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SOURCES, BIODISPONIBILITÉ ET TRANSFORMATION DE LA MATIÈRE ORGANIQUE DISSOUTE EN ZONE LITTORALE DU LAC SAINT-PIERRE : IMPORTANCE DE LA CONNECTIVITÉ HYDROLOGIQUE

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RÉSUMÉ

La fluorescence en spectroscopie tridimensionnelle combinée à l'analyse parallèle de facteurs (PARAFAC) a été utilisée dans les deux volets de ce projet de recherche. Dans un premier temps, cet outil a permis de caractériser les sources de carbone (terrestres et aquatiques) soutenant la production bactérienne dans la masse d'eau sud du lac Saint-Pierre (Québec, Canada). Ce lac fluvial peu profond est densément couvert de macrophytes durant l'été et est bordé de terres humides qui augmentent fortement la connectivité entre les zones littorales et pélagiques. Au cours de la saison estivale, la fluorescence de la matière organique dissoute (MOD) autochtone a diminué significativement; alors que celle de la MOD allochtone a augmenté. Ces changements étaient reliés aux fluctuations des niveaux d'eau qui affectent la connectivité hydrologique entre les zones pélagiques et littorales et entre le tributaire et le plan d'eau. Dans un gradient amont-aval d'écoulement de l'eau, l'intensité de fluorescence de la MOD terrestre a diminué de la mi-juillet à la mi-août. Les processus de transformation qui s'opèrent le long de ce gradient ont pu contribuer à modifier la quantité et la qualité de la MOD dans la masse d'eau. La production bactérienne était positivement reliée à la MOD allochtone, mais la biodisponibilité relative de la MOD était positivement associée à une origine autochtone. Ces résultats illustrent la capacité de la communauté bactérienne à utiliser différentes sources de carbone et soulignent l'importance de la MOD terrestre pour le réseau alimentaire microbien dans un lac fluvial. La connectivité hydrologique entre les terres humides, les tributaires et le lac fluvial a joué un rôle écologique clé en structurant la quantité et la qualité de la MOD circulant dans l'écosystème.

Dans un deuxième temps, l'importance combinée des processus photochimiques et microbiens dans la dégradation de la MOD et sur sa biodisponibilité a été évaluée dans un échantillon d'eau provenant de la rivière Saint-François, tributaire sud du lac Saint-Pierre (Québec, Canada). Cette fois-ci, la fluorescence tridimensionnelle combinée avec des mesures en absorbance ont révélé des transformations photochimiques, particulièrement en présence du rayonnement ultraviolet, de la MOD terrestre en composés plus petits. Aucun changement correspondant dans les caractéristiques chimiques de la MOD n'a pu être identifié à l'aide d'analyse en résonance magnétique nucléaire (H^1 RMN). Le fractionnement chimique de la MOD a révélé une abondance élevée en acides hydrophiliques biodisponibles dans la rivière Saint-François. Ceci n'était pas visible par les mesures en fluorescence, qui ont sous-estimé la portion non humique de la MOD. Les composantes fluorescentes représentatives d'une origine autochtone étaient dominantes dans les acides hydrophiliques mais aussi dans les acides humiques plus réfractaires. Cette étude a ainsi permis de souligner la complémentarité de l'information obtenue à l'aide des outils optiques et chimiques et l'importance d'utiliser les deux approches pour obtenir une vision complète de la dynamique de la MOD dans les écosystèmes aquatiques.

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AVANT-PROPOS

En vertu des articles 136 à 138 du règlement des études de cycles supérieurs, le présent mémoire a été rédigé sous forme d'articles scientifiques. Il comporte deux articles : 1) « Fluorescence analyses of sources and bioavailability of DOM in the littoral zone of fluvial Lac Saint-Pierre: importance of hydrological connectivity » dont les auteurs sont Geneviève Trudel et Jean-Jacques Frenette et qui sera soumis au périodique « *Aquatic Microbial Ecology* » et 2) « Contribution of light exposure and microbial processes to the degradation of dissolved organic matter in river environment : comparison between optical and chemical characterization » dont les auteurs sont Geneviève Trudel, Jean-Jacques Frenette et Benoît Daoust et qui sera soumis au périodique « *Aquatic Ecology* ».

Le mémoire contient **1)** une introduction générale, rédigée en français (Chapitre I) **2)** le premier article rédigé en anglais (Chapitre II) **3)** le deuxième article, également rédigé en anglais (Chapitre III). Les directives aux auteurs pour les deux périodiques sont présentées en annexe (A et B). Le manuscrit a été préparé selon le document intitulé « *Exigences et modalités liées à la présentation du mémoire ou de l'essai présentés sous forme d'article sous la forme d'article(s) scientifiques(s) pour les programmes de maîtrises en sciences de l'environnement (3403 et 3893)* ».

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$a\text{CDOM}_{375}$	Coefficient d'absorption à 375 nm
BP	Production bactérienne
CDOM	Carbone organique dissous chromophorique
Chl	Chlorophylle
DIC	Carbone inorganique dissous
DOC	Carbone organique dissous
FA	Acide fulvique
H^1 RMN	Résonance magnétique nucléaire sur le proton H
HA	Acide humique
HyA	Acide hydrophilique
MEE	Matrice d'excitation-émission
MOD	Matière organique dissoute
PAR	Lumière disponible pour la photosynthèse (400-700 nm)
PARAFAC	Analyse parallèle de facteurs
P_{max}	Taux de photosynthèse maximal
TN	Azote total
TP	Phosphore total
UV	Rayonnement ultraviolet

CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Revue de la littérature

Afin de comprendre et d'expliquer la productivité des écosystèmes aquatiques, un nombre croissant de recherches s'attardent à cerner l'importance du réseau microbien dans le transfert de la matière organique dissoute (MOD) vers les niveaux trophiques supérieurs (Pomeroy 1974, Azam et al. 1983). Autrefois restreintes à un rôle de décomposition, les bactéries sont de plus en plus perçues comme des organismes clés dans la dynamique des réseaux trophiques aquatiques. Il est maintenant bien connu que les bactéries hétérotrophes utilisent la MOD et la remettent en circulation vers les niveaux supérieurs de la chaîne alimentaire en étant elles-mêmes consommées par les bactérivores (Pomeroy & Wiebe 1988). Toutefois, l'implication relative du réseau microbien dans la production secondaire des écosystèmes aquatiques reste peu comprise.

La vision traditionnelle des réseaux trophiques aquatiques accentue l'importance de la production primaire autochtone comme support à la production hétérotrophe, minimisant ainsi l'implication des échanges entre le réseau microbien et la chaîne alimentaire classique dans la productivité secondaire. Récemment, certaines évidences suggérant l'hétérotrophie nette des écosystèmes aquatiques ont remis en question cette vision (del Giorgio & Peters 1994, Duarte & Agusti 1998, Cole et al. 2000). En effet, pour qu'un système soit hétérotrophe, la respiration doit être supérieure à la production, ce qui implique l'utilisation par des organismes hétérotrophes d'apports externes en matière organique. Cette matière organique terrestre est connue pour sa contribution à la respiration de l'écosystème, toutefois, l'importance de sa contribution pour la production secondaire reste peu étudiée (Meili et al. 1996, Grey et al. 2001).

L'efficacité du recyclage de la MOD dépend nécessairement de sa qualité, intimement liée à sa source. La MOD aquatique d'origine terrestre, dite allochtone, est davantage réfractaire à la dégradation que la MOD autochtone originaire du plan d'eau. Ainsi, certaines études ont montré que les bactéries utilisent de façon préférentielle la MOD autochtone plus labile (Cole et al. 1982, Kritzberg et al. 2004). Toutefois, des processus physiques et biologiques altèrent la MOD et favoriseraient son utilisation par le réseau microbien (Wetzel et al. 1995, Bertilsson & Allard 1996).

1.1.1 Matière organique dissoute

La matière organique dissoute (MOD) est un mélange complexe composé de substances organiques aromatiques et aliphatiques à divers stades de décomposition. Cette matière représente une portion substantielle de la totalité du carbone organique dans les écosystèmes aquatiques (Wetzel 2001). La MOD joue un rôle significatif dans les écosystèmes aquatiques de par sa quantité et ses nombreuses interactions avec les autres processus et composantes de l'écosystème. Elle affecte, par exemple, la disponibilité des nutriments dissous et des métaux et modifie les propriétés optiques de la colonne d'eau. Par son importance dans le budget en carbone, en énergie et en nutriments des écosystèmes aquatiques, elle peut affecter les chaînes alimentaires, l'hétérotrophie et le recyclage des nutriments (Findlay & Sinsabaugh 2003).

En particulier, la MOD influencera le transfert trophique du carbone via son utilisation par les bactéries et la consommation subséquente de ces dernières par les bactérivores. L'assimilation de la MOD par les bactéries est fonction de sa biodisponibilité qui elle-même varie selon sa source, sa composition et les processus de transformation qui s'opéreront sur elle. Aussi, la considération des gradients spatiaux et temporels est primordiale puisqu'ils expliquent, en grande partie, les changements que subira la matière organique dissoute au niveau écosystémique (Wetzel 1992).

1.1.1.2 Source et composition

La matière organique dissoute peut être acheminée à l'écosystème aquatique par des sources internes (autochtones) ou externes (allochtones). La MOD d'origine autochtone provient principalement de l'activité photosynthétique des algues et des macrophytes. La majorité des études se sont attardées à la portion algale (d'origine majoritairement phytoplanctonique en milieu lentique et périphytique en milieu lotique) dans l'excrétion de MOD. Pourtant, l'habitat qu'occupent les macrophytes, soit la zone littorale, constitue plus de 95% de la surface lacustre de presque 99,8% de tous les lacs du monde (Wetzel 2001). De plus, le maximum de biodiversité des écosystèmes d'eau douce a lieu à l'interface des terres humides et de la zone littorale avec les régions pélagiques (Wetzel 1999b). Les macrophytes constitueraient ainsi une source importante de MOD pour les lacs peu profonds où la zone littorale est importante; alors que le phytoplancton serait davantage significatif en lacs profonds et en milieu marin.

Les algues et les macrophytes captent l'énergie solaire et la transforment en composés carbonés réduits. Différents processus permettent la remise en circulation de ce carbone emmagasiné dans les cellules : la mort cellulaire, via la sénescence naturelle des cellules ou l'infection virale, l'activité de broutage ainsi que l'excrétion des cellules vivantes par des processus passifs et actifs. L'importance relative de ces processus de relâchement de la MOD varie en fonction du caractère biologique et chimique de l'écosystème. La différence dans la prépondérance de ces processus influencera les échelles spatiales et temporelles auxquelles s'opèrent l'apport de la MOD et influencera également la quantité et la qualité de la biomasse algale transférée au pool de MOD (Bertillsson & Jones 2003).

Il est connu depuis longtemps que la MOD d'origine autochtone constitue un substrat de haute qualité pour les bactéries (Cole et al. 1982). En effet, cette matière est composée en majorité de substances labiles à faible poids moléculaire (sucre monomère, acides carboxyliques, acides aminés et aldols). Toutefois, malgré la

prédominance de composés biodisponibles, une fraction substantielle de la MOD algale serait récalcitrante à la dégradation microbienne (Fry et al. 1996). Cette récalcitrance peut provenir des propriétés originales de la matière (ex. présence de composés à haut poids moléculaire) ou peut dériver de changements diagénétiques apportés par le milieu (photolyse, dégradation enzymatique).

Dans les écosystèmes d'eau douce, la proximité des régions littorales et des terres humides ainsi que l'apport important de MOD provenant de la décomposition du matériel végétal dans le bassin versant expliquent la prépondérance de la MOD de nature allochtone (Wetzel 1992). Cette matière allochtone, principalement composée de substances à haut poids moléculaire et à forte aromaticité (substances humiques), était considérée comme largement non disponible pour les niveaux trophiques supérieurs (Aiken 1985, Geller 1986). Toutefois, plusieurs études ont montré que la MOD allochtone peut être assimilée par les bactéries. Elle pourrait ainsi participer significativement à la productivité des écosystèmes aquatiques (Jones 1992, DeHaan & de Boer 1992, Thomas 1997) et parvenir aux niveaux trophiques supérieurs (ex. poissons) en milieu lacustre (Cole et al. 2002, Pace et al. 2004) par l'intermédiaire du réseau alimentaire microbien.

1.1.1.3 Processus de transformation

Les principales voies d'altération de la matière organique dissoute sont les processus microbiens et photochimiques (Weigner & Seitzinger 2001). Par leur action sur la biodisponibilité de la MOD, ils jouent un rôle essentiel dans l'incorporation du carbone organique dissous dans le réseau microbien.

Il est maintenant bien connu que la lumière altère la disponibilité de la MOD par la photodégradation des macromolécules du pool de MOD. Les substances humiques constituent la majeure portion de la partie chromophorique de la MOD (CDOM) qui absorbe la radiation ultraviolette (Wetzel 2001). La lumière, particulièrement les courtes longueurs d'onde énergétiques, augmente la biodisponibilité de la MOD par la dégradation de grosses molécules aromatiques en plus petites entités (Strome &

Miller 1978) qui sont efficacement assimilées par le bactérioplancton (Kieber et al. 1989, Bertilsson & Tranvik 1998, Obernosterer et al. 1999a). Les processus photochimiques peuvent être perçus comme un mécanisme catalytique pour les processus microbiens (Wetzel et al. 1995, Bertilsson & Allard 1996). La production de MOD moins réfractaire par les UV facilite l'utilisation bactérienne de cette source d'énergie et entraîne l'installation d'une voix séquentielle de dégradation photochimique-microbienne. Les bactéries et le phytoplancton peuvent également altérer directement la biodisponibilité de la MOD par la sécrétion d'exoenzymes et par l'oxydation de la surface cellulaire (Pantoja & Lee 1994).

Cependant, certaines études ont montré une absence d'effet (Amon & Benner 1996) et même la présence d'effets négatifs (Tranvik & Bertilsson 2001) sur le transfert du carbone exposé à la lumière vers les bactéries. Pour expliquer ces effets contrastés des processus photochimiques sur la biodisponibilité de la MOD, des hypothèses mettant en relief l'importance de la source et de l'âge de la MOD ont été mises de l'avant. Les différentes études (ex. Benner & Biddanda 1998, Obernosterer et al. 1999, Tranvick & Bertilsson 2001) ont convergé vers l'hypothèse selon laquelle la MOD d'origine terrestre, plus âgée et davantage aromatique sera plus labile suite à l'irradiation; alors que la MOD d'origine algale deviendra plus récalcitrante.

Ainsi, à l'échelle écosystémique, l'importance pour les bactéries des substances produites par photodégradation sera influencée par l'importance des apports allochtones et autochtone de la MOD en ce que l'origine influencera la bioréactivité des molécules. De plus, à cette même échelle, l'importance des processus photochimiques variera nécessairement en fonction de la quantité de lumière pénétrant dans la colonne d'eau. Cette quantité est elle-même influencée par la composition en matière interagissant avec la lumière (particulièrement le CDOM) et par les caractéristiques hydrologiques du système (niveaux d'eau, phénomène brassage, temps de rétention de l'eau, etc.).

1.1.2 Couplage entre production bactérienne et production primaire

En milieu où les éléments minéraux ne sont pas limitants, la croissance et la production bactérienne seront majoritairement influencées par la qualité du carbone assimilé. Comme mentionné dans la section précédente, la biodisponibilité de la MOD, principalement constituée de carbone organique dissous (DOC), variera principalement en fonction de son origine. Le carbone issu de la photosynthèse du phytoplancton (carbone autochtone), de nature plus labile et contenant plus d'énergie par unité de masse que le carbone d'origine terrestre (carbone allochtone), constituera un substrat de plus grande qualité pour les bactéries. Ainsi, plusieurs études ont montré une corrélation significative entre la production bactérienne et la production phytoplanctonique (Cole et al. 1988, Reche et al. 1996, Carillo et al. 2002), suggérant une dépendance des bactéries pour le carbone d'origine algale. Ces études ont été réalisées dans des milieux où la portion autochtone du DOC était dominante.

Dans des systèmes qualifiés d'hétérotrophes, c'est-à-dire où la respiration est alimentée par des apports externes (allochtones) en carbone organique, des études ont montré une absence de couplage entre la production primaire et les microorganismes hétérotrophes. Findlay et al. (1991) ont mesuré, pour la rivière Hudson, un taux absolu de production de carbone bactérien supérieur à la production primaire phytoplanctonique, indiquant l'utilisation par les bactéries d'une source de carbone non phytoplanctonique. Également, les études de Jansson et al. (2000), Drakare et al. (2002) ont montré l'importance d'apports allochtones dans la production bactérienne de petits lacs humiques.

Ainsi, les relations entre la production bactérienne et la production primaire phytoplanctonique peuvent donner un indice quant à la composition du DOC présent dans un écosystème donné. Il est important de souligner cependant que la production phytoplanctonique ne correspond pas à la production primaire totale du milieu. En l'occurrence, l'absence de corrélation entre la production phytoplanctonique et la production bactérienne n'implique pas nécessairement la contribution d'apport

allochtone, particulièrement dans un système où les macrophytes sont abondants (Stanley et al. 2003).

1.2 Problématique et Objectifs de recherche

Le lac Saint-Pierre (Québec, Canada) est le plus grand lac fluvial le long du fleuve Saint-Laurent et constitue le dernier élargissement d'importance du fleuve (13,1 km de largeur à niveau d'eau moyen) avant l'estuaire. Il est caractérisé par une faible profondeur (3,17 m de profondeur moyenne à niveau d'eau moyen) et sa forte biodiversité lui a valu la classification de «réserve écologique de la biosphère» par l'UNESCO en 2000. Ce milieu hautement productif présente la plus grande plaine d'inondation en eaux douces du Québec (Burton 1991) et est couvert par d'importants bancs de macrophytes submergées et émergentes des mois de juin à septembre. Les macrophytes sont particulièrement abondantes sur la rive sud du lac, dans la masse d'eau de la rivière Saint-François. Cette masse d'eau est influencée par un bassin versant agricole et présente des niveaux élevés en nutriments (Huggins et al. 2004) et une concentration relativement élevée en CDOM (Frenette et al. 2006).

Les larges rivières ou lacs fluviaux bordés de plaines inondables, tel que le lac Saint-Pierre, sont particulièrement intéressants pour l'étude des apports allochtones latéraux en matière organique. En effet, la connectivité hydrologique, entre les plaines d'inondation et le plan d'eau, favorise l'apport de subsides terrestres vers le milieu aquatique sur une base temporelle. Ainsi, il est bien connu que la quantité de matière allochtone apportée au milieu aquatique est directement liée au degré de connectivité avec les milieux terrestres avoisinants (Wetzel 2001). Situé en zone tempérée, le lac Saint-Pierre est soumis à des fluctuations saisonnières de niveaux d'eau. L'apport en matière organique allochtone peut ainsi être relié au degré de connectivité terrestre-aquatique variant dans le temps : forte connectivité en périodes de niveaux d'eau élevés associés à la crue printanière et aux pluies automnales abondantes et faible connectivité, durant la période d'étiage des mois de juillet et

d'août. Ces variations temporelles dans le lien unissant les plaines inondables au lac fluvial permettent d'évaluer l'importance, non seulement en terme de qualité mais aussi de quantité, de la matière organique allochtone pour le réseau microbien et la productivité de l'écosystème.

Également, par la discontinuité physique que représente le lac Saint-Pierre dans le système fluvial du Saint-Laurent, la connectivité peut être regardée sous un axe longitudinal d'écoulement des eaux. Le lac est caractérisé par une forte hétérogénéité principalement associée à la présence de nombreux tributaires sur les deux rives. Ces tributaires contribuent à la formation de masses d'eau distinctes, tant sur le plan biologique que physico-chimique, qui s'écoulent le long du lac sans se mélanger. La rive sud du lac est reconnue pour être plus productive que sa rive nord (Frenette et al. 2003, Huggins et al. 2004). Cette plus grande productivité pourrait être le résultat d'une plus large proportion de la colonne d'eau exposée au rayonnement solaire. En effet, les niveaux d'eau sur cette rive étant plus bas, la lumière disponible pour la photosynthèse pénètre sur une plus grande proportion de la colonne d'eau, ce qui favoriserait la croissance des plantes et la productivité phytoplanctonique. La masse d'eau située la plus près de la berge sud, et donc la plus susceptible d'être influencée par cette dernière, est formée par l'apport du tributaire de la rivière Saint-François. Cette masse d'eau, ayant un temps de résidence d'environ sept jours dans le lac Saint-Pierre en période estivale, est influencée par un bassin versant agricole expliquant des concentrations élevées en éléments nutritifs (Huggins et al. 2004). Alors que la masse d'eau circule, des transformations photochimiques et microbiennes de la matière organique dissoute génèrent une hétérogénéité spatiale sur un axe longitudinal. Ici encore, les variations dans les niveaux d'eau affecteront la connectivité, cette fois longitudinale, entre le tributaire et le milieu fluvial. En intervenant à la fois au niveau de l'importance des apports de la rivière Saint-François ainsi qu'au niveau du temps de transport de l'eau, la connectivité hydrologique influencera les propriétés de la MOD, du réseau microbien ainsi que des niveaux trophiques supérieurs.

Évaluer les sources de carbone utilisées par les bactéries hétérotrophes reste difficile puisque la MOD est composée de substances dissoutes hétérogènes qui lui confèrent une nature complexe. Plusieurs études ont utilisé les isotopes stables afin d'attribuer les sources de carbone consommées par les organismes hétérotrophes (Coffin et al. 1989, Jones et al. 1998, McCallister et al. 2004, Kritzberg et al. 2004). Cette méthode, très puissante, est basée sur une différence isotopique entre les sources de carbone. Toutefois, les caractéristiques isotopiques ne sont pas toujours distinctes dans les écosystèmes d'eau douce (Cole et al. 2002, Pace et al. 2004). Les outils bio-optiques, combinant les propriétés d'absorbance et de fluorescence de groupes de molécules, ont été utilisés avec succès comme traceurs des sources de MOD (Coble et al. 1990, McKnight et al. 2001, Stedmon & Markager 2001). Récemment, une nouvelle méthode en fluorescence spectroscopique, combinant des matrices d'excitation-émission (MEE) et l'analyse parallèle de facteurs (PARAFAC) a été développée pour caractériser la portion fluorescente de la MOD et tracer ses différentes fractions dans les écosystèmes (Stedmon et al. 2003).

À l'aide de cette méthode en fluorescence, le premier chapitre de ce mémoire visait 1) à caractériser les sources de carbone utilisées par les bactéries hétérotrophes et 2) à évaluer la relation entre la qualité des sources de carbone et la production bactérienne et ce, sur un axe longitudinal d'écoulement de l'eau et sur un axe latéral liant le plan d'eau aux plaines inondables. L'échantillonnage s'est déroulé dans la masse d'eau formée par la rivière Saint-François dans le lac Saint-Pierre, à différents niveaux d'eau durant la saison estivale.

Dans un deuxième temps, afin de cerner le rôle des processus de transformation de la MOD sur sa biodisponibilité, une expérience en milieu contrôlée a été effectuée. Le deuxième chapitre de ce mémoire avait ainsi pour objectifs 1) de déterminer l'importance de la radiation solaire et de l'activité microbienne sur la dégradation et la biodisponibilité de la MOD provenant de la rivière Saint-François et 2) de relier les analyses optiques et chimiques à la biodisponibilité de la MOD, telle que mesurée par la production bactérienne.

1.3 Aire d'étude et Méthodologie

1.3.1 Chapitre II

Les échantillons d'eau ont été récoltés le long de trois transects parallèles à la berge et situés dans la masse d'eau de la rivière Saint-François, sur la rive sud du lac Saint-Pierre (l46°12'N, 72°50'W) (Fig. 2.1, Tableau 2.1). La position de ces stations a été déterminée par modélisation hydrodynamique afin de s'assurer que chacun des transects se situait dans une même isoligne d'écoulement de l'eau. Six campagnes d'échantillonnage ont été effectuées entre la mi-juin et la mi-août 2004, en raison d'une fois par semaine pendant les trois premières semaines et d'une fois aux deux semaines pour la période subséquente. Puisque la répartition des masses d'eau dans le lac Saint-Pierre change au cours de la saison, une modélisation hydrodynamique était effectuée avant chaque campagne d'échantillonnage pour assurer le positionnement des stations dans la masse d'eau de la rivière Saint-François. Afin de prendre en considération un mélange latéral potentiel des masses d'eau, une image Landsat 5 a été utilisée pour valider le positionnement des stations dans la masse d'eau de la rivière Saint-François (Fig.2.1a et b).

Les différentes sources de MOD ont été évaluées par spectrofluorométrie à l'aide de matrices d'excitation-émission (MEEs) combinée à l'analyse parallèle de facteurs (PARAFAC) (Stedmon et al. 2003). Brièvement, l'utilisation de PARAFAC a permis de caractériser la partie fluorescente de la MOD en décomposant les matrices de fluorescence en différentes composantes dont la contribution relative dans la MOD fluorescente est dépendante de sa source. Différents groupes de composants fluorescents ont alors été assignés à une origine allochtone ou autochtone. En plus des données de cette étude, les MEEs de l'étude traitée dans le deuxième article (Chapitre III) ont été incluses dans le modèle. Ceci a permis d'élargir le spectre des signatures de la MOD et d'améliorer la modélisation avec PARAFAC. Les MEEs ont été combinées en une matrice tridimensionnelle et une série de modélisation, de 2 à 6 composantes, a été appliquée aux données. Le modèle à cinq composantes s'est

avéré le plus approprié (Fig. 2.3). Des analyses de variance (ANOVA) à mesures répétées ont été effectuées afin de vérifier les différences dans la MOD autochtone et allochtone, dans la production bactérienne et la biodisponibilité relative de la MOD entre les périodes d'échantillonnage et la position géographique (gradients latéraux et longitudinaux).

Dans le but d'étudier l'importance relative des différentes sources de carbone pour les bactéries hétérotrophes, des mesures de production bactérienne (BP) et phytoplanctonique (taux de photosynthèse maximal (P_{max})) ont été effectuées à plusieurs stations lors de niveaux d'eau variés. En plus de ces mesures, des analyses de nutriments (phosphore total (TP) et azote total (TN), de chlorophylle a (Chl *a*) et de carbone organique et inorganique dissous (DOC et DIC respectivement) ont été réalisées. L'impact des sources de carbone sur la production bactérienne a été évalué à l'aide d'un indice de biodisponibilité de la MOD. Cet indice était calculé en divisant la production bactérienne par la concentration en DOC (BP:DOC, Ziegler & Benner 2000). Des régressions linéaires et deux analyses en composantes principales ont permis d'illustrer les relations entre les variables environnementales, les sources de carbone, la production bactérienne et phytoplanctonique (P_{max}) et la biodisponibilité de la MOD.

1.3.2 Chapitre III

Dans ce chapitre, une expérience de photo-biodégradation en milieu contrôlé a été réalisée. Les échantillons d'eau ont été récoltés au mois de mai 2005 à l'embouchure de la rivière Saint-François, située à environ 5,5 km en amont du lac Saint-Pierre. Cette rivière est caractérisée par d'importants apports en matière organique terrestre provenant d'un bassin versant agricole qui explique des concentrations élevées en nutriments (Tableau 2.1).

Afin d'étudier l'importance de la radiation solaire sur la dégradation de la MOD, des échantillons d'eau filtrés sur 0,2µm ont été placés dans des contenants de

polyéthylène et exposés à trois différents traitements de lumière: la lumière solaire (la lumière disponible pour la photosynthèse (PAR) + le rayonnement ultra-violet (UV)), la lumière solaire sans le rayonnement ultra-violet (PAR seulement) et un contrôle à l'obscurité. Les échantillons ont été exposés au soleil du 20 au 31 mai.

Dans le but d'évaluer l'effet de transformations photochimiques et microbiennes de la MOD sur la production bactérienne, la moitié des échantillons d'eau filtrée ont été inoculés avec une communauté naturelle de bactéries provenant de la rivière Saint-François. Ces échantillons ont été soumis aux mêmes traitements de lumière ci-haut mentionnés pour la même durée d'exposition. Des mesures de production bactérienne étaient effectuées aux deux jours.

La caractérisation de la dégradation de la MOD a été évaluée par analyses spectroscopiques et chimiques. En optique, l'absorbance ainsi que la spectrofluorométrie associée à l'analyse parallèle de facteurs (PARAFAC) ont été utilisées. Le même modèle PARAFAC utilisé dans le chapitre 1 a été appliqué aux données de cette expérience. Ceci a permis de différencier les composantes d'origine autochtone des composantes d'origine allochtone. En chimie, les échantillons initiaux non-incubés et les échantillons à la fin de la période d'incubation ont été fractionnés en acides humiques (HA), fulviques (FA) et hydrophiliques (HyA). Des analyses en résonance magnétique nucléaire sur H^1 (H^1 RMN) ont été effectuées sur ces fractions afin de caractériser les changements chimiques qui se sont opérés sur la MOD exposée à la lumière et aux bactéries. Avant le début des expériences, des mesures de phosphore total, d'azote total et de chlorophylle *a* ont été réalisées pour caractériser les conditions chimiques initiales de l'eau de la rivière Saint-François.

1.4 Résultats et Conclusions

1.4.1 Caractérisation des sources de carbone

La spectrofluorométrie combinée avec la modélisation PARAFAC a été utilisée avec succès pour attribuer l'origine de la MOD fluorescente circulant dans une masse

d'eau du Lac Saint-Pierre (chapitre II) et pour caractériser l'impact des processus de transformation sur la MOD (chapitre III). Cinq composantes fluorescentes ont été identifiées et caractérisées par comparaison avec la littérature (Tableau 1.2). Les composantes 1 et 2 étaient positivement corrélées (Tableau 1.3) et correspondaient à des groupes de fluorescence de type protéinique et d'origine autochtone (Coble et al. 1990, Mopper & Scultz 1993, Chen et al. 2003, Yamashita & Tanoue 2003). Les composantes 3 à 5, corrélées entre elles (Tableau 3), correspondaient à de la MOD d'origine allochtone (Stedmon et al. 2003, 2005a, 2005b et les références citées). Le site d'étude était typique des écosystèmes aquatiques d'eau douce où le pool de MOD est composé majoritairement de substances humiques (Wetzel 1992).

1.4.2 Chapitre II

1.4.2.1 Variations spatiales et temporelles de la MOD

La MOD autochtone et allochtone a montré des variations significatives en intensité de fluorescence au cours de la saison estivale, illustrant la grande hétérogénéité des sources de carbone dans le Lac Saint-Pierre (Fig. 2.4). Au cours du temps, une diminution linéaire significative de la fluorescence de la MOD autochtone a été observée (Tableau 1.4, $p < 0,001$) en opposition à une augmentation linéaire significative de la fluorescence de la MOD allochtone (Tableau 1.4, $p < 0,05$). Les caractéristiques du bassin versant du lac Saint-Pierre associées aux évènements de précipitations ont pu contribuer à l'augmentation de la MOD terrestre dans le plan d'eau au cours de l'été. En effet, le plus haut niveau d'eau pour une date d'échantillonnage correspondait à la plus forte concentration de MOD allochtone et de DOC (Fig. 2.4). La hausse de la connectivité hydrologique entre le milieu aquatique et les terres humides a ainsi contribué à modifier la composition de la MOD dans l'écosystème. Également, la plus faible biodisponibilité pour les bactéries hétérotrophes de la MOD allochtone par rapport à la MOD autochtone (Wetzel 2001) a pu contribuer à son accumulation dans la masse d'eau au cours de la saison estivale. En contrepartie, la nature très labile de la MOD autochtone explique son haut taux de renouvellement et sa faible concentration dans les écosystèmes

d'eau douce (Wetzel 2001). La diminution observée de la MOD autochtone, particulièrement au mois de juillet, pourrait s'expliquer par l'augmentation des niveaux d'eau pour cette période. En effet, la hausse des niveaux aurait contribué à diminuer à diminuer le temps de résidence de l'eau dans le lac Saint-Pierre, ce qui défavorise la croissance des producteurs primaires (Vis et al. 2006).

L'intensité de fluorescence de la MOD allochtone a diminué significativement (Tableau 1.4, $p < 0,05$) le long du gradient amont-aval (Fig. 2.4c). Ce patron peut être expliqué par trois principaux facteurs : 1) l'augmentation de la distance par rapport à la source de la rivière Saint-François 2) les processus photochimiques et microbiens 3) la présence de macrophytes.

La diminution de la MOD allochtone le long d'un gradient longitudinal peut être reliée, en partie, à l'éloignement géographique de la source de la rivière Saint-François. En effet, cette rivière, par les caractéristiques agricoles de son bassin versant, est une source de nutriments, de MOD et de matière particulière dans le lac Saint-Pierre. L'augmentation de la distance par rapport à la source le long du gradient amont-aval a pu résulter en une diminution de l'apport de matière terrestre provenant de cette source. La rive sud du lac Saint-Pierre est caractérisée par la présence de petits canaux reliant les terres humides au lac. Cependant, l'absence de variation significative de la MOD le long du gradient latéral suggère que l'apport latéral provenant des terres humides était bien mélangé avec la contribution amont-aval de la masse d'eau de la rivière Saint-François.

En plus des variations dans l'apport de MOD des terres humides et des tributaires, des processus *in situ* physiques, chimiques et biologiques se produisant dans l'écosystème contribuent à altérer la quantité et la composition de la MOD. Les processus photochimiques et microbiens contribuent à l'incorporation du DOC dans le réseau trophique microbien en modifiant la biodisponibilité de la MOD (Weigner & Seitzinger 2001). Le long du gradient longitudinal, des transformations photochimiques et microbiennes de la MOD ont pu contribuer à la diminution de la

fluorescence de la MOD allochtone. En contrepartie, la MOD autochtone a pu devenir plus réfractaire à la dégradation microbienne, engendrant son accumulation en aval. Cependant, l'influence de ces transformations sur la fluorescence du CDOM requiert davantage d'investigations.

Également, la présence d'importants bancs de macrophytes, particulièrement en aval de la masse d'eau de la rivière Saint-François, peut influencer grandement la nature et la quantité de MOD. Les macrophytes sont reconnues pour jouer un rôle important dans les écosystèmes aquatiques en structurant et en modifiant les propriétés physiques, chimiques et biologiques de la colonne d'eau. Entre autres, elles contribuent à réduire la vélocité du courant, augmentant le temps de résidence de l'eau (Sand-Jensen & Mebus 1996, Morin et al. 2000). L'étude de Martin et al. (2005) a montré une diminution du ratio CDOM sur DOC le long d'un transect amont-aval à l'intérieur d'un banc de macrophytes du lac Saint-Pierre. Cette diminution était expliquée, en partie, par une plus grande période d'exposition de la MOD à la lumière solaire. Ainsi, dans notre étude, l'abondance de macrophytes dans la masse d'eau de la rivière Saint-François a pu contribuer à la transformation photochimique de la MOD, en augmentant le temps d'exposition au soleil. De plus, plusieurs études ont suggéré que les activités photosynthétiques des macrophytes contribuent aux majeures sources de DOC, particulièrement de faible poids moléculaire, dans la majorité des écosystèmes aquatiques peu profonds (Mann & Wetzel 1996, Bertilsson & Jones 2003). L'augmentation de la MOD autochtone en aval, quoique non significative, pourrait être également attribuée à l'exsudation et/ou le relâchement de COD autochtone provenant des macrophytes vivantes.

1.4.2.2 Variations de la production bactérienne et de la biodisponibilité de la MOD

Comme pour les sources de MOD, la production bactérienne, le P_{max} et la biodisponibilité relative de la MOD ont varié de façon importante dans la même masse d'eau, à la fois dans l'espace et dans le temps (Table 1.5). Des endroits ayant des ratios élevés et faibles de PB:P_{max} ont été observés dans le lac Saint-Pierre. Des valeurs élevées de ce ratio (>0,5) sont caractéristiques de lacs humiques alors que

des valeurs faibles (<0,5) sont représentatives de lacs plus oligotrophes (Ktrizberg 2005). Également, la biodisponibilité de la MOD, variant entre 0,05 et 0,29, était caractéristique de lacs aux statuts trophiques différents (Ziegler & Benner 2000). Ces résultats mettent en lumière la grande dynamique et l'hétérogénéité des lacs fluviaux comme le lac Saint-Pierre, qui sont soumis à diverses sources de carbone sur une base annuelle.

1.4.2.3 Relation entre la qualité du carbone et la production bactérienne

La production bactérienne s'est révélé être significativement corrélée (au seuil de 5%) avec seulement deux variables : soit une relation positive avec les niveaux d'eau ($r^2 = 0,5$, $p < 0,05$) et une relation négative avec le P_{max} ($r^2 = 0,32$, $p < 0,05$). Cependant, les résultats d'une analyse en composante principale (ACP) ont permis d'illustrer une association positive entre la production bactérienne, les nutriments et la MOD d'origine allochtone (Fig. 2.5a). Cette matière organique constituerait ainsi un élément majeur de la diète des bactéries. Ici encore, la connectivité hydrologique entre les plaines inondables du lac Saint-Pierre et la masse d'eau de la rivière Saint-François a pu jouer un rôle clé dans l'utilisation de la MOD par les bactéries. En effet, une hausse des niveaux d'eau a pu contribuer à un apport en nutriments et en matière organique terrestre « jeune » qui aurait favorisé la production bactérienne. De plus, des études récentes ont montré que des groupes phylogénétiques de bactéries ont une utilisation différentielle de molécules à faible et haut poids moléculaires (Cottrel & Kirchman 2000, Covert & Moran 2001, Kirchman et al. 2004). En plus de l'apport en MOD terrestre, la hausse de la connectivité hydrologique a pu favoriser l'introduction d'une communauté de bactéries, provenant des terres humides, spécialisées dans l'assimilation de MOD d'origine allochtone. Finalement, l'association négative entre la production bactérienne et la production phytoplanctonique (P_{max}) est en accord avec la vision d'un écosystème prédominé par les sources allochtones où les bactéries hétérotrophes utilisent le carbone provenant d'autres sources que celle de la production phytoplanctonique. Cependant, dans cette étude, la productivité des autres sources de MOD autochtone (macrophytes, épiphytes, etc.) n'a pas été mesurée.

Toutefois, la MOD terrestre est composée principalement de substances humiques à haut poids moléculaire, ce qui rend son utilisation par les bactéries moins efficace que l'utilisation de la MOD autochtone de plus faible poids moléculaire (Amon & Benner 1996, Kritzberg et al. 2005). La seconde ACP a montré cette différence dans la qualité alimentaire des sources de carbone par une association positive entre la biodisponibilité de la MOD et le carbone dérivé de sources autochtones (Fig. 2.5b). Ainsi, un petit pourcentage du carbone autochtone peut participer à un plus grand pourcentage dans la biomasse des bactéries. Cependant, puisque plus de 80% de la MOD dans la rivière Saint-François provient de source allochtones, ce carbone contribuait nécessairement à la majorité de la biomasse des bactéries.

Les résultats de cette étude sont en accord avec d'autres recherches montrant une contribution significative de la MOD terrestre dans la production bactérienne de lacs non-eutrophes et humiques (Jansson et al. 2000, Drakare et al. 2002, Steinberg 2003). Des évidences pour la contribution relative du carbone allochtone à la production bactérienne pour des lacs eutrophes et humiques restent cependant peu nombreuses (Steinberg 2003). La production primaire étant élevée dans ces lacs, le carbone autochtone est traditionnellement vu comme étant la principale source d'énergie pour les niveaux trophiques supérieurs. Cependant, dans le contexte d'un écosystème comme un lac fluvial, la connectivité hydrologique joue un rôle dans la structure des écosystèmes et peut influencer la composition de la MOD et l'utilisation des différentes sources de carbone par les communautés bactériennes. Toutefois, la biodisponibilité relative de la MOD était reliée davantage à la MOD d'origine autochtone, ce qui suggère que l'efficacité de croissance des bactéries était supérieure en présence de MOD autochtone. Il reste donc à évaluer si la MOD allochtone joue un rôle clé dans la production secondaire ou si sa contribution est limitée à la respiration de ses consommateurs.

1.4.3 Chapitre III

Les résultats de cette expérience sont basés sur des outils optiques et chimiques et impliquent des processus photochimiques et microbiens dans la dégradation et sur la biodisponibilité de la MOD provenant d'une rivière influencée par des apports terrestres. Puisque la filtration tangentielle n'a pas permis d'éliminer assez de bactéries pour prévenir une croissance bactérienne importante, il n'a pas été possible d'isoler l'effet photochimique de l'effet microbien. Cependant, la comparaison des traitements à l'obscurité avec les traitements exposés à la lumière a permis d'évaluer la contribution de la lumière à la dégradation de la MOD.

1.4.3.1 Changements optiques de la MOD exposée à la lumière solaire et à l'activité microbienne

Les analyses spectrales de la MOD ont révélé des changements significatifs dans la composition au cours de l'exposition à la lumière. Une diminution de l'absorbance à 375 nm (Fig. 3.2a) ainsi qu'une augmentation du ratio en absorbance $a250{:}a365$ (Fig. 3.2b) observées pour les traitements UV-PAR et PAR seulement sont consistants avec les résultats d'autres chercheurs, qui suggèrent une photodégradation de substances à haut poids moléculaire en substances à faible poids moléculaire (Lindell et al. 1995, Wetzel et al. 1995, Obernoster & Herndl 2000, Osburn et al. 2001, Eugelhaupt et al. 2003). Une plus grande diminution du coefficient d'absorption a été observée pour le traitement avec UV, en accord avec d'autres études soulignant la nature plus énergétique du rayonnement ultra-violet (e.g. Moran & Zepp 1997).

La spectrofluorométrie en trois-dimensions (MEEs) combinée avec la modélisation PARAFAC utilisée dans le précédent chapitre a permis de différencier l'effet des processus photochimiques et microbiens sur les composantes reliées à une origine allochtone et autochtone. Seule la fluorescence des composantes d'origine allochtone a montré une variation significative au cours de l'incubation à la lumière (Fig. 3.3). Cette variation était similaire à celle observée en absorbance, soit une diminution constante de l'intensité de fluorescence, particulièrement en présence

d'UV, indiquant une transformation de grosses molécules en molécules plus petites. L'absence de variation significative des composantes autochtones exposées à la lumière a confirmé la nature moins aromatique et donc moins photoréactive de la MOD d'origine algale (Wetzel 2001).

1.4.3.2 Changements en chimie et en fluorescence des fractions de la MOD exposée aux processus photochimiques et microbiens

Les analyses chimiques de l'eau provenant de la rivière Saint-François ont révélé une concentration importante de la fraction des acides hydrophiliques (HyA) (Fig. 3.4), démontrant le caractère non-humique de la rivière, basé sur le faible contenu en composés aromatiques des HyA. Des résultats similaires ont été observés dans les différentes masses d'eau du lac Saint-Pierre (données non publiées). Cependant, les analyses optiques suggèrent plutôt l'eau de la rivière Saint-François comme étant humique, considérant la forte absorbance du DOC dans les UV (Tranvik & Bertilsson 2001). Les résultats ont donc reflété la forte absorptivité des UV par la portion humique (acides humiques et fulviques) de la MOD, même si ces fractions ne forment pas la majeure partie du pool de MOD.

Les résultats chimiques obtenus avec la résonance magnétique nucléaire (H^1 RMN) sur les fractions d'acide fulvique (FA), d'acide humique (HA) et d'acide hydrophilique (HyA) n'ont pas appuyé les résultats observés en optiques (Tableau 2.3). Le pourcentage de composés aromatiques, associés à des molécules réfractaires, n'a pas diminué significativement sous les traitements de lumière et de bactéries. De plus, le pourcentage en poids relatifs n'a révélé aucun changement significatif dans les fractions de la MOD (Fig. 3.4). Utilisant ^{13}C RMN, Eugelhaupt et al. (2003) n'ont également pas observé de patron constant dans le contenu en carbone aliphatique et aromatique suite à une exposition ;a la lumière, même si des changements en absorbance ont été observés. Il a été suggéré que la photolyse agit sur des types de chromophores spécifiques plutôt que sur des liens carbonés généraux (carbone aromatique et aliphatique, glucides, chaînes saturées). Ceci pourrait expliquer la différence entre nos résultats en optique et en chimie.

Les MEEs en fluorescence ont permis d'approfondir les caractéristiques des fractions de la MOD (FA, HA et HyA). La fluorescence des cinq composantes pour chacune des fractions de MOD a montré la plus grande contribution générale en fluorescence des acides humiques (Fig. 3.5), ce qui est consistant avec la nature aromatique de cette fraction. Les fractions HyA et HA étaient dominées par la composante reliée à la tyrosine (C2) (Fig. 3.5b et c). Dans l'étude de Chen et al. (2003), les auteurs ont observé la même dominance des composantes protéiniques dans la fluorescence des fractions HyA et HA et ce, pour différentes sources d'eau. Ainsi, dans notre étude et celle de Chen et al. (2003), les molécules représentatives d'une origine autochtone sont des constituants majeurs de la fraction hydrophilique biodisponible mais également de la fraction humique plus réfractaire à la consommation bactérienne. Ceci peut s'expliquer par la structure de ces molécules qui diverge entre les HyA et les HA. Les HyA sont probablement composés de protéines simples et solubles et de glucides qui contribuent à la forte biodisponibilité des HyA. Les HA comportent des protéines plus complexes et non solubles qui sont moins biodisponibles (Steinberg 2003). Ainsi, les HyA et les HA seraient composés de molécules ayant des MEEs en fluorescence similaires, mais possédant des structures et des biodisponibilités différentes. Au contraire des autres fractions, les composantes 4 et 5, correspondant à une fluorescence humique et fulvique respectivement, ont contribué le plus à la fluorescence des FA (Fig. 3.5a).

1.4.3.3 Importance de la phototransformation de la MOD sur la production bactérienne

Les transformations photochimiques de la MOD n'ont pas résulté en une augmentation de la production bactérienne. En effet, l'incubation de l'eau de la rivière Saint-François filtrée sur 0,2µm et inoculée avec des bactéries sous la lumière solaire (UV-PAR), sous le PAR seulement et à l'obscurité pour une période de 12 jours n'a révélé aucun patron constant dans la production bactérienne par rapport aux conditions initiales (Fig. 3.6). La production bactérienne pour les traitements UV-PAR et PAR seulement n'a jamais été significativement plus élevée comparé au

contrôle noir, excepté pour le jour 8, où le traitement PAR a montré une augmentation par rapport au noir. Du début à la fin de l'incubation, la production bactérienne pour le traitement UV-PAR n'a montré aucune différence significative par rapport au traitement PAR ($p > 0,05$; test de t non-pairé avec un ajustement Bonferroni).

En fait, ces transformations semblent avoir réduit la biodisponibilité de la MOD pour les bactéries. La biodisponibilité de la MOD, calculée comme le ratio de la production bactérienne sur la concentration en DOC (Ziegler & Benner 2000), a diminué significativement du jour 2 à la fin de l'incubation pour le traitement PAR et du jour 8 à la fin pour le traitement au noir ($p < 0,05$, ANOVA) (Fig. 3.7). Aucune différence significative entre les trois traitements n'a été observée à aucun moment de l'incubation ($p > 0,05$, test de t non-pairé avec un ajustement Bonferroni).

Les facteurs qui influencent principalement la production bactérienne et son utilisation de la MOD sont 1) la composition de la communauté bactérienne, 2) la biodisponibilité des nutriments et 3) la composition chimique de la MOD (del Giorgio & Davis 2003). Considérant que le même inoculum de bactéries provenant de la rivière Saint-François a été utilisé dans tous les échantillons d'eau, les explications possibles pour les résultats de l'activité bactérienne sont les nutriments et la composition de la MOD. La possibilité d'une limitation en azote ou en phosphore des bactéries ne peut être totalement mise de côté. Cependant, les concentrations en azote total et phosphore total sont très élevées dans la rivière Saint-François (Tableau 2.1) causée par l'apport important de nutriments du bassin versant. De façon générale, aucune évidence de limitation en nutriments pour les films biologiques du lac Saint-Pierre n'a été observée précédemment (Huggins et al. 2004). Il serait ainsi surprenant que les bactéries aient été soumises à une limitation en nutriments au cours des 12 jours d'incubation.

Plusieurs études ont montré une augmentation de la production bactérienne dans des échantillons d'eau exposés à la lumière solaire naturelle ou artificielle ((Lindell et al.

1995, Wetzel et al. 1995, Miller & Moran 1997, Benner & Biddanda 1998, Engelhaupt et al. 2003, Smith & Benner 2005). Ces études ont été effectuées avec de l'eau influencée par d'importants apports de matière terrestre. La croissance bactérienne peut être limitée par la faible biodisponibilité de cette eau riche en substances humiques et les transformations photochimiques semblent avoir un impact positif sur l'activité bactérienne (Lindell et al. 1995). Dans notre étude, la transformation photochimique de la MOD n'a pas résulté en une plus grande production bactérienne et, de façon générale, a même résulté en une baisse de la biodisponibilité de la MOD pour les bactéries. Le traitement UV-PAR a entraîné la plus forte transformation de la MOD et les bactéries soumises à ce traitement ont montré une diminution dans l'utilisation de la MOD au cours du temps. La MOD de la rivière Saint-François a une biodisponibilité relative de 0,03 (Fig. 3.7). Ziegler et Benner (2000) ont présenté le ratio BP:DOC de différents environnements, variant entre 1,01 pour la Lagune Madre et <0,06 pour l'eau provenant d'un étant humique. Selon cette étude, nos échantillons d'eau initiaux de la rivière Saint-François peuvent être classés comme étant hautement réfractaires à la transformation biologique. Ainsi, les processus photochimiques étaient sensés avoir une influence positive sur la production bactérienne et la biodisponibilité, ce qui n'a pas été observé. De plus, Tranvik et Bertilsson (2001) ont appliqué un modèle de régressions multiples à une banque de données de 30 lacs différents, reliant l'augmentation de la croissance bactérienne suite à une exposition aux UV aux concentrations en carbone autochtone vs allochtone et à la chlorophylle *a*. Comme prédit par ce modèle, la production bactérienne de l'eau de la rivière Saint-François aurait dû être positivement influencée par l'irradiation de la MOD par les UV. Alors, comment peut-on expliquer l'effet négatif des UV sur la production bactérienne observé dans notre expérience? La biomasse bactérienne et non seulement la MOD ont été exposées à la lumière durant l'incubation. Ainsi, les bactéries ont pu être affectées négativement par l'exposition à la lumière directe, ce qui aurait pu empêcher tout effet bénéfique pour les bactéries de la transformation de la MOD (Herndl et al. 1993, Wilhelm & Smith 2000, Maranger et al. 2003). Également, la seconde mesure de production bactérienne a été faite après 48 heures d'incubation,

ce qui représente une longue période de temps dans le cycle de vie d'une bactérie. Il est possible que l'effet positif de la transformation de la MOD ait été visible sur une échelle de temps plus courte. Il serait intéressant de refaire l'expérience sur une échelle de temps plus courte en s'assurant 1) qu'il n'y a pas de limitation en nutriments pour les bactéries 2) que la filtration sur 0,2µm soit efficace à enlever la majorité des bactéries et à prévenir une croissance trop importante de ces dernières et 3) que l'inoculation de bactéries dans l'eau filtrée soit effectuée après l'exposition à la lumière afin d'éviter des dommages aux bactéries causés par la lumière. Ainsi, les mesures de production bactérienne refléteront les changements dans la biodisponibilité de la MOD et permettront de relier les caractéristiques optiques et chimiques de la MOD à son utilisation par les bactéries.

1.5 Conclusions générales

Les mesures optiques, en absorbance et en fluorescence tridimensionnelle, se sont avérées une option très intéressante à la fois pour identifier les sources de carbone présentes dans un milieu lotique et pour suivre la dégradation (photochimique et biologique) de la MOD. Elles ont permis d'examiner la connectivité hydrologique sur un plan horizontal dans un lac fluvial et de mettre en relief l'importance de la MOD d'origine terrestre dans la production bactérienne d'un tel système (Chapitre II). Également, les analyses optiques ont révélé une transformation de la MOD d'origine allochtone au cours de l'exposition à la lumière et aux microbes, montrant une dégradation de grosses molécules en plus petites molécules (Chapitre III). Le long de la circulation de la masse d'eau de la rivière Saint-François dans le lac Saint-Pierre, des transformations photochimiques et biologiques peuvent ainsi modifier la composition de la MOD et influencer par le fait même son utilisation par les bactéries hétérotrophes. Malheureusement, des problèmes méthodologiques ont empêché d'établir des conclusions claires quant aux répercussions de ces changements sur la biodisponibilité de la MOD pour les bactéries.

Cependant, l'expérience en milieu contrôlé a permis de cerner certaines limites des analyses optiques. Puisque l'approche utilisée n'est valide que pour la partie fluorescente de la MOD, elle sous estime la contribution des composés non aromatiques tels que les HyA, qui ont une nature faiblement photoréactive. Les analyses chimiques ont révélé une forte abondance de ces acides dans le milieu étudié, ce qui n'a pu être visible à l'aide de la fluorescence. Dans la masse d'eau de la rivière Saint-François, une importante partie de la MOD était donc formée de HyA biodisponibles qui jouent un rôle écologique potentiellement majeur dans la productivité de l'écosystème. Les analyses chimiques sont donc essentielles afin d'identifier la composition moléculaire de la MOD totale, et non seulement sa portion chromophorique. De plus, davantage de recherches sont nécessaires dans le but d'identifier la signature en fluorescence des différentes sources de carbone (périphyton, épiphytes, végétation ligneuse) contribuant au pool de MOD. L'utilisation conjointe des analyses chimiques, des isotopes stables et de la fluorescence permettrait d'avoir une vision plus complète de la dynamique de la MOD dans les écosystèmes aquatiques.

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CHAPITRE II

Fluorescence analyses of sources and bioavailability of DOM in the littoral zone of the fluvial Lac Saint-Pierre: importance of hydrological connectivity

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Running head: Fluorescence analyses of DOM sources and bioavailability

2.1 ABSTRACT

Three-dimensional fluorescence spectroscopy combined with parallel factor (PARAFAC) data analysis was used to assess the different sources of carbon (terrestrial and aquatic) sustaining bacterial production in the south shore water mass of the fluvial Lac Saint-Pierre (Québec, Canada). This shallow lake is densely covered with macrophytes during the summer, and surrounded by wetlands which largely modify the connectivity between the littoral and pelagic zones. The lake is also strongly influenced by tributaries, which contribute to the make up of different water masses with distinct physical and chemical characteristics. Significant decreases in autochthonous and increases in allochthonous DOM fluorescence were observed during the summer. Theses changes were related to fluctuations in water levels which in turn affected hydrological connectivity between the littoral and pelagic zones and the tributary and the main water body. With regards to the upstream-downstream gradient of water circulation, fluorescence intensity of terrestrial DOM decreased from mid-July onwards. Transformation processes as well as presence of macrophytes are likely to have modified the DOM as the Saint-François water mass flowed into the lake. Bacterial production was positively associated to allochthonous DOM which fluctuated with water levels, but the DOM relative bioavailability was positively related to autochthonous DOM. These results illustrate the capacity of the bacterial community to use different carbon sources and highlight the importance of terrestrial DOM in the microbial food web of a fluvial lake. Hydrological connectivity between wetlands, tributaries and the fluvial lake

played a key ecological role in structuring the quantity and quality of DOM flowing into the ecosystem.

KEY WORDS: Fluorescence, DOM, Bacterial production, Bioavailability, Fluvial lake, Hydrological connectivity

2.2 INTRODUCTION

Many studies have recognized the importance of microbial food webs in the transfer of energy within pelagic ecosystems. It is now well known that heterotrophic bacteria utilize dissolved organic matter (DOM) and transfer it to higher levels via their consumption by predators (Pomeroy & Wiebe 1988). Traditionally, *in situ* primary production has been recognised as supporting heterotrophic production, minimising the implication of interactions between the microbial food web and the algal-grazer pathway for secondary production. However, evidence underlining the net heterotrophy of most freshwater ecosystems has called this view in question (del Giorgio & Peters 1994, Cole et al. 2000). In heterotrophic ecosystems, respiration exceeds gross primary production which has led to the generalization that heterotrophic organisms utilize external sources of organic matter. Terrestrial organic matter is known to contribute to ecosystem respiration, but the importance of its contribution to secondary production is still poorly understood (Meili et al. 1996, Grey et al. 2001).

The efficiency of DOM recycling depends on its quality, which is intimately linked to its source. DOM of terrestrial origin (allochthonous) is characterized by

large molecular size and high aromaticity (Aiken 1985, Geller 1986) and is more recalcitrant to microbial degradation than autochthonous DOM originating from aquatic primary producers. Thus, several studies demonstrated that heterotrophic bacteria preferentially utilize more labile autochthonous DOM (Cole et al. 1988, Reche et al. 1996, Carillo et al. 2002). In these studies, where primary production contributes to the main part of the DOM, bacterial abundance and production were strongly correlated to phytoplankton production, indicating bacterial dependence on algal derived carbon. However, in humic and oligotrophic lakes, where primary production is low, studies have shown that allochthonous DOM participated substantially to ecosystem productivity (Jansson et al. 2000, Drakare et al. 2002, Kritzberg et al. 2004, Pace et al. 2004, Daniel et al. 2005) although autochthonous DOM is used preferentially. Hence, heterotrophic bacteria are clearly able to depend on autochthonous and allochthonous carbon sources. Variation in the relative extent of the utilization of each of these sources may depend on the trophic status of the ecosystem.

Evaluating the carbon sources utilized by bacteria remains difficult because of the complex nature of DOM which is composed of a heterogeneous mixture of dissolved substances. These substances are subsequently influenced by microbial and photochemical degradation processes. Several studies have utilized stable isotopes to attribute the source of carbon consumed by heterotrophic organisms (Jones et al. 1998, Coffin et al. 1989, McCallister et al. 2004, Kritzberg et al. 2004). Although a powerful tool, this method is based on isotopic differences between carbon sources and isotopic characteristics are not always distinct in freshwater

ecosystems (Cole et al. 2002, Pace et al. 2004). On the other hand, bio-optical tools combining the absorbance and fluorescence properties of groups of molecules have been successfully used as tracers of DOM sources (Coble et al. 1990, McKnight et al. 2001, Stedmon & Markager 2001). Recently, a new fluorescence spectroscopy method, combining fluorescence excitation-emission matrix (EEM) spectroscopy and parallel factor (PARAFAC) data analysis have been developed to characterize fluorescent DOM and trace its different fractions in the ecosystem (Stedmon et al. 2003). This approach is based on the assumption that the optically active fraction of DOM, called chromophoric dissolved organic matter (CDOM), is a proxy for the whole DOM pool.

The aim of this study was to apply this fluorescence method to characterize carbon sources (autochthonous and allochthonous) supplying bacterial production in Lac Saint-Pierre (Québec, Canada) along spatio-temporal gradients. Lac Saint-Pierre is the largest fluvial lake in the St. Lawrence River where various sources of DOM can be found. This lake is extensively covered by a large macrophytes biomass and connected to tributaries which introduce water masses characterized by distinct physical and chemical properties, including a large supply of DOM (Frenette et al. 2006). Hydrological connectivity between the surrounding floodplain and the lake favours transport of terrestrial subsidies toward the aquatic ecosystem on an annual basis. This study relates to the south shore water mass which receives terrestrial material from the wetland and from the Saint-François River tributary. As the water mass circulates into the lake, photochemical and microbial transformations of DOM create spatial heterogeneity on the longitudinal axis (Frenette et al. 2006).

The goals of the study were 1) to document the spatial and temporal changes in the DOM characteristics (allochthonous and autochthonous) of a water mass circulating in fluvial Lac Saint-Pierre during water level fluctuations and 2) to evaluate the importance of different DOM sources to sustain bacterial productivity.

2.3 MATERIALS AND METHODS

2.3.1 Study site. Lac Saint-Pierre (l46°12'N, 72°50'W) is a large (~400 km^2) fluvial lake formed by the broadening of the St. Lawrence River and is the last major enlargement (13.1 km width at mean discharge) of the river before the St. Lawrence estuary. Lac Saint-Pierre is shallow (mean depth of 3.17 m during the period of mean discharge) and has been recognised as an "ecological reserve of the biosphere" by UNESCO in 2000 due to its high biodiversity. This highly productive ecosystem represents the largest freshwater wetland ecosystem in Quebec (Burton 1991) and is covered with extensive submerged and emergent macrophyte beds from June to September. Macrophytes are especially abundant on the south shore within the Saint-François River water mass. This water mass is influenced by an agricultural watershed characterized by relatively high concentrations of nutrients (Huggins et al. 2004) and CDOM (Frenette et al. 2006).

2.3.2 Sampling stations within the Saint-François River water mass. Water samples were collected at 15 stations distributed along the Saint-François River water mass (Fig. 1a-c) from mid-June to mid-August 2004. Bacterial and phytoplankton primary production were measured at 6 and 5 stations respectively (5 of these were the same) from mid-July to mid-August (Fig. 1c). Table 1 summarizes

the station number assignment in the longitudinal and lateral transects as well as the environmental properties associated with the stations during the sampling period.

The location of the stations within the same water mass was determined with a 2D hydrodynamic model (horizontal). The currents and the water levels were simulated with HYDROSIM that uses the conservative form of the quantity of movement from Saint-Venant equations and takes into account the local frictions associated with the substratum and the submerged plants (see Morin et al. 2000). Further details about water mass distribution in Lac Saint-Pierre using this approach can be found in Martin et al. (2005) and Frenette et al. (2003). As the water mass distribution changes over the season, hydrodynamic modeling was conducted before each of the six sampling campaigns to ensure that the selected sites were located within the Saint-Francois River water mass. Consequently, the position of the stations changed between the campaigns (Fig. 1a-b).

To take into account potential lateral mixing between water masses, Landsat 5 image from the 27 June 2005 (Fig. 1a-b) was used to validate the position of the stations within the Saint-François River mass for the six sampling campaign of summer 2004. The water levels were similar in June 2004 and 2005 and were about equivalent or higher to those of mid-June 2005 for the remaining of summer 2004. CDOM and suspended particulate inorganic matter (SPIM) are responsible for generating color producing agents (CPA) in inland waters. Optical satellite sensors can be utilized to quantify these agents (Jerome et al. 1994, Bukata et al. 1995). The limits of the Saint-François River mass distribution in the lake was determined by assessing the contribution of the green (520-600 nm) and red (630-690 nm) bands on

the Thematic Mapper (TM) images. When compared to the other water masses of Lac Saint-Pierre, waters from the Saint-François River have a lower reflection in the green band, caused by a relatively high concentration of CDOM which absorbs in green, and a lesser reflection in red due to low SPIM levels (see Frenette et al. 2006).

2.3.3 Water collection, DOC, nutrients and Chl a. Water samples were collected with an acid-washed acrylic tube allowing integral water column sampling and were pre-filtered on 63 μm (Nitex, Filmar inc.). They were placed in acid-washed 2 l polyethylene bottles and transported on ice to the laboratory, where they were immediately treated.

DOC (dissolved organic carbon) and DIC (dissolved inorganic carbon) samples were filtered through Milli-Q rinsed 0.22 μm Isopore membrane (Millipore). Their concentrations were estimated using high temperature catalytic oxidation on a Shimadzu TOC 5000A instrument. TN (total nitrogen) and TP (total phosphorous) concentration were measured spectrophotometrically following persulfate digestion. Chlorophyll *a* (Chl *a*) samples were filtered through a 25 mm GF/F filter (Whatman). Hot ethanol method was used for the extraction of Chl *a* according to Marker et al. (1980b). Extractions continued in the dark at 4 °C for 1 h after which sample absorption measurements were taken at 665 and 750 nm (Shimadzu spectrophotometer, UV-Probe, Columbia, MD, USA) before and after acidification to correct for phaeopigments.

2.3.4 Absorbance measurements. Water samples were filtered through rinsed Milli-Q 0.22 μm Isopore membrane (Millipore) before analysis. An

absorbance spectrum (190-900 nm) was performed on the samples using a Shimadzu UV-2401 PC spectrophotometer in 1 cm quartz cuvette with NANOpure water as reference. Absorption coefficients at 375 nm (aCDOM$_{375}$) were calculated using: $a_{375} = A_{375} \times 2.303/b$, where A_{375} is the absorbance of the sample at 375 nm and b is the path length of the cuvette in meters (Kirk 1994). The absorbance at 690 nm (where temperature dependency is near zero) was used to correct the UV-absorptivity values (Laurion et al. 2000).

2.3.5 Fluorescence measurements. The fluorescence measurements were performed using a spectrofluorometer (Cary Eclipse, Varian Co., Palo Alto, CA, USA) in a 1 cm quartz cuvette. An excitation-emission matrix (EEM) for each sample was obtained by combining a series of emission scans (230-600 nm, 2 nm increments) while exciting at wavelengths between 220 and 450 nm every 5 nm. The excitation and emission bandwidths were 5 nm. The EEMs were corrected for inner-filter effects using the measured absorbance spectra (Mobed et al. 1996, McKnight et al. 2001). The EEMs were also Raman calibrated and corrected for instrument biases according to the methods described in Stedmon et al. (2003).

2.3.6 PARAFAC modeling. PARAFAC modeling was performed in MATLAB using "PLS_Toolbox from Eigenvector" (Anderson & Bro 2000, see Stedmon et al. 2003). In addition to the data from this study, EEMs from a microbial and photochemical degradation experiment and from isolated fractions of DOM from Lac Saint-Pierre were included in the model. This increased the range of DOM signatures and improved the PARAFAC modeling (see Stedmon & Markager 2005a). The EEMs were combined in a three-dimensional data array (288 samples x

186 emission wavelengths x 46 excitation wavelengths) and a series of PARAFAC modeling, from 2 to 6 components, were fitted to the data set. In order to determine the appropriate number of components, a split-half analysis was performed, comparing the excitation and emission spectra of the components between the calibration and validation data arrays. Up to five components were validated using this technique (Fig. 3). Examination of residuals indicated little signal information which warranted the use of the five component model (data not shown).

As Stedmon & Markager (2005b), fluorescence of each component is stated as Fmax (RU), corresponding to the fluorescence at the excitation and emission maximum (Table 2).

2.3.7 Bacterial production and DOM bioavailability. Bacterial production was estimated using ^{3}H-leucine incorporation as described by Kirchman in Kemp et al. (1993) and adapted to the use of microtubes according to Smith & Azam (1992). Sample water (1.5 ml, 5 replicates) was incubated in darkness at the in situ station temperature for 30 min with L-4,5 ^{3}H-leucine (PerkinElmer, 59.5 Ci mmol^{-1} ^{3}H-leucine, 10 nM final leucine concentration). Three killed controls (blanks) for every three live incubations were obtained by adding 50% trichloracetic acid (TCA) to determine abiotic sorption of the ^{3}H-leucine. Incubations were terminated by addition of 50% TCA. The centrifugation, vortex, and wash sequence were carried out as described in Smith & Azam (1992) with an addition of a final 80% ethanol wash. Microtubes were then exposed to the air for one night, allowing ethanol to evaporate. Scintillation cocktail was added to the tubes at least two days before scintillation counting of the samples.

In order to evaluate the impact of carbon sources on bacterial production, an index of DOM bioavailability was used. It was calculated as the ratio of bacterial production to the concentration of DOC (BP:DOC, Ziegler & Benner 2000).

2.3.8 Phytoplankton primary production. Phytoplankton primary production was measured using photosynthesis rate-irradiance curves, realized in a radial incubator modified by Babin et al. (1994). The incubator was composed of six incubation chambers arranged radially around a 230-W Optimarc lamp (Duro-Test Crop) that provided an irradiance spectrum into the visible similar to that of the solar light. All walls of the incubation chamber were composed of black Plexiglas, except the one facing the light, which was made of white-diffusing Plexiglas. Each chamber was designed to contain twelve 70 ml culture-flask (BD Falcon tubes) minimizing light refraction. Ten flasks were incubated along a light gradient varying from 7 to 996 μmol quanta m^{-2} s^{-1} depending on stations. Two dark flasks were used as control. Irradiance (PAR, 400-700 nm) was measured in each incubation bottle with a Biospherical quanta meter (QSL-2100, Biospherical Instrument Inc., San Diego, CA). The chamber has a false-bottom and in and out entries of controlled-temperature water allowing a constant temperature.

Twelve 25 ml subsamples were taken from the sample bottles and dispensed into the flasks. They were inoculated with a solution of $NaH^{14}CO_3$ to obtain a final concentration of 0.1 μCi ml^{-1}. Initial activity was measured in triplicates by adding 1 ml of sample to 1 ml of ethanolamine. Samples were then incubated for 60 min. Incubations were terminated by adding 830 μl of 6 N HCL in each flask. Subsamples of 5 ml were taken and added to scintillation vials containing 10 ml of

Universol ES scintillation cocktail (ICN). Samples were counted using a liquid scintillation counter. The maximum photosynthetic rate (P_{max}) in $\mu g\ C\ l^{-1}h^{-1}$ was estimated according to the equation of Platt et al. (1980).

2.3.9 Statistical analyses. Correlations between the five fluorescence components were investigated by Pearson correlation and Bonferroni tests of significance. Repeated-measure ANOVAs were performed in SYSTAT to test for differences in autochthonous and allochthonous DOM, bacterial production and DOM relative bioavailability between sampling date and geographical position (lateral and longitudinal gradients). The univariate approach was used because the smallest group did not have more cases than the dependant variables, which is required for the multivariate approach. Univariate repeated-measure ANOVA requires sphericity. Having "time" as a within-subjects independent variable, the assumption of homogeneity of covariance was likely to be violated. Thus, the Greenhouse-Geisser (G-G) significance test which is adjusted for the violation of this assumption was used.

Linear regression analyses (SYSTAT) and two principal component analyses (PCA; CANOCO program 4.0) were done to evaluate the association between environmental variables, carbon sources, bacterial and phytoplankton primary production and DOM bioavailability. For all analyses, variables were log-transformed before analysis when necessary to achieve the assumptions of normality and homoscedasticity.

2.4 RESULTS

2.4.1 Characterization of carbon sources

Five fluorescence components were identified using EEMs combined with PARAFAC modeling. The five components were significantly correlated with each other (Table 3). Components 1 and 2 were positively correlated and both of these were negatively correlated with components 3, 4 and 5. These latter components (3, 4 and 5) were highly and positively correlated. All the components were characterized by comparison with those found in the literature (Table 2). Components 1 and 2 correspond to protein-like fluorescence groups, with a fluorescence peak almost identical to tryptophan (component 1) and free tyrosine dissolved in water (component 2) (Coble et al. 1990, Mopper & Scultz 1993, Chen et al. 2003, Yamashita & Tanoue 2003), known to be derived from autochthonous processes. Components 3 to 5 correspond to allochthonous DOM (Stedmon et al. 2003, 2005a and b and refs therein). Component 4 is common to a wide range of freshwater environments, mostly influenced by agricultural catchments (Stedmon et al. 2003) and component 5 is known to be highly concentrated in forest stream and wetlands (Stedmon et al. 2003). Further in the analyses, we considered the carbon from autochthonous origin as the sum of components 1 and 2 and the carbon from allochthonous origin, as the sum of components 3, 4 and 5.

2.4.2 Spatial and temporal variation in DOM

Results from the repeated-measure ANOVAs showed significant variations in both autochthonous and allochthonous fluorescence from mid-June to mid-August

(Table 4). A significant linear decrease in autochthonous DOM fluorescence was observed with time (Fig. 4b, $p < 0.001$), as opposed to a significant linear increase in allochthonous DOM fluorescence (Fig. 4c, $p < 0.05$). Given that allochthonous carbon formed the majority of the DOM fluorescence pool (average ± SD: 82% ± 6), its fluorescence followed the same pattern as the DOC concentration, which varied between 5 mg l^{-1} and 13 mg l^{-1} (Fig. 4d).

DOM fluorescence from both autochthonous and allochthonous origins did not vary significantly between the three lateral transects, implying no significant variation along the lateral inshore-offshore gradient (Table 4). From upstream to downstream along the Saint-François River water mass, DOM from terrestrial origin showed significant variations in fluorescence throughout the season ($p < 0.05$) (Table 4; Fig. 4c). In early summer, florescence from allochthonous DOM increased along the longitudinal gradient; from mid-July onward, a constant decrease in fluorescence was observed in the downstream portion of the water mass. Even though the effect of the longitudinal gradient was not significant for autochthonous DOM, a pattern of increased fluorescence from upstream to downstream was discernable from mid-July onward (Fig. 4b).

2.4.3 Spatial and temporal variation in bacterial production and P_{max}

Bacterial production and DOM relative bioavailability (BP:DOC) did not vary significantly neither spatially (along the longitudinal and lateral transect) nor temporally (from mid-July to mid-August) (Table 5, $p > 0.05$, repeated-measure ANOVAs). However, bacterial production and the ratio of bacterial production to P_{max} ($BP:P_{max}$) tended to be higher upstream (Table 5). Not enough data were

collected to evaluate spatial variation in P_{max}. However, it was significantly lower on July 29 in comparison to mid-July and mid-August (Table 5, $p < 0.05$, ANOVA). This decrease in P_{max} corresponded to the increase in water level at the same period (Fig. 4a).

2.4.4 Relationships between carbon quality and bacterial production

Bacterial production was not correlated to autochthonous carbon sources ($p = 0.41$) and was positively, but not significantly (at the 5% level), associated to allochthonous carbon ($p = 0.14$). It showed a weak association with TN but no relationship with TP ($p = 0.06$ and $p = 0.27$ respectively). Bacterial production was positively related to water levels ($r^2 = 0.5$, $p < 0.05$) and negatively related to P_{max} ($r^2 = 0.32$, $p < 0.05$). There was a weak positive correlation of BP:DOC with autochthonous carbon ($p = 0.111$) but no significant relationship with allochthonous carbon, TN and TP ($p = 0.254$, $p = 0.262$ and $p = 0.625$ respectively).

Two principal component analyses (PCA) were conducted in order to gain more information about gradients in associations between environmental variables and 1) bacterial production (Fig. 5a) and 2) DOM relative bioavailability (Fig. 5b). In the former PCA, the first factor (47.2% of the variation explained) was mainly represented by carbon origin and the second factor (23.5% of the variation explained), by TN. From this analysis, bacterial production increased with DOM of allochthonous origin and with nutrients, while being negatively associated with DOM of autochthonous origin and DIC. The second PCA illustrated the positive association between DOM relative bioavailability, autochthonous derived carbon and TN. The first factor (47.6% of the variation explained) was represented by

carbon sources and the second factor (23.8% of the variation explained), by TN and DOM relative bioavailability Also, both PCAs clearly underscored the separation between sampling sites on the basis of the spatial gradient: the upstream stations were related to the allochthonous DOM and the downstream stations to the autochthonous DOM, in accordance with results observed in Fig. 4b-c.

2.5 DISCUSSION

2.5.1 Characterization of carbon sources for bacteria

Fluorescence spectroscopy combined with PARAFAC modelling was successfully used in this study to define the origins of the DOM flowing into a specific water mass of Lac Saint-Pierre. Amongst the five modeled components, two were attributable to autochthonous processes and three to terrestrial (allochthonous) origin. The study site was typical of freshwater ecosystems, where the DOM pool was primarily composed of humic substances (Wetzel 1992). In fact, the percentage of allochthonous carbon comprising the fluorescent DOM pool in Lac Saint-Pierre (about 80%) is similar to that in the Amazon River ecosystem, where more than 80% of the DOC belonged to the high-molecular weight fraction (Amon & Benner 1996). Both river systems are strongly influenced by terrestrial exchanges with wetlands and transport of matter from inflowing tributaries.

2.5.2 Temporal variations in DOM

Both allochthonous and autochthonous DOM showed significant variations in fluorescence intensity over the summer season (Fig. 4a-b), illustrating the

heterogeneity in carbon sources within Lac Saint-Pierre. The watershed characteristics are recognized to have a great influence on DOM composition in freshwater ecosystems (Steinberg 2003). The quantity and quality of the materials flowing into the freshwater bodies will depend on the catchment's size, its type of soil and vegetation as well as the microbial activity and the hydrological conditions (Steinberg 2003). Lac Saint-Pierre is markedly influenced by inflowing tributaries and wetlands, which contribute to the spatial and temporal variation in the lake's optical and chemical properties (Frenette et al. 2006). In general, DOC concentrations are positively correlated with discharge (Steinberg 2003) and precipitation events are likely to increase the concentration of terrestrial DOC into the water body. The observed increase in allochthonous carbon fluorescence over the course of the sampling campaign could thus be attributed to the increase in water levels in response to higher discharge rates from inflowing tributaries. Higher water levels corresponded to higher concentrations in allochthonous DOM and DOC (Fig. 4b-c).

This increase in allochthonous carbon loading could also be explained by the accumulation of organic material through time. DOM of terrestrial origin is abundant and generally less bioavailable than DOM of algal autochthonous origin (Wetzel 2001). Throughout the summer, fresh allochthonous DOM arriving from the watershed could have overwhelmed the uptake capacity of heterotrophic bacteria, leading to a net accumulation of detritus material in the lake. In counterpart, the highly labile nature of autochthonous DOM explains its high turnover rate and weak concentration in freshwater ecosystems (Wetzel 2001). In general, autotrophic

primary production is controlled by light and nutrient availability (Wetzel 2001). In large nutrient-rich rivers as the Lac Saint-Pierre, primary producers are mainly controlled by physical factors, such as water level and residence time (Sellers & Bukaveckas 2003), which also affect the light climate of the ecosystem (Frenette et al. 2006). The study of Vis et al. (2006) revealed that phytoplanktonic biomass doubled in the southern water mass of the Lac Saint-Pierre following a 1 m drop in mean water level between 2000 and 2001. This increase was mainly explained by a longer water transit time in Lac Saint-Pierre which allowed the development of a more extensive phytoplankton community. In the month of July of our sampling campaign, the increase in water levels may have contributed to a decrease in water residence time and thereby, to limit growth of primary producers.

2.5.3 Spatial (horizontal) variation in DOM

DOC concentration as well as the fluorescence intensity of allochthonous DOM (mostly composed of humic substances) decreased along the upstream-downstream gradient from mid-July onwards. This pattern can by explained by three main factors: 1) the influence of the Saint-François River decreasing from its point source into the lake, 2) photochemical and microbial processes, and 3) the presence of macrophytes.

The decrease in allochthonous DOM along the longitudinal gradient can be related, in part, to the decreased influence of the Saint-François River with increasing distance from the source. The study of Frenette et al. (2006) demonstrated the importance of horizontal (longitudinal and lateral) connectivity in driving chemical and physical processes of a fluvial lake. The Saint-François River, by the

agricultural characteristics of its watershed, acts as a source of nutrients, DOM and particulate matter to Lac Saint-Pierre. Increased distance from the source could have resulted into a decreased loading of terrestrial material due to sedimentation of particulate material and dilution from the water mass in place. The south shore of Lac Saint-Pierre is characterized by small canals connecting the wetlands to the main water body. However, the absence of significant variation in DOM along the lateral transect (Table 4) suggested that the lateral input from the wetlands was well mixed with the upstream-downstream contribution from Saint-François water mass.

In addition to variations in DOM loading from wetlands and tributaries, *in situ* physical, chemical as well as biological processes occurring in the ecosystem, may contribute to alter the DOM content and composition. Photochemical and microbial processes contribute to the incorporation of DOC into the microbial food web by changing the bioavailability of DOM (Weigner & Seitzinger 2001). Through the action of sunlight, particularly the UV radiation absorbed by CDOM, high molecular weight molecules are cleaved into smaller entities (Strome & Miller 1978) which are efficiently taken up by bacterioplankton (Kieber et al. 1989, Bertilsson & Tranvik 1998, Obernosterer et al. 1999a). The impact of photochemical processes on DOM bioavailability will depend on the origin and chemical composition of DOM. As summarised by Moran and Covert (2003), DOM of terrestrial origin, which is older and more aromatic, is more labile to bacteria under irradiation, while DOM from algal origin is more recalcitrant to bacterial processing after irradiation. During the downstream transport of the Saint-François water mass, photochemical and microbial transformations of DOM occurred and may have contributed to the

observed decrease in allochthonous DOM along the longitudinal axis. On the other hand, autochthonous DOM may have become more recalcitrant to microbial degradation, leading to its accumulation downstream. The influence of these transformations on the CDOM fluorescence signal requires further investigation.

Also, presence of large macrophytes beds situated downstream of the Saint-François water mass may greatly influence the nature and quantity of DOM. Macrophytes are recognized to play a key role in structuring and modifying the physical, chemical and biological properties of the water column. Among other things, they contribute to reduce current velocity, thereby increasing the water residence time (Sand-Jensen & Mebus 1996, Morin et al. 2000). The study of Martin et al. (2005) showed a decrease in the CDOM to DOC ratio along an upstream-downstream transect within a macrophyte bed of Lac Saint-Pierre. This decrease was explained in part by the exposition of DOM to sunlight for longer periods of time. Thus, in this study, the large amount of macrophytes in the Saint-François River water mass may have contributed to the photochemical transformation of DOM by increasing the solar exposure time. Furthermore, several studies have suggested that the photosynthetic activity of macrophytes contributed to the DOC pool, mainly composed of low molecular weight compounds in shallow aquatic ecosystems (Mann & Wetzel 1996, Bertilsson & Jones 2003). Thus, the increase in autochthonous DOM downstream could also be attributable to the exudation and/or the leachate release of autotrophic DOC from living macrophytes. However no significant increase in autochthonous DOM could be observed with time during the study period where increase biomass of macrophytes occurred.

2.5.4 Bacterial production and relative bioavailability of DOM

As for DOM sources, bacterial production, P_{max} and DOM relative bioavailability varied greatly within the same water mass, both in time and space (Table 5). Patchiness of high as well as low values of BP:P_{max} could be observed in Lac Saint-Pierre. High BP:P_{max} are characteristic of humic lakes (>0.5) and low values (<0.5) are characteristic of more oligotrophic lakes (Kritzberg 2005). The DOM relative bioavailability, ranging from 0.05 to 0.29, was also characteristic of lakes of different trophic status (Ziegler and Benner 2000). These findings emphasize the very dynamic and heterogeneous nature of fluvial lakes such as Lac Saint-Pierre, which are submitted to various sources of carbon input on an annual basis.

2.5.5 Relationships between carbon quality and bacterial production

No clear significant relationship between bacterial production, DOM relative bioavailability and environmental variables could be observed. However, given that bacterial production was associated with allochthonous DOM (Fig. 5a), terrestrial carbon sources proved to be a major component of the bacterial community diet. The linear regression analyses and the first PCA suggested that bacterial production was, in addition to allochthonous carbon, related to nutrients and water levels. Considering that bacterial production was not limited in nutrients, which were abundant in the study site (Huggins et al. 2004), the results can be explained by the dynamic movements of carbon and the bacterial community. Increases in water levels are likely to favour hydrological connectivity between the wetlands, the tributaries and the main water body, and to consequently contribute to the input of

fresh nutrients and terrestrial DOM from the flood plain. This large contribution of potentially "young" DOM could have favoured bacterial production. Also, recent studies demonstrated that major phylogenetic groups of bacteria have a differential utilisation of high and low molecular weight DOM (Cottrel & Kirchman 2000, Covert & Moran 2001, Kirchman et al. 2004). In addition to the input of terrestrial DOM, a rise in hydrological connectivity could have introduced a different bacterial community from the wetlands and the tributary that specialises in allochthonous carbon uptake. Finally, the negative association between bacterial and phytoplankton production (P_{max}) into the Saint-François water mass, agreed with the view of a predominantly allochthonous ecosystem where heterotrophic bacteria utilize carbon substrates originating from sources other than phytoplankton production. However, this study did not measure the productivity of the other potential sources of autochthonous DOM such as macrophytes or epiphyton.

Terrestrial DOM is largely composed of humic substances of high molecular weight and thus its utilization by bacteria is less efficient than on autochthonous DOM, of lower molecular weight. This was illustrated by the relation obtained from the second PCA, relating DOM bioavailability to DOM from autochthonous origin. Carbon from algal- and macrophytes-derived origin is known to be of higher nutritional value (Wetzel 2001) and of higher bioavailability (Amon & Benner 1996, Kritzberg et al. 2005). Thus, a small percentage of autochthonous carbon may participate to a much greater percentage of the bacterial biomass, but considering that over 80% of the DOM in the Saint-François River mass is from allochthonous sources, this carbon necessarily contributed to the major part of the bacterial

biomass. In Kritzberg et al. (2006b), the authors proposed the use of the CDOM to Chl *a* ratio to predict the contribution of allochthonous carbon to bacterial production. In this study, the CDOM:Chl *a* ratio of 0.6 suggested a relative contribution of 80% by allochthonous carbon to the bacterial biomass. However, this ratio doesn't consider the leachate and exudation contribution by macrophytes and may therefore underscore the autochthonous contribution. Yet, our results showed the negative association of bacterial production to autochthonous DOM, which included macrophytes-derived carbon. In a parallel experiment, we incubated macrophytes from the Saint-François water mass in controlled lab conditions and the fluorescence characteristics showed to be autochthonous (component 1) (data not published). Allochthonous carbon sources were hence likely to support the major portion of bacterial biomass in our study site. Our results are consistent with several studies showing a significant contribution of terrestrial DOM to bacterial production in humic, non-eutrophicated lakes (Jansson et al. 2000, Drakare et al. 2002, Steinberg 2003). Evidence for the relative contribution of allochthonous carbon to bacterial production is scarce for eutrophic, humic lakes as Lac Saint-Pierre (Steinberg 2003). Primary production being high in these lakes, autochthonous carbon is traditionally viewed as the major supply of energy to higher trophic levels. However, in the context of a river ecosystem such as a fluvial lake, hydrological connectivity plays a dominant role in structuring the ecosystem and may influence the availability of carbon and hence, the utilisation of the different carbon sources by the bacterial communities.

2.6 CONCLUSION

Fluorescence spectroscopy has revealed to be a simple and powerful tool to identify carbon sources present in a lotic ecosystem. It allowed us to examine horizontal connectivity in a fluvial lake: on a lateral axis, through the terrestrial-aquatic exchanges between the wetlands and the lake and on a the longitudinal axis, through the water masses exchanges between inflowing tributaries and the lake. In our study site, terrestrial DOM seemed to contribute the most to bacterial production which may be transferred to higher secondary production. However, respiration rates having not been measured, we can interpret the results in terms of production, but not in terms of growth efficiency. It remains to be evaluated if allochthonous carbon plays a key role in secondary production or if its contribution is limited to the respiration of its consumers. The combined use of fluorescence tools and stable isotopes would provide pertinent information about the sources and composition of DOM contributing to aquatic ecosystem productivity.

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2.9 TABLE TITLES

Table 1. Station distribution along the lateral and longitudinal transects and average (min-max) optical and chemical properties at theses stations sampled between 17 June and 12 August 2004. Chl *a*, chlorophyll *a*; TP, total phosphorus; TN, total nitrate; DOC, dissolved organic carbon; $a\text{CDOM}_{375}$, absorption coefficient at 375 nm wavelength

Table 2. Fluorescence maxima for the five identified components and their corresponding characteristics. Secondary maxima are shown in brackets.

Table 3. Correlation matrix for the five fluorescent components. The degree of significance is $p < 0.001$, except indicated by asterisk:* $p < 0.01$. Data are log-transformed.

Table 4. Univariate repeated-measures ANOVA testing the temporal and spatial variations in autochthonous and allochthonous DOM sources. GG-P, Greenhouse-Geisser significance test adjusted for sphericity. Long, longitudinal transect; Lat, lateral transect

Table 5. Bacterial production (BP), maximum photosynthetic rate (P_{max}) and relative bioavailability calculated as BP divided by concentration of DOC

2.10 LIST OF FIGURES

Fig. 1. The 27 June 2005 Landsat 5 satellite image of Lac Saint-Pierre, St. Lawrence River, Canada. Location of the sampling stations was determined with the HYDROSIM model on (a) the 16 June 2004 and (b) the 22 June 2004. The modelisation in (a) was used for the first sampling campaign and the modelisation in (b) was used for the other 5 sampling campaigns. (c) 15 stations distributed along the Saint-François River water mass were sampled from mid-June to mid-August. Bacterial production was measured at six stations (circled) and primary production at five stations (*), from mid-July to mid-August (location of the stations is not at scale).

Fig. 2. Daily mean water levels in Lac Saint-Pierre in 2004 and 2005.

Fig. 3. Validation of the five-component model and the spectral characteristics of each component. Graphs (a-e) show the excitation (dotted line) and the emission (solid line) loadings for each component, obtained from two independent PARAFAC models on random halves of the data array. The fact that they overlap validates the five-component model. The contour plots (f-j) show the spectral characteristics of each component 1-5, respectively. Ex., excitation wavelength; Em., emission wavelength.

Fig. 4. Water levels (a); autochthonous (b) and allochthonous (c) DOM fluorescence and DOC concentration (d) along the summer season and the longitudinal gradient. Error bars indicate ± 1SD of the mean. R.U., relative unit.

Fig. 5. Principal component analysis illustrating the association between environmental variables (TP, total phosphorous; TN, total nitrogen; DIC, dissolved inorganic carbon; DOC, dissolved organic carbon) carbon sources (Allo, allochthonous carbon; Auto, autochthonous carbon) and (a) bacterial production (BP) and (b) DOM relative bioavailability (BP:DOC) for upstream and downstream stations, from mid-July to mid-August.

Station numbers	Lateral transect	Longitudinal transect	Chl *a* (µg l^{-1})	TN (mg l^{-1})	TP (mg l^{-1})	DOC (mg l^{-1})	*a*CDOM$_{375}$ (m^{-1})
1	1	Upstream	5.42 (1.38-9.65)	0.721 (0.66-0.773)	0.041 (0.036-0.047)	8.81 (7.45-12.9)	9.1 (5.9-16.9)
2	1	Upstream	3.46 (1.08-5.45)	0.92 (0.537-1.876)	0.051 (0.036-0.074)	7.89 (5.79-12.56)	8.4 (4.2-16.9)
3	1	Upstream-2	1.14 (0.26-1.82)	0.853 (0.585-1.682)	0.037 (0.027-0.047)	7.83 (5.57-12.60)	8.0 (4.1-16.8)
4	1	Middle	0.93 (0.67-1.6)	0.455 (0.334-0.711)	0.015 (0.008-0.024)	7.67 (6.49-9.63)	6.7 (5.5-9.4)
5	1	Downstream	1.43 (1.13-2.05)	0.411 (0.298-0.772)	0.029 (0.018-0.061)	6.93 (5.76-7.81)	5.5 (4.5-6.7)
6	1	Downstream	1.51 (0.49-2.8)	0.406 (0.303-0.698)	0.025 (0.018-0.033)	7.16 (5.61-7.95)	5.4 (4.2-6.4)
7	1	Downstream	1.5 (0.77-3.89)	0.676 (0.383-1.514)	0.024 (0.017-0.036)	6.8 (4.82-9.51)	6.2 (4-10.8)
8	2	Upstream	4.77 (1.27-6.45)	0.918 (0.588-1.911)	0.045 (0.013-0.072)	7.86 (5.75-12.72)	8.3 (4.2-17.2)
9	2	Upstream-2	1.66 (0.91-2.36)	0.887 (0.53-1.597)	0.034 (0.027-0.044)	7.82 (5.52-12.93)	8.0 (3.9-17)
10	2	Middle	1.3 (0.43-2.31)	0.454 (0.323-0.734)	0.021 (0.013-0.04)	7.54 (6.41-8.4)	6.3 (5.6-7.7)
11	2	Downstream	1.47 (0.9-3.21)	0.441 (0.3-0.829)	0.021 (0.016-0.029)	6.9 (5.95-7.99)	5.4 (4.5-6.3)
12	3	Upstream	5.52 (1.63-8.82)	0.710 (0.519-1.011)	0.041 (0.033-0.052)	8.18 (6.4-12.75)	8.6 (4.6-16.8)
13	3	Upstream-2	1.95 (1.45-2.28)	0.747 (0.84-1.365)	0.032 (0.027-0.037)	7.79 (5.56-12.42)	8.1 (4.3-16.3)
14	3	Middle	1.01 (0.56-1.65)	0.423 (0.356-0.649)	0.019 (0.016-0.024)	7.52 (6.56-8.41)	6.2 (5.4-7.6)
15	3	Downstream	1.28 (0.57-2.05)	0.400 (0.34-0.567)	0.028 (0.018-0.053)	7.06 (6.38-7.52)	5.5 (4.5-6.4)

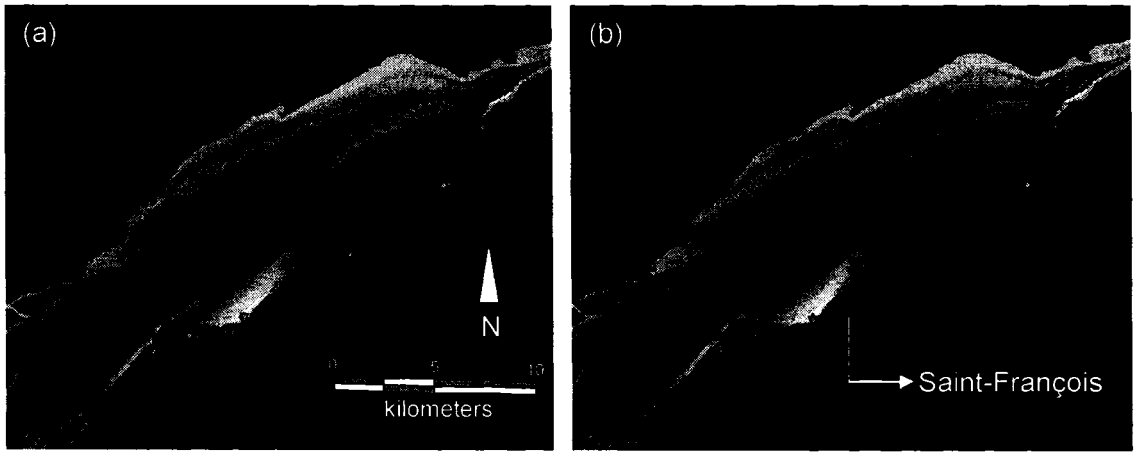
Component	Excitation maximum	Emission maximum	Origin (Stedmon et al. 2005a and b)	Characteristics (Stedmon et al. 2005a and b; Chen et al. 2003)
1	305	374	Autochthonous	Tryptophan and protein-like fluorescence*
2	<250 (275)	305	Autochthonous	Tyrosine-like fluorescence Derived from autochthonous processes
3	355 (270)	471	Allochthonous (terrestrial)	Humic-like fluorescence.
4	330	428	Allochthonous (terrestrial/anthropogenic)	Humic-like fluorescence Exported from agricultural subcatchments
5	<250	438	Allochthonous (terrestrial)	Fulvic-like fluorescence Exported from natural and agricultural catchments

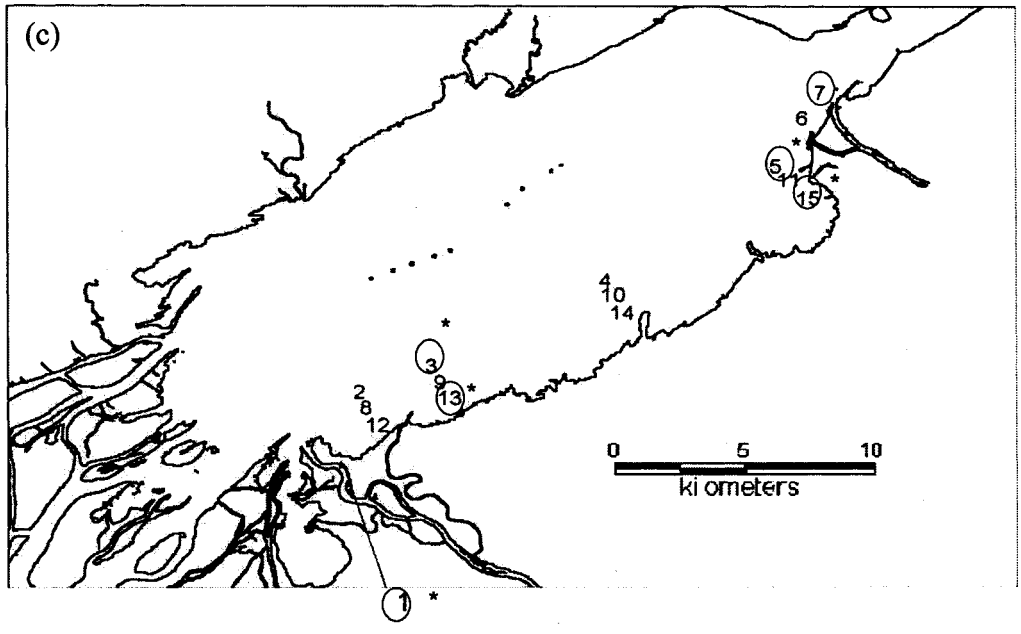
	Comp 1	Comp 2	Comp 3	Comp 4
Comp 5	-0.37*	-0.63	0.94	0.98
Comp 4	-0.42*	-0.67	0.98	
Comp 3	-0.54	-0.72		
Comp 2	0.59			

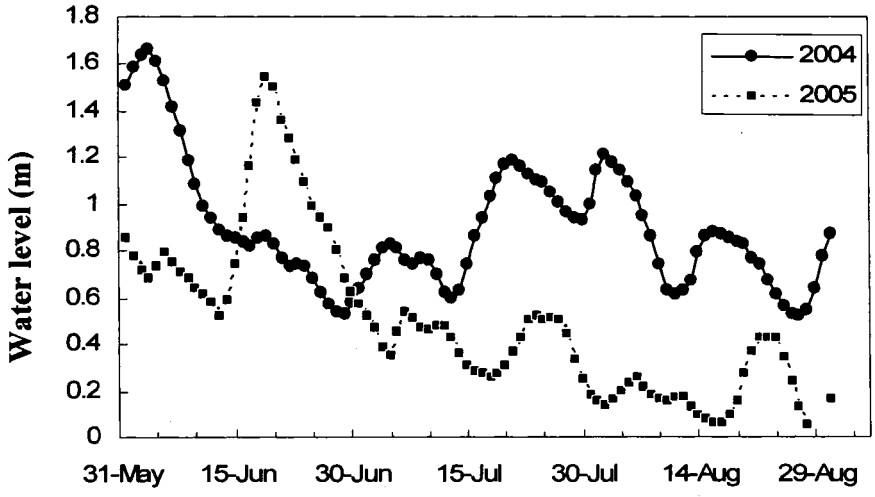
		Autochthonous						Allochthonous					
	Sources	**SS**	**df**	**MS**	**F**	**P**	**GG-P**	**SS**	**df**	**MS**	**F**	**P**	**GG-P**
BETWEEN SUBJECTS	**Long**	0.42	3	0.14	6.405	ns		0.31	3	0.103	21.026	*	
	Lat	0.26	2	0.13	5.865	ns		0.01	2	0.004	0.905	ns	
	Long * Lat	0.27	6	0.045	2.043	ns		0.01	6	0.002	0.347	ns	
	Error	0.07	3	0.022				0.02	3	0.005			
WITHIN SUBJECTS	**Time**	3.52	5	0.704	58.441	**	**	1.52	5	0.304	20.292	**	*
	Error	0.18	15	0.012				0.23	15	0.015			

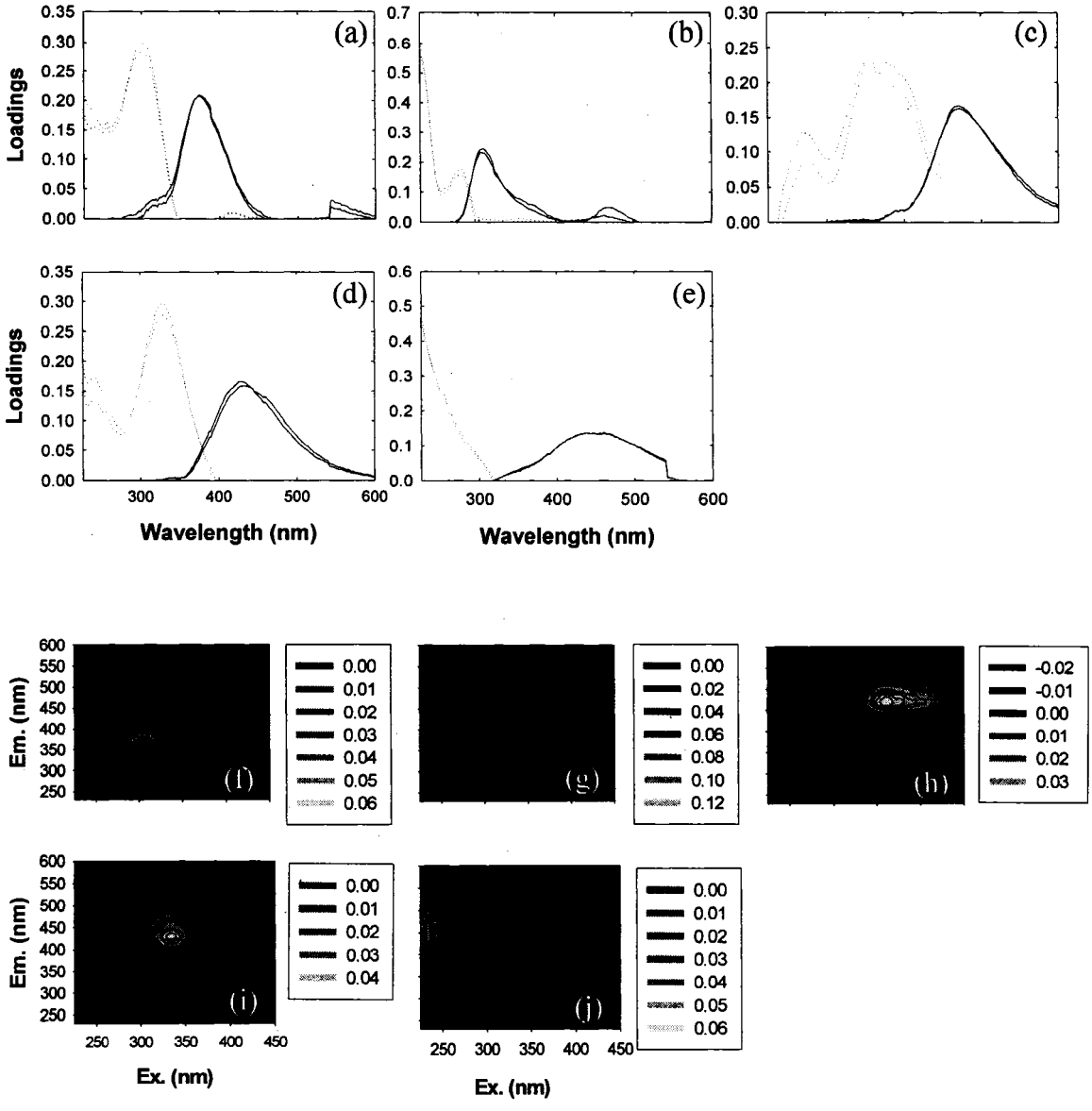
* $p < 0.05$
** $p < 0.001$

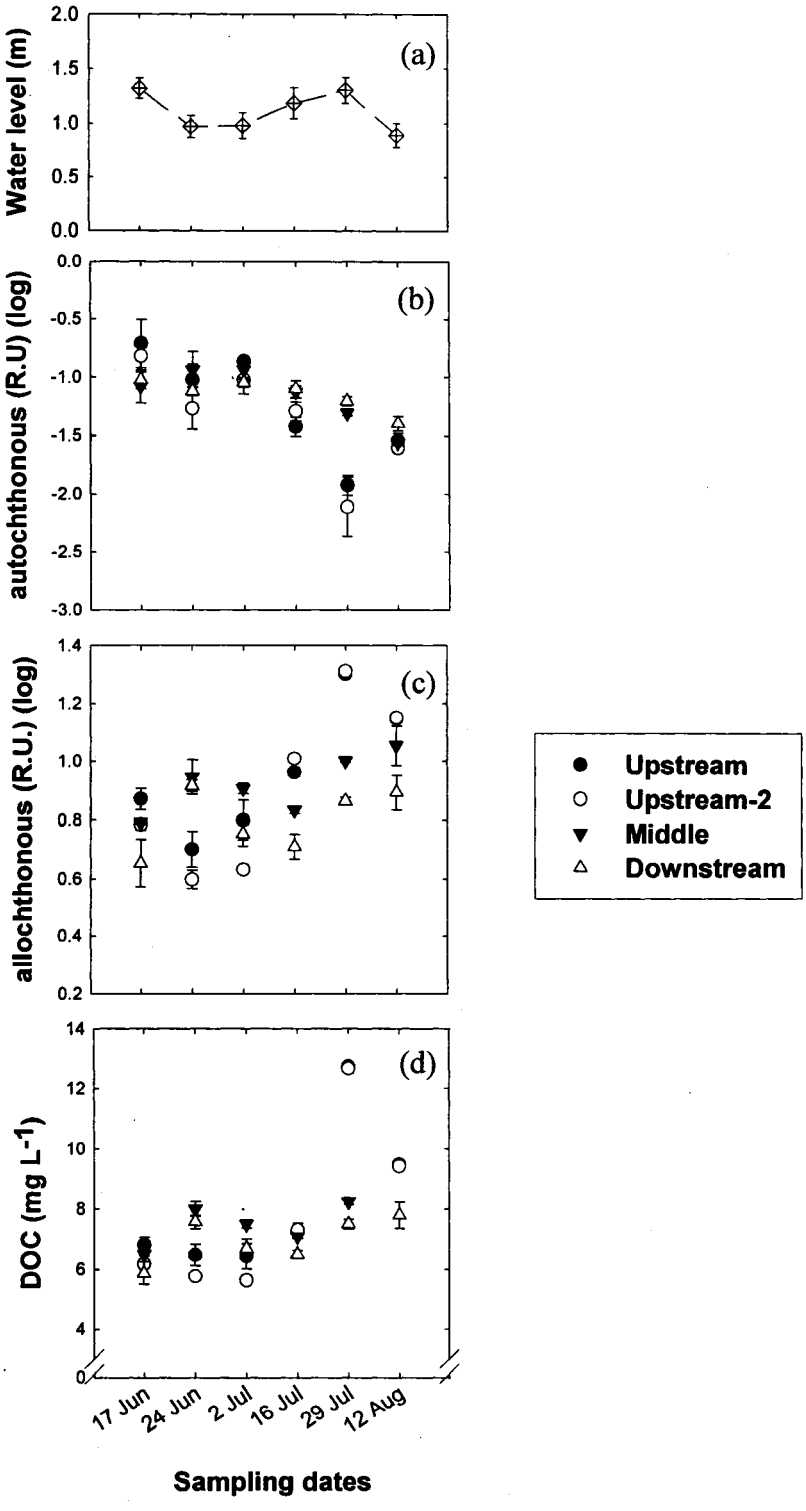
Date	Site	Longitudinal transect	Lateral transect	BP ($\mu gC\ l^{-1}h^{-1}$)	Pmax ($\mu gC\ l^{-1}h^{-1}$)	BP:Pmax	BP:DOC ($g\ C\ h^{-1}$)/(mg C)
16 July	1	Upstream	1	0.57	15.13	0.04	0.07
	3	Upstream-2	1	1.72	3.17	0.54	0.24
	5	Downstream	1	0.16	4.82	0.03	na
	7	Downstream	1	1.64	na	na	0.24
	13	Upstream-2	3	1.95	3.74	0.52	0.26
	15	Downstream	3	1.88	4.74	0.40	0.29
29 July	1	Upstream	1	0.70	na	na	0.05
	3	Upstream-2	1	2.24	0.41	5.42	0.18
	5	Downstream	1	0.81	2.18	0.37	0.1
	7	Downstream	1	1.60	na	na	0.22
	13	Upstream-2	3	2.15	0.74	2.90	0.17
	15	Downstream	3	1.49	0.72	0.86	0.21
12 August	1	Upstream	1	1.32	12.22	0.11	0.13
	3	Upstream-2	1	1.26	2.06	0.61	0.13
	5	Downstream	1	0.72	4.17	0.17	0.1
	7	Downstream	1	0.79	na	na	0.08
	13	Upstream-2	3	2.25	2.65	0.85	0.24
	15	Downstream	3	0.88	4.52	0.19	0.12

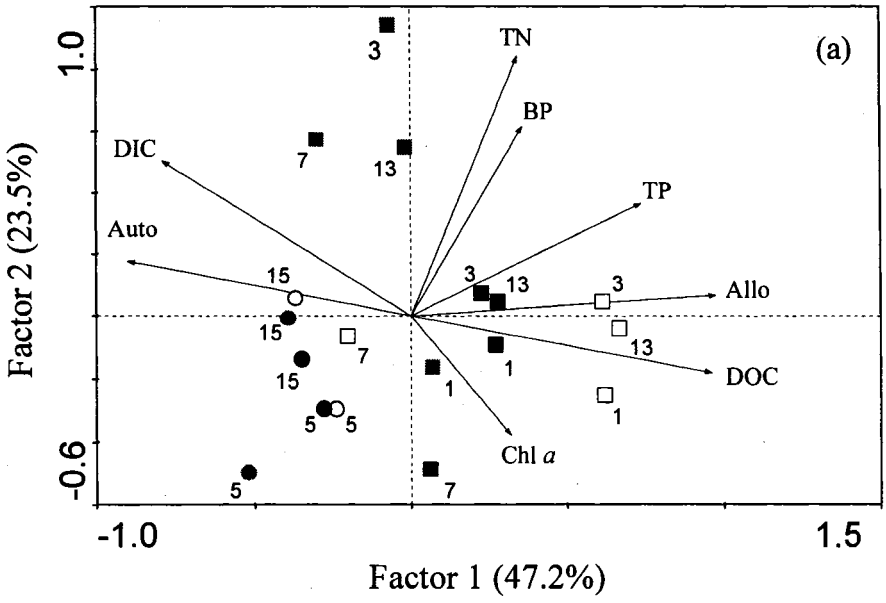

(a)
N
kilometers
(b)
Saint-François

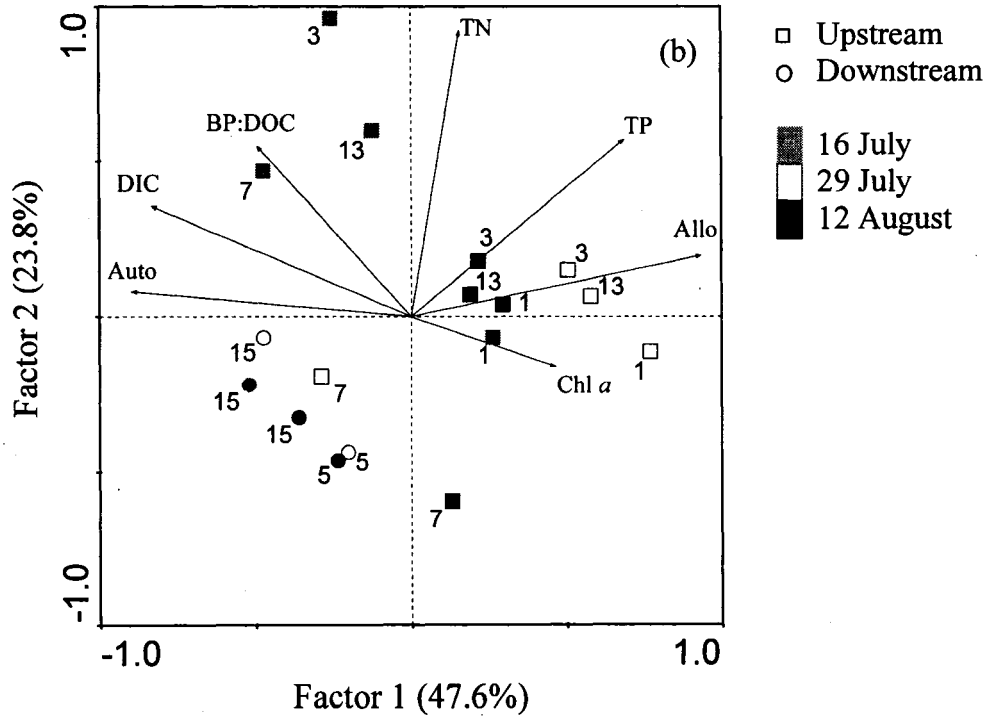

(c)
7
6
5
15
4
10
14
3
9
13
2
8
12
1
0
5
10
ki ometers


Water level (m)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
31-May
15-Jun
30-Jun
15-Jul
30-Jul
14-Aug
29-Aug
2004
2005


(a)
(b)
(c)
(d)
(e)
Loadings
Wavelength (nm)
(f)
(g)
(h)
(i)
(j)
Em. (nm)
Ex. (nm)


Water level (m)
(a)
autochthonous (R.U) (log)
(b)
allochthonous (R.U.) (log)
(c)
DOC (mg L-1)
(d)
Upstream
Upstream-2
Middle
Downstream
17 Jun
24 Jun
2 Jul
16 Jul
29 Jul
12 Aug
Sampling dates


(a)
TN
BP
TP
DIC
Auto
Allo
DOC
Chl a
Factor 2 (23.5%)
Factor 1 (47.2%)
1.0
-0.6
-1.0
1.5


(b)
TN
BP:DOC
TP
DIC
Auto
Allo
Chl a
Upstream
Downstream
16 July
29 July
12 August
Factor 2 (23.8%)
Factor 1 (47.6%)
1.0
-1.0
-1.0
1.0

CHAPITRE III

Contribution of light exposure and microbial processes to the degradation of DOM in river environment : comparison between optical and chemical characterization

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Key words: bacteria, bioavailability, DOM, fluorescence, Nuclear magnetic resonance, solar radiation

3.1 Abstract

We investigated the combined importance of photochemical and microbial processes in the degradation and bioavailability of dissolved organic matter (DOM) in a water sample from a river influenced by large terrestrial inputs (Saint-François River, Québec, Canada). Absorption coefficient (aCDOM$_{375nm}$), optical absorbance index of molecular weight (a_{250}:a_{365}) and three-dimensional fluorescence spectroscopy combined with PARAFAC modeling revealed photochemical transformation, mostly in presence of UV, of allochthonous DOM into more labile compounds. No corresponding changes in chemical characteristics of DOM could be identified using ^{1}H NMR analyses. Chemical fractionation of DOM revealed a high abundance of labile hydrophilic acids in the Saint-François River. This was not visible with fluorescence measurements which underestimated the non-humic portion of DOM. Fluorescent components representative of an autochthonous origin were the major contributors in the hydrophilic acids but also in the more refractory humic acids. The fulvic acid fraction was mostly dominated by components corresponding to fulvic and humic fluorescence. This study highlighted the complementary information that can be gained from optical and chemical tools in DOM characterization and the importance of using both of them to get the whole picture of the DOM dynamic in aquatic ecosystem.

3.2 Introduction

Dissolved organic matter (DOM) is a complex mixture of dissolved substances at diverse stages of decomposition and represents a substantial portion of total organic carbon in aquatic ecosystems (Wetzel, 2001). In open oceans, DOM originates principally from planktonic photosynthesis (Wetzel, 1984; Hedges et al., 1993; Meybeck, 1993) and this autochthonous DOM is largely composed of labile, non-humic substances found at low concentrations. In freshwater ecosystems, the nature of DOM is mostly allochthonous owing to the proximity of littoral and wetland regions as well as large loadings of DOM from the decomposition of plant material within the drainage basin. Freshwater DOM is thus primarily composed of humic substances (Wetzel, 1992). These substances are found in large concentrations in inland waters and were first considered to be recalcitrant to microbial and photochemical degradation because of their terrestrial and higher aquatic plant origin (Frimmel and Christman, 1988; Perdue and Gjessing, 1990). Allochthonous DOM was assumed to be largely unavailable to higher trophic levels because of their large molecular size and high aromaticity (Aiken, 1985; Geller, 1986). However, several studies have shown that allochthonous DOM may participate in good part to the productivity of aquatic ecosystems (DeHaan and de Boer, 1992; Jones, 1992; Thomas, 1997) and was found to reach higher trophic levels (e.g. fish) in lake environments (Cole et al., 2002; Pace et al., 2004).

Microbial and photochemical processes are the main pathways for altering and removing DOM from aquatic ecosystems (Weigner and Seitzinger, 2001). By their action, they can alter the bioavailability of DOM, and thus contribute to the

incorporation of the dissolved organic carbon (DOC) into the microbial food web. The potential increase of solar ultraviolet-B radiation (Kerr and McElroy, 1993) caused by the depletion of stratospheric ozone has generated much concern about photochemical processes. While the negative impacts of ultra-violet radiation (UVR) on the physiology of micro-organisms has been widely addressed (damage to structural and physiological components (Karentz et al., 1994)), little is known about their positive effects on the bioavailability of DOM in freshwater ecosystems. Humic substances constitute a major portion of the chromophoric DOM (CDOM) which absorbs UV radiation (Wetzel, 2001). Sunlight, particularly the UV region of the spectrum, may increase the bioavailability of DOM by the cleavage of large and aromatic molecules into smaller entities (Strome and Miller, 1978) which are then taken up more efficiently by bacterioplankton and transferred up in the food web (Kieber et al., 1989; Bertilsson and Tranvik, 1998; Obernosterer et al., 1999a). However, photochemical transformations can also produce biorefractory compounds resulting in a net negative effect on the carbon flux (Benner and Biddanda, 1998; Obernosterer et al., 1999; Tranvik and Bertilsson, 2001). Moran and Covert (2003) further suggested that irradiation effects varied according to the source of DOM. DOM of terrestrial origin, which is older and more aromatic, is more labile to bacteria under irradiation, while DOM from algal origin is more recalcitrant to bacterial processing.

Production of less refractory DOM by UVR facilitates bacterial utilization of this important energy source and leads to the instalment of a sequential photochemical-microbial degradation pathway. Bacteria and phytoplankton can also directly alter

DOM availability by secretion of exoenzymes and cell surface oxidation (Pantoja and Lee, 1994).

In this study, we investigate the impacts of photochemical and microbial processes on the degradation of terrestrial DOM which characterize the Saint-François River (Québec, Canada). The watershed of this river is influenced by large input of terrestrial organic matter mostly from agricultural activity.

Numerous studies have utilised either chemical analysis, such as ^{1}H RMN (Huizong et al., 2001) or optical properties, like absorbance and fluorescence (Whitehead et al., 2000; Belzile et al., 2002b) to evaluate the changes in DOM caracteristics when exposed to stress. However, few studies have examined the relationship between these two approaches to evaluate the bioavailability of DOM (Bertilsson and Tranvik, 1998; Osburn et al., 2001). Optical and chemical analyses can both be used to evaluate the changes in molecular size of DOM. Chemical analyses give direct information about the size and lability of the moieties of DOM. Yet, the chemical approches is long and complex and requires specialized apparatus. In counterpart, optical analyses are simple, rapid to execute and are based on the easy-to-use absorbance and fluorescence approaches. By using bacterial production as mesure of bioavailability, we expect to validate the simpler optical method with the chemical analyses as an adequate indication of molecular size of DOM.

The goals of this study were 1) to determine the impacts of spectral radiation and microbial activities on the degradation and bioavailability of DOM from a river largely influenced by agriculture 2) to compare the optical and chemical approaches as tools to understand the degradation and bioavailability of DOM.

3.3 Materials and Methods

3.3.1 Study site and sample collection

Water samples were collected on May 17 2005 at Saint-François River, a major tributary of Lac Saint-Pierre, Québec, Canada. This river is characterized by a relatively large component of chromophoric DOM (CDOM) and high concentrations of nutrients (Table 1). Sampling site was located at about 5.5 km upstream of the Lac Saint-Pierre. Water was collected near the surface in acid-washed 20 l polyethylene cubitainers and transported on ice to the laboratory, where they were immediately filtered by tangential flow filtration on a 0.2 μm through fluorocarbon polymer filter (Millipore Pellicon). Filtered water was stored in the dark at 4 °C for 2 days until used in the experiments.

3.3.2 Photodegradation and biodegradation experiments

The filtered water samples were exposed to 3 different light treatments: natural sunlight (UVR + photosynthetically available radiation (PAR)), sunlight minus UV (PAR only) and a dark control (wrapped in aluminium foil). UVR was blocked (<400 nm) by placing a box made of acrylic (Acrylite® OP-3, CYRO Industries, Manchester, U.S.A.) over the polyethylene container (Figure 1). For each treatment, duplicate samples were incubated in two acid-washed 20 l polyethylene cubitainers, which transmit >50% of UVR from 280-400 nm (Figure 1). The samples were exposed to natural sunlight from May 20 to May 31. The irradiance in the UV (313, 320 and 340 nm) and in the visible (443, 550 nm and PAR: 400-700 nm) was

monitored each day at the site of incubation with a spectroradiometer (Model PUV 2546, Biospherical Instruments, San Diego, USA). During the incubation period, the average UV-B level was 11 W/m^2, the average UV-A was 28 W/m^2 and the average PAR was 67 W/m^2. Fluctuations of temperature in the cubitainers (18-22 °C) were moderated by placing them in a small pool filled with tap water.

In order to evaluate the effect of photochemical transformation and biodegradation of DOM on bacterial production, half of the water samples filtered on 0.2 µm fluorocarbon polymer filter were inoculated with a natural bacterial inoculum (1% of the final volume) prepared by concentrating bacteria from the Saint-François River over the 0.2 µm filter. Those samples were placed in polyethylene cubitainers and exposed to the same light treatment as above (UVR-PAR, PAR only and a dark control). Every two days, bacterial carbon production was measured by ^{3}H-leucine incorporation in the dark (see below).

For both experiments, subsamples (10 ml) were collected for optical analysis and bacterial abundance every day, and for DOC measurements every two days. Water samples for the ^{1}H NMR analyses were collected at the end of the experiments. At least 40 l of water were necessary to recover enough biomass of fractionated DOM for this analysis. Before the beginning of the experiments, samples for total phosphorous (TP), total nitrogen (TN) and chlorophyll *a* (Chl *a*) were collected to determine the initial chemical characteristics of the water.

3.3.3 Bacterial abundance

Water samples for bacterial abundance (10 ml) were preserved with 0.2 µm filtered formaldehyde (2% final concentration) and stored at 4 °C until counting. Total

bacterial abundance was determined by flow cytometry, using a FACScan (Becton Dickinson, Mountain View, CA, USA) as described in del Giorgio et al. (1996).

3.3.4 Bacterial production

Bacterial production was estimated using ^{3}H-leucine incroporation as described by Kirchman in Kemp et al. (1993) and adapted for microtubes according to Smith and Azam (1992). Sample water (1.5 ml in triplicate) was incubated in darkness at the temperature of the cubitainers (between 18 and 20 °C) for 30 min with L-3,4,5 ^{3}H-leucine (PerkinElmer, 117 Ci mmol^{-1}^{3}H-leucine, 10 nM final leucine concentration). Two killed controls (blanks) for every three live incubations were obtained by adding 50% trichloracetic acid (TCA) to determine abiotic sorption of the ^{3}H-leucine. Incubations were terminated by the addition of 50% TCA. The centrifugation, vortex, and wash sequences were carried out as described in Smith and Azam (1992) with an addition of a final 80% ethanol wash. Microtubes were then exposed to the air for one night, allowing ethanol to evaporate. Scintillation cocktail was added to the tubes at least two days before scintillation counting of the samples.

3.3.5 DOC, TP, TN and Chl a analyses

DOC samples were filtered through Milli-Q rinsed 0.22 μm Isopore membrane (Millipore) and its concentration was estimated using high temperature catalytic oxidation on a Shimadzu TOC 5000A instrument. TP and TN concentration were measured spectrophotometrically following persulfate digestion. Chlorophyll *a* (Chl *a*) samples were filtered throught a 25 mm GF/F filter (Whatman). Hot ethalnol

method for the extraction of Chl *a* was used according to Marker et al. (1980b). Extractions continued in the dark at 4 °C for 1 h after which samples absorption measurements were taken at 665 and 750 nm (Shimadzu spectrophotometer, UV-Probe, Columbia, MD, USA) before and after acidification to correct for phaeopigments.

3.3.6 Optical analyses

Absorbance and fluorescence analyses were conducted on water samples and on the isolated DOM fractions in solution at pH 7 (see below).

Aliquots of 10 ml were collected in each 20 l cubitainers for each treatment. Optical analyses data were averaged for the 2 cubitainers exposed to the same treatment. Water samples were filtered through rinsed Milli-Q 0.22 μm Isopore membrane (Millipore) before analysis. Absorbance spectra (289-900 nm) were performed with a Shimadzu UV-2401 PC spectrophotometer in 1 cm quartz cuvettes with NANOpure water as reference. In order to estimate the change in DOM characteristics, absorption coefficients at 375 nm (aCDOM$_{375}$) were calculated using: $a_{375} = A_{375} \times 2.303/b$, where A_{375} is the absorbance of the sample at 375 nm and b is the path length of the cuvet in meters (Kirk, 1994). The absorbance at 690 nm (where the temperature dependency is near zero) was used to correct the UV-absorptivity values (Laurion et al., 2000). Also, a 250 nm : 365 nm absorbance ratio was calculated (Strome and Miller, 1978). An increase in the ratio represents a decrease in molecular weight.

The fluorescence measurements were performed using a spectrofluorometer (Cary Eclipse, Varian Co., Palo Alto, CA, USA) in a 1 cm quartz cuvette. An

excitation-emission matrix (EEM) for each sample was obtained by combining a series of emission scans (230-600 nm, 2 nm increments) while exciting at wavelengths between 220 and 450 nm every 5 nm. The excitation and emission bandwidths were 5 nm. The EEMs were corrected for inner-filter effects using the measured absorbance spectra (Mobed et al., 1996, McKnight et al., 2001). The EEMs were also Raman calibrated and corrected for instrument biases according to the methods described in Stedmon et al. (2003).

3.3.7 PARAFAC modeling

PARAFAC modeling was performed in MATLAB using "PLS_Toolbox from Eigenvector" (Anderson and Bro, 2000; see Stedmon et al., 2003). In addition to the data from this study, EEMs from DOM samples and isolated fractions of DOM from Lac Saint-Pierre were included in the model. This allowed to increase the range of DOM signatures and improve the PARAFAC modeling (see Stedmon et al., 2005a). The EEMs were combined into a three-dimentional data array (288 samples x 186 emission wavelengths x 46 excitation wavelengths) and a series of PARAFAC modeling, from 2 to 6 components, were fitted to the data set. In order to determine the appropriate number of components, a split-half analysis was effectuated, comparing the excitation and emission spectra of the components between the calibration and validation data arrays. Up to five components were validated using this technique and related to literature (Table 2). Examination of residuals, indicating little signal information, also assessed the five components model (data not showed). As Stedmon and Markager (2005b), the fluorescence of each component is stated as Fmax (RU), corresponding to the fluorescence at the

excitation and emission maxima (Table 2). For DOM fractions, fluorescence was divided by the concentration in mg ml^{-1} of the acids in the sample.

3.3.8 Chemicals and materials

Unless noted otherwise, all chemicals were analytical grade purchased from Aldrich and used without purification. DOWEX-50 cation exchange resin and XAD-8 resin were supplied by Aldrich Chemicals. Prior to use, the resins were successively washed with methanol and acetonitrile in a Soxhlet apparatus for 16 hrs. They were then rinsed with 5 portions of 300 ml of deionised (DI) water. Cation exchange resin was conditioned by passing 500 ml of HCl 3 M with a 5 ml min^{-1} flow. The resin was reconditioned after each sample. All glassware was systematically washed with vitriol (HCl + HNO_3) and thoroughly rinsed with DI water. D_2O and NaOD 40% were purchased from CDN isotopes (Pointe-Claire, Canada).

3.3.9 Chemical fractionation of DOM

The initial water sample (non-incubated sample) and incubated samples were fractionated according to the following procedure. Water samples were first filtered by tangential flow filtration on 0.2 μm and then concentrated by reverse osmosis. The concentrates were then acidified to pH 1-2 using concentrated HCl and allowed to sit overnight in the refrigerator to induce humic acids precipitation. The next morning, the samples were filtered onto 0.7 μm GF/F filters and residuals were thoroughly washed with HCl 0.1 M. The filtrate contained non-desalted fulvic (FA) and hydrophilic (HyA) acids (Huizhong et al. 2001). To obtain humic acids, a solution of NaOH 0.1 N was added to the paper filter, provoking an acid-base

reaction that dissolved the humic acids. pH of this new filtrate was then ajusted to 7 with concentrated HCl affording non-desalted humic acids. FA and HyA were separated using an XAD-8 resin column (Leenheer, 1984; Thurman, 1985; Huizhong et al., 2001). All acid fractions were desalted by passing through an H^+-saturated cation exchange resin (Dowex 50WX8 resin), rotary concentrated and freeze-dried (Huizhong et al., 2001).

3.3.10 ^1H NMR analysis

Approximately 10-50 mg of dry sample were suspended in 1.5 ml of D_2O to which was added 4 drops (about 1 ml) of NaOD 40 % in D_2O to enhance solubility of the organic matter. The sample was then filtered through cotton wool directly in a NMR tube. The signal of D_2O was used as reference and set to 4.79 ppm chemical shift. NMR spectra were recorded on a Varian Mercury 200, using a 5 second pulse delay. The spectra were recorded immediately after sample preparation to avoid basic hydrolysis of certain functional groups (e.g. esters).

Spectra were divided in two major regions: sp^3-hybridized or aliphatic (0.0 – 4.4 ppm) and sp^2-hybridized or aromatic/olefinic (6.0 – 8.4 ppm). We also subdivided the aliphatic region into 3 distinctive portions (Peuravuori and Pilahja, 1998a; 1998b): 0.0-1.6 ppm (protons on saturated chain (or paraffinic) carbons), 1.6-3.0 ppm (mainly attributed to hydrogens on carbons flanked by a carbonyl function) and 3.0-4.2 ppm (mainly attributed to hydrogens on carbohydrate moieties (Malcolm, 1990)). The percentages of each of these protons were calculated by integrating peak areas of specific resonance signals. ^1H NMR spectroscopy was chosen for this work because the low organic content of our samples precluded the

isolation of sufficient material for ^{13}C NMR analysis, which requires important amounts of sample than the more sensitive ^{1}H NMR technique.

3.4 Results

Treatments with 0.2 μm filtered water (no bacteria) showed no difference in bacterial abundance with the inoculated treatments (data not shown) indicating a methodological problem with the filtration efficiency of our tangential filtration flow system. Thus, the 0.2 μm filtration was not successful in removing enough bacterial biomass to prevent important regrowth. All incubation treatments were therefore systematically influenced by presence of bacteria in all light treatments.

3.4.1 Bio-optical analyses of water samples

There was no significant difference ($p > 0.05$, unpaired *t*-test with Bonferroni adjustment) in DOC concentration between either the initial or final dark control as compared with samples exposed to light (data not showed). However, in the course of the incubation period, phototransformation of DOM resulted in a decrease of absorption coefficient at 375 nm ($a\text{CDOM}_{375}$) for both light treatments (UV-PAR and PAR only) (Figure 2a). No significant decrease was observed for the dark control between the first and the last day of the incubation. Between these days, the treatment with UV-PAR showed a greater decrease in absorption coefficient (37.4%) compared to the treatment with PAR only (no UV) in which absorption coefficient diminished by 19.1%.

The increase in a_{250} : a_{365} absorbance ratio, considered as an indicator of the relative importance of high molecular weight (HMW) over low molecular weight (LMW) molecules (Strome and Miller, 1978), showed a daily chemical degradation of large molecules for all light treatments during the 12 days incubation period (Figure 2b). Similarly to $a\text{CDOM}_{375}$, the UV-PAR treatment showed a stronger effect than the PAR treatment with a 25.3% increase in the ratio.

In the case of the fluorophores, only the components corresponding to an allochthonous origin (components 3 to 5) showed a clear pattern of change over the incubation time when exposed to light (Figure 3). For all these components, UV-PAR treatment had the strongest effect on fluorescence, with a decrease of 45.7%, 41.8% and 29.3% for components 3, 4 and 5 respectively. The PAR treatment showed different pattern of change according to the component: a decrease in fluorescence of 28.6% for component 3, an increase of 17.8% for component 4 and no significant variation between the first and the last day of incubation for component 5 ($p > 0.05$, ANOVA). No significant changes were observed for the dark control in the three allochthonous components ($p > 0.05$, ANOVA).

3.4.2 Chemical and fluorescence analyses of DOM fractions

H^1 NMR spectra for all fractions of DOM (FA, HA, and HyA) were dominated by broad humps representative of DOM complex macromolecular compounds (Malcolm, 1990), although some sharp signals were also observed. Spectrum of the blank (D_2O + NaOD without any organic substance) showed a single peak with a chemical shift at 4.79 ppm.

In relative weight, hydrophilic acids dominated the DOM pool (79%), followed by fulvic acids (19%) and humic acids (2%) (Figure 4). There were no significant variations ($p > 0.05$, ANOVA) in relative and absolute weight (data not shown) of the DOM fractions between the initial non-incubated sample (reference) and the samples at the end of the 12 days incubation (Figure 4).

Table 3 presents the relative peak areas of the DOM fractions ^{1}H RMN spectra before the incubation (reference) and at the end of the incubation for the different treatments. No significant changes were observed in these peak areas at the end of the incubation period as compared to the reference and this, for the light treatments and the dark control and for the three DOM fractions ($p > 0.05$, ANOVA).

The five components fluorescence signatures issued from the DOM fractions in the reference sample revealed additional information on the nature of these fractions (Figure 5). Humic acids had the overall largest fluorescence, followed by the fulvic and the hydrophilic acids. The fulvic acids were the only fraction dominated by the allochthonous components (C3 to C5). Besides, the tyrosine-like fluorescence component (C2) dominated the humic and hydrophilic acids.

3.4.3 Effect of DOM and bacteria irradiation on bacterioplankton

Incubation of 0.2 µm filtered water from Saint-François River inoculated with bacteria under full solar irradiation (UV-PAR), under PAR only (PAR) and in darkness for a 12 days period revealed no consistent pattern in bacterial production relative to the initial condition (Figure 6). Bacterial production for the UV-PAR and the PAR treatments was never significantly higher as compared dark control, except for the day 8, where the PAR treatment showed an increase compared to dark

control. At days 0, 6 and 12, a decrease was observed for all treatments. From the beginning to the end of the incubation, bacterial production for the UV-PAR treatment showed no significant difference with the PAR treatment ($p > 0.05$, unpaired *t*-test with Bonferroni adjustment).

Bioavailability of DOM, calculated as a ratio of bacterial production to concentration of DOC (BP:DOC, Ziegler and Benner 2000), significantly decrease from day 2 to the end of incubation for the PAR treatment and from day 8 to the end for the dark control ($p < 0.05$, ANOVA) (Figure 7). UV-PAR treatment had no significant effect on bioavailability over the incubation time ($p > 0.05$, ANOVA) (Figure 7). No significant differences between the three treatment were observed at any of the time measured ($p > 0.05$, unpaired *t*-test with Bonferroni adjustment).

3.5 Discussion

Our experimental results are based on optical and chemical tools and address the implications of photochemical and microbial activity on the degradation and bioavailability of DOM from a river influenced by terrestrial inputs. As the tangential flow filtration on 0.2 μm was not successful in removing most bacteria to prevent a significant regrowth, it was impossible to isolate the photochemical from the microbial effect. However, by comparing the dark control with the treatments exposed to light, we could identified the major contribution of light to DOM degradation.

3.5.1 Optical changes in DOM exposed to solar radiation and microbial processes

The spectral analyses of DOM revealed significant changes in composition along the exposure to light. In absorbance analyses, the decrease of absorption coefficient at 375 nm as well as the increase in the a_{250} : a_{365} absorbance ratio observed for UVR treatments are consistent with results of other researchers that suggest a photodegradation of high molecular weight (HMW) molecules into low molecular weight (LMW) molecules (Lindell et al., 1995; Wetzel et al., 1995; Obernoster and Herndl, 2000; Osburn et al., 2001; Eugelhaupt et al., 2003). As light is absorbed by the chromophores of DOM (CDOM), large molecules are photodegraded into smaller components that do not absorb to the same extend as the primary DOM (Strome and Miller, 1978). When compared to PAR, the higher decrease in absorption coefficient observed with the UVR treatment is consistent with other studies which underline the high energetic nature of UVR (e.g. Moran and Zepp, 1997). In our study, the lack of significant differences in DOC concentration observed during the time course of light exposure (PAR only and UV-PAR), contrasted with the large decrease in $a\text{CDOM}_{375}$ which suggest that the bulk of DOM from the Saint-François river is not photoreactive. This supports the finding of Smith and Benner (2005) who concluded that photoreactive DOM has a greater absorptivity than that of bulk DOM pool.

The fluorescence EEM spectroscopy combined with PARAFAC modeling has recently been used to characterise composition, origin and transformation of DOM (Stedmon et al., 2003; Stedmon and Markager, 2005a; 2005b). In our study, the allochthonous fluorescent components identified with PARAFAC modeling,

revealed a similar trend that in absorbance: transformation with UV treatments of larger and recalcitrant molecules into smaller and labile ones. The absence of significant variation for autochthonous components when exposed to light confirmed the less aromatic, and thus less photoreactive nature of algal-derived DOM (Wetzel, 2001).

3.5.2 Chemical and fluorescence changes in DOM fractions exposed to photochemical and microbial processes

Chemical analyses of the water from Saint-François River revealed a high content in HyA fraction which demonstrated the non-humic character of the river based on the very low aromatic content. Similar results were observed in the other water masses of Lac Saint-Pierre (unpublished). However, the optical analyses rather suggest the Saint-François River to be considered as humic, regarding the strong DOC absorbance in UV (Tranvik and Bertilsson, 2001). High abundance in hydrophilic acid is common in groundwater (Steinberg, 2003) and wastewater (Huizong et al., 2001; Imai et al., 2002). These types of water are not likely to represent the major pool of the water in the Saint-François River and the Lac Saint-Pierre. Hence, our results indicate the high UV-absorptivity of the humic part (fulvic and humic acids) of the DOM even thus they do not constitute the major portion of the DOM pool.

Our chemical results obtained with the ^{1}H RMN spectra regarding the FA, HA and HyA fractions of the DOM did not agree with our optical observations. The percentage of aromatic compounds, which are associated to recalcitrant molecules, did not significantly decrease under the light and bacterial treatments. Furthermore, relative weight percentages revealed no changes in any DOM fractions. Using ^{13}C

NMR, Eugelhaupt et al. (2003) neither observed consistent pattern in aliphatic and aromatic carbon after exposure to light, even though changes in absorptivity were observed. It was suggested that photolysis act on specific types of chromophores rather than on general carbon bond type (aliphatic and aromatic C, carbohydrates and saturated chain). This could explain the difference between our chemical and optical results.

Fluorescence EEMs gave further insight on the characteristics of DOM fractions (FA, HA and HyA). Fluorescence of the five components on each DOM fractions showed the largest overall contribution of humic acids in fluorescence, which is consistent with the aromatic nature of this fraction, as confirmed by ^{1}H RMN. The relative abundance of the fluorescent components in DOM fractions from the Saint-François River is similar to that found by Chen et al. (2003) on DOM fractions from wastewater effluents and rivers in the south western United States. In our study, the HyA and HA fractions were dominated by the tyrosine-like component (C2) which correspond to the aromatic-protein region identified by Chen et al. (2003). These authors observed the same pattern in hydrophobic and hydrophilic acids fractions isolated from different water sources. Therefore, in the present case, molecules representative of an autochthonous origin are major contributors in the labile hydrophilic fraction but also in the more refractory humic acid pool. This can be explained by the structure of these molecules that is likely to differ between the HyA and the HA. The former are mostly composed of simple soluble proteins and carbohydrates contributing to a high bioavailability and the latter are formed by more complex non soluble proteins that are less bioavailable

(Steinberg, 2003). Thus, HyA and HA would be composed of molecules with similar fluorescent EEMs possessing different structures and bioavailability. In contrast to the other fractions, the components 4 and 5, corresponding to humic and fulvic fluorescence respectively, contributed the most to the FA fluorescence, which was also observed by Chen et al. (2003) for a standard fulvic acid from the International Humic Substance Society (IHSS).

3.5.3 Importance of phototransformation of DOM on bacterial productivity

Factors that mainly influence bacterial production and utilization of DOM are 1) the composition of the bacterial community, 2) the biodisponibility of nutrients and 3) the chemical composition of DOM (del Giorgio and Davis, 2003). Considering that the same inoculum of Saint-François River bacteria was utilized in all water samples, the remaining explanations for the results in bacterial activity are the nutrients and the composition of DOM. The possibility of a nitrate or phosphorous limitation in bacterial production cannot be totally discarded. However, the concentration in TP and TN for the Saint-François River are very high (Table 1) due to large nutrient input from its watershed. Moreover, no evidence of nutrient limitation in biofilms from Lac Saint-Pierre has been observed previously (Huggins et al., 2004). Thus it would be surprising that bacteria underwent nutrient limitation over the 12 days incubation period.

Several studies have documented an enhancement in bacterial production in water samples exposed to natural sunlight or simulated light (Lindell et al., 1995; Wetzel et al., 1995; Miller and Moran, 1997; Benner and Biddanda, 1998; Engelhaupt et al., 2003; Smith and Benner, 2005). These studies were conducted

with water influenced by large input of terrestrial matter. Bacterial growth can be limited by the low bioavailabilty of this humic-rich water and photochemical transformations seem to have positive impacts on bacterial activity (Lindell et al., 1995). In our study, the photochemical transformation of DOM did not result into a higher bacterial productivity and moreover, seemed actually to reduce the bioavailability of DOM to bacteria. The UV-PAR treatment showed the highest transformation of DOM and bacteria from this treatment exhibited a decrease in DOM utilization with time. The water from the Saint-François River has a relative bioavalabitlity of 0.03 (Figure 7). Ziegler and Benner (2000) present the BP:DOC ratio of different environment, ranging from 1.01 for Laguna Madre to less than 0.06 for humic pond water. According to theses authors, our initial water sample can be classified as highly recalcitrant to biological transformation. Thus, photochemical processes were expected to have a positive influenced on bacterial production or bioavailability, which was not the case. In addition, Tranvik and Bertilsson (2001) applied a multiple regression model to the data from 30 different lakes relating UV enhancement of bacterial growth to concentration in autochthonous vs. allochthonous DOC and chlorophyll *a*. As stated by this model, bacterial production of the Saint-François River water should have been positively influenced upon DOM irradiation by UVR. Hence, how can we explain the negative effects of UVR on the bacterial production observed in our experiment? Bacterial biomass, not only DOM, was exposed to light during incubation. Thus, bacteria could have been affected negatively by direct light exposure which could have prevented any benefit effect of DOM transformation to bacteria (Herndl et al., 1993; Wilhelm and Smith, 2000;

Maranger et al., 2002). However the second measurement of bacterial production was done after 48 hours of incubation which is a long period in bacteria's life cycle. It is thus possible that the positive impacts of DOM transformation were visible on a shorter time scale.

3.6 Conclusions

Optical measurements were showed to be an interesting option to monitor DOM degradation and revealed information about DOM transformation that were not visible with the chemical analyses. They are simple and rapid to execute and provide pertinent information via absorbance and fluorescence measurements. However, they underestimate the contribution of labile components such as the hydrophilic acids due to the low absorptivity nature of these acids. Chemical analyses are thus essential in identifying the molecular composition of the whole DOM, not only its chromophoric part. This is of great ecological importance considering the high percentage of hydrophilic acids in the Saint-François River and its potentially high contribution to bacterial production and subsequently higher trophic level production. The combined use of optical and chemical tools gives a more complete picture of the DOM dynamic in aquatic ecosystems.

Unfortunately, methodological problems prevented us to have clear conclusions on potential changes in bioavailability underwent by DOM exposed to sunlight and microbes. It would be interesting to start again the experiment using shorter time scale and making sure that 1) there is no limitation in nutrients 2) the

0.2 μm filtration is efficient in removing most bacteria to prevent significant regrowth 3) the inoculation of bacteria is done after light exposition to prevent light-damage to bacteria. In doing so, measurements of bacterial production would reflect changes in DOM bioavailability and would eventually permit to relate optical and chemical characteristics of DOM to its utilization by bacteria.

3.7 Acknowledgments

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3.9 Table titles

Table 1. Optical and chemical caracteristics of the Saint-François River water sample used in this experiment. Chl *a*, chlorophyll *a*; TP, total phosporus; TN, total nitrate; $a\text{CDOM}_{375}$, absorption coefficient at 375 nm wavelength.

Table 2. Fluorescence maxima for the five identified components and their corresponding characteristics. Secondary maxima are shown in brackets.

Table 3. Relative peak areas of fractionated DOM ^{1}H NMR spectra at the end of the incubation period for the photochemical and microbial degradation experiments. FA: fulvic acid; HA: humic acid; HyA: hydrophilic acid. Reference: initial non-incubated sample; UV-PAR: samples exposed to total solar radiation; PAR: samples exposed to PAR only (> 400 nm); Dark: dark control. Bacteria were present in all treatments

3.10 List of figures

Figure 5. Relative fluorescence of the five components (C1 to C5) in a) fulvic acids b) humic acids and (c) hydrophilic acids of the non-incubated (reference) DOM fractionated sample.

Figure 6. Results of experiments designed to assess the effect of photochemical transformation of DOM on bacterial production in 0.2 μm filtered water inoculated with natural bacterial inoculum. Water was exposed to total solar radiation (UV-PAR), solar radiation > 400 nm (PAR) and a dark control. Values are plotted as the ratio of bacterial production measured in UV-PAR and PAR treatments relative to bacterial production in the dark control. Error bars indicate ± SD of the mean.

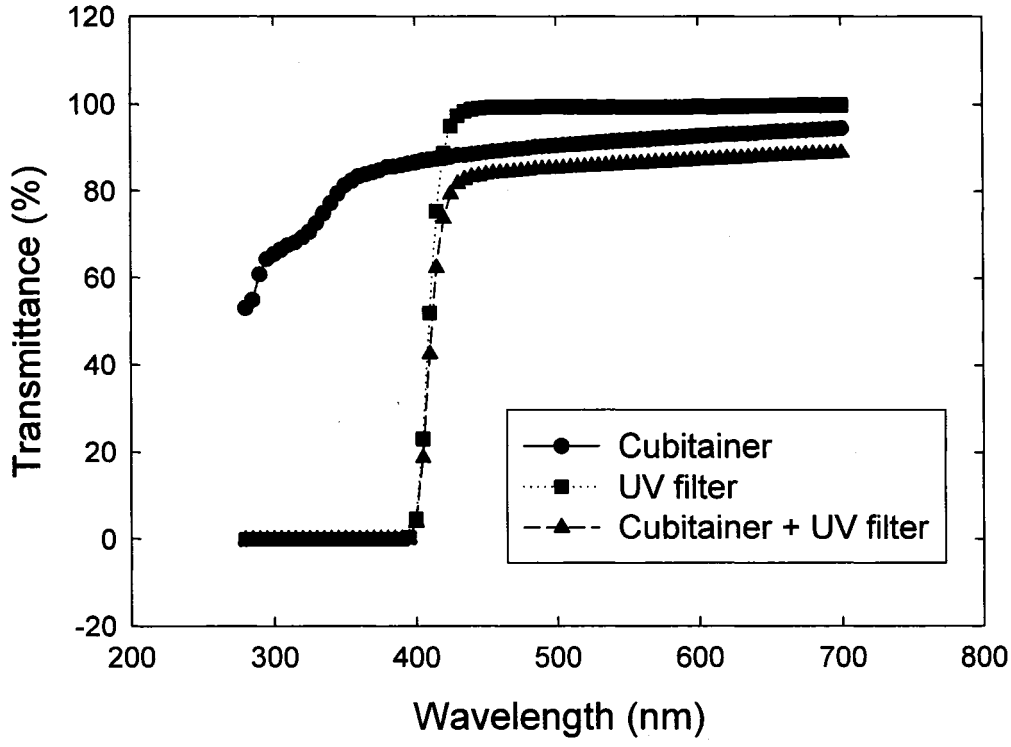
Figure 7. The relative bioavailability of DOM calculated as the average bacterial production (BP; μg C h^{-1}) divided by the concentration of DOC (mg) in 0.2 μm filtered water inoculated with natural bacterial inoculum. Water was exposed to total solar radiation (UV-PAR), solar radiation > 400 nm (PAR) and a dark control. Error bars indicate ± SD of the mean.

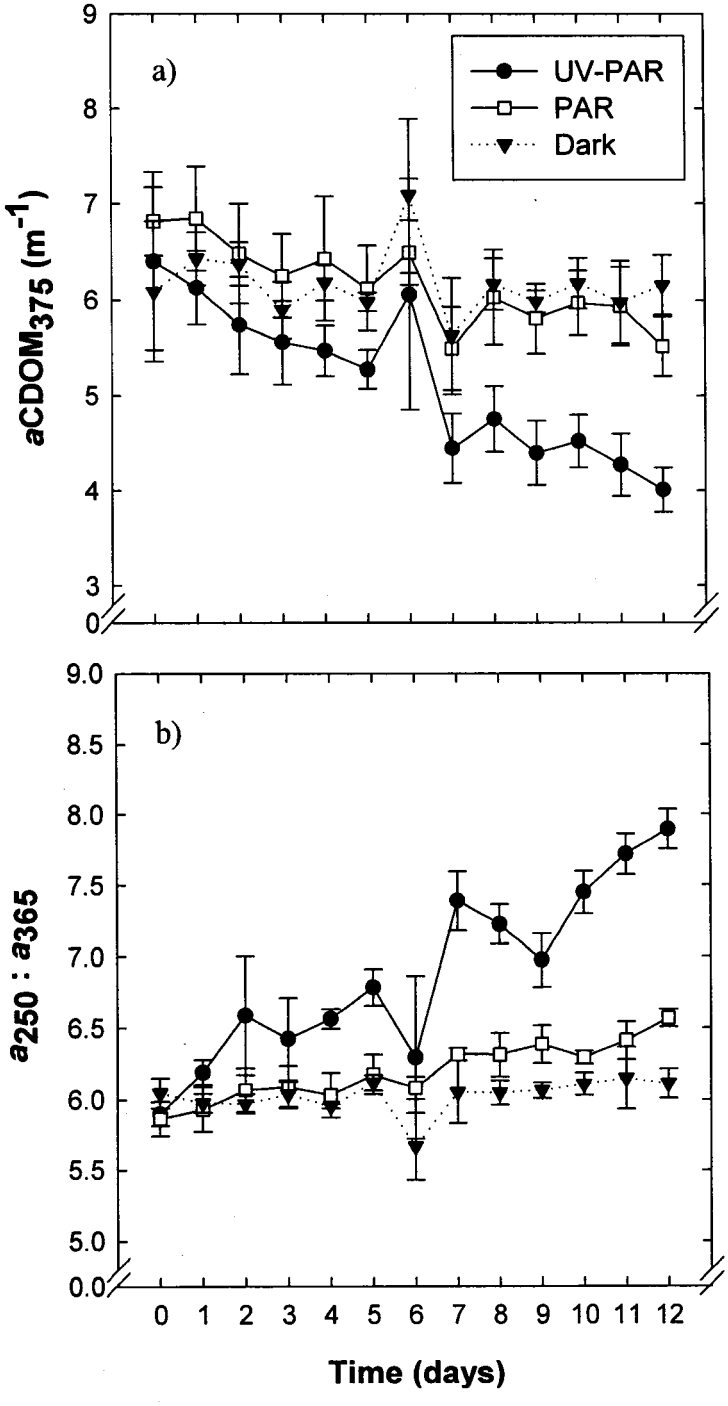
Variable	Saint-François River
Chl *a* ($\mu g\ l^{-1}$)	6.79
DOC ($mg\ l^{-1}$)	5.91
TP ($mg\ l^{-1}$)	0.03
TN ($mg\ l^{-1}$)	0.5
$a\mathrm{CDOM}_{375}$ (m^{-1})	8.7

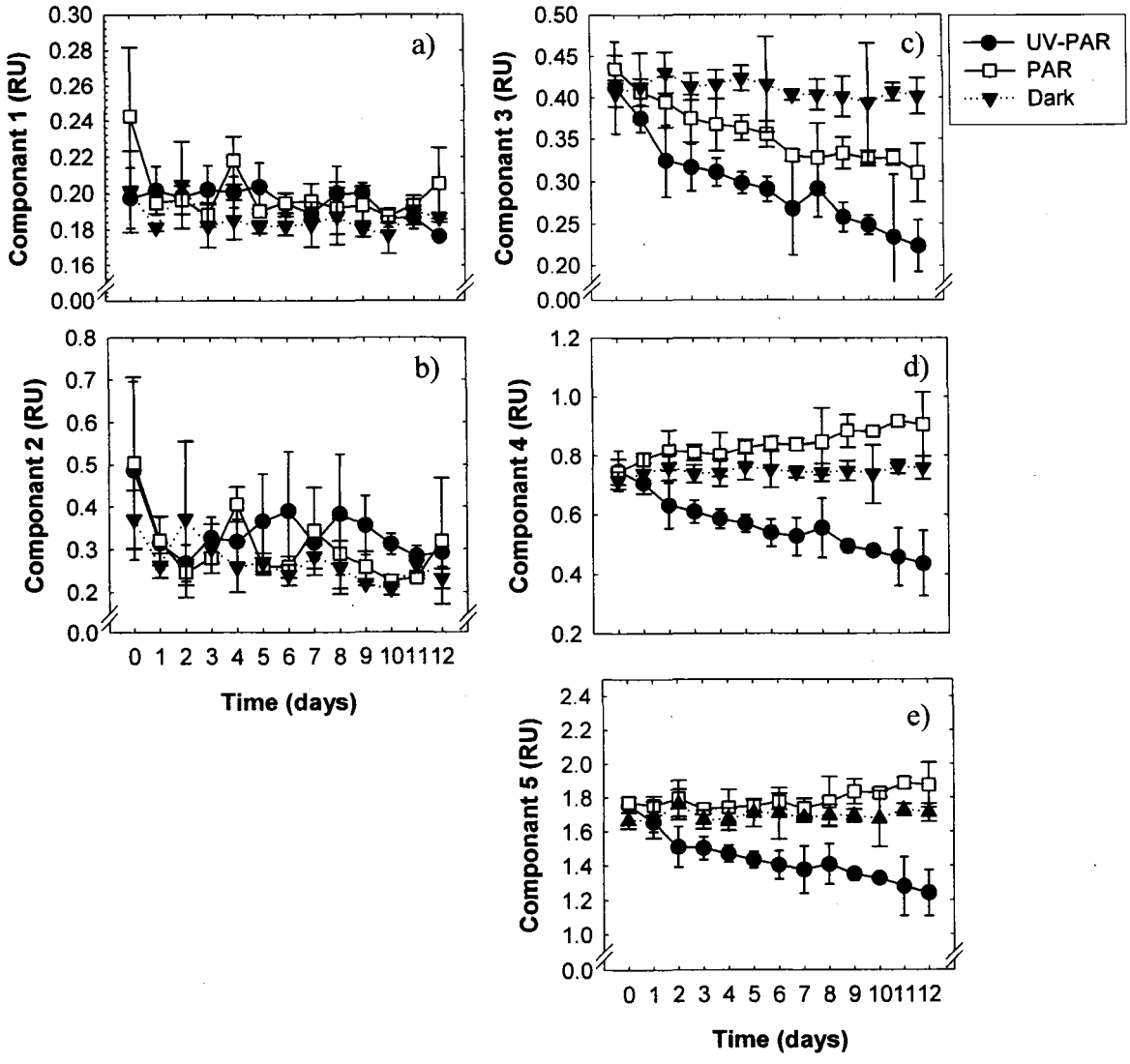
Component	Excitation maximum	Emission maximum	Origin (Stedmon et al. 2005a and b)	Characteristics (Stedmon et al. 2005a and b; Chen et al. 2003)
1	305	374	Autochthonous	Tryptophan and protein-like fluorescence*
2	<250 (275)	305	Autochthonous	Tyrosine-like fluorescence Derived from autochthonous processes
3	355 (270)	471	Allochthonous (terrestrial)	Humic-like fluorescence.
4	330	428	Allochthonous (terrestrial/anthropogenic)	Humic-like fluorescence Exported from agricultural subcatchments
5	<250	438	Allochthonous (terrestrial)	Fulvic-like fluorescence Exported from natural and agricultural catchments

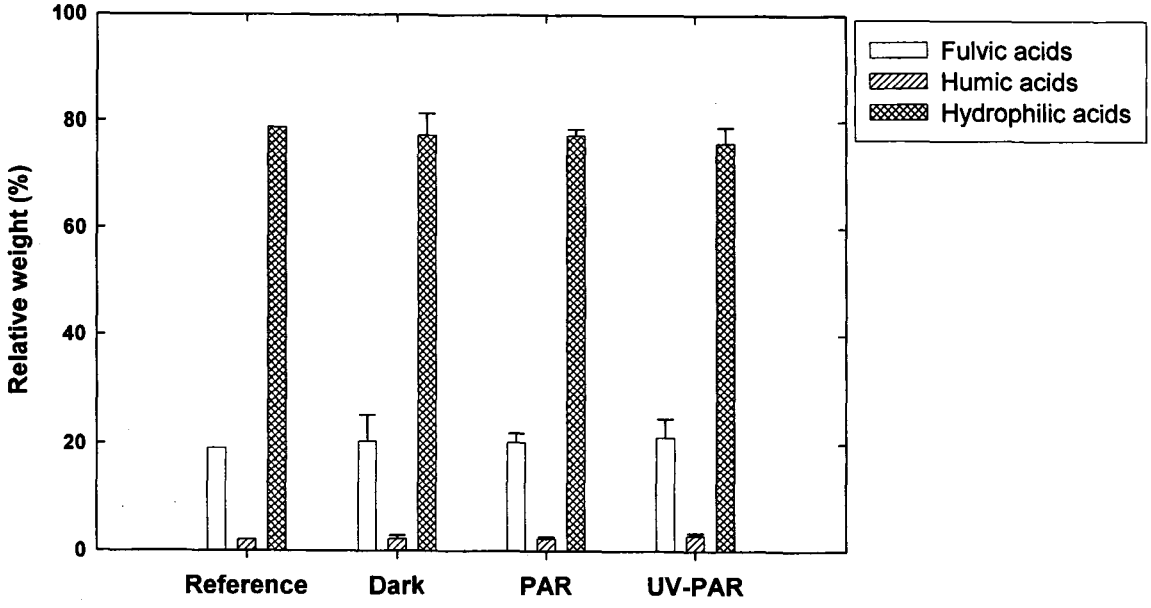
		Aromatic (%)	Aliphatic (%)	Carbohydrate (%)	Saturated chain (%)
FA	Reference	2.9	97.1	16.0	35.0
	UV-PAR	5.4±0.6	94.7±0.6	13.4±3.7	34.5±4.5
	PAR	9.4±3.5	90.7±3.5	13.0±1.7	33.5±2.4
	Dark	9.0±4.7	91.0±4.7	13.2±5.0	35.9±1.8
HA	Reference	55.6	44.4	41.4	33.4
	UV-PAR	21.0±21.9	79.0±21.9	46.3±5.1	4.7±1.1
	PAR	13.1±0.1	87.0±0.1	32.4±8.3	27.5±9.9
	Dark	45.9±53.4	54.2±53.4	21.9±13.9	14.5±16.3
HyA	Reference	3.1	96.9	42.4	33.4
	UV-PAR	1.7±0.1	98.4±0.1	40.9±7.4	27.8±6.8
	PAR	0.8±0.4	99.2±0.4	46.6±4.7	19.8±0.1
	Dark	0.6±0.3	99.4±0.3	41.3±5.4	19.3±4.7

[a] Aromatic/olefinic : signals in the 6,0-8,4 ppm region.
[b] Aliphatic : signals in the 4,4-0,0 ppm region.
[c] Carbohydrate : signals in the 4,4-3,0 ppm region.
[d] Saturated chain : signals in the 1,6-0,0 ppm region.


120
100
80
60
40
20
0
-20
200
300
400
500
600
700
800
Transmittance (%)
Wavelength (nm)
Cubitainer
UV filter
Cubitainer + UV filter


a)
UV-PAR
PAR
Dark
aCDOM375 (m-1)
b)
a250 : a365
Time (days)


a)
b)
c)
d)
e)
Componant 1 (RU)
Componant 2 (RU)
Componant 3 (RU)
Componant 4 (RU)
Componant 5 (RU)
Time (days)
Time (days)
UV-PAR
PAR
Dark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80
60
40
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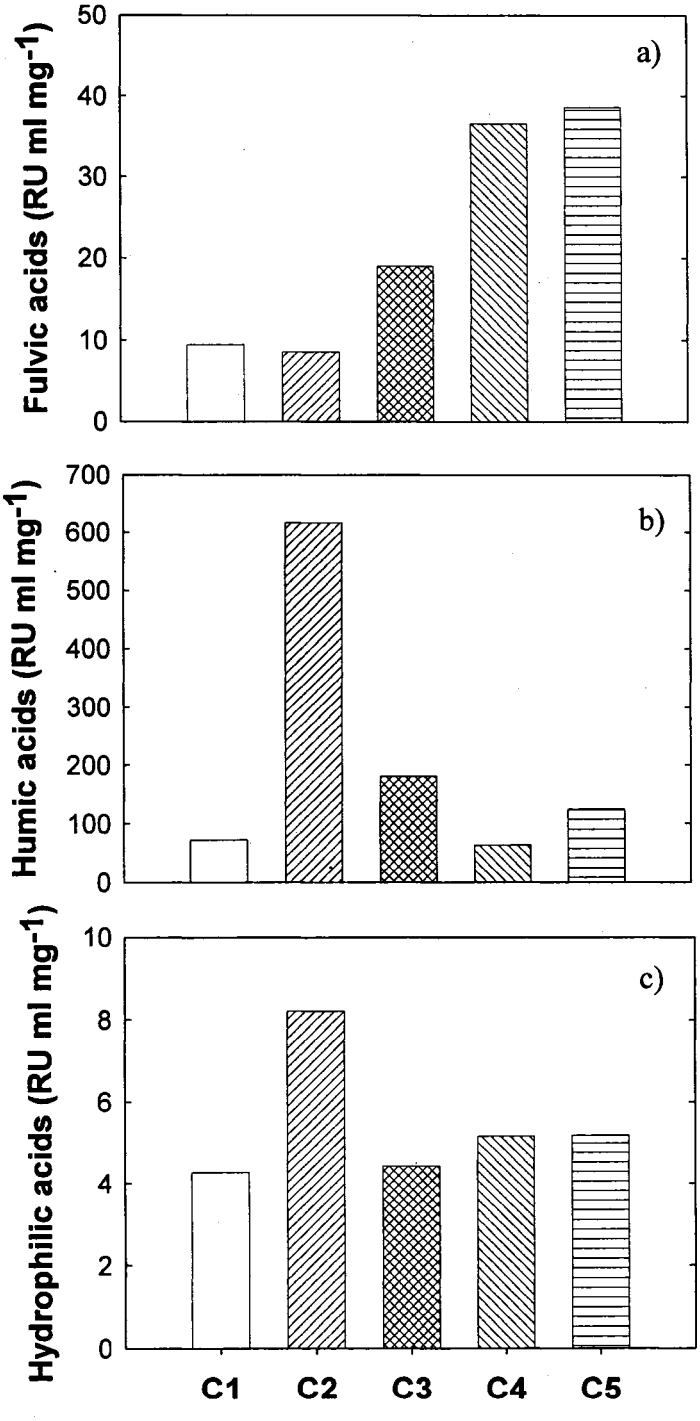
0
Relative weight (%)
Reference
Dark
PAR
UV-PAR
Fulvic acids
Humic acids
Hydrophilic acids


a)
Fulvic acids (RU ml mg-1)
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40
30
20
10
0
b)
Humic acids (RU ml mg-1)
700
600
500
400
300
200
100
0
c)
Hydrophilic acids (RU ml mg-1)
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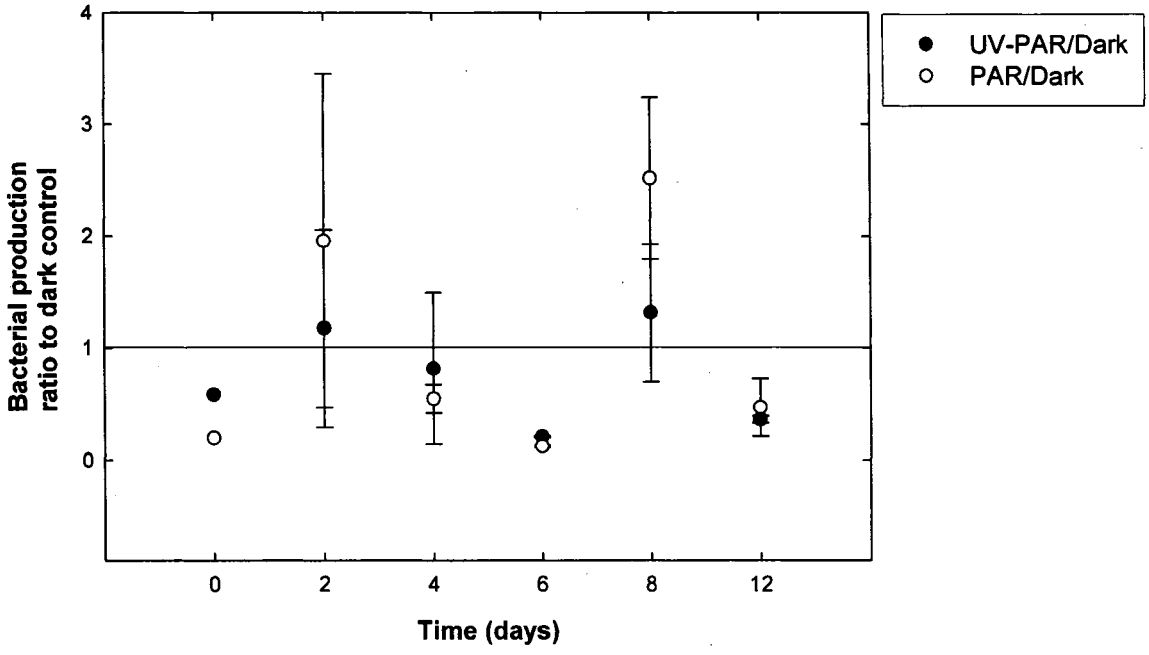
8
6
4
2
0
C1
C2
C3
C4
C5


Bacterial production
ratio to dark control
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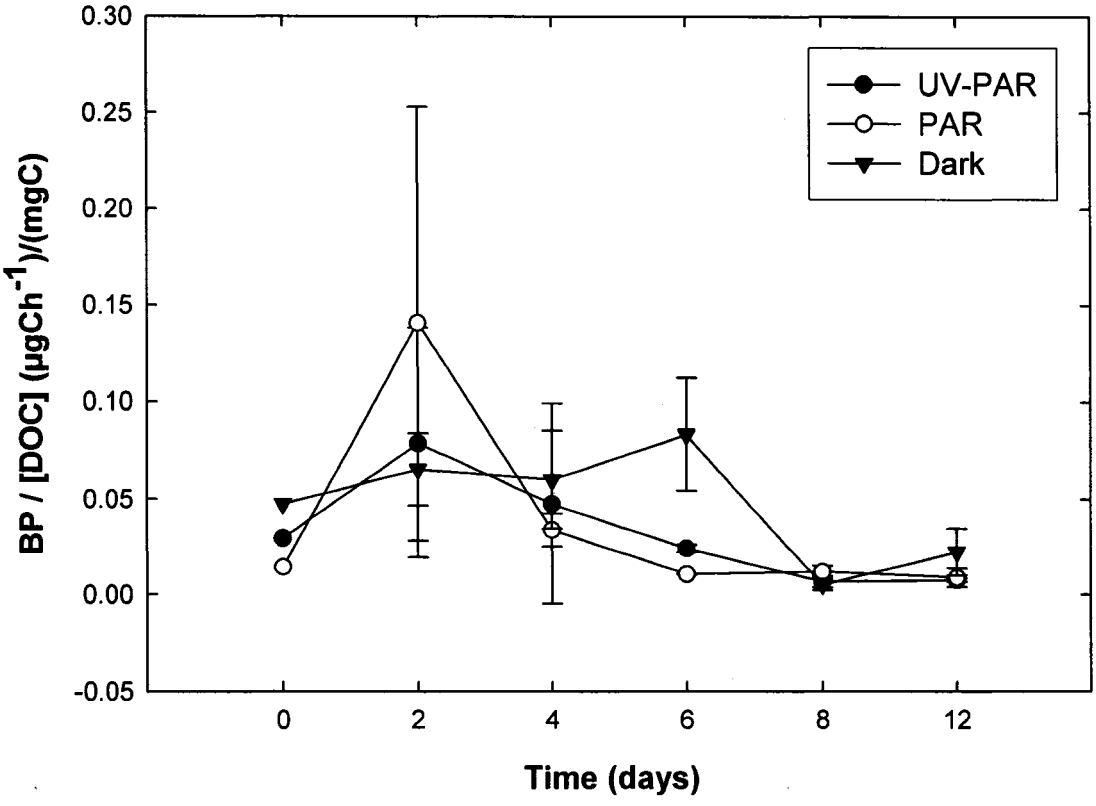
3
2
1
0
0
2
4
6
8
12
Time (days)
UV-PAR/Dark
PAR/Dark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0.20
0.15
0.10
0.05
0.00
-0.05
BP / [DOC] (µgCh-1)/(mgC)
0
2
4
6
8
12
Time (days)
UV-PAR
PAR
Dark

ANNEXE A

Instructions for authors: Aquatic Microbial Ecology

We publish: Research Articles (preferably not more than 12 printed pages); Reviews, state-of-the-art evaluations of important current research areas (up to 25 printed pages); Invited Reviews, authored by prominent experts; Notes, brief reports of important new information deserving priority publication (up to 4 printed pages); Comments, critical, fair assessments of published works and Reply Comments, replies to Comments (normally 2 to 3 printed pages; for more details on Comments/Reply Comments click here); Theme Sections, integrated multiauthor analyses and syntheses initiated and coordinated by acknowledged experts. They highlight cutting-edge research areas or problems (as brief as possible); As I See It, important, not peer-reviewed, *personal* views on hot topics (brief and fair).

Authors will be offered the option of publishing their article as Open Access. For further details see Open Access Initiative.

MANUSCRIPT SUBMISSION

Submitted manuscripts must (1) not be submitted simultaneously to any other publication outlet; (2) be original, i.e. not published before; (3) have been approved by all immediately involved, e.g. authors, institutional authorities.

Authors should submit manuscripts and revisions electronically. Acceptable electronic formats are Adobe pdf and MS-Word. Manuscripts must be transmitted in a single file that contains all text, tables, and figures. Pages and lines should be numbered. All fonts must be embedded in the file, which must not contain any security settings.

Submissions should consist of: (1) a cover letter with a brief description of the main goals and findings contained in the manuscript, and the response to reviews if the submission is a revision, (2) the manuscript itself. The files should be attached to an email message addressed to the Editors-in-Chief via the Inter-Research editorial office (ame-submissions@int-res.com). Authors may specify which Editor they would like to have conduct the review process for their manuscript, and identify 3 or more suitable referees. Manuscripts must be submitted via Inter-Research, and never to individual Editors.

Hard copies are NOT required unless electronic submission is impossible. In that case, manuscripts may be submitted by post - 1 hard copy and an electronic file on disc - to the Inter-Research editorial office.

PROCESSING

Manuscripts are critically evaluated by at least 3 reviewers. The Editor decides on acceptance or rejection. Acceptable manuscripts are usually returned to the author for consideration of comments and criticism.

On acceptance, titles of manuscripts are added to 'Forthcoming publications' on the Inter-Research Web site. The first, or corresponding, author receives a paper or electronic proof. Printing errors must be carefully corrected. At this stage, stylistic changes are not acceptable without compensatory payment. Tables of contents for each issue appear on the Web shortly before publication. Abstracts and .pdf versions of full articles are added on the day of publication.

For each article published in AME a free copy of the journal volume or number will be mailed to the first, or corresponding, author. Orders for offprints must be made when returning the proof (use the form provided).

PREPARATION

Submit revised mss on 3.5" diskette, Zip disk, CD (formatted as Mac/PC hybrid) or per email as a word-processing file (e.g. MS Word), together with figure files (if any). Large files (>1 MB) can be uploaded to our ftp site (ftp.int-res.com). The ftp site can be freely accessed, but please inform us if you upload anything.

To facilitate and accelerate the production process, please make sure that the ms conforms to the IR style. **For the appropriate format please refer to recent issues of AME.** Poor mss incur extra costs and delays; this applies particularly to figures and tables. If a ms requires excessive changes, we may have to return it, or charge you for the extra work involved in copy editing, typesetting and proofreading. To avoid this, please bring your ms in line with the following guidelines:

Cover page

Title: Avoid the use of 'A', 'An', 'The', 'On', etc. at the beginning, eliminate unnecessary modifiers, and make the title as logical, specific and concise as possible. The title should preferably have up to 100 characters (ca. 15 words, 2 lines in print), and 150 characters at most. Compare

'A novel method for the production of monoclonal antibodies (MAbs) specific to an envelope protein (28kDa) of white spot syndrome virus (WSSV) of shrimp and detection of WSSV by MAb-based antigen-capture enzyme-linked immunosorbent assay'

(236 characters, 37 words)

vs.

'Detection of white spot syndrome virus (WSSV) of shrimp by means of monoclonal antibodies (MAbs) specific to an envelope protein (28 kDa)'
(137 characters, 22 words).

Provide a running head with 3 to 6 words; e.g. 'Detection of shrimp WSSV'.

Authors and addresses: If a ms has several authors from different institutions,

use superscript numerals for identification;
provide a full valid street address or PO Box for each institution;
use * to refer to footnotes that identify the corresponding author and provide her/his e-mail.

Abstract: Limit the abstract (max. 250 words) to concise information on your work and its principal results. It should not contain literature cites, reams of data, or meaningless clauses such as *'the results are discussed'*.

Key Words: Supply 3 to 8 key words, listed in order of importance; these may be composites (e.g. 'environmental assessment', 'population dynamics'), but they should not be phrases or sentences.

Text

Please use numbered pages and lines, 12 point font, and double spacing. Do your very best to use correct English grammar, spelling and punctuation; if you are not a native speaker, you should have the text edited by someone who is, before sending the ms to IR. You may also wish to consult a 'How to' book such as Day (1998) *How to write and publish a scientific paper.* (Oryx, Phoenix, AZ).

Headings: Our main headings are in capital letters. Subheadings are bold type lower case, usually centered. Further subheadings can be used and you need not worry about details as long as their order is clear; they should be kept short and in the same style as described under 'Title'. We do not accept solitary subheadings, i.e. any section must contain at least 2 subheadings, or none at all.

Verbosity: Please eliminate verbiage; examples (verbiage underlined) with improved versions:

'Numerous studies in recent years, such as those by Miller (1995) and Smith (1998), have shown that low salinities enhance oyster recruitment'.

'Low salinities enhance oyster recruitment (Miller 1995, Smith 1998)'.

'Nevertheless, it seems likely that fur seal lactation success could be influenced by ...'

'Fur seal lactation success may depend on ...'.

Species names must be in italics, the genus is written in full at the first mention in each paragraph and abbreviated whenever mentioned again in the same paragraph. When referring to a species, do not use the genus name alone, unless you have previously defined it that way; be precise when using 'sp.' (singular) and 'spp.' (plural).

Abbreviations: Define unusual abbreviations and acronyms in the 'Abstract' (if used there) and at first mention in the main text, and thereafter use only the abbreviation / acronym.

Lists of items in the text should be run-on with numerals in parentheses; e.g. 'This study on mussels was conducted to: (1) assess their distributional range, (2) determine their population density, (3) collect specimens for culinary experiments'.

Equations and units: Use standard SI units. Relations or concentrations (e.g. mg per l) must be given as 'mg l^{-1}' (not mg/l); this applies to text, tables and graphs (e.g. axis labels). Variables are usually italicised (except for Greek letters). Italicisation should be consistent in text, figures and equations, and kept the same whether the symbols are in normal, superscript or subscripted text. Leave one blank space on either side of '=', '>', ± etc. where these denote equalities or inequalities.
Example: '$p < 0.05$, $r^2 = 0.879$' (*not* '$p<0.05$, $r^2=0.879$')
but: 'we studied organisms of size <0.5 µm'

Acknowledgements: Do not give first names in full, only initials (with period and space), e.g. 'We thank M. A. Smith and R. F. G. Miller'. Authors of the current ms should be given as initials only, e.g. 'We acknowledge a grant to M.A.S. from ...'.

Figures and tables

Figures: Please see Guidelines to Authors on Figure Preparation.

These should be self-explanatory; they must be referred to in correct numerical order in the text. Please prepare them very carefully; poor figures are a principal source of delay and additional work in the production process. High quality laser printouts, photographic prints (i.e. created by a camera), and electronic files in standard formats are acceptable.

Legends: Table legends should be given above each table; figure legends should be supplied as a list, and not placed with the individual figures. Captions should be brief and precise; they should not contain text in bold or italic, except for species names. If a figure or table provides data on biological species, its legend should begin with the full Latin name of that species. Example:

'Fig. 3. *Crassostrea gigas* and *Mytilus edulis*. Larval growth rates (mm d^{-1}; mean ± SD) at (a) 20°C and (b) 25°C'

Tables: Keep tables as simple and short as possible. Make sure the layout is clear. Preferably, write the rows as normal text lines and use tabs to indicate the columns (rather than using the 'Table' (cells) option in a word-processing program). For table footnotes, use superscripted lower case letters; asterisks can be used to indicate statistical significance. Tables too long to be printed in the journal can be published on our Website as an electronic supplement.

Literature cited

Limit the number of citations to a maximum of about 1 page of citations for every 5 pages of text. Use IR format (e.g. no periods or spaces with authors' initials, nor periods within journal names; examples below). All quoted literature must be listed, and all listed literature must be quoted. If in doubt with regard to abbreviations or how much information the cite should contain, provide all of it and let us shorten it.

Periodicals: Use standard abbreviations according to 'BIOSIS Serial Sources'. You may download a list of journal abbreviations from www.int-res.com/misc/journallist.txt or use the bibliographic database software 'EndNote' to import the list and obtain styles for IR journals at www.endnote.com/support/enstyles.asp. Example:

Blackburn N, Fenchel T (1999) Influence of bacteria, diffusion and shear on micro-scale nutrient patches, and implications for bacterial chemotaxis. Mar Ecol Prog Ser 189:1-7

Books: Please write the title of the book in lower case, and give the publisher and place of publication. In the case of book series, give the series editor as well. Examples:

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River, NJ

Carpenter (2003) Regime shifts in lake ecosystems: pattern and variation. In: Kinne O (ed) Excellence in ecology, Book 15. International Ecology Institute, Oldendorf/Luhe

Papers from books, conference reports, symposium proceedings, etc.: Please give the title of the cited chapter, the editor(s) and title of the volume, the publisher and place of the publisher (not the location where the conference was held), and the pages of the chapter. The date of the cite must be the year of publication (not the year in which the conference was held). Example:

Levin LA, Tolley D (2000) Influences of vegetation and abiotic enviromental factors on salt marsh invertebrates. In: Weinstein MP, Kreeger DA (eds) Concepts and controversies in tidal marsh ecology. Kluwer Acedemic Publishers, Dordrecht, p 661-707

West TL, Amrose WG (1992) Abiotic and biotic effects on population dynamics of oligohaline benthic invertebrates. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine eutrophication and population dynamics. Proc 25th Eur Mar Biol Symp. Olsen & Olsen, Fredensburg.

Certain conference proceedings/symposiums may be cited as a journal.

Bambach RK, Knoll AH, Sepkoski JJ Jr (2002) Anatomical and ecological constraints on Phanerozoic animal diversity in the marine realm. Proc Natl Acad Sci USA 99:6854-6859

Dissertations: Please write the title in lower case, 'MS / PhD thesis / dissertation' (no spaces or periods in 'MS' or 'PhD'), and give the university and its location. Example:

Eve TM (2001) Chemistry and chemical ecology of Indo-Pacific gorgonians. PhD dissertation, University of California, San Diego, CA

Inter-Research and International Ecology Institute mailing address:
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Research published in IR journals must have been conducted in accordance with institutional, national and international guidelines concerning the use of animals in research and/or the sampling of endangered species.

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ANNEXE B

Instructions for Authors

Aquatic Ecology

Online Manuscript Submission

Springer now offers authors, editors and reviewers of Aquatic Ecology the option of using our fully web-enabled online manuscript submission and review system. To keep the review time as short as possible (no postal delays!), we encourage authors to submit manuscripts online to the journal's editorial office. Our online manuscript submission and review system offers authors the option to track the progress of the review process of manuscripts in real time. Manuscripts should be submitted to: http://aeco.edmgr.com

The online manuscript submission and review system for Aquatic Ecology offers easy and straightforward log-in and submission procedures. This system supports a wide range of submission file formats: for manuscripts - Word, WordPerfect, RTF, TXT and LaTex; for figures - TIFF, GIF, JPEG, EPS, PPT, and Postscript.

NOTE: By using the online manuscript submission and review system, it is NOT necessary to submit the manuscript also in printout + disk.

In case you encounter any difficulties while submitting your manuscript on line, please get in touch with the responsible Editorial Assistant by clicking on "CONTACT US" from the tool bar.

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.

Manuscripts should be written in correct English. Manuscripts should be typed clearly, double-spaced throughout 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.

Electronic figures
Electronic versions of your figures must be supplied. For vector graphics, EPS is the preferred format. For bitmapped graphics, TIFF is the preferred format. The following resolutions are optimal: line figures - 600 - 1200 dpi; photographs - 300 dpi; screen dumps - leave as is. Colour figures can be submitted in the RGB colour system. Font-related problems can be avoided by using standard fonts such as Times Roman, Courier and Helvetica.

Colour figures

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Language
We appreciate any efforts that you make to ensure that the language is corrected before submission. This will greatly improve the legibility of your paper if English is not your first language.
Arranging the manuscript
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

Figures

All photographs, graphs and diagrams should be referred to as a 'Figure' and they should be numbered consecutively (1, 2, etc.). Multi-part figures ought to be labelled with lower case letters (a, b, etc.). Please insert keys and scale bars directly in the figures. Relatively small text and great variation in text sizes within figures should be avoided as figures are often reduced in size. Figures may be sized to fit approximately within the column(s) of the journal. Provide a detailed legend (without abbreviations) to each figure, refer to the figure in the text and note its approximate location in the margin. Please place the legends in the manuscript after the references.

Tables

Each table should be numbered consecutively (1, 2, etc.). In tables, footnotes are preferable to long explanatory material in either the heading or body of the table. Such explanatory footnotes, identified by superscript letters, should be placed immediately below the table. Please provide a caption (without abbreviations) to each table, refer to the table in the text and note its approximate location in the margin. Finally, please place the tables after the figure legends in the manuscript.

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 ((++)31–78–6392555).

Fifty offprints will be supplied free of charge.

References

1. Journal article:
Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

2. Inclusion of issue number (optional):
Saunders DS (1976) The biological clock of insects. Sci Am 234(2):114–121

3. Journal issue with issue editor:
Smith J (ed) (1998) Rodent genes. Mod Genomics J 14(6):126–233

4. Journal issue with no issue editor:
Mod Genomics J (1998) Rodent genes. Mod Genomics J 14(6):126–233

5. Book chapter:
Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York

6. Book, authored:
South J, Blass B (2001) The future of modern genomics. Blackwell, London

7. Book, edited:
Smith J, Brown B (eds) (2001) The demise of modern genomics. Blackwell, London

8. Chapter in a book in a series without volume titles:
Schmidt H (1989) Testing results. In: Hutzinger O (ed) Handbook of environmental chemistry, vol 2E. Springer, Berlin Heidelberg New York, p 111

9. Chapter in a book in a series with volume title:
Smith SE (1976) Neuromuscular blocking drugs in man. In: Zaimis E (ed) Neuromuscular junction. Handbook of experimental pharmacology, vol 42. Springer, Berlin Heidelberg New York, pp593–660

10. Proceedings as a book (in a series and subseries):
Zowghi D et al (1996) A framework for reasoning about requirements in evolution. In: Foo N, Goebel R (eds) PRICAI'96: topics in artificial intelligence. 4th Pacific Rim conference on artificial intelligence, Cairns, August 1996. Lecture notes in computer science (Lecture notes in artificial intelligence), vol 1114. Springer, Berlin Heidelberg New York, p 157

11. Proceedings with an editor (without a publisher):
Aaron M (1999) The future of genomics. In: Williams H (ed) Proceedings of the genomic researchers, Boston, 1999

12. Proceedings without an editor (without a publisher):
Chung S-T, Morris RL (1978) Isolation and characterization of plasmid deoxyribonucleic acid from Streptomyces fradiae. In: Abstracts of the 3rd international symposium on the genetics of industrial microorganisms, University of Wisconsin, Madison, 4–9 June 1978

13. Paper presented at a conference:
Chung S-T, Morris RL (1978) Isolation and characterization of plasmid deoxyribonucleic acid from Streptomyces fradiae. Paper presented at the 3rd international symposium on the genetics of industrial microorganisms, University of Wisconsin, Madison, 4–9 June 1978

14. Patent:
Name and date of patent are optional

Norman LO (1998) Lightning rods. US Patent 4,379,752, 9 Sept 1998

15. Dissertation:
Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

16. Institutional author (book):
International Anatomical Nomenclature Committee (1966) Nomina anatomica. Excerpta Medica, Amsterdam

17. Non-English publication cited in an English publication:
Wolf GH, Lehman P-F (1976) Atlas der Anatomie, vol 4/3, 4th edn. Fischer, Berlin. [NB: Use the language of the primary document, not that of the reference for "vol" etc.!]

18. Non-Latin alphabet publication:
The English translation is optional.
Marikhin VY, Myasnikova LP (1977) Nadmolekulyarnaya struktura polimerov (The supramolecular structure of polymers). Khimiya, Leningrad

19. Published and In press articles with or without DOI:
19.1 In press
Wilson M et al (2006) References. In: Wilson M (ed) Style manual. Springer, Berlin Heidelberg New York (in press)
19.2. Article by DOI (with page numbers)
Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med 78:74–80. DOI 10.1007/s001090000086
19.3. Article by DOI (before issue publication with page numbers)
Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med (in press). DOI 10.1007/s001090000086
19.4. Article in electronic journal by DOI (no paginated version)
Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. Dig J Mol Med. DOI 10.1007/s801090000086

20. Internet publication/Online document

Doe J (1999) Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry.Available via DIALOG. http://www.rsc.org/dose/title of subordinate document. Cited 15 Jan 1999

20.1. Online database
Healthwise Knowledgebase (1998) US Pharmacopeia, Rockville. http://www.healthwise.org. Cited 21 Sept 1998
Supplementary material/private homepage
Doe J (2000) Title of supplementary material. http://www.privatehomepage.com. Cited 22 Feb 2000
University site
Doe J (1999) Title of preprint. http://www.uni-heidelberg.de/mydata.html. Cited 25 Dec 1999
FTP site
Doe J (1999) Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt. Cited 12 Nov 1999
Organization site
ISSN International Centre (1999) Global ISSN database. http://www.issn.org. Cited 20 Feb 2000

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