aware that both visible light and UV radiation exert strong effects on the biochemical composition of both attached (biofilm) and free-floating (sestonic) algae.

Why did NWRI do this study?

Lake Saint-Pierre has recently been classified by UNESCO as an "ecological reserve of the biosphere" and is now officially recognized as a world heritage site. This recognition arose, in part, because of its high biodiversity; the lake supports 83 fish species and harbors 288 bird species. In Lake Saint-Pierre, the areas adjacent to the south shore are known to be the most productive. For example, there are higher densities of zebra mussel larvae (Dreissena polymorpha) in the south compared with the north water mass. Perch (Perca flavescens) and American eel (Anguilla rostrata) growth rates are highest in the south water mass are densities perch and other fish species. Finally, most of the commercial fisheries are preferentially located along the south shore. This study was conducted, in part, to determine the underlyng reasons for the high biodiversity in this lake and also to provide an explaination for the higher productivity in the southern areas of the lake.

What were the results?

We designed, tested and proved a new incubation device that permits biofilms to be grown in highly hydrodynamic environments such as the shallow waters of rivers and fluvial lakes. The device maintains the artificial substrates at a fixed depth with respect to the surface despite rapid, and often unpredictable, changes in water level. It ably resists waves and strong currents. This design permits the operator the option of using optical and neutral density cut-off filters for precise control of the light climate impinging on the artificial substrates.
Distinct algal communities and fatty acid groups characterized each site; the biofilms that grew in the south contained a greater relative abundance of green and blue-green algae, along with greater amounts of low nutritional quality saturated fatty acids (SAFA) at the expense of greater quality poly-unsaturated fatty acids (PUFA). Conversely, the northern biofilms had a greater relative

abundance of diatoms, along with, greater PUFA, including two fatty acids implicated in the physiological competency of grazers, i.e., eicosapentaneoic acid (EPA) and docosahexaneoic acid (DHA). However, the low quality food effect in the south was compensated for by a much higher biomass of biofilms. This explains why the southern areas of the lake have higher overall productivity.

How will these results be used?

The results will be published in an International, peer-reviewed journal. The incubation device should prove broadly useful to regulators/researchers interested in the effects of toxicants, nutrients, and/or light climate on the growth of aquatic biofilms in harsh environments. These results will be used by the Lac Saint-Pierre Fishermen's Association to help establish harvests limits for commercially important fish species in the lake. They will also be used by GRIL (compendium of Quebec universities conducting research on aquatic systems) to plan future studies on Lake Saint-Pierre.

Who were our main partners in the study?

University of Quebec, NSERC, Lac Saint-Pierre Fishermen's Association

Publishing Information

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