UNIVERSITÉ DU QUÉBEC À MONTRÉAL

PRODUCTION DE CARBONE ORGANIQUE DISSOUS PAR LE COMPLEXE MACROPHYTES-ÉPIPHYTES : EFFETS DE FACTEURS PHYSICO-CHIMIQUES, IMPLICATIONS SUR LA PRODUCTIVITÉ DES COMMUNAUTÉS DE MACROPHYTES ET INCIDENCE À L'ÉCHELLE DE L'ÉCOSYSTÈME

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COD	Carbone organique dissous
DIC	Dissolved inorganic carbon
DM	Dark benthic chamber on macrophytes
DS	Dark benthic chamber on sediments
DOC	Dissolved organic carbon
DOCme	Dissolved organic carbon released by macrophyte-epiphyte complex
DN	Dissolved nitrogen
DP	Dissolved phosphorus
GPP	Gross primary production
NCP	Net community production
NPPme	Net primary productivity of the macrophyte-epiphyte complex
R	Respiration
TM	Transparent benthic chamber on macrophytes
TS	Transparent benthic chamber on sediments
TP	Total phosphorus

INTRODUCTION GENERALE

De manière classique, la représentation schématique du cycle global de l'eau sur la Terre comprend l'océan, les nuages, la pluie et les rivières et fleuves qui ramènent cette eau vers l'océan. Les lacs en sont exclus et sont de ce fait perçus comme de simples réservoirs d'eau. D'autre part, dans l'imaginaire collectif ce sont des lieux qui ont une forte connotation négative puisque liés à d'innombrables monstres et légendes, et cela sur tous les continents. Or la science est depuis toujours guidée par la fascination, le désir des hommes de comprendre leur environnement. Etant donné la faible popularité des lacs, on comprend pourquoi leur étude a longtemps été mise de côté par des scientifiques qui avaient tout à découvrir. L'idée est cependant apparue à certains que ces systèmes pouvaient servir à comprendre les phénomènes océaniques à petite échelle. Ainsi en 1892, Forel a consacré une nouvelle science, la limnologie, qu'il a définie comme étant « l'océanographie des lacs » et qui consistait alors plus en des études hydrologiques que biologiques. Cette science a pris son essor au début du 20^{e} siècle avec la fondation en 1922 de la Société Internationale de Limnologie et est à l'origine d'importantes contributions en écologie théorique (i.e. travaux de Odum et Hutchinson).

Dans le contexte mondial actuel où les ressources en eau et l'excès de CO_2 font partie des débats quotidiens, les limnologistes sont au cœur des préoccupations scientifiques et populaires. En effet, il a non seulement été démontré que les eaux continentales jouent un rôle non négligeable dans la production de gaz à effets de serre (Cole *et al.*, 2007 ; Duarte et Prairie, 2005) mais aussi que jusqu'ici le nombre de petits lacs avait été largement sous-estimé (Downing *et al.*, 2006). Finalement il a été possible d'estimer que la contribution globale des lacs à l'échelle planétaire est du même ordre de grandeur que celle des forêts et des océans (Prairie, communication personnelle). La reconnaissance de la limnologie comme science permettant de comprendre des phénomènes d'échelle planétaire appuie la thèse d'une planète-écosystème (Lovelock, 1979) et démontre que le sectarisme en science entraîne la négligence de phénomènes pourtant majeurs. Malgré cette évidence on trouve un cloisonnement des idées au sein même des disciplines et également en limnologie. En effet il semble que les origines océanographiques de cette discipline aient conduit à une inclination

des études pour la zone pélagique (zone plus profonde) au détriment des études portant sur la zone benthique (zone littorale, définie par la présence d'algues ou plantes aquatiques ; Vadeboncoeur, 2002). Par exemple, dans le contexte d'étude des émissions de gaz à effet de serre, des mesures du métabolisme de lacs ont mis en évidence une production de CO_2 liée à une hétérotrophie de la zone pélagique (Del Giorgio, 1993), c'est à dire une consommation de matière organique par le bactérioplancton plus importante que la production par le phytoplancton. Il a été montré que le régime métabolique des lacs (autotrophe vs. hétérotrophe) est lié à leur concentration en Carbone Organique Dissous (COD ; Prairie *et al.* 2002), et que les apports de COD d'origine terrestre participent au régime métabolique des lacs (Tranvik 1992; Pace *et al.* 2004; Carpenter *et al.* 2005; Kritzberg *et al.* 2006) ce qui appuie les observations d'hétérotrophie. Cependant ces études ont été faites dans un compartiment spécifique, l'épilimnion de la zone pélagique, étant la couche chaude de surface. De plus ces études considèrent que tout ce qui n'y est pas produit dans ce compartiment est d'origine allochtone, terme référant à une provenance extérieure au lac. On ne tient donc pas compte ici de la zone benthique des lacs qui, dans certains systèmes, est pourtant reconnue pour dépasser la productivité de la zone pélagique par la présence de macrophytes (Wetzel et Søndergaard, 1998).

Les macrophytes, terme regroupant macroalgues, plantes vasculaires (Angiospermes) ou non (Bryophytes, Ptéridophytes), sont réparties selon leur habitat : émergentes, flottantes ou submergées (Kalff, 2002). Les lits de macrophytes, quand ils sont présents, peuvent être les principaux producteurs primaires des zones littorales lacustres et font partie des systèmes les plus productifs de la biosphère, c'est pourquoi certains auteurs proposent même que la zone littorale des lacs soit déterminée par la présence de macrophytes (Canfield *et al.*1983). La productivité s'élève par exemple à 5000 à 7000 g poids sec/m^2/an pour les espèces émergées *Phragmites australis* et *Typha spp.* (milieux tempérés tropicaux). En comparaison la productivité des forêts équatoriales s'élève à 2200 g poids sec/m^2/an (Ramade, 1994). La productivité des macrophytes submergées des eaux douces des zones tempérées est moins importante (de 500 à 1000g de poids sec/m^2/an) cependant elles ont un fort potentiel de colonisation qui peut être estimé par la profondeur de Secchi et la pente littorale, déterminant

respectivement leur profondeur maximale de colonisation (Chambers et Kalff, 1985, Duarte *et al.*, 1986) et la profondeur de la biomasse maximale (Duarte et Kalff, 1986).

Leur présence affecte globalement le fonctionnement des lacs en modifiant leur structure (e.g. zone filtrante), leur métabolisme (e.g. augmentation de la productivité), leur chimie (e.g. concentrations en éléments nutritifs), et leur physique (e.g. turbidité de l'eau) (Jeppensen *et al.*, 1998). Les macrophytes représentent également une source importante de matière organique utilisable à divers niveaux trophiques et ceci à différents stades de leur vie. En effet, plusieurs espèces de macrophytes submergées présentent un développement en cohorte, ce qui se traduit par la présence simultanée de tissus vivants à différents stades de vieillissement et de tissus en décomposition. La période de sénescence des macrophytes constitue une source de carbone sous deux formes : d'une part du COD provenant de la libération des contenus cellulaires dans le milieu et pouvant s'agréger en particules (Otsuki et Wetzel, 1974 ; Alber et Valiela, 1994), d'autre part les tissus végétaux constitués de cellulose et de lignine, indigestes pour les invertébrés brouteurs, et dont la dégradation est essentiellement bactérienne (Mann 1988). Wehr *et al.* (1998) ont d'ailleurs montré en mésocosmes que le carbone issu des macrophytes sénescentes entraîne, dans la colonne d'eau, une augmentation de la teneur en chlorophylle *a*, une augmentation du taux de multiplication des cyanobactéries ainsi qu'une augmentation de l'abondance des hétérotrophes non flagellés, des rotifères, des *Daphnia pulicaria* et des copépodes *nauplii*; cette source de carbone favorise également la production bactérienne (Findlay S. *et al.* 1986; Findlay et Sinsabaugh, 2002, Wehr *et al.* 1999).

D'autre part, depuis les années 1960, il est connu que les macrophytes submergées en croissance perdent une partie non négligeable de leur photosynthétat sous forme de COD (37,3% de la production primaire brute de la communauté, Kailov et Burlakova, 1969; Wetzel, 1969), phénomène également mis en évidence chez le phytoplancton (Fogg *et al.*, 1965). Rapidement, des études ont porté sur la nature de ce COD relâché dans le milieu et ont montré qu'il s'agissait de sucres simples et d'acides aminés (Wetzel et Manny, 1972; Godmaire et Nalewajko, 1989). Pour certaines espèces, *in vitro*, il a été montré que le taux de relargage à la lumière est significativement supérieur à celui observé lors d'incubation à

l'obscurité (Sieburth 1969, Sondergaard 1981, Pregnall 1983, Nalewajko et Godmaire 1993). Il semble donc que pour ces espèces le relargage de COD soit lié à la photosynthèse. Cependant d'autres études sur des espèces différentes ont abouti à des conclusions opposées (Hough et Wetzel 1975, Barron *et al* 2003). Puisqu'il semblait anormal qu'un organisme dépense 1/3 de son énergie à fabriquer des molécules destinées à être relâchées dans le milieu, Sharp en 1977 s'est demandé si ce phénomène n'était pas du à des cellules en mauvais état (« Do healthy cells do it ? »). Il s'en est suivi la démonstration du contraire et l'arrivée de l'hypothèse suivante : la production de COD par les cellules vivantes en santé serait un phénomène d'évacuation des photosynthétats excédentaires en conditions limitantes en nutriments et donc inappropriées pour la synthèse de molécules plus complexe et l'accumulation de biomasse (« Overflow mechanism », Fogg 1983 ; Jensen 1984). Cette hypothèse suggère donc qu'en conditions limitantes en nutriments : (1) plus il y a de photosynthèse, plus il y de COD relâché dans le milieu ; (2) il n'existe pas de rétro-inibition de la photosynthèse dans ces conditions. Suite à ces expériences en laboratoire ayant posé les bases théoriques du phénomène de COD relâché, plusieurs études *in situ* ont démontré que ces observations n'étaient pas des artéfacts dus à l'expérimentation *in vitro* et qu'il était possible de mesurer des taux de COD relâché et de les inclure dans les bilans de carbone des écosystèmes (Wetzel et Sondergaard, 1998; Ziegler et Benner, 1999 ; Barrón *et al.*, 2003 ; Marañón *et al.*, 2004). Cependant ce type d'étude n'a jamais été effectué dans des systèmes dulcicoles, et les mécanismes influençant la production de COD restent très peu documentés (Godmaire et Nalewajko, 1990).

En nous plaçant dans le cadre de l'étude de la contribution des communautés de macrophytes submergées en croissance sur le métabolisme lacustre, nous avons donc non seulement quantifié leur production de COD *in situ* mais aussi étudié les facteurs influents en se basant sur l'hypothèse d'un lien positif entre photosynthèse et COD relâché (« Overflow mechanism »). En tant que communauté de macrophytes submergées, nous considérons le complexe macrophytes-épiphytes. En effet, les macrophytes submergées offrent une grande diversité d'habitats pour des espèces épiphytes aussi bien dans l'espace (surface foliaire importante) que dans le temps. Sur la surface de la plante une matrice muccopolysaccharidique d'origine bactérienne permet la colonisation par des espèces

autotrophes (diatomées, cyanobactéries) et hétérotrophes (bactéries et champignons); il en résulte un biofilm à métabolisme très élevé utilisant le COD relargué (Carpenter et Lodge, 1986) et les nutriments provenant de la plante mais aussi du milieu (Wetzel et Sondergaard, 1998). Pour étudier la physiologie des macrophytes il est nécessaire de s'affranchir de l'effet des épiphytes; par contre pour une étude comme la nôtre qui vise à comprendre le rôle des lits de macrophytes à l'échelle de l'écosystème, on considèrera le complexe épiphytes-macrophyes puisque ces organismes sont étroitement liés.

Notre cheminement a consisté en 5 étapes :

Dans le premier chapitre nous avons comme objectifs de mesurer la production de COD par les macrophytes des lacs du sud –est du Québec ; d'étudier une possible différence entre espèces dominantes dans ces lacs ; de savoir si des facteurs physiques affectant la photosynthèse, comme la photopériode, la quantité de lumière reçue et la température moyenne, ont un effet sur la production de COD. Selon notre hypothèse, nous nous attendons à voir (1) une différence entre espèces de par leurs différentes capacités photosynthétiques, (2) une augmentation du taux de COD relâché avec l'augmentation de la luminosité et de la température ambiantes. Notre dispositif expérimental de chambres benthiques *in situ* disposées sur les macrophytes nous a effectivement permis de mesurer une augmentation de COD durant le jour mais pas la nuit. Nos résultats montrent cependant que quantité de lumière reçue et température n'influencent pas les taux de COD relâché. De plus, les trois espèces de macrophytes étudiées (*Myriophyllum spicatum, Potamogeton amplifolius, Potamogeton richardsonii*, angiospermes dominant dans les lacs étudiés) ne présentent pas de taux de COD relâché différents.

Pour le deuxième chapitre nous avons comme objectif de tester des facteurs chimiques comme la concentration en nutriments ou en CO_2 sur les taux de COD relâché. Selon notre hypothèse nous nous attendons à observer une relation négative entre taux de COD relâché et les concentrations en phosphore et/ou azote dans les sédiments (principale source de nutriments pour les macrophytes), et des relations positives avec le ratio C:N:P dans les plantes qui reflète les conditions de croissance des plantes, et la teneur en CO_2 dans l'eau

(pCO_2). Nos résultats ne montrent aucune influence des nutriments sur les taux de COD relâché. Par contre il apparaît clairement une relation négative entre teneur en CO_2 et taux de COD relâché. Ces résultats nous permettent de rejeter l'hypothèse de départ et nous conduisent à une hypothèse alternative selon laquelle ce phénomène de production de COD serait une réponse physiologique visant à parer une limitation en CO_2 en agissant comme un système de concentrateur de carbone (Carbon Concentrating Mechanism). Cette hypothèse est conservée dans les chapitres III et IV.

Le troisième chapitre vise à démontrer clairement l'effet de la teneur en CO_2 dans l'eau sur la production de COD par les communautés de macrophytes en croissance, en étudiant ce phénomène le long d'un gradient naturel de CO_2 dans une rivière de Floride (Ichetucknee River). Dans cet écosystème très différent des lacs étudiés au Québec et avec une espèce spécifique à ce milieu (*Sagittaria kurziana ;* United State Department of Agriculture), nous démontrons à nouveau que les taux de COD relâché dans nos chambres benthiques par les communautés de macrophytes diminuent avec l'augmentation de la teneur en CO_2. Ces résultats corroborent donc notre hypothèse alternative.

Dans le quatrième chapitre, nous présentons la synthèse des données de productivité mesurées dans les lacs du Québec et la rivière Ichetucknee (Floride) et nous étudions les éventuelles relations entre taux de COD relâché et productivité. Nous montrons que la productivité nette des communautés étudiées au Québec est limitée par les concentrations en CO_2 dissous, alors que pour les communautés étudiées en Floride la productivité est soutenue par la prise de HCO_3^-. Finalement nous trouvons une relation générale entre l'utilisation du HCO_3- comme source de carbone et les taux de carbone relâché par les macrophytes, ce qui corrobore notre hypothèse.

Finalement, dans un cinquième chapitre, nous appliquons les taux de COD relâché calculés aux biomasses totales de macrophytes submergées mesurées dans nos écosystèmes. Nous obtenons une valeur de COD relâché extrapolable à l'échelle mensuelle et ainsi comparable à des données de COD apportées dans les systèmes par le bassin versant (allochtone). Nous montrons que la contribution du COD provenant des macrophytes par

rapport au COD allochtone est généralement faible mais peut par contre devenir majeure en cas de faibles précipitations (se traduisant par des apports allochtones réduits). Cet exercice d'extrapolation de nos résultats nous permet d'avoir une vision globale du phénomène étudié à l'échelle de l'écosystème et ainsi d'appréhender son impact pour le métabolisme global.

CHAPITRE I

IN SITU DOC RELEASE BY SUBMERGED MACROPHYTE-EPIPHYTE COMMUNITIES IN SOUTHERN QUEBEC LAKES

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Context: Questioning the possible implication of submerged macrophytes in carbon pool of lakes, we focus our research on the role of DOC release by macrophyte-epiphyte communities and more particularly on the factors influencing DOC release, considering the hypothesis of the « Overflow mechanism ». The objectives of the first chapter are (1) to measure the DOC release by macrophyte-epiphyte complex in south-eastern Quebec lakes; (2) to compare the release by different dominant macrophytes species; (3) to study the effect of factor known to influence photosynthesis on the release.

1.1 ABSTRACT (RÉSUMÉ)

We studied the *in situ* release of dissolved organic carbon (DOC) by growing submerged freshwater macrophytes. Incubations with benthic chambers in five south-eastern Quebec lakes show a net DOC production for different communities of *Myriophyllum spicatum* and *Potamogeton spp.* Daytime DOC release rates range from undetectable to 9.7 $mgC.m^{-2}.h^{-1}$. Although DOC release was restricted to daylight hours and thus suggestive of a photosynthesis-related process, we found no strong link between DOC release rates and concurrent illumination or temperature. We found no difference in DOC release rates between the 3 main colonizing species of the studied region. The overall mean DOC release rate was $4.57mgC.m^{-2}.h^{-1}$ (SD ± 0.65) or 56 $\mu gC.g$ (dry weight)$^{-1}.h^{-1}$ (SD ± 8) which, we suggest, can be used for extrapolations at the lake scale.

Keywords: macrophytes, lakes, DOC release, photoperiod, *Myriophyllum spicatum, Potamogeton spp.*

Nous avons étudié la production *in situ* de carbone organique dissous (COD) par des macrophytes d'eau douce en croissance. Des incubations ont été réalisées avec des chambres benthiques dans cinq lacs du sud-est du Québec, ce qui a mis en évidence une nette production de COD par différentes communautés de *Myriophyllum spicatum* et de *Potamogeton spp*. La production diurne de COD variait entre des valeurs indétectables et des taux de 9.7 $mgC.m^{-2}.h^{-1}$. Bien que cette production soit uniquement diurne et de ce fait certainement liée à des processus photosynthétiques, nous n'avons pas pu mettre en évidence de lien entre production et quantité de lumière ou température. Par ailleurs nous n'avons pas trouvé de différence entre les productions de COD des trois principales espèces étudiées dans la région. Le taux moyen global de production de COD est de $4.57 mgC.m^{-2}.h^{-1}$ (SD ± 0.65) soit 56 $\mu gC.g$ (poids sec)$^{-1}.h^{-1}$ (SD ± 8), taux que nous pensons pouvoir utiliser pour des extrapolation à l'échelle des lacs.

1.2 INTRODUCTION

The loading of terrestrial dissolved organic carbon (DOC) can contribute significantly to the energy pathways of lake ecosystems (Tranvik 1992; Pace *et al.* 2004; Carpenter *et al.* 2005; Kritzberg *et al.* 2006). The concept of net heterotrophy in lakes whereby ecosystem respiration exceeds gross primary production has considerably changed our view of how lakes support their living biota. This pattern of excess respiration is believed to be found mostly in oligotrophic systems with DOC concentrations > 5-6mg.L^{-1} (Del Giorgio and Peters 1993 and 1994; Del Giorgio *et al.* 1997; Prairie *et al.* 2002, Hanson *et al.* 2003) and is believed to be largely responsible for the CO_2 supersaturation observed in most lakes of the world (Cole *et al.* 1994, Sobek *et al.* 2003; Del Giorgio *et al.* 1999; Duarte and Prairie, 2005). However, recent studies have also shown that, in some lakes at least, pelagic net heterotrophic metabolism can be observed simultaneously with CO_2 undersaturation, i.e. with a net CO_2 influx from the atmosphere (Prairie *et al.* 2002). Such discrepant properties would occur if a source of organic carbon metabolized in epilimnetic waters originated from elsewhere within the ecosystem. In such cases, net heterotrophy of the water column would not necessarily reflect the metabolic status of the whole ecosystem. Freshwater systems are among the ecosystems where this situation is most likely to occur because benthic primary production often dominates the overall productivity of the system, particularly in shallow oligotrophic lakes (Vadeboncoeur *et al.*, 2003). Indeed, studies of lake metabolism that do not consider the potential role of the benthic zone are likely to yield a biased assessment of the overall metabolic balance. In particular, if benthic organic matter production is translocated in some way to the water column and respired there, it could lead to a net heterotrophy of the pelagic zone but not necessarily of the whole ecosystem. To our knowledge, this hypothesis has been seldom explored in freshwater systems, largely because of the common misconception that benthic zone is not often quantitatively significant (Vadeboncoeur *et al.*, 2002). Yet, this situation has been described in marine systems by Gazeau *et al.* (2005) who argued that benthic community dominated by macrophytes (*Posidonia oceanica*) could partly balance the net heterotrophy of the planktonic compartment, an idea supported by other studies as well (Duarte and Cebrian, 1996; Gattuso *et al.* 1998; Wetzel and Sondergaard, 1998; Ziegler and Benner, 1999).

Macrophyte beds represent a potential DOC source to the open water both from plant decay (Otsuki et Wetzel, 1974; Mann 1988; Alber et Valiela, 1994) and also from DOC release by living plants. Dissolved organic matter release by macrophytes has been demonstrated several decades ago by Kailov and Burlakova (1969) who worked with five marine species of macroalgae. Further studies confirmed the release of dissolved organic carbon (DOC) both on marine (Brilinsky 1977) and freshwater species (Wetzel and Manny, 1972; Godmaire and Nalewajko, 1989). Reported rates of DOC vary enormously, ranging from 0.006 and 9.1mgC.g (dry weight)$^{-1}$.h^{-1}, and this release can represent a non negligible part of aquatic plant production (1.1% to 67%). Some studies described higher level of DOC release in the light than in the dark (Sieburth 1969, Sondergaard 1981, Pregnall 1983, Nalewajko et Godmaire 1993), thus underlying a possible link between the release and the photoperiod. Similarly, DOC release has been described in marine phytoplankton and it was viewed as an overflow mechanism linked to the photosynthesis in nutrient limiting conditions (Jensen, 1984).

In this *in situ* study, our main objective was to quantify DOC release by the macrophyte-epiphyte complex in a series of natural lakes, to explore whether DOC release as an overflow mechanism for photosynthate in nutrient limiting conditions. This hypothesis supposes that when CO_2 and light are not limiting factors, photosynthesis will produce carbohydrates even if nutrient limiting conditions prevent the production of biomass from these simple molecules. According to this hypothesis, DOC release occurs only during photosynthesis and the rate of DOC release should be related to factors such as light and temperature which are known to influence positively macrophyte photosynthetic rates (Kirk, 1994; Madsen and Brix, 1997). As photosynthetic capacity differs among macrophyte species (Nielsen and Sand-Jensen, 1989), we also tested whether DOC release rates vary consistently among 4 different species (*Myriophyllum spicatum, Potamogeton amplifolius, Potamogeton richardsonii, Potamogeton robinsii*). The hypotheses were tested both as rates per unit area (in milligrams of carbon per square meter per hour, mgC.m^{-2}.h^{-1}) and per unit plant biomass (in milligrams of carbon per gram of dry weight per hour, mgC.gdw^{-1}h^{-1}), respectively representing responses at the community and the plant level. Finally, we examined the temporal variability in DOC release over the growing season.

1.3 MATERIAL AND METHODS

Study site and experimental design

The measurements were undertaken during the summers of 2004, 2005 and 2006 in six lakes of the Eastern township region ~ 100km east of Montréal Québec (Table 1.1). These lakes are of glacial origin and are influenced by the alluvial sedimentary geology of the Saint Lawrence River.

To test our hypothesis we used *in situ* benthic chambers inserted in the sediments. Chambers were made with a polyvinyl chloride cylinder (20 cm high, 20 cm diameter) covered hermetically with a polyethylene transparent plastic bag equipped with a sampling port (Barron *et al.*, 2003). At each study site, we randomly selected monospecific macrophyte beds coupled with a nearby unvegetated sediment location as control (except in shallow L. Trois Lacs where *Potamogeton richardsonii* covered the entire benthic surface). The macrophyte species studied are listed in Table 1.2. Depending on the experiment, 2, 3 or 4 replicate transparent benthic chambers were placed on macrophytes (TM) and 2 on unvegetated sediments (TS). Testing the effect of light on DOC release first implied a comparison between daily and nightly rates. Therefore, all *in situ* incubations lasted 24h or 36h from sunrise or sunset (T0). Water samples from chambers were taken in duplicates for DOC at the beginning (T0), then just before sunset (or just after sunrise; T1) and finally just after the following sunrise (or just before sunset; T2) for the 24h incubations; one more sampling was made 12h after (before sunset or just after sunrise; T3) for the 36h incubations made in L. Stukely.

We also tested the effect of mean light and temperature received during the daytime on concurrent DOC release rates. To obtain a wider and more continuous range in light and temperature conditions we carried out additional experiments with transparent benthic chambers containing macrophytes covered with one or two neutral screens (noted TM+screen). Daytime averaged temperature in TM chambers ranged between 17.09 °C and 28.01 °C, and daytime averaged light received ranged between 612 LUX and 19 523 LUX.

Table 1.1

Lakes characteristics. TP: total phosphorus. TN: Total nitrogen. Chl*a*: Chorophylle *a*. DOC: Dissolved organic carbon. MB: total macrophyte biomass, in grams of dry weight per square meter (gdw.m-2). ND: undetermined. -: unvisited site. *:averaged data from Prairie and Parkes, 2006

Lake	Longitude	Latitude	Surface (km2)	Mean depth (m)	Mean Secchi depth (m)	TP ($\mu g.L^{-1}$)	SD	TN ($mg.L^{-1}$)	SD	Chl*a* ($\mu g.L^{-1}$)	SD	DOC ($mg.L^{-1}$)	SD	MB 2004	MB 2005	MB 2006
D'Argent	72°18 W	45°18 W	0.96	4.6	2.5	12,4	4,4	0,4	0,1	3,4	1,6	7,7	1,5	24	4 4	ND
Bowker	72°12 W	45°25 W	2.3	25.9	7.9	4,9	3,0	0,2	0,0	0,9	0,3	2,6	0,3	0	-	-
Peasley	72°16 W	45°16 W	0.23	10.5	2.9	10,5	6,4	0,3	0,0	3,2	1,5	6,9	1,0	-	-	27
Stukely	72°15 W	45°21 W	3 86	13.6	5.4	7,4	6,2	0,3	0,0	1,7	0,6	4,6	0,3	17	0	0
Trois Lacs	71°53 W	45°48 W	2.85	1.26	1.5	30,9	17,4	0,7	0,1	4,4	2,4	9,9	1,8	68	90	ND
Waterloo	72°31 W	45°20 W	1.15	2.9	0.9	34,1	14,4	0,6	0,2	16,8	10,4	7,3	1,4	10	15	-

Taking TM+ screen chambers into account, the lowest temperature and the lowest light received became 16.68 °C and 78 LUX respectively.

According to our hypothesis, we should observe the maximum release at the highest photosynthesis, i.e. at maximum light and temperature. To further explore this prediction, we followed DOC concentrations over a 24-hour cycle at a much higher time resolution by taking samples every one or two hours during the day for 2 TM chambers in L. Stukely. Light intensity and temperature were measured every 10sec but averaged for every minute, in each transparent benthic chamber with HOBO Pendant Temp/Light® logger, placed at the top of the chambers.

Water samples for DOC measurements were drawn through the sampling ports with 60mL (polyethylene) acid-washed (HCl 10%) syringes, filtered on 0.45µm (filtropur Starsted®) and kept refrigerated (4°C) in 40 mL acid-washed tubes with silicone-Teflon caps to prevent gas exchange during analysis. The sampling led to a volume reduction inside the chambers of around 1%. DOC analysis were made with a 1010 TIC TOC analyser, O.I. Analytical, by high temperature wet oxidation (sodium persulfate; 100g/L) after dissolved inorganic carbon (DIC) elimination by acidification (sulphuric acid 5%) and sparging; analytical triplicate samples were taken in 2004, but only in duplicates in 2005 and 2006 given the high reproducibility observed. Analytical replicates had a coefficient of variation 0.7% and sample replicates had a coefficient of variation of 1%.

At the end of the 24h incubation, the water volume within each benthic chamber was estimated by injecting a 10mL weak fluorescein solution (absorption peak at 552nm) and drawing two samples after mixing for 5 min. Calculated volumes ranged between 4 and 11L. At the end of each experiment, macrophyte shoots within the PVC ring were harvested to measure their aboveground biomass after rinsing and drying at 55°C during 24h to 36h.

Macrophyte biomass was also quantified at the whole lake scale. Random quadrat samples were taken the second week of August in 2004 and the first week of August in 2005 and 2006 within the zone confined by the maximum depth for macrophytes colonisation

estimated from the Secchi depth (Chambers and Kalff, 1985). For each lake except L. Trois Lacs where macrophytes colonized the entire benthic surface, we randomly choose sampling points in the colonisation zone from digital bathymetric map; in the field these sampling point were reached with a GPS. We used a 25x25cm quadrat and we estimated the number of replicates needed after Downing and Anderson (1985). Between 10 and 22 replicates were taken in each lake. Macrophyte shoots inside the quadrat were harvested to measure their aboveground biomass after rinsing and drying at 55°C during 24h to 36h.

DOC release by macrophytes communities

Diurnal and nocturnal DOC variations were calculated as the simple difference in DOC concentrations between sunrise and sunset (photoperiod) and sunset and sunrise respectively, in both TM and TS chambers. DOC changes within each chamber were expressed both per unit area ($mgC.m^{-2}.h^{-1}$) and per unit plant biomass ($mgC.gdw^{-1}h^{-1}$).

The presence of macrophytes is known to alter their own environments by increasing the sedimentation rate (Barko and James, 1998; Rooney *et al.*, 2003; Schulz and Köhler, 2006) and the nature of the sedimenting material (Duarte *et al.*, 1999; Barron and Duarte, 2006), and by providing efficient mechanisms for solute transport within the sediment or at the interface (Carpenter and Lodge, 1986). Thus, basic calculations of DOC production or consumption from DOC concentration changes in chambers containing both water and sediments provide estimates of the net effect of the presence of macrophytes on DOC release patterns (termed net bulk DOC release). Indeed, daily and nightly DOC variations measured in transparent chambers containing macrophytes (TM) are the sum of several processes occurring simultaneously. In an attempt to further disentangle these processes, we made additional measurements with benthic chambers on bare sediments near the macrophyte bed, thus providing the closest control in terms of sediments as well as with the phytoplanktonic and heterotrophic communities of the surrounding water. The production and consumption of DOC can be derived from several potential compartments: macrophyte-epiphyte complex (me), phytoplankton (Ψ), heterotrophic community (h) and the sediments (sed). In transparent chambers placed on sediments (TS), the same processes are presents except for the

contribution of macrophyte-epiphyte complex. While an imperfect control, these experiments provide valuable clues by estimating, by subtraction, the net DOC release attributable to the macrophyte-epiphyte complex itself (termed DOCme). Equations 1.1 to 1.4 describe the processes occurring in transparent macrophytes (TM) and sediments (TS) chambers:

$$\Delta DOC(TM)day = DOCme(d) + DOC_{\psi}(d) + DOC_{h}(d) + DOC_{sed}(d) - R \qquad (eq.1.1)$$

$$\Delta DOC(TM)night = DOCme(n) + DOC_{\psi}(n) + DOC_{h}(n) + DOC_{sed}(n) - R \qquad (eq.1.2)$$

$$\Delta DOC(TS)day = DOC_{\psi}(d) + DOC_{h}(d) + DOC_{sed}(d) - R' \qquad (eq.1.3)$$

$$\Delta DOC(TS)night = DOC_{\psi}(n) + DOC_{h}(n) + DOC_{sed}(n) - R' \qquad (eq.1.4)$$

where R is the DOC respired in TM chambers and R' the DOC respired in TS chambers. By successive subtractions we obtained net DOCme:

$$\Delta DOC(TM)day - \Delta DOC(TM)night - \Delta DOC(TS)day + \Delta DOC(TS)night = DOCme(d) - DOCme(n) \qquad (eq.1.5)$$

and

$$DOCme(d) - DOCme(n) = DOCme \qquad (eq.1.6)$$

both expressed in $mgC.m^{-2}.h^{-1}$ and in $mgC.gdw^{-1}.h^{-1}$.

Uncertainty estimates for these compound calculations were derived from classical error propagation formulas.

1.4 RESULTS

Macrophyte biomass data

In the benthic chambers, areal macrophytes biomass varied between 6 to 336gdw.m^{-2}, with a mean of 95gdw.m^{-2} (SE ± 3.4, n=246) which is in the same range than other reported macrophyte biomass in south-eastern Quebec lakes (Chambers and Kalff, 1984; Rooney and Kalff, 2000; Duarte and Kalff, 1990). We naturally observed significant increases in mean biomass through the summertime (ANOVA, $p<0.05$) with average growth rates of 37 gdw.$month^{-1}$ in June-July, and 10 gdw.$month^{-1}$ in July-August. Estimated macrophytes biomasses at the whole lake scale are given in Table 1.1 for each lake.

Day-night comparison in DOC release rates

Our macrophyte incubations clearly showed that the DOC release is linked to the photoperiod since it occurred during day but stopped at night (fig. 1.1; table1.2). This was the case whether experiments were begun in the evening or in the morning showing that the observed DOC release was tied to the photoperiod and not to the initiation time of the incubations. In all cases, we observed an increase in DOC concentration during the day but not during the night in benthic chambers containing macrophyte(fig.1.2; one way ANOVA for each lake, $p<0.05$), except in one of the sampling dates in L. Trois Lacs (20/06/05) where DOC was apparently consumed faster than it was produced. Considering different TM chambers used for each experiment (per date and lake) as replicates we obtained mean daily DOC release rates varying from undetectable to 9.7 mgC.m^{-2}.h^{-1} (SE ± 1.1; n=2) in L. Waterloo or from undetectable to 157 µgC.gdw^{-1}.h^{-1} (SE ± 32; n=3) L. Stukely. Nightly variations in DOC were mostly negative, thus representing DOC consumption. They varied from -3.8 mgC.m^{-2}.h^{-1} (SE ± 1.4; n=3) in L. Trois Lacs to 1.5 (SE ± 0.8; n=3) in L. Stukely or from -63 µgC.gdw^{-1}.h^{-1}, (SE ± 23; n=4) to 12 µgC.gdw^{-1}.h^{-1}, (SE ± 5; n=3) both in L. Stukely. In chambers without macrophyte (TS), we observed no significant DOC changes in all our

Table 1.2

Mean daily and nightly DOC release rates for five studied lakes with corresponding daily temperature and received light inside the benthic chamber. Myrio, P. amp, P. rich, P. rob, are abbreviations for *Myriophyllum spicatum, Potamogetton amplifollius, Potamogetton richardsonii and Potamogetton robinsii* respectively.

				DAY				NIGHT							
Lake	Sampling date	Species	n	mean DOCr	SE	mean DOCr	SE	mean DOCr	SE	mean DOCr	SE	Temp.	SE	Light	SE
				$mg.m^{-2}.h^{-1}$		$ug.gdw^{-1}.h^{-1}$		$mg.m^{-2}.h^{-1}$		$ug.gdw^{-1}.h^{-1}$		?C		LUX	
Bowker	07/07/2004	*P. rob*	2	9,7	1,1	92	7	-0,8	0,1	-8	1				
D'Argent	28/06/2004	*P. amp*	2	3,2	0,9	49	12	-2,1	0,5	-33	10				
D'Argent	06/06/2005	*Myrio*	4	0,4	0,2	4	3	0,6	0,3	6	4	21,11	0,05	10673	2352
D'Argent	08/06/2005	*P. amp*	4	0,4	0,3	22	20	-0,7	0,3	-74	51	21,00	0,07	3090	57
D'Argent	27/06/2006	*Myrio*	3	3,8	1,7	50	32	-3,3	0,8	-26	3	22,04	0,05	7000	350
D'Argent	28/06/2006	*Myrio*	3	4,5	1,3	36	10	1,5	0,9	10	6	21,58	0,11	3140	1087
D'Argent	03/08/2006	*P. amp*	3	1,0	1,4	6	12	-1,5	2,2	-10	19	22,49	0,07	1746	263
D'Argent	05/09/2006	*P. amp*	3	-0,4	1,4	-6	23	-0,4	0,2	-6	3	18,27	0,05	1076	233
Peasley	11/07/2006	*P. amp*	3	5,5	2,0	47	17	-0,6	0,2	-5	1	24,72	0,04	5578	589
Peasley	07/08/2006	*P. amp*	3	0,1	2,0	7	19	-0,8	1,2	-8	15	24,85	0,06	9731	1694
Stukely	22/06/2005	*Myrio*	4	1,7	0,5	48	11	-1,7	0,3	**-63**	23	19,00	0,10	9412	1670
Stukely	28/06/2005	*P. amp*	3	8,1	1,0	**157**	32	-2,3	0,8	-46	19	27,93	0,04	17528	658
Stukely	06/07/2005	*Myrio*	2	3,4	0,6	75	36	-0,1	2,0	15	41	21,44	0,01	11322	1146
Stukely	09/08/2005	*P. amp*	3	5,8	1,7	75	24	-0,3	0,6	-4	8				
Stukely	12/06/2006	*P. amp*	3	2,6	1,3	47	23	-2,2	0,9	-42	19	17,16	0,07	12312	182
Stukely	21/06/2006	*P. amp*	3	9,1	0,9	75	11	0,1	2,7	-2	18	21,62	0,07	2661	377
Stukely	17/07/2006	*P. amp*	3	7,8	0,4	68	8	**1,5**	0,8	**12**	5	27,24	0,05	14830	1486
Stukely	20/07/2006	*Myrio*	2	8,0	0,6	79	13	-2,8	3,4	-36	41	26,69	0,06	19446	77
Stukely	18/08/2006	*P. amp*	3	3,6	0,4	43	9	1,5	1,6	13	16	22,47	0,03	16198	776
Trois Lacs	19/07/2005	*P. rich*	2	5,3	1,1	87	32	-1,9	1,5	-33	28	27,16	0,08	7791	1598
Trois Lacs	28/07/2005	*P. rich*	3	2,8	0,4	25	2	-1,9	0,5	-18	5	24,82	0,04	5079	1609
Trois Lacs	20/06/2006	*P. rich*	3	8,1	0,3	47	3	-3,8	1,4	-20	7	22,89	0,01	2899	213
Trois Lacs	01/08/2006	*P. rich*	3	2,1	0,8	16	11	0,6	1,3	9	11	24,41	0,04	3339	664
Waterloo	06/07/2004	*Myrio*	2	9,7	2,6	112	56	-0,6	1,8	-26	32				
Waterloo	15/06/2005	*Myrio*	3	8,0	3,2	141	54	-0,5	0,3	-16	14	20,81	0,04	5021	362

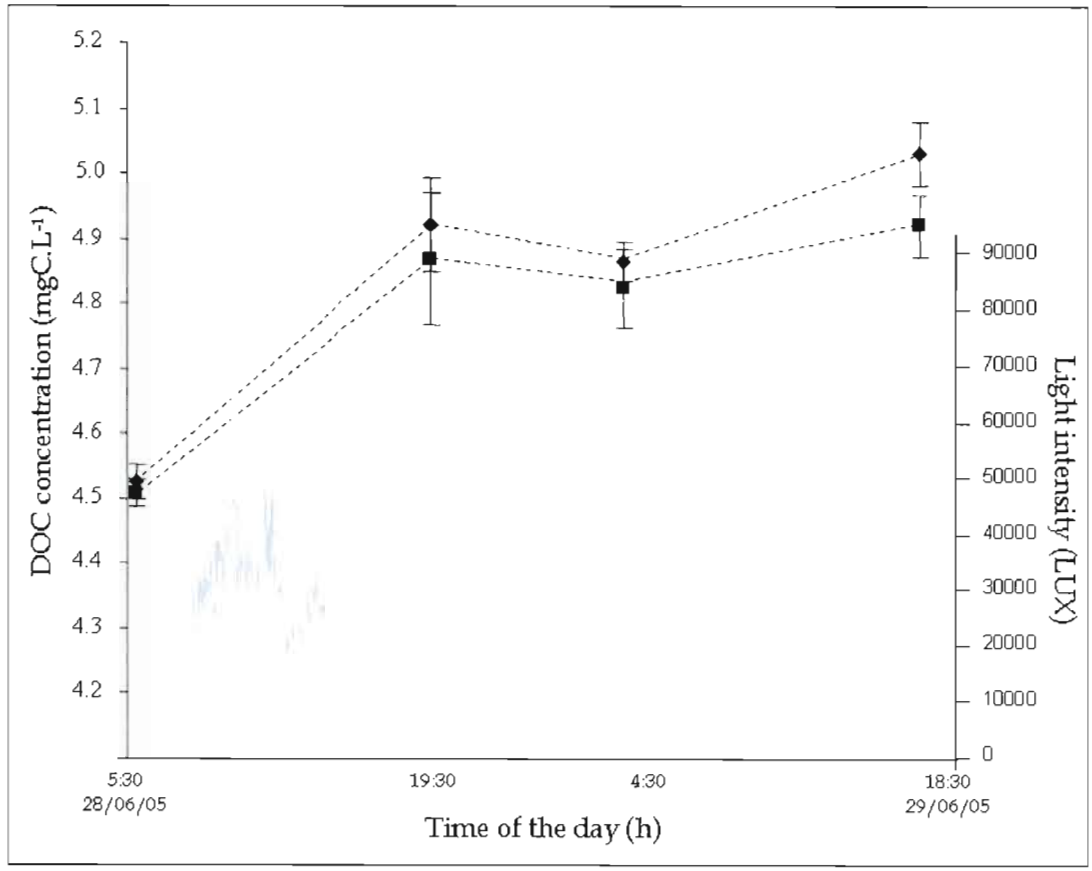

Figure 1.1 Trend in DOC concentration (left axis) and light intensity (right axis) during 36 hours in two transparent chambers containing macrophytes (■ and ♦ respectively) in L. Stukely in 2005. Bars represent standard error (n=4).

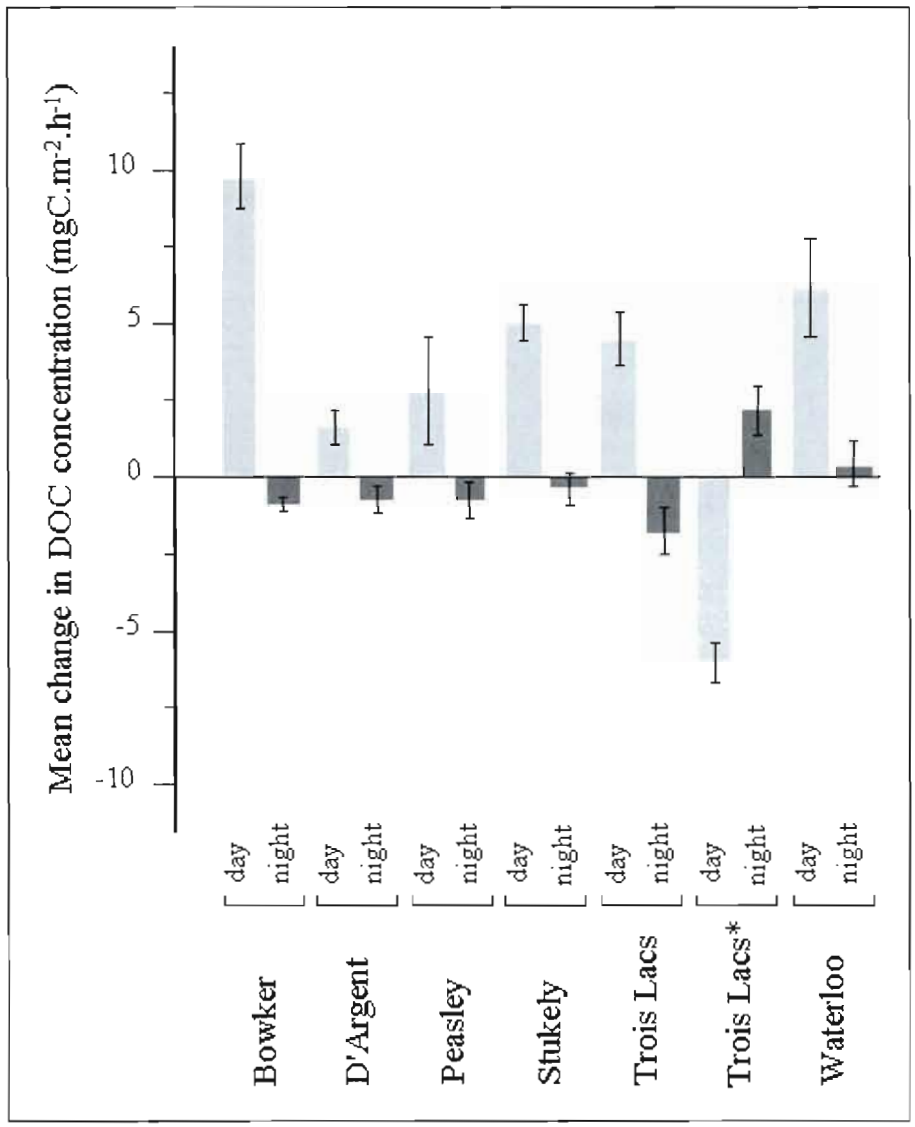

Figure 1.2 Day-time and night-time general mean DOC rates (with data from all years, in mgC.m-2.h-1) for transparent chambers containing macrophyte, at L. d'Argent, L. Bowker, L. Peasley, L. Stukely, L. Trois lacs (data of 20/06/05 apart, L. Trois lacs*) and L. Waterloo. Bars represent standard error.

experiments (ANOVA, $p>0.05$), meaning that the planktonic community of the benthic zone and naked sediments do not release DOC and that therefore changes in DOC concentration observed in TM chambers are attributable to the presence of macrophyte community.

Estimation of net DOC release rates by the macrophyte-epiphyte communities (DOCme).

As we explained earlier, day-time DOC rates obtained from direct measurements in chambers containing macrophyte take into account not only the effect of the macrophyte-epiphyte complex but also the effect of the overall metabolic activity occurring within the chamber, including DOC consumption occurring simultaneously with the release. In some cases (e.g. L. Trois Lacs, 20 June 05), this consumption can be strong enough to totally mask the diurnal DOC release. Equation 1.5, developed earlier, allows the estimation of the DOC release rate more closely attributable to the macrophyte-epiphyte complex itself, rather than by the entire community found in the benthic chambers. Because there were no significant DOC variations in the chambers devoid of macrophyte (TS), we considered the DOC release by phytoplankton and/or sediments to be quantitatively negligible. As a result, calculation of the net DOC release from macrophyte (DOCme, eq.1.5) can be simplified to:

$$DOC\ me = \Delta DOC(TM)day - \Delta DOC(TM)night \qquad (eq.1.7)$$

From individual DOC release rates we calculated corrected rates of DOC release by macrophyte-epiphyte complex (DOCme) using eq.1.7. Once averaged (per date and lake) obtained rates varying from undetectable to 11.9 $mgC.m^{-2}.h^{-1}$ (SE ± 1.72; n=3) for L. Trois Lacs and from undetectable to 203 $\mu gC.gdw^{-1}.h^{-1}$, (SE ± 50; n=3) in L. Stukely. For the following analysis, we excluded the data obtained on 20 June 05 in L. Trois Lacs, which still pointed to a strong net consumption even after correction (mean DOCme of $-8.7 mgC.m^{-2}.h^{-1}$, SD ± 2.5 or $-218 \mu gC.gdw^{-1}.h^{-1}$, SD ± 154). In all cases, these corrections (from subtractions) compounded the uncertainty of our measurements and therefore decreased the statistical power of our analysis. Nevertheless, we performed the two sets of calculations to

examine both the robustness of our conclusions and the potential importance of these corrections. We therefore will be able to conclude on the implication of the pelagic and sedimentary community versus macrophyte-epiphyte complex on the DOC release.

Short-term daytime evolution of DOC concentration in macrophytes-epiphytes communities

In 2005 and 2006, we followed diurnal DOC concentrations more intensively in L. Stukely on *P. amplifolius* incubations: DOC measurements were taken at sunrise and then every 2.5 hours on average. We observed an increase in DOC, from the first hours and occurring all day through (fig. 1.3A and 1.3B for 2005 and 2006 respectively). In each replicate benthic chambers, rates of DOC changes were not statistically different between sampling steps (ANCOVA, time*step: $p>0.05$, data not shown). It therefore suggests that DOC release is a continuous process that begins at sunrise but that is not clearly related to the rate of photosynthesis. Taking the two sampling year separately, we found no relationship between the increase in DOC concentration for each step and temperature or amount of light received during that same period. Thus, at this short temporal scale, DOC release is independent of temperature and light, pointing to a decoupling between the rates of release and the main factors regulating photosynthesis.

Influence of light and temperature and temporal patterns in DOC release rates

Combining all our measurements (2004, 2005, 2006; TM and TM+screen, n=108), we found no temporal pattern through summertime in the mean daily illumination in the benthic chambers, whereas temperature clearly increased from early June, to the middle of July and then decreased until the end of our experiments in the beginning of September. Similarly, it was clear that DOC release rates were not constant over the growing season (ANOVA, $p<0.05$): as for temperature, rates reached a peak in late June-early July and declined to minimal values in Late August. Once the seasonality effect extracted using sampling day as a fixed factor, we found that temperature had a weak positive effect on DOC release rates (with corrected rates, per unit plant biomass) explaining 7% of the variation, whereas light did not (ANCOVA: sampling time * mean daily temperature* mean illumination; model R^2: 0.56, $p<0.05$). Expressed on areal basis, neither light nor

temperature had a significant effect on DOC release rates. Seasonality in corrected DOC release rates was less clear and tests showed no effect of combined sampling time, mean daily temperature and mean illumination (ANCOVA, $p>0.05$). The weak association between release rates and physical factors does not point to a strong coupling between photosynthesis and release rates.

Variation in release rates among macrophyte species

According to our hypothesis and since photosynthetic capacity differs among macrophyte species, mean DOC release rate should also differs among species. Given the temporal trend noted above, we tested a possible difference among species by using the residuals of the polynomial function. An ANOVA detected no difference in the temporally detrended mean DOC rates (with uncorrected or corrected rates) between species ($p>0.05$, $n=25$,). DOC release measurements grouped by genera instead of species also showed no difference in mean DOC release rates (ANOVA $p>0.05$).

As such, this lack of difference from results presented in Table 2, the overall mean rates of $4.57 mgC.m^{-2}.h^{-1}$ (SD ± 0.65) or 56 $\mu gC.gdw^{-1}.h^{-1}$ (SD ± 8) can be used as a general estimates of DOC release which may be useful for extrapolations to the lake scale.

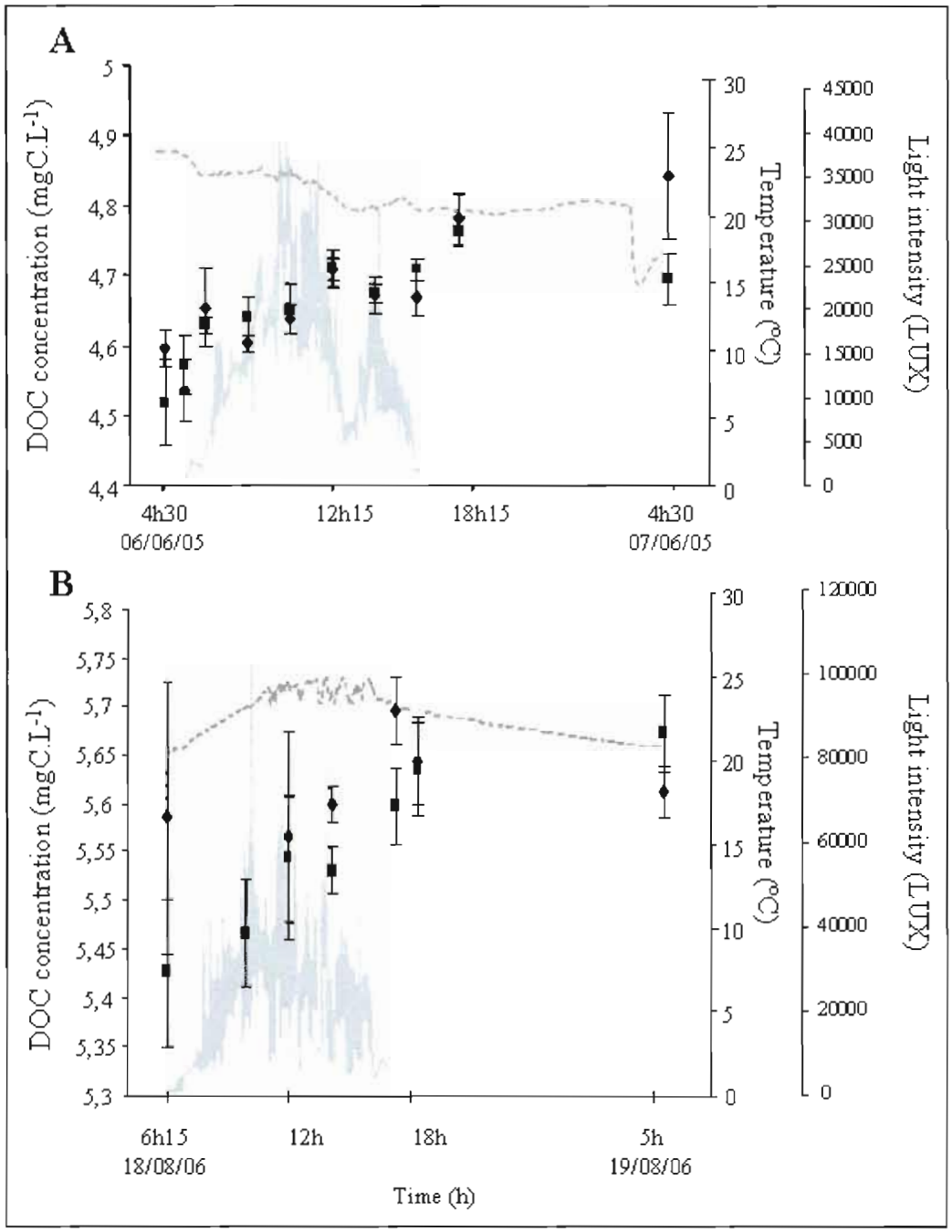

Figure 1.3 Trends in DOC concentration (left axis), light intensity and temperature (right axis) for benthic chambers containing macrophytes, during time courses experiment in L. Stukely, in 2005 (A) and 2006 (B). Bars represent standard deviation.

1.5 DISCUSSION

Relevance of correcting rates

Water column heterotrophic organisms can be efficient at controlling the pool of DOC compounds and therefore may obscure actual rates of DOC release by the macrophyte-epiphyte complex. The corrections increased/reduced the observed DOC released rates ranging from undetectable to 9.7mgC.m^{-2}.h^{-1} before corrections, to corrected rates varying from undetectable to 11.9mgC.m^{-2}.h^{-1} after corrections. Although the correction rate was 2.55 in average (i.e. corrected rates are on average 2.55 higher than uncorrected), it was highly variable (SD ± 12.67), suggesting that it was quite important in individual measurements.

These corrections make several assumptions regarding the metabolism of the heterotrophic community such as the similarity between day-time and night-time respiration, both in water column and sediments. It also assumes that the presence of macrophyte does not alter the rates of these background metabolic processes. Naturally, respiration is enhanced by warmer temperature in these compartments (see review in Pace and Prairie, 2005) and can therefore be higher during the day than at night. Thus, the amount of DOC consumed by the heterotrophic community during the day may be higher than we assumed, leading to conservative DOCme rates. However, we observed that the corrections did not lead to changes in relationships between release rate and light or temperature. As well, the global release rates proposed to upscale our result at the lake scale were not statistically different with or without correction. Finally, this exercise allowed us to underline the dominant role of macrophyte-epiphyte complex in DOC release, and the fact that variations in environmental factors do not induce a shift from DOC production by macrophyte-epiphyte complex to production by the rest of the community (planktonic and/or heterotrophic communities or sediments).

Patterns in DOC release by macrophyte-epiphyte complex

To our knowledge, this study is the first to provide *in situ* evidence of a substantial DOC release by living freshwater macrophyte (together with their epiphyte). DOC release has been reported for macrophyte and epiphyte separately in laboratory experiments (e.g. Penhale and Smith, 1977). However, because we were more interested in quantifying this release in natural conditions, we could not separate these two components and therefore attribute the release to the macrophyte-epiphyte complex. As previously shown in laboratory experiments for macrophytes (Nalewajko and Godmaire, 1993) and for marine phytoplankton (Marañón *et al.* 2004), DOC release occurred during the day-time only and is thus closely associated to the photosynthetic process. As the same patterns were observed for incubations beginning at sunset or at sunrise, it rules out the possibility that the release was due to the initial experimental stress associated with plant manipulation.

The DOC release rates we measured (from undetectable to 9.7 $mgC.m^{-2}.h^{-1}$ or from undetectable to 157 $\mu gC.gdw^{-1}.h^{-1}$, for macrophyte biomass varying between 6 to 336 $gdw.m^{-2}$) are lower to those of Barrón *et al.* (2003) for marine macrophyte communities. This is likely attributable to the much higher macrophyte biomass in their systems ($0.14gC.m^{-2}.d^{-1}$ to $0.6\ gC.m^{-2}.d^{-1}$ for macrophyte biomass varying between 242 to $4841gdw.m^{-2}$). Ziegler and Benner (1999) also found net daily DOC flux from seagrass higher than our DOC release rates. Qualitatively, these comparisons show that freshwater macrophyte communities act as their marine equivalent and that the release is likely not associated to osmotic stresses induced by the environment. However, to allow future comparisons of DOC release rates from various ecosystems and species, we clearly see the importance of expressing DOC release rate not only as a function of the colonized surface (per m^2, reflecting the phenomenon at the community scale), but also per unit of macrophyte biomass (per gdw, reflecting the role of the organism itself).

To examine the relative importance of macrophyte DOC release to other internal DOC sources to the ecosystem, we compared our rates with those obtained for phytoplankton DOC release. Because phytoplankton and macrophyte processes operate in different dimensional

setting, we integrated our observed areal rates over the water column above the macrophyte to yield commensurable volumetric rates. For marine phytoplankton, Marañón *et al.* (2005) obtained DOC production rates varying between 1 and $3mg.m^{-3}.d^{-1}$ in an oligotrophic environment. From rates obtained in our benthic chambers and the water column depth at the sampling site, we calculated volumetric DOC production rates by macrophytes ranging between 0.4 and $98mgC.m^{-3}.d^{-1}$ in the oligotrophic L. Stukely. Thus, living macrophytes can constitute a major internal source of organic carbon, even in oligotrophic systems. Therefore, we can hypothesize that the proportion of DOC contributed by macrophytes in relation to that released by phytoplankton should vary, along a trophic gradient, simply as a function of the predictable switch of major source of primary production, from the benthic to the pelagic zone (Vadeboncoeur *et al.*, 2003).

Light and temperature effects: do they confirm our hypothesis?

Because both light and temperature are known to affect photosynthetic rates, we expected that, if DOC release can be viewed as an overflow mechanism when an important element is limiting (such as nutrients), DOC release rates should increase with both light intensity and temperature. We conducted two follow-up types to test the effects of light and temperature on DOC release rates, one at short-time scale in one chosen lake (L. Stukely) with samples taken every 2.5 hours and the other at summertime scale in the six studied lakes, using rates calculated on a daily basis. Neither of them showed strong relationship between mean light received and concurrent DOC release rates. This leads to reject the link between DOC release and photosynthesis since, if it was the case, release rates would increase with increasing light. Moreover we observed that DOC release began with sunrise which means, according to the overflow mechanism hypothesis, that even at low light nutrients are limiting to biomass production. This observation therefore support the critic of this hypothesis since it would not be evolutionary advantaging for macrophytes to maintain high photosynthetic capacity in environment that cannot lead to maximum growth efficiency (the DOC release being a loss for macrophytes).

About temperature effect, even if the short time scale study concludes that there was no relationship between temperature and DOC release, the large scale study showed a general weak positive effect of temperature, stronger at lake scale particularly for L. Stukely. Two explanations can be proposed for this relationship: firstly and according to the hypothesis of an overflow mechanism, higher temperature could induce a higher DOC release because of a higher photosynthesis efficacy (light being constant, Madsen and Brix, 1997) leading to higher accumulation of photosynthates considering that middle could be nutrient limiting. This explanation is acceptable but we do not support it since our study of light effect just rejected the hypothesis of an overflow mechanism. The second explanation is suggested by the work of Madsen and Brix (1997), who observed that in inorganic carbon limiting conditions *E. canadensis* and *R. aquatilis* increased their carboxylation efficiency when temperature increased (higher Q_{10} than waited if CO_2 diffusion rate was the only implicated factor). And to increase this carboxylation efficiency enzymatic, morphologic or anatomic changes could be implied. Therefore we can suggest that DOC release could be partly implied in increasing carboxylation efficiency of macrophytes in response to increased temperature in inorganic carbon limiting conditions, as would do an increase in extra-cellular carbonic anhydrase. This hypothesis therefore implies that in non-limiting inorganic carbon condition, DOC release rates would be lower. This proposition has to be tested as well as the link between nutrients availability and DOC release to surely reject the hypothesis viewing the DOC release as an overflow mechanism.

Implication to the whole ecosystem

To our knowledge, our study is the first one comparing *in situ* DOC release rates, and we found no difference between species or among lakes. Perhaps such differences exist but are too weak to be measurable with our technique. However, until these estimates can be further refined, we suggest that the use of our overall average DOC release rate ($4.57 mgC.m^{-2}.h^{-1}$ (SD ± 0.65) or 56 $\mu gC.gdw^{-1}.h^{-1}$ (SD ± 8)) constitute the best approximation to estimate the ecosystem implication of submerged macrophyte in lake DOC dynamics. Estimation of the potential contribution of macrophytes to the DOC budget of the whole ecosystem can be

calculated as the simple product of the average release rate with average macrophyte biomass in the colonisable zone of lakes (fifth chapter).

In conclusion, we demonstrated the occurrence of day time DOC release by macrophyte-epiphyte complex in South-eastern Quebec lakes. We reject the hypothesis presenting DOC release as an overflow mechanism for recent photosynthates produced in limited environments since we demonstrated no link between physical factors influencing photosynthesis and DOC release rates. Finally, since we observed no difference in averaged DOC release rates between the 3 main colonizing macrophytes of our lakes, we proposed a mean DOC release rate useful for quantitative extrapolation of the process at the whole lake scale.

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CHAPITRE II

NUTRIENT AND CO_2 EFFECTS ON DOC RELEASE RATES BY SUBMERGED MACROPHYTES-EPIPHYTES COMPLEX IN SOUTHERN QUEBEC LAKES.

Context: In the first chapter we studied the effect of physical factors such as photoperiod, light intensity and temperature on DOC release, because these are known to influence photosynthesis and so possibly DOC release (« Overflow mechanism »). Since photosynthesis rates are also influenced by chemical factors, in this second chapter we decided to study the effect of nutrient concentrations and CO_2 availability on DOC release.

2.1 ABSTRACT (RÉSUMÉ)

We studied the effect of nutrient and CO_2 availability on DOC release rates by macrophyte-epiphyte complex in five eastern Quebec lakes. Using measurements of DOC release rates by *Myriophyllum spicatum* and *Potamogeton spp* previously described (first chapter), we showed that nutrient availability (corresponding to lakes tropic statuses, exchangeable phosphorus, dissolved phosphorus and nitrogen in sediments) had no influence on DOC release rates. We also found that, despite some of the studied macrophyte communities presented a deficiency in nutrient content, no relationship were observed between nutrient content and DOC release rates, this result supporting the reject of the hypothesis presenting the DOC release as an overflow mechanism in nutrient limiting condition. In another hand, we demonstrated that highest DOC release rates occurred in lowest CO_2 concentrations, leading to a new hypothesis presenting the DOC release as a response to limiting CO_2 conditions.

Keywords: Macrophytes, DOC release, nutrients, CO_2.

Nous avons étudié l'effet de la disponibilité en nutriments et en CO2 sur les taux de production de COD par les macrophytes en croissance dans cinq lacs du sud-est du Québec. La disponoibilité en nutriments, exprimée en terme de statut trophique, de phosphore échangeable dans les sédiments, de phosphore et d'azote dissous dans les sédiments, n'a pas d'influence sur les taux de production de COD. Le contenu en nutriments des communautés étudiées n'influençait pas les taux de production de COD, ceci même pour les communautés carencées. Ces observations nous permettent de réfuter l'hypothèse décrivant le processus de production de COD par les macrophytes comme un système d'évacuation des surplus de photosynthétats en condition de nutriments limitants. Par conte nous montrons que la production de COD apparaît lorsque les concentrations en CO2 sont faibles, d'où l'hypothèse alternative présentant cette production de COD comme une réponse à des carences en CO2.

2.2 INTRODUCTION

Aquatic angiosperms are derived from terrestrial ancestors and have conserved many morphological characteristics of these organisms. Nevertheless, a number of physiological changes have occurred during their evolution to adapt to the particular aquatic environment (Duarte *et al.* 1994; Wetzel, 2001; Rascio, 2002). For example, water being no longer limiting, protective structures against desiccation, such as a waxy cuticle or stomata, disappeared in submerged angiosperms (Maberly and Madsen, 2002). In contrast, other resources became more limiting in the aquatic environment such as the CO_2 necessary for photosynthesis (Ludlow and Wilson, 1971). Interestingly, aquatic angiosperms and more specifically submerged macrophytes, developed carbon concentrating mechanism that strongly resemble those present in other aquatic photosynthetic organisms, such as micro and macro algae and cyanobacteria (Badger, 2003). Another shared attribute among aquatic photosynthetic organisms has been their propensity to release extracellular dissolved organic matter observed in marine phytoplankton (Fogg *et al.*1965; Marañón *et al.* 2004), macroalgae (Khailov et Burlakova (1969), and both marine and freshwater submerged macrophytes (Barrón *et al.* 2003; Wetzel et Manny, 1972; Godmaire et Nalewajko, 1989; first chapter).

Yet, factors influencing DOC release by submerged macrophytes are poorly understood. For example, while some laboratory experiments showed a link between DOC release and photoperiod (Sieburth, 1969; Sondergaard, 1981; Pregnall, 1983; Nalewajko and Godmaire, 1993), others did not (Hough and Wetzel, 1975). The same inconsistency was observed in *in situ* experiments: no clear relationship between light intensity and DOC release was observed in the marine macrophyte *Fucus serratus* (Barrón *et al.* 2003) However, in six Quebec lakes (Canada), DOC release by four submerged macrophytes species (and their epiphytes, henceforth called macrophyte-epiphyte complex) occurred only during the day (first chapter). The possible link between DOC release and photoperiod brought some authors to hypothesize that the release would be linked to photosynthesis as an overflow mechanism of excess photosynthetates for cells limited by nutrients (Fogg 1983; Jensen 1984). An alternative

interpretation is that DOC release is simply a passive diffusion of small compounds occurring in all photosynthetic cells whatever the conditions (Bjornsen 1988; Marañón *et al.* 2004).

In a previous study on *Myriophyllum. spicatum* and three species of *Potamogeton* in Quebec lakes, we observed that the release occurred only during the photoperiod, but no link were found between either the amount of light or the mean temperature received by the macrophyte-epiphyte complex and DOC release rates suggesting that DOC release is independent from photosynthesis which question the validity of the overflow mechanism hypothesis (first chapter). In this study, we examine further this hypothesis in evaluating the implication of nutrients and pCO_2, factors implied in photosynthesis and subsequent biomass production. Accordingly, we should observe a DOC release rate in proportion to the degree of limitation by nutrients. Since macrophytes can use nutrients from both the water column and the sediments, we examined the possible dependence of DOC release rate on several measures of nutrient availability.

As a corollary, we also tested the effect of pCO_2 on DOC release rate. It is well known that most aquatic photosynthetic organisms are CO_2 limited. We hypothesized that with an increase in water pCO_2 in our experimental systems, macrophyte-epiphyte photosynthesis rate should increase (Kirk, 1994), and if nutrients conditions remain unchanged, we should therefore observe an increase in DOC release rates.

2.3 MATERIAL AND METHODS

Study site and experimental design

The measurements were undertaken during the summers of 2004, 2005 and 2006 in five lakes of the Eastern township region of Quebec, ~ 100km east of Montréal (Table 1.1). These lakes of different trophic status are from a glacial origin and are influenced by the alluvial sedimentary geology of Saint Lawrence River. We studied the DOC release by macrophyte-epiphyte complex using *in situ* benthic chambers consisting of PVC cylinders inserted in the sediments covered by a transparent plastic bag (clear or dark) equipped with a sampling port (first chapter). Briefly, incubations with transparent benthic chambers were made during 24h on *Myiophyllum spicatum*, *Potamogeton amplifolius* and *P. richardsonii*. Transparent benthic chambers on unvegetated sediments (TS) were used as control. Water samples from chambers were taken in duplicates for DOC at the beginning (T0), then just before sunset (or just after sunrise; T1) and finally just after the following sunrise (or just before sunset; T2). Samples were drawn through the sampling ports with 60mL (polyethylene) acid-washed (HCl 10%) syringes, filtered on 0.45µm (filtropur Starsted®) and kept refrigerated (4°C) in 40 mL acid-washed tubes with silicone-Teflon caps to prevent gas exchange during analysis. DOC analyses were made with a 1010 TIC TOC autoanalyser, O.I. Analytical. At the end of the 24h incubation, the water volume within each benthic chamber was estimated by injecting a 10mL weak fluorescein solution (absorption peak at 552nm) and drawing two samples after mixing for 5 min. Calculated volumes ranged between 4 and 11L. At the end of each experiment, macrophyte shoots inside the PVC ring were harvested to measure their aboveground biomass after rinsing and drying at 55°C during 24h to 36h.

DOC release rates by macrophyte-epiphyte complex.

Diurnal and nocturnal DOC variations were calculated as the simple difference in DOC concentrations between sunrise and sunset (photoperiod) and sunset and sunrise respectively, in both TM and TS chambers. DOC changes within each chamber were expressed both per unit area ($mgC.m^{-2}.h^{-1}$) and per unit plant biomass ($mgC.g.dry\ weight^{-1}h^{-1}$). Basic calculations of DOC production or consumption from DOC concentration changes in chambers containing

both water and sediments provide estimates of the net effect of the presence of macrophytes on DOC release patterns (termed net bulk DOC release). Indeed, daily and nightly DOC variations measured in transparent chambers containing macrophytes (TM) are the sum of several processes occurring simultaneously. To estimate the net DOC release of the macrophytes and their epiphytes only (termed DOCme), corrections were made to avoid the potential contributions of phytoplankton, heterotrophic community and the sediments (see first chapter). Finally the release rates are calculated from daily DOC rates minus nightly DOC rates. Relationships between DOCme rates and other variables were examined using release rates expressed on an areal basis ($mgC.m^{-2}.h^{-1}$) and per unit biomass ($mgC.gdw^{-1}.h^{-1}$). In an earlier study (see first chapter), we showed that conclusions about effect of light and temperature on DOC release were the same using uncorrected or corrected rates, thus demonstrating the predominant role of macrophyte-epiphyte complex in the release. Here we also test both uncorrected and corrected rates to better understand the implication of macrophyte-epiphyte complex in this process.

Lakes trophic status and sediment nutrient content

Lake trophic status were determined for the six studied lakes (Table 1.1) using both total phosphorus concentration and chlorophyll *a* in epilimnion of each lake (Prairie and Parkes, 2006).

In 2005, three sediments cores were taken randomly inside the studied macrophyte bed for L. D'Argent, L. Stukely, L. Trois Lacs and L. Waterloo. Surface (0cm), 5cm, 10cm, 15cm and 20 cm depth sediments were collected from each core. Two samples (0.5g) were taken for each depth and exchangeable phosphorus were extracted with 1M $MgCl_2$ (first step of the P fractionation with Sedex method (Ruttenberg, 1992; Kassila *et al.*, 2000). For each extraction, two analytical replicates were conduced to measure phosphorus by ascorbic acid reduction method (Murphy and Riley, 1962). The significant difference in phosphorus concentrations between sampling depth and between cores were analysed by ANOVA.

In 2006, three peepers (membrane porewater samplers, passive diffusions) were randomly inserted in sediments of the studied macrophyte bed for L. D'Argent, L. Stukely, L.

Peasley and L. Trois Lacs to analyze sediment porewater. These Plexiglas peepers presented twenty rows of 2 cells each, one every centimeter, filled with distilled water and covered with biologically inert membranes (Supor®-200 PALL). For each sampler, one row of sampling cell was used for dissolved phosphorus (DP) analysis and the second one was used for dissolved nitrogen (DN) analysis. Dyalizers were placed in sediments during 14 days at least to allow equilibration of nitrogen and phosphorus concentrations (Carignan, 1985). Four milliliters of water were sampled from cells corresponding to sediment surface and then every 2cm in depth. Two analytical replicates were conduced for both dissolved phosphorus and nitrogen analysis in diluting 2mL of samples with distilled water (40mL final). DP concentration was measured by ascorbic acid reduction method (Murphy and Riley, 1962); DN analyses were carried out on an ALPKEM RFA 300 auto analyzer. Differences in concentration with depth were studied with one way ANOVA.

Phosphorus and nitrogen in macrophytes

In 2006, macrophytes from transparent chambers experiment (TM) in L. D'Argent, L. Peasley, L. Stukely and L. Trois Lacs were kept after dry weight measurements to measure their total phosphorus and nitrogen content. Dried macrophytes (at 55°C, during 24h to 36h) of each TM were cut; then, three samples of each macrophyte community were reduced in powder and a subsample (around 0.05g) was used to determined total phosphorus concentrations. This was done either in triplicates directly by persulfate oxidation and ascorbic acid reduction method (Murphy and Riley, 1962), or in duplicates by ignition and hot acid digestion followed by phosphorus concentration determination in duplicates by persulfate oxidation and ascorbic acid reduction method to ensure orthophosphate measurement (Andersen, 1976). In parallels, subsamples (around 2mg of macrophyte powder) were used to measure nitrogen and carbon content in triplicates (Carlo Erba EA 1108 Elemental Analyser).

pCO_2 modification and measurement inside the benthic chambers

To study the effects of pCO_2 on DOC release, we took two separated approaches. First, we measured the ambient pCO_2 concurrently with our DOC sampling during the incubations

(at sunrises and sunsets). To measure the pCO_2 inside the benthic chambers, 30mL of water taken with a syringe from the chamber were mixed with 30mL of ambient air, during one minute. The pCO_2 of ambient air and the pCO_2 of air in the syringe after mixing (noted pCO_2eq) were measured with CO2 infrared gas analyzer EGM-4 ® (PP-system). The pCO_2 of air after mixing represented the equilibrium state but was not the real pCO_2 of the sampled water; the following equation was used to calculate it:

$$pCO_2\text{water} = \Delta\, pCO_2 / \text{Vmol} + pCO_2\text{eq}^* K_H\text{eq}/K_H\text{w} \qquad \text{(eq.2.1)}$$

where, $\Delta\, pCO_2$ is the difference between pCO_2 of ambient air and pCO_2 of air after mixing; Vmol is the molal volume; $K_H$eq and K_Hw are Henry's constants for water after and before mixing respectively.

Second, submerged macrophytes incubations with transparent benthic chambers were conducted not only in natural condition (TM) but also in CO_2 enriched water (TM+ CO_2). At the beginning of the 24h incubations (sunrise, T0), after pCO_2 measurement of water inside the chambers (original pCO_2), around 120mL of CO_2 -enriched distilled water (obtained by melting 250g of dry ice in 2L of distilled water, pCO_2around 60 000ppm) were injected in 4 to 9 transparent benthic chambers containing macrophytes in L. D'Argent, L. Peasley, L. Stukely and L. Trois Lacs. After 10minutes, the homogenized resulting pCO_2 were between 5 to 20 times the original pCO_2; these last measurements with those of TM chambers were used to study pCO_2 between ambient pCO_2 in the water and DOC release rates by macrophyte-epiphyte complex. In preliminary laboratory experiments, we found no significant gas diffusion across these plastic bags.

2.4 RESULTS

All DOC release rates used in the present chapter were already described in our first chapter and are again used to test the hypothesis presenting the DOC release as an overflow mechanism but with different factors. About the release rates, we underline that we previously observed an increase in DOC concentration during the day but not during night in all chambers containing macrophytes for all studied lakes. Nightly variations in DOC were mostly negative, thus representing DOC consumption. From there, we calculated rates of DOC release by macrophyte-epiphyte complex per unit area or per unit biomass.

Lakes trophic statuses and DOC release rates

Because the five studied lakes spanned from oligotrophic L. Stukely to eutrophic L. Waterloo (Table 1.1), we were able to assess whether the rate of DOC release was related to the availability of nutrients in the water column. Averaged DOC release rates obtained showed significant differences among lakes either on an areal or per unit biomass basis (fig.2.1; one way ANOVA $p<0.0001$). However, mean DOC release rates were not related to the trophic status of lakes since ultra-oligotrophic L. Bowker and eutrophic L. Waterloo did not have significantly different mean DOC release rates. So as we were suspecting, nutrients in water column did not influence the release. The same conclusion was obtained using corrected DOC release rates.

Nutrients in sediments and DOC release rates

Coring made in 2005 in L. D'Argent, L. Stukely, L. Trois Lacs and L. Waterloo allowed us to measure exchangeable phosphorus in sediments of macrophyte beds where DOC release measurements were made. For each lake, the three cores were statistically indistinguishable (one way ANOVA, $p>0.05$), we therefore averaged phosphorus concentrations measured for each sampling depth: surface concentrations were higher than deeper concentrations for L. d'Argent and L. Trois Lacs, whereas the reverse was true for L. Stukely and L. Waterloo (fig.2.2). We also observed a strong difference between concentrations measured in L. Trois Lacs and the three other lakes. In this particular lake, sediments contained on average10

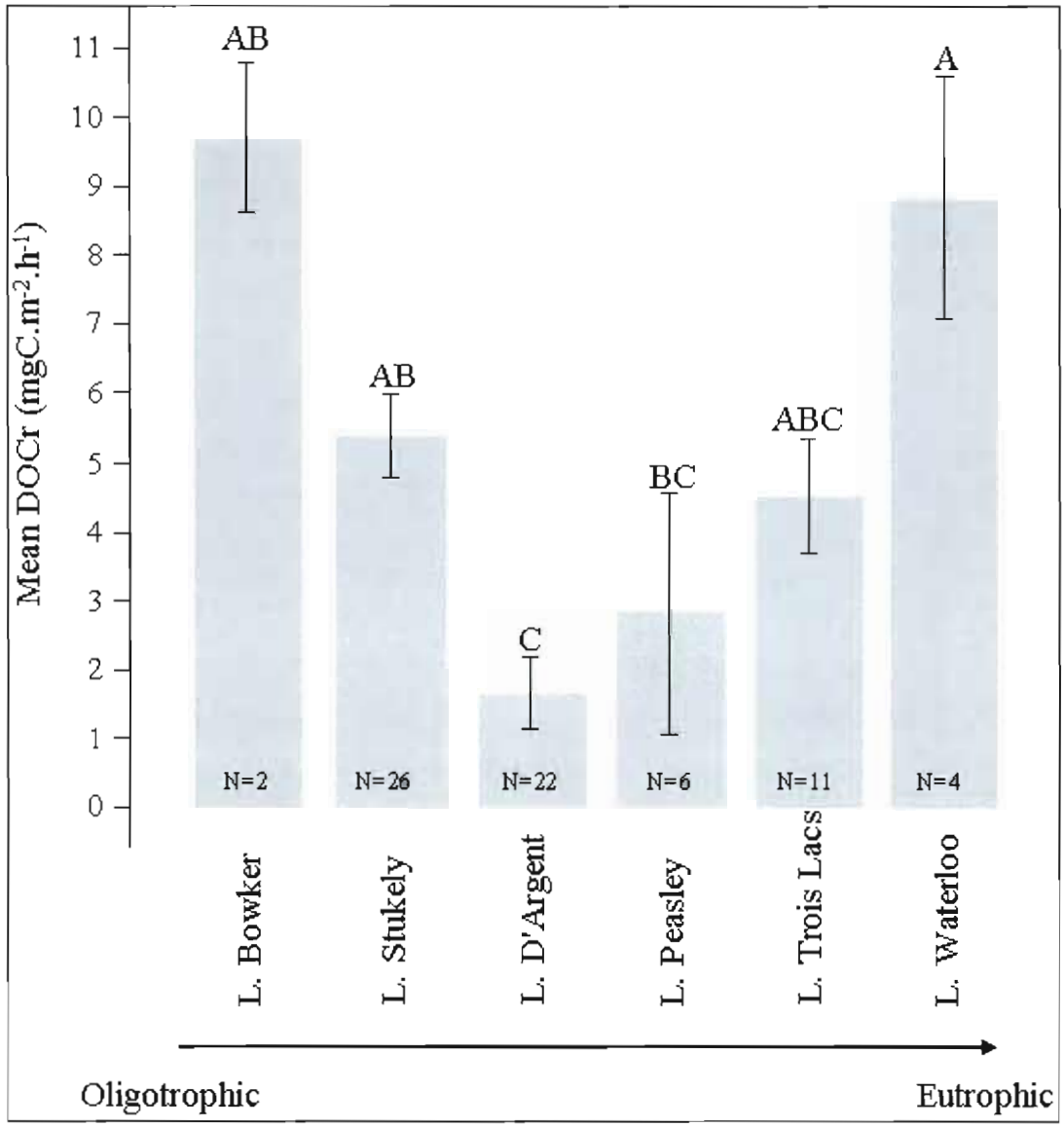

Figure 2.1 Averaged uncorrected DOC release rates by macrophyte-epiphyte complex by lakes along the trophic status gradient. Bars represent standard error and letters correspond to Tukey-Kramer HSD groups.

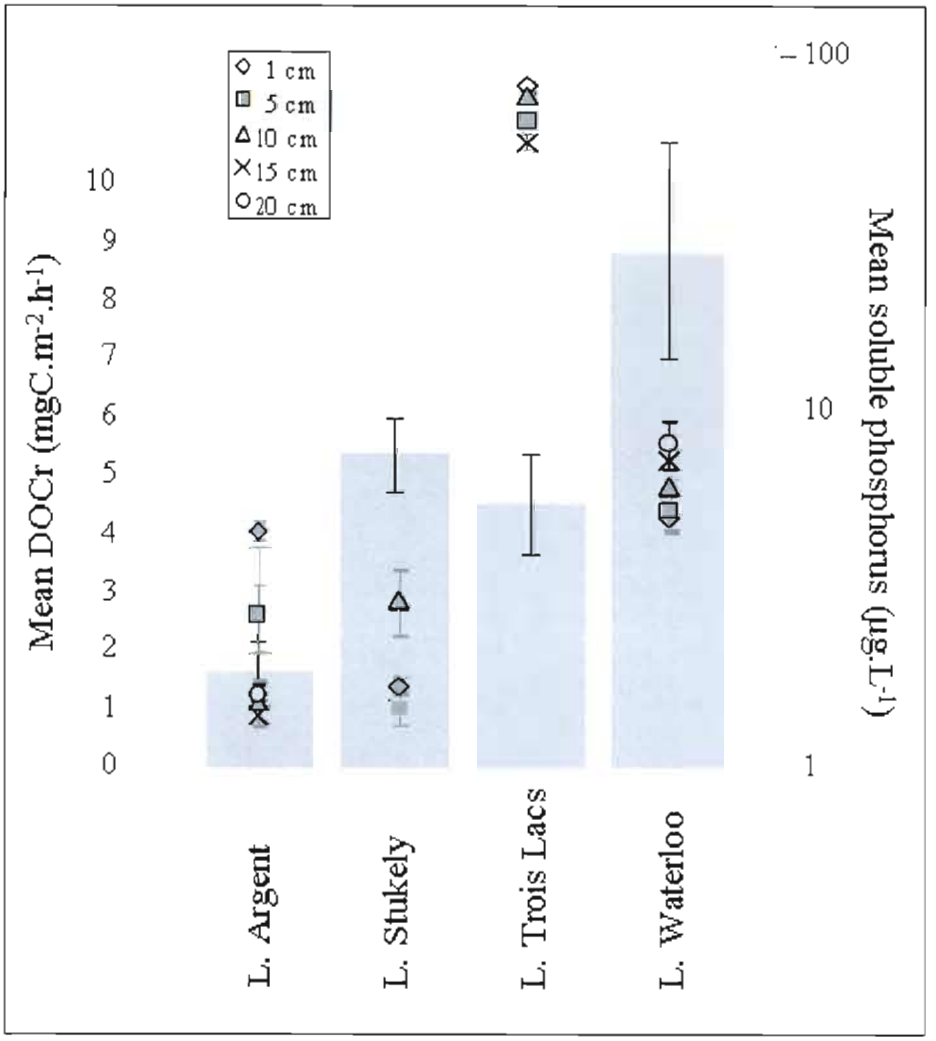

Figure 2.2 Mean DOC release rates (left axis, histogram) and mean soluble phosphorus measured from sediment cores (right axis, ♦, ■, ▲, X, and ● representing the different sampling depth) for four studied lakes. Bars represent standard error.

times more exchangeable phosphorus than the other lakes. To detect a possible link between these exchangeable phosphorus concentrations obtained in 2005 and the DOC release rates, we averaged DOC release rates obtained for all TM chambers (experiments of 2004, 2005 and 2006) for each lake . We clearly observed no qualitative link between these two variables (fig.2.2): the very high phosphorus concentration in L. Trois Lacs did not correspond to the higher DOC release rate; at the opposite, in L. Stukely, we measured about the same exchangeable phosphorus concentration than in L. D'Argent but a DOC release rates two times higher. In short we found no evidence of a link between DOC release rates by macrophyte-epiphyte complex and exchangeable phosphorus in the sediments. Thus this first experiment tends to reject the hypothesis of an overflow mechanism.

In parallel, dyalizers' experiments showed that there were not even clearer link between DP concentrations in sediments and DOC release rates. For L. D'Argent, L. Peasley and L. Stukely we averaged the DP concentrations of the three dyalizers by sampling depth. There were no statistical differences between DP concentrations at different depth (except in surface for L. Stukely). Then, we averaged all the values to obtain a mean DP concentration per lake (147 $\mu g.L^{-1}$, 109 $\mu g.L^{-1}$and 136 $\mu g.L^{-1}$ for L. D'Argent, L. Peasley and L. Stukely respectively). In L. Trois Lacs two of the three dyalizers were lost after strong increase of the water level; result (437 $\mu g.L^{-1}$) is therefore the average of analytical duplicates made at each sampling depth for only one dyalizers. Figure 3 shows that the highest DP concentration is found in L. Trois Lacs where we observed the highest DOC release rate. But the lowest DP concentration observed in L. Peasley did not correspond to the lowest DOC release rates observed in L. D'Argent. The same discordances were observed between DN concentrations and DOC release rates (fig.2.3), and the same conclusions were obtained with corrected release rates. So, in our experiments there were no evidences of the influence of nutrients on DOC release rates.

Effect of phosphorus and nitrogen content in macrophytes on DOC release

Direct measurement by ascorbic acid reduction gave values for macrophyte communities per benthic chamber varying between 0.52$\mu gP.mg(plant)^{-1}$ (SD ± 0.01) in L.

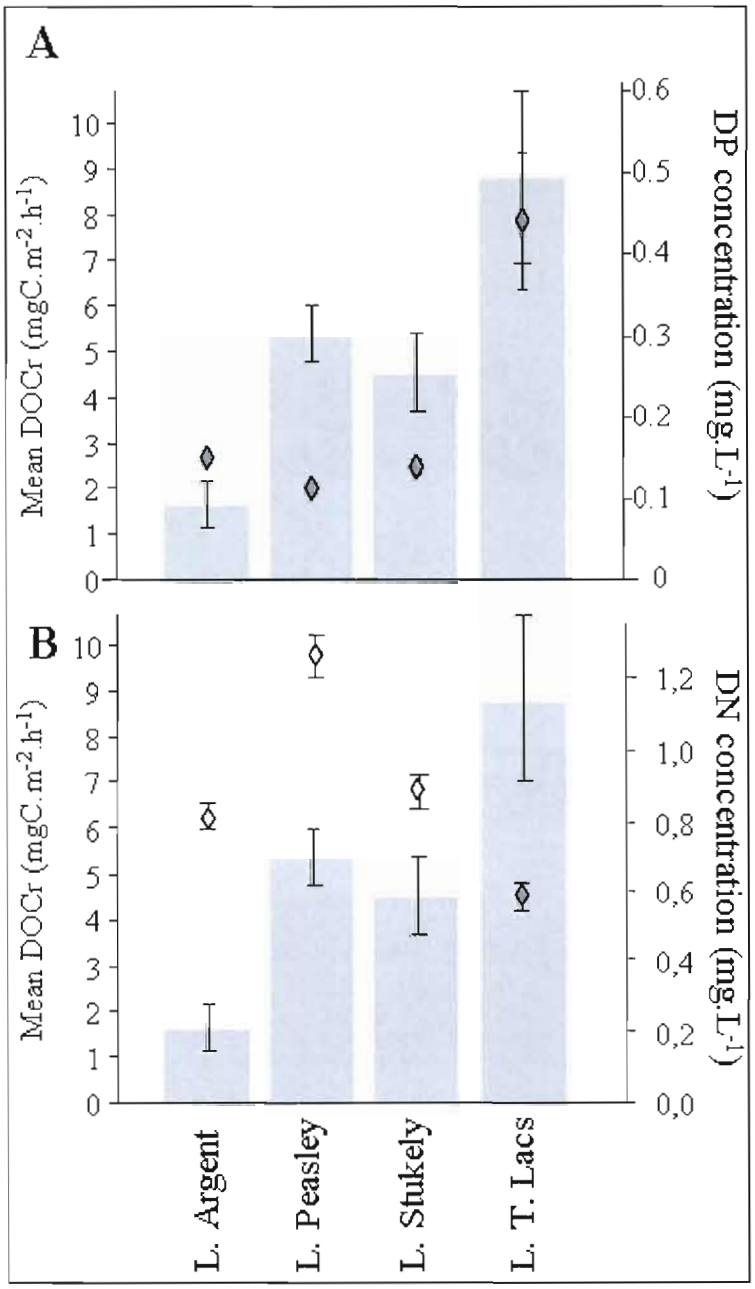

Figure 2.3 Mean DOC release rates (left axis and histogram) in comparison with dissolved phosphorus (A, right axis and ♦) and dissolved nitrogen (B, right axis and ♦) of the sediments interstitial water for four studied lakes. Bars represent standard error.

Peasley and 1.88 µgP.mg(plant)$^{-1}$ (SD ± 0.07) in L. D'Argent. Analytical error estimates from the triplicates was 11% in average (SD ± 10%). With the ignition and hot acid method, the lowest value measured was for the same community in L. Peasley but the value was higher with 0.72 µgP.mg(plant)$^{-1}$ (SD±0.01); the highest value was 3.2 µgP.mg(plant)$^{-1}$ (SD±0.02) for L. Trois Lacs. Analytical error estimates from the four replicates of each community was 15% in average (SD ± 21%). Globally values from the ignition and hot acid method were statistically higher (Student test, fig. 2.3) and were therefore used to calculate nutrients ratios. We observed that the mean phosphorus contents were statistically different among lakes (ANOVA, $p<0.05$) and representative but not linked to the DP concentration in sediments (fig.2.4).

About nitrogen content, we found C:N ratio varying between 10.67 and 24.64 with a mean value of 17.44. When averaged by lakes, the 4 values obtained were strongly related to the corresponding concentrations of dissolved nitrogen in sediments (results not shown; R^2= 0.97; $p<0.05$). Considering all our macrophyte communities, we found a good relationship between phosphorus and nitrogen content (fig.2.5; regression, R^2= 0.55; $p<0.0001$). The mean N:P ratio was 16.74 (SD ± 4.53).

In our context of studying variables implied in DOC release, we found no global relationship between either phosphorus or nitrogen content (in % of dry weight) or C:N or N:P ratios and DOC release rates either uncorrected or corrected (regressions, $p>0.05$). We neither found relationship when results were examined by species or lake. Thus, in general we found no effect of nutrients on DOC release by macrophyte-epiphyte complex.

Influence of water pCO_2 on DOC release rates

We first examined the pCO_2 influence on DOC release rates by macrophyte-epiphyte complex in natural conditions (transparent chambers simply put on macrophytes, TM). We found that pCO_2, varying between 25 and 152 µM (equivalent to 650 and 3950 ppm), was inversely related to DOC release rates (fig.2.6A), expressed either in mgC.m^{-2}.h^{-1} (regression, R^2 = 45%, $p<0.0001$, n = 40) or in µgC.gdw^{-1}.h^{-1} (regression, R^2 = 32% $p<0.001$, n = 40). These negative relationships were also observed at the scale of individual lakes. In both L.

D'Argent and L. Trois-Lacs, pCO_2 significantly explained about half of the variation in DOC release rates, either expressed per unit area (R^2 = 47% and 66%; $p<0.05$; n = 12 and n = 7 for L. D'Argent and L. Trois Lacs respectively) or per unit biomass. (R^2 = 52% and 53%; $p<0.05$; n = 12 and n = 7 for L. D'Argent and L. Trois Lacs respectively).

Our CO_2 enrichment experiments confirmed our results. We first verified that we had higher pCO_2 in enrichment benthic chambers (one way ANOVA, $p<0.05$, in general and per lake, fig.2.7A): general analysis showed us a higher mean DOC release rate in TM than in TM+ CO_2 chambers (4.5 $mgC.m^{-2}.h^{-1}$ SE±0.6 and 1.3 $mgC.m^{-2}.h^{-1}$ SE±0.6 respectively). At the scale of individual lakes, we observed a lower DOC release rate in TM + CO_2 chambers of L. Stukely. Moreover, in L. D'Argent and L. Trois Lacs we observed no more release but DOC consumption (fig.2.7B). Thus, in high pCO_2 condition the DOC release is inhibited therefore leading to the observation of the heterotrophic DOC consumption usually hidden by the release.

Globally found that pCO_2, varying between 25 and 770 µM (equivalent to 650 and 20060 ppm) negatively influenced DOC release rates (regression, R^2 = 18%, $p<0.0001$, n = 83, fig.2.6B). The relationship observed in natural (without CO_2 enrichment) conditions in L. D'Argent was confirmed (R^2 = 35%, $p<0.01$, n = 20, fig.2.6B). Including the results of the enrichment experiences, the link between pCO_2 and DOC release became statistically significant in L. Stukely (regression, R^2 = 22%, $p<0.01$, n = 44, fig.2.6B), mostly because of the 2005 data which present a good relationship (R^2 = 52%, $p<0.01$, n = 17). Once again relationships were the same using corrected DOC release rates.

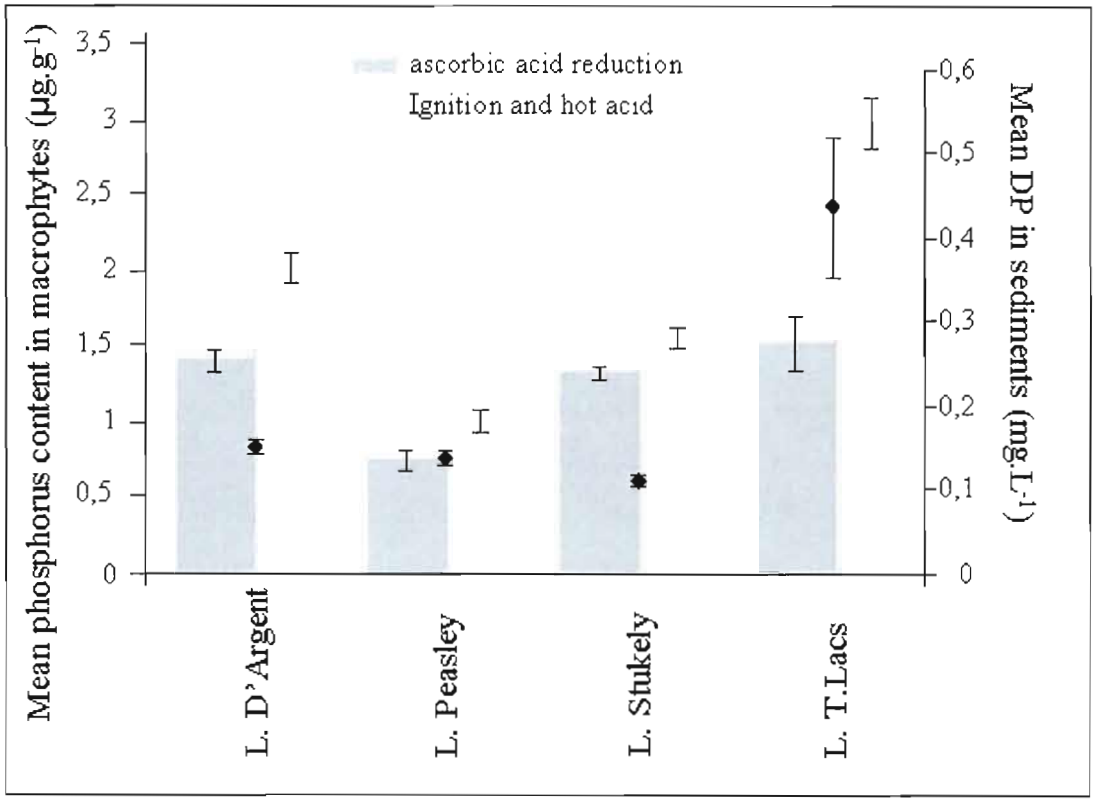

Figure 2.4 Mean phosphorus content in macrophytes measured either by ascorbic acid reduction or by ignition and hot acid method (left axis, histograms) in comparison with mean dissolved phosphorus in sediments (right axis, ♦) for four studied lakes. Bars represent standard error.

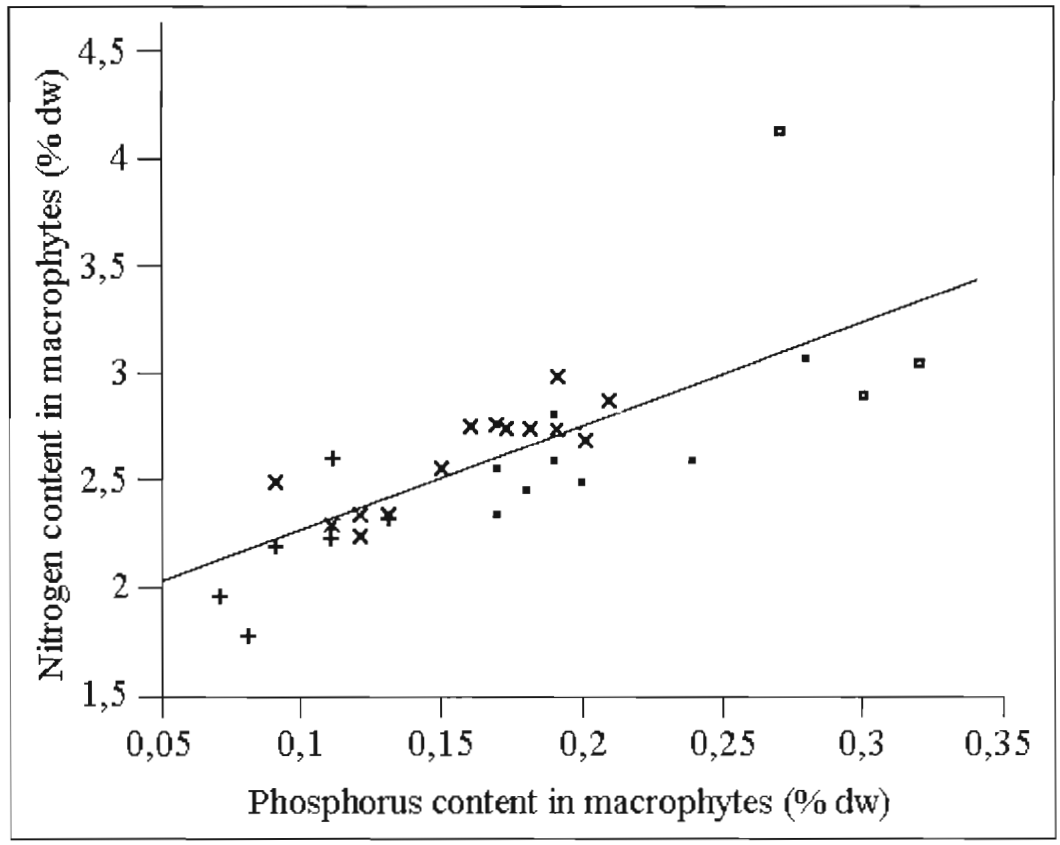

Figure 2.5 Relationship between nitrogen and phosphorus content in macrophytes. ■, +, X, and □ representing samples of L. d'Argent, L. Peasley, L. Stukely, and L. Trois Lacs respectively.

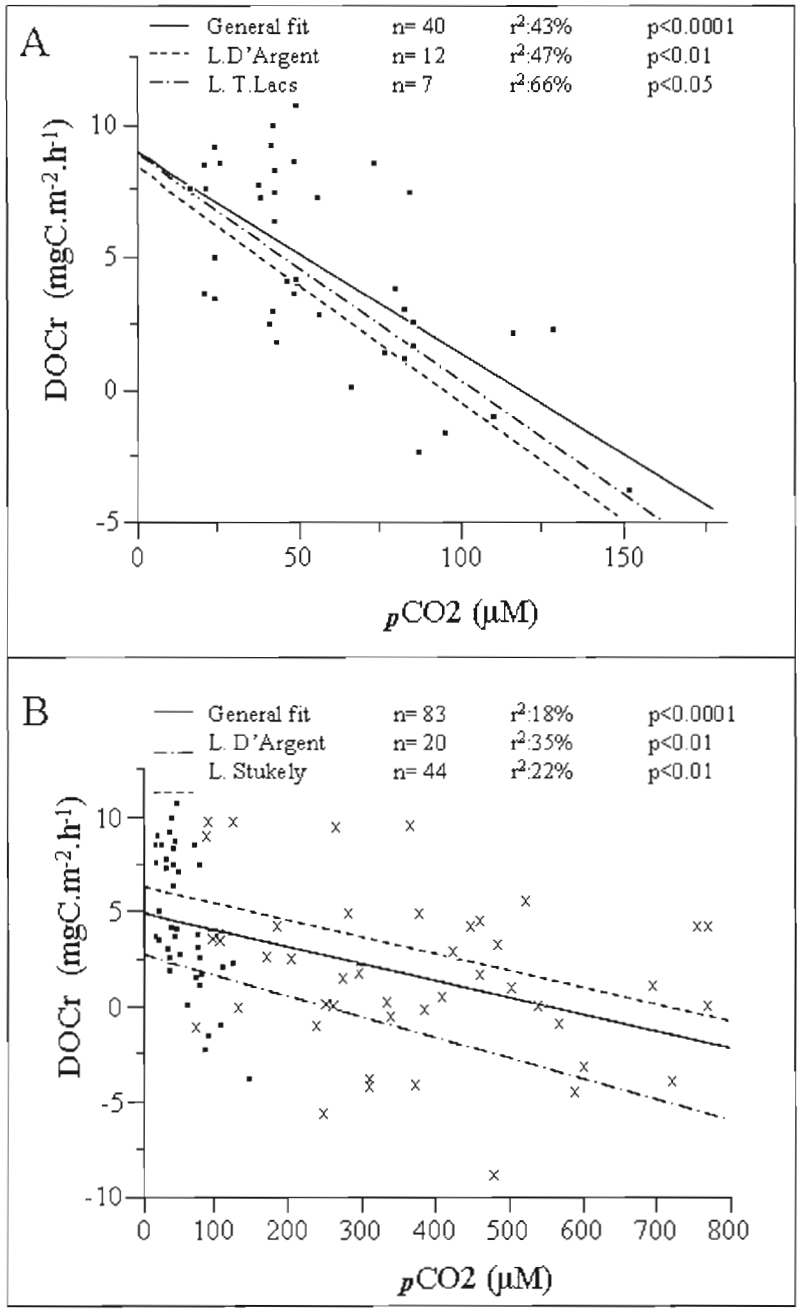

Figure 2.6 Relationships between DOC release rates (left axis) and pCO_2 measured at the beginning of the experiment (right axis) with either data from transparent benthic chambers in natural pCO_2 condition only (TM, fig.6 A), or with data from TM chambers (■) and TM chambers enriched in CO_2 (TM+CO_2, X , fig.6 B).

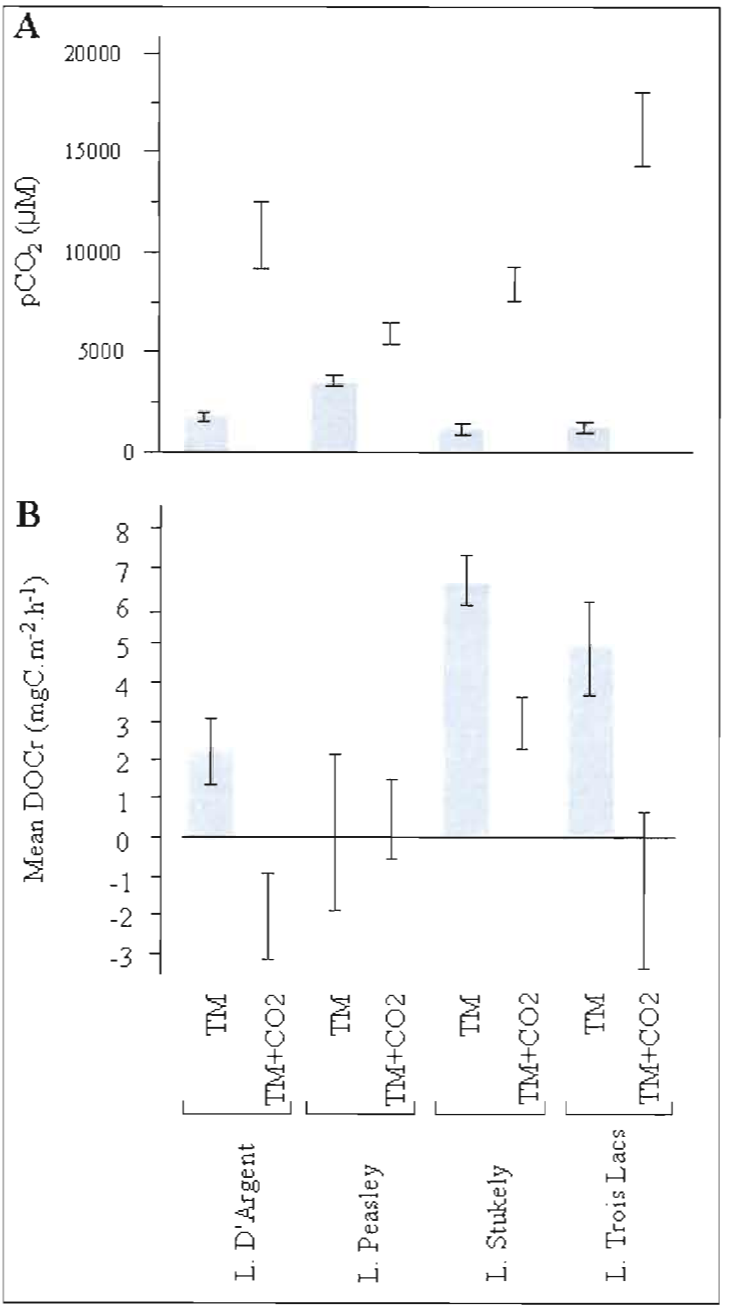

Figure 2.7 Mean pCO_2 (left axis fig.7A) and mean DOC release rates (left axis fig.7B) for transparent benthic chambers in natural pCO_2 condition only (TM) and TM chambers enriched in CO_2 (TM+CO_2) for four studied lakes. Bars represent standard error

2.5 DISCUSSION

Independence of DOC release rates in relation to nutrients in sediments

To understand what drives DOC release, it was necessary to test factors that affect the plant physiology and more precisely factors implied in photosynthate production and plant growth. Among these factors are nutrients. Several authors have proposed that the release of DOC could be due to imbalance between carbon assimilation by photosynthesis and available nutrients necessary to produce complex molecules. According to Jensen (1984), the fewer nutrients available, the higher the DOC release. Yet, marine studies showed that there was no link between trophic status and DOC release by microalgae (Marañón *et al.*, 2005), and we also observed no pattern between DOC release rates in trophic status in the lake we studied. However, this last result was not surprising since macrophytes are known to acquire phosphorus and nitrogen mostly from the sediments (Carignan, 1982 and 1985; Nichols and Keeney, 1976).

In order to go further in the study of the effect of nutrient on DOC release by macrophyte it was then necessary to measure nutrients in sediments and concurrent DOC release rates. In this study, we showed that no clear link exist between exchangeable porewater phosphorus, dissolved phosphorus or nitrogen in sediments and the concurrent DOC release rates by macrophyte-epiphyte complex. These finding corroborate our results on independence of DOC release rates by macrophyte-epiphyte complex rapport to trophic status and therefore imply that other factor induce this process. However, to confirm this, more systematic measurements would be necessary. In fact we analyzed the sediment extractable phosphorus and nitrogen and porewater phosphorus of only four lakes, which is no enough to fairly affirm that there is no relationship between DOC release and nutrients at larger scale, since we do not have enough data to make reliable statistics.

Did nutrient content of macrophytes reflect a limiting environment influencing DOC release?

From our measurements of total phosphorus in macrophytes, we only considered results obtained by the ignition and hot acid method since this method is more akin to detect

orthophosphate than simple ascorbic acid reduction method. In 1992 chapter, Duarte reviewed the carbon, nitrogen and phosphorus content of aquatic plants and our results strongly reflect the majority of the observation. 11 macrophyte communities on 32 had phosphorus content below or equal to 0.13% dry weight, which is the critical level for maximum growth for angiosperms (Gerloff and Krombholz 1966). These communities presented C:N:P ratios distant from the Atkinson ratio of 550:30:1 (Atkinson and Smith 1984), reflecting a depletion in phosphorus and nitrogen relative to carbon. Yet, we observed that nitrogen and phosphorus contents were linked and that the mean N:P ratio was close to the value of 12 found by Duarte (1992) thus confirming a potential characteristic of plant kingdom. Despite the observed depletion in phosphorus and nitrogen, we could not find any relationship between nutrients content and DOC release rates in our communities, leading to the rejection of the hypothesis of an overflow mechanism (Jensen, 1984).

Influence of pCO_2 on DOC release rates

The purpose of this experiment was to test in an alternative manner the hypothesis saying that DOC release is an overflow mechanism (Jensen, 1984) for recent photosynthate. This hypothesis suggests that photosynthesis is not regulated by plants and therefore produce as many photosynthates as CO_2 concentration permits. If this was the case, DOC release rates should be positively related to CO_2 concentration in water, since photosynthesis rate is itself positively linked to it. Yet, this is not what we demonstrated. In fact, either in natural or modified conditions we globally observed that the higher CO_2 in the water, the lower DOC release rates. With our precedent results showing that DOC release were not linked to nutrients in our systems, we are now able to refute the proposed hypothesis. From our point of view, the DOC release by macrophyte-epiphyte complex is a process linked to the photoperiod and not derived from the photosynthesis process. However, we think that it could partly be extra cellular release of enzymes implied in photosynthesis function. In fact, some laboratory studies on chemical nature of the DOC released by macrophytes demonstrated the presence of glycoprotein and amino acids in a smaller fraction than carboxylic acids (Godmaire and Nalewajko, 1989; Wetzel et Manny, 1972). Presence of amino acids were also demonstrated in phytoplankton exudates (Watt, 1969; Mague *et al.* 1980; Sondergaard and

Shierup, 1982). These findings suggest the presence of proteins and from then it is evident to suggest that these proteins could be enzymes.

In parallel, studies on photosynthesis by macrophytes, and more precisely on *M. spicatum* and *Potamogeton spp.*, have shown that in CO_2 limiting conditions (alkaline systems), these organisms are able use HCO_3^- to compensate low CO_2 concentration (Maberly and Madson, 2002); among the process involved in HCO_3^- use is the production of extra cellular carbonic anhydrase which transform HCO_3^- in CO_2 (Badger, 2003). It was also demonstrated on micro-algae that the production of extra cellular carbonic anhydrase is stopped in the dark. These findings suggested us the hypothesis that DOC release could partly be production of carbonic anhydrase to enhance photosynthesis since macrophytes are generally limited by CO_2 (Kirk, 1994).

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CHAPITRE III

ROLE OF PCO_2 IN *IN SITU* DOC RELEASE BY THE SUBMERGED MACROPHYTE-EPIPHYTE COMPLEX IN ICHETUCKNEE RIVER, FLORIDA

Context: the two precedent chapters demonstrated that the current physical and chemical factors known to influence photosynthesis rate are not related to DOC release rates, except for CO_2 availability. We therefore rejected the hypothesis of the « Overflow mechanism » and proposed an alternative hypothesis presenting the DOC release as part of the concentrating mechanisms macrophytes could use in CO_2 limiting environment. In the following chapter, we therefore test this hypothesis in studying DOC release in a natural ecosystem presenting high range of dissolve CO_2 concentrations.

3.1 ABSTRACT (RÉSUMÉ)

We studied the *in situ* release of dissolved organic carbon (DOC) by growing submerged freshwater macrophytes. Incubations with benthic chambers in Ichetucknee River, Florida (USA) show a net DOC production for different communities of *Sagittaria kurziana*. Daytime DOC release rates range from undetectable to 54.8mgC.m^{-2}.h^{-1}. Although DOC release was restricted to daylight hours and thus suggestive of a photosynthesis-related process, we found no strong link between DOC release rates and concurrent illumination or temperature. We observed a negative relationship between pCO_2 and DOC release suggesting that the process of DOC release could be a response to CO_2-limiting environments.

Keywords: macrophytes, DOC release, pCO_2, CO_2 limitation, Florida.

Nous avons effectué des incubations de *Sagittaria kurziana* dans la rivière Ichetucknee en Floride, grâce à des chambres benthiques. Nous avons mis en évidence une production de COD par ces macrophytes en croisaance atteignant jusqu'à 54.8mgC.m^{-2}.h^{-1}. Nous n'avons pas trouvé de lien entre production de COD et température ambiante ou quantité de lumière reçue par les macrophytes. Par contre nous avons trouvé une relation négative entre production et pCO_2 suggérant une réponse des plantes aux conditions limitantes en pCO_2.

3.2 INTRODUCTION

Macrophyte in marine and freshwater systems has been shown to release DOC (Dissolved Organic carbon) at the same time they do photosynthesis (for the most recent studies: Barron et al, 2003; Ziegler and Benner, 1999; first chapter). This phenomenon was also demonstrated for phytoplankton and it was hypothesized that DOC release could be an overflow mechanism of photosynthetic cells when photosynthesis exceeds capacity to synthesize complex molecules in nutrients limiting conditions (Fogg 1983; Jensen 1984). This hypothesis was rejected following studies on phytoplankton (Bjornsen 1988; Marañón *et al.* 2004), and we also demonstrated in a previous study that DOC release by macrophytes (hereafter termed macrophyte-epiphyte complex since our experimental design included the two communities hardly separable) in lakes was not linked to nutrients concentration, either in water or in sediments (second chapter). Yet, our study and others on freshwater macrophytes (Sieburth, 1969; Sondergaard, 1981; Pregnall, 1983; Nalewajko and Godmaire, 1993) reported that DOC release occur only during the day. A link with photosynthesis processes is therefore highly suspected. Since the principal determinants of photosynthesis efficacy are light and CO_2 availability, we tested their effect on DOC release rates in a study in Southestern Quebec lakes, Canada. We reported no light effect but we found relationships indicating that DOC release is inhibited by increasing water pCO_2. So in this chapter we first verify the negative link between water pCO_2 and DOC release by macrophyte-epiphyte complex in a system presenting a naturally high pCO_2 gradient in water. Since our observations pointed out the importance of DOC release in CO_2 limiting conditions, we hypothesize that DOC release would actively influence the CO_2 availability for macrophyte-epiphyte complex.

3.3 MATERIAL AND METHODS

Study site

The measurements were undertaken during March 2007 in Ichetucknee River, Florida USA (Lat. 29° 59' N, Long. 82° 45' W). The river is formed by nine named and many unnamed springs coming from the Floridan Aquifer System. This karst system is recharged by rainwater of which $CaCO_3$ and CO_2 concentration increases through the lixiviation of the soils thus resulting in high alkalinity (150 $mg.L^{-1}$ $CaCO_3$ in average in Ichetucknee Spring Group; Scott *et al.*, 2004) and CO_2 supersaturation.

We made our measurements in 6 different sites along the 4 miles of the river located in the Ichetucknee Spring State Park: Site I: Head Spring output (Lat. 29° 983' N, Long. 82° 761' W); Site II: Blue Hole Spring output (Lat. 29° 979' N, Long. 82° 759' W); Site III: Mission Spring output (Lat. 29° 976' N, Long. 82° 759' W); Site IV: Devil Eye Spring output (Lat. 29° 973' N, Long. 82° 759' W); Site V: Grassy Hole Spring (Lat. 29° 967' N, Long. 82° 761' W); Site VI: Take out point (Lat. 29° 954' N, Long. 82° 784' W).

DOC release by macrophytes communities and pCO_2 measurements

We studied the net DOC production by macrophyte-epiphyte complex using transparent *in situ* benthic chambers inserted in the sediments. We made our study on Sagittaria kurziana which is the dominant macrophyte species in Ichetucknee River. At least 4 chambers were placed on macrophyte beds (termed TM chambers) and when possible 2 chambers were placed on naked sediments (termed TS chambers). DOC samples were taken with 60mL acid-washed syringes in duplicates at sunrise and sunset and analysed in laboratory with a 1010 TIC TOC analyser, O.I. Analytical. At the end of the 24h incubation, the water volume within each benthic chamber was estimated by injecting a 10mL weak fluorescein solution (absorption peak at 552nm) and drawing two samples after mixing for 5 min. Calculated volumes ranged between 4.4 and 23.5L. Macrophyte shoots inside the PVC ring were harvested to measure their aboveground biomass after rinsing and drying at 55°C during 24h to 36h. DOC changes within each chamber were expressed both per unit area ($mgC.m^{-2}.h^{-1}$)

and per unit plant biomass ($\mu gC.g(dry\ weight)^{-1}.h^{-1}$). To estimate the net DOC release of the macrophytes and their epiphytes only (henceforth termed net DOCme), in other words to avoid the effect phytoplankton, heterotrophic community and the sediments, we used the following equation developed in the first chapter:

$$\Delta DOC(TM)day - \Delta DOC(TM)night - \Delta DOC(TS)day + \Delta DOC(TS)night = DOCme \quad (eq.3.1)$$

Uncertainty estimates for these compound calculations were derived from classical error propagation formulas.

At sunset and sunrise, in addition to DOC samples, water samples were taken in the chambers with 60mL acid-washed syringes to measure pCO_2 (CO_2 infrared gas analyzer EGM-4 ® (PP-system), see first chapter),

Light intensity was measured in each benthic chamber with HOBO Pendant Temp/Light® logger every 10sec but averaged for every minute.

3.4 RESULTS

DOC release rates by macrophyte-epiphyte complex

First, we clearly demonstrated the phenomenon of DOC release in Ichetucknee River related to the presence of Sagittaria kurziana and linked to the photoperiod, since it occurred only during the day whenever the experiments began (fig.3.1). Yet, the DOC release rates were not linked to the amount of light received during the day (regression, $p>0.05$) suggesting a process uncoupled with photosynthesis. We obtained diurnal DOC variations ranging from undetectable to 54.8mgC.m^{-2}.h^{-1} (SD ± 12.4) with a mean rate of 16.6mgC.m^{-2}.h^{-1} (SD ± 14) and we did not detect significant variations at night. Expressed per unit plant biomass found in each chamber, we observed a maximum diurnal DOC variation of 126µgC.gdw^{-1}.h^{-1}(SD ± 12), with a mean rate of 46.4µgC.gdw^{-1}.h^{-1}(SD ± 35.7).

Because there were no significant DOC variations in the chambers devoid of macrophyte (TS) for site I, II, IV, V and VI, we considered the DOC release by phytoplankton and/or sediments to be quantitatively negligible. As a result, calculation of the net DOC release by macrophyte-epiphyte complex (DOCme, eq.3.1) can be reduced to:

$$DOC\ me = \Delta DOC(TM)day - \Delta DOC(TM)night \qquad (eq.3.2)$$

Using this equation, we obtained maximum net DOCme production in transparent chambers containing macrophytes of 29.1mgC.m^{-2}.h^{-1} (SD ± 2.7) and 162µgC.gdw^{-1}.h^{-1}, (SD ± 15). For site III we measured a significant release of DOC in chambers installed on sediments. We therefore used eq.1 to calculate DOCme, and we obtained maximum rates of 39.3mgC.m^{-2}.h^{-1} (SD ± 18.2) and 84µgC.gdw^{-1}.h^{-1}, (SD ± 39). Corrected rates were in average 0.53 times higher than uncorrected but the correction rate was highly variable (SD ± 154%), suggesting that it was quite important in individual measurements.

Influence of water pCO_2 on DOC release rates

The choice of the Ichetucknee River as experimental site proves to be good to study the effect of a pCO_2 gradient on DOC release. In fact, we observed a significant difference in the

averaged sunrise pCO_2 measured in chambers containing macrophytes for the 6 different sites(one way ANOVA, $p<0.05$), ranging from 130 µM (3398ppm) in site III to 168 µM (4929ppm) in site IV. We found that the DOC release was inhibited by increasing pCO_2: in fact we observed a negative relationship between pCO_2 and both DOC release by the entire community (DOCr in $mgC.m^{-2}.h^{-1}$)) and by the macrophyte-epiphyte complex (DOCme in $mgC.m^{-2}.h^{-1}$; regressions, $R^2 = 43\%$ and $R^2 = 29\%$ respectively, $p<0.05$, $n = 23$; fig.2A and 2B). When expressed on a plant biomass basis (in $\mu gC.gdw^{-1}.h^{-1}$), these relationships are no more significant (regressions; $p>0.05$), suggesting a response of the entire community (rather than individual) to this particular environmental condition.

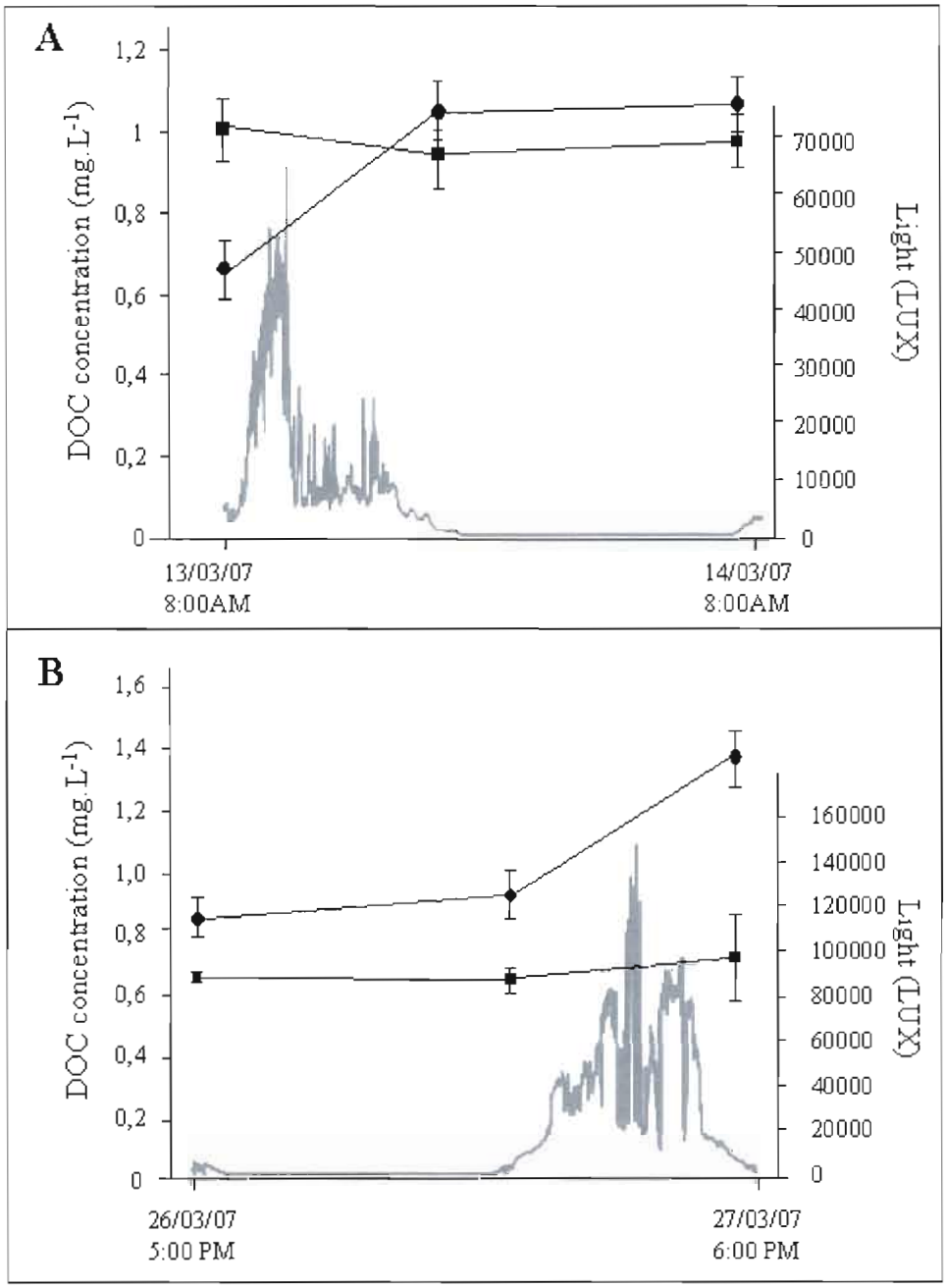

Figure 3.1 Trends in DOC concentration (left axis) and light intensity (right axis) for benthic chambers containing either macrophytes (●) or naked sediments (■), during 24h experiments beginning at sunrise (A) or sunset (B). Bars represent standard deviation

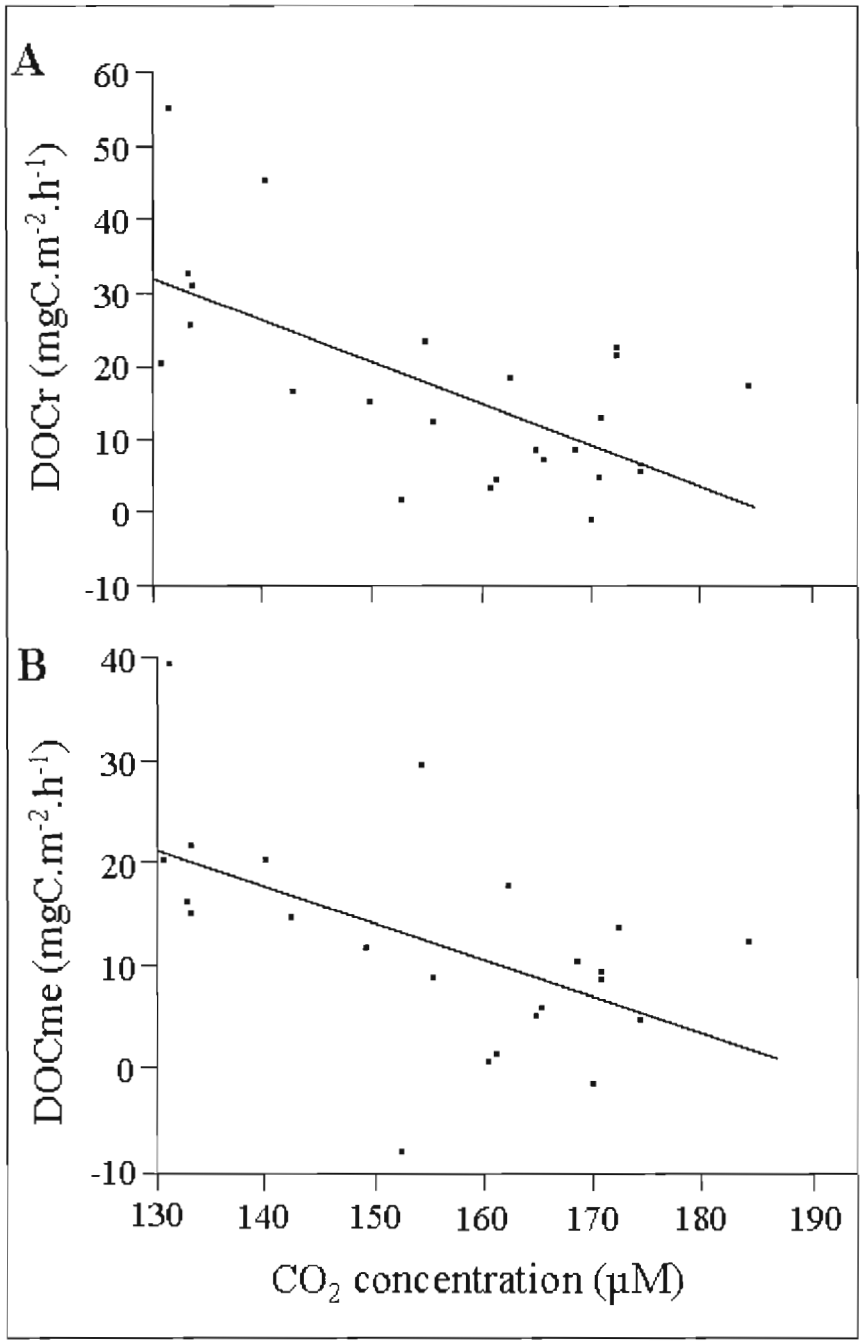

Figure 3.2 Relationships between DOC release rates by the entire community (DOCr) or by the macrophyte epiphyte complex (DOCme) and CO_2 concentration (µM) measured at sunrise (A and B respectively).

3.5 DISCUSSION

DOC release as a response to limiting CO_2

Our results first show an *in situ* DOC release by Sagittaria kurziana linked with the photoperiod. We already observed such an *in situ* DOC release by Myriophyllum spicatum and Potamogetton. sp in southeastern Quebec lakes (first chapter). Yet, the two studies presented different macrophyte classes (monocotyledonous in Ichetucknee river vs. dicotyledonous in Quebec lakes), different hydrologic systems (river vs. lakes) and different trophic status (from oligotrophic to eutrophic). Moreover, DOC release was also observed in several marine macrophytes (Barrón *et al.*, 2003) and phytoplankton (Marañón *et al.* 2004). We therefore reinforce the idea that this phenomenon is a general property of photosynthetic cells.

More importantly, our study points out the influence of water pCO_2 in the morning (beginning of the photosynthesis) on daily DOC release rates, even in supersaturated systems. In fact, we found a negative relationship between these two variables in Quebec lakes, where the pCO_2 is 5 to 6 times lower than in Ichetucknee River. In this river, we had the opportunity to verify the relationship in naturally high pCO_2 range. We discovered the same negative relationship than in Quebec lakes (fig.3), but in Ichetucknee River the DOC release rates were higher in average (mean DOCr: 16.6mgC.m^{-2}.h^{-1} (SE ±2.9, n= 23) vs. 4.5 mgC.m^{-2}.h^{-1} (SE ±0.6, n= 40); mean DOCme: 11.7mgC.m^{-2}.h^{-1} (SE ±2.2, n= 23) vs. 5.2 mgC.m^{-2}.h^{-1} (SE ±0.8, n= 40) for Florida and Quebec respectively; One Way ANOVA, $p<0.05$) and the slope were different (ANCOVA, $p<0.05$). Remarkably, we found that for Ichetucknee River and Southeastern Quebec lakes, the decrease in pCO_2 explained the same proportion of the DOC release variance ($R^2_{(Floride)}$ = 43%, $p<0.001$, n=23; $R^2_{(Québec)}$ = 43%, $p<0.001$, n=40, for both DOCr and DOCme). These results suggest that the same physiological process occurs for all studied communities but with reaction time depending on the system. Because in Florida there was no relationship between pCO_2 and DOC release rates at the plant scale (rates expressed on a biomass basis), we can suppose that the release is a phenomenon of response from the community facing a stress, here the limiting pCO_2. Knowing that Wetzel and Manny reported the presence of amino acids in the released DOC by freshwater macrophyte (1972),

we can conclude that DOC release would not be a passive diffusion of simple molecules as carbohydrates but an active synthesis of enzymes as carbonic anhydrase resulting of macrophyte adaptation to their environment.

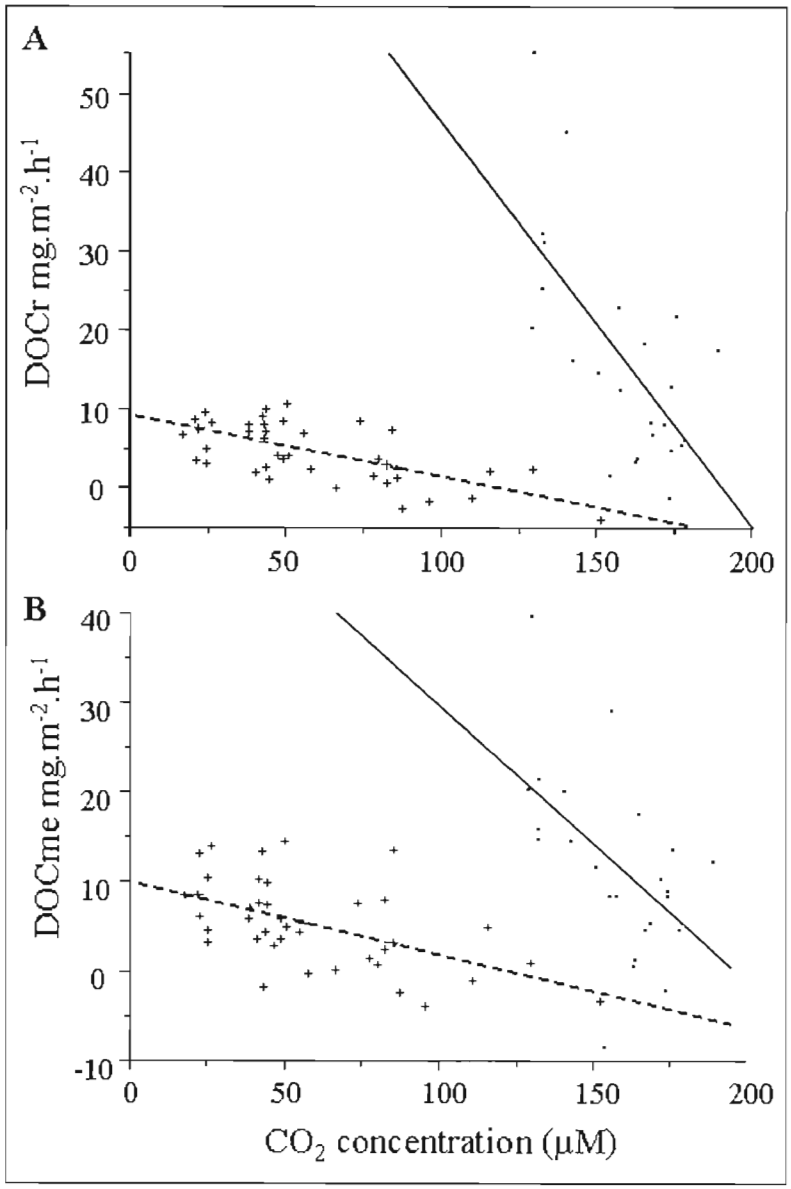

Figure 3.3 Comparison of the relationships obtained for Ichetucknee River (solid lines) and Quebec lakes (dashed lines), between DOC release rates by the entire community (DOCr) or DOC release by the macrophyte-epiphyte complex (DOCme) and CO_2 concentration (µM) measured at sunrise (A and B respectively).

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CHAPITRE IV

LINK BETWEEN DISSOLVED ORGANIC CARBON RELEASE, BICARBONATE UPTAKE AND RESULTING PRODUCTIVITY BY FRESHWATER MACROPHYTE-EPIPHYTE COMPLEX

Context: We demonstrated a link between dissolve CO2 availability and DOC release rates in the different studied ecosystems, and proposed a hypothesis presenting the DOC release as a carbon concentrating mechanism in CO2 limited environments. To confirm it, we have to study the effect of CO2 availability on macrophytes primary productivity and the possible positive link between DOC release and productivity.

4.1 ABSTRACT (RÉSUMÉ)

We studied the *in situ* release of dissolved organic carbon (DOC) by growing submerged freshwater macrophytes in relation to their metabolism, in Quebec Lakes (Canada) and Ichetucknee River, FL (USA). With experimental CO_2 enriched waters in Quebec, we demonstrated that increase in CO_2 water concentration led to increase in net community productivity (NCP) calculated as dissolved inorganic carbon uptake. Calculating the portion of the productivity attributable to either CO_2 or HCO_3^- uptake, we found a global positive relationship between increase in CO_2 water concentration and increase in CO_2 uptake, while CO_2 water concentration had no influence on HCO_3^- uptake. Surprisingly, in CO_2 supersaturated Ichetucknee R., NCP was sustained by HCO_3^- uptake. Studying the link between DOC release and productivity, we found a good general relationship between DOC release and HCO_3^- uptake. These observations are discussed regarding various hypotheses on the physiologic role of DOC release for macrophytes. We conclude that DOC release could be the result of a carbon concentrating mechanism acting in promoting higher productivity in CO_2-limited environments.

Keywords: Macrophytes, DOC release, Net Community productivity, CO_2, HCO_3^-, CCM.

Nous avons étudié la production *in situ* de COD par des macrophytes en croissance en relation avec leur métabolisme dans des lacs du Québec et dans la rivière Ichetucknee en Floride. Au Québec, les incubations artificiellement enrichies en CO_2 ont abouti à des productions nettes (NCP, calculées comme prise de carbone inorganique dissous - CID) plus élevées. En découplant le CID en CO_2 et HCO_3^-, nous montrons une augmentation de la prise de CO_2 (mais pas de HCO_3^-), par les macrophytes des incubations enrichies. Nous avons trouvé que dans la rivière Ichetucknee, naturellement enrichie en CO2, la production nette est soutenue par la prise de HCO_3^-. Nous avons également trouvé une relation positive entre prise de HCO_3^- et production de COD par les macrophytes, ce qui nous permet de discuter le rôle physiologique de cette production.

4.2 INTRODUCTION

Availability of inorganic carbon is determinant for photosynthetic organisms and, for aquatic ones, this is more constraining than for terrestrial plants. Physical and chemical processes regulating CO_2 diffusion through air/water interface and transformation are more complex in water, resulting in smaller CO_2 availability in aquatic environments. Physical restriction to CO_2 availability for photosynthetic organisms include a 10^4 times lower diffusion rate in water than in the air (Rascio, 2002), permanent or temporary thermal stratification of water column which highly reduce CO_2 penetration from the surface, and the presence of a boundary layer around organism which impose slow CO_2 exchanges governed by molecular diffusion (Wetzel, 2001). At the chemical level, the highly soluble CO_2 is transformed to carbonic acid and is in equilibrium with bicarbonate and carbonate forms, the proportions of these different dissolved inorganic carbon (DIC) forms depending on water pH (eq.4.1). In circumneutral waters, bicarbonate (HCO_3^-) is the dominant form. It is therefore not surprising that photosynthetic organisms have developed the capacity to use it. The transformation of bicarbonate in CO_2 is made by carbonic anhydrase, an enzyme universally present in photosynthetic cells, but particularly useful in CO_2 limited systems (Wetzel, 2001). This usually intra-cellular enzyme (direct HCO_3^- uptake and intracellular conversion in CO_2) can also be excreted in the environment to increase the CO_2 concentration around the organisms (indirect HCO_3^- uptake via transformation in CO_2; Falkowski and Raven, 1997, Maberly and Madsen, 2002) and thus increase the photosynthesis. Therefore when measuring primary production of aquatic organisms, the measurement of CO_2 variations is not always sufficient since bicarbonate can also be involved in photosynthesis.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \leftrightarrow CH_2O + O_2 \quad \text{(eq.4.1)}$$

At pH<8, photosynthetic organisms able to use HCO_3^- can uptake both CO_2 and HCO_3^- as carbon source, with preference for CO_2 for which they usually have a higher affinity (Durako, 1993). According to the carbonate buffering system, at pH<8 CO_2 consumption uptake leads to increase in pH, while HCO_3^- uptake leads to a decrease in pH. However, CO_2 uptake being dominant, carbon uptake globally leads to an increase in pH and so in prevalence of the bicarbonate form; thus at pH 8, CO_2 proportion is close to zero. In closed systems with no renewal source of CO_2 the only carbon source for photosynthesis is then HCO_3^-. CO_2 restricted organisms are therefore unable to photosynthesize, which is demonstrated by pH

drift experiments (stop in pH increase reflecting a stop in CO_2 uptake); Maberly and Madsen, 1998). HCO_3^- uptake will in turn leads to pH increase by the action of the carbonate buffering system; HCO_3^- affinity will then determine the capacity of organisms to leave at increasing pH (Spence and Maberly, 1985). HCO_3^- uptake will also lead to a decrease in alkalinity formed by carbonate, borate and silicate buffering systems (Stumm and Morgan, 1996). However, to a minor extent, changes in alkalinity can be due to nutrients uptake, which complicate the interpretation of the variation (Brewer and Goldman, 1976). In this chapter, where we present close system experiments with natural evolution of DIC concentration, pH and alkalinity with time, we will thus consider change in alkalinity as a proxy for HCO_3^- uptake by the studied macrophytes.

In the previous chapters (II and III), we found that the DOC release may be a response to CO_2 limited environments and we propose that the release could be linked to the transformation of HCO_3^- into CO_2, as would carbonic anhydrase. The present study aims to explore this hypothesis by examining wether: (1) the limitation in CO_2 in our systems leads to low production rates; (2) the positive relationships between DOC release and productivity of macrophyte communities expressed either as total dissolved in inorganic carbon (DIC) or only as HCO_3^- and CO_2 uptake.

4.3 MATERIAL AND METHODS

Study site and experimental design

Quebec lakes - The measurements were undertaken during the summers 2005 and 2006 in six lakes of the Eastern township region ~ 100km east of Montréal, Québec Canada (Table 1.1). Incubations were made on *Myiophyllum spicatum*, *Potamogeton amplifolius* and *P. richardsonii.*

Ichetucknee River, FL - The measurements were undertaken during March 2007 in Ichetucknee River, Florida USA (Lat. 29° 59' N, Long. 82° 45' W). The river is formed by nine named and many unnamed springs coming from the Floridan Aquifer System. This karst system is recharged by rainwater of which $CaCO_3$ and CO_2 concentration increases through the lixiviation of the Karstic soils thus resulting in high alkalinity (2.99meq$CaCO_3$ in average in Ichetucknee Spring Group; Scott *et al.*, 2004; Kurz *et al.*, 2004) and CO_2 supersaturation. We made our measurements in 6 different sites along the 6.4 km of the river located in the Ichetucknee Spring State Park: Site I: Head Spring output (Lat. 29° 983' N, Long. 82° 761' W); Site II: Blue Hole Spring output (Lat. 29° 979' N, Long. 82° 759' W); Site III: Mission Spring output (Lat. 29° 976' N, Long. 82° 759' W); Site IV: Devil Eye Spring output (Lat. 29° 973' N, Long. 82° 759' W); Site V: Grassy Hole Spring (Lat. 29° 967' N, Long. 82° 761' W); Site VI: Take out point (Lat. 29° 954' N, Long. 82° 784' W). Incubations were made on *Sagittaria kurziana.*

For both Quebec and Florida sites, we studied the DOC release by macrophyte-epiphyte complex and communities' metabolism using *in situ* benthic chambers inserted in the sediments for 24h incubations. Transparent benthic chambers on unvegetated sediments (TS) were used as control. Complete materiel and sampling methods and results about DOC release by macrophyte-epiphyte complex are described in the first chapter.

Net community production and pCO_2 measurements

At sunset and sunrise, water samples were taken in the chambers with 60mL acid-washed syringes to measure partial pressure in CO_2 (pCO_2 ; CO_2 infrared gas analyzer EGM-4 ®, PP-system), and alkalinity both in duplicates. At pH values found in our systems, alkalinity values are considered as equal to HCO_3^- concentrations, CO_3^{2-} concentrations being negligible. CO_2 concentration is obtained from pCO_2. DIC concentration is thus the sum of CO_2 and HCO_3^- concentrations.

DIC changes within each chamber were expressed both per unit area ($mgC.m^{-2}.h^{-1}$) and per unit plant biomass ($mgC.g(dry\ weight)^{-1}.h^{-1}$), to examine how release rates may be affected by plant density. Community gross primary production (GPP), respiration (R) and net community production (NCP) were estimated from the DIC concentration changes in the benthic chambers between sunset and sunrise samples: hourly rates of R and NCP were calculated from the difference in DIC during the night and the day respectively. Hourly rates of GPP were given by the sum of the hourly rates of R and NCP. Daily rates of GPP were then obtained by multiplying the hourly rates of GPP by the photoperiod (estimated as the time between the sunset and sunrise sampling hour). Daily rates of respiration were calculated by multiplying hourly rates of R by 24h. Daily rates of NCP were estimated as the difference between daily rates of GPP and R.

Although the calculations described above allowed the usual estimation of community metabolism inside the different chambers, it does not necessarily reflect the metabolic status of the isolated macrophyte-epiphyte community but also includes the effects of the incubated sediments and the water column as well. To estimate the net primary production of the macrophyte and their epiphyte only (termed NPPme), corrections were necessary. Daily DIC variations measured in chambers containing macrophyte are the sum of the biological processes occurring simultaneously: in one hand, gross primary productions of the macrophyte- epiphyte complex and phytoplankton, GPPme and GPP_ψ respectively; in the other hand, respiration of the macrophyte-epiphyte complex, phytoplankton, heterotrophic community and sediments. DIC variations measured from the chambers on sediments (SC) without macrophyte reflect processes linked to phytoplankton, heterotrophic community and

sediments; in parallel, DIC variations measured from the chambers on macrophyte (MC) reflect not only the last processes but also the effect of macrophyte. Therefore in subtracting the DIC variations obtained in the different chambers we obtained the net primary production of the macrophyte-epiphyte complex alone. Equation 4.2 and equation 4.3 describe the processes occurring in macrophyte (MC) and sediments (SC) chambers during the day:

$$\Delta \text{ DIC (MC)} = \text{GPPme} + \text{GPP}_{\psi} - \text{Rme} - \text{R}_{\psi} - \text{Rh} - \text{Rsed} \qquad \text{(eq.4.2)}$$

$$\Delta \text{ DIC (SC)} = \text{GPP}_{\psi} - \text{R}_{\psi} - \text{Rh} - \text{Rsed} \qquad \text{(eq.4.3)}$$

where Δ DIC is the DIC variation during the photoperiod, are macrophyte-epiphyte GPP and phytoplankton GPP respectively, and Rme ,R_{ψ}, Rh, Rsed are the respiration terms for macrophyte-epiphyte complex, phytoplankton, heterotrophic and sediments respectively. Subtracting (eq.4.2) from (eq.4.3) yields:

$$\Delta \text{ DIC (MC)} - \Delta \text{ DIC (SC)} = \text{GPPme} - \text{Rme} = \text{NPPme} \qquad \text{(eq.4.4)}$$

Δ DIC (SC) rates obtained for each lake were the average of the two bare sediment chambers. For L. Trois Lacs, where we had no TS chambers, we subtracted a mean DIC (SC) rate obtained from all other lakes. This allowed us to compare the diurnal productions of DOC attributed to the macrophyte-epiphyte complex (DOCme) with the net plant production during the same period.

4.4 RESULTS

Community metabolism

In chambers containing macrophyte (TM), we obtained average rates of DIC change that differed considerably and significantly between day and night for all lakes and the Ichetucknee River (one-way ANOVA, $p<0.05$; Fig.4.1). During the day we observed a decrease in DIC which is used by photosynthetic organisms inside the chamber; at night we observed an increase in DIC reflecting whole community respiration. For clear chambers devoid of macrophyte (TS), diurnal and nocturnal DIC variations were not significantly different in all lakes (one way ANOVA, $p>0.05$; results not shown). From variations in DIC concentrations, we calculated net community production (NCP) and respiration (R) (table 4.1). From these values, we calculated daily GPP rates. Twelve of the 19 sampled Quebec lake communities were net heterotrophic, implying that their metabolisms were partly sustained by external carbon sources. In Ichetucknee R. 19 of the 23 communities studied were autotrophic.

From NCP and R values we estimated net primary production of the macrophyte-epiphyte complex (NPPme). For Quebec lakes, corrected rates were in average 1.39, 1.27, 0.79, 7.01 and 0.53 times higher than uncorrected for L. d'Argent, L. Peasley, L. Stukely, L. Trois Lacs and Ichetucknee R. respectively. The correction rates being quite stable among lakes (except one higher value at L. Trois Lacs, which increase the multiplying factor), it suggests that net effect of planktonic, heterotrophic and sedimentary communities had a constant effect on the metabolism of chamber. Therefore, relationships with NCP or NPPme do not lead to different conclusions.

Influence of pCO_2 on net community productivity

During the experiments, we measured pCO_2 in benthic chambers to verify that higher CO_2 concentrations correspond to higher production rates. Our experiments being first designed to test the effect of pCO_2 on DOC release by macrophyte-epiphyte complex, we conducted two types of experiments: either in natural conditions spanning a natural gradient

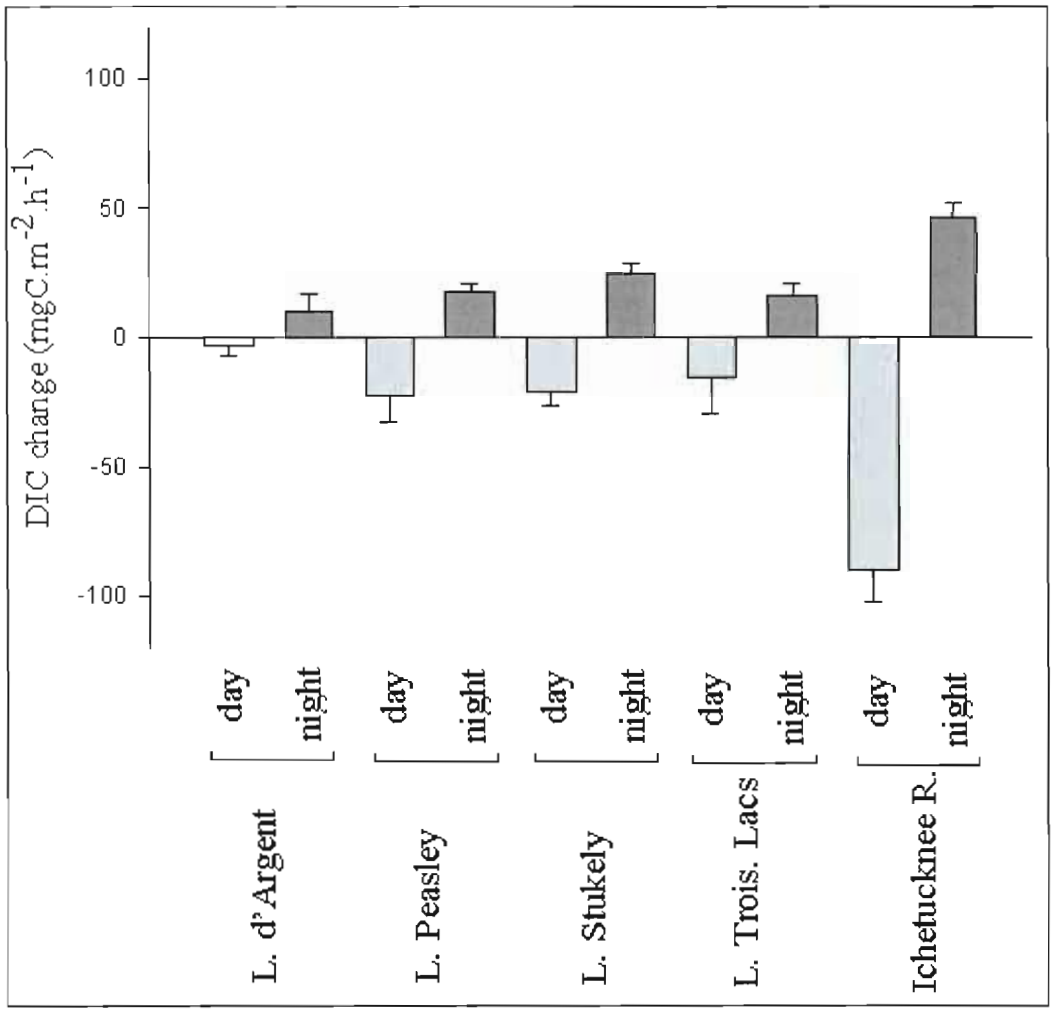

Figure 4.1 Daytime and nighttime changes in dissolved inorganic carbon (DIC; in $mgC.m^{-2}.h^{-1}$) for L. d'Argent, L. Peasley, L. Stukely, L. Trois Lacs (Quebec) and Ichetucknee R. (Florida).

Table 4.1

Maximum, minimum, means and standard deviation (SD) of daily and nightly DIC variations (negative values represent DIC consumption), gross primary productivity (GPP), respiration (R) and net primary productivity of macrophyte-epiphyte complex (NPPme), and percentage of dissolved inorganic carbon (DIC) variation due to variation in either CO_2 or HCO_3^- for Quebec (QC) and Florida (FL) studied systems

Ecosystem	Variable	Units	Maximum value	Minimum value	Mean	SD	$\%HCO_3^-/\Delta DIC$	$\%CO_2/\Delta DIC$
QC	Daily ΔDIC (hourly NCP)	$mg.m^{-2}.h^{-1}$	10.12	-86.98	17.11	0.94	14.8%	85.2%
		$ug.gdw^{-1}.h^{-1}$	99.50	-665.36	170.51	9.92	16.4%	83.6%
	Nightly ΔDIC (hourly R)	$mg.m^{-2}.h^{-1}$	59.61	4.97	-24.39	1.67	18.1%	81.9%
		$ug.gdw^{-1}.h^{-1}$	456.08	38.00	-240.15	12.61	16.4%	83.6%
	GPP	$g.m^{-2}.d^{-1}$	1.30	0.13	-0.54	0.03		
		$mg.gdw^{-1}.d^{-1}$	12.69	1.25	-5.31	0.19		
	R	$g.m^{-2}.d^{-1}$	1.43	0.12	-0.59	0.04		
		$mg.gdw^{-1}.d^{-1}$	10.95	0.91	-5.76	0.27		
	NPPme	$mg.m^{-2}.h^{-1}$	100.56	-7.27	23.77	2.68		
		$ug.gdw^{-1}.h^{-1}$	802.03	-44.26	244.90	66.81		
FL	Daily ΔDIC (hourly NCP)	$mg.m^{-2}.h^{-1}$	-226.63	-35.10	118.16	4.78	62.8%	37.2%
		$ug.gdw^{-1}.h^{-1}$	-854.93	-103.42	411.77	17.30	62.2%	37.8%
	Nightly ΔDIC (hourly R)	$mg.m^{-2}.h^{-1}$	106.66	49.13	-49.13	4.20	40.0%	60.0%
		$ug.gdw^{-1}.h^{-1}$	421.23	175.56	-175.56	16.90	41.0%	59.0%
	GPP	$g.m^{-2}.d^{-1}$	3.07	0.55	-1.62	0.07		
		$mg.gdw^{-1}.d^{-1}$	12.23	1.82	-5.90	0.25		
	R	$g.m^{-2}.d^{-1}$	2.56	-0.04	-1.18	0.11		
		$mg.gdw^{-1}.d^{-1}$	10.11	-0.09	-4.21	0.42		
	NPPme	$mg.m^{-2}.h^{-1}$	253.08	-87.12	85.41	5.85		
		$ug.gdw^{-1}.h^{-1}$	670.30	-348.86	258.22	74.88		

of pCO_2 (transparent benthic chamber with macrophyte community; TM), or with artificially increased pCO_2 (by addition of CO_2-enriched distilled water; TM+CO_2) in Quebec lakes only. With the data obtained in natural conditions only, we detected no effect of water CO_2 concentration on NCP for both Quebec lakes and Ichetucknee R. (regression, $p>0.05$, n=25 and n= 23 respectively). When including data of CO_2-enriched chambers (TM+CO_2) for Quebec lakes (which increased the water CO_2 concentration range by 10 fold), we observed, as expected, a positive general relationship between water CO_2 concentration and NCP (all lakes included, $R^2=0.29$, $p<0.0001$, n=45). This relationship was particularly strong in L. Stukely where we had the most replicates ($R^2=0.91$, $p<0.0001$, n=22). These relationships were essentially the same with corrected (NPPme, results not shown) or uncorrected rates and therefore conclusions are valid at the whole community and at the macrophyte-epiphyte complex scales. These results strongly suggest that our macrophyte communities in natural conditions were CO_2–limited.

The method we used to calculate NCP allowed us to calculate the portion of the productivity attributable to the uptake of HCO_3^- and CO_2 (table 4.1). Minimum and maximum rates for HCO_3^- and CO_2 uptake (reflecting photosynthesis) or release (equivalent to respiration) are presented in table 4.2. Globally, considering the two regions pooled, we observed a general positive relationship between increase in CO_2 uptake and increase in CO_2 water concentration ($R^2=0.23$, $p<0.0001$, n=67). This relationship is also found for Quebec lakes ($R^2=0.31$, $p<0.0001$, n=44), but this was not the case for Ichetucknee R. ($p>0.05$, n=45), where CO_2 uptake was independent of CO_2 water concentration, with a mean rate of 46.9 $mg.L^{-1}.h^{-1}$(±18.7). Surprisingly, HCO_3^- uptake rates was also independent of both CO_2 water concentration and CO_2 uptake (regressions, $p>0.05$), with a mean rate of 2.9 $mg.L^{-1}.h^{-1}$(±7.7) for Quebec lakes and 74.2 $mg.L^{-1}.h^{-1}$(±46.3) for Ichetucknee R. In this latter system, CO_2 uptake was about half of the HCO_3^- uptake, and so productivity was mostly sustained by bicarbonate.

Productivity and DOC release

We found a good general relationship between net community production and DOC release when expressed on an areal basis (regression, n=68, $R^2=0.40$, $p<0.0001$, fig.2A),

Table 4.2
Maximum, minimum, means and standard deviation (SD) of daily and nightly HCO_3- and CO_2 variations for Quebec (QC) and Florida (FL) studied systems

Ecosystem	Variable	Units	Max	Min	Mean	SD
QC	Daily ΔHCO_3-	$mg.m^{-2}.h^{-1}$	-19.79	13.79	-2.39	0.94
		$ug.gdw^{-1}.h^{-1}$	-249.74	105.46	-26.44	9.92
	Daily ΔCO_2	$mg.m^{-2}.h^{-1}$	-100.76	8.10	-13.67	$<10^{-3}$
		$ug.gdw^{-1}.h^{-1}$	-770.82	84.50	-133.47	$<10^{-3}$
	Nightly ΔHCO_3-	$mg.m^{-2}.h^{-1}$	-2.30	12.97	4.25	1.67
		$ug.gdw^{-1}.h^{-1}$	-23.84	145.24	37.95	12.61
	Nightly ΔCO_2	$mg.m^{-2}.h^{-1}$	0.43	55.20	19.41	$<10^{-3}$
		$ug.gdw^{-1}.h^{-1}$	3.31	411.07	194.99	$<10^{-3}$
FL	Daily ΔHCO_3-	$mg.m^{-2}.h^{-1}$	-166.43	-10.24	-74.24	4.78
		$ug.gdw^{-1}.h^{-1}$	-613.23	-30.17	-255.97	17.30
	Daily ΔCO_2	$mg.m^{-2}.h^{-1}$	-80.25	-18.99	-43.91	$<10^{-3}$
		$ug.gdw^{-1}.h^{-1}$	-391.06	-55.65	-155.80	$<10^{-3}$
	Nightly ΔHCO_3-	$mg.m^{-2}.h^{-1}$	-9.81	51.98	19.65	4.20
		$ug.gdw^{-1}.h^{-1}$	-25.78	220.60	72.03	16.90
	Nightly ΔCO_2	$mg.m^{-2}.h^{-1}$	5.60	74.28	29.48	$<10^{-3}$
		$ug.gdw^{-1}.h^{-1}$	14.91	269.05	103.53	$<10^{-3}$

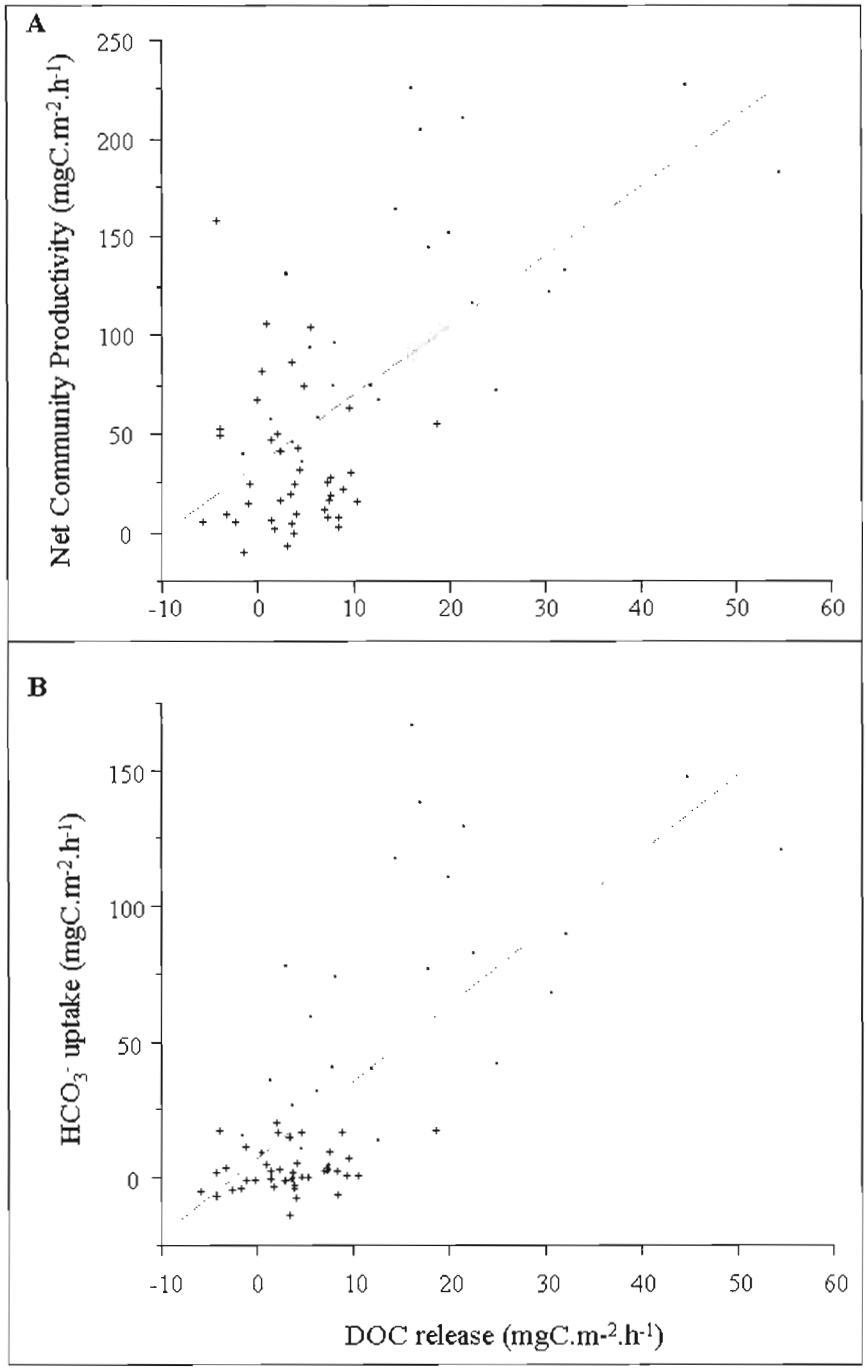

Figure 4.2 Relationships between net community productivity (A; Y= 33,72 + 3,56X) or HCO_3^- uptake (B; Y= 3,89 + 2,9X) and DOC release rates. (+)and ■ for QC and FL results respectively.

which was also found when using corrected data (NPPme and DOCme, regression, n=67, R^2=0.40, p<0.0001). We found weak or not significant relationships using data on a biomass plant basis (data not shown). Decoupling NCP in HCO_3^- and CO_2 uptake, we found a good general relationship between DOC release and HCO_3^- uptake (regression, n=68, R^2=0.52, p<0.0001; fig.4.2B), but not with CO_2 uptake (regression p>0.05). The same observations were made with corrected DOC release rates (DOCme vs. HCO_3^- uptake: regression, n=68, R^2=0.22, p<0.0001; DOCme vs. CO_2 uptake: regression p>0.05).

In the perspective that DOC release represent a loss for the plants, for communities presenting both positive DOC release rate and productivity we have calculated that for Quebec lakes DOC release represented in average 26.2 % (SD ± 24.4; n=30) of the hourly NCP and 13.2% (SD ± 8.4) of the hourly GPP. For Ichetucknee R., we found that DOC release represented in average 14.2 % of the hourly NCP (SD ± 8.7; n=22) and 9.7% (SD ± 6) of the hourly GPP.

4.5 DISCUSSION

Our experiments confirmed that although they are naturally supersaturated in CO_2 (58 µM in average, while systems in equilibrium with air exhibit water CO_2 concentrations of about14 µM), the Quebec lakes appear CO_2-limited for macrophyte-epiphyte primary production. In fact, artificially increased pCO_2 led to higher productivity and higher CO_2 use for photosynthesis, which is not surprising since the high CO_2 requirement of macrophyte for maximal photosynthesis compared to what is generally available in lakes has already been described (Kirk, 1994). Surprisingly, in Ichetucknee R., where natural CO_2 concentration is about 10 times higher than the concentration in equilibrium (158 µM in average vs. 14 µM), we observed that productivity was sustained mostly by HCO_3^- instead of CO_2. In this ecosystem, we could not observe the shift to CO_2 sustained productivity at high CO_2 concentrations as we had observed in our experimental CO_2 enriched waters from Quebec lakes.

CO_2 is directly used by photosynthesis process, whereas HCO_3^- needs to be converted in CO_2 by carbonic anhydrases to be used as carbon source, which imply a cost for cells (Raven and Lucas, 1985; Jones, 2005). Clearly, since HCO uptake appears to dominate productivity in some systems, it is a worthwhile cost. Carbonic anhydrases are widely spread in photosynthetic organisms, suggesting it is necessary to obtain an efficient photosynthesis (Badger, 2003).

In addition to the positive link between water CO_2 concentration and macrophyte-epiphyte complex productivity, we demonstrate a strong link between DOC release and productivity which is general across ecosystems. At the moment, we can not conclude which variable influence the other, two alternative hypotheses can be proposed: (1) DOC release is driven by productivity and acts as an overflow mechanism (Jensen, 1984); (2) productivity is positively influenced by the release. In the first case, factors positively influencing photosynthesis would induce DOC release rates.

In previous studies, we found that DOC release is not affected by factors influencing photosynthesis except by water CO_2 concentration (see first, second and third chapters). We also found that increase in water CO_2 concentration leaded to decrease in DOC release rates

in both Quebec lakes and Ichetucknee R. (see second and third chapters). These findings are thus in opposition with the Jensen hypothesis. Moreover, given that DOC release is associated with bicarbonate assimilation, it is hard to reconcile with the hypothesis that release is simply an overflow mechanism. Indeed, the cost of using HCO_3^- would be increased by its excretion.

The alternative hypothesis considers the DOC release as a process promoting higher productivity. We showed that productivity is low and sustained by HCO_3^- uptake at low CO_2 concentrations, and that DOC release is positively linked to HCO_3^- uptake. So DOC release could be the result of a carbon concentrating mechanism acting in CO_2 limiting environments. In fact, the use of HCO_3^- requires intra or extracellular carbonic anhydrase production (Badger, 2003). Studies on carbonic anhydrase by freshwater macrophytes being scarce, most are based on other aquatic photosynthetic organisms (phytoplankton to macroalgae). Since different classes of carbonic anhydrase are spread widely across phylogenetic groups (Badger, 2003), we can therefore suppose that mechanisms would be widespread (Wilbur and Anderson, 1948; Rigobello-Masini *et al.*, 2003; Haglund *et al.*, 1992; Flores-Moya and Ferńandez, 1998). Moreover the same chemicals are widely used to inhibit intracellular (acetazolamide and 6-ethoxyzolamide) or extracellular carbonic anhydrase (dextran-bound sulfonamide), thus demonstrating the same action (Flores-Moya and Ferńandez, 1998; Andría *et al.*, 2000; Yu *et al.*, 2007; references also available for studies on animal cells). Moreover, we demonstrated in previous studies (first, second and third chapters) that illumination was necessary to observe DOC release and that maximum rates occurred at low-CO_2 concentrations. Similarly, extracellular carbonic anhydrase activity has been shown to be inducible by light (Haglund *et al.*, 1992; Rigobello-Masini, 2003), and in low-CO_2 concentrations (Szabo and Cloman, 2007; Andría *et al.*, 2000) by both micro and macroalgae.

Wetzel and Manny (1972) estimated that 11% of DOC released by aquatic macrophyte corresponds to nitrogenous compounds and that most of compounds were glucose and other carbohydrates that have very low molecular weight. In the same way, Chen and Wangersky (1996) demonstrated by phytoplankton in growth phase that only 10% of the DOC released had a molecular weight higher than 10kDa. From our knowledge, the molecular weight of carbonic anhydrases produced by macrophyte is not known yet, but has

been estimated at 24kDa in to the marine diatom *Phaeodactylum tricornutum* (Szabo and Colman, 2007). If we assume that about 10-11% of the DOC release found to be nitrogenous high weight compounds correspond to carbonic anhydrase, our average DOC release rate of 56 µgC.g (dry weight)$^{-1}$.h^{-1} (SD ± 8) by macrophyte in studied Quebec lakes (first chapter), would suggest a maximum extracellular carbonic anhydrase production of a few µgC.g (dry weight)$^{-1}$.h^{-1}. This hypothesis should be further tested experimentally using extra-cellular inhibiters. Further investigations are also required to explain the release of simple carbohydrates which would represent 90% of the release. Furthermore, because we studied macrophyte species that were HCO_3^- users, the same experiments on organisms restricted to CO_2 uptake would yield valuable information.

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CHAPITRE V

QUANTITATIVE CONTRIBUTION OF DOC RELEASE BY THE MACROPHYTE-EPIPHYTE COMPLEX IN DIFFERENT ECOSYSTEMS

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Context: Epilimnetic heterotrophy has been observed in several lakes, leading the question of the origin of the DOC surplus allowing this heterotrophy. We measured DOC release by macrophyte-epiphyte complex in several Quebec lakes and in Ichetucknee River (FL). This source of carbon could have an implication in the heterotrophy of lakes, according to its quantitative importance compared to allochtonous DOC pool. We therefore use our mean DOC release rates to conduct an up-scaling exercise in order to address this question.

5.1 ABSTRACT (RÉSUMÉ)

Dissolved organic carbon (DOC) release by living submerged macrophytes-epiphytes complex has been quantified *in situ* in five southern Quebec lakes of various trophic status and in Ichetucknee River (FL). Incubations with benthic chambers showed a net DOC production for different communities. We found different rates between lakes but not linked with trophic status, soluble or dissolved phosphorus and dissolved nitrogen in the interstitial waters of the sediments. Since we found no difference in DOC between species, the overall mean DOC release rate of 56 $\mu gC.gdw^{-1}.h^{-1}$ can be used for extrapolations at the lake scale for Quebec and the rate of 46.4 $\mu gC.gdw^{-1}.h^{-1}$ can be used for Ichetucknee River. At the whole ecosystem level, the global DOC from macrophyte-epiphyte release is compared to the allochthonous DOC load from the watershed. We estimated that DOC release represent between 1 to 43 % of the total DOC available. In shallow lakes with dense macrophyte beds, the implication of macrophytes-epiphytes complex in carbon budget is significant.

Nous avons étudié la production de COD par diverses communautés de macrophytes au Québec et en Floride. Nous n'avons pas montré de différence entre les espèces pour chaque région et nous avons donc calculé un taux moyen de 56 $\mu gC.gdw^{-1}.h^{-1}$ pour le Québec et de 46.4 $\mu gC.gdw^{-1}.h^{-1}$ pour la Floride. Connaissant les biomasses de macrophytes dans chaque système, nous avons pu estimer la contribution de la production de COD par rapport au COD entrant dans les systèmes par les tributaires. Nous avons calculé que la production de COD représente 1 à 43% du COD disponible dans les lacs du Québec et seulement 10% dans la rivière Ichetucknee. Cette contribution est discutée par rapport aux conditions climatiques.

5.2 INTRODUCTION

The loading of terrestrial dissolved organic carbon (DOC) can contribute significantly to the energy pathways of lake ecosystems (Tranvik 1992; Pace et al. 2004; Carpenter et al. 2005; Kritzberg et al. 2006). However, water column metabolism could also be sustained by benthic production since it often dominates the overall productivity of the system, particularly in shallow lakes (Vadeboncoeur et al. 2003). Living macrophytes are known to release DOC (Wetzel & Manny 1972; Godmaire & Nalewajko 1989, Barrón et al. 2003) and are therefore a potential source of carbon for water column metabolism. So, we determined the relative contribution of DOC released by living macrophytes to the entire DOC pool in order to estimate its potential contribution to metabolism at the ecosystem scale. We measured *in situ* DOC release rates by the macrophyte-epiphyte and then compared them to DOC loads from the watersheds.

5.3 MATERIAL AND METHODS

South-eastern Quebec Lakes

The following study was performed in five lakes in south-eastern Quebec, Canada (Table 1.1) and their watersheds. This work stems from a study of DOC release by the macrophyte-epiphyte complex in a series of Quebec lakes of different trophic status. In this previous study, all lakes showed an increase in DOC concentration in benthic chambers containing macrophytes during the day but not during the night. We found that calculated rates of DOC release by the macrophyte-epiphyte complex were not significantly different among species. We therefore proposed a mean DOC release rate of 56 $\mu gC.gdw^{-1}.h^{-1}$ that could be extrapolated to the whole-lake scale (see first chapter). In the present study, we combined this average rate with the lake macrophyte biomass data to compute an average lake-wide macrophyte-derived internal organic carbon loading rate. We then compared this internal load to estimates of allochtonous DOC loads from the lakes' watersheds, obtained from a separate project on the same lakes. Our macrophyte biomass data (Table 2) are only representative of the conditions at the time of sampling. However, our field observations showed that macrophyte senescence began around August 20th in 2004 and around August 10th in 2005. Our biomass measurements made in August are therefore representative of the maximum standing biomass of macrophytes reached in each lake. We extrapolated standing biomass to daily biomass for each lake for July and August, assuming a macrophyte biomass doubling time of about one month, as we observed in our benthic chambers. We then used our estimate of the biomass-based DOC release rate of 56 $\mu gC.gdw^{-1}.h^{-1}$ (see first chapter) to calculate the daily and then monthly DOC released in the whole lake (in $gC.lake^{-1}.month^{-1}$), accounting for differences in daily photoperiod among months. These monthly DOC release rates were then compared with the monthly estimation of DOC loads from watersheds for each lake (Prairie and Parkes, 2006.).

Ichetucknee River, Florida

This ecosystem is interesting to study because the river if formed exclusively by several high discharge springs, presenting very low DOC concentrations. The DOC measurements were

taken in March 2007 in the Ichetucknee River, Florida, USA (Lat. 29° 59' N, Long. 82° 45' W). From our incubation with benthic chambers on macrophytes, we estimated a mean DOC release rate by the macrophyte-epiphyte complex of 46.4 $\mu gC.gdw^{-1}.h^{-1}$ (SD ± 35.7; see third chapter). In order to calculate the daily DOC release, we used the average value of total macrophyte biomass measured by Kurz et al. (2004) at the end of May 2003 and 2004 (Table 3). As the biomass was measured in May, we used the corresponding photoperiod. We also used their estimation of river surface area.

In addition, we measured DOC concentration at the head of seven of the nine principal springs forming the Ichetucknee River; DOC concentrations varied between 0.17 and 0.65 $mgC.L^{-1}$, the mean DOC concentration being 0.45 $mgC.L^{-1}$ (±0.2) (one sampling date and one sampling station per spring, two samples analysed in duplicates). Knowing the water discharge for the entire group of springs (Scott et al. 2004, value of October 2001, variability of the discharge not included), we were able to calculate the daily DOC load in the river and compare it to the DOC load from the macrophyte-epiphyte complex.

5.4 RESULTS AND DISCUSSION

South-eastern Quebec Lakes

The relative importance of DOC released by the macrophyte-epiphyte complex at the lake scale is expressed as a percentage of the total DOC load (Table 5.1). Clearly, DOC released by the macrophyte-epiphyte complex is probably of limited quantitative significance to whole-lake metabolism, except in shallow lakes where macrophyte biomass is high (i.e. macrophytes cover the majority of the benthic zone). In these lakes, DOC release can represent up to half of the total DOC budget when the DOC load from the watershed is low. Since drier conditions cause the DOC load from the watershed to be lower, Schindler & Curtis (1997) suggested that global warming, and its associated decrease in precipitation, would lead to lake metabolism being sustained more by autochthonous than allochtonous carbon sources. Similarly, Schippers et al. (2004) modeled the response of macrophytes to atmospheric CO_2 increases and showed an increase in lake colonisation by these organisms. Therefore, we propose that global warming would cause an increase in the relative quantitative contribution of DOC released by the macrophyte-epiphyte complex to whole-lake metabolism. A weak quantitative contribution is however not synonymous of weak qualitative contribution; in fact the nature of the DOC released by macrophytes (carbonic acids and amino acids; Wetzel et Manny, 1972; Godmaire et Nalewajko, 1989) suggest a high lability and thus an important role for heterotrophic community.

Ichetucknee River

As in Quebec lakes, our estimates clearly show that the contribution of DOC released by macrophytes at the whole-river scale is low: DOC release by macrophyte-epiphyte complex only represent 10% of what is loaded from the springs (Table 5.2). It is worth to note that we considered only two sources of DOC in the river, the inputs from the springs and the DOC releases from living macrophytes. This is not sufficient to build up a DOC budget, which is not our goal. However, this exercise demonstrates the small importance of DOC release compared to DOC load from the springs (which are representative of the watershed). In 2004, Kurz et al. reported flow rates ranging from 0.01 to 0.55$m.s^{-1}$ in the Ichetucknee River,

indicating a highly variable water residence time. We can also expect the macrophyte biomass to be higher with low flow (colonization should thus be easier). Therefore in such systems, we propose that the relative importance of DOC release by macrophytes will depend on flow rate, ranging from highly significant in areas of low flow with high macrophyte biomass and high water residence time, to insignificant in areas of high flow with fewer macrophytes and low water residence time.

Table 5.1

Relative importance of DOC released by the macrophyte-epiphyte complex relative to DOC loads from watersheds in Quebec lakes. Estimations for July and August, in 2004 and 2005. MB corresponds to total standing macrophyte biomass density in lakes (in g.m-2), DOCm and DOCin are total DOC released by the macrophyte-epiphyte complex at the whole-lake scale (in $kg.m^{-1}$) and total DOC inflowing from the watershed (in $kg.m^{-1}$), respectively. P is the percentage of total DOC load attributable to macrophyte release

				July			August	
Lake	SD	MB	DOCme	DOC in	P	DOCme	DOC in	P
Argent	15/08/2004	24	268	26860	1%	528	51460	1%
Stukely	15/08/2004	17	792	6620	11%	1559	12800	11%
Trois Lacs	16/08/2004	68	2257	161440	1%	4444	ND	ND
Trois Lacs	21/07/2005	90	5306	68130	7%	8297	13570	38%
waterloo	15/08/2004	10	133	14919	1%	263	20830	1%

Table 5.2

Estimations of the DOC load from release by the macrophyte-epiphyte complex versus from the DOC load from the springs in the Ichetuknee River (FL). Macrophyte biomass and river surface area from Kurz *et al.* (2004). Spring discharge from Scott *et al.* (2004)

Macrophyte-epiphyte complex	Biomass ($gdw.m^{-2}$)	Surface (m^2)	DOCrelease rate ($ugC.gdw^{-1}.h^{-1}$)	Photoperiod (h)	DOC load ($kgC.d^{-1}$)
	493 ± 215	142000	46.4 (± 35.7)	11.5	**37.36**
Springs	DOC concentration ($mgC.L^{-1}$)		Springs discharge ($m^3.scc^{-1}$)	DOC load ($kgC.d^{-1}$)	
	0.45		5.27	**204.78**	

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CONCLUSION GÉNÉRALE

L'étude de la production de carbone organique dissous par les macrophytes submergées et le phytoplancton a débuté à la fin des années 1960, avec la vision que ce phénomène représentait une perte pour l'organisme. En effet une partie de la production primaire se retrouve libérée dans l'eau et n'est donc pas utilisée pour la production de biomasse. Les mécanismes sous-jacents ont été abordés sans que des études approfondies les décrivent. Récemment, la problématique des bilans de carbone dans les systèmes aquatiques a entraîné un nouvel intérêt pour ce phénomène puisque l'implication des macrophytes dans le métabolisme global des écosystèmes devait être prise en compte. Ce bref résumé de la problématique globale montre que les champs d'étude potentiels sont divers puisque tous peu documentés: aspect physiologiques du processus, quantification, implication à l'échelle de l'écosystème. Or l'étude de ces aspects requiert des méthodes et approches très différentes. Notre objectif de départ était d'appréhender l'implication quantitative de la production de COD par les macrophytes submergées à l'échelle de l'écosystème et pour ceci de déterminer les facteurs influençant la production. Or ces deux objectifs sont difficilement conciliables puisqu'une approche *in situ* permet une meilleure quantification du phénomène (nous étudions la communauté macrophytes-épiphytes, ainsi que la communauté phytoplanctonique et hétérotrophe environnante), alors que les facteurs sont plus rigoureusement étudiés en laboratoire. Nous avons donc fait le compromis de faire un étude *in situ* avec le plus de réplicats possibles dans des conditions variables (différents lacs).

Notre hypothèse de départ était que production de COD et photosynthèse étaient liées. Ceci a déterminé notre plan d'échantillonnage puisque nous souhaitions tester les facteurs

influençant la photosynthèse pour voir si ils influençaient la production de COD. Notre première approche portait sur la possibilité de quantifier le phénomène mais était également centrée sur l'étude de l'effet de la lumière et de la température sur la production de COD (premier chapitre). Nous avons non seulement montré que ces facteurs n'ont pas d'influence, mais aussi que la production n'était pas différente chez les différentes espèces dominant les lacs étudiés. Par la suite nous avons étudié les effets des nutriments et du CO_2, éléments connus pour faire varier les taux de photosynthèse. La mise en évidence d'une relation négative entre concentration en CO_2 et production de COD par les macrophytes a entraîné la remise en question de notre hypothèse de départ. En effet le CO_2 est connu pour être limitant pour la photosynthèse (particulièrement en milieu aquatique à cause de sa faible dissolution dans l'eau) et une augmentation de sa concentration entraîne une augmentation de productivité comme nous l'avons démontré dans le 4^e chapitre. Puisque nous avons observé de plus grandes productions de COD lorsque le CO_2 est en faible concentration (chapitres 2 et 4), nous avons posé une hypothèse alternative présentant la production de COD comme une réponse à une limitation en CO_2. L'étude de la productivité des communautés de macrophytes nous a d'ailleurs montré que la production de COD est positivement liée aux taux de disparition du HCO_3^- dans le milieu. Or, il est connu qu'en cas de limitation en CO_2, les organismes photosynthétiques développent des mécanismes de concentration du carbone afin d'augmenter leur concentration intracellulaire en CO_2. Parmi ces mécanismes, il existe des enzymes extra et intracellulaires, les anhydrases carboniques, qui transforment le HCO_3- en CO_2. Dans le 4^e chapitre nous discutons donc la possibilité qu'une partie du COD produit par les macrophytes soit des enzymes ou fasse partie d'un complexe enzymatique ayant pour but d'augmenter la concentration en CO_2 dissous autour des communautés. Ces découvertes sur

le lien entre CO_2 et production de COD nous ont mené vers une compréhension plus physiologique du phénomène et nous ont éloigné de l'étude du rôle du COD produit dans le métabolisme global des écosystèmes. Cependant nous avons démontré que l'utilisation d'un taux moyen de COD produit peut raisonnablement être appliqué aux diverses espèces présentes dans un écosystème (1er chapitre). Ainsi nous avons pu comparer des estimations de production globale de COD par les macrophytes submergées à des estimations d'apport de COD allochtone (dont la production se fait à l'extérieur de l'écosystème). Il en ressort que cette production est faible par rapport aux apports des bassins versants. La question de la labilité du COD produit par rapport au COD allochtone reste posée, ce qui nous empêche de conclure sur le fait que le COD produit par les macrophyte est négligeable dans le métabolisme global des écosystèmes.

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