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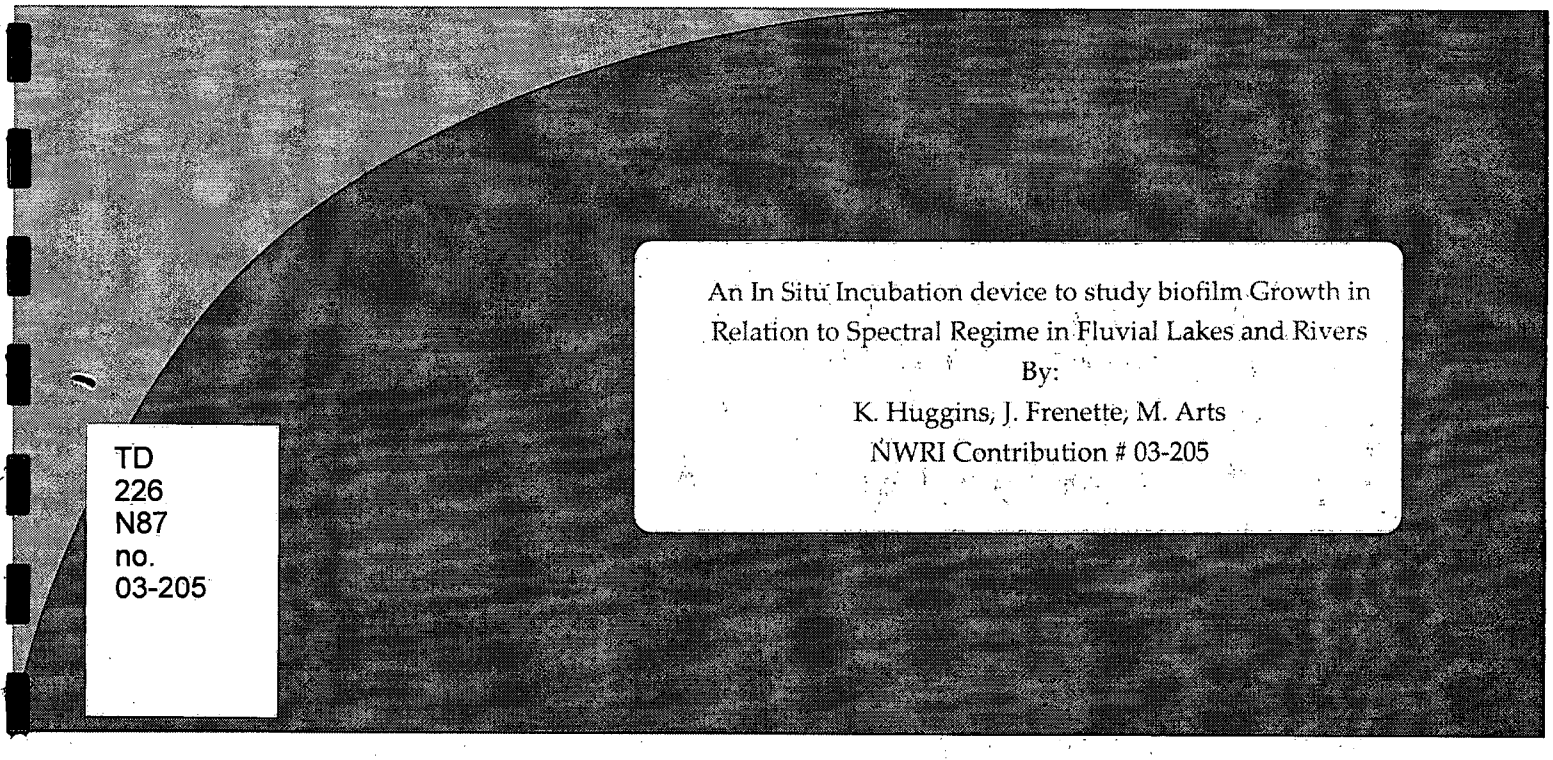

An In Situ Incubation device to study biofilm Growth in Relation to Spectral Regime in Fluvial Lakes and Rivers

By:
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An *In Situ* Incubation Device to Study Biofilm Growth in Relation to Spectral Regime in Fluvial Lakes and Rivers

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Running head: *In situ* light effects on biofilms

Abstract

Biofilms are increasingly being used to monitor and study the effects of light, nutrients and/or toxicants on aquatic systems. However, methodological differences amongst these studies render it difficult to make comparisons or establish rigorous protocol standards. We designed a specialized incubation device for growing biofilms on artificial substrates in highly hydrodynamic fluvial and riverine systems. The new incubation device was tested in shallow, fluvial, Lake Saint-Pierre (lat: 46°12'; long: 72°50'), where harsh hydrodynamic conditions exist. The device allows researchers to, a) grow biofilms at fixed incubation depths with respect to the surface, b) sample the biofilms and monitor light conditions throughout the experiment, c) design specific experiments to compare the developing biofilm communities within and amongst sites, under different light regimes and, d) perform reciprocal transfers of substrates amongst sites and light regimes. Within the sites, different light regimes were constructed using wavelength-selective filters such that the developing biofilms were exposed to, a) three different ultraviolet radiation (UVR) regimes: with UVR, no UVR (neither ultraviolet A (UV-A) nor ultraviolet B radiation (UV-B)), and no UV-B and, b) three different intensities of photosynthetically active radiation (PAR: 400-700 nm): 90%, 70%, and 50% transmission of ambient solar radiation. The success of this new incubation device, in the relatively harsh environment of Lake Saint-Pierre, suggests that it can readily be used in many other types of aquatic environments where aquatic biofilms grow.

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Abstract

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Incubateur *in situ* permettant l'étude de la croissance des biofilms dans des lacs fluviaux et des rivières par rapport au régime spectral

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

Résumé

Les biofilms sont de plus en plus utilisés pour l'observation et l'étude des effets de la lumière, des éléments nutritifs et/ou des substances toxiques sur les systèmes aquatiques. Toutefois, parmi ces études, des différences méthodologiques rendent difficiles les comparaisons ou l'établissement de normes de protocole rigoureuses. Nous avons conçu un incubateur destiné à la culture de biofilms sur des substrats artificiels dans des systèmes fluviaux fortement hydrodynamiques. Le nouvel incubateur a été mis à l'épreuve dans des eaux fluviales peu profondes à fort hydrodynamisme, soit au lac Saint-Pierre (46°12' de latitude N.; 72°50' de longitude O.). L'incubateur permet aux chercheurs : a) de faire la culture de biofilms à des profondeurs fixes par rapport à la surface; b) de prélever des échantillons de biofilms et d'observer les conditions lumineuses durant l'expérience; c) de concevoir des expériences précises visant à comparer les communautés du biofilm en croissance à l'intérieur d'un même site et d'un site à l'autre, selon diverses conditions d'éclairement; d) de réaliser des transferts réciproques de substrats d'un site à l'autre et d'un régime lumineux à l'autre. À l'intérieur d'un même site, nous avons aménagé diverses conditions lumineuses à l'aide de filtres photosélectifs, de manière à ce que les biofilms en croissance soient exposés : a) à trois conditions de rayonnement ultraviolet différentes : avec rayonnement, sans rayonnement (ni rayonnement ultraviolet A [UVA], ni rayonnement ultraviolet B [UVB]) et sans UVB; b) à trois intensités différentes de rayonnement photosynthétiquement utilisable (400-700 nm) : 90 %, 70 % et 50 % du rayonnement solaire du milieu ambiant. Le succès de ce nouvel incubateur, dans le milieu relativement hostile qu'est le lac Saint-Pierre, nous permet de croire que le dispositif peut être utilisé sans délai dans d'autres milieux aquatiques où les biofilms se développent.

NWRI RESEARCH SUMMARY

Plain language title

Description and testing of a new incubation device to grow aquatic biofilms

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

Growing aquatic biofilms in hydro-dynamically-harsh conditions is difficult. There are no satisfactory devices at present to deal with such conditions.

Why did NWRI do this study?

Biofilms are increasingly being used as tools for monitoring the health of aquatic ecosystems but there are logistic problems associated with growing them in hydro-dynamically harsh conditions (e.g. strong currents, waves, lack of control over light climate, poor statistical design considerations).

What were the results?

The incubation device design went through several prototypes until a final design was selected. The final design was tested in Lake Saint-Pierre, a large fluvial lake in the Saint Lawrence River archipelago. The device enabled NWRI researchers and researchers at the University of Quebec to successfully grow aquatic biofilms over an extended period (8 weeks) and to test the effects of light climate on community (algal) composition of the biofilms.

How will these results be used?

The device should prove useful to other researchers interested in monitoring the community structure and physiological competency of aquatic biofilms re: effects of contaminants, nutrients and/or light climate.

Who were our main partners in the study?

University of Quebec in Trois Rivieres.

Sommaire des recherches de l'INRE

Titre en langage clair

Description et mise à l'essai d'un nouvel incubateur pour la culture de biofilms aquatiques

Quel est le problème et que savent les chercheurs à ce sujet?

Il est difficile de faire croître des biofilms aquatiques dans de mauvaises conditions hydrodynamiques. Actuellement, on ne dispose pas de dispositifs appropriés.

Pourquoi l'INRE a-t-il effectué cette étude?

Les biofilms sont de plus en plus utilisés pour surveiller la santé des écosystèmes aquatiques, mais leur croissance pose des problèmes logistiques lorsque les conditions hydrodynamiques sont défavorables (p. ex. forts courants, vagues, éclairement non contrôlé, conception statistique déficiente).

Quels sont les résultats?

Plusieurs prototypes d'incubateurs ont été mis à l'essai. Le prototype retenu a été mis à l'essai dans le lac Saint-Pierre, vaste lac fluvial de l'archipel du Saint-Laurent. Grâce à l'incubateur, les chercheurs de l'INRE de l'Université du Québec ont été en mesure de faire croître des biofilms aquatiques pendant huit semaines et d'étudier les effets de l'éclairement sur la composition des communautés (algales) dans les biofilms.

Comment ces résultats seront-ils utilisés?

L'incubateur devrait se révéler utile aux chercheurs qui s'intéressent à la structure des communautés et à la compétence physiologique des biofilms aquatiques à contrer les effets des contaminants, des nutriments et de l'éclairement.

Quels étaient nos principaux partenaires dans cette étude?

Université du Québec à Trois-Rivières.

Introduction:

A major portion of aquatic biofilms biomass consists of photosynthetic organisms that depend on ambient light conditions for growth. However, increased exposure to photosynthetically active radiation (PAR) is often associated with increased levels of UVR, which may have varied physiological and morphological effects on biofilms and, directly or indirectly, the organisms that depend on them. Enhanced UVR as a result of ozone depletion is of mounting concern, especially in shallow, optically-clear aquatic systems where changes in water levels (e.g. as a function of climate change) could have important impacts on primary productivity. T his topic h as been the subject of many field- and laboratory-based studies, especially over the last two decades (e.g. Bothwell et al., 1993; Francoeur and Lowe, 1998).

Exposure to UVR (280-400 nm) radiation can lead to a series of disturbances in phytoplankton communities, such as, reductions in cellular division rates (Goes et al., 1994), DNA damage (Vincent and Neale, 2000; Bothwell et al., 1994), and inhibition of photosynthesis (Vincent and Neale, 2000; Helbling et al., 1992). However, most studies dealing with the effects of UVR and/or high light exposure on primary productivity have been conducted using mono- or batch-cultured algal species in the laboratory, (e.g. DeNicola and McIntire, 1990; Vadeboncoeur and Lodge, 2000). This makes it difficult to extrapolate to natural systems where species diversity is so much higher and where algal cells are continuously transported through the water column by the action of waves, horizontal advection, micro-scale turbulence, and other mixing processes. In addition, lab-based experiments cannot easily replicate natural diurnal and seasonal fluctuations in underwater light climate (UVR and PAR). Thus, experiments on light quality and/or

quantity effects have proven to be quite difficult to conduct *in situ*; especially in lotic systems. In contrast to phytoplankton, algae contained in biofilms colonize and grow on various substrates at fixed positions with respect to the bottom, and are less capable of large scale movements, relying instead on active or passive displacement within the microbial mat to decrease their exposure to UVR and/or high light irradiances. Unlike phytoplankton, biofilms algae are unable to find refuge in deeper water (Hill et al., 1997) or behind physical shelters (Vinebrooke and Leavitt, 1999).

There are substantial differences in methodology and protocol (e.g. incubation depths, substrate type, incubation devices) amongst experiments conducted on biofilms. Where experiments have been conducted in littoral zones of lakes or ponds (Chételat et al. 1999; Hill et al., 1997, Fairchild, 1985; Vinebrooke & Leavitt, 1999), substrates are most commonly placed directly on the sediments or within incubation chambers located on or near the sediment surface. However, complexity in the interpretation of results increases markedly when the study sites consist of fluvial and/or shallow water systems. In such systems hydrodynamic conditions usually cause difficulties, not only with the installation/deployment of incubation apparatus, but also because, a) artificial substrates used to grow biofilms may be displaced by currents and become lost and/or, b) substrates may be heavily impacted by deposition of sediments. For such reasons, studies dealing with light effects on fast-flowing fluvial aquatic biofilms have mostly been conducted in laboratory incubation chambers (Sommer, 1996) or artificial streams (Bothwell et al., 1993; Stelzer & Lamberti, 2001; Hill et al., 1992), which could introduce their own biases (e.g. nutrient concentrations, increased competition between the algae, divergence from the natural successional patterns and species present). Such biases are even more

pronounced for incubation periods >1 month; corresponding to the minimal incubation time required for colonization and community development of biofilms in most systems (Aloi, 1990). Therefore, *in situ* experiments within such extreme environments are few (Vinebrooke & Leavitt, 1999). For example, Francoeur and Lowe (1998) designed an incubation device for epilithon growth that was anchored to the lake bottom in 1 m water column depth and noted that their substrates temporarily emerged above the water surface as a result of strong winds and waves.

The Saint Lawrence River ecosystem harbors a vast community of biofilms which serve as a crucial food source for consumers at higher trophic levels. Other than the studies conducted by De Sève and Goldstein (1980), who collected biofilms on rocks located at depths of 10-30 cm and Vis et al. (1998a, 1998b) who sampled biofilms growing on navigational buoys, we are not aware of any other studies that have sampled biofilms in the St. Lawrence River or its fluvial lakes. Their methods suffer from some drawbacks in that they do not allow researchers to select and/or alter the locations of their sampling sites and do not permit manipulation of the incubation depth, in order to monitor the underwater light field reaching the biofilms. Furthermore, they involve the use of very different substrate materials, which could influence the colonization and growth of algae. Ceramic tiles are commonly used in research to grow aquatic biofilms because of their, a) uniform and homogeneous surface characteristics, b) low costs including reduced sampling costs (Aloi, 1990; Lamberti & Resh, 1983), c) ease of removal which facilitates sampling and, d) suitability for experiments planned with robust statistical design elements e.g. randomized block and/or reciprocal transfer protocols. Such tiles are typically made of unglazed ceramic clay (DeNicola and

McIntire, 1991; Lamberti and Resh, 1983; Hill et al., 1997). Although we adopted tiles as our substrate material to grow biofilms within our incubation rafts we also examined how biofilm algal community structure differed between tiles and macrophytes (collected directly from the lake) and between tiles and rocks (both placed on simple platforms mounted on wooden stakes).

Materials and Methods

Incubation rafts

The rafts consisted of 2 main decks (Fig.1): a lower story supporting the substrates and an upper story on which light filters were fixed. The frame was made of non-corrosive, slightly flexible, aluminum (6 mm thick; Metal Lamine D.R. Inc., Trois Rivieres, Quebec). The platform (1.52 m by 1.80 m) was strengthened with a square frame built with 7.6 cm inner-diameter acrylnitrile butadene styrene tubing (hereafter ABS tubing) fastened underneath. Perforations in these ABS tubing support structures helped to prevent the platforms from floating.

The distance between the incubation platforms and the floats were set so as to maintain a pre-determined incubation depth with respect to the surface throughout the experimental period. Previously measured UVR and PAR penetration in Lake Saint-Pierre allowed us to determine a suitable incubation depth for the purposes of this experiment (Frenette et al. 2003). We decided to deploy the incubation devices at 0.25 m because significant intensities of UVB and UVA were still present at this depth. However, the incubation depth can easily be modified according to the objectives of other study designs. The incubation platforms were free to move vertically with respect to the

surface owing to their attachment mechanisms. These consisted of sturdy ABS rings (2.5 cm thick and 10.2 cm inner diameter) slipped loosely over, and positioned around, the wooden (spruce) stakes at each of the four corners. The 4 m long (5 X 10 cm) pointed stakes were driven into the sediments in order to anchor the rafts in position.

This freedom to move in the vertical dimension, thereby maintaining a fixed distance between the tiles and the surface, allowed us to maintain a light environment whose characteristics were only indirectly affected by changes in water level. Of course, seasonal variation in the chemical composition of the overlying waters would still have an impact on the attenuation characteristics of the overlying waters. Such effects would be generally more pronounced in highly dynamic fluvial environments such as Lake Saint-Pierre and less pronounced in other systems where such incubation rafts could be deployed (e.g. low DOC lakes on the Canadian Shield). This play in the movement of the rafts had, in addition to maintaining a more constant light climate, the desirable effect of permitting the flotation device to move slightly with the waves rather than to resist them. In addition, the incubation rafts were fixed at a 45° angle with respect to the current direction in order to minimize the effects of water turbulence and drag (Fig. 3).

Artificial substrata

The artificial substrata consisted of sediment-colored, unglazed ceramic tiles (Céramique Des Rochers, Trois-Rivières, Canada) that were glued on polypropylene sheets (1.22 m X 2.4 m X 4 mm; Laird Plastics,Montreal, Canada) using aquarium-grade silicone adhesive. Two sizes (232.3 cm^2 & 25.8 cm^2) of tiles were used depending on the biomass available and the quantity of material needed to perform the different analyses.

One propylene sheet contained three 232.3 cm^2 and nine 25.8 cm^2 tiles. Three of these sheets were aligned (side-by-side) underneath one light filter.

Substrate testing

The community composition of periphyton growing on the tiles was compared to natural substrates by allowing these communities to develop naturally on tiles versus rocks and macrophytes. For this particular experiment, three series of 0.3 m^2 platforms were fixed at different heights on wooden stakes were used to support rocks and tiles. Three stakes were deployed at both the north and the south incubation sites. The stakes were removed after 28 d (July 30 to Aug. 26). The substrates (rocks and tiles) were cut loose from the platforms and immediately placed in individual plastic bags filled with lakewater which were then placed 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 future identification. Water was added to the plastic bags containing the macrophytes 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 macrophytes were placed in pre-weighed aluminum trays and oven-dried at 60°C for 24 h. Identification of algae was done to the level of class and, when possible, to the level of genus. 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 plankton counting package (program version 4.1; developed by Hamilton and Gosselain 2001, see: http://ibelgique.ifrance.com/algamica) for appropriate biovolume calculations of periphytic cells according to their shape (Hillebrand and Kahlert 2001).

Light quantity and quality

Light filters were randomly placed into slots in the upper story. The upper story was fixed at a height of 0.25 m above the lower one containing the substrata. The short distance between these two stories reduced stray light contamination and shading without drastically modifying water flow. The filter types consisted of acrylic sheeting and/or polyester film (Mylar-D®) with different UVR cutoffs (Fig. 2). Acrylite® OP-3 (CYRO Industries, Manchester, U.S.A.) transmits 90% of PAR with 0% transmission <390 nm. Acrylite® OP4 (CYRO Industries, Manchester, U.S.A.) also transmits 90% of PAR and most of the UV-A and UV-B (75% transmission at 275 nm). UV-B was removed by layering a film of Mylar-D® (50% transmission at 318 nm) onto a sheet Acrylite® OP4. In addition, neutral density filters, made of opaque window screen material varying in pore size, were used to reduce ambient light by 50%, 70%, or 90%. For maintenance purposes (for cleaning or replacement as appropriate), the six filters (0.23 m X 1.52 m) were mounted such that they could be easily removed by slipping them in and out of their compartments, like drawers (Fig. 3) at roughly 2 d intervals.

Flotation device

Each platform was chained to a flotation system designed to limit shading (Fig. 2). This system was easy to manipulate and could support a heavy (~110 kg) load. Preliminary tests with other materials (wooden planks fastened to flotation buoys, polyvinyl chloride tubing (PVC-tubing), and ABS tubing confirmed that ABS tubing with Styrofoam flotation buoys (0.25 m X 0.51m X 2.44 m; The Dow Chemical Company, Michigan, U.S.A.) were the most advantageous materials, with respect to performance, cost, and weight (buoyancy). A square frame (1.87 m X 1.87 m) was constructed using 7.6 cm inner-diameter ABS tubing and 1.06 m long flotation buoys were fitted along each side. Once again, perforations were made along the length of the ABS tubing to assure that only the buoys provided flotation.

Periphyton sampling

The incubation rafts were removed from the lake after 47 to 49 d. The stakes were pulled from the sediments and the incubation sections with floats were hauled into the boats for processing. The polypropylene sheets supporting a series of tiles located in the middle position were cut free from the rafts and put into plastic bags in a cooler filled with lake water. In the laboratory, biofilms growing on the tile surfaces were then gently scraped off using razor blades and spatulas and set aside for later analyses (e.g. community structure, chl *a*, lipids, nitrogen, phosphorus, etc...).

Assessment

Study area

Lake Saint-Pierre is a large (480 km^2) fluvial lake with a complex hydrodynamic regime, where downstream currents can be strong reaching mean flow rates of 0.5-1 $m \cdot s^{-1}$ (Basu et al., 2000). It features three main water masses (north, central, and south) composed of inflows from the, a) Ottawa, Du Loup, and Maskinongé rivers, b) St. Lawrence River (maritime channel), and, c) Richelieu, Saint-François and Yamaska rivers, respectively (Frenette et al., 2003).

Six incubation devices were deployed in the north (lat: 46°12', long: 72°55') and south (lat: 46°8', long: 72°51') water masses of this lake for a period of 47 d to 49 d (from July 24 & 25, 2001 until Sept. 9, 10, & 11, 2001). The two water masses differed in their physico-chemical characteristics. These site-specific characteristics (discussed in detail in Frenette et al. 2003) affected attenuation rates and, hence, ratios amongst PAR, UV-A and UV-B radiation. We measured irradiance at ~0.05 m intervals at peak wavelengths of 313, 320, 340, 443, and 550 nm and also PAR (400-700 nm) using a spectroradiometer (Model PUV-2545, Biospherical Instruments, San Diego, USA). We found that biofilms in the north received ca. 3X less UVR (UVA and UVB) and 1.3X less PAR at the incubation depth (0.25 m) compared to biofilms in the south (Table 1). However, ratios of PAR:UVB and PAR:UVA were ~2.3X greater in the north.

The tiles were abundantly colonized by biofilms by the end of the experiment. Ceramic tiles are widely used as substrates for studying biofilm growth and community structure. In our study sites the most widely available natural substrate consisted of submerged macrophytes and, to a lesser extent, rocks. We tested the suitability of tiles as

substrates by comparing the community structure of the algae growing on tiles, macrophytes (*Vallisneria* sp.), and rocks (Fig. 4). A one-way ANOVA, with substrate as the independent variable, revealed a greater abundance of diatoms growing on macrophytes compared to tiles, which were greater than rock substrates. The abundance of chlorophytes was equivalent on plants and tiles, which were both greater than rocks.

The main differences in community structure of the biofilms growing on the tiles fixed to our incubation platforms were between the north and south water masses, with little to no detectable effects caused by the varying light treatments within the respective water masses. In the north, the lower UVB and UVA irradiances (and lower PAR:UVR ratio) probably contributed to the greater proportion of more UV-sensitive diatoms in the biofilms (Fig. 5). Conversely, the greater UVB and UVA irradiances coupled to the lower PAR:UVR in the south, favored a community structure dominated by chlorophytes and cyanobacteria which are more highly adapted to high light and UVR. The chlorophytes in the north biofilms consisted mainly of *Cladophora* sp., *Oedogonium* sp. and *Stigeoclonium* sp., while *Cladophora* sp. and *Cloeochaete* sp. dominated in the south. Absolute diatom biomass did not differ significantly between the two sites, however, diatom species richness and relative abundance was 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.

Discussion

Biofilms colonize virtually every type of substrate in aquatic systems (Hillebrand & Kalhert, 2001). They are increasingly used in an array of studies (Morin & Cattaneo, 1992; Aloi, 1990), dealing with nutrient status (Hillebrand & Kahlert, 2001), trophic interactions (Napolitano et al., 1996), and water quality monitoring (Vis et al., 1998a; Reavie & Smol, 1998). These organisms represent an important food source for herbivores. Therefore, variations in the growth rate or nutritional status of biofilms could have serious implications for aquatic ecosystems (Napolitano, 1994). This need for understanding where and how quickly essential nutrients and energy flows through aquatic systems has precipitated an ever-increasing number of studies on various aspects of grazer activity (Lamberti, 1993).

Difficulties exist for those wishing to study biofilms *in situ* and, even more so in harsh hydrodynamic environments such as Lake Saint-Pierre; a large, shallow fluvial lake exposed to high winds and waves and divided down its long axis by a fast-flowing river (Frenette et al. 2003). In part, because of this, very little is known about biofilms which play a crucial role as food for higher trophic levels in such productive and diverse environments.

The incubation device we designed is built with sturdy non-corrosive materials and stays anchored in the sediments despite being exposed to strong currents and waves. The design was such that all artificial substrates could be incubated at the same depth independent of changes in water level. The modular placement of tiles and flexibility in the choice of optical filters allows for rigorous statistical testing (e.g. randomized block designs) of various hypotheses of interest to different researchers. Taken together, these

key design features will allow researchers to test for differences in the growth, physiological condition (e.g. pigments, lipids, nutrients, amino acids) and, as our results demonstrate, community structure of biofilms amongst locations differing in their inherent underwater optical properties while simultaneously providing the opportunity to survey and regularly monitor underwater light exposures within each site.

The underwater light climate has important consequences for the food quality of primary producers through its varied effects on metabolic processes (e.g. Wang and Chai 1994; Arts and Rai, 1997; Frenette et al. 1998; Vincent and Neale 2000). However, many studies on the effects of UVR on primary production are performed under laboratory conditions (e.g. De Lange et al., 1999; Cullen & Lesser, 1991), not necessarily reflecting the natural growth conditions of algae nor the ambient UVR:PAR ratios. While such experiments have allowed us to study, in detail, the impacts of high intensities of UVR, our proposed protocol allows for *in situ* observation of the effects of ambient UVR and PAR intensities while also allowing experimental modifications of these wavelengths (e.g. neutral density screens, wavelength-specific cut-off filters) to test specific hypotheses of interest. Our incubation device provides an efficient way to obtain integrated effects of light climate on the life histories, community structure and physiological condition of aquatic biofilms. Such measurements provide crucial input for the formulation of bio-optical models designed to accurately describe and predict the effects of light climate and water level fluctuations on aquatic biofilms.

Light regime is known to be one of the major factors regulating biofilm growth Our device will therefore be helpful for performing future studies on biofilms because it provides researchers the opportunity to manipulate the light regime reaching the biofilms

in a controlled way. The modular design of our experimental platform, and the ease with which the tiles can be removed, could be useful for experiments that incorporate a reciprocal transfer design element(s). In addition to being useful in various aquatic systems (e.g. lakes, ponds, shallow marine environments) it is especially suited for use in hydrodynamically-harsh environments such as fluvial lakes and rivers.

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Figure 1: Side view of the incubation platform within the water. Square perforated ABS tubing maintained the rigidity of the incubation platform and supported the flotation buoys. The incubation raft maintained a constant distance between the tiles and the water surface throughout the incubation period.

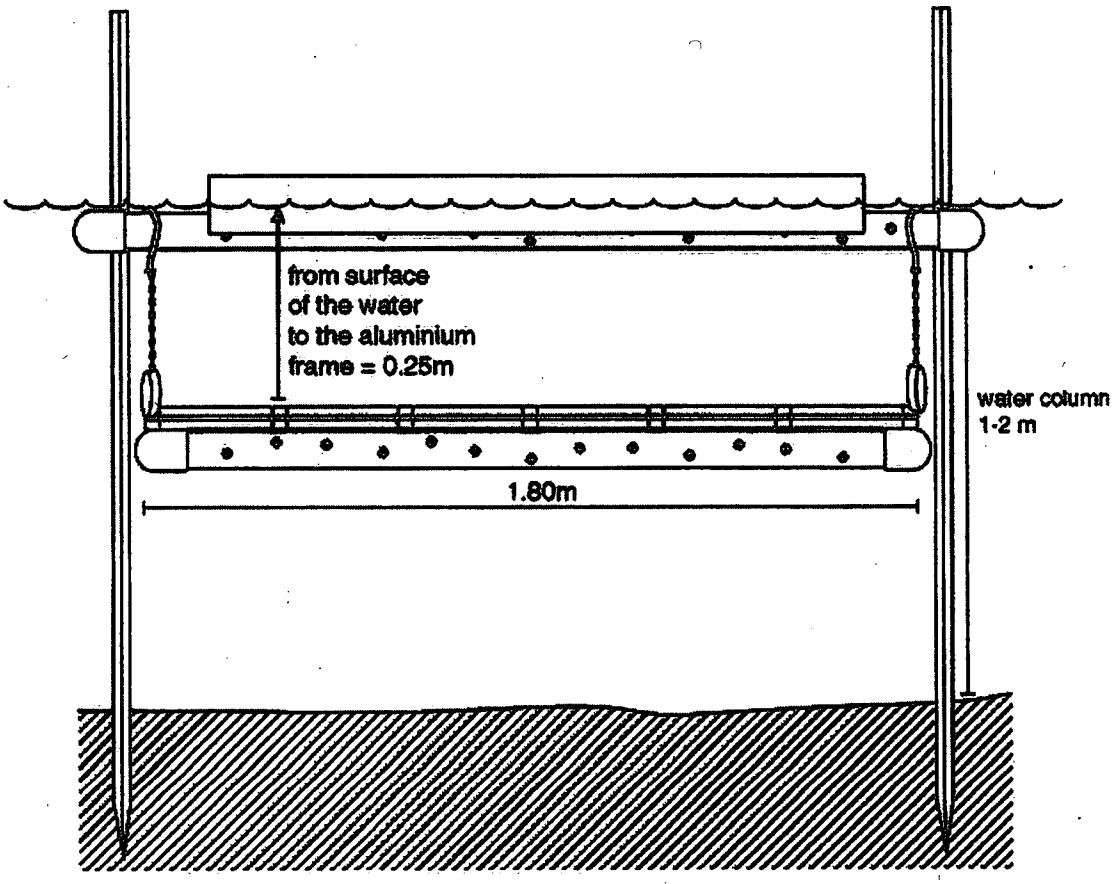


Figure 2: Diagram of one incubation raft with the two main decks: a lower story supporting the substrates and an upper story with the light filters. The platform was attached to a square float and was anchored to the bottom sediments with wooden stakes. The rings at the corners allowed for it to move vertically with the water movements.

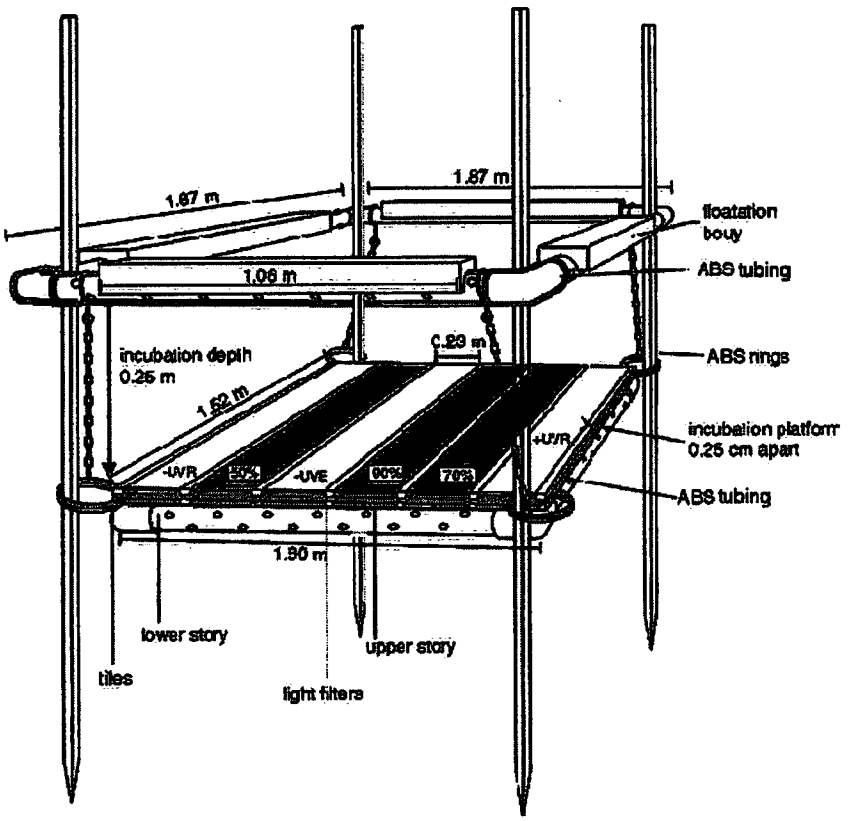


Figure 3: Set-up of the light filters within the aluminum frame. Filters were easily removable for maintenance.

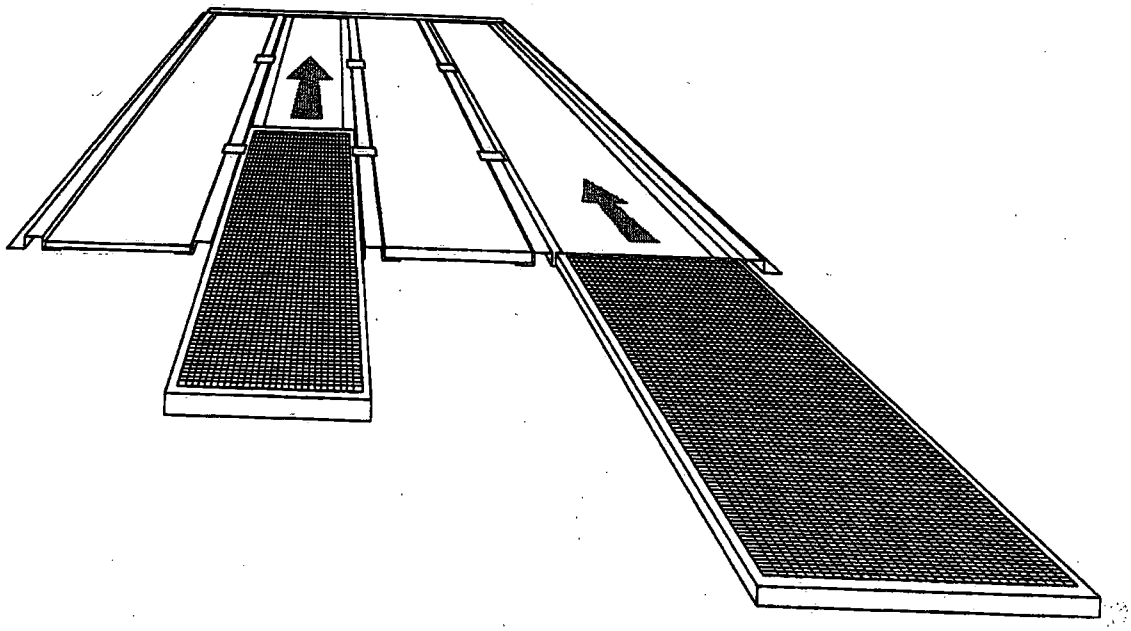


Figure 4: Differences in community structure amongst the three substrates (macrophytes, rocks, and tiles) examined in this study.

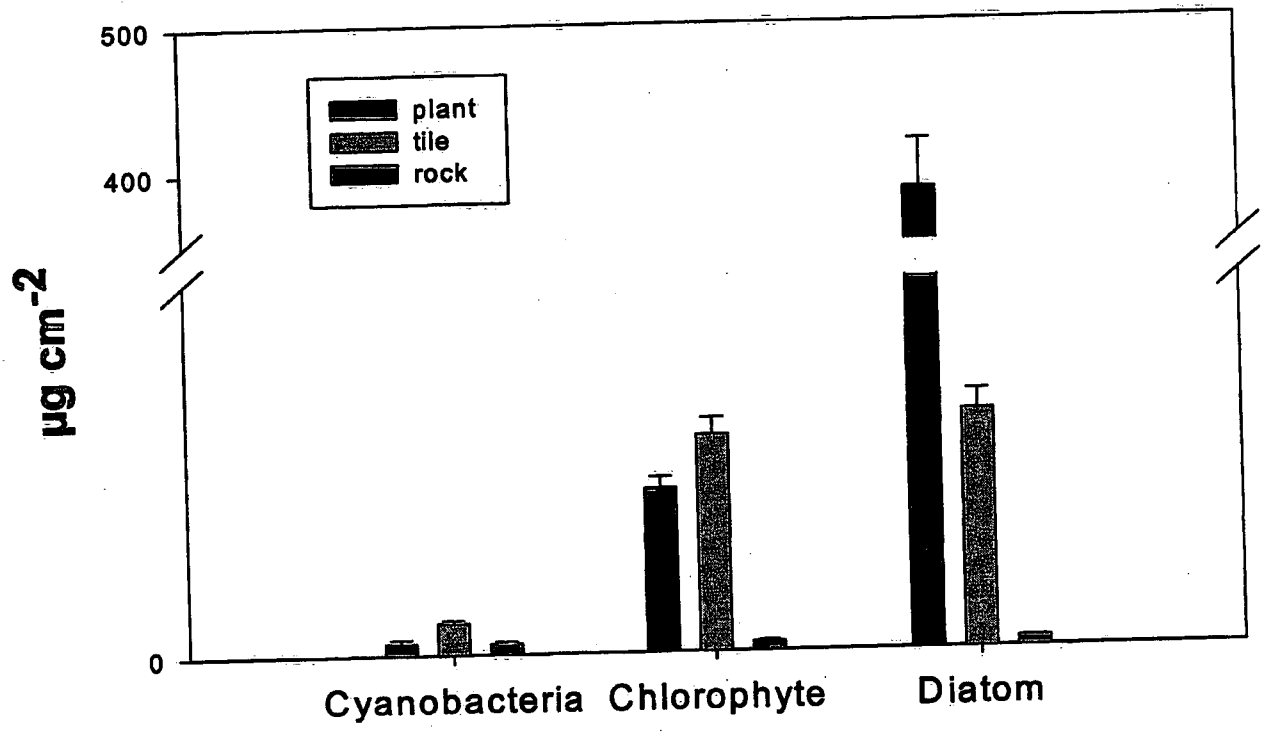
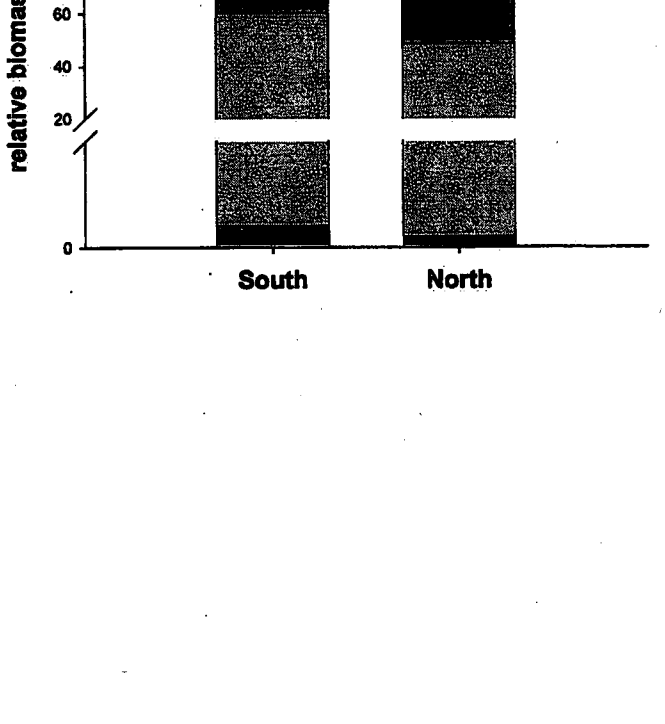


Figure 5: Differences in community structure (% of total biomass) of the main algal taxa found growing within aquatic biofilms on ceramic tiles harvested after 45-49 days from incubation rafts deployed in the north and south study sites.



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