UNIVERSITÉ DU QUÉBEC

MÉMOIRE PRÉSENTÉ À
L'UNIVERSITÉ DU QUÉBEC À TROIS-RIVIÈRES

COMME EXIGENCE PARTIELLE
DE LA MAÎTRISE EN SCIENCES DE L'ENVIRONNEMENT

PAR
KIM HUGGINS

LA QUALITÉ NUTRITIVE DES FILMS BIOLOGIQUES DANS LE LAC SAINT-PIERRE, QUÉBEC (CANADA), EN FONCTION DE LA QUANTITÉ ET DE LA QUALITÉ DE LUMIÈRE

JUILLET 2003

AVANT-PROPOS

Le présent mémoire a été rédigé conformément à l'article D45-46-47 du règlement des études de cycles supérieurs. Selon ces normes, le mémoire du programme de 2^{e} cycle en sciences de l'environnement peut-être présenté sous forme d'article scientifique plutôt que sous forme de mémoire traditionnel.

Avec l'accord de mon directeur de recherche, le professeur Jean-Jacques Frenette, les deux articles issus de mon projet de maîtrise ont été (ou seront bientôt) soumis à deux périodiques scientifiques différents. Le présent mémoire contient 1) un résumé substantiel rédigé en français (Chapitre 1), 2) le premier article soumis à « Aquatic Ecology » et rédigé en anglais (Chapitre 2), et 3) le deuxième article soumis au Journal Canadien des Sciences Halieutiques et Aquatiques, aussi rédigé en anglais (Chapitre 3). Les directives aux auteurs pour les deux périodiques sont présentées en annexe (A et B). Le résumé substantiel présente la situation globale du projet et comprend l'introduction, la problématique et les objectifs qui avaient été présentés dans le cadre du séminaire I (ECL-6005), ainsi que les résultats obtenus et les conclusions de la recherche.

REMERCIEMENTS

Comme tout projet de grande envergure, cette maîtrise aurait été inconcevable sans l'aide précieuse d'un grand nombre d'intervenants. Je profite donc de cette section pour mentionner toute l'estime que j'ai pour ces personnes qui ont rendu cette expérience si agréable.

Premièrement, je suis redevable à mon directeur de recherche, le Professeur Jean-Jacques Frenette, qui n'a cessé de m'encourager durant ce long processus. Il m'a démontré à quel point il est agréable de travailler avec une équipe dynamique. Il est un excellent mentor rempli de connaissances et d'enthousiasme contagieux et je le remercie énormément pour cette belle aventure. Deuxièmement, je tiens à souligner mon appréciation envers mon co-directeur, Dr Michael Arts, qui m'a accueilli dans son laboratoire pendant deux mois et qui m'a encadré et encouragé pendant ma maîtrise. Ce fut un privilège de bénéficier de ses brillantes connaissances et de sa grande expertise. Je tiens aussi à remercier les Professeurs Stéphane Campeau et Warwick Vincent qui ont accepté de participer à la correction de mon mémoire. Je suis très chanceuse d'avoir pu profiter de leurs critiques et de leurs judicieux commentaires.

En plus de m'enrichir sur le plan professionnel, cette maîtrise m'a également dotée de nouvelles amitiés qui m'ont comblé de bonheur tout au long de ce processus. Premièrement, la joie de se retrouver parmi un groupe d'étudiant(e)s partageant les mêmes passions et désirant s'entraider m'a enchanté du début à la fin. C'est donc dans cette perspective que je remercie tous les gens du laboratoire (Geneviève Trudel, Sylvain Thélème, Marie-Audrey Livernoche, Carl Martin et Didier M'Radamy) pour leur aide sur le terrain, leur encouragement et leurs conseils qui m'ont aidé à tous les points de vue. Je me dois de souligner la force et la persévérance de Pascal Lavallée; sans qui, tout ce projet aurait été irréalisable. J'ai aussi été fortuné de travailler en parallèle avec une amie très chère (Christine Barnard), dont la persévérance et le perfectionnisme sont de grandes sources d'inspiration. Je remercie aussi tous les professeurs et étudiants du GRÉA (Groupe de Recherche sur les Écosystèmes Aquatiques) qui m'ont stimulé par leur

passion pour leurs projets respectifs de recherche, démontrant toutes les possibilités excitantes qu'offre la recherche en écologie. Enfin, j'ai côtoyé des personnalités absolument charmantes et agréables, entre autres, Isabelle Villemure et Claudie Latendresse, dont leur amitié demeurera à jamais très précieuse.

Finalement, je suis extrêmement reconnaissante envers une foule de personnes pour leur dévouement, leur patience, leur amitié et leur professionnalisme. Je tiens donc à remercier mon père (Michael Huggins) qui était au poste à tous les matins, Patrick Murphy qui n'a cessé d'être une source d'encouragement et d'énergie, Karen Raphaël, Sandra Raphaël, Line Dejean, Benjamen Clancy, Hugues Boulanger, Jennifer Carley, Jean-Louis Benoît, Jonathan Rouette, Roger Thibeault, Guy Marchand, Mark Milot, René Béland, Roger Gladu et Yves Maillot.

Vous avez tous, à un moment ou un autre de ma maîtrise, participé à des moments d'intense satisfaction, de désespoir, de bonheur et de fous rires, de tristesse, de détente et de stress. Ces souvenirs sont parmi mes plus précieux…merci à tous!

LISTE DES SYMBOLES ET ABBRÉVIATIONS

Acides gras : AG

Acides gras poly-insaturés : PUFA

Acides gras saturés : SAFA

Acides gras mono-insaturés : MUFA

Acide α-linolénique : ALA ou 18 :3ω3

Acide linoléique : LA ou 18 :2ω6

Acide γ-linoléique : GLA ou 18 :3ω6

Acide arachadonique : ARA ou 20 :4ω6

Acide docosahexanaenoique : DHA ou 22 :6ω3

Acide docosapentanaenoique : EPA ou 20 :5ω3

CDOM : carbone organique dissous chromophorique

COD : carbone organique dissous

Nomenclature des acides gras utilisée est n : mωp, où le n = le nombre de carbones dans la molécule, m = le nombre de doubles liaisons, et ω p = la position de la première double liaison par rapport à la terminaison méthylée. Par exemple, l'acide gras 16 :0 (l'acide palmitique) est un acide gras saturé possédant 16 carbones dans la molécule, alors que l'acide gras poly-insaturé, 20 : 5ω3 (l'acide eicosapentanaenoique), contient 20 carbones et cinq doubles liaisons, dont la première se situe à 3 carbones, inclusivement, de la terminaison méthylée.

PAR : lumière disponible pour la photosynthèse (400-700 nm)

PC : carbone particulaire

PN : azote particulaire

PP : phosphore particulaire

TN : azote total

TP : phosphore total

RUV : rayonnement ultra-violet (280-400 nm)

RUV-B : rayonnement ultra-violet B (280-320 nm)

RUV-A : rayonnement ultra-violet A (320-400 nm)

TABLE DES MATIÈRES

LISTE DES FIGURES

LISTE DES TABLEAUX

CHAPITRE I : Document synthèse

Résumé

Ce projet de recherche comportait deux volets principaux: le premier consistait à développer un protocole expérimental *in situ* pour permettre la croissance de films biologiques exposés à divers régimes lumineux. L'expérience ayant lieu dans le fleuve Saint-Laurent était soumise aux contraintes des grandes forces hydrodynamiques engendrées par les débits et courants élevés. Ceci nécessitait donc la conception et la fabrication de montages spécialisés. Ces montages étaient constitués de deux étages: 1) l'étage supérieur supportait une série de filtres de lumière intervenant au niveau du rayonnement ultra-violet (RUV) (avec RUV, sans RUV, sans UV-B) et de la lumière disponible pour la photosynthèse (PAR) (90%, 70% et 50% de lumière transmise), 2) l'étage inférieur contenait plusieurs séries de substrats artificiels, constitués de tuiles en céramique non-glaisées, qui permettaient la colonisation et la croissance des films biologiques. Ces montages ont été installés dans les masses d'eau du nord et du sud du lac Saint-Pierre, dont les caractéristiques optiques diffèrent grandement.

Le deuxième volet de cette recherche impliquait l'analyse de la qualité alimentaire des films biologiques, en terme de stoechiométrie et de lipides, en fonction des divers régimes lumineux. Les variations les plus marquées étaient attribuables aux différences entre les structures de communautés des algues présentes dans les masses d'eau du sud et du nord. La masse d'eau de sud est caractérisée par une eau moins turbide et contenant moins de carbone organique dissous (COD), notamment de carbone organique chromophorique (CDOM) qui est une matière colorée contribuant fortement à l'absorption des RUV. En terme de biomasse absolue, la masse d'eau du sud supportait une plus grande biomasse de tous les taxons (chlorophycées, cyanobactéries et diatomées) et ceci se reflétait par des contenus en azote, phosphore, carbone, poids sec et lipides totaux plus élevés. Il n'y avait cependant aucune différence en terme de stoechiométrie (ratios carbone : azote, carbone : phosphore et azote : phosphore) et en terme de biomasse relative, les diatomées étaient supérieures dans le nord. La proportion relative en acides gras essentiels (acides gras poly-insaturés (PUFA),

notamment l'acide eicosapentaenoique (EPA) et l'acide docosahexaneoique (DHA) indiquait que les acides gras de plus haute qualité nutritive se retrouvaient en proportions plus élevées, par rapport aux autres acides gras, dans le nord que dans le sud. En quantité absolue cependant, les valeurs de PUFA et de SAFA étaient plus élevés dans le Sud et constituaient donc une valeur énergétique plus élevée pour les consommateurs potentiels.

Introduction : Revue de littérature

Lac Saint-Pierre

Le lac Saint-Pierre est le plus grand lac fluvial du Fleuve Saint-Laurent (superficie : 472 km² (Environnement Canada, 2000)). En novembre 2000, l'Organisation des Nations Unies pour l'éducation, la science et la culture (UNESCO) lui a conféré le titre de réserve écologique de la biosphère en raison de sa grande productivité et biodiversité. On peut y retrouver 80 espèces de poissons d'eau douce et 288 espèces d'oiseaux, ce qui correspond à près de 70% des espèces présentes au Québec (Langlois et al. 1992).

Ce lac est caractérisé par diverses masses d'eau possédant des propriétés chimiques, physiques et biologiques distinctes. Ces différences sont occasionnées en partie par l'origine de leurs eaux (principalement, les Grands Lacs dans le centre et La Rivière Outaouais dans le nord) qui sont, de plus, alimentées par différents tributaires à caractères distincts. Ces tributaires irriguent plusieurs types de terres agricoles, fermes et usines caractéristiques de leurs bassins versants (Frenette et al., 2003). Les eaux situées dans le centre du lac circulent dans le chenal maritime qui débute son parcours depuis les Grands Lacs. Cette eau est claire et verte et contient une faible quantité de nutriments (Germain et Janson, 1984). Le dragage que subit ce chenal depuis 1959, lui confère une profondeur de 11 m ainsi qu'une vitesse de courant variant entre 0,6-1 $m \cdot s^{-1}$ (Langlois et al., 1992). Ces caractéristiques agissent comme barrière physique qui ne permettent pas aux eaux adjacentes, soit au nord et au sud, de s'entremêler entre elles. La masse d'eau du nord reçoit son eau principalement de la rivière Outaouais et se différencie, sur l'axe transversal, progressivement de la masse d'eau centrale. L'observation visuelle permet de constater qu'elle est brune et possède une turbidité élevée (Frenette et al., 2003).

Cette masse reçoit les eaux provenant de plusieurs tributaires, les principaux étant la rivière Maskinongé, Du Loup et Yamachiche. En comparaison, la masse d'eau du sud reçoit les eaux de quatre tributaires, soit des rivières Richelieu, Yamaska, Saint-François et en aval, Nicolet. Comme au nord, la transition entre cette masse d'eau et celle du centre s'effectue de manière progressive. Ces eaux brunes sont plus transparentes que celles du nord (Frenette et al., 2003).

Les études menées par Frenette et al. (2003) démontrent l'importance de la portion colorée du carbone organique dissous (COD), le CDOM (chromophoric dissolved organic matter) dans l'atténuation des RUV (rayons ultra-violets) au niveau du Lac Saint-Pierre. Le CDOM est une matière jaune d'origine allochtone composé de matières fulviques et humiques produite lors de la dégradation de matières végétales. Il peut contribuer significativement à la coloration de l'eau et diminuer la quantité de lumière disponible pour la photosynthèse (PAR). Les analyses chimiques de l'eau dans le lac démontrent que la concentration en CDOM est généralement plus élevée dans la masse d'eau du nord que celle du sud (Frenette et al., 2003)

Les lacs fluviaux ont dans l'ensemble des caractéristiques typiques à eux, soit une faible profondeur, une réduction du courant et un temps de résidence plus élevé (Hudon et al. 1996), ce qui en font des zones de rétention importantes. Les organismes présents sont soumis aux propriétés intrinsèques du plan d'eau dans lequel ils ont le temps de s'établir et de se développer. Dans le Lac Saint-Pierre, le temps de résidence dans le chenal est d'environ 0,5 jours, comparativement aux eaux situées de part et d'autre qui varient entre 2.5 jusqu'à plus de 13 jours selon les années (Hudon et al., 1996) où les vitesses de courant sont < 0.3 $m \cdot s^{-1}$ (Environnement Canada 1981). La faible profondeur de ces habitats permet une pénétration de la lumière jusqu'au fond de la colonne d'eau, ce qui favorise le développement extensif de macrophytes aquatiques (Hudon et al., 1996; Basu et al., 2000) et qui sera influencé par des fluctuations en niveaux d'eau. Ces plantes peuvent abriter et retenir de grandes quantités de zooplancton et d'algues épiphytiques à l'intérieur de ces zones.

Films biologiques

Les films biologiques (aussi appelés périphyton) sont constitués d'une matrice de micro-organismes, majoritairement des algues, qui colonisent et croissent sur des substrats submergés. Ils ont été choisis pour étudier les effets de la lumière car ils sont sédentaires, et ne peuvent donc pas se déplacer au travers la colonne d'eau. Comparativement, le phytoplancton est majoritairement soumis aux mouvements de l'eau et peut être exposé à de très hautes doses de rayonnement ultraviolet et de PAR pour des périodes de temps variables. Ainsi, cette étude concernera les films biologiques qui sont soumis à une exposition relativement constante de toutes les composantes lumineuses.

La colonisation de substrats par des espèces d'algues repose sur le taux d'immigration des propagules algales dans le milieu, sur un minimum de stabilité de la colonne d'eau, sur la présence de substrats appropriés et sur les facteurs physico-chimiques environnants (Maltais, 1994). Après l'étape de la colonisation, la croissance des films biologiques est régit principalement par la lumière (Hill et Boston, 1991), les nutriments et le broutage. Puisque la lumière s'avère la source d'énergie essentielle pour accomplir les processus de photosynthèse, elle est un des facteurs principaux régissant la structure de communauté, la productivité et la croissance des films biologiques (Wellnitz et Ward 2000, Hill 1996, Steinman et Mcintire 1987, Hill, Ryon et Schilling 1995). Ainsi, la croissance sera limitée en profondeur lorsque la lumière est atténuée trop fortement dans la colonne d'eau et en surface si les intensités lumineuses et l'exposition aux RUV sont trop élevées et induisent de la photo-inhibition et/ou des dysfonctions cellulaires (Maltais, 1994). De plus, la lumière a une influence directe sur l'assimilation des nutriments et les transformations biochimiques, mais peu d'études démontrent les effets des régimes lumineux *in situ* sur la qualité nutritive des algues en eau douce (Sterner et Hessen, 1994; Urabe, 1995). À notre connaissance, aucune étude n'a encore tenté de caractériser la structure de communauté des films biologiques à l'intérieur de chaque masse d'eau dans le lac Saint-Pierre malgré leurs divergences en qualité spectrale.

Qualité nutritive

La valeur nutritive des algues peut sévèrement affecter la croissance des producteurs secondaires, même à des distances trophiques importantes (Sterner et Hessen, 1994). Comparativement aux carnivores, les herbivores utilisent peu de temps et d'énergie pour la cueillette de nourriture, ce qui augmente la probabilité de déséquilibres alimentaires (Sterner et Hessen, 1994). De plus, il existe des différences intrinsèques entre les divers groupes d'algues en terme de qualité nutritive qui varient inter- et intra-spécifiquement selon les conditions du milieu. Puisque la lumière est la source d'énergie majeure pour les algues, elle influence le taux d'assimilation des nutriments et les processus biochimiques nécessaires à la synthèse de macromolécules.

Les principaux nutriments présents dans les algues sont le carbone (C), l'azote (N) et le phosphore (P). La forme principale de carbone assimilée lors des processus de photosynthèse est le carbone inorganique dissous (DIC). Puisque le DIC est abondant dans les régions pélagiques et bien mélangées des lacs, il est rarement un élément limitant pour la croissance des films biologiques (Wetzel, 2001). L'N est assimilé majoritairement sous forme d'ammonium (NH_4^+) et utilisé pour la synthèse d'acides aminés et de protéines (Wetzel, 2001; Sterner et Hessen, 1994). Lorsque le nitrate (NO_3^-) est la forme d'azote le plus disponible dans le milieu, les algues doivent utilisé de l'énergie sous forme d'ATP pour le transformer en NH_4^+. Le P est assimilé sous forme inorganique dissoute, soit d'orthophosphate (PO_4^{3-}) présent dans le phosphore soluble réactif (SRP). Avec la photosynthèse, il est utilisé pour former l'ATP. Il joue un rôle très important dans la majorité des processus métaboliques et il est aussi une composante majeure des acides nucléiques (ADN, ARN), des phosphoprotéines, des phospholipides et il est associé à certaines enzymes et vitamines (Wetzel, 2001; Sterner et Hessen, 1994). Puisqu'il est généralement l'élément le moins abondant dans les systèmes d'eau douce, il est souvent limitant pour les algues (Sterner et al., 1997).

Comme l'équilibre entre les nutriments permet de détecter lesquels pourraient être potentiellement limitants, les concentrations sont souvent exprimées sous forme de ratios stoechiométriques (C : N : P). Le rapport C:P est devenu un indicateur grandement utilisé pour décrire la qualité nutritive dans les milieux aquatiques où, par exemple, un

ratio de C : P ≥ 300 est indicateur d'une faible valeur nutritive (Urabe et Sterner, 1996; Sterner et al., 1997; Sterner and Schulz, 1998).

Certaines études ont aussi démontré que lorsque les concentrations en phosphore dans l'eau ne sont pas limitantes, le contenu en AG poly-insaturés à longue chaîne (> 20 carbones) (PUFA) pourrait devenir limitant et intervenir sur la qualité nutritive (Müller-Navarra, 1995; Bret et Müller-Navarra, 1997; Weers et Gulati, 1997). Les classes de lipides principales dans les algues sont 1) les lipides neutres, qui sont majoritairement constitués d'acides gras saturés (SAFA) et d'acides gras mono-insaturés (MUFA) et 2) les lipides polaires qui sont constitués principalement d'acides gras poly-insaturés (PUFA). Les lipides neutres agissent spécialement comme réservoirs d'énergie et ils augmentent en présence de stress environnementaux (changement de température, lumière, nutriments). Les lipides polaires sont présents dans les membranes cellulaires et thylakoïdes, assurant la fluidité et jouant un rôle essentiel dans les processus métaboliques cellulaires.

Les PUFA sont essentiels chez les animaux car ils sont impliqués dans le développement et le fonctionnement du cerveau (capacités d'apprentissage et de mémorisation) et du système optique. Ils réduisent le taux de maladies associées aux conditions neurologiques (maladies bipolaires et de dépression) et ils diminuent la fréquence de maladies cardio-vasculaires en favorisant une meilleure circulation sanguine (Wang et Chai, 1994; Arts et al., 2000). Puisqu'il y a peu de synthèse *de nova* de PUFA chez le zooplancton, le contenu en PUFA de leur nourriture joue un rôle critique dans leur survie (Goulden et Pace, 1990; Arts, 1998). Parmi les PUFA qui contribuent à optimiser la croissance et la survie des herbivores dans les systèmes aquatiques, l'acide eicosapentanaeoique (EPA) et l'acide docosahexanaeoique (DHA) sont particulièrement importants (Müller-Navarra et al., 1995)

Les processus bio-synthétiques chez les algues débutent par l'insaturation des SAFA (16 :0 et 18 :0) en MUFA (16 :1ω7 et 18 :1ω9), qui eux sont ensuite transformés en PUFA selon une série de d'insaturations et d'élongations. Les dernières étapes menant à la synthèse de PUFA chez les algues sont beaucoup plus complexes que celles des plantes supérieures (Khozin-Goldberg et al., 2002), et l'influence des facteurs

environnementaux, tels que la quantité et la qualité de la lumière ambiante sur leur synthèse demeure très peu connue (Bigogno et al., 2002).

Les trois groupes d'algues étudiées dans la structure des films biologiques de cette étude sont les cyanpbactéries, les chlorophycées et les diatomées. Le contenu en acides gras des cyanobactéries et des chlorophycées est généralement considéré de faible qualité nutritive en raison de leur faible contenu en PUFA (Ahlgren et al., 1997; Sterner, 1997). Comparativement, les diatomées présentent un intérêt particulier pour la survie et la croissance des consommateurs puisque leur contenu en acides gras essentiels est généralement plus élevé (Gulati et Demott, 1997; Ahlgren et Gunnel, 1992; Arts, 2000), notamment en terme d' EPA (Müller-Navarra et al., 1995).

Lumière

Plusieurs études ont été menées dans le but de comprendre comment les hautes intensités lumineuses (e.g. Napolitano, 1994; Thompson et al., 1990; Wellnitz et Rinne, 1999; Frost et Elser, 2002; Hessen et al., 2002) ou le rayonnement ultra-violet (RUV) (Wang et Chai, 1994; McNamara et Hill, 2000; Frost et Xenopoulos, 2002), influencent la qualité nutritive des communautés algales; par contre, aucune d'entre elles n'a démontré les effets de la lumière en milieu naturel sur la qualité nutritive (P et PUFA) en tenant compte simultanément de l'aspect quantitatif (intensité de la lumière disponible pour la photosynthèse (PAR)) et qualitatif (RUV) de la lumière.

Quantité

La quantité de lumière dans la colonne d'eau fait référence à la densité de flux de photons disponibles pour la photosynthèse. Ceci correspond au nombre de photons (quanta) par unité de temps par unité de surface (la densité de flux de photons de la lumière disponible pour la photosynthèse (PAR) en unités de μmolphotons • m^{-2} • s^{-1}) présents dans le spectre de 400-700nm (Wetzel, 2001). La pénétration de la lumière dans la colonne d'eau est dépendante de la quantité et de la nature de la matière particulaire et dissoute en suspension lesquelles régissent les phénomènes de dispersion et d'absorption de la lumière.

Chez les producteurs primaires, l'intensité de la lumière contrôle les processus de photosynthèse qui génèrent l'énergie sous forme d'ATP nécessaire au bon fonctionnement cellulaire. L'ATP est utilisée pour assimiler les nutriments et elle est nécessaire pour les transformations biochimiques de ces nutriments en macro-molécules (e.g. lipides, hydrates de carbone, protéines) (Urabe et Sterner, 1996; Wetzel, 2001). De façon générale, la photosynthèse augmente linéairement avec l'irradiance jusqu'à une valeur seuil, où le niveau maximal est maintenu. En présence d'irradiance qui ne cesse d'augmenter, la photo-inhibition s'installe dans les cellules, provoquant une chute progressive de la photosynthèse engendrée par le surplus d'électrons reçu par rapport au potentiel d'utilisation.

En présence de fortes intensités lumineuses, il y a accroissement de la biomasse algale qui engendre des augmentations dans le ratio C :P; ces accumulations de carbone dans les cellules s'effectuent au niveau des lipides neutres, soit sous forme de SAFA et de MUFA. Comparativement, des intensités lumineuses trop faibles occasionnent une réduction du ratio C : P, qui se reflète par une biomasse plus faible mais de plus grande qualité nutritive. Par conséquent, en présence d'une quantité de lumière intermédiaire, il devrait y avoir un équilibre entre la biomasse algale et la qualité nutritive de celles-ci (Sterner et Hessen, 1997).

Les intensités lumineuses déterminent aussi en partie la présence d'AG puisque les premières étapes de synthèse nécessitent l'ATP généré par la photosynthèse pour le fonctionnement de l'enzyme CoA carboxylase (Wainman et al, 1998). Certaines études ont démontré qu'en conditions de forte lumière, la synthèse de DHA était favorisée, comparativement à EPA qui était favorisée par des conditions de faible lumière. C'est en instance d'intensités lumineuses intermédiaires (73-77% d'ombrage) que la synthèse de ces deux PUFAs s'est avérée optimale (Napolitano, 1994). Tel la photo-inhibition, il est possible que la synthèse de PUFA soit stimulée en fonction du taux d'irradiance jusqu'à un seuil d'intensité lumineuse (300-800 μmolphotons • m^{-2} • s^{-1}) après laquelle les processus d'insaturation et d'élongation diminuent (Wainman et al., 1998). Au-dessus de cette limite, le carbone aurait tendance à s'accumuler dans les cellules sous forme de lipides neutres.

Qualité

La qualité de la lumière fait référence aux longueurs d'onde qui composent le spectre de lumière. Le spectre solaire est caractérisé par les rayonnements UVC (100-280nm), UVB (280-320nm), UVA (320-400nm), la radiation utilisée pour la photosynthèse (PAR : 400-700nm) et le rayonnement infra-rouge (IR>700nm). Les rayons UV-C sont complètement absorbés par l'ozone et l'oxygène atmosphérique (Madronich, 1995; Diaz et al., 2000) et le rayonnement infra-rouge se transforme majoritairement en énergie thermique à la rencontre de la surface de l'eau (Wetzel, 2001).

Le rayonnement ultra-violet (RUV), incluant les rayons UVB et UVA, provoque une diminution du taux de croissance des algues et une diminution de la production primaire (Goes et al., 1994; Vincent et Neale, 2000). Ils occasionnent une réduction en ATP dans les cellule, ce qui inhibe les mécanismes d'assimilation de N (Dohler, 1992) et de P (Frost et Xenopoulos, 2002). Ces processus sont accompagnés d'accumulations intracellulaires de carbone, majoritairement sous forme de lipides neutres (Hessen et al., 1997).

Plusieurs études démontrent les effets néfastes des RUV sur les taux de synthèse d'acides gras essentiels dans les cellules. Malgré de légères incohérences entre celles-ci, il existe une tendance générale vers une diminution des acides gras essentiels, soit d'EPA et de DHA en fonction du rayonnement UVB (Wang et Chai, 1994). Les effets sur les SAFA et les MUFA semblent cependant négligeables (Goes et al., 1994). Les RUV engendre une péroxidation des acides gras provoquée par l'action conjuguée de hautes concentrations en oxygène avec des RUV plus élevés dans les eaux de surface. Les acides gras sans double liaison ou avec une ou deux doubles liaisons sont plus résistantes à ce phénomène (Hessen et al., 1997). De plus, les UVB modifient la synthèse d'ATP dans les cellules algales, ce qui induit des changements au niveau de l'enzyme CoA carboxylase, qui est nécessaire en grande quantité pour les processus d'élongation et d'insaturation (Thompson et al., 1990).

Problématique, objectifs et hypothèses de travail

Puisque le lac Saint-Pierre est caractérisé par diverses masses d'eau, dont les propriétés physiques, chimiques et biologiques varient grandement, il constitue un site idéal pour étudier les effets de régimes lumineux variables (quantité et qualité) sur la qualité nutritive *in situ*. Des séances d'échantillonnages préalables effectuées sur le lac Saint-Pierre par l'équipe du Dr. Frenette ont démontré que la profondeur de pénétration de PAR et des RUV était supérieure dans la masse d'eau du sud que celle du nord (Frenette et al., 2003). Ceci était attribuable à une décroissance du CDOM dans la masse d'eau du sud, ce qui permet aux RUV de pénétrer plus profondément dans cette masse d'eau. Ainsi, les communautés de film biologique qui s'y retrouvent devraient subir des pressions de croissance plus difficiles qui seraient attribuables à une exposition trop élevée de PAR et de RUV que ceux se retrouvant dans la masse d'eau du nord. Paradoxalement, pendant la période d'échantillonnage et les années précédentes, les activités de pêche commerciales et sportives étaient concentrées majoritairement sur la rive sud. Ceci mène à trois suppositions : 1) il y a une plus grande quantité de nourriture disponible dans la masse d'eau du sud dû à la quantité suffisante de PAR, 2) que les organismes vivant dans la masse d'eau du sud ont développé des adaptations aux RUV et 3) puisque les RUV peuvent photo-dégrader le CDOM en des molécules de plus petites tailles, ils augmentent la bio-disponiblilité du carbone pour les bactéries et rendent le système plus productif à la base.

Pendant l'été 2001, le manque de précipitation conjugué aux températures anormalement élevées tout au long de l'été a provoqué une des plus grandes baisses du niveau de l'eau dans le lac Saint-Pierre depuis cinquante ans. Une continuation de ce phénomène pourrait engendrer des conséquences importantes au niveau de la vie aquatique dans ce lac à cause d'expositions plus élevées de PAR et de RUV non graduelles.

Dans le cadre de ce projet nous nous sommes intéressés plus particulièrement à l'effet engendré par les différences de lumière sur la qualité de la nourriture disponible pour les réseaux trophiques supérieurs. En manipulant le broutage, nous avons aussi vérifier si l'absence ou la présence de broutage affecte la qualité nutritive. À notre connaissance, celle-ci est une des premières études qui examine l'effet de la lumière, en

terme de quantité et de qualité, sur la qualité nutritive des algues (P et PUFA) en milieu naturel.

Méthodologie

Aire d'étude

Six montages expérimentaux (Figure 2) ont été placés dans les masses d'eau du nord (lat: 46°12', long: 72°55') et du sud (lat: 46°8', long: 72°51') du lac Saint-Pierre pour une période de 47 à 49 jours (du 24 et 25 juillet au 9, 10 et 11 septembre, 2001).

Montages expérimentaux

Une description détaillée des montages expérimentaux est fournie dans le premier article (Chapitre 2). Des radeaux en aluminium (1.52 m X 1.80 m) furent spécialement conçus pour la culture de films biologiques en milieu naturel. Ils comportaient deux étages séparés de 0.25 m de manière à minimiser l'influence des traitements de lumière avoisinants. L'étage supérieur était constitué de six filtres (0.3 m X 1.52 m) correspondant aux traitements de lumière et à l'étage inférieur était disposées une série de tuiles en céramiques non-glaisées (Rouge Sima). Parmi les filtres, trois intervenaient au niveau de l'intensité de la lumière et permettaient une transmission de 90%, 70% et 50% de la lumière ambiante. Les trois autres filtres intervenaient au niveau des RUV : 1) traitement avec RUV (+RUV), permettant une transmission de 93% de PAR et la plupart des UV-A et UV-B (50% transmission à 275 nm), 2) traitement sans RUV (-RUV), constitué de Acrylite® OP3 qui transmet 93% de PAR et aucune radiation <390 nm, et 3) traitement sans UVB (-UVB) constitué d'une feuille de Mylar®D, qui permet 50% de transmission à 318 nm ; celle-ci était fixé à une feuille d'Acrylite® OP4.

Ces radeaux étaient fixés à des systèmes de flottaison (1.77 m X 1.77 m) permettant de conserver une distance constante par rapport à la surface de l'eau. Le cadre carré était constitué de tuyaux d'ABS (7.62 cm de diamètre interne) sur lesquels étaient fixés des billots de flottaison longitudinalement sur les quatre côtés du tuyau. Pour assurer une flottaison égale de tous les systèmes d'incubation, les tuyaux d'ABS étaient perforés à plusieurs endroits. Ce système de flottaison permettait un mouvement vertical libre. Quatre perches en bois, à l'intérieur des coins du système de flottaison

étaient enfoncées dans les sédiments pour empêcher la dérive des radeaux. Six montages étaient placés dans la masse d'eau du sud et six dans la masse d'eau du nord. Pour trois des six radeaux dans chaque masse d'eau, il y avait exclusion des gros brouteurs à l'aide de filets maillants (ouvertures de 4 mm^2) entourant les montages.

Ce design expérimental était conçu pour étudier 1) l'effet des sites (nord vs. sud), 2) l'effet de régimes lumineux variables à l'intérieur même d'un site (+RUV, -RUV, -UVB, 90%, 70% et 50%), et finalement 3) de vérifier l'impact du broutage, sur la qualité nutritive des films biologiques (présence, absence).

Les communautés algales sur les tuiles, les macrophytes et les roches, furent analysées dans le but de comparer l'efficacité de nos substrats tuilés à ceux naturellement présents dans le lac. Les roches avaient été ramassées préalablement sur les berges du lac et, avec les tuiles, nettoyées à l'HCl. Trois tuiles et trois roches furent collées sur des plates-formes fixées à une perche en bois à des intervalles de 0.5 mètre à partir des sédiments du fond. Les perches ont été retirées 27 jours après leur installation (30 juillet au 26 août). Les substrats rocheux et les tuiles ont été grattés en laboratoire et les films biologiques placés dans des contenants avec 1% de solution Lugol. Les plantes (*Vallisneria* sp.), arrachées des sédiments avec leur racines intactes, furent placées dans des sacs de plastiques contenant de l'eau distillée. Après 1 minute de brassage, l'eau des sacs a été transférée dans les contenants avec du Lugol. Les plantes ont été placées dans un four à 60°C pendant 24 heures dans le but d'évaluer leur poids sec.

Échantillonnage et analyses

Une description plus détaillée des analyses chimiques, physiques, biologiques et statistiques effectuées est disponible dans le deuxième article scientifique (Chapitre 3).

Caractéristiques de la colonne d'eau

Pendant la période d'incubation, à tous les 2 à 3 jours, des échantillons de 2L d'eau provenant de chaque site (nord et sud) furent analysés pour déterminer le contenu en phosphore total (TP), phosphore réactif soluble (SRP), nitrites-nitrates (NO_3-NO_2), ammoniac (NH_3-N), azote total (TN), silice (SiO_2) et carbone organique dissous (COD). Ces analyses ont été effectuées au Laboratoire National des Essais Expérimentaux

(LNEE, Burlington, Ontario). La concentration de matière organique dissous chromophorique (CDOM) a été déterminée à l'aide d' un spectrophotomètre (Cary 100Bio, Varian Co., Palo Alto, CA, USA). Celle-ci correspondait à l'absorption obtenue à 340 nm lors d'un spectre d'absorption de 290 à 750nm, selon Frenette et al. (2003).

Les valeurs d'irradiance ont été obtenues à l'aide d'un spectroradiomètre (Model PUV-2545, Biospherical Instruments, San Diego, USA) qui a été descendu lentement de la surface jusqu'au fond et qui mesurait les longueurs d'onde spectrales: 313, 320, 340, 443, 550 nm, et PAR (400-700 nm). Ces données nous ont permis de connaître l'irradiance dans la colonne d'eau à toutes les profondeurs (Ed_z) et de calculer les coefficients d'atténuation (Kd) selon Frenette et al., 2003.

Films biologiques

À la fin de la période d'échantillonnage, les radeaux ont été retirés de l'eau et les tuiles détachées et ramenées au laboratoire. Celles-ci étaient grattées à l'aide de lames de rasoir et le matériel périphytique conservé selon l'analyse à effectuer. Pour l'analyse du contenu lipidique, une portion a été placée dans des vials cryogéniques et immédiatement conservés à –80°C. Pour la détermination de la structure de communauté, une autre portion a été placée dans des bouteilles ambrées contenant 1% de solution Lugol. Finalement, la portion résiduelle a été placée dans un cylindre gradué dont le volume a été complété à 750 mL avec de l'eau nanopure. Le tout fut légèrement broyé pour former un homogénat. Celui-ci a été filtré sur des filtres 1) GF/F 25 mm préalablement brûlés pour l'analyse de la chlorophylle *a*, le poids sec sans cendre, le carbone et azote particulaire, et 2) GF/F 45mm préalablement brûlés et lavés à l'HCl afin de mesurer le contenu en phosphore particulaire.

Structure de communauté

L'identification des algues a été faite jusqu'au genre, dans la majorité des cas et sinon jusqu'au groupe algal principal (diatomées, chlorophycées, cyanpbactéries). Après 12 heures de sédimentation dans une chambre Utermöhl, des décomptes de 600 individus ou plus ainsi que les mesures de biovolumes ont été faits à l'aide d'un microscope inversé (Nikon, Diaphot 300) à de grossissements de 100X, 200X et 400X.

Biomasse et stoechiométrie

Les concentrations de chlorophylle *a* ont été mesurées après extraction des filtres dans l'éthanol 90% pendant 1 heure et les lectures prises au spectrophotomètre (Shimadzu, UV-Probe, Columbia, MD, USA) (Hanson 1988; Cattaneo unpublished), mesurant l'absorption à 665 et 750 nm avant et après acidification à l'HCl (Wetzel et Likens, 2001). Les analyses du contenu en nutriments dans les algues (carbone, azote et phosphore particulaire) ont été effectuées au NLEE.

Acides gras - Extraction et fractionnement

Après lyophilisation, les échantillons ont été extraits trois fois dans une solution de chlorofome : methanol (1:1, v/v). Trois rinçages au sel ont été effectués, après quoi, les échantillons ont été méthylés avec du BF3-méthanol à 70°C pendant une heure. Suivant un lavage à l'eau ultra-pure, une extraction à l'hexane nous a permis de récolter uniquement les esters de méthyles d'acides gras (FAME), dont la composition exacte fut déterminée à l'aide d'un chromatographe à phase gazeuse (Hewlett-Packard 6890).

Approche statistique

Les comparaisons des variables chimiques et optiques de l'eau entre les deux sites ont été effectuées avec des tests de t de Student. La dominance des groupes d'algues en fonction du site (nord, sud) et du broutage (présence, absence) a été déterminée à l'aide d'une ANOVA à deux voies pour les structures de communautés retrouvées à l'été 2001. Cette même analyse a été utilisée pour tester les différences entre les substrats (tuiles, macrophytes, roches) et entre les sites (nord, sud) à l'été 2002.

L'effet du site (nord, sud), de la lumière (+RUV, -RUV, -UVB, 90%, 70%, 50%) et du broutage (présence, absence) a été testé au moyen d'une analyse SPLIT-PLOT pour chacune des variables dépendantes transformées (log, racine, puissance). Le terme d'erreur a été corrigé pour le regroupement des traitements de lumières à l'intérieur d'un même radeau. Puisque nous avons perdu un radeau expérimental, le nombre d'échantillons (n) = 66 au lieu de 72 pour les traitements principaux (2 sites X 6 lumières X 2 broutages X 3 réplications), et de 2 ou 3 lorsque les interactions à trois

voies étaient significatives. Pour augmenter la taille de celles-ci et diminuer l'influence des données extrêmes, nous avons regroupé les traitements de 100% et 90% de lumière ambiante sous un nouveau traitement, nommé hautes intensités (HI), et les traitements 70% et 50% sous le traitement de faibles intensités (FI). En conséquence, nos traitements principaux étaient constitués du site (M), avec deux niveaux (nord (N), sud (S)), de la lumière (L), avec 4 niveaux (-UVB, -RUV, HI et FI) et du broutage (B), avec deux niveaux (présence, absence).

Résultats

Plan expérimental

L'utilisation de tuiles dans les études menées sur les films biologiques est répandue. La comparaison de ces substrats aux substrats naturels dans le lac (macrophytes et roches) nous a permis de constater que les tuiles permettaient une plus grande croissance de chlorophycées que les roches et que les macrophytes. Les biomasses relatives de diatomées étaient plus faibles sur les roches. Il n'y avait aucune différence significative entre les diatomées présentes sur les tuiles et les macrophytes. La biomasse de diatomées était plus élevée dans le nord que dans le sud pour tous les substrats utilisés.

Analyse de l'eau

La profondeur de pénétration de 1% de RUV et de PAR était significativement plus élevée dans le sud que dans le nord. Ceci est attribuable aux plus grandes concentrations moyennes de CDOM dans le nord. Les données d'irradiance à la profondeur d'incubation démontrent que les substrats étaient exposés, en moyenne, à 3.4 fois plus d'UVB, 3 fois plus d'RUV et 1.3 fois plus de PAR dans le sud que dans le nord. Le contenu en TN (moyenne = 0.24 $mg \cdot L^{-1}$), NO_2-NO_3 (moyenne = 0.055 $mg \cdot L^{-1}$), NH_3 (moyenne = 0.015 $mg \cdot L^{-1}$), SRP (moyenne = 0.0065 $mg \cdot L^{-1}$), et en TP (moyenne = 0.030 $mg \cdot L^{-1}$) de l'eau ne variaient pas de manière significative entre le nord et le sud selon le test de t de Student. Par contre, SiO_2 était supérieur dans le nord (moyenne = 1.47 ± 0.40 au nord et 0.74 ± 0.20 au sud).

Effet du site

Parmi les trois groupes d'algues étudiés (diatomées, cyanpbactéries, chlorophycées), l'abondance relative des chlorophycées était plus élevée dans le nord et le sud. Dans le nord, l'abondance des diatomées n'était pas significativement différente de celle des chlorophycées. Dans le sud, la Chl*a*, C, N, P, et AFDW étaient plus élevés que dans le nord ($p < 0.001$), indiquant une plus grande biomasse de film biologique. Le ratios stoechiométriques ne variaient pas de manière significative entre les deux sites.

La moyenne des SAFA:MUFA:PUFA était de 32:16:52, comparativement à 28:17:55 dans le nord, démontrant une augmentation des SAFA au détriment des PUFA dans le sud. Tous les acides gras, excluant 20:3ω6 et 22:1ω9 variaient significativement entre les deux sites. Les films biologiques du sud étaient associés aux PUFA : ALA, LA, ARA, DPA, et 20:3ω6, aux MUFA: 24:1ω9 et 18:1ω9, et les SAFA: 12:0, 14:0, 16:0 et 17:0. En comparaison, les films biologiques du nord étaient associés aux PUFA: EPA, DHA, 18:3ω6, 20:3ω3, 20:2 et 22:2, au MUFA: 16:1ω7 et aux SAFA: 20:0, 24:0, 22:0, 18:0 et 15:0.

Effet de la lumière

Les régimes de lumière variable à l'intérieur de chaque site ne correspondaient pas à des différences en terme de biomasse, de nutriments et de ratios stoechiométriques. Parmi les AG, seulement quelques uns étaient affectés par l'effet des filtres : 12:0, ($p = 0.007$), 16:1ω7 ($p = 0.045$), 20:3 ω6 ($p = 0.035$), ω3 ($p = 0.004$), ω3/ ω6 ($p = 0.007$) et les MUFA ($p = 0.015$). Par contre, ces différences engendrées par les filtres de lumière étaient variables et ne permettaient pas de discerner d'effets possibles causés par le retrait des UVB, du RUV et entre les HI et les FI de lumière.

Effet du broutage

En présence de broutage, il y avait un légère augmentation dans l'abondance relative des diatomées ($p = 0.049$), le PC ($p = 0.057$), le PN ($p = 0.025$), et le PP, mais ces différences n'étaient pas hautement significatives. La présence de broutage avait un

effet variable sur le contenu en acide gras, mais tout comme la lumière, ces effets étaient variables.

Interaction triple (PP, PUFA)

L'interaction triple (site X lumière X broutage) était significative pour PP ($p = 0.027$) et PUFA ($p = 0.038$). PP était en moyenne plus élevé au sud qu'au nord, sauf pour les traitements –UVB et –RUV (absence). Contrairement, le %PUFA était plus élevé au nord qu'au sud, et ceci, sous les traitements de FI (présence et absence), -RUV (présence) et –UVB (présence).

Discussion

Parmi les divers effets auxquels les films biologiques étaient soumis dans cette étude (site, lumière et broutage), celui du site était le plus déterminant au niveau du contenu en nutriments et en AG. L'analyse de la structure de communauté nous a permis de vérifier qu'il y avait des différences initiales au niveau de l'abondance relative des groupes d'algues présents, soit une plus grande quantité de diatomées au nord qu'au sud. Ceci nous permet de constater que le régime de lumière présent dans chacune des masses d'eau agissait à un premier niveau de sélection sur la structure de communauté présente. La manipulation de la lumière et du broutage à l'intérieur de ces habitats, où les structures de communauté étaient déjà établies par les pressions de sélection naturelle, ne permettait pas de détecter les effets moindres engendrés par ces deuxièmes niveaux de sélection.

Premier niveau de sélection - Effet du site

Dans le nord, les plus grandes concentrations de CDOM et de particules en suspension étaient responsables de l'absorption des longueurs d'onde à plus haute énergie du spectre, soit des UVB (313 et 320 nm) et des UVA (340 nm) (Frenette et al. 2003; Rae et Vincent 1998). Ces différences en terme de régime spectral avaient des influences significatives sur la structure de communauté, la biomasse, le contenu en nutriments et le contenu en AG. Dans le sud où les expositions aux RUV et PAR étaient plus élevées, les biovolumes des films biologiques étaient plus grands pour tous les

taxons étudiés. De façon relative, l'abondance des chlorophycées demeure supérieure dans le sud et le nord, mais dans le nord l'abondance relative des diatomées se rapproche de celle des chlorophycées. En quantité absolue, les valeurs de PUFA et de SAFA étaient aussi plus élevées dans le sud; par contre, la proportion relative des PUFA par rapport aux SAFA était plus élevée dans le nord. Donc, malgré la croissance des algues plus élevée dans le sud, une plus grande exposition aux RUV pourrait expliquer la réduction de la proportion de diatomées par rapport aux cyanobactéries et aux chlorophycées. Ceci corrobore d'autres études ayant démontré que les diatomées sont particulièrement sensibles aux effets néfastes des RUV (Vinebrooke et Leavitt 96, Rae et Vincent 1998, Francoeur et Lowe 98, Watkins et al. 2001).

Il n'y avait aucune différence entre le nord et le sud dans les ratios de C :P, C :N et N :P. Les moyennes de C :P, soit de 204 +/- 37 et de 236.96 +/- 67 dans le nord et le sud respectivement, étaient inférieures au seuil de qualité nutritive de 300 utilisé dans plusieurs études et ne permettaient pas, à elles seules, de détecter des différences en terme de qualité nutritive. Plusieurs études, incluant la nôtre, démontrent que lorsque P est suffisant dans le milieu (C :P < 300), la qualité nutritive repose essentiellement sur la composition biochimique (Urabe et al. 1997).

L'irradiance moyenne de PAR reçu par les films biologiques se situait entre 300-800 $\mu mol \bullet m^{-2} \bullet s^{-1}$ dans le nord et dans le sud, ce qui est optimal pour la production de lipides (Wainman 1998). Par contre, la composition spécifique en AG des lipides était largement variable entre les sites. Selon les études existantes, la plus grande quantité de SAFA (12:0, 14:0, 16:0 et 17:0) au détriment des PUFA est probablement une résultante des plus hautes intensités de RUV et de PAR, ce qui correspond aux résultats obtenus pour le sud. De plus, il y avait une quantité plus élevée de 18:1ω9, ALA, LA, ARA et 20:3ω6 qui sont des pré-curseurs dans la synthèse d'EPA et de DHA. Ce résultat très intéressant d'une inhibition dans les processus d'insaturation et d'élongation juste avant la formation d'EPA et de DHA, n'a pas été démontré antérieurement dans le contexte d'autres études et demande d'être exploré davantage. Comparativement, dans le nord, les films biologiques avaient un contenu plus élevé en PUFA , notamment d'EPA et de DHA.

Deuxièmes niveaux de sélection

Effet de la lumière

Dans cette étude, les effets délétères engendrés par les RUV et/ou de hautes intensités de PAR à l'intérieur de chacune des masses d'eau ont été observés sur seulement quelques AG. Nous croyons que les écarts entre les RUV-B, RUV-A et PAR étaient trop minimes pour enclencher des dommages irréversibles au cellules. Les RUV-A et des longueurs d'onde plus énergétiques du PAR contribuent à réparer les effets dommageables des RUV-B (Quesada et al., 1995). Une réduction de la couche d'ozone modifie les proportions de RUV-B par rapport aux autre longueurs d'onde atteignant la surface de la terre. Si le taux d'ozone continue à diminuer à des rythmes supérieurs à ce dont les algues peuvent s'adapter, ceci pourrait avoir des effets majeurs sur la qualité nutritive des communautés algales. Cependant, certaines études, incluant celle-ci, démontrent que les communautés algales ne subissent que des modifications mineures ou indétectables en présence de RUV à court terme (Rae et Vincent 1996, McNamara et Hill, 2000). Les expériences ayant démontré des effets significatifs s'adressaient à des films biologiques qui avaient été exposés aux RUV pour une durée > 130 jours (Watkins et al., 2001), comparativement à notre étude qui s'est échelonnée de 58 à 60 jours.

Effet du broutage

Le broutage a eu un effet significatif sur la structure de communauté, où il y avait augmentation dans l'abondance relative des diatomées dans les traitements « présence ». Le broutage sur les cellules de plus grandes tailles augmente les taux de succession, permettant un renouvellement constant des cellules sénescentes par la colonisation et le développement de nouvelles cellules. Ceci permet aux petites cellules situées plus profondément dans la matrice périphytique, telles les diatomées, de recevoir suffisamment de lumière pour une croissance maximale (Lamberti et al. 1987). L'effet inverse du broutage dans le nord et le sud au niveau du contenu de certains AG suggère que le broutage affecte de manière différente les communautés algales selon leur habitat et le type de communautés algales et des consommateurs déjà présents.

Quantité et qualité de la nourriture

Les études existantes sur la quantité et la qualité de nourriture disponible se sont attardées principalement au contenu en nutriments (Sterner et Hessen 1994), à la stoechiométrie (Sterner 1997), ou au contenu en protéines (Cruz-Rivera et Hay 2000), même si les végétaux sont reconnus comme étant une source alimentaire pauvre en protéines comparativement à d'autres sources de nourriture. Nous ne connaissons aucune étude ayant utilisé le contenu en PUFA pour déterminer la valeur nutritive de la nourriture, malgré les nombreuses évidences de leur caractère essentiel pour tous les vertébrés étudiés jusqu'à maintenant (Sergant et al. 1995 in Gulati et Demott 1997). Un supplément en PUFA dans la diète augmente la croissance, la fécondité et la survie de plusieurs espèces de zooplancton (Demott et Muller-Navarra 1997; Weers et Gulati 1997).

Nos résultats démontrent que la biomasse des films biologiques était supérieure dans le sud. Par conséquent, le contenu total en acides gras et en nutriments était aussi plus élevé. En comparaison, il y avait une quantité plus faible de nourriture disponible dans le nord mais celle-ci, pour une même quantité de nourriture, contenait une proportion plus concentrée en PUFA par rapport aux SAFA. Les brouteurs dans le sud seront en présence d'une quantité de nourriture plus abondante mais pour la même quantité de nourriture consommée, le rapport PUFA : SAFA ingéré sera plus faible que dans le nord. En présence de nourriture de plus faible qualité, les consommateurs ont développé des comportements compensatoires, notamment en augmentant les taux de broutage (Cruz-Rivera 2000). Le manque d'informations sur les effets des ces différences au niveau quantité/qualité entre le nord et le sud soulève plusieurs questions que nous planifions aborder dans un future rapproché : Quelles sont les communautés de brouteurs présents dans chaque site? Comment les organismes des réseaux trophiques supérieurs s'adaptent-ils aux différences dans la disponibilité ou dans la qualité de la nourriture selon les milieux? Comment ces différences se reflètent-elles sur les taux de croissance, de fécondité, de survie et de productivité sur tous les maillons de la chaîne trophique, incluant herbivores et carnivores? Les organismes possèdent-ils des mécanismes biochimiques leur permettant de synthétiser leurs propres acides gras essentiels?

Conclusion

En conclusion, nos résultats démontrent que de plus grandes expositions aux RUV et PAR engendrent des différences importantes dans la qualité nutritive (% des AG essentiels) des films biologiques les provenant de sites relativement semblables dans leur quantité de P disponible. Ceci a des effets significatifs sur la disponibilité de la nourriture dans les écosystèmes aquatiques, soit en terme de quantité ou de qualité ce qui ultimement pourrait déterminer la productivité des réseaux trophiques à des niveaux supérieurs. Nos radeaux d'incubation constituaient des structures idéales permettant d'étudier les effets engendrés par des différences de régime spectral sur les films biologiques en milieu fluvial soumis à de fortes contraintes hydrodynamiques.

Références non-citées dans les articles

Ahlgren G, Gustafsson I-B, Boberg M (1992) Fatty Acid Content and Chemical Compositioon of Freshwater Microalgae. Journal of Phycology 28:37-50

Arts MT (2001) Lipids in Freshwater Zooplankton: Selected Ecological and Physiological Aspects. *Lipids in Freshwater Ecosystems.* Springer-Verlag, New York, Inc. 71-90

Arts MT, Robarts RT, Evans MS (1997) Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Biology 38:525-537

Arts MT, Kasai F, Waiser MJ, Tumber VP, Plante AJ, Rai H, de Lange HJ (2000) The attenuation of ultraviolet radiation in high dissolved organic waters of wetlands and lakes on the northern Great Plains. Limnology and Oceanography 45:292-299

De Lange HJ, Van Donk E (1997) Effects of UVB-irradiated algae on life history traits of *Daphnia pulex*. Freshwater biology 38:711-720

Désilets L, Langlois C (1989) Variabilité spatiale et saisonnière de la qualité de l'eau du fleuve Saint-Laurent, Centre Saint-Laurent, Environnement Canada, Montréal. 122 pp.

Diaz SB, Morrow JH, Booth CR (2000) Spectral weighting functions for quantifying effects of UV radiation in marine ecosystems. *The effects of UV radiation in the marine environment.* Cambridge University Press, 35-71

Germain A, Janson M (1984) *Qualité des eaux du fleuve Saint-Laurent de Cornwall à Québec (1977-1981).* Environnement Canada, Direction générale des eaux

intérieures, Longueil, Québec, 220p.

Graham MD, Vinebrooke RD (1998) Trade-offs between herbivore resistance and competitiveness in periphyton of acidified lakes. Canadian Journal of Fisheries and Aquatic Science 55:806-814

Gurr MI, James AT (1971) *Lipide Biochemistry*. Cornell University Press, New York. 231 pp

Hader DP (1993) Risks of enhanced solar ultrviolet radiation for aquatic ecosystems. Progress in phycological research 9: 1-45

Harisson PJ, Thompson PA, Calderwood GS (1990) Effects of nutrient and light limitation on the biochemical composition of phytoplankton. Journal of Applied Phycology 2:45-56

Hepinstall JA, Fuller RL (1994) Periphyton reactions to different light and nutrient levels and the response of bacteria to these manipulations. Arch. Hydrobiol. 131:161-173

Hill WR, Boston HL (1991) Community development alters photosynthesis-light relations in stream periphyton. Limnol. Oceanogr. 36: 1375-1389

Hill WR, Harvey BC (1990) Periphyton responses to higher trophic levels and light in a shaded stream. Canadian Journal of Fisheries and Aquatic Sciences 47: 2307-2314

Hudon C (2000) Phytoplankton assemblages in the St. Lawrence River, downstream of its confluence with the Ottawa River, Québec, Canada. Canadian Journal of

Fisheries and Aquatic Sciences 57:16-30

Karentz D, Cleaver JE, Mitchell DL (1991) Cell survival characteristics and molecular responses of antarctic phytoplankton to ultra-violet-B radiation. Journal of phycology 27: 326-341

Langlois C, Lapierre L, Léveillé M, Turgeon P, Ménard, C (1992). *Synthèse des connaissances sur les communautés biologiques du lac Saint-Pierre.* Groupe de travail sur les zones d'intérêt prioritaire, Centre Saint-Laurent. 236 p.

Lombardi AT, Wangersky PJ (1991) Influence of phosphorus and silicon on lipid class production by the marine diatom Chaetoceros gracilis grown in turbidostat cage cultures. Marin Ecology Progress Series 77:39-47

Madronich S, McKenzie RL, Caldwell MM, Björn LO (1995) Changes in ultraviolet radiation reaching the earth's surface. Ambio. 24:143-152

Maltais M-J (1994) Le périphyton épilithique observé dans un lac oligotrophe nordique: le lac à l'Eau Claire. Mémoire de maîtrise. Département de biologie, Université Laval.

McIntire. D.C. (1973) Periphyton Dynamics in laboratory streams; a simulation model and its implications. Ecological Monographs 43:399-420

Napolitano GE (1998) Fatty Acids as Trophic and Chemical Markers in Freshwater Ecosystems. *Lipids in Freshwater Ecosystems.* Springer-Verlag, New York, Inc. 21-44

Rondeau B (1993) Qualité des eaux du fleuve Saint-Laurent 1985-1990. Tronçon Cornwall-Québec. Centre Saint-Laurent. Environnement Canada. 150 pp.

Parrish CC (1988) Dissolved and particulate marine lipid classes: a review. Marine Chemistry 23:17-40

Saravia LA, Momo F, Boffi Lissin LD (1998) Modelling periphyton dynamics in running water. Ecological Modelling 114:35-47

Shifrin NS, Chisholm SW (1981) Phytoplankton lipids: interspecific differences and effects of nitrate, silicate, and light-dark cycles. Journal of Phycology 17:374-384

Smith RC, Bidigare RR, Prézelin BB, Baker KS, Brooks JM (1987) Optical characterization of primary productivity accross a coastal front. Marine Biology 96:575-591

Smith REH, Furgal JA, Charlton MN, Greenburg BM, Hiriart V, Marwood C (1999) Attenutaion of ultraviolet radiation in a large lake with low dissolved organic matter concentrations. Canadian Journal of Fisheries and Aquatic Science 56:1351-1361

Thompson PA, Harrison PJ, Whyte JNC (1990) Influence of irradiance on the fatty acid composition of phytoplankton. Journal of Phycology 26:278-288

Urabe J (1995) Direct and indirect effects of zooplankton on seston stoichiometry. Écoscience 2:286-296

Vadeboncoeur Y, Lodge DM (2000) Periphyton production on wood and sediment: substratum-specific response to laboratory and whole-lake nutrient manipulations. Journal of North American Benthological Society 19:68-81

Vinebrooke RD, Graham MD (1997) Periphyton assemblages as indicators of recovery in acidified Canadian Shield lakes. Canadian Journal of Fisheries ans Aquatic Science 54:1557-1568

Whitehead RF, deMora SJ, Demers S (2000) Enhanced UV radiation – a new problem for the marine environment. *The effects of UV radiation in the marine environment.* Cambridge University Press, 1-34

Chapitre 2: An *In Situ* Incubation Device to Study Biofilm Growth in Relation to Spectral Regime in Fluvial Lakes and Rivers

Kim Huggins[1,2], Jean-Jacques Frenette[2], and Michael T. Arts[3]

[1]*Author to whom correspondence should be addressed (Kim_Huggins@uqtr.ca)*
[2]*Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Québec, Canada G9A 5H7*
[3]Aquatic Ecosystem Impacts Research Branch, National Water Research Institute, 867 Lakeshore, Road, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6

KEY WORDS: Lake St. Pierre, light, microbial mats, periphyton, UVR, fluvial lake

Abstract

A specialized incubation device was designed for biofilm growth on artificial substrates in fluvial and riverine systems. The device was tested in Lake Saint Pierre (lat: 46°12'; long: 72°50'), the largest (480 km^2) fluvial lake along the St. Lawrence River and its last major enlargement (13.1 km wide at mean discharge) before reaching the estuary. This unique device allowed us to grow biofilms at fixed depths, despite the influences of seasonally changing water levels, strong currents and waves. We deployed several of these devices in two water masses that differed in terms of chromophoric dissolved organic matter content and hence, their underwater light climates (Frenette et al., 2003). Using wavelength-selective filters we exposed developing biofilms to three different ultraviolet radiation (UVR) regimes: 1) with UVR, 2) neither ultraviolet A (UV-A) nor ultraviolet B radiation (UV-B), 3) no UV-B, and to different intensities of photosynthetically active radiation (PAR: 400-700 nm): 90%, 70%, and 50% transmission of ambient solar radiation. This device provides a stable underwater incubation platform for measuring the effects of different underwater light climates on biofilms in the shallow regions of fluvial lakes and rivers, which are considered extreme environments subject to particularly harsh hydrodynamic conditions. Our device will allow researchers to obtain integrated life-history data of biofilms in response to light climate effects. Such measurements provide crucial input for the formulation of bio-optical models designed to accurately describe and predict the effects of light climate on aquatic biofilms.

Introduction

A major portion of aquatic biofilms consists of photosynthetic organisms that depend on ambient light exposure 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, either directly or indirectly, on organisms that are dependent on them. Enhanced UVR as a result of ozone depletion is of mounting concern, especially in shallow, optically clear aquatic systems where reductions in water levels (e.g. as a function of climate change) could exacerbate the effects of ozone thinning. This topic has been the subject of extensive studies, both in the field and in the laboratory, especially over the last two decades (e.g. Bothwell et al., 1993; Francoeur and Lowe, 1998). Exposure to UV-B (280-320 nm) radiation can lead to reductions in bacterioplankton activity (Herndl et al., 1993), increases in zooplankton mortality (Williamson et al., 1999), as well as a series of disturbances in phytoplankton communities: 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).

Many studies dealing with the effects of UV-B on primary productivity have been conducted using phytoplankton species (e.g. Hessen et al., 1997; Goes et al., 1995). Since these organisms are highly influenced by light quality and/or quantity, these experiments have proven to be quite laborious to conduct *in situ*; especially in lotic systems where cells are continuously transported through the water column by the action of waves, horizontal advection, micro-scale turbulence, and other mixing processes thus exposing them to continually varying UVR and PAR intensities. In contrast, biofilms

colonize and grow at fixed positions with respect to the bottom, and are less capable of using active or passive displacement outside the microbial mat, i.e. by finding refuge in deeper waters (Hill et al., 1997) or behind physical shelters (Vinebrooke and Leavitt, 1999) to decrease their exposure to UVR.

There are inconsistencies amongst results obtained through testing the effects of UVR on epilithon (periphyton which grows on rocky substrates). The findings range from long-term deleterious effects (Vinebrooke & Leavitt, 1999), to short-term effects (Francoeur et Lowe, 1998), to no effects at all (Hill et al., 1997). Some studies have been conducted in the laboratory (e.g. DeNicola and McIntire, 1990; Vadeboncoeur and Lodge, 2000) where diurnal light fluctuations (UVR and PAR) are not easily replicated. Many others have been performed *in situ* but there is substantial divergence amongst the methods used.

Few studies however have been carried out *in situ* within extreme environments, such as shallow lakes (Vinebrooke & Leavitt, 1999) or high-order fluvial systems. Other than the studies conducted by DeSève and Goldstein (1980) who collected periphyton on rocks located at depths of 10-30 cm, and Vis et al. (1998a, 1998b) who sampled periphyton growing on navigational buoys, we are not aware of any other studies that have sampled epilithon in the St. Lawrence River. The use of buoys is an interesting concept, however, it suffers from serious drawbacks in that it does not allow researchers to select and vary the locations of their sampling sites and does not permit ready manipulation of the underwater light field.

In fluvial and/or shallow water 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. The deposition problem is often seen when artificial substrates are deployed on, or just above, the sediment surface. Because of such problems, studies on light climate effects on 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). Where experiments have been conducted in littoral zones of lakes or ponds, substrates (Chételat et al. 1999; Hill et al., 1997, Fairchild, 1985; Vinebrooke & Leavitt, 1999) are most commonly placed directly on the sediments or within incubation chambers located on or near the sediment surface.

In studies on biofilms, incubation depth is a crucial factor that has often been overlooked in previous experimental designs. Accurate quantitative and qualitative light (PAR & UVR) penetration profiles are fundamental in deciding appropriate and realistic intensities to which biofilms should be exposed; this, in turn, determines the appropriate incubation depth(s) to use. Exposure to light will not be constant if the attachment mechanism is fixed either with respect to the sediment surface. 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.

In addition, the incubation system proposed here allows researchers to more precisely monitor the quality and quantity of light actually reaching the biofilm. Several of our devices could be deployed at the same location and at various fixed depths with

respect to the surface; an essential feature for modeling the effects of fluctuating water levels.

Wind plays a key role in the mixing and hydrodynamic regimes of shallow lakes, (Scheffer, 1998). In the majority of such systems, the euphotic zone extends to the bottom, such that light penetration depth exceeds that of the water column ($Ze/Zt > 1$) (Cloern, 1987). This allows for photosynthesis to take place in the pelagic and benthic zones, permitting phytoplanktonic and periphytic organisms as well as extensive macrophyte beds to develop throughout the water column. Lake St. Pierre is a large (480 km^2) fluvial lake with a complex hydrodynamic regime. It features three main water masses 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). These three water masses are characterized by distinctly different underwater light climates. Since lateral mixing is restricted on either side of the channel, incoming tributaries basically conserve their biophysical properties along the longitudinal axis of the lake (Frenette et al., 2003). Downstream currents can be very strong in the lake with mean flow rates varying between 0.5-1 m s^{-1} (Basu et al., 2000). The design of the new experimental incubation device demonstrated here allowed us to grow biofilms within the desired water masses despite strong hydrodynamic regimes and ensured complete submergence of the artificial substrates for the duration of the experiment. These were designed with some basic requirements in mind: They had to be sturdy and stay anchored in the sediments in the face of strong currents and waves; they had to allow unrestricted vertical movement of the incubation platform so that a constant depth could be maintained with respect to

the surface; the floatation device had to be made of materials that were readily available and relatively inexpensive; it had to be designed such that shading of the incubation platform was minimized; the incubation substrates had to be removable to allow for transfers to take place between incubation sites; the light filters were designed to be replaceable and/or easily pulled out for cleaning purposes; all construction materials had to be non-corrosive.

Materials and Methods

Study area

The incubation devices were deployed in the north (lat: 46°12', long: 72°55') and south (lat: 46°8', long: 72°51') water masses of Lake St. Pierre for a period of 47 d to 49 d (from July 24 & 25, 2001 until Sept. 9, 10, & 11, 2001). The two water masses differ in their physico-chemical and biological characteristics thus affecting attenuation rates and, hence, ratios amongst PAR, UV-A and UV-B radiation (Frenette et al., 2003). The hydrodynamic conditions of these sites are comparable since both were situated in bays with similar depths that ranged from 3 m at the beginning of the experiment to about 1 m by the end. Data obtained by the Canadian Hydrological Data Center (Environment Canada, 2001) demonstrated drastic decreases in the Lake St. Pierre water levels over the summers of 1995, 1999, 2000, and 2001.

Incubation rafts

Design (Fig. 1.1 and 1.2)

The rafts consisted of 2 main decks (Fig. 1.2): a lower story supporting the substrates and an upper story on which light filters were fixed. The frame was made of rust-free, slightly flexible, aluminium of 0.06 cm thickness (Metal Lamine D.R. Inc.,

Trois Rivières, Quebec). The platform (1.52 m by 1.80 m) was strengthened with 7.6 cm inner-diameter ABS tubing fastened underneath. Holes were drilled along the ABS tubing to prevent floatation.

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. Because the rafts were free to move in the vertical dimension they maintained a light environment whose characteristics were dictated only by the seasonal variation in the chemical composition of the overlying waters but was independent of the effects of changes in water level on attenuation. In addition to the desirable effect this had on the maintenance of an appropriate light climate, this freedom of movement minimized the effects of waves because the incubation platform and the floatation device would momentarily "give" when struck by a wave.

Artificial substrates

The artificial substrates consisted of unglazed ceramic tiles (Céramique Des Rochers, Trois-Rivières, Canada) that were glued on cut polypropylene sheets (1.22 m X 2.4 m X 0.4 cm) (Laird Plastics,Montreal, Canada) using aquarium-grade silicone. Researchers commonly use tiles to grow aquatic biofilms because they, a) are uniform and homogenous in their surface characteristics and dimensions, b) are inexpensive and, c) reduce sampling costs (Aloi, 1990; Lamberti & Resh, 1983). Two sizes (232.3 cm^2 & 25.8 cm^2) of tiles were used depending on the biomass available and the quantity of material required to perform the different analyses.

Light quantity and quality

Light filters were randomly placed into slots in the upper story. The upper story was fixed at a height of 2.5 cm above the lower one, which contained the substrates. 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. 1.2). 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 of 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. 1.3). This was done at roughly two-day intervals.

Floatation device

Each platform was chained to a floatation system designed to limit shading (Fig. 1.2). This system was easy to manipulate and could support a heavy (~110 kg) load. Preliminary tests with other materials (wooden planks fastened to floatation buoys, polyvinyl chloride tubing (PVC-tubing), acrylonitrile-butadiene-styrene tubing (hereafter ABS tubing) confirmed that ABS tubing with Styrofoam floatation buoys (0.25 m X 0.51m X 2.44 m) (The Dow Chemical Company, Michigan, U.S.A.) were the

Figure 1.1: Side view of the incubation platform within the water. A square perforated ABS tubing maintained the rigidity of the incubation platform and supported the floatation buoys. The incubation raft maintained a constant distant with that of the water surface for the entirety of the incubation period.

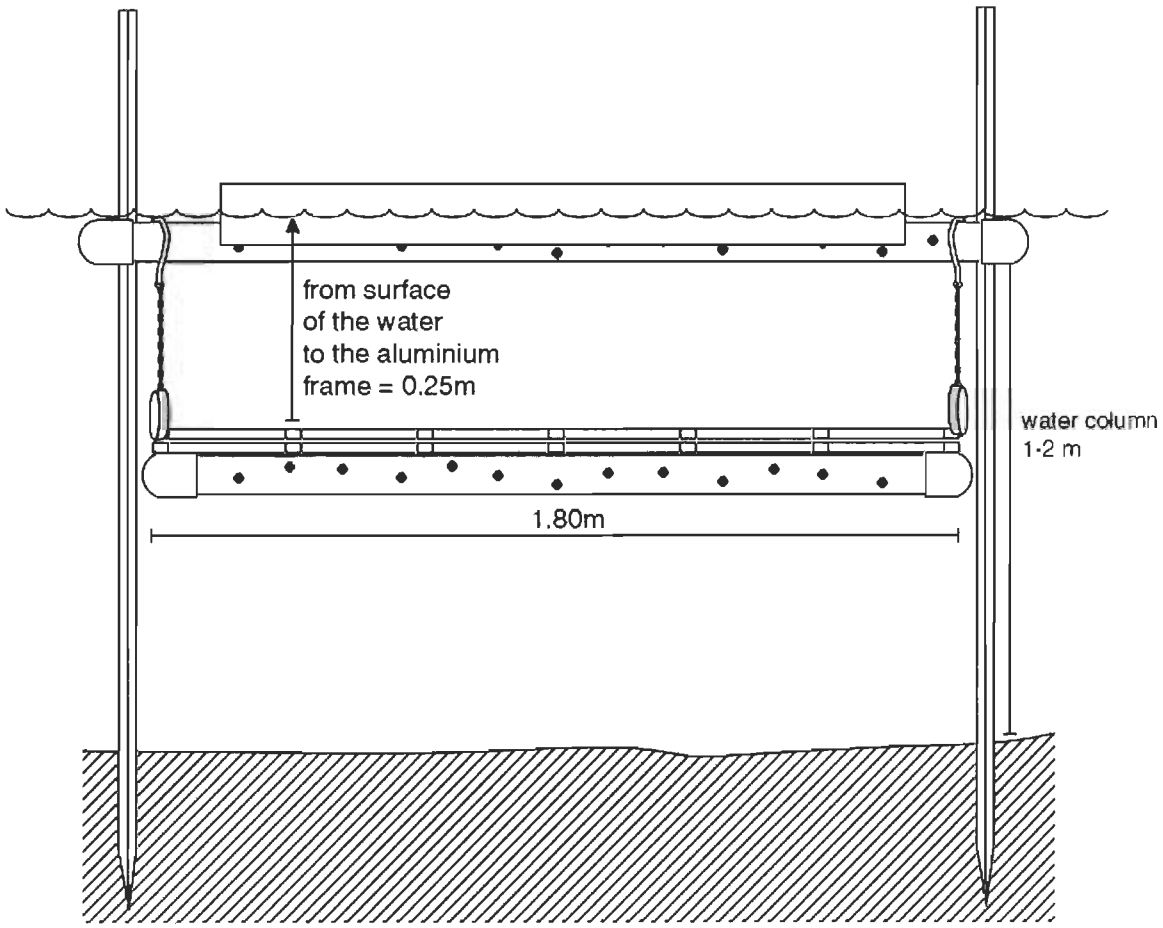

from surface
of the water
to the aluminium
frame = 0.25m
water column
1·2 m
1.80m

Figure 1.2: Diagram of one incubation raft with the two main decks: a lower story supporting the substrates (clay tiles) and an upper story with the light filters (no UVR, noUVB, with UVR, 90%, 70%, 50%). 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.

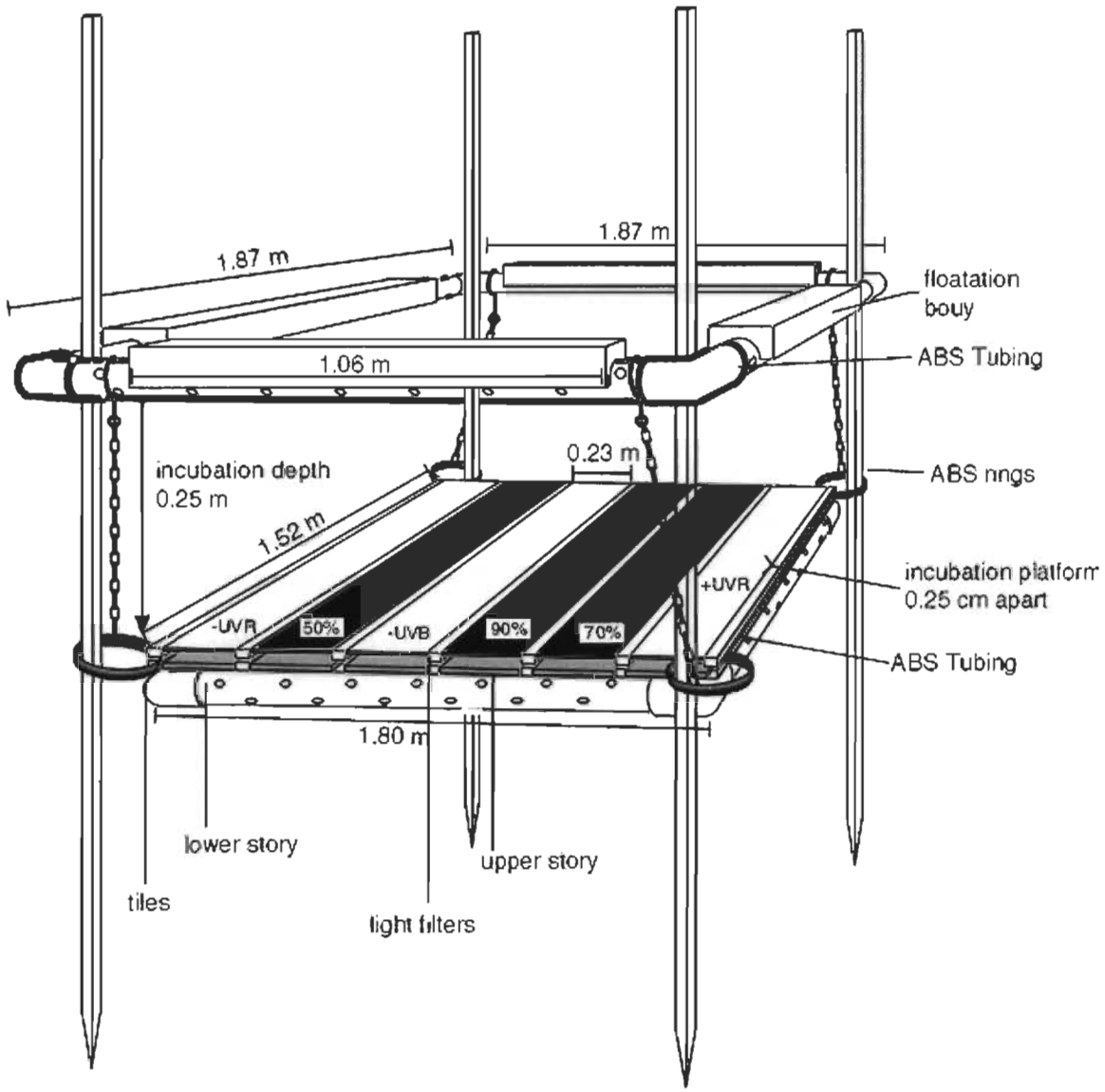
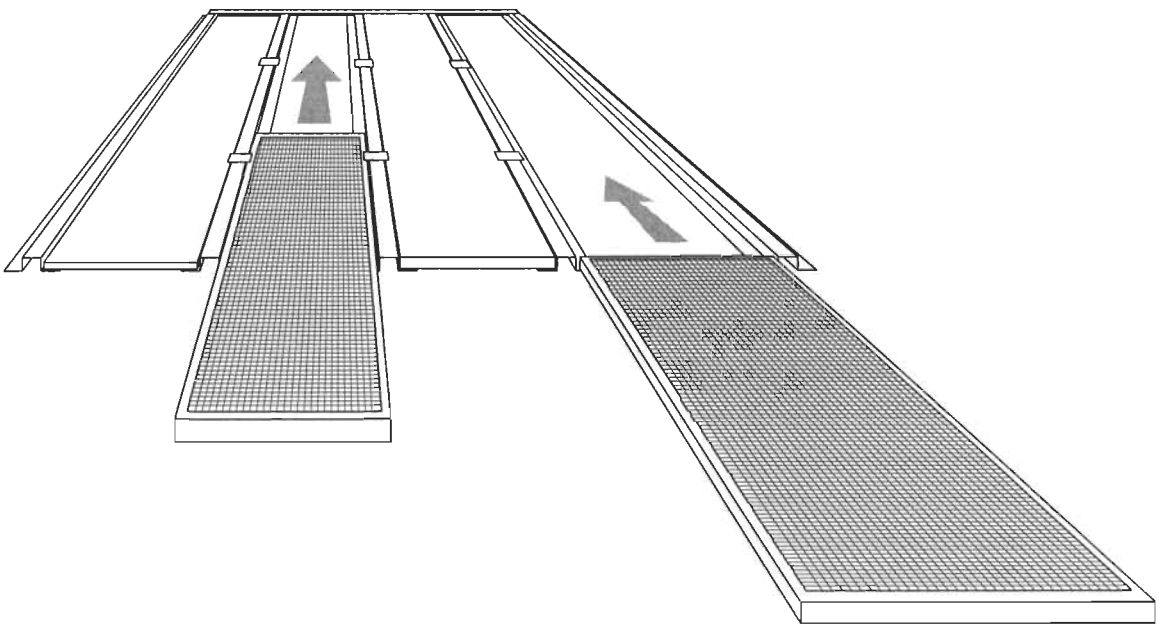

1.87 m
1.87 m
floatation
bouy
1.06 m
ABS Tubing
0.23 m
incubation depth
0.25 m
ABS rings
1.52 m
incubation platform
0.25 cm apart
+UVR
-UVR
50%
-UVB
90%
70%
ABS Tubing
1.80 m
lower story
upper story
tiles
light filters

Figure 1.3: Illustration of the light filters within the aluminium frame. The neutral density filters (screening material) were fixed to aluminium frames. All filters were easily retractable for maintenance access.



most advantageous materials, with respect to cost and weight. A square frame (1.87 m X 1.87 m) was constructed using 7.6 cm inner-diameter ABS tubing and floatation buoys were fitted along 1.06 m length of each side. Holes were drilled along the length of the ABS tubing so that floatation was provided solely by the buoys.

The incubation platforms were free to move vertically with respect to the water surface. 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 made this vertical movement possible. These 3.66 m long (5.08 cm X 10.16 cm) stakes were sharpened at one end and driven into the sediments in order to anchor the rafts in position. The incubation platforms were fixed at 45° with respect to the direction of the current in order to minimize the effects of water turbulence and drag.

Grazer nets

Nets with a mesh size of 4 mm^2 were installed on three of the six rafts to provide us with macro-grazer exclusion treatments. These nets were made from plastic and aluminum screening cut in strips that were sewn together using large, solid, elastic bands at the corners. They were tightly fitted around the incubation sections so they could regularly be removed and re-installed (e.g. for cleaning). The fact that the artificial substrates were suspended above the sediments further limited the access of non-swimming grazers in particular (Hill et al., 1997). A pore size that would provide complete grazer exclusion would have unrealistically reduced flow rates.

Biofilm sampling

The incubation rafts were removed from the lake after 57 to 59 d. The stakes were pulled from the sediments and the incubation sections with floats were hauled, one by one, into our boats. The polypropylene sheets supporting a series of tiles were cut free from the rafts and put into pre-identified plastic bags in a cooler filled with lake water. Biofilms growing on the tile surfaces were scraped off using razor blades and spatulas back in the laboratory. Large or small tiles were scraped depending on the biomass available and analyses required. Biofilm samples were collected for lipid, stoichiometry, chlorophyll *a*, and community structure analyses (Huggins et al., in prep.).

Discussion

Our experimental incubation device was highly successful in cultivating biofilms in fluvial Lake St. Pierre where particularly harsh hydrodynamic conditions (strong currents, high waves, and fluctuating water levels) are the norm. Few studies on epilithon have been conducted in the St. Lawrence River where decreases in water level due to global warming are predicted (Schindler, 2001). Such changes would likely alter the biological, chemical and physical properties of this crucial waterway. This river is one of the major freshwater systems of North America, extending from the Laurentian Great Lakes to the Atlantic Ocean and draining 25% of the world's freshwater supply (Vincent and Dodson, 1999).

Biofilms colonize virtually every type of substrate in aquatic systems (Hillebrand & Kalhert, 2001). Biofilm organisms 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 monitoring water quality (Vis et al., 1998; Reavie & Smol, 1998). These organisms represent an important food source and variations in their growth rate or nutritional status could have serious implications for aquatic ecosystems (Napolitano, 1994). This need for understanding how and where energy flows through aquatic systems has precipitated an ever-increasing number of studies on various aspects of grazer activity (Lamberti, 1993). For example, grazing rates on periphyton tend to be greater in experiments performed in laboratory or in artificial outdoor channels (Cattaneo & Mousseau, 1995). Because of such biases, such experiments should, preferably, be conducted in the field.

We strongly suggest that future experiments, designed to investigate the effects of underwater light climate and/or grazing activity on biofilms, be carried out with careful consideration/measurements of the natural underwater light climates and at fixed incubation depths with respect to the water surface (rather than with respect to the sediment surface). The incubation design discussed here is based on such principles, and will be helpful for performing future studies on biofilms. Further, our incubation device is especially suited for use in locations such as fluvial lakes and rivers exposed to severe hydrodynamic conditions; situations where past studies have been limited. Because of its modular design and the ease with which tiles can be removed we suggest that this apparatus will be especially useful for experiments that incorporate a reciprocal transfer design element(s).

The underwater light climate has important consequences for the food quality of primary producers through its varied effects on metabolic processes (e.g. Arts and Rai,

1997; Frenette et al. 1998; Vincent and Neale 2000). Many studies on the effects of UVR on primary producers are performed under laboratory conditions (e.g. De Lange et al., 1999; Cullen & Lesser, 1991), not necessarily reflecting the natural growth environment 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 effects of ambient UVR as well as the ability to manipulate UVR:PAR rations, different light intensities, and grazing activity on biofilms under near-natural conditions. Our incubation device provides an efficient way to obtain integrated life-history effects of light climate on 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 on aquatic biofilms.

Acknowledgements

We are grateful to Pascal Lavallée, Sylvain Thélème, Geneviève Trudel, Marie-Audrée Livernoche, Carl Martin, Christine Barnard, Jean-Louis Benoît, Roger Thibeault, Guy Marchand, and Marc Milot, from the Université du Québec à Trois-Rivières, as well as, Yves Mailhot, Daniel Dolan et Denis Bourbeau from the FAPAQ. We also thank Michael Huggins, Karen Raphaël, Sandra Raphaël, Benjamen Clancy, Line De Jean, Patrick Murphy, Hugues Boulanger, and Jennifer Carley for help with the construction of the incubation rafts. This research was supported by the Fonds pour la formation des Chercheurs et l'Aide à la Recherche (FCAR) and the National Science Research Council (NSERC) to J-J. F., as well as the National Water Research Institute

(Environment Canada) to M.T.A. Michael Donnelly of the Graphics Arts Unit of NWRI, Environment Canada provided the schematic figures of the incubation device.

References

Aloi JE (1991) A critical review of recent freshwater periphyton field methods. Can J Fish Aquat Sci 47: 656—670

Arts MT, Rai H (1997) Effects of ultraviolet radiation on the production of lipid, carbohydrate, and protein in three freshwater algal species. Fresh Biol 38: 597—610.

Basu BK, Kalff J, Pinel-Alloul B (2000) Midsummer plankton development along a large temperate river: the St. Lawrence River. Can J Fish Aquat Sci 57 (Suppl. 1): 7—15

Bothwell ML, Sherbot D, Roberge AC, Daley RJ (1993) Influence of natural ultraviolet radiation on lotic periphyton diatom community growth. J Phycol 29: 24—35

Bothwell ML, Sherbot DMJ, Pollock CM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic-level interactions. Science 265: 97—100

Cattaneo A, Mousseau B (1995) Empirical analysis of the removal rate of periphyton by grazers. Oecologia 103: 249—254

Chételat J, Pick FR, Morin A, Hamilton PB (1999) Periphyton biomass and community composition in rivers of different nutrient status. Can J Fish Aquat Sci 56: 560—569

Cloern JE (1987) Turbidity as a control on phytoplankton biomass and productivity in estuaries. Cont Shelf Res 7: 1367—1381

Cullen JJ, Lesser MP (1991) Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar Biol 111: 183—190

De Lange HJ, Verschoor AM, Gylstra R, Cuppen JG, Van Donk E (1999) Effect of artificial ultraviolet-B radiation on experimental aquatic microcosms. Freshw Biol 42: 545—560

DeSève MA, Goldstein ME (1980) The structure and composition of epilithic diatom communities of the St Lawrence and Ottawa rivers in the Montreal area. Can J Bot 59: 377—387

DeNicola DM, McIntire CD (1990) Effects of substrate relief on the distribution of periphyton in the laboratory streams. II. Interactions with irradiance. J Phycol 26: 634—641

Fairchild GW (1985) Algal periphyton growth on nutrient -diffusing substrates: an in situ bioassay. Ecology 66: 465—472

Francoeur SN, Lowe RL (1998) Effects of ambient ultraviolet radiation on littoral periphyton: biomass accrual and taxon-specific responses. J Freshwater Ecol 13: 29—37

Frenette J-J, Arts MT, Morin J (2003) Bio-optical spectral gradients of downwelling light in a fluvial lake (Lake Saint-Pierre, St.-Lawrence River). Aquat Ecol 37: 77-85

Frenette J-J, Vincent WF, Legendre L (1998) Size-dependent C:N uptake by phytoplankton as a function of irradiance: ecological implications. Limnol Oceanogr 43: 1362—1368

Goes JI, Handa N, Taguchi S, Hama T (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter *Tetraselmis* sp.: relationship to cellular pigments. Mar Ecol Progr Ser 114: 259—274

Goes JI, Handa N, Taguchi S, Hama T, Saito H (1995) Impact of UV radiation on the production patterns and composition of dissolved free and combined amino acids in marine phytoplankton. J Plank Res 17: 1337—1362

Helbling EW, Villafane V, Ferrario M, Holm-Hansen O (1992) Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. Mar Ecol Progr Ser 80: 89—100

Herndl GJ, Muller-Niklas G, Frich J (1993) Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature 361: 717—719

Hessen DO, DeLange HJ, Van Donk E (1997) UV-induced changes in phytoplankton cells and its effects on grazers. Freshw Biol 38: 513—524

Hill WR, Boston HL, Steinman AD (1992) Grazers and nutrients simultaneously limit primary productivity. Can J Fish Aquat Sci 49: 504—512

Hill WR, Dimick SM, McNamara AE, Branson CA (1997) No effect of ambient UV radiation detected in periphyton and grazers. Limnol Oceanogr 42: 769—774

Hillebrand H, Kahlert M (2001) Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. Limnol Oceanogr 46: 1881—1898

Lamberti GA, Resh VH (1983) Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. Ecology 64: 1124—1135

Lamberti GA (1993) Grazer-Algal interactions. In: Lamberti GA, Steinman AD (eds) *Research in artificial streams; applications, uses, and abuses*. J N Am Benthol Soc 12: 337—342

Morin A, Cattaneo A (1992) Factors affecting sampling variability of freshwater periphyton and the power of periphyton studies. Can J Fish Aquat Sci 49: 1695—1703

Napolitano GE (1994) The relationship of lipids with light and chlorophyll measurements in freshwater algae and periphyton. J Phycol 30: 943—950

Napolitano GE, Shantha NC, Hill WR, Luttrell AE (1996) Lipid and fatty acid compositions of stream periphyton and Stoneroller Minnows (*Campostoma anomalum)*: trophic and environmental implications. Arch Hydrobiol 137: 211—225

Reavie ED, Smol JP (2000) Epilithic diatoms of the St. Lawrence River and their relationship to water quality. Can J Bot 76: 251—257

Scheffer M (1998) Ecology of shallow lakes. Chapman & Hall, London, UK. pp. 346

Schindler DW (2001) The cumulative effects of climate warming and other human stresses on Canadian freshwaters in the new millennium. Can Fish Aquat Sci 58: 18—29

Sommer U (1996) Nutrient competition experiments with periphyton from the Baltic Sea. Mar Ecol Progr Ser 140: 161—167

Stelzer RS, Lamberti GA (2001) Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnol Oceanogr 46: 356—367

Vadeboncoeur Y, Lodge DM (2000) Periphyton production on wood and sediment: substratum-specific response to laboratory and whole-lake nutrient manipulations. J N Am Benthol Soc 19: 68—81

Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. *In* the effects of UV radiation in the marine environment. *Editor:* Cambridge University Press. pp. 149—176

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

Vinebrooke RD, Leavitt PR (1999) Differential responses of littoral communities to ultraviolet radiation in an alpine lake. Ecology 80: 223—237

Vis C, Hudon C, Cattaneo A, Pinel-Alloul B (1998a) Periphyton as an indicator of water quality in the St. Lawrence River (Québec, Canada). Env Pollut 101: 13—24

Vis C , Cattaneo A, Hudon C (1998b) Periphyton in the clear and colored water masses of the St. Lawrence River (Québec, Canada): A 20-year overview. J Great Lakes Res 24: 105—117

Wang K. S., Chai T-J (1994) Reduction in omega-3 fatty acids by UV-B irradiation in microalgae. J Appl Phycol 6: 415—421

Williamson CE, Hargreaves BR, Orr PS, Lovera PA (1999) Does UV play a role in changes in predation and zooplankton community structure in acidified lakes? Limnol Oceanogr 44: 774—783

Chapter 3: Nutritional Quality and Biofilms with respect to light regime in Lake St. Pierre (Québec, Canada)

Kim Huggins[1,2], Jean-Jacques Frenette[2], and Michael T. Arts[3]

[1]*Author to whom correspondence should be addressed (Kim_Huggins@uqtr.ca)*

[2]*Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Québec, Canada G9A 5H7*

[3]Aquatic Ecosystem Impacts Research Branch, National Water Research Institute, 867 Lakeshore, Road, P.O. Box 5050, Burlington, Ontario, Canada L7R 4A6

Abstract

In situ experiments were conducted using specialized incubation devices for the growth of biofilms to compare the effects of varying light regimes between sites, within each site, and of grazer exclusion on food quality in Lake St. Pierre. Differing *in situ* UVR and PAR 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 none on stoichiometry. 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 selection exerted on community structure, which occurred naturally owing to the existing environmental conditions. The secondary level treatments (light and grazing treatments) had little to no detectable effects on food quality on the already existing community structures.

Abbreviations: ABS, absent; ALA, γ-linolenic acids (18:3ω3); ARA, arachadonic acid (20:4ω6); CDOM (chromophoric dissolved organic carbon); EFA, essential fatty acids; EPA, eicosapentaenoic acid (20:5ω3); DHA, docosahexaenoic acid (22:6ω3); DOC

(dissolved organic carbon); LA, linoleic acid (18:2ω6); GLA, α-linolenic acid (18:2ω3); DPA, docosapentaenoic acid (DPA); N, north; PUFA, poly-unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; PC, particulate carbon; PN, particulate nitrogen; PP, particulate phosphorus; PRES, present; S, south; SAFA, saturated fatty acids; TN, total nitrogen; TP, total phosphorus; m:pωx, fatty acid with "m" carbon atoms, "p" double bonds (if more than one) situated "x" carbons from the terminal methyl group of the molecule.

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 (Bret and Müller-Navarra 1997; Weers and Gulati 1997). The majority of these studies were conducted in laboratory conditions, directly on algal cultures (e.g. Boersma and Schöps 2001), or indirectly, by studying effects on consumers (e.g. Wacker and von Elert 2001), and very few *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 most authors agree 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 diatoms which would 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 largely responsible for variations in 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.

Studies have shown that 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 and al. 1998), most information on food quality involves the C:P ratio owing to its greater predictive power on zooplankton growth (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 assuring many metabolic processes (Wetzel 2001). However, the power of C:P as a food quality indicator is of limited value when the P content in the environment is sufficient and/or similar between sites compared (von Elert and Stampfl 2000).

Another useful indicator of food quality is the fatty acid content of the biofilms. FA belong to one of three groups depending on their degree of desaturation: SAFA, MUFA, and PUFA. 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 banks, which increase as a result of

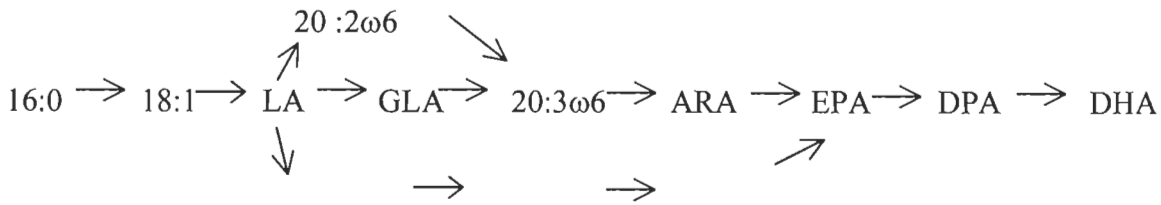
exposures to stressful environmental conditions, such as variations in temperature, nutrients, and light climate. In contrast, PUFA are major constituents of polar lipids, which are present in cellular and chloroplast membranes in algae. In organisms, PUFA function as neural transmitters, they are necessary for proper vision, and ensure thermal protection in cold environments (Arts and al. 2000). Certain PUFA can be defined as essential (EFA) meaning that they cannot be synthesized de novo from linoleic acid (18:3ω6 or LA) and α-linolenic acid (18:3ω3 or ALA) in sufficient amounts to optimize physiological performance (Arts et al. 2001, Cunnane 1996). They must therefore be obtained by dietary sources, such as algae, which correspond to important sources of EFA. 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). The biosynthetic pathways (Fig. 2.1) 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 of PUFA in algae is less understood than that of higher plants and little is known about the mechanisms influencing their synthesis. However, strong evidences indicate that it is influenced by light quality and quantity (Bigogno et al. 2002; Khozin-Goldberg et al. 2002). In fact, lipid content of biofilms should be greater when lipid synthesis is light saturated, i.e. between 300-800 $\mu mol \bullet m^{-2} \bullet s^{-1}$ (Wainman 1998).

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 is still unclear. We hypothesize that grazers will selectively remove cells of richer food quality, thus decreasing biofilm EFA content.

Lake St. Pierre is characterized by three main water masses that differ in optical properties (Frenette et al. 2003), thus constituting a natural experimental site for intra-lake comparison 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 community structure amongst the north and south water masses of Lake Saint-Pierre. These waters are separated by the fast-flowing maritime channel and are respectively altered by their incoming tributaries that irrigate lands highly influenced by agricultural, industrial, and farm activities. They correspond to important retention zones in which organisms may have ample time to adapt and develop. The algal communities which develop in these habitats are likely to have experienced a natural selection process owing in part to their light environment. Furthermore, present environmental changes, such as, ozone deterioration and global warming will alter light regimes available to algae, modifying the present communities and urge us to fully understand how foodwebs will be affected by these changes.

Figure 2.1 : 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. Diagram modified from Bigogno et al. (2002) and Khozin-Goldberg (2002).


20 :2ω6
16:0
18:1
LA
GLA
20:3ω6
ARA
EPA
DPA
DHA

Materials and Methods

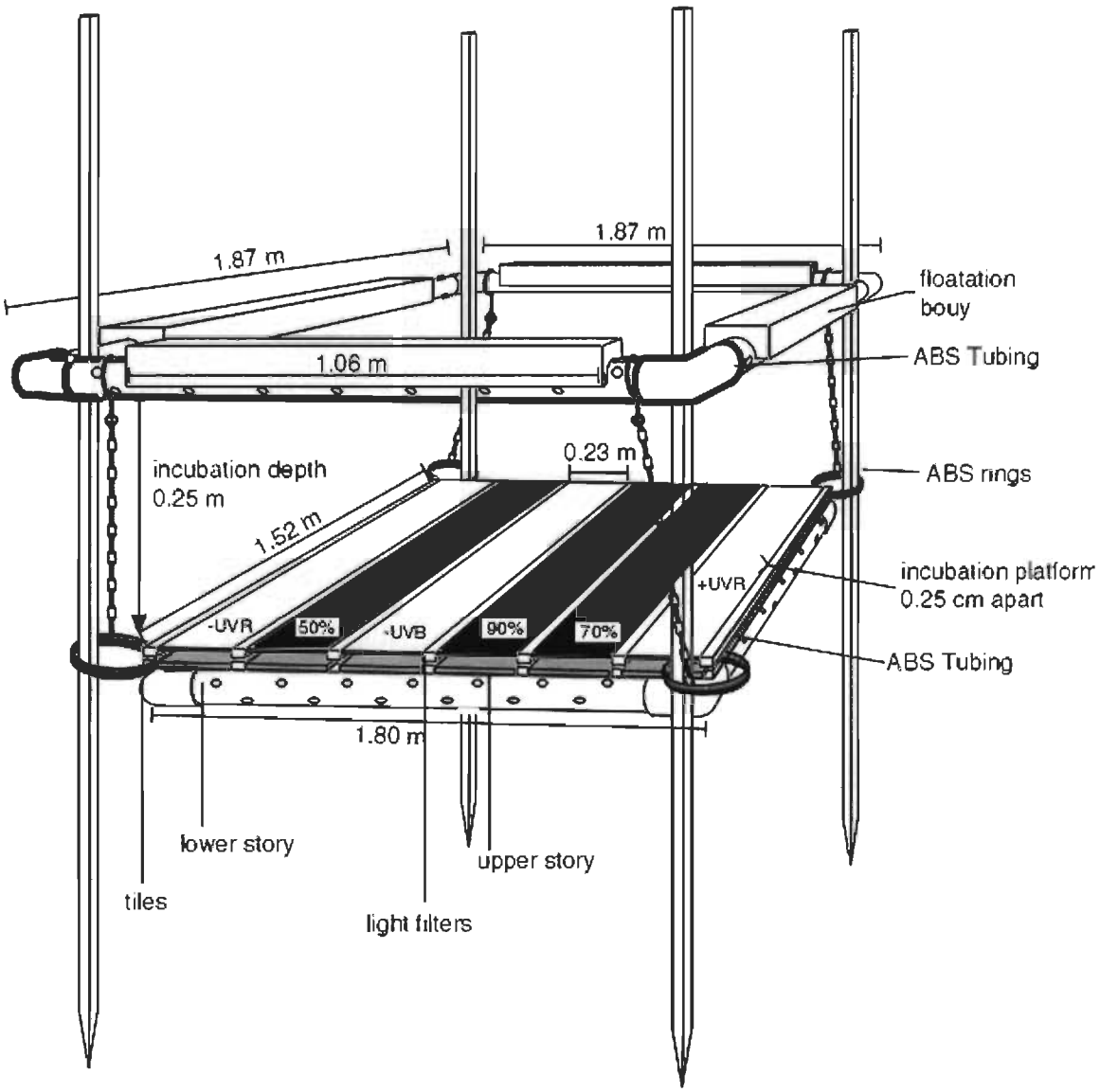
Study area

Incubation devices (Fig. 2.2) were deployed in the north (lat: 46°12', long: 72°55') and south (lat: 46°8', long: 72°51') water masses of Lake St. 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 along the St. Lawrence River and occupies a mean annual area of 472 km^2 (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. Furthermore, water chemical analyses at these two sites showed no significant differences in terms of total nitrogen and phosphorus.

Experimental setup

Aluminum rafts were designed, consisting of 2 stories: a lower one 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 six light filters were fixed. Three of these consisted of acrylic sheeting and/or polyester film (Mylar) with different UVR cutoffs: 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

Figure 2.2: 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
1.80 m
lower story
upper story
tiles
light filters

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 window screen material varying in pore size, and 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 to a square 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 in summer 2002 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 placed in the north and in the south at the incubation sites of the previous summer.

The stakes were removed after 28 d of incubation (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 similarly 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 solution. The plants were placed in pre-weighed aluminium trays in the oven at 60°C for 24 h for dry weight information. Identification of algae was done to the main algal groups (diatoms, chlorophytes, and cyanpbacteria). 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 50mL 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 and soluble reactive phosphorus (SRP) were obtained after acid digestion, followed by the addition of ammonium molybdate, which is reduced with stannous chloride to form a molybdenum blue complex measured spectrophotometrically at 660 nm. Total N, nitrites-nitrates (NO_3-NO_2), and ammoniac (NH_3-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) for the two sites 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 by descending a spectroradiometer (Model PUV-2545, Biospherical Instruments, San Diego, USA) through the water column every

2 to 3 d at midday, 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 periphytic material was placed in cryogenic 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 periphytic material, which was 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 (PC) and nitrogen (PN), and 3) pre-combusted and acid pre-washed 47 mm Millipore glass fibre filters for particulate organic phosphorus (PP) contents. PC, PN, 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 PC, PN, and PP, were carried out at the National Laboratory for Environmental Testing (NLET, Burlington, Ontario). PC and PN 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 chromatographer (Hewlett Packard 6890 Series GC) using a splitless injection Supelco column (SP-2560) of 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. 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 (cyanobacteria, chlorophytes, and diatoms) as the dependant variables. The differences in taxonomic composition between tile, rock and plant substrates were 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 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 once the experiment had begun, 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 thus reduced. On account of this, as well as high standard deviations caused by unexplainable 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 so remained as was. Consequently, the main effects were: site with two levels (north (N), south (S)), light (L) with four levels (no UVB, noUVR, HL, LL), and grazing (G), with two levels (grazer presence (PRES), 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 evaluate further the

associations between nutrients, biomass, stoichiometry, and lipids, we used a principal components ordination biplot (Fig. 2.3).

Results

Experimental setup

The clay tiles were abundantly colonized by biofilms. Their regulated incubation depth with respect to surface water allowed for a thorough monitoring 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. 2.4). 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 St. 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.

Chemical and physical 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. 2.5) and total suspended particles concentrations. The measurements of downward irradiance at

the incubation depths (0.03 m from the surface) measured at midday every 2 to 3 d were averaged over the incubation period along with the light irradiances corresponding to the neutral density filters (Table 2.1). 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 $mg \cdot L^{-1}$), NO_2-NO_3 (average = 0.045 $mg \cdot L^{-1}$), NH_3 (average = 0.015 $mg \cdot L^{-1}$), SRP (average = 0.007 $mg \cdot L^{-1}$) and P (average = 0.03 $mg \cdot L^{-1}$) did not vary significantly between the two sites (north and south). However silicates (SiO_2) were greater in the north (average = 1.47 ± 0.40 $mg \cdot L^{-1}$ and 0.74 ± 0.20 $mg \cdot L^{-1}$, north and south respectively). The values for TN and TP corresponded to aquatic trophic states 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, cyanpbacteria, chlorophytes), relative abundance of chlorophytes was greatest in the north and south (Fig. 2.6), 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 equalled 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 2.2) revealed greater biomasses ($p < 0.001$) in the south biofilms, with respect to Chl*a*, PC, PN, 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 2.2, Fig. 2.6). 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 2.2). Amongst the linolenates, DPA ($p < 0.001$) and ALA (p-value = 0.019) were greater in the south, while greater contents of EPA, DHA, and 20:3ω3 ($p < 0.001$) were found in the north. The ω3:ω6 ratio revealed that linolenates were 4X and 3X greater than linoleates in the north and south, respectively (p-value < 0.001) (Table 2.2, Fig. 2.6).

All the individual FA varied significantly with site, with the exception of 20:3ω6 and 22:1ω9 (Table 2.2; Fig. 2.3). The biofilms in the south were associated with greater biomass (Chl*a*, and AFDW) and utrient (PC, PN, PP)values, along with these UFA: ALA, LA, ARA,DPA, and 20:3ω6, whereas the north biofilms contained the following PUFA: EPA, DHA, 18:3ω6, 20:3ω3, 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.

Figure 2.3: Ordination biplot illustrating the association between biomass, nutrient content, stoichiometry and fatty acids for the two sites (north and south) and for the grazer treatments (PRES and ABS). All the variables were mainly influenced by the effect of site (primary order of selection). 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.

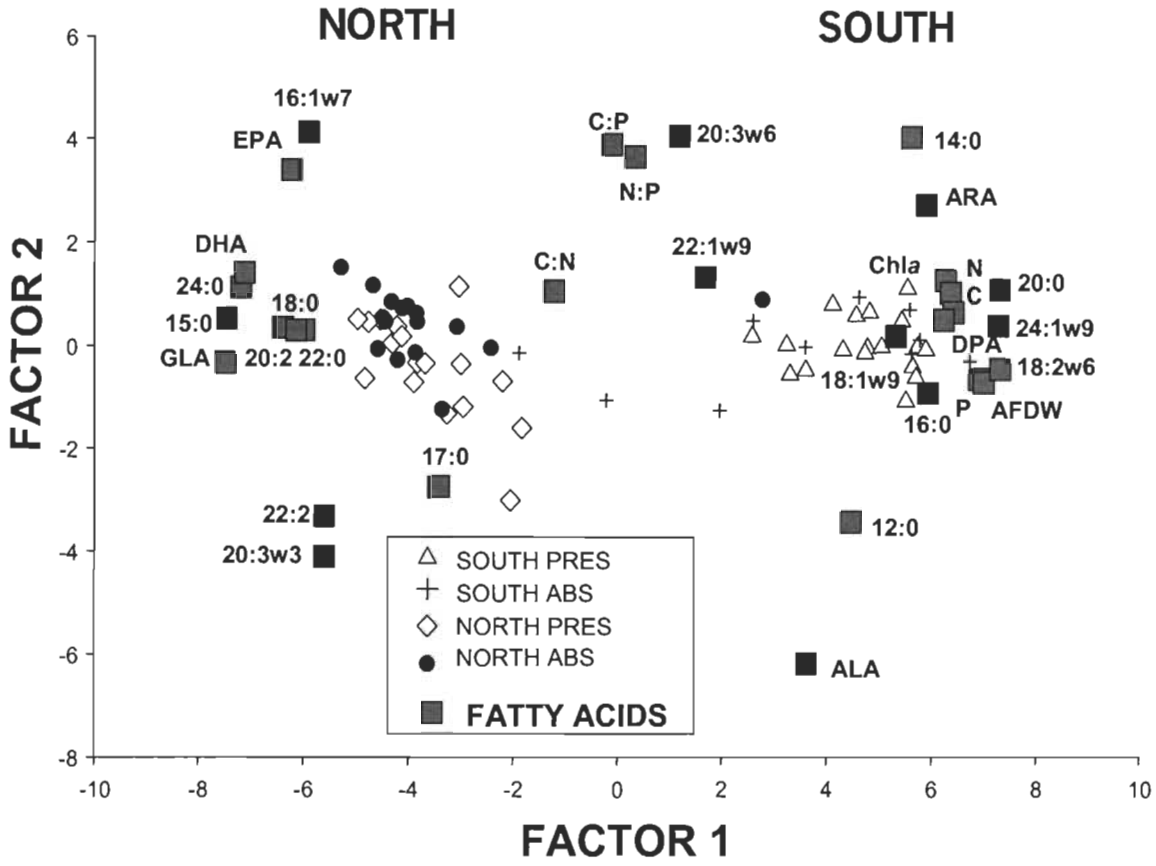

NORTH
SOUTH
16:1w7
EPA
C:P
20:3w6
N:P
14:0
ARA
DHA
24:0
15:0
GLA
18:0
20:2
22:0
C:N
22:1w9
Chla
N
C
20:0
24:1w9
DPA
18:2w6
18:1w9
P
AFDW
16:0
17:0
22:2
20:3w3
12:0
ALA
SOUTH PRES
SOUTH ABS
NORTH PRES
NORTH ABS
FATTY ACIDS
FACTOR 2
FACTOR 1
6
4
2
0
-2
-4
-6
-8
-10
-8
-6
-4
-2
0
2
4
6
8
10

Figure 2.4: Relative biomass (%) of cyanobacteria, chlorophytes, and diatoms growing on natural substrates (plants, rocks) and artificial substrates (tiles) in the north and south for an incubation period of 28 d (July 30 to August 26).

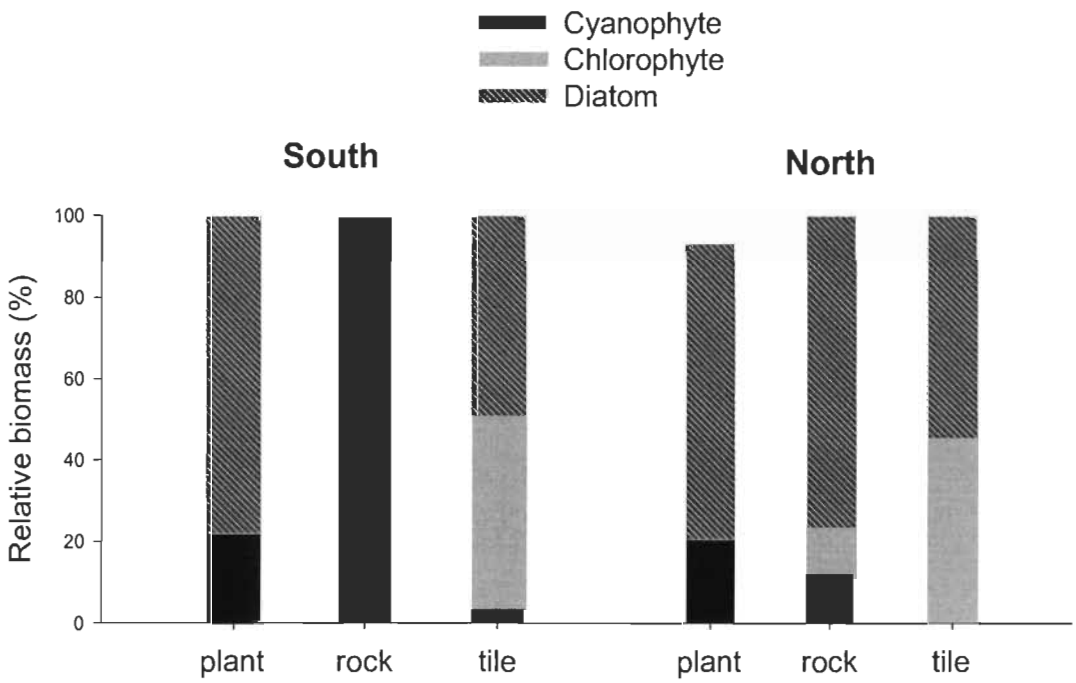

Cyanophyte
Chlorophyte
Diatom
South
North
Relative biomass (%)
100
80
60
40
20
0
plant
rock
tile
plant
rock
tile

Figure 2.5: 1% UVB (○), UVA (∇), and PAR (□) penetration depths (on left y-axis) and CDOM (▼) concentrations (absorption at 340nm) (on right y-axis). CDOM concentrations are missing for certain dates.

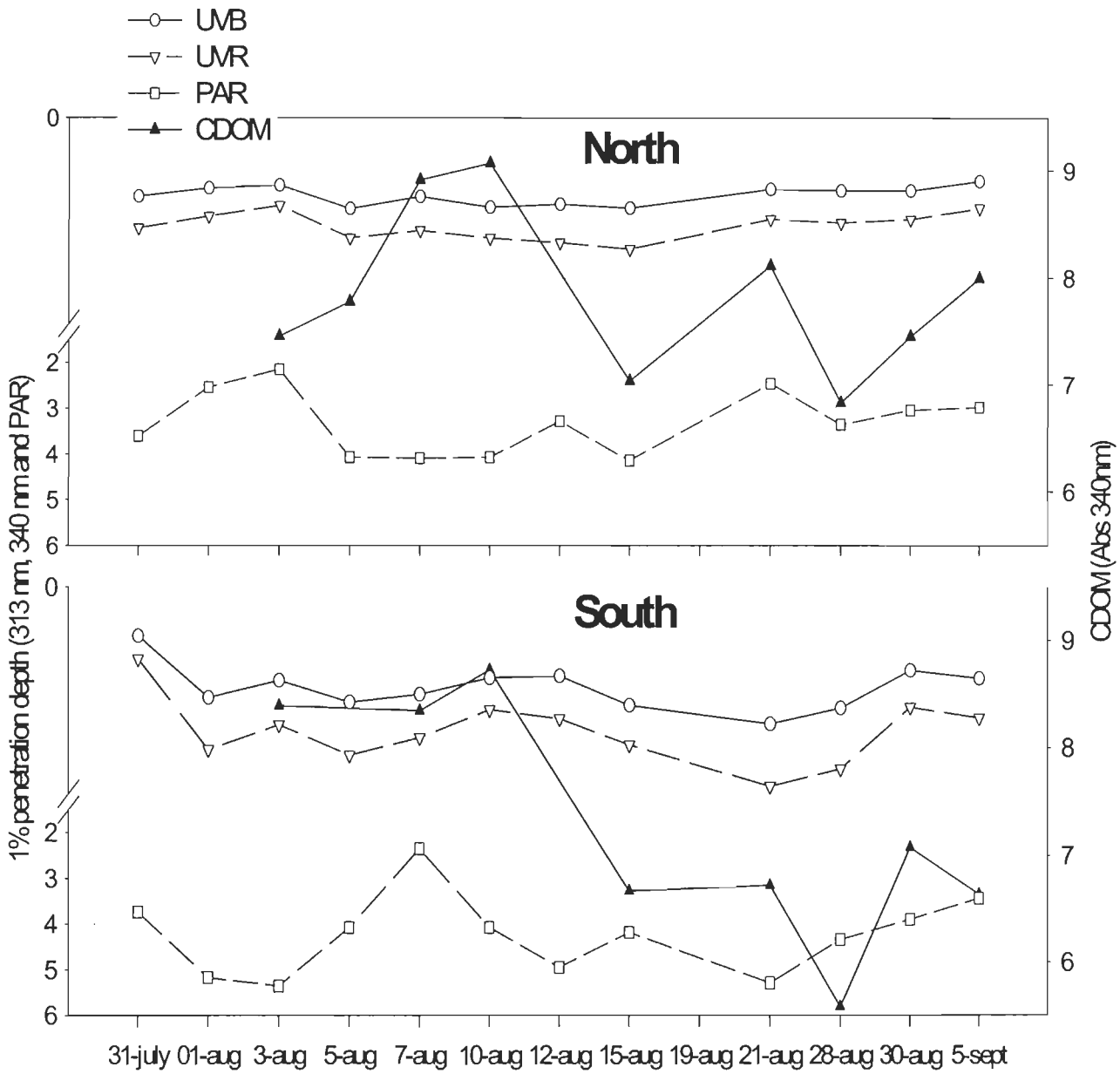
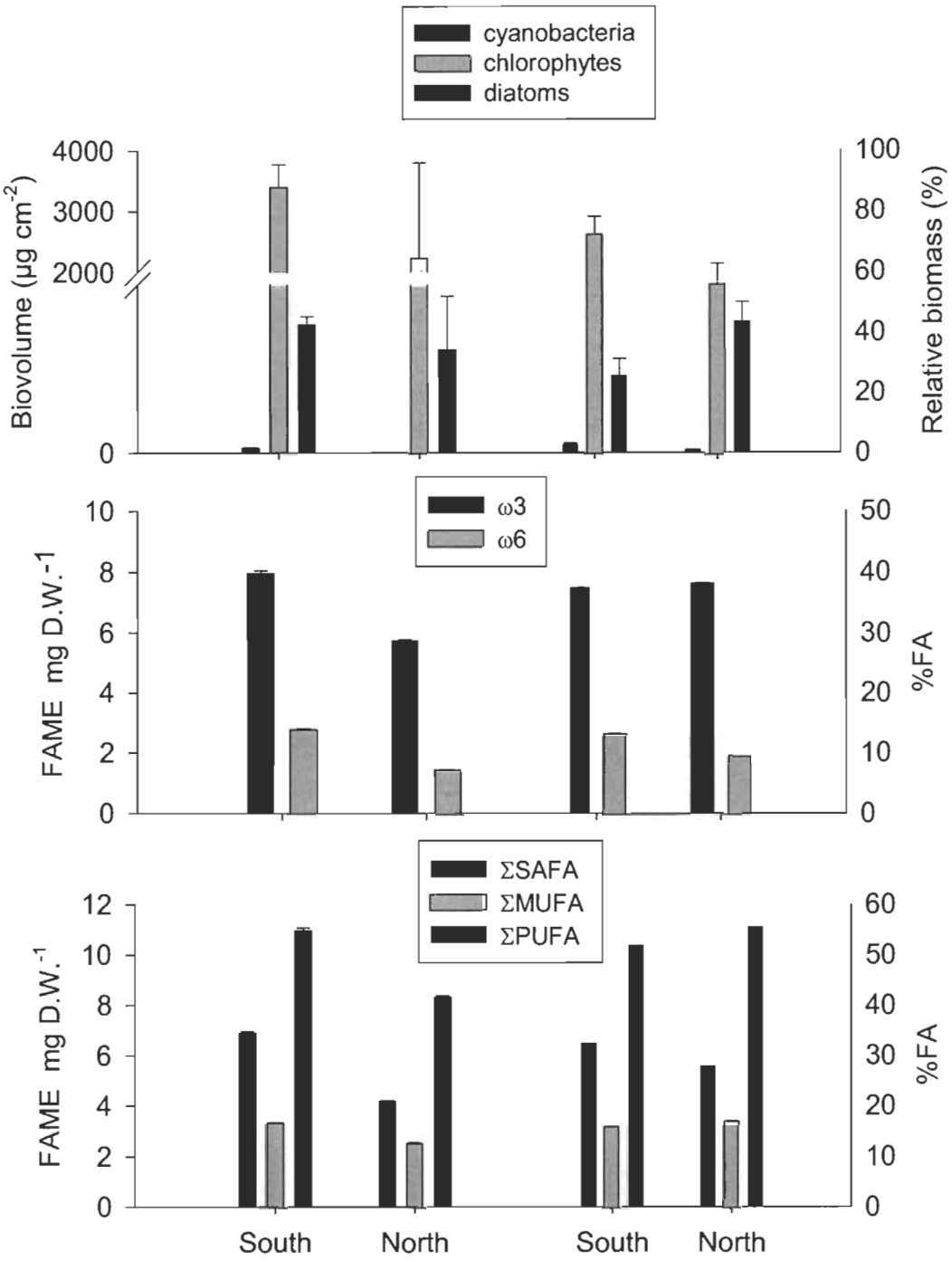

UVB
UVR
PAR
CDOM
North
South
1% penetration depth (313 nm, 340 nm and PAR)
CDOM (Abs 340nm)
0
2
3
4
5
6
9
8
7
6
31-july
01-aug
3-aug
5-aug
7-aug
10-aug
12-aug
15-aug
19-aug
21-aug
28-aug
30-aug
5-sept

Table 2.1: Average UVB ($W \cdot m^{-2}$), UVA ($W \cdot m^{-2}$), and PAR ($\mu mol \cdot m^{-2} s^{-1}$) irradiances measured at midday at the incubation depth every 2 to 3 d.

		North	South
	UVB	0.02	0.06
	UVA	0.13	0.41
HL treatment ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	100% PAR	601	796
	90% PAR	540	716
LL treatments ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	70% PAR	420	557
	50% PAR	300	398

Figure 2.6: Absolute and relative biomass (%) of cyanobacteria, chlorophytes, and diatoms growing in the artificial substrates (tiles) and absolute and % FA content of SAFA, MUFA, PUFA, ω3 and ω6 at the two study sites (south and north) incubated for a period of 27 to 29 d (July 24 & 25 until Sept. 9, 10, & 11).


cyanobacteria
chlorophytes
diatoms
Biovolume (µg cm-2)
Relative biomass (%)
4000
3000
2000
0
100
80
60
40
20
0
ω3
ω6
FAME mg D.W.-1
%FA
10
8
6
4
2
0
50
40
30
20
10
0
ΣSAFA
ΣMUFA
ΣPUFA
FAME mg D.W.-1
%FA
12
10
8
6
4
2
0
60
50
40
30
20
10
0
South
North
South
North

Effect of light

The differing light regimes within each site did not account for any significant variations 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 the sole effects of altering light regimes within each site; i.e., 12:0, (p-value = 0.007), 16:1ω7 (p-value= 0.045), 20:3ω6 (p-value = 0.035), ω3 (p-value = 0.004), ω3/ω6 (p-value = 0.007), and MUFA (p-value = 0.015). The LL treatment was significantly greater than HL in the case of 12:0, greater than noUVB for ω3/ω6 and greater than HL and noUVB for ω3. However, LL was lower than HL for 20:3ω6 and lower than noUVB in the case of 16:1ω7 and MUFA (Table 2.2). 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 responsible for increasing linoleate content in the north.

Effect of grazing

The two-way ANOVA revealed that presence of grazers slightly increased diatom relative biomass (p = 0.049). PC (p = 0.057), PN (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-value = 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

Table 2.2: 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 significantly different values under the light treatments are differentiated from the others with * or **.

	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	**27.67 (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 (1.08)**	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)
20:3ω3	**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)
ω3	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)****
ω3: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)

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-value = 0.062)and 14:0 (p-value = 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. For 20:3ω3 and 17:0, the effects of grazing were little to undetectable in the south, while it increased their content in the north (Fig. 2.7). 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 (site X light X grazing) was significant for PP (p-value = 0.027) and PUFA (p-value = 0.038) content (Fig. 2.8, Table 2.3). 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, four-fold greater than other samples 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 2.3).

Discussion

The effect which most strongly influenced food quality of the biofilms corresponded to that of site, i.e., their location in the lake, while the effects caused by the light filters and grazers within each water mass had lesser of an 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 of selection on the algal communities, while the treatments of light and grazing within each 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 days, depending on yearly 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.

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. 2.5), 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 (PC, PN, PP) and

Figure 2.7: % FA (14:0, 20:3ω3, ω3, 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).

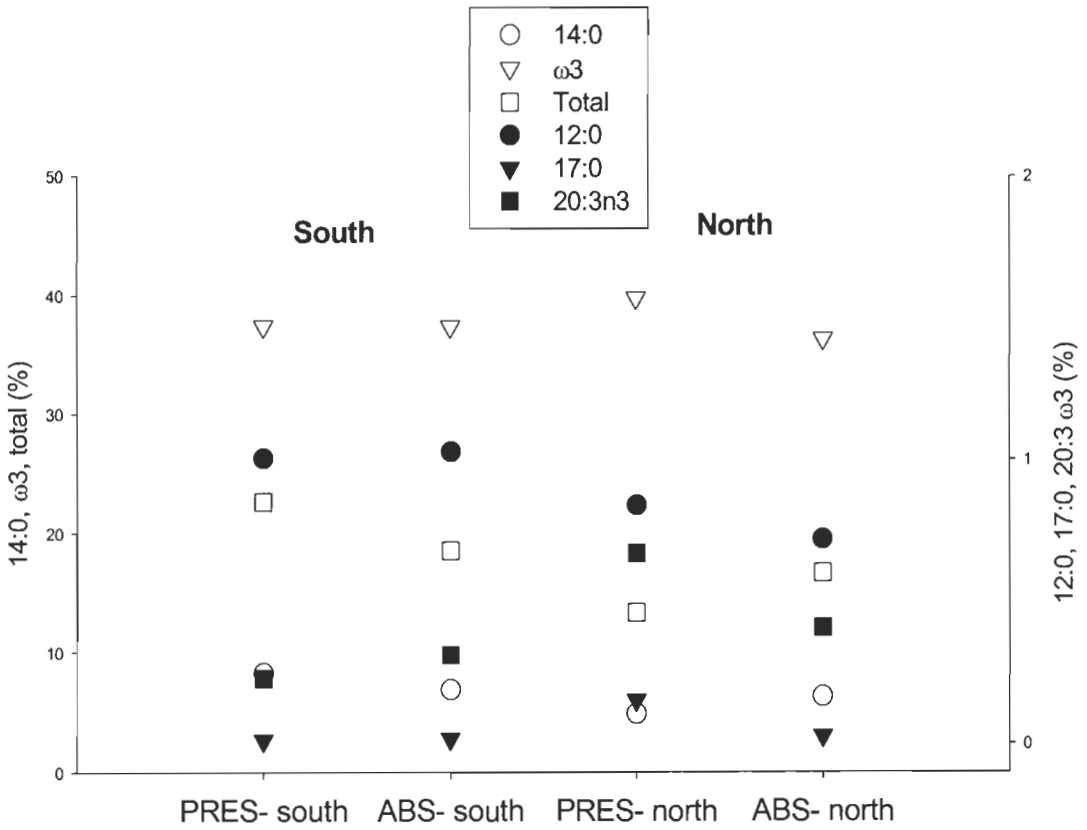
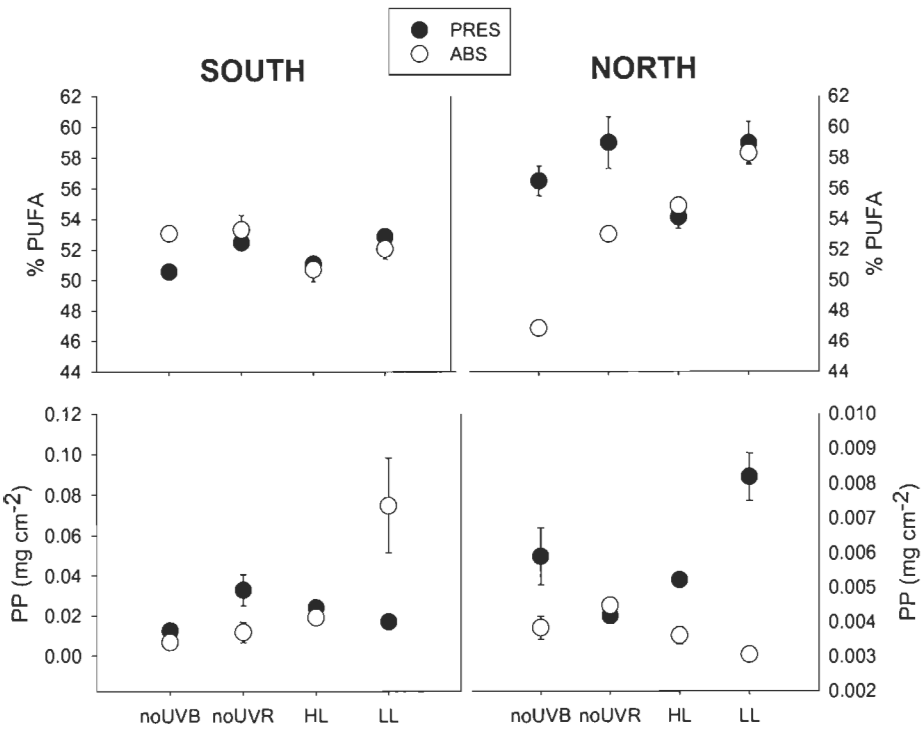

14:0
ω3
Total
12:0
17:0
20:3n3
South
North
14:0, ω3, total (%)
12:0, 17:0, 20:3 ω3 (%)
0
10
20
30
40
50
0
1
2
PRES- south
ABS- south
PRES- north
ABS- north

Table 2.3: 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, while 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)**		*

Figure 2.8: Significant triple interaction (site X light X grazing) for PUFAs (p-value = 0.038) and PP (p-value = 0.027).


PRES
ABS
SOUTH
NORTH
% PUFA
PP (mg cm-2)
noUVB
noUVR
HL
LL

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 adversely to high light and UVR intensities by producing a shift towards a more 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 in terms of absolute quantities the differences in the underwater light environment between sites resulted in greater biovolumes in the south for all classes examined. This was probably a result of greater PAR which allowed for a greater growth of the algal cells. However, we hypothesize that the greater UVR exposure in the south was the most probable cause for the decrease in diatom relative abundance and the increase in larger, filamentous chlorophytes and cyanobacteria (Fig. 2.6). This supports recent findings that diatoms are particularly sensitive to UVR exposure (e.g. Vinebrooke and Leavitt 96, Rae and Vincent 1998, Francoeur and Lowe 98, 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 and 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. 2.1), 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 accumulations 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 present in polar lipids (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, thus bringing about subsequent decreases in %PUFA, notably EPA and DHA (Wang and Chai 1994).

The composition in FA of the lipids analyzed varied largely amongst the sites. Based on absolute quantities, total lipids, ΣPUFA, ΣMUFA, ΣSAFA were greater in the south. However, based on 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 FA content in the south biofilms contained greater relative contents of

18:1ω9, ALA, LA, ARA, and 20:3n6, which are precursors involved 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 pre-dominance by diatoms with respect to other algal groups (Léveillé et al. 1997), supporting our community structure analysis of greater relative abundance of 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 of site and the light climate present at each site were primarily responsible for the differing algal community structures. Physiological mechanisms probably allowed them to adjust their fatty acid biosynthetic pathways in order to attain FA profiles to optimize their survival in each respective environment, 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 the more energetic wavelengths of PAR or high light intensities 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 modify the proportion of UVB radiation, with that

of other wavelengths, reaching the earth's surface. If the rate of these changes exceeds that to which the algal communities may adapt, this could have serious implications on autotrophic nutritional quality and quantity.

The few effects observed were in terms of FA; two were part of the PUFA group (sum of linolenates and 20:3ω6) along with 12:0, the sum of all MUFA, and 16:1w7. These fine alterations in cell FA biosynthesis 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. 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 grazing increased nutrient content of the cells (C, N, and usually P) and biomass (Chl *a*, AFDW). Similar increases in PC and PN 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 initial hypotheses, grazing did not significantly decrease PUFA content. No other studies that we know of have examined in detail how grazing affects the 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). Few amongst these have discussed issues of quantity vs. quality based on fatty acid 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 (Demott and Muller-Navarra 1997; Weers and Gulati 1997; von Elert 2002). Although some are capable of desaturation and elongation of shorter chained, 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). With respect to our results, we have demonstrated that the biofilms in the south contained a greater quantity of food owing to the greater biomass, nutrient and fatty acid content. However, on the basis of relative fatty acids, the biofilms in the north contained greater proportions of good quality fatty acids in relation to ones of lesser quality.

In the presence of lower quality food, certain grazers have developed feeding compensation behaviours 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 quantifications of grazer densities were performed in this study, personal observations allowed us to confirm the dominance of *Gammarus lacustris* as the main grazing community on our tiles in the north and south. 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), the quality of their ingested food could have important repercussions on the productivity of this lake.

Our findings clearly suggest that light regime within the water column, which is directly affected by fluctuating water levels, plays a major role 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 verify these effects *in situ*, and more precisely, in a fluvial lake where harsh hydrodynamic conditions persisted, which is unprecedented to our knowledge. Future studies on the determinant of food quality in aquatic systems should emphasize natural communities of algae. Our upcoming research will consist of detailed analyses of feeding behaviours and resulting effects on the fitness of key species within each habitat to fully determine the effects of food quality and/or quantity in trophic transfers.

References

1. Ahlgren, G., Lundstedt, L., Brett, M., and Forsberg, C. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research **12**: 809-818.

2. Arts, M. T., Ackman, R. G., and Holub, B. J. 2001. "Essential fatty acids" in aquatic ecosystems: a crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. **58**: 122-137.

3. Arts, M. T., Robarts, R. D., and Evans, M. S. 1997. Seasonal changes in particulate and dissolved lipids in a eutrophic prairie lake. Freshwater Ecology. **38**: 525-537.

4. Arts, M. T., Rai, H. 1997. Effects of enhanced ultraviolet-B radiation on the production of lipid, polysaccharide and protein in three freshwater algal species. Freshwater Biology. **38**: 597-610.

5. Bigogno, C., Khozin-Goldberg, I., and Cohen, Z. 2002. Accumulation of arachidonic acid-rich triacylglycerols in microalga *Parietochloris incisa* (Trebuxiophyceae, Chlorophyta). Phytochemistry **60**: 135-143.

6. Bligh , E. G. and Dyer, D. L. 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911-917

7. Boersma, M. and Schöps, C. 2001. Nutritional quality of seston for freshwater herbivore *Daphnia galeata* X *hyalina*: biochemical versus mineral limitations.

Oecologia **129**: 342-348.

8. Bothwell, M.L., Sherbot, D., Roberge, A.C., and Daley, R.J. 1993. Influence of natural ultraviolet radiation on lotic periphyton diatom community growth. Journal of Phycology **29**: 24-35.

9. Brett, M.T. and Müller-Navarra, D.C. 1997. The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biology **38**: 483-500.

10. Cruz-Rivera, E. and Hay, M.E. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology **81**: 201-219.

11. Cunnane, S.C. 1996. Recent studies on the synthesis, β-oxidation, and deficiency of linoleate and a-linolenate: are essential fatty acids more aptly named indispensable or conditionally dispensable fatty acids?. Canadian Journal of Physiology and Pharmacology **74**: 629-639.

12. DeMott, W.R. and Müller-Navarra, D. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia,* a cyanobacterium and lipid emulsions. Freshwater Biology **38**: 649-664.

13. DeMott, W.R., Gulati, R.D., and Siewertsen, K. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of Daphnia magna. Limnology and Oceanography **43**: 1147-1161.

14. Francoeur, S.N. and Lowe, R.L. 1998. Effects of ambient ultraviolet radiation on littoral periphyton: biomass accrual and taxon-specific responses. Journal of

Freshwater Ecology **13**: 29-37.

15. Frenette J.-J., Arts M.T., and Morin J. 2003. Spectral gradients of downwelling light in a fluvial lake (Lake St. Pierre, St-Lawrence River). Aquatic Ecology **37**: 77-85.

16. Frenette, J.-J., Vincent, W.F., and Legendre, L. 1998. Size-dependent C : N uptake by phytoplankton as a function of irradiance: Ecological implications . Limnology and Oceanography **43** : 1362-1368.

17. Frost, P. C. and Elser, J.J. 2002. Growth responses of littoral mayflies to the phosphorus content of their food. Ecology Letters **5**: 232-240.

18. Goes, J.I., Handa, N., Taguchi, S., and Hama, T. 1994. Effect of UV-B radiation on the fatty acid composition of the marine phytoplanktoer *Tetraselmis* sp.: realtionship to cellular pigments. Marine Ecology Progress Series **114**: 259-274.

19. Goulden, C.E. and Place, A.R. 1993. Lipid-accumulation and allocation in daphniid-cladocera. Bulletin of Marine Science **53** (1): 106-114.

20. Gulati, R.D. and Demott, W.R. 1997. The role of food quality for zooplankton: remarks on the state-of -the-art, perspectives and priorities. Freshwater biology **38**: 753-768.

21. Hessen, D.O., Faerovig, P.J., and Andersen, T. 2002. Light, nutrients, and P : C ratios in algae: grazer performance related to food quality and quantity. Ecology **83**: 1886-1898.

22. Hill, W.R., Ryon, M.G., and Schilling, E.M. 1995. Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology **76**: 1292-1309.

23. Hillebrand, H. and Kahlert, M. 2001. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. American Society of Limnology and Oceanography **46**: 1881-1898.

24. Hudon, C., Paquet, S., and Jarry, V. 1996. Downstream variations of phytoplankton in the St. Lawrence River (Québec, Canada). Hydrobiologia **337**: 11-26.

25. Khozin-Goldberg, I., Didi-Cohen, S., Shayakhmetova, I., and Cohen, Z. 2002. Biosynthesis of eicosapentaeneoic acid (EPA) in the freshwater Eustigmatophyte *Monodus subterraneous* (Eustigmatophyceae). Journal of Phycology **38**: 745-756.

26. Kirk, J.T.O. (1994) Light and Photosynthesis in Aquatic Ecosystems. Cambridge, University Press.

27. Klyachko-Gurvich, G.L., Tsoglin, L.N., Doucha, J., Kopetskii, J., Shebalin (Ryabykh), I.B., and Semenenko, V.E. 1999. Desaturation of fatty acids as an adaptive response to shifts in light intensity. Physiologia Plantarium **107**: 240-249.

28. Lamberti, G.A., Ashkenas, L.R., Gregory, S.V., and Steinman, A.D. 1987. Effects of three herbivores on periphyton communities in laboratory streams. Journal of North American Benthological Society **62**: 92-104.

29. Léveillé, J.-C., Amblard, C., and Bourdier, G. 1997. Fatty acids as specific algal markers in a natural lacustrian phytoplankton. Journal of Plankton Research **19**: 469-490.

30. McNamara, A.E. and Hill, W.R. 2000. UV-B irradiance gradient affects photosynthesis and pigments but not food quality of periphyton. Freshwater Biology **43**: 649-662.

31. Müller-Navarra, D.C. 1995. Biochemical versus mineral limitation in *Daphnia*. Limnology and Oceanography **40**: 1209-1214.

32. Neale, P.J., Banaszak, A.T., and Jarriel, C.R. 1998. Ultraviolet sunscreens in *Gymnodinium sanguineum* (dinophyceale): mycosporine-like amino acids protect against inhibition of photosynthesis. Journal of Phycology **34**: 928-938.

33. Quesada, A, Mouget, J.-L., Vincent, W. F. 1995. Growth of Antarctic cyanobacteria under ultra-violet radiation: UVA counteracts UVB radiation. Journal of Phycology. **31**: 242-248.

34. Rae, R. and Vincent W.F. 1998. Effects of temperature and ultraviolet radiation on microbial foodweb structure: potential responses to global change. Freshwater Biology **40**: 747-758.

35. Steinman, A.D., Mc Intire, Gregory, S.V., Lamberti, G.A., and Ashkens, L.R. 1987. Effects of herbivore type and density on taxonomic structure and physiognomy of

algal assemblages in laboratory streams. J. N. Am. Benthol. Soc. **6**: 175-188.

36. Sterner, R.W. 1997. Modeling interactions of food quality and quantity in homeostatic consumers. Freshwater Biology **38**: 473-481.

37. Sterner, R.W., Elser, J.J., Fee, E.J., Guildford, S.J., and Chrzanowski, T.H. 1997. The light : Nutrient Ratio in Lakes: The balance of Energy and Materials Affects Ecosystem Structure and Process. The American Naturalist **6**: 663-684.

38. Sterner, R.W. and Hessen, D.O. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Ann. Rev. Ecol. Syst. **25**: 1-29.

39. Sterner, R.W. and Schulz, K.L. 1998. Zooplankton nutrition: recent progress and a reality check. Aquatic Ecology **32**: 261-279.

40. Urabe, J., Clasen, J., and Sterner, R.W. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? Limnology and Oceanography **42**: 1436-1443.

41. Urabe, J. and Sterner, R.W. 1996. Regulation of herbivore growth by the balance of light and nutrients. Ecology **93**: 8465-8469.

42. Vinebrooke, R.D. and Leavitt, P.R. 1996. Effects of ultraviolet radiation on periphyton in an alpine lake. Limnology and Oceanography **41**: 1035-1040.

43. von Elert, E. 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnology and Oceanography **47**: 1764-1773

44. von Elert, E. and Stampfl, P. 2000. Food quality for *Eudiaptomus gracilis*: the importance of particular highly unsaturated fatty acids. Freshwater Biology **45**: 189-200.

45. Wacker, A. and von Elert, E. 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. Ecology **82**: 2507-2520.

46. Wainman, B. C., Smith, R. E. H., Rai, H., and Furgal, J. A. (1998) Irradiance and Lipid Production in Natural algal Populations in Lipids in Freshwater Ecosystems. Springer-Verlag, New York.

47. Wang K.S. and Chai T.-J. 1994. Reduction in omega-3 fatty acids by UV-B irradiation in microalgae. Journal of applied phycology **6**: 415-421.

48. Watkins, E.M., Schindler, D.W., Turner, M.A., and Findlay, D. 2001. Effects of solar ultraviolet radiation on epilithic metabolism and nutrient community composition in a clear-water boreal lake. Can. J. Fish. Aquat. Sci. **58**: 2059-2070.

49. Weers, P.M.M. and Gulati, R.D. 1997. Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnology and Oceanography **42**: 1584-1589.

50. Wellnitz, T.A. and Ward, J.V. 1998. Does light intensity modify the effect mayfly grazers have on periphtyon? Freshwater Biology **39**: 135-149.

51. Wetzel, R. G. (2001) Limnology. Lake and River Ecosystems. Third Edition.

Academic Press, San Diego.

52. Wetzel, R. G. and Likens, G. E. (2000) Limnological Analyses. Third Edition. Springer-Verlag, New York.

53. Zhekisheva, M., Boussiba, S., Khozin-Goldberg, I., Zarka, A., and Cohen, Z. 2002. Accumulation of oleic acid in *Haematococcus pluvialis* (Chlorophyceae) under nitrogen starvation or high light is correlated with that of astaxanthin esters. Journal of Phycology **38**: 325-331.

ANNEXE A

Manuscripts submitted for publication and communications concerning editorial matters should be sent to:
Editorial Office
Aquatic Ecology
P.O. Box 990
3300 AZ Dordrecht
The Netherlands
Fax +31-78-6576254
No page charges are applicable, but prospective authors should condense their pages as much as possible.
Aquatic Ecology welcomes research papers, review papers and proceedings of thematic symposia/workshops.
General papers describing original research should not exceed twenty pages of printed text, including tables, figures and references (one page of printed text = approximately 600 words). These will usually be published within five months after acceptance.
Short Communications should not exceed 4 printed pages in total. A short communication does not contain a separate introduction and materials and methods section, but instead results and references directly follow a short abstract.
Papers already published or in press elsewhere will not be accepted. If any part of the subject matter or experiments included in a manuscript submitted to the journal has been the subject of any prior publication, this prior publication must be identified.
The journal encourages the submision of manuscripts on floppy disk (see `Preparing Text on Floppy Disk' below). Submitting your text on floppy disk should expedite publication.
Conventionally typed manuscripts should conform to the following guidelines:
Manuscripts should be written in correct English. Four copies should be submitted (the original manuscript plus three carbon or photocopies, each including all tables, figures and references). Besides, a hard copy, a text on diskette is highly appreciated. Four sets of original halftones are required. The author should retain a complete copy of the manuscript. Manuscripts should be typed clearly, double-spaced throughout on one side of A4 paper with margins of 3--5 cm. All pages (including the tables, figures, legends and references) should be numbered consecutively. As a guide for acceptable style please consult: Council of Biology Style Manual, 6th edition (1987), available from the American Institute of Biological Sciences, 9650 Rockville Pike, Bethesda, MD 20814, USA.
The manuscript should be arranged in the following order.
Title page (page 1)
the title should be brief but informative.
a subtitle may be used to supplement and thereby shorten an excessively long main title.
the Author's full name (if more than one, use `and' before the last author's name and indicate to whom correspondence should be addressed).
Affiliation(s)/Address(es) should be complete, and should include a fax number and/or e-mail address for correspondence.
Key words/Abstract/Abbreviations (page 2)

Key words (a maximum of 6, in alphabetical order, suitable for indexing). Key words should differ from words mentioned in the title.
Abstract (brief and informative, not to exceed 250 words). No abbreviations should be used in the abstract.
Abbreviations (arranged alphabetically; only those which are not familiar and/or commonly used).

Main Text

The text should, if possible, be developed under the following headings:
Introduction
Materials and Methods
Results
Discussion
Conclusions
The relative importance of headings and subheadings should be clear. The approximate location of figures and tables should be indicated in the margin.
New paragraphs should be indicated by clear indentations.
The use of footnotes should be avoided if possible. However, if essential, they should be typed on the appropriate page, but clearly separated from the text with a line above them.

After the main text

Acknowledgements (also grants, support, etc., if any) should follow the text and precede the references.
Notes should be numbered consecutively with superscript numerals and listed in numerical order after Acknowledgements.

References

Literature references should be listed alphabetically, typed double-spaced, and in the text referred to by author name and year of publication enclosed in parentheses, e.g. (Smith, 1990).
Citations of personal communications and unpublished data should be avoided unless necessary. Such citations should in text appear only as: (D. Wilman, pers. comm.), (C.S. Andrew, unpubl.), and not in the reference list.
Abbreviate titles of periodicals according to the style of the Bibliogrpahy Guide for Editors and Authors (Biosis, Chemical Abstract Service and Engineering Index, Inc. 1974).
References should contain: author(s), name(s) followed by author(s) initials, year, title of article (only first word and proper nouns capitalized), journal (not inderlined), volume number and inclusive page numbers. Books must include the location and name of the publisher.
Examples
Periodicals
van Gijsegem F, Somssich IE and Scheel D (1995) Activation of defense-related genes in parsley leaves by infection with *Erwinia chrysanthemi*. Eur J Plant Pathol 101: 549--559
Books (edited by someone other than author of article)
Smith EL, Austem BM, Blumenthal KM and Nyc JF (1975) Glutamate dehydrogenases. In: Boyer PD (ed.) The Enzymes. Vol. 11 (pp. 293--367) Academic Press, New York
Books (monographs)

Hicks CR (1973) Fundamental Concepts in the Design of Experiments. Holt, Rinehard and Winston, New York

Tables

Each table should be typed on a separate page

Tables should be numbered with Arabic numerals, followed by the title. Horizontal rules should be indicated; vertical rules should not be used. Table footnotes should be marked with superscript numbers.

Each table must be mentioned in the text.

Tables may be edited by the publisher to permit more compact typesetting.

Do not use abbreviations in the title.

Figures

Each figure must be mentioned in the text.

Line drawings must be in black ink on white paper or blue graph paper, and should not contain shading. Extremely small type should be avoided, since figures are often reduced in size.

Halftone reproductions must be clear well-contrasted glossy prints trimmed at right angles.

Original halftones must be supplied with each copy of the manuscript.

Colour plates will be inserted only at the author's expense. Quotes will be provided for each individual case. Colour transparancies (diapositives) give better colour plates than do photographs.

Figures as well as legends should be identified by Arabic numbers and headed `Figure 1', etc.

Where multi-part figures are used, each part should be clearly identified in the legend, preferably with lower case letters.

The top of the figure should be indicated on the back. Each figure should be identified by lightly writing the author's name and figure number on the back.

Do not give magnification or scales in the figure legends: instead draw bar scales directly, on the figures.

Do not use abbreviations in the figure legends.

Abbreviations and units

SI units should be used, e.g.: mg, g, km, m, cm, mm, ppm, cpm, Ci (Curie), l (litre), ml, s(second), min(minute), h (hour), mol, m^{-3}, kg per ha or kg ha^{-1}. The minus index form is always to be used in tables.

Use mg l^{-1}, not mg/l.

If a non-standard abbreviation is to be used extensively, it should be defined in full on page 2 and follow the abstract.

The author will be sent an offprint order form and proofs, which should be returned to the Publisher without delay. If there are typesetting problems, e.g. misplaced figures or tables, it is the responsibility of the author to contact the Publisher urgently by fax ((++)31-78-6392254). Fifty offprints will be supplied free of charge.

Manuscripts on Diskette

Format

We strongly prefer manuscripts typed on IBM-compatible computers, with operating system MS DOS (version 3.2 or higher), and word processing package WordPerfect (4.2 or higher).

We also accept files in most other word processing packages that run under MS DOS, and Apple Macintosh diskettes.
If this combination is not available to you, please contact us as soon as possible.
If you work with the Graphical User Interface *Windows* or on a Macintosh computer, use only regular fonts like Courier, Times, Helvetica or standard Symbol.

Do's

File. Identify your file clearly on the label with a sensible name. Make absolutely sure that you send us your final version, and that the print-out is identical to what you have saved on the diskette.

Consistency. Be absolutely consistent and check the use of punctuation, abbreviations, capitals and lower case in headings, spelling, etc. If possible, use the spelling checker on your computer.

Special characters. If the ASCII character set or the character set(s) of your word processing package does not contain the special characters you need, key in a code between angle brackets, < >, and use this each and every time you want the character to appear. You could, for example, use <gamma> for a lower case Greek gamma and <Gamma> for an upper case Greek gamma. Make the code self-explanatory. *Note:* Always supply us with a list of the codes that you have used!

Headings. Start headings etc. flush left, with two lines space above (i.e. three Hard Returns) and one line space below (two Hard Returns). Distinguish different levels of headings and be consistent.

Paragraphs. Indent all paragraphs with a [TAB] code, and separate them from one another with one Hard Return. Do not indent the first paragraph under a heading or subheading.

Block quotations should be indented with an [Indent] code and should have one line space (i.e. two Hard Returns) above and below.

Figures should be submitted in camera-ready form. The position of the figure in the text should be indicated in the margin of the hard copy. Figure legends should be placed at the end of your file.

Tables. We prefer tables to be submitted in camera-ready form. If you also put your tables on diskette, please separate columns with [TAB] codes (not with spaces) and, consequently, adjust the tabular stops to position the columns.

Equations. One-line equations without fractions can be typeset from the diskette when they are keyed in as plain text. Other equations cannot be used from the diskette: they will be typeset manually from the hard copy.

References and Notes. Strictly follow the Instructions for Authors of the journal in which the article will be published for the style of referencing and the use of notes.

Don'ts

Hyphenation. Do not hyphenate words at the end of a line. Use only one hyphen for words such as ``well-being", and ``re-do" and use two hyphens for sequences of dates and years such as ``conference dates are 12--15 September, 1992", ``age groups between 20--30 years are welcome", and page number indications in References, e.g. ``pp. 240--243".

Hard Returns. Do not use Hard Returns except when absolutely necessary, such as at the end of paragraphs, headings, etc. Otherwise, let the word wrap feature of your word processor do this work for you.

TAB feature and Space Bar. If you need more than one space between two items, e.g. when you write in columns, always use the [TAB] feature of your word processing package. Use the space bar only for separating words from one another. Do not use the space bar to format tables, for centering or laying out texts, or for any other form of line or page formatting.

Delivering your article

Always supply us with both the hard-copy (printout) version of your final text preferably with a diskette.

Label your diskette properly, giving exact details of operating system and software used.

Always retain a back-up copy of your diskette.

Further information is available from:

Kluwer Academic Publishers
P.O. Box 990
3300 AZ Dordrecht
The Netherlands
Telephone: (++)31 78 6576000
Fax: (++)31 78 6576254

ANNEXE B

Canadian Journal of Fisheries and Aquatic Sciences

Instructions to Authors

Introduction

The Journal welcomes manuscripts reporting significant new knowledge and understanding of fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, communities, ecosystems, or processes that affect aquatic systems. They may cover a range of disciplines including biology and ecology of marine and freshwater organisms, limnology, oceanography, physiology, toxicology, genetics, economics, disease, and management. Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to manuscripts that emphasize under standing of observed phenomena and interpretation of experimental results.

We encourage papers that lead from clearly stated purpose or rationale, and from testable hypotheses, concepts, or questions, to identifiable conclusions or syntheses. Such papers may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Rationale for the study and interpretation of the results should be set in a broad disciplinary or interdisciplinary context. Methodological and modeling papers should include applications and provide verification of enhanced performance.

We continue to discourage papers that are essentially descriptive, except in emerging disciplines; have only site-specific or local applications (a certain year, place, taxon, chemical compound, etc.); confirm only previously established principles; or apply standard techniques without breaking new methodological ground. Studies that are clearly preliminary or fragmentary, or whose relevance to broader issues is not demonstrated, and interpretations solely of an unsupported speculative nature will not be entertained.

Manuscripts submitted should be as comprehensive as possible; if a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together.

The Journal considers the following types of contributions:

Articles — Studies of broad scope that are original contributions to science.

Perspectives — Syntheses, critiques, and re-evaluations of current concepts and paradigms.

Discussions — Comment and Reply on subjects recently published in the Journal. **Rapid Communications** — New concepts, methodology, and topical or controversial subjects.

Guidelines for these contribution types are available from the Editorial Office' s Web site.

The publication process

The Editorial Office **encourages authors to submit their manuscripts by e-mail** to cjfas@uoguelph.ca in Word, WordPerfect, or PDF format. For further details on electronic submission, including other acceptable file formats, please contact the Editorial Office directly.

Alternatively, submit manuscripts **in triplicate** to the Editors, *Canadian Journal of Fisheries and Aquatic Sciences*, University of Guelph, Department of Zoology, Building #006, Room 107, Guelph, ON N1G 2W1, Canada. Enclose the manuscript text, tables, and figures on diskette, specifying the software package(s) and version(s) used; Microsoft Word is the preferred word processing

package for **review purposes. Packaging of manuscripts and illustrations should be robust enough to resist damage in transit.**

All submissions must be accompanied by a covering letter that (1) states the main points and significance of the work, (2) avows that all coauthors fully participated in and accept responsibility for the work, (3) avows that the manuscript is not being considered for publication elsewhere, (4) suggests potential referees, (5) identifies other manuscripts, including "extended abstracts", containing the same, similar, or related information, and (6) includes the telephone and fax numbers, as well as e-mail and current mailing addresses of all authors. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal, before final evaluation by the Editors and (or) Associate Editors. However, the Editors will return unreviewed those manuscripts that do not fall within the Journal' s scope or character, and those that exceed the Journal' s guidelines for prior publication as " extended abstracts" (guidelines available from the Editorial Office' s Web site). Papers submitted for inclusion in Journal Supplements are treated with the same rigor of review as articles in regular issues.

As general guidelines, manuscripts should not exceed 36 double-spaced pages, inclusive of references, tables, and figures; the number of references should not normally exceed 36; the number of tables plus figures should not normally exceed 12. Shorter, efficiently written papers are encouraged. Longer Articles (e.g., with more tables and figures) and longer Perspectives (e.g., with more references) will certainly be accepted, provided that their significance and interpretation of the new knowledge are commensurate with their length.

Responses to referees and revisions to manuscripts should normally be completed within 90 days. Manuscripts not returned within 90 days of receipt may be treated as new submissions unless the authors contact the Editorial Office.

Copyright material — Whenever a manuscript contains material that is protected by copyright (aerial photographs, figures, tables, etc.), it is the obligation of the

author to secure **written permission from the holder of the copyright** to reproduce the material **for both the print and electronic formats**. These letters must accompany the submitted manuscript; otherwise, publication may be delayed. All material designated as " taken from..." must be accompanied by a letter of permission. If the material is not to be reproduced exactly as in the original, it should be designated as " modified from..." In either case the source of the material must be included in the reference list.

Submission of final accepted manuscript — Authors are requested to submit the **final accepted manuscript only, both in hard copy format and on disk**. Text files and figure files should be submitted on separate disks. All disks must be labeled clearly with the authors' names. **Text** (including tables) should be provided in a word-processing format (any form of WordPerfect, Microsoft Word, or TeX is preferable, IBM compatible or Macintosh). TeX macros for preparing papers for submissions are available at ftp://ftp.tex.ac.uk/tex-archive/macros/latex/contrib/supported/nrc/, ftp://ftp.dante.de/tex-archive/macros/latex/contrib/supported/nrc/, and ftp://ctan.tug.org/tex-archive/ macros/latex/contrib/supported/nrc/. Identify the word-processing software, version number, and type of computer used (IBM or Macintosh). For **figures**, see the section " Preparation of electronic illustration files." Include a statement in the letter accompanying the manuscript that the version on the disk exactly matches the final hard copy version.

Galley proofs — A galley proof, illustration proofs, the copy-edited manuscript, and a reprint order form are sent to the corresponding author. **Galley proofs must be checked very carefully, as they will not be proofread by NRC Research Press**, and must returned within 48 h of receipt. The proof stage is not the time to make extensive corrections, additions, or deletions. The cost of changes introduced at the proof stage by the author, if deemed to be excessive, will be charged to the author.

Reprints — If reprints are desired, the reprint order form must be filled out completely and returned with payment (cheque, credit card number, purchase

order number, or journal voucher) together with the corrected proofs and manuscript. Orders submitted after the Journal has been printed are subject to considerably higher prices. **The Journal does not provide free reprints and reprints are not mailed until a purchase order number or payment is received.**

Copyright transfer — All authors are required to complete a copyright transfer form assigning all rights to NRC. Copyright transfer forms are available from the Editors, in the first issue of each volume, or on the Web site of NRC Research Press.

Requests for permission to republish the paper, in whole or in part, should be sent to NRC Research Press.

Preparation of manuscripts

General guidelines

Movement of manuscripts through review channels and the Editorial Office is greatly expedited if manuscripts are prepared in Journal style and format. General guidelines follow.

Type the manuscript on white paper (21.6 × 27.9 cm (8.5 × 11 in.)) on only one side of the page. Leave all margins at least 2.5 cm (1 in.) wide. Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations. **Double-space all parts of the manuscript**, including tables, captions for tables and figures, footnotes, and the reference list. Use italic font if available; when not available, underline material that is to be set in italics. Do not use all capitals anywhere in the manuscript. Use the name-and-year system for literature citations.

Editorial practices

Spelling should follow that of *Webster's Third New International Dictionary* or the *Oxford English Dictionary*. Authors are responsible for consistency in spelling. The *CBE Manual for Authors, Editors, and Publishers: Scientific Style and Format* (6th ed., 1994) published by the Council of Biology Editors, Inc., Chicago, IL 60603, U.S.A., is used as the authority in matters of form. Titles of periodicals

are abbreviated as in *BIOSIS® Serial Sources* (BIOSIS, 2100 Arch Street, Philadelphia, PA 19103-1399, U.S.A.). Authors are responsible for ensuring the accuracy and completeness of their reference list. The Journal follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed., 1991, Spec. Publ. No. 20, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990, Trans. Am. Fish. Soc. **119**: 2– 15). **SI** units (Système international d' unités) should be used or SI equivalents should be given. This system is explained and other useful information is given in the *Canadian Metric Practice Guide* (1989) published by the Canadian Standards Association (178 Rexdale Blvd., Etobicoke, ON M6N 3T3, Canada). For practical reasons, some exceptions to SI units are allowed (noted in the editorial in the Journal, Vol. 40, No. 12).

Checklist for manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data depository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the **title** encompass the content of the report? Does the Abstract give the essentials of the new knowledge? Is the **Introduction** largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of **Materials and methods** limited to what readers need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the **Results** supported by the data provided? Are findings distinguished from inferences? Is the **Discussion** limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

(1) Limit abstract to one paragraph of about 175 words.

(2) Double-space all elements of the manuscript, including references, table captions, and figure legends.

(3) Avoid exclusive use of capitals anywhere in the manuscript, including headings, table captions, and figure legends.

(4) Italicize only Latin names of organisms and appropriate statistical and mathematical notations.

(5) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

(6) Avoid ambiguous forms such as g C/m^2/day; use g C $\cdot m^{-2} \cdot day^{-1}$.

(7) Identify the test used to test statistical significance and give the probability value. No reference is needed for common statistical tests.

(8) In the text, generalize from tables and figures; avoid repeating all of the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

(9) Include an acknowledgement section at manuscript stage, not when page proofs arrive.

(10) Provide the person' s initials and mailing address when referring to personal communications.

(11) Delete unnecessary references that do not apply directly to the problem.

(12) Check references carefully against text citations and vice vers a to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g., available from Department of Economics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

(13) Delete commas between name and date in citations such as (Smith 1990) and do not underline or italicize " et al" .

(14) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints, laser printouts, or original artwork/electronic files of figures (see " Illustrations" and " Preparation of electronic illustration files") on request for publication. Use the same type of lettering throughout if possible.

Parts of the manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists.

Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph is relevant to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximal amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g., X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as " On" or " Towards" or expressions such as " A contribution to…" or " Investigations on…" Good titles greatly assist scientists and librarians in using scientific literature and aid indexers in preparing titles for keyword indexes. Series titles should be avoided.

Abstract

An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded. Like the title, the abstract enables readers to determine the paper' s content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as " …is discussed" or " …was found" ; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper' s contents and be

completely self-contained (i.e., any *essential* references) in a retrievable form (e.g., R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. **37**: 268– 282).

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375– 500 words.

Materials and methods

Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as " To determine…we…" If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made so as to avoid confusion between facts and inferences. State note-worthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly

applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straight-forward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence.** Nearly every manuscript submitted to the Journal contains errors in the references.

Footnotes

Footnotes to material in the text should not be used unless they are unavoidable, but their use is encouraged in tables. Where used in the text, footnotes should be cited in the manuscript by superscript Arabic numbers (except in the tables, see below) and should be numbered serially beginning with any that appear on the title page. Each footnote should be typed on the manuscript page on which the reference to it is made; **footnotes should not be included in the list of references.**

Equations

Equations should be clearly typed; triple-spacing should be used if superscripts and (or) subscripts are involved. Superscripts and subscripts should be legible and carefully placed. Distinguish between lowercase *l* and the numeral *one*, and between capital *O* and the numeral *zero*. A letter or symbol should represent only one entity and be used consistently throughout the paper. Each variable must be defined in the text or in a **List of symbols** to appear after the reference list. Variables representing vectors, matrices, vector matrices, and tensors must be clearly identified. Numbers identifying equations must be in parentheses and placed flush with the **left margin**. In numbering, no distinction is made between mathematical and chemical equations.

Tables

Tables are used to present repetitive data and should be as economical of space as possible. Design tables to fit a one- or two-column width of the Journal. Type each on a separate page and number with Arabic numerals. Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra space instead. Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own. Indicate table footnotes by superscript lowercase letters and type them below the table. Place the tables after the list of references. Note that *text tables* are not numbered, are typed within the text, and seldom need horizontal lines.

Appendices

Figures and tables used in an appendix should be numbered sequentially but separately from those used in the main body of the paper, for example, Fig. A1, Table A1, etc.

Supplemental material

The National Research Council of Canada maintains a depository in which supplementary material may be placed, either at the request of the author or suggestion of the Editors. Such material may include extensive tables of data,

detailed calculations, and maps not essential for understanding and evaluating the paper. Such material must be clearly marked when the manuscript is submitted. Tables and figures should be numbered in sequence separate from those published with the paper (e.g., Fig. D1, Table D1). The supplemental material should be referred to by footnotes. Copies of material in the depository may be purchased from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, ON K1A 0R6, Canada.

Illustrations

Provide photographic reproductions, laser printouts, or the original artwork (no larger than 27.9 × 43.2 cm (11 × 17 in.)) of each illustration. Provide three sets of illustrations for review purposes. Each figure or group of figures should be planned to fit, after appropriate reduction, into the area of either one or two columns of text. The maximum finished size of a one-column illustration is 8.8 × 23.9 cm (3.5 × 9.4 in.) and that of a two-column illustration is 18.2 × 23.9 cm (7.2 × 9.4 in.). The figures (including halftones) must be numbered consecutively in Arabic numerals, and each one must be referred to in the text and must be self-explanatory. All terms, abbreviations, and symbols must correspond with those in the text. Only essential labelling should be used, with detailed information given in the caption. Each illustration must be identified by the figure number and the authors' names on the back of the page or in the left-hand corner, well away from the illustration area.

Line drawings should be made with black ink or computer-generated in black on high-quality white paper or other comparable material. For computer-generated graphics, supply a laser print at the highest resolution available. **Photocopies are not acceptable.**

All lines must be sufficiently thick (0.5 points minimum) to reproduce well, and all symbols, superscripts, subscripts, and decimal points must be in good proportion to the rest of the drawing and large enough to allow for any necessary reduction without loss of detail. Avoid small open symbols; these tend to fill in upon reproduction. **Lettering produced by dot matrix printers or typewriters, or by**

hand, is not acceptable. The same font style and lettering sizes should be used for all figures of similar size in any one paper.

Maps must have very clear, bold patterns and must show longitudes and latitudes (or UTM coordinates) and a scale. All place names and geographic features on Quebec maps must be in French only, with proper accents and capitalization.

Photographs should be *continuous tone* on glossy paper. Prints must be of high quality, on glossy paper, with strong contrast. The copies for reproduction should only show essential features, be trimmed, and mounted on **thin** flexible white bristol board with no space between those arranged in groups. A photograph, or group of them, should be planned to fit into the area of either one or two columns of text **with no further reduction**. Electron micrographs or photomicrographs should include a scale bar directly on the print. The best results will be obtained if the authors match the contrast and density of all figures arranged as a single plate.

Color illustrations will be at the author' s expense. Further details on prices are available from Cecily Pearson, Managing Editor of the Journal (613-993-9099; fax: 613-952-7656; e-mail:cecily.pearson@nrc-cnrc.gc.ca).

NRC Research Press prefers the submission of electronic illustration files for accepted manuscripts and will use these electronic files whenever possible. If electronic files are not available, paper versions of the figures will be scanned. Note that the scanner will easily reproduce flaws (e.g., correction fluid, smudges). Submission of noncontinuous (screened) photographs and scanned illustrations printed out on laser printers is not recommended as moirés develop; a moiré is a noticeable, unwanted pattern generated by rescanning or rescreening an illustration that already contains a dot pattern.

Preparation of electronic illustration files

Authors must supply electronic graphic files and **high-quality, hard-copy originals. Electronic files (i.e., figure number and figure content) should match the originals.** On the disk label, identify (1) the software application and version and

(2) file name(s), size, and extension. If you have compressed your files, indicate what compression format was used. PC or Macintosh versions of True Type or Type 1 fonts should be used. **Do not use bitmap or nonstandard fonts.** Electronic graphics can be accepted on the following disks: 3.5-in. disks, 100-MB Zip cartridge, and CD-ROM.

The preferred graphic application of NRC Research Press is CorelDraw! For other applications that can be used, see the Electronic graphics list.

All figures should be submitted at their final published size. For figures with several parts (e.g., *a*, *b*, *c*, *d*, etc.) created using the same software application, assemble them into one file rather than sending several files. **Remember** that the more complex your artwork becomes, the greater the possibility for problems at output time. Avoid complicated textures and shadings, especially in vector illustration programs; this increases the chance for a poor-quality final product.

Bitmap (raster) files — Bitmaps are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid.

The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art and finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

All *colour* files submitted must be as CMYK (cyan, magenta, yellow, and black). These colours are used in full-colour commerical printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector files — Vector files are image files produced using element s such as lines and shapes. Typically these files are used for *line drawings*.

Bitmaps inside vector files — Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

Format conventions

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel (Bq)
calorie (cal)
centimetre (cm)
centimetre, square (cm^2)
centimetre, cubic (cm^3)
centimetres per gram per second ($cm\ g^{-1}\ s^{-1}$)
coulomb (C)
decimetre (dm)
degree Celcius (°C)
degrees of freedom (df)
gram (g)
hectare (ha)
hertz (Hz)
hour (h)
joule (J)
kilometre (km)
litre (L)
lumen (lm)
lux (lx)
metre (m)
metre, square (m^2)
metre, cubic (m^3)
micrometre (µm)
milligram (mg)
millilitre (mL)
millimetre (mm)
millimetre, square (mm^2)
minute (min)
molar mass (M)
mole (mol)
moles per litre (mol/L, M)
Pascal (Pa)
second (s)
standard deviation (SD)

standard error (SE)
tonne (metric ton) (t)
volt (V)
volume (vol)
watt (W)

Dates

Dates should be written in the sequence day — month — year without internal punctuation (On 9 October 1983 the...).

Reference citations in text

Name-and-year system

The Journal uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: " Brown (1983) compared..." or " ...were compared (Brown 1983)" . If the reference has more than two authors, include only the surname of the first author followed by " et al." (not italicized): " Brown et al. (1983) compared..." or " ...were compared (Brown et al. 1983)" .

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by " personal communication" .

Unpublished data

If an unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation " In press" . Only those manuscripts that are in galley or page proof stage or for which there is an acceptance letter can be considered in press. If an article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation " unpublished data" in the text and do not include it in the reference list.

Reference lists

References should be listed at the end of the paper in alphabetica l order according to surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in

chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *BIOSIS® Serial Sources*) for references.

Journal article

Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. **39**: 1444–1452.

Entire issue of journal

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Book in a series

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Book not in a series

LeBlond, P.H., and Mysak, L.A. 1978. Waves in the ocean. Elsevier, New York.

Part of book

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. Neil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 203– 229.

Corporate author

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C.

Theses

Kutty, M.N. 1999. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. No. 1032.

Translation

Koike, A., and Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1– 11. [Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols " h," " min," and " s" are not used because they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, " 12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

NRC Research Press
National Research Council of Canada
Ottawa, ON K1A 0R6
Canada