UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LE RÔLE DES PRODUCTEURS SECONDAIRES DANS LES RÉSEAUX TROPHIQUES PLANCTONIQUES ET LE CYCLE DU CARBONE DES LACS ET RÉSERVOIRS DU NORD DU QUÉBEC

THÈSE
PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE

PAR
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JANVIER 2007

Il fallait tout rebâtir sur nouveaux frais. Faire le vide. Tout reprendre à la base, en partant des choses les plus élémentaires. Quelques années plus tard, lors d'une conversation sur ce thème, l'écrivain Yvon Taillandier me rappela cette formule de Lao Tseu, que j'approuve sans réserve : « Les rayons de la roue sont nombreux, mais c'est le vide qu'il y a au milieu qui fait avancer la charrette ».

TÀPIES

REMERCIEMENTS

Je comptais prendre un peu de poids en venant faire une thèse en Amérique du Nord. Mais cet objectif n'a pas été atteint et je reste toujours aussi squelettique. Serge a dit que je suis un vrai organisme hétérotrophe, malgré que mon zooplancton ne le soit pas. Pourtant, au cours des 4 dernières années, j'ai reçu un support constant me permettant d'assurer une bonne alimentation nutritive et intellectuelle. La vérité est que je suis aussi autotrophe car mes efforts de production ont été orientés vers d'autres organismes dont la biomasse augmente aujourd'hui plus vite que la mienne. Vous êtes nombreux à avoir contribués de près ou de loin à cette thèse et je vous remercie pour votre support, votre aide, votre patience, votre confiance et votre temps. Dolors a été une super directrice parce qu'elle m'a laissé conduire cette thèse avec beaucoup de supports et de liberté. Le groupe de l'Uqam forme un environnement formidable pour y étudier les sciences aquatiques et c'est en le quittant que je le réalise le plus. Notre labo forme un groupe plutôt rigolo, formé de personnalités très différentes, favorisant quelques bonnes étincelles. Aujourd'hui encore, je ne suis pas tout à fait sur d'avoir compris le fonctionnement du GÉOTOP mais sa contribution pour les analyses isotopiques a été précieuse. J'ai eu la chance de recevoir le support financier du Fond de Recherche sur la Nature et les Technologies du Québec, du GRIL et du GÉOTOP. Je remercie les membres des jurys qui sont intervenus au cours de ma scolarité et ont donné de leur temps et produit des commentaires permettant d'améliorer mes travaux.

Je remercie Mariame Touré qui a gardé mes enfants (avec Nathalie) durant ces dernières années, Hélène Thibault pour son amitié et support, Bernadette Pinel-Alloul et Antonella Cattaneo pour leur support; mes amis de l'université de longue date Alain Patoine, Olivier Perceval, Rémy Tadonleke, Anik Brind'Amour et Hélène Limèn. Serge Paquet a apporté un soutien énorme dans mon travail et je tiens à reconnaître ici que cette thèse ne serait pas la même sans lui. Marie-Laure Querrec a

relu l'introduction. Alain Tremblay m'a aidé à participer à des congrès et Jean Louis Fréchette m'a offert quelques heures de vols au dessus du réservoir SM-3. Patricia Chambers a facilité mon accès au NWRI en fin de rédaction. Merci à tous.

Je remercie ma famille et en particulier mes parents et ma sœur pour avoir permis à l'aventure canadienne d'avoir lieu, malgré les difficultés causées par l'éloignement. Finalement, cette thèse est dédiée à Chantal, Gwen et Clara pour leur énorme support et sans qui je n'aurai pas trouvé l'énergie pour poursuivre ce travail. Cette fois, c'est pour de bon, vous pouvez prendre vos ciseaux et découper ces feuilles de papier « de qualité supérieure » d'après l'emballage.

AVANT PROPOS

Cette thèse est constituée de 4 chapitres présentés sous forme d'articles scientifiques. Le premier chapitre vise à déterminer quelle est la meilleure méthode permettant d'obtenir la composition isotopique en carbone des algues et est actuellement soumis pour publication dans la revue *Limnology and Oceanography-Methods*. Les résultats de cette comparaison de méthodes ont ensuite été utilisés afin d'évaluer l'importance des sources de carbone d'origine terrestre et algale dans la diète du zooplancton. Ce second chapitre est actuellement soumis pour publication dans la revue *Limnology and Oceanography*. L'importance du zooplancton comme maillon de la chaine trophique ainsi que les sources de variabilités influançant la signature en azote des organismes sont examinées dans le troisième chapitre.
Le chapitre quatre traite de la dynamique du zooplancton au cours de la mise en eau du réservoir LG-2. Pour cette étude, une base de données temporelles a été utilisée et les résultats ont été comparés avec des données prélevées dans le cadre de cette thèse. Cette étude est publiée dans un ouvrage monographique. Finalement, une première annexe contient un protocole développé pour l'analyse isotopique d'échantillons de zooplancton de faible poids (Helene Limén and Jérôme Marty, 2004, Application Note GV Instruments AN13). Une seconde annexe présente les résultats préliminaires des sources de carbone pour le zooplancton des lacs et réservoirs du Nord du Québec (Marty, J. and Planas, D. 2005. Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie. 29:342-344)

Cette thèse est une contribution du projet « Assessment and modeling of the production and emission of greenhouse gases from reservoir » qui a reçu l'octroi d'une subvention stratégique du Conseil de Recherche en Sciences Naturelles et en Génie du Canada, impliquant un partenaire industriel, Hydro-Québec (No. STP224191-99).

J'ai réalisé l'ensemble de l'échantillonnage des données présentées, l'analyse des données et la rédaction des chapitres. Jean Nicolas Jasmin a contribué au second chapitre en déterminant les taux d'assimilation du zooplankton. Marie-Andrée Gagnon et Jean-François Ouellet ont participé aux campagnes d'échantillonnage. L'utilisation de données extérieures à la thèse est précisée dans les chapitres concernés.

TABLE DES MATIÈRES

AVANT PROPOS.....v
LISTE DES FIGURES.....xii
LISTE DES TABLEAUX.....xv
RÉSUMÉ.....xvii
INTRODUCTION GÉNÉRALE.....1
Contexte de la thèse.....1
L'utilisation des isotopes stables comme outil écologique.....3
Structure de la thèse.....4

CHAPITRE I: A COMPARISON OF METHODS TO DETERMINE CARBON ALGAL SIGNATURES IN FRESHWATER.....8

1.1 Résumé/Abstract.....9
1.2 Introduction.....10
1.3 Materials and procedures.....12
1.3.1 Study area and sampling.....12
1.4 Methods to determine carbon algal signature.....13
1.4.1 Method 1: δ^{13}C-algae as particulate organic carbon (δ^{13}POC).....13
1.4.2 Method 2: δ^{13}C-algae based on particulate organic matter and algal proportion (δ^{13}algae-POC).....13
1.4.3 Method 3: δ^{13}C-algae determination from δ^{13}C of dissolved inorganic carbon (δ^{13}algae-DIC).....15

1.4.3 Method 4: δ^{13}C-algae as primary consumers signature (δ^{13}*Daphnia* sp.)....17

1.4.4 Method 5: δ^{13}C-algae determination with enriched phytoplankton samples (δ^{13}algal-samples)....17

1.5 Assessment....18

1.5.1 Determination of $\delta^{13}C_{terr.}$, C:Chl ratio and ε_p....18

1.5.2 Algal signatures for each approach....20

1.5.3 Effect of variation in C:Chl ratio on the proportion of algal carbon in POM....20

1.5.4 δ^{13}algal-POC and assumptions in mixing model....24

1.5.5 Is δ^{13}algal-sample the best estimate of algal carbon signature....25

1.5.6 Comparisons between methods....26

1.6 Discussion....29

1.7 Conclusion and recommendations....31

1.8 Acknowledgements....32

1.9 References....33

CHAPITRE II: AUTOCHTHONOUS CARBON AS THE MAIN SUBSIDY FOR ZOOPLANKTON IN OLIGOTROPHIC LAKES AND RESERVOIRS FROM THE BOREAL ECOREGION....39

2.1 Résumé/Abstract....40

2.2 Introduction....41

2.3 Materials and methods....43

2.3.1 Study sites....43

2.3.2 Sampling procedures and analysis....45

2.3.3 Stable isotopes analysis (SIA)....45

2.3.4 Calculations and zooplankton carbon source....47

2.3.5 Algae versus bacteria as food source for zooplankton.....48
2.4 Results.....49
2.4.1 Carbon isotopic signature of dissolved inorganic and organic carbon.....50
2.4.2 Carbon stable isotope of particulate organic matter and phytoplankton.....50
2.4.3 Zooplankton carbon signatures.....54
2.4.4 Labeling experiment.....58
2.5 Discussion.....60
2.5.1 Sources of carbon available to zooplankton and utilization.....60
2.5.2 Heterotrophy and isotopic composition of major carbon pools.....64
2.5.3 Variations in autochthony versus allochthony in oligotrophic ecosystems.....66
2.6 Conclusions.....68
2.7 Acknowledgements.....68
2.8 References.....69

CHAPITRE III: SURFACE TEMPERATURE AND TAXONOMY EXPLAIN REGIONAL $\delta^{15}N$ ZOOPLANKTON VARIABILITY IN OLIGOTROPHIC ECOSYSTEMS.....75

3.1 Résumé/Abstract.....76
3.2 Introduction.....77
3.3 Study sites.....78
3.4 Methods.....80
3.4.1 Sampling and chemical analysis.....80
3.4.2 Stable isotope analysis (SIA).....80
3.4.3 Sources of variability in zooplankton $\delta^{15}N$.....81

3.5 Results.....82
3.5.1 $\delta^{15}N_{ZOO}$ variability.....83
3.5.2 Ecosystems and sites effect in the $\delta^{15}N$-$\delta^{13}C$ relationship.....86
3.5.3 Taxonomy effect in the $\delta^{15}N$-$\delta^{13}C$ relationship.....87
3.5.4 Taxonomy effect in the $\delta^{15}N$-temperature relationship.....88
3.5.5 Cross-studies validation.....89
3.6 Discussion.....90
3.7 Conclusions.....94
3.8 Acknowledgements.....95
3.9 References.....96

CHAPITRE IV: PLANKTONIC COMMUNITY DYNAMICS OVER TIME IN A LARGE RESERVOIR AND THEIR INFLUENCE ON CARBON BUDGETS.....98

4.1 Résumé/Abstract.....99
4.2 Introduction.....100
4.3 Materials and methods.....102
4.3.1 Long-term data set (1978-1984).....102
4.3.1.1 Study area.....102
4.3.1.2 Material and methods.....103
4.3.2 Recent data set.....106
4.3.2.1 Study area.....106
4.3.2.2 Zooplankton biomass estimates.....106
4.4 Results.....108
4.4.1 Long-term variation in zooplankton community (1978-1984).....108
4.4.2 Relation with water quality and trophic status.....110
4.4.3 Recent data set: A comparison between reservoirs.....114
4.4.3.1 Limnoplankton.....114

4.4.3.2 Zooplankton specific weight.....117
4.5 Discussion.....119
4.6 Conclusions.....123
4.7 References.....123

CONCLUSIONS GÉNÉRALES.....129

RÉFÉRENCES INTRODUCTION ET CONCLUSION GÉNÉRALE.....134

ANNEXE A: STABLE CARBON AND NITROGEN ISOTOPE ANALYSIS ON SMALL-SIZED SAMPLES: A PROTOCOL FOR PREPARATION AND ANALYSING MICROSCOPIC ORGANISMS.....140

ANNEXE B: SOURCE OF CARBON FOR ZOOPLANKTON IN LAKES AND RESERVOIRS FROM NORTHERN QUEBEC (CANADA).....151

LISTE DES FIGURES

Figures

Page

1.1 Phytoplankton fractionation (εp) (‰), as a function of the ratio between phytoplankton growth rate and CO_2 concentration ($\mu/[CO_2]$, $L.\mu mol^{-1}.d^{-1}$), for reservoirs stations (black circles) and lakes (grey circles), as modified after Karlsson et al. (2003) (open squares). Dashed lines indicate regressions obtained for the marine species described in Popp et al (1998). 16

1.2 Fractionation values (ε_p) calculated to match *Daphnia* sp. signatures and δ^{13}algae-POC, assuming CO_2 as the only source of carbon. One outlier (open circle) was excluded from analysis.....19

1.3 Percentage of algal carbon in POM based on minimum Chl. a (Chl.a =0.6 µg.L-1; POC=431.2 µg.L-1), maximum Chl. a (Chl.a =4.4 µg.L-1; POC=266 µg.L-1) and median conditions (Chl.a =1.6 µg.L-1; POC=273.5 µg.L-1) (dashed line), as a function of C:Chl. a ratio. Grey area indicates the range of C:Chl. a ratio calculated for enriched algal samples.....22

1.4 The relationship between δ^{13}algae-POC and algal carbon proportion in POM. The left axis is the theoretical relationship between δ^{13}algae-POC and the proportion of algal carbon in particulate organic carbon, for a range of δ^{13}POC values ($\delta^{13}C_{terr.}$ was set at -27‰). The right axis is the frequency in the proportion of algal carbon in POM (C:Chl was set at 80).....23

1.5 Correlation matrix between each method considered as algal carbon signatures. Dashed line indicates equal δ^{13}C isotopic signatures.....27

2.1 Mean values (± SE) of (A) $\delta^{13}C_{POM}$, (B) percentage of algal carbon in seston, (C) $\delta^{13}C_{ALG}$ and (D) $\delta^{13}C_{ZOO}$ per ecosystem in spring (dashed) and summer (gray).....51

2.2 Correlation matrix between $\delta^{13}C_{POM}$, $\delta^{13}C_{ZOO}$ and $\delta^{13}C_{ALG}$. Dashed line indicates equal δ^{13}C isotopic composition. White points were identified as outliers and excluded from statistical analysis.....56

2.3 Relationship between mean $\delta^{13}C_{ZOO}$ and ecosystems surface area. Regression line was fitted on reservoirs data only.....58

2.4 Relationship between algal and bacterial assimilation rates. DA =*Daphnia* sp., CY =Cyclopoids, CA =Calanoids, EP =*Epischura* sp. and HO = *Holopedium* sp.....59

3.1 Mean (± 1 SE) $\delta^{15}N$ and $\delta^{13}C$ of zooplankton (‰) within each ecosystem and season.....84

3.2 Frequency distributions of length food web (‰) within zooplankton community and corresponding number of trophic levels.....85

3.3 $\delta^{15}N$ of zooplankton taxonomic groups or species as a function of $\delta^{13}C$ (A) and surface temperature (B). Open diamonds in the $\delta^{15}N$-$\delta^{13}C$ relationship indicate outliers.....88

3.4 Observed versus predicted $\delta^{15}N$ (‰) values obtained from the surface water temperature model corrected for taxonomic effects based on the temporal series from Lake Loch-Ness (open circles), and two sets of lakes from Estrie (Canada) (black triangles) and Sweden (black circles). See methods for data sources.....90

3.5 Mean zooplankton $\delta^{15}N$ (‰) as a function of nitrate and nitrite concentration ($\mu g \cdot L^{-1}$) in the SM-3 reservoir (Left) and all other ecosystems (Right).....93

4.1 Localisation of the sampling sites in LG-2 reservoir, for the long-term data set and for 2001 sampling.....103

4.2 Annual variations (1978-1984) in zooplankton biomass in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE.....109

4.3 Annual variations (1978-1984) in total phosphorus and Chl.a in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE.....112

4.4 Annual variations (1978-1984) in carbonic acid (H_2CO_3), pCO_2, oxygen and pH in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE.....113

4.5 Ash free dry weight (mg. AFDW.L^{-1}) of seston for a group of northern Quebec lakes and reservoirs. Levels not connected by the same letter are significantly different. Data are shown as the mean ± SE.....115

4.6 Comparison of zooplankton weight, phytoplankton biomass and evasive CO_2 flux for a group of northern Quebec lakes and reservoirs. Data are presented as the mean ± SE.....118

A.1 Stable carbon and nitrogen signatures of *S. quadrispinosus* and *P. sulfincola* from Explorer ridge. Error bars represent SE.....148

A.2 Stable carbon and nitrogen isotopic composition of zooplankton organisms (n=3) in lake Jean-Marie (Northern Quebec). Error bars represent standard error.....149

LISTE DES TABLEAUX

Figures

Page

1.1 Physical, chemical and biological characteristics of 13 lakes (L) and 6 reservoirs (R) in Northern Canada for the summers of 2001–2003. T, temperature; μ, phytoplankton growth rate; ε_p, phytoplankton fractionation..14

1.2 Stable carbon isotopic composition ($\delta^{13}C$, ‰) of DIC and algal signatures, according to the 5 methods compared in this study.....21

1.3 Mean carbon signature (and std. error) (‰) for each approach and comparison of each pair based on Tukey-Kramer HSD test. Variables not connected by same letter are significantly different.....28

2.1 Morphometric and limnological properties of reservoirs (Res.) and lakes situated in James Bay (JB), Manicouagan (M) and Sainte-Marguerite (SM) regions. Mean value (±SE) is indicated when several sites (n) were visited per ecosystem in spring (Sp) and summer (Sm).....44

2.2 Mean (± S E.) $\delta^{13}C$ values per ecosystem (‰) of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), particulate organic carbon (POC) and zooplankton groups or taxa.....52

2.3 Multiple regression models for prediction of $\delta^{13}C_{POM}$ and $\delta^{13}C_{ZOO}$.....53

2.4 Partition of variance of $\delta^{13}C_{ZOO}$ for 2002 (spring/summer) and 2001-2003 (summer) periods. Because of unbalanced data set, Residual maximum likelihood (REML) was used to determine variance components as described in Matthews and Mazumder (2003).....55

3.1 $\delta^{15}N$ values (‰) obtained for zooplankton taxonomic groups or genus, in reservoirs (Res.) and lakes. Mean and standard error are indicated when several sites (n) were visited in reservoirs. Spring and summer data are identified as Sp and Sm respectively.....79

3.2 Variance partition of zooplankton $\delta^{15}N$ for 2002 (spring/summer) and 2001-2003 (summer) data sets.....86

4.1 Physical and chemical characteristics in lacustrine and riverine stations of LG-2 and in a reference lake, from 1978 to 1984. Data represents the annual means for each station, with minimal and maximal values.....105

4.2 Multiple regression model for prediction of total zooplankton biomass during flooding period of LG-2 reservoir.....110

4.3 Limnoplankton biomass expressed as ash free dry weight (mg. AFDW.L^{-1}) according to size fraction, in a series of 4 reservoirs from Northern Quebec and in natural lakes. Data are presented as the means over sampling period and stations, with minimal and maximal values.....116

A.1 Copepod (*Stygiopontius quadrispinosus*) and polychaete (*Paralvinella sulfincola*) carbon and nitrogen isotopic composition. Sample weight (mg), type of tin capsule, trap current (μA), and standard (C6 sucrose from IAEA for carbon and N-1 from IAEA for nitrogen) precision (±SD) are also presented.....144

A.2 Carbon and nitrogen content (means±SD, n=44) of freshwater zooplankton organisms and the number of individuals required for dual $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis, using the 600 trap current (μA).....146

A.3 Precision of stable carbon and nitrogen isotopic composition of freshwater organisms presented as the variation (SD) of standards used (C6 sucrose from IAEA for carbon and N-1 from IAEA for nitrogen).....147

B.1 Main characteristics of sample reservoirs and lakes.....153

B.2 Partition of variance for zooplankton carbon signatures data.....155

RÉSUMÉ

L'objectif général de la thèse était de déterminer le rôle du zooplancton dans le cycle du carbone et les réseaux trophiques planctoniques des lacs et réservoirs oligotrophes du Nord du Québec. Une approche isotopique a été appliquée pour répondre à cet objectif.

Dans le premier chapitre, nous avons comparé plusieurs estimés de la signature en carbone des algues obtenus en appliquant une série de méthodes utilisées dans la littérature. La signature en carbone algal obtenue à partir de la signature de la matière particulaire, corrigée pour la biomasse algale était similaire à celle d'un organisme herbivore du zooplancton tel que *Daphnia*. De plus, les signatures de ces deux approches étaient comparables à celles obtenues pour une série d'échantillons d'algue concentrée. Par contre, nous avons montré que la signature du phytoplancton calculée à partir de la signature du carbone inorganique dissout et du fractionnement algal était significativement différente des autres méthodes. Ces résultats impliquent que la forme du carbone fixé lors de la photosynthèse ainsi que le fractionnement algal doivent être précisément identifiés afin de déterminer la signature algale. Nos résultats montrent que les modèles prédictifs du fractionnement algal développés pour des espèces marines ne peuvent être appliqués en milieux d'eaux douces.

Dans le second chapitre, les résultats de la comparaison de méthode pour déterminer la signature du carbone algal ont été appliqués afin d'évaluer l'importance des apports allochtones versus autochtone pour la communauté du zooplancton, dans des écosystèmes source de CO_2 pour l'atmosphère tels que les réservoirs et les lacs boréaux. Nous avons montré que l'ensemble de la communauté du zooplancton dépendait des apports d'origine autochtone comme principale source d'alimentation. Cette conclusion était supportée par la similarité entre la signature en carbon du zooplancton et celle des algues. De plus, à partir d'une approche expérimentale, nous avons montré que le taux d'assimilation algal par le zooplancton était de six fois supérieur au taux d'assimilation bactérien. Ce chapitre montre que les producteurs primaires supportent la production du zooplancton, même dans les écosystèmes peu productifs où le carbone d'origine terrestre est dominant.

Le chapitre 3 vise à déterminer les sources de variation de la signature en azote des organismes du zooplancton. Une partition de la variance a mis en évidence l'importance de la taxinomie et de la saisonalité pour expliquer les variations en $\delta^{15}N$ du zooplancton. Nous avons montré que la signature en carbon des organismes peut prédire les valeurs de $\delta^{15}N$, mais ne permet pas de tenir compte des variations saisonnières. Par contre, la température de surface, en tenant compte des variations taxinomiques, a permis de prédire la signature le $\delta^{15}N$ des organismes pour l'ensemble de nos données. Nous avons vérifié la validité de ce modèle en

l'appliquant à d'autres données de la littérature. Cette analyse a indiqué que la température de surface permet de prédire les variations inter- et intra-écosystèmes dans les milieux oligotrophes et révèle l'existence d'une signature de base régionale qui ne peut être prédite par le modèle. De plus, cette étude montre que le zooplancton est une communauté complexe, représentant plusieurs niveaux trophiques dans la chaîne alimentaire. Cette caractéristique a des conséquences importantes pour la description de l'ensemble des réseaux trophiques.

Le dernier chapitre de la thèse examine la dynamique du zooplancton lors de la mise en eau du réservoir LG-2. Les organismes dont le taux de reproduction est rapide (Rotifères et Cladocères) répondent en premier face à l'augmentation des ressources nutritives. Les variations de la biomasse totale du zooplancton lors des premières années de formation du réservoir étaient expliquées par une combinaison de variables physiques (temps de résidence, température et turbidité), chimiques (phosphore total) et biologiques (chlorophylle a). A partir des données récentes, nous avons montré les effets descendants du zooplancton sur la biomasse algale et sur ses conséquences sur les flux de carbone observés à l'interface eau-atmosphère.

Mots clés : zooplancton, carbone allochtone, carbon autochtone, isotopes stables, réseaux trophiques planctoniques, cycle du carbone, réservoirs, lacs, région boréale

Key words: zooplankton, allochthonous and autochthonous carbon, stable isotopes, planktonic food webs, carbon cycling, boreal lakes and reservoirs.

INTRODUCTION GÉNÉRALE

Contexte de la thèse

La forêt boréal occupe 3% de la surface émergée de notre planète, correspond à un quart de la surface boisée et couvre 35% du territoire canadien. Cette étendue de verdure est clairsemée de millions de lacs dont le nombre exact n'est pas encore connu à ce jour. Environ 9% du territoire canadien est recouvert par les lacs, atteignant jusqu'à 15 % pour le nord québécois. Par conséquent les écosystèmes aquatiques des régions boréales contribuent significativement au cycle de l'eau et du carbone, tant à l'échelle régionale que planétaire.

Les zones riches en lacs sont aussi les plus riches en réservoirs. A l'échelle mondiale, la surface des réservoirs ne cesse d'augmenter (Downing et al. 2006). On compte au Canada 10 des 40 plus grands réservoirs du monde. La capacité de rétention d'eau des grands réservoirs du pays est équivalente à deux années de ruisellement national ou encore à un quart du volume des Grands Lacs (Prowse et al. 2004). Le Québec possède parmi les plus grands réservoirs du pays (LG-2 et Manicouagan) et l'hydro-électricité représente 97 % de la production énergétique de la province. Les réservoirs sont nombreux et marquent le paysage des régions boréales, et pourtant, que savons nous de leur fonctionnement ? La contamination en mercure des populations du nord du Québec suite à la création des barrages de la Baie James n'était pas prévue. Aujourd'hui encore, il n'existe qu'un seul livre portant sur la limnologie des réservoirs (Thornton 1990).

Cette thèse apporte de nouvelles connaissances sur le fonctionnement des lacs et des réservoirs des régions boréales. En particulier, deux grands thèmes sont abordés : le cycle du carbone et les caractéristiques de la structure des réseaux

trophiques planctoniques. Le rôle des écosystèmes aquatiques dans le cycle global du carbone est un sujet recevant une attention grandissante de la part des écologistes (Cole et al. 1994; Del Giorgio and Duarte 2002; Sobek et al. 2003; Duarte and Prairie 2005). Un des buts de ces études est de déterminer si les écosystèmes aquatiques agissent comme des sources ou des puits de carbone pour l'atmosphère. La capacité d'un écosystème à absorber ou relarger du carbone dépend du ratio entre la quantité de matière produite via la production primaire et la quantité de matière minéralisée via la respiration bactérienne (Cole et al. 1994). Les écosystèmes aquatiques de la forêt boréale sont généralement peu productifs à cause des faibles apports nutritifs provenant des sols de cette région et par conséquent sont généralement considérés comme des sources de carbone pour l'atmosphère (Jonsson et al. 2003; Planas et al. 2005).

Afin de compendre le cycle du carbone des écosystèmes aquatiques, il est nécessaire de déterminer les processus qui stucturent les producteurs primaires et la communauté bactérienne. Une communauté en particulier joue un rôle clé pour les premiers niveaux des réseaux trophiques : le zooplancton.

La communauté du zooplancton occupe une position centrale dans les réseaux trophiques à cause de son double rôle écologique : celui de prédateur et de proie (Galbraith 1967; Hutchinson 1971). Les organismes du zooplancton peuvent être séparés en différents groupes fonctionnels (herbivores, carnivores, détritivores) ou taxinomiques (Cladocères, Copépodes, Rotifères). Cette communauté contrôle par effets descendants les producteurs primaires (Lampert et al. 1986; Kerfoot et al. 1988) et l'ensemble des communautés microbiennes (Sherr and Sherr 1984; Zöllner et al. 2003; Sanders and Wickham 1993). Finalement, la stucture de la communauté du zooplancton influence l'état d'un écosystème à agir comme une source ou un puits de carbone pour l'atmosphère (Schindler et al. 1997).

L'utilisation des isotopes stables comme outil écologique.

L'application des techniques isotopiques en écologie aquatique est relativement récente (Schindler and Lubetkin 2004). A partir de la signature en carbone et en azote des organismes, les flux de matière et d'énergie au sein des réseaux trophiques peuvent être quantifiés. L'intérêt de l'utilisation de l'isotope stable du carbone ($\delta^{13}C$) repose sur le fait que la signature d'un consommateur reflète celle de sa source alimentaire (DeNiro and Epstein 1978). Dans les écosystèmes aquatiques, la signature en carbone permet d'évaluer l'importance des apports d'origine benthique et pélagique pour les consommateurs (France 1995; Hecky and Hesslein 1995). Plus récemment, de nombreuses études ont appliqué les techniques isotopiques afin de déterminer l'importance du carbone allochtone et autochtone pour les réseaux trophiques (Jones et al. 1998; Grey et al. 2000; Martineau et al. 2004; Karlsson et al. 2003; Pulido-Villena et al. 2005; Carpenter et al. 2005). Afin de déterminer l'importance de plusieurs sources de carbone à partir de la signature d'un consommateur, des modèles de mélange sont appliqués et requièrent la distinction de la signature de chaque source (Phillips and Gregg 2003). Dans les écosystèmes aquatiques d'eau douce, cette condition peut limiter l'application des techniques isotopiques car il arrive fréquemment que la signature du carbone terrestre soit similaire à celle du carbone algal (Cole et al. 2002). Dans ce cas, des approches expérimentales visant à éloigner la signature des producteurs primaires de la signature allochtone représente une solution pour palier à ce problème (Pace et al. 2004).

La composition isotopique en azote ($\delta^{15}N$) est utilisée comme outil afin de déterminer la position trophique d'un consommateur au sein de la chaîne alimentaire. Ceci est due au fait que la signature d'un consommateur est enrichie, comparée à sa source alimentaire (DeNiro and Epstein 1981; Minagawa and Wada 1984). L'utilisation de l'isotope de l'azote en écologie aquatique a permis de décrire la

complexité des réseaux trophiques (Peterson and Fry 1987), de détecter des perturbations reliés aux activités humaines (Cabana and Rasmussen 1996; Vander Zanden et al. 1999) et de prédire la bioaccumulation de contaminants (Cabana and Rasmussen 1994). Dans les écosystèmes aquatiques, la signature en azote des producteurs primaires et des consommateurs varie spatialement et temporellement au sein d'un même écosytème ou parmi plusieurs écosystèmes d'une même région (Yoshioka and Wada 1994; Gu et al. 1996; Leggett et al. 2000).

Structure de la thèse

Dans cette thèse, j'ai appliqué une approche isotopique afin de répondre aux objectifs suivants :

1 Comment déterminer une signature du carbone algal pour des écosystèmes oligotrophes?

Afin d'interpréter la signature des consommateurs, il est nécesaire de connaître la signature des principale sources de carbone intervenant dans la diète. Dans les milieux aquatiques d'eau douce, les sources de carbone d'origine allochtone et autochtone représentent les principales sources de carbone particulaires. Dans les eaux colorées des écosystèmes oligotrophes du bouclier Canadien, les apports d'origine terrestre sont les plus abondants (Jones 2005). Par conséquent, il est difficile d'isoler physiquement la matière d'origine autochtone dans ces écosystèmes. Afin pallier à ce problème de séparation, des méthodes indirectes sont utilisées pour inférer la signature en carbone des algues. Dans ce chapitre, j'ai comparé les signatures du carbone algal obtenues à partir d'une série de méthodes basées sur la signature du carbon particulaire, du carbon inorganique dissout et d'un organisme herbivore du zooplancton. J'ai déterminé la concordance entre ces méthodes et,

lorsque des différences étaient présentes, j'ai identifié les raisons possibles supportant ces différences.

2 Quelle est l'importance du carbone d'origine allochtone et autochtone pour les organismes du zooplancton dans les écosystèmes hétérotrophes ?

Cette étude constitue une application des résultats du chapitre précédent. Le carbone d'origine terrestre joue, via le processus de la respiration, un rôle important dans le cycle du carbone des écosytèmes aquatiques. Lorsque la respiration excède la production, l'écosystème est alors considéré hétérotrophe (Cole et al. 1994). Cependant, l'utilisation de cette ressource par les organismes métazoaires demeure peu connue. Si le carbone d'origine allochtone représente une part importante de la diète des organismes dans des petits lacs (Jones et al. 1999; Karlsson et al. 2003; Carpenter et al. 2005; Pulido-Villena et al. 2005), les algues représentent la principale source de carbone dans plusieurs types d'écosystèmes hétérotrophes (Thorp 2002; Bunn et al. 2003; Martineau et al. 2004; Sobczak et al. 2005). A partir d'une approche isotopique, j'ai ainsi déterminé, dans une série de lacs et de réservoirs oligotrophes, l'importance des apports allochtones versus autochtones pour les groupes taxinomiques du zooplancton (ex : Calanoïdes, Cyclopoïdes) ou les principaux genres (ex : *Daphnia* sp, *Epischura* sp.). L'hypothèse posée était que les apports de carbone d'origine allochtone jouent un rôle important dans la diète des organismes du zooplancton des écosystèmes oligotrophes, en particulier dans les réservoirs dont le budget de carbone est influencé par des apports provenant de la décomposition de la matière organique inondée.

3 Quelles sont les sources de variation de la signature en azote ($\delta^{15}N$) du zooplancton?

En écologie aquatique, les sources de variabilité de la signature en azote sont moins connues que celles du carbone. Les variations inter-écosystèmes sont généralement reliées à la signature des apports des composés azotés inorganiques et aux processus de transformation du cycle de l'azote qui affectent la signature de base de l'écosystème (Vander Zanden and Rasmussen 1999; Leggett et al. 2000; Post 2002; Karlsson et al. 2004). D'importantes variations en $\delta^{15}N$ peuvent être aussi observées au sein d'un même écosystème (Leggett et al. 2000; Syväranta et al. 2006). Dans les systèmes perturbés, la signature en $\delta^{15}N$ d'un consommateur est reliée à l'influence de sources ponctuelles d'origine anthropique (Cabana and Rasmussen 1996; Vander Zanden et al. 2005) alors que dans les milieux oligotrophiques, les variations intra-écosystèmes reflètent généralement des différences d'habitats (profond, pélagique ou littoral) (Vander Zanden and Rasmussen 1999; Post 2002). Au sein d'un même site, les variations en $\delta^{15}N$ des organismes sont reliées à leur position trophique dans la chaîne alimentaire (DeNiro and Epstein 1981; Peterson and Fry 1987). Finalement, la signature en $\delta^{15}N$ des organismes à courte durée de vie varie temporellement car elle suit les variations de la signature de base du système ou incorpore les variations de diète dans le cas des consommateurs (Zohary et al. 1994; Matthews and Mazumder 2005).

Dans ce troisième chapitre, j'ai quantifié les sources de variance inter-écosystème, intra-écosystème et intra-site de la signature en $\delta^{15}N$ du zooplancton pour une série d'écosystèmes oligotrophes. Les variations temporelles en $\delta^{15}N$ du zooplancton ont été déterminées à partir de données prélevées au printemps et en été dans deux réservoirs et douze lacs. J'ai développé des modèles prédictifs de la signature en azote de ces organismes. Finalement, à partir de données de la littérature, j'ai vérifié si les relations observées à partir des données de cette étude pouvaient être appliquées généralement aux écosystèmes oligotrophes.

4 Quelle est la dynamique du zooplancton lors de la mise en eau du réservoir LG-2 et quelle est l'influence de cette communauté sur le cycle du carbone ?

Lors de la mise en eau d'un réservoir, un nouvel écosystème est créé. Il remplace des rivières, des lacs et des milieux terrestres et permet le développement de communautés qui n'étaient pas présentes à l'origine (Marzolf 1990). Peu de données permettent de quantifier les effets de la création de réservoir car, en général, les connaissances des écosystèmes avant l'inondation sont manquantes. Dans ce chapitre, j'ai utilisé une base de données à long terme pour décrire la dynamique de la biomasse du zooplancton lors de la mise en eau du réservoir LG-2. J'ai déterminé l'influence des variables physiques et biologiques responsables des variations dans la biomasse de cette communauté. A partir de données modernes prélevées dans le cadre de cette thèse, j'ai déterminé les effets de la taille des organismes du zooplancton sur la biomasse algale et sur le flux de carbone observé à l'interface eau-atmosphère d'une série de réservoirs et lacs boréaux.

CHAPITRE I

A COMPARISON OF METHODS TO DETERMINE CARBON ALGAL SIGNATURES IN FRESHWATER

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1.1 RÉSUMÉ/ ABSTRACT

Afin d'appliquer les techniques isotopiques en écologie et déterminer les flux de masse et d'énergie pour les niveaux supérieurs des réseaux trophiques, la signature de base de l'écosystème est nécessaire. Pour les écosystèmes aquatiques, les algues représentent le premier niveau trophique mais il demeure difficile d'obtenir leur composition isotopique à cause des problèmes de séparation de ces organismes de petite taille. Dans cette étude, nous comparons plusieurs approches utilisées dans la littérature pour déterminer la signature en carbone des algues d'eau douce. Les résultats indiquent que la signature d'un consommateur primaire tel que *Daphnia* sp., la signature de la matière particulaire corrigée pour la biomasse algale ou la signature d'un échantillon concentré d'algues sont comparables. Par contre, la signature algale déterminée à partir de celle du carbone inorganique dissout et du fractionnement algal était significativement plus basse comparée aux autres approches. Cette différence était attribuée à l'incorporation possible de bicarbonate et surtout aux problèmes associés à la détermination du fractionnement algal en milieu d'eau douce.

When applying stable isotopes approaches in aquatic ecology, the signature of basal sources is required to accurately assess the flux of mass and energy to higher trophic levels of food webs. In the case of algae, it is difficult to get such information because of the complications associated with isolating small organisms from a bulk sample. In this study, we compare several approaches currently used in the literature to determine algal carbon signatures in freshwater ecosystems. The results indicated that the signature of a primary consumer such as *Daphnia* sp., the signature of particular organic carbon with a correction for algal biomass and the signature of algal samples were comparable. In contrast, algal signatures derived from dissolved inorganic carbon were significantly lower than from other approaches. This discrepancy was attributed to a potential uptake of bicarbonate and to problems in determining fractionation values based on current models.

Key words: algal carbon signatures, algal fractionation, carbon stable isotope.

1.2 INTRODUCTION

Bulk particulate organic carbon (POC) represents a mixture of live and detrital organic matter of terrestrial and aquatic origin. Terrestrial organic carbon signatures exhibit little variations in the boreal region (Junger & Planas 1994, Jones et al. 1999), thus implying that most of the variance of δ^{13}POC is related to that of the algae. Because of the difficulty in separating living from non-living organisms, POC signatures have been directly considered as equivalent to that of the algae based on the assumption that most of the bulk POC is composed of algal material. However, in small lakes, terrestrial organic matter may represent a significant portion of bulk POC (Pace et al. 2004) and therefore must be considered when calculating algal signatures by including algal carbon to total POC ratio in the mixing models. Finally, inconsistencies in the interpretation of algal signatures between various aquatic studies based on POC approaches (Hamilton & Lewis 1992, France et al. 1996) has led to the use of primary consumers organisms (i.e. mussels, *Daphnia sp.*) rather than primary producers as the baseline signature for benthic and pelagic food webs, respectively (Vander Zanden et al. 1999, Matthews & Mazumder 2003).

Carbon algal signatures are also determined by the isotope ratio of carbon dioxide and the amount of fractionation occurring during photosynthesis (ε_p). In such calculations, carbon dioxide is assumed to be the only form of carbon incorporated, because it is believed to be the most abundant form of carbon in freshwaters and because of the lower energy costs associated with uptake by passive diffusion (Burkhardt et al. 1999). Based on experimental studies on marine algal taxa, dynamic models predicted carbon fractionation as a function of growth rate, CO_2 concentration and cell geometry (Laws et al. 1997, Popp et al. 1998, Burkhardt et al. 1999). Because of consistent fractionation values obtained from laboratory and in situ measurements, models developed experimentally were applied in nature (Bidigare et al. 1997).

To our knowledge, little has been done to compare the various approaches used to determine carbon algal signatures, despite reports of inconsistencies and extreme caution in interpretations based on these tools (Raven et al. 1994, France 1996, del Giorgio & France 1996). The determination of accurate basal signatures is crucial to the interpretation of consumer's isotopic composition. For instance, most recent studies in freshwaters that have determined the source of carbon for aquatic consumers through $\delta^{13}C$ analysis have continued to generate mixed results as to whether or not allochthonous carbon is entering the food web (Jones et al. 1998, Bunn et al. 2003, Karlsson et al. 2003, Martineau et al. 2004, Pace et al. 2004, Marty & Planas 2005). It is therefore important to assess if conclusions from such studies reflect differences in the functioning of communities or are instead attributable to the methods used, which may have led to bias conclusions. Further, a comparison of the various methods used to determine carbon algal signature is important to identify factors that may be responsible for differences between them. The purpose of this study was to evaluate if carbon signatures of algae obtained from several approaches from freshwater ecosystems were similar. Specifically, we compared algal signatures which were 1) derived from the bulk POC signature, 2) calculated from the POC signature with a correction for algal biomass, 3) calculated from carbon dioxide signature and algal fractionation and 4) derived from the signature of grazer organisms such as *Daphnia* sp. As some of these approaches required calculations, sensitivity analyses were performed to determine the effect of variations in parameters entered into mixing models. The estimates $\delta^{13}C$ from each approach were further compared to a series of phytoplankton signatures obtained by directly separating algal material from the bulk particulate organic matter. Comparison of algal carbon signatures obtained from the various approaches revealed important discrepancies, with significantly different signatures obtained when using CO_2. Further, such differences between methods raise questions on the assumptions

supporting calculations, especially regarding the form of carbon incorporated by algae and fractionation factor (ε_p).

1.3 MATERIALS AND PROCEDURES

1.3.1 Study area and sampling

Samples used in this paper were collected from a series of 13 pristine lakes and 6 reservoirs situated on the Canadian Shield, in three areas (James Bay territories, Manicouagan and Ste-Marguerite), visited between 2001 and 2003. Ecosystem characteristics are described in detail in Planas et al. (2005). Sampling included several stations per reservoir depending on its surface area (286 to 2646 km^2) and one station per lake (deepest point). Data presented in this study were collected once, during mid-summer. Temperature, oxygen and pH profiles were measured in situ with a YSI-6600 multiprobe. Integrated water samples (60 L) were collected from the euphotic zone of the water column (Li-Cor LI193SA and LI-190SA) or from the epilimnion if deeper than photic zone in the case of stratified water column, using a 4 L Van Dorn bottle. This water was used to determine the concentration in particulate organic matter (see below), Chlorophyll a concentration (Nusch 1980) and primary production (PP). Methods and results for PP measurements are reported in Planas et al (2005). Zooplankton were collected from the entire water column (max. 30 m) using a 110µm mesh size plankton net and were kept alive in filtered water to allow gut evacuation, until arrival in the laboratory. Dissolved inorganic carbon concentration and signature were measured on water samples collected at 1 m depth. CO_2 concentration was measured in the field using a non-dispersive infrared analyzer (Li-Cor LI-7000) and a gas chromatograph in the case of LG-2 reservoir (Varian Star-3400), following the headspace technique described in Cole et al. (1994). The concentration of each carbon form was calculated based on carbonate thermodynamic equilibrium (Stumm & Morgan 1996). The signature of DIC was determined for 20

stations, on water samples collected in glass bottles at 0.5 m depth, preserved with $HgCl_2$, sealed and kept at 4°C until analysis. A summary of main physical, chemical and biological characteristics used in this study is presented in Table 1.1.

1.4 METHODS TO DETERMINE CARBON ALGAL SIGNATURES

1.4.1 Method 1: δ^{13}C-algae as particulate organic carbon (δ^{13}POC)

Particulate organic matter (POM) was collected on pre-combusted glass fibre filters (GF/C-Whatman), by filtering 0.5 to 1 L of water, sampled as described above. Filters were stored frozen in liquid nitrogen and dried at 45°C prior to C/N and SI analysis, performed on a GV Instruments Isoprime™ mass spectrometer coupled to a Carlo Erba Elemental Analyser (NA 1500 series 2).

1.4.2 Method 2: δ^{13}C-algae based on particulate organic matter and algal proportion (δ^{13}algae-POC)

A variation of the approach described above consisted in the calculation of phytoplankton carbon signature considering POM as a mixture of algae and detrital material. The following mixing model was used:

$$\delta^{13}\text{-POC} = x\,(\delta^{13}\text{algae-POC}) + (1-x)\,(\delta^{13}C_{terr.}) \quad (1)$$

and modified to determine phytoplankton signature as:

$$\delta^{13}\text{algae-POC} = [\delta^{13}\text{POC} - (1-x)\,(\delta^{13}C_{terr.})]/x \quad (2)$$

Table 1.1: Physical, chemical and biological characteristics of 13 lakes (L) and 6 reservoirs (R) in Northern Canada for the summers of 2001–2003. T, temperature; μ, phytoplankton growth rate; ε_p, phytoplankton fractionation.

Sites	Stations	pH	T (°C)	DIC ($\mu mol.L^{-1}$)	CO2 ($\mu mol.L^{-1}$)	μ (d^{-1})	μ/CO_2 ($m^3.mol^{-1}.d^{-1}$)	ε_p (‰)	POC ($\mu g.L^{-1}$)	Chl. a ($\mu g.L^{-1}$)
L	Berté	6.1	16.0	43.5	25.7	0.6	22.9	16.6	86.9	0.6
L	Desaulnier	6.8	18.3	91.3	25.4	1.0	37.9	9.9	262.9	1.5
L	Duchaunay	-	16.5	-	-	0.3	-	-	116.9	0.8
L	Aux Cèdres	-	18.0	-	-	1.0	-	-	340.5	1.1
L	Germain	-	16.0	-	-	0.8	-	-	431.2	0.6
L	Jean-Marie	6.8	18.2	68.4	19.3	0.3	13.5	20.8	453.9	2.1
L	Km 12	7.5	16.6	259.8	19.2	0.5	27.3	14.7	214.8	1.3
L	Km 17	6.8	17.7	112.6	32.9	0.5	13.8	20.7	430.5	1.9
L	Km 380	7.5	18.0	246.0	19.5	1.0	49.9	4.6	320.4	1.2
L	Matonipi	-	11.0	-	-	0.3	-	-	202.5	0.9
L	Patukami	6.9	16.6	95.2	26.6	0.9	34.2	11.6	213.0	1.2
L	Polaris	6.5	17.8	35.4	16.6	0.6	34.0	11.7	288.4	1.1
L	Yasinsky	6.9	17.5	130.0	33.3	1.0	31.0	13.0	102.9	1.8
R-LA1	LA1-02	6.4	15.4	74.3	38.5	0.7	18.2	18.7	424.5	2.0
R-LA1	LA1-03	6.3	15.3	54.7	31.4	0.7	20.7	17.6	394.5	2.1
R-LA1	LA1-04	6.4	16.2	78.5	41.3	0.5	10.9	22.0	501.2	2.6
R-LA1	LA1-05	6.5	16.7	59.7	27.9	0.5	18.8	18.5	385.0	2.1
R-LA1	LA143C (2001)	6.3	19.2	35.2	24.7	0.9	36.6	10.5	689.1	3.9
R-LA1	LA143C	6.8	14.9	109.7	34.1	0.6	17.8	18.9	428.1	2.2
R-LA1	LA1903 (2001)	6.4	17.9	48.7	31.5	0.5	16.1	19.6	281.1	2.4
R-LA2	LA2-01	6.3	11.8	41.6	25.6	0.5	20.9	17.5	455.0	2.2
R-LA2	LA2-02	6.5	12.6	59.3	27.9	0.5	18.0	18.8	367.2	2.3
R-LA2	LA2-03	6.3	12.9	44.7	27.3	0.6	23.1	16.5	383.8	2.3
R-LA2	LA2-04	6.0	12.7	43.8	32.3	0.5	14.2	20.5	358.6	1.9
R-LA2	LA2-05	6.1	12.9	42.0	29.4	0.4	14.8	20.2	368.9	2.3
R-LG2	LG2 #1	6.4	13.8	33.0	35.7	0.6	16.0	19.7	195.3	1.6
R-LG2	LG2406	6.2	7.2	71.6	64.3	1.6	24.5	15.9	130.2	0.9
R-LG2	LG2039	6.5	15.9	54.1	32.5	0.6	18.5	18.6	-	1.9
R-LG2	LG2509	6.6	14.4	64.9	37.6	0.7	19.3	18.2	246.9	1.9
R-LG2	LG2018	6.1	18.5	60.1	50.5	0.6	11.5	21.7	252.3	2.2
R-LG2	LG2336	6.8	16.2	69.8	27.0	1.0	36.3	10.6	114.6	1.6
R-LG2	LG2604	6.4	15.7	55.7	31.1	0.9	27.4	14.6	153.9	1.5
R-LG2	LG2610	6.6	17.2	-	-	0.5	-	-	218.4	1.6
R-LG2	LG2615b	6.4	12.0	79.9	70.4	0.5	6.8	23.8	461.5	1.1
R-LG4	LG4-01	6.1	11.4	56.7	39.4	0.8	20.8	17.5	314.4	1.0
R-LG4	LG4-02	6.2	11.6	58.6	39.2	0.4	11.1	21.9	265.6	1.4
R-LG4	LG4-03	6.1	11.2	55.8	38.8	0.6	15.4	20.0	342.0	1.4
R-LG4	LG4-04	6.2	12.0	63.5	42.4	0.4	9.9	22.4	357.8	1.7
R-LG4	LG4-05	7.6	15.9	413.8	26.0	0.7	25.2	15.6	305.0	1.2
R-MA5	MA5-0400	6.2	11.5	55.7	38.8	0.4	10.1	22.3	98.4	0.7
R-MA5	MA5-0600	6.6	16.0	87.6	35.2	0.5	15.3	20.0	149.6	1.0
R-MA5	MA5-0800	6.3	17.0	65.2	37.1	0.5	12.8	21.1	105.2	1.1
R-MA5	MA5-1200	6.6	14.0	87.1	35.6	0.4	11.0	21.9	206.3	1.3
R-SM3	SM3-5057	5.9	18.0	73.1	56.0	0.5	9.5	22.6	266.0	4.4
R-SM3	SM3-5107	-	13.0	-	-	0.4	-	-	196.4	2.1
R-SM3	SM3-5121	6.1	15.0	70.7	48.3	0.5	10.5	22.1	179.7	1.7
R-SM3	SM3-5124	6.0	12.5	94.5	69.7	0.6	8.1	23.2	335.2	4.3
R-SM3	SM3-5146	6.0	16.5	69.8	50.7	0.3	6.3	24.1	183.9	1.3

Where x represents the proportion of algal carbon in the particulate organic matter pool, which was calculated from the ratio between phytoplankton biomass and POC concentration both expressed in $\mu gC.L^{-1}$. The ratio of organic carbon to chlorophyll a was derived from the same mixing model, using algal signatures obtained from pure algal samples (methods described below).

$$C{:}Chl.\ a = [(\delta^{13}POC - \delta^{13}C_{terr.}).(POC)]\ [(\delta^{13}algae\text{-}POC - \delta^{13}C_{terr.})\cdot(Chl.\ a)]^{-1} \quad (3)$$

Terrestrial signature was determined based on the relationship between $\delta^{13}POC$ and Chl. a., assuming that POC contains only terrestrial carbon when Chl. a concentration tends to zero.

1.4.3 Method 3: $\delta^{13}C$-algae determination from $\delta^{13}C$ of dissolved inorganic carbon ($\delta^{13}algae\text{-}DIC$)

DIC stable isotope compositions were determined using a TIC-TOC analyser (1010 O-I-Analytical) connected to a Finnigan Mat Delta Plus Mass Spectrometer following the methods described in St-Jean (2003). Phytoplankton signature was considered as a function of carbon dioxide signature and the photosynthetic fractionation parameter epsilon (ε_p):

$$\delta^{13}algae\text{-}DIC = \delta^{13}CO_{2(aq)} - \varepsilon_p \quad (4)$$

In this mixing model, we assumed that carbon dioxide was the main form of DIC incorporated during photosynthesis and the signature of this form of carbon was calculated according to Mook et al. (1974). Phytoplankton fractionation (ε_p) was calculated from the relation between phytoplankton growth rate divided by carbon

dioxide concentration ($\mu/[CO_2]$) and (ε_p), as described by Laws et al. (1995) and Popp et al. (1998). Phytoplankton growth rate (μ) was estimated as the ratio between algal biomass and primary production, measured in each ecosystem at the same time as SIA sampling (see data in Planas et al. (2005)). Maximum ε_p was set at -26.8‰ (Goericke et al. 1994) and minimum ε_p was determined according to Karlsson et al. (2003), assuming zooplankton carbon signature as autochthonous signature for the highest $\mu/[CO_2]$ ratio (Lake Km. 380, $\mu/[CO_2]$=0.05 and calculated ε_p=4.1; Fig. 1.1).

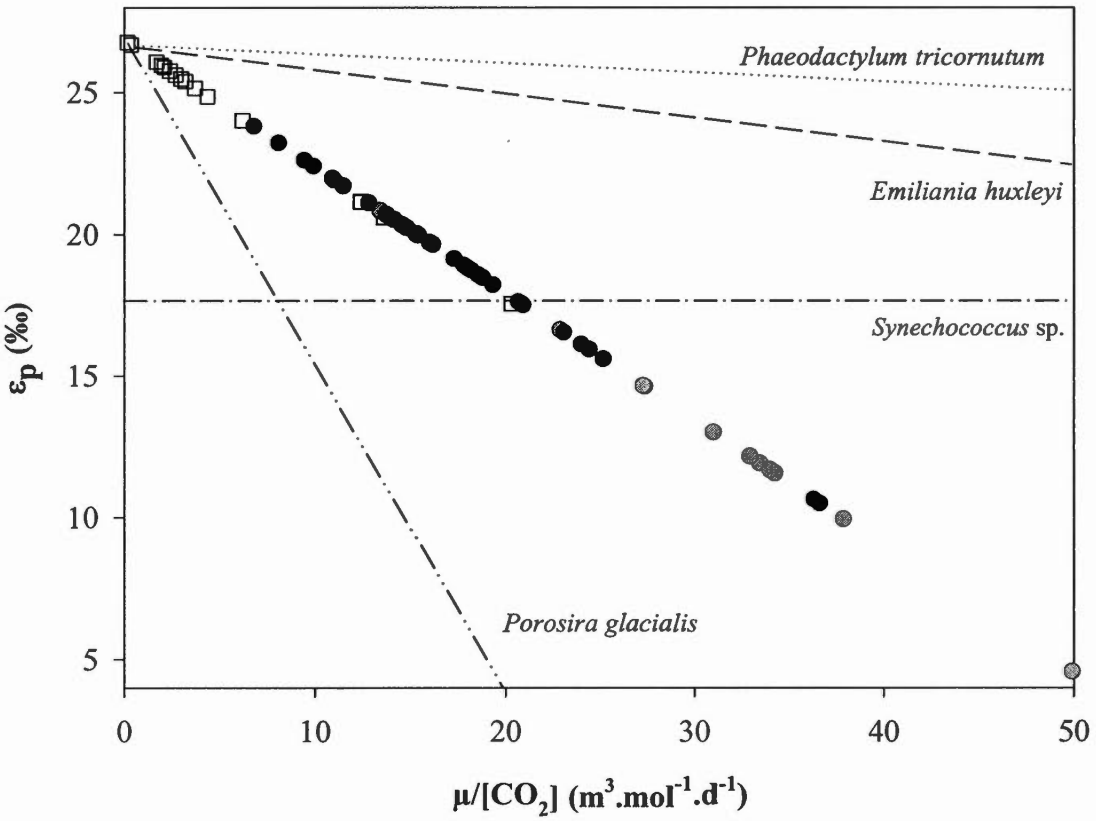

Fig. 1.1: Phytoplankton fractionation (εp) (‰), as a function of the ratio between phytoplankton growth rate and CO_2 concentration ($\mu/[CO_2]$, $L.\mu mol^{-1}.d^{-1}$), for reservoirs stations (black circles) and lakes (grey circles), as modified after Karlsson et al. (2003) (open squares). Dashed lines indicate regressions obtained for the marine species described in Popp et al (1998).

1.4.4 Method 4: δ^{13}C-algae as primary consumers signature (δ^{13}Daphnia sp.)

In this study, *Daphnia* sp. was considered as the primary consumer organism and was isolated from zooplankton bulk under a binocular and preserved as for POC samples. Carbon SIA was determined according to methods developed for small sized samples (Limén & Marty 2004).

1.4.5 Method 5: δ^{13}C-algae determination with enriched phytoplankton samples (δ^{13}algal-samples)

For a limited number of stations from both lakes and reservoirs, we were able collect sufficient algal material, allowing for SIA. Phytoplankton samples were collected from the entire water column (max. depth: 30m) by vertical tows with a 110µm mesh plankton net. Non-algal organisms, visible under a binocular, were manually removed from samples. Organisms were then concentrated onto a 28µm mesh size nitex filter and stored in cryotubes in liquid nitrogen. Centrifugation (1 min., 14,000 rpm) was performed to further separate the algal fraction from other organic particles. The top-green fraction of samples was then isolated, observed under binocular to remove non-algal material and processed for SIA following the same protocol as for zooplankton. Additional microscopic observations revealed that sampled phytoplankton consisted mainly of large diatoms such as *Tabellaria* sp.

All particulate samples (POM and zooplankton) were freeze-dried and SIA were performed in triplicates at GÉOTOP-UQAM and none of the samples were acidified prior to combustion because of the relatively low concentration of inorganic carbonates in circumneutral Canadian Shield waters. One sample per site was analyzed for the determination of δ^{13}-DIC, at G.G. Hatch Isotopes Laboratory (University of Ottawa, Canada). Results are given using the standard δ notation with

$\delta=[(R_{sample}/R_{reference})-1] \times 1000$, expressed in units per thousand (‰) and $R=^{13}C/^{12}C$ (Verardo et al. 1990). Secondary standard (Leucine) of known relation with the international standard of Pee Dee Belemnite was used as reference material. Precisions on SI measurement were on average 0.08 ‰.

1.5 ASSESSMENT

1.5.1 Determination of $\delta^{13}C_{terr.}$, C:Chl ratio and ε_p

δ^{13}POC was negatively related to Chl. a concentrations and this relationship was used to calculate region-specific terrestrial signatures, assuming the absence of algal material when Chl. a reaches zero (for details, see Marty (2006)). Precisely, $\delta^{13}C_{terr.}$ value was -27.5, -29 and -28.1‰ for James Bay, Manicouagan and Ste. Marguerite regions, respectively. C:Chl. ratio was calculated for stations where algal samples were collected and ranged from 37 to 103. The mean value (C:Chl.=80) was applied to calculate the proportion of algal carbon in POC at each station. Based on this value, algal carbon exceeded POC concentration for 4 sites and in these cases, POC was considered as 100% algal. Algal carbon represented on average 51% of POC and ranged from 10 to 100%.

Phytoplankton fractionation (ε_p) obtained as a function of μ/CO_2 was in the range of values observed for marine species (Popp et al. 1998) and followed the same line as calculated values for other lakes (Karlsson et al. 2003) (Fig. 1.1). ε_p was generally lower in lakes than in reservoirs stations (means: 13.7 and 18.9‰, respectively) as a result of both higher CO_2 concentration and lower growth rate in reservoirs (Table 1.1). Fractionation values were also calculated in order to match δ^{13}algae-DIC with δ^{13}algae-POC and δ^{13}*Daphnia* sp., based on the assumption that CO_2 was the only carbon form assimilated by algae (Fig. 1.2). Mean fractionation

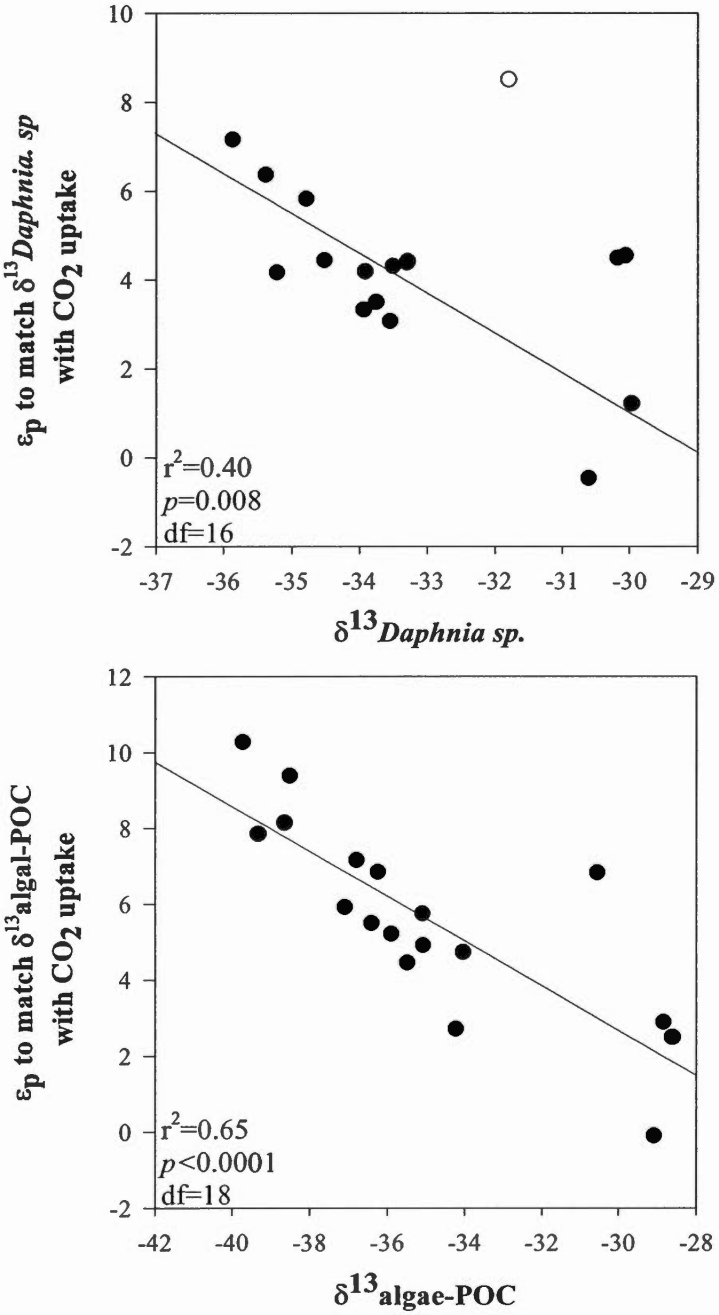

Fig. 1.2: Fractionation values (ε_p) calculated to match *Daphnia* sp. signatures and δ^{13}algae-POC, assuming CO_2 as the only source of carbon. One outlier (open circle) was excluded from analysis.

values were lower compared to those obtained with the μ/CO_2 approach (4.3 and 5.6‰ to match δ^{13}algae-POC and δ^{13}*Daphnia* sp., respectively) and ranged from almost 0 to 10‰.

1.5.2 Algal signatures for each approach

Stable isotope data also suggested that particulate organic matter was based one a mixture of both algal and detrital material. δ^{13}POC ranged from -34.6 to -28‰ (mean: -30‰) and after accounting for the proportion of algal carbon in POM, δ^{13}algae-POC was on average lower than that of bulk POC (mean: -32.2 ‰) and ranged from -36.3 to -28 ‰.

DIC signatures ranged from -35.9‰ to -16.6‰. Based on CO_2 signatures and fractionation values calculated as function of μ/CO_2 (Table 1.1, Fig. 1.1), mean δ^{13}algal-DIC was -47.0‰ and showed a range of variations between -52.3‰ to -30.7‰. The δ^{13}C values of *Daphnia* sp. ranged from -39.6 to -29.2 ‰ (mean: -32.9‰) and the enriched phytoplankton samples isolated from 7 stations had δ^{13}C values ranging from -34.6‰ to -29.2‰ (mean: -32.7‰) (Table 1.2).

1.5.3 Effect of variation in C:Chl ratio on the proportion of algal carbon in POM

The effect of variations in C:Chl. ratio on the proportion of algal carbon in POC was examined for maximum, median and minimum chlorophyll concentrations (Fig. 1.3). A positive linear relationship was found between C:Chl. and the proportion of algal carbon in POC. Under low chlorophyll concentrations in POM, the majority of POM was terrestrial, independent of the C:Chl. ratio. However, increases in Chl.a positively influenced the slope of this relationship, illustrating the

Table 1.2: Stable carbon isotopic composition ($\delta^{13}C$, ‰) of DIC and algal signatures, according to the 5 methods compared in this study.

Sites	Stations	δ^{13}DIC	Method 1 δ^{13}POC	Method 2 δ^{13}algae-POC	Method 3 δ^{13}algae-DIC	Method 4 δ^{13}*Daphnia* sp.	Method 5 δ^{13}algal-samples
L	Berté	-	-29.3	-29.5	-	-29.6	-
L	Desaulnier	-	-29.3	-31.6	-	-	-
L	Duchaunay	-	-28.5	-28.1	-	-31.1	-
L	Aux Cèdres	-	-28.0	-	-	-31.2	-
L	Germain	-	-28.5	-31.2	-	-30.3	-
L	Jean-Marie	-28.9	-29.4	-32.8	-52.3	-30.6	-
L	Km 12	-16.6	-28.2	-29.1	-40.6	-30.1	-
L	Km 17	-34.3	-28.2	-29.6	-49.9	-30.0	-32
L	Km 380	-17.0	-28.1	-29.5	-30.7	-30.2	-
L	Matonipi	-	-29.2	-29.6	-	-29.3	-
L	Patukami	-	-29.6	-32.3	-	-	-
L	Polaris	-33.0	-28.2	-29.7	-45.0	-	-29.2
L	Yasinsky	-	-29.1	-30.7	-	-	-
R-LA1	LA1-02	-18.7	-30.1	-34.3	-48.4	-33.5	-33.3
R-LA1	LA1-03	-32.8	-30.8	-35.3	-48.1	-34.5	-33.6
R-LA1	LA1-04	-31.8	-29.9	-33.1	-51.3	-33.3	-32.7
R-LA1	LA1-05	-30.6	-30.2	-33.9	-49.4	-33.5	-33.3
R-LA1	LA143C (2001)	-	-29.9	-32.8	-	-32.5	-
R-LA1	LA143C	-19.0	-29.5	-32.5	-48.2	-33.3	-
R-LA1	LA1903 (2001)	-	-30.6	-32.0	-	-	-
R-LA2	LA2-01	-25.7	-30.8	-35.9	-49.0	-35.2	-
R-LA2	LA2-02	-29.0	-30.3	-33.2	-49.9	-33.9	-34.6
R-LA2	LA2-03	-24.7	-30.7	-34.2	-47.7	-	-
R-LA2	LA2-04	-30.3	-29.9	-33.1	-50.7	-33.9	-
R-LA2	LA2-05	-35.9	-30.4	-33.5	-50.9	-33.8	-
R-LG2	LG2 #1	-	-29.6	-30.8	-	-30.9	-
R-LG2	LG2406	-	-28.6	-29.5	-	-32.0	-
R-LG2	LG2039	-	-	-	-	-30.7	-
R-LG2	LG2509	-	-29.2	-30.3	-	-30.7	-
R-LG2	LG2018	-	-32.3	-34.4	-	-33.1	-
R-LG2	LG2336	-	-28.3	-28.2	-	-30.5	-
R-LG2	LG2604	-	-29.9	-30.5	-	-31.0	-
R-LG2	LG2610	-	-29.3	-30.5	-	-	-
R-LG2	LG2615b	-	-28.4	-32.2		-31.2	-
R-LG4	LG4-01	-31.1	-29.3	-34.7	-46.9	-34.8	-
R-LG4	LG4-02	-33.9	-29.9	-33.4	-51.3	-35.1	-
R-LG4	LG4-03	-28.3	-30.5	-36.3	-49.4	-35.4	-
R-LG4	LG4-04	-29.8	-30.5	-35.4	-51.6	-35.9	-
R-LG4	LG4-05	-25.8	-28.5	-30.7	-39.3	-31.8	-
R-MA5	MA5-0400	-	-30.1	-30.9	-	-32.5	-
R-MA5	MA5-0600	-	-30.3	-31.6	-	-33.8	-
R-MA5	MA5-0800	-	-30.0	-30.2	-	-33.3	-
R-MA5	MA5-1200	-	-31.2	-34.5	-	-33.3	-
R-SM3	SM3-5057	-	-34.6	-33.0	-	-38.1	-
R-SM3	SM3-5107	-	-33.1	-34.0	-	-39.6	-
R-SM3	SM3-5121	-	-30.6	-31.4	-	-31.2	-
R-SM3	SM3-5124	-	-34.5	-34.3	-	-38.2	-
R-SM3	SM3-5146	-	-32.3	-35.6	-	-38.4	-

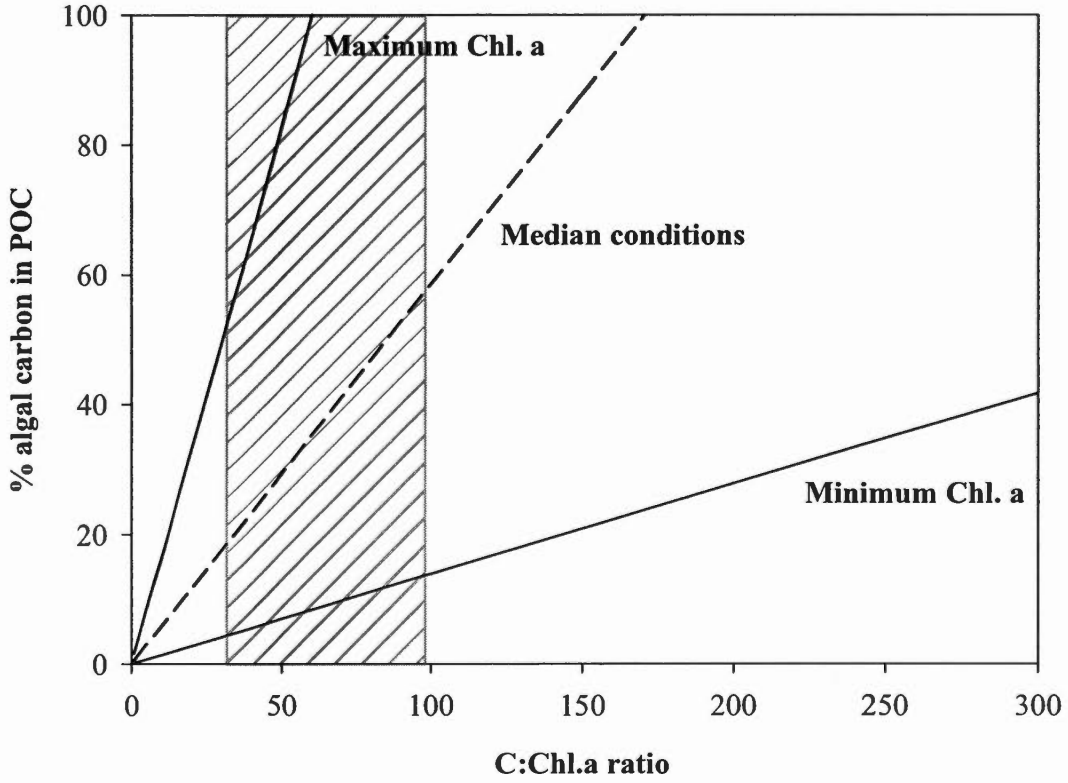

Fig. 1.3: Percentage of algal carbon in POM based on minimum Chl. a (Chl.a =0.6 µg.L-1; POC=431.2 µg.L-1), maximum Chl. a (Chl.a =4.4 µg.L-1; POC=266 µg.L-1) and median conditions (Chl.a =1.6 µg.L-1; POC=273.5 µg.L-1) (dashed line), as a function of C:Chl. a ratio. Grey area indicates the range of C:Chl. a ratio calculated for enriched algal samples.

importance of the C:Chl. ratio in the calculation of the proportion of algal carbon (and in turn, that of δ^{13}algae-POC). The slope obtained for the median chlorophyll value was 0.6, implying that estimates in the proportion of algal carbon were generally sensitive to C:Chl. ratios. As a consequence, we also looked at the effect of variations in such parameters on δ^{13}algae-POC, for a range of δ^{13}POC (Fig 1.4). When δ^{13}POC was similar to $\delta^{13}C_{terr.}$, δ^{13}algae-POC was relatively insensitive to

variations in the proportion of algal carbon in POC when higher than 20-30%. However, δ^{13}algae-POC was highly sensitive to changes in the proportion of algal carbon when POC was mostly terrestrial. Similar trends were observed for lighter values of δ^{13}POC, with the difference being that algal signatures tended to be sensitive over a larger range of carbon algal proportion. Therefore, with about 50% of POC originating from algae (Fig. 1.4) and mean δ^{13}POC at -30 ‰, algal signatures resulting from our calculations were strongly influenced by the estimate of the algal carbon proportion in POC.

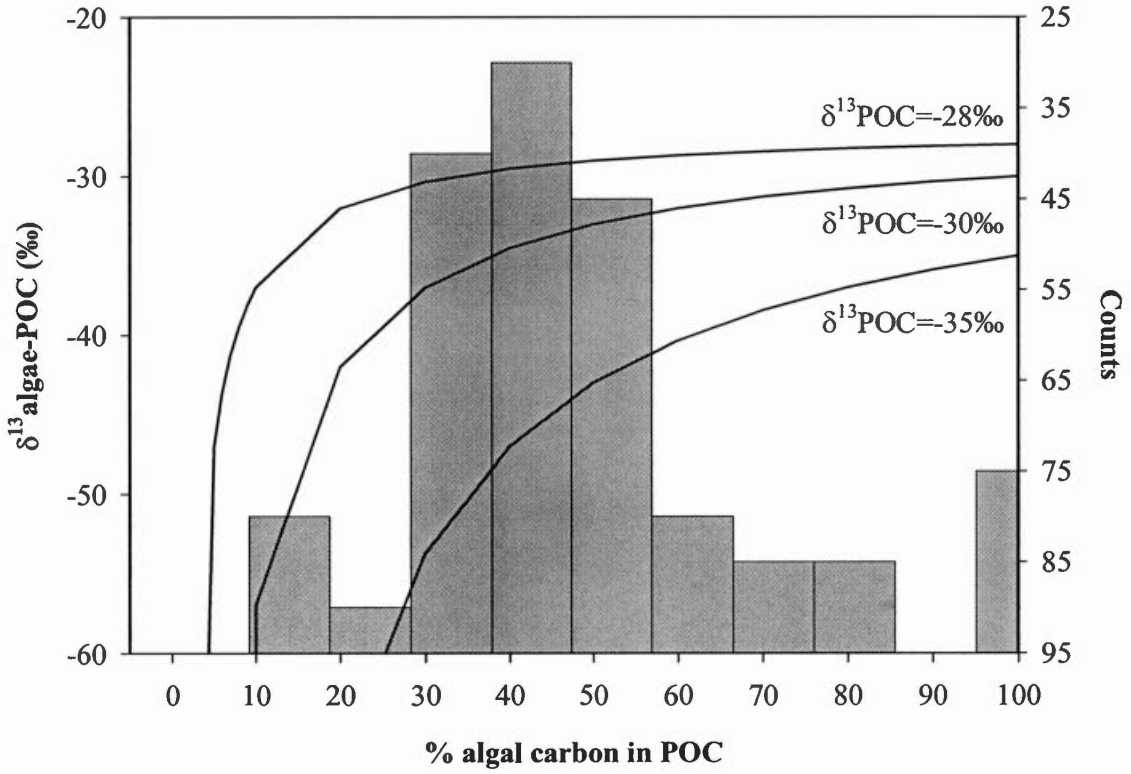

Fig. 1.4: The relationship between δ^{13}algae-POC and algal carbon proportion in POM. The left axis is the theoretical relationship between δ^{13}algae-POC and the proportion of algal carbon in particulate organic carbon, for a range of δ^{13}POC values ($\delta^{13}C_{terr.}$ was set at -27‰). The right axis is the frequency in the proportion of algal carbon in POM (C:Chl was set at 80).

1.5.4 δ^{13}algal-POC and assumptions in mixing model

The reliability of mixing models depends primarily on the difference in the isotopic signatures of end members entered into the model. In this study, a mixing model was used to determine δ^{13}algae-POC, based on the assumption that POC is composed of algal carbon associated with chlorophyll and terrestrial carbon. Non-algal POC could be considered of terrestrial origin in our study since macrophytes were not present in reservoirs and very scarce in the sampled oligotrophic lakes. Because $\delta^{13}C_{terr.}$ is rather uniform on the boreal ecoregion, the proportion of algal carbon in POC had the most influence on algal signatures and therefore must be accurately determined to get correct algal signatures. As shown in the sensitivity analysis, δ^{13}algae-POC varies widely with changes in the proportion of algal carbon when POC is dominated by terrestrial organic carbon. Algal signatures thus have to be extremely light to account for a depletion in δ^{13}POC when terrestrial carbon dominated the bulk POC.

Two main sources of error can influence the proportion of algal carbon. First, Chl. a concentration was considered as a proxy allowing for the calculation of algal signatures, with the assumption that algal carbon present in POC contains chlorophyll. Although Chl.a can be related to the signature of various organic fractions such as POC (this study, Gu et al. (1996)), zooplankton (Jones et al. 1999, Pulido-Villena et al. 2005) and sediments (Gu et al. 1996), we cannot exclude the possibility that POC contained dead autochthonous carbon, with no chlorophyll, leading to underestimation of algal signatures. This bias will, however, particularly affect algal signatures when POC is primarily terrestrial in origin.

In addition to the bias arising from the presence of dead algae, the proportion of algal carbon in POC and therefore the algal signature, is ultimately influenced by the ratio of organic carbon to Chl a. Leavitt and Carpenter (1990) found that C:Chl.

ratio varied between 20 to 300 according to season, irradiance and productivity. Since we were not able to measure the ratio *in situ*, a constant value of 80 for all stations was assumed, based on calculations from algal samples. Additionally, a limited amount of material for isotopic analyses restricted our ability to measure the chlorophyll content of enriched algal samples, which would have provided a more direct measurement of C:Chl. compared to calculations from mixing models. Nonetheless, a C:Chl. of 80 is a realistic value for oligotrophic ecosystems (Westlake 1980, Leavitt & Carpenter 1990) and if underestimated, would have had only a small effect on algal signatures as shown by sensitivity analysis. The concordance of signatures obtained for algae-POC, *Daphnia* sp. and algae samples in this study provides additional evidence that the ratio we used was appropriate. However, C:Chl. ratio in more productive ecosystems should be accurately determined as it will likely be lower than in oligotrophic systems (Leavitt & Carpenter 1990).

1.5.5 Is δ^{13}algal-sample the best estimate of algal carbon signature?

Ideally, the best estimate of algal signatures could be obtained on pure algal material separated from POM because it represents a direct measurement, independent of all other variables. We were able to collect a few direct samples and this was possible only because large algal organisms dominated the community at these sites, allowing for the use of a simple net tow and for the separation of non-algal material from bulk POM. Further, the separation of algae from POM was simplified in our samples because a single species dominated the algal community. Unfortunately, this method is difficult to apply in all systems, since the collection of smaller algae will require smaller mesh size net and therefore will be accompanied with other particles of various sizes, hard to simply remove. In addition, separation will be further complicated as algal communities become more diverse. As a result, improved separation techniques or compound specific analyses are currently being developed to

obtain direct measurements of algal community signatures. For example, based on the combination of fluorescent activated cell sorting (FACS) and $\delta^{13}C$ measurements of cellular fatty acids (FA), Bontes et al. (2006) successfully determined the signature of different phytoplankton groups in a eutrophic lake. However, the carbon signatures of specific compounds are often variable for a given algal group (Pel et al. 2003, Finlay 2004, Boschker et al. 2005, Bontes et al. 2006), and the results are currently limited because of low availability of data and poor knowledge of factors determining the $\delta^{13}C$ of specific biomarker molecules (Pond et al. 2006). In addition, such new techniques require additional equipment (gas chromatogragh) and sample preparations compared to the other approaches presented, which ultimately translate into higher costs per analysis.

1.5.6 Comparisons between methods

In order to avoid transformation of non-normally distributed data, non-parametric correlation (Spearman's ρ) was used to test the relationship between each pair of approaches (Fig. 1.5), and slopes and intercepts were compared to the 1:1 line by entering equality line parameters into a custom test. All correlations between each pairs of approaches were significant, excepted for pairs involving δ^{13}algae-DIC. Also, all relationships were characterized by parameters significantly different than 1:1 line with the exception of δ^{13}*Daphnia* sp./δ^{13}algae-POC relationship (Fig. 1.5). δ^{13}POC was enriched compared to signatures for δ^{13}algae-POC, and similar enrichment was observed with the signatures of algal samples and *Daphnia* sp. The depletion in *Daphnia* sp. carbon signatures compared to those of POC illustrates selective feeding on isotopically light phytoplankton whose signatures become masked by a larger pool of particulate terrestrial organic matter. This explanation is the most probable in our ecosystems compared to differential feeding according to depth, because of homogenous POM composition over the entire water column due to

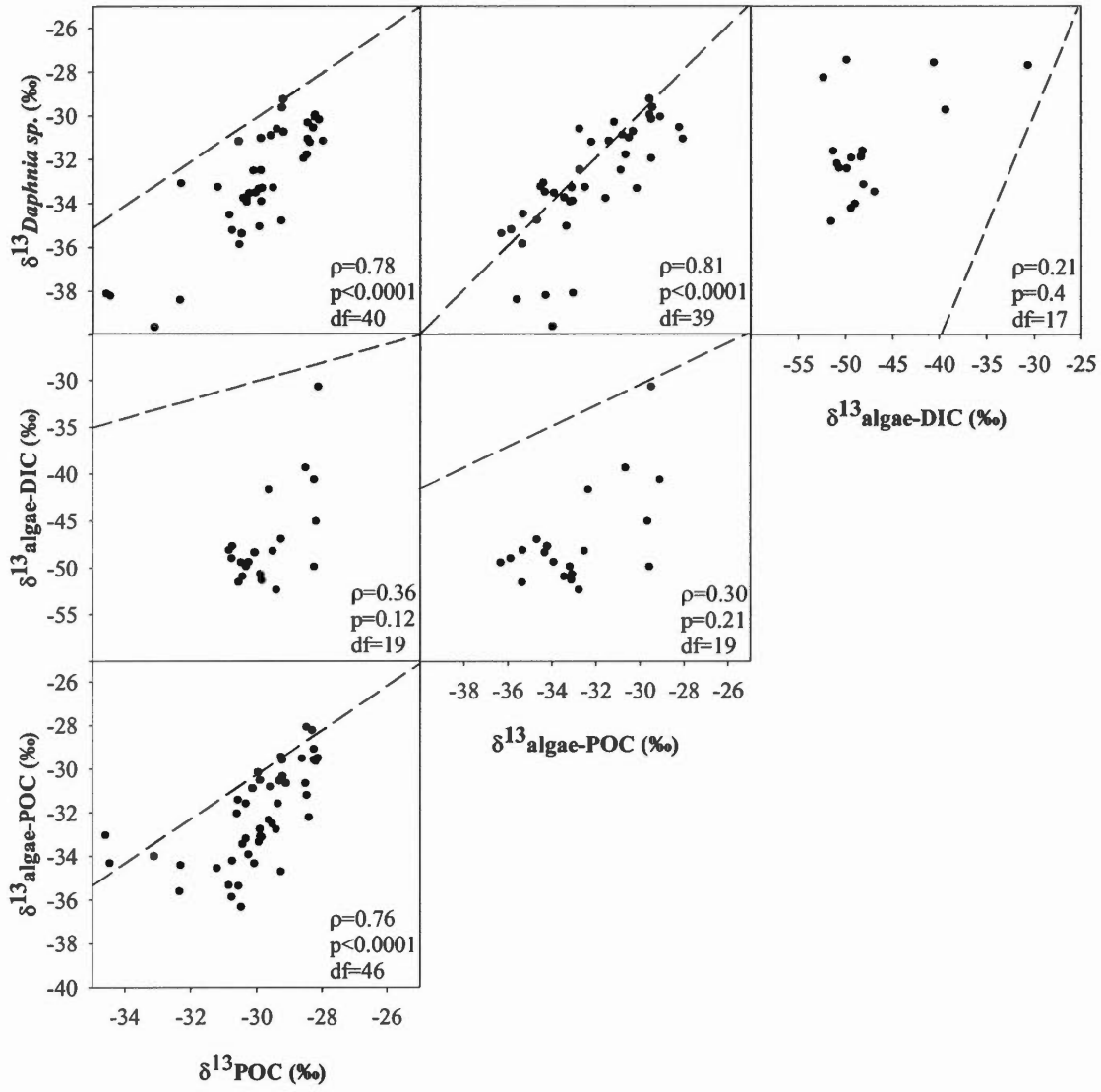

Fig. 1.5: Correlation matrix between each method considered as algal carbon signatures. Dashed line indicates equal $\delta^{13}C$ isotopic signatures.

the absence of stratification in most cases. Further, our result was supported by the strong correlation obtained between δ^{13}algae-POC and δ^{13}*Daphnia* sp. and this relationship was not different from the 1:1 line.

Although the number of enriched algal samples was small and despite the large mesh size of the net (110μm), algal samples included the most abundant taxa in terms of biomass (Marty-unpublished) and thus were representative of the phytoplankton community in our ecosystems. Analysis of variance (Welch-ANOVA for unequal variances) revealed significant differences between means of the 5 approaches (r^2= 0.79, df=161, $p<0.0001$) and Tukey Kramer HSD test on each pair showed that mean δ^{13}algae-POC, δ^{13}*Daphnia* sp. and δ^{13}algal-samples were statistically similar. In addition, mean δ^{13}POC was also similar to that of δ^{13}algal-samples but mean δ^{13}algae-DIC was significantly different from all others (Table 1.3).

Table 1.3. Mean carbon signature (and std. error) (‰) for each approach and comparison of each pair based on Tukey-Kramer HSD test. Variables not connected by same letter are significantly different.

Variables	**n**	**Mean**	**Std. Error**	**HSD test**
δ^{13}POC	47	-30.0	0.4	A
δ^{13}algal-samples	7	-32.7	1.0	AB
δ^{13}*Daphnia* sp.	41	-32.9	0.4	B
δ^{13}algae-POC	46	-32.2	0.4	B
δ^{13}algae-DIC	19	-47.0	0.6	C

1.6 DISCUSSION

Algal signatures based on $\delta^{13}CO_2$ were lighter than those of any other approach, and to our knowledge, no other studies have reported such low signatures. The reasons for such depletion are related to both δ^{13}DIC and ε_p. Algal carbon uptake mechanisms and photosynthetic fractionation are still poorly known in freshwaters systems compared to marine environments in which little variation in CO_2 concentration and DIC signature are observed (Finlay 2004). In fact, the increasing number of studies reporting DIC signatures clearly illustrates that δ^{13}DIC could exhibit a wide range of variation in freshwater ecosystems (-35.6 ‰ to equilibrium values) (see Fig. 5 in Bade et al. (2004), Prokopenko and Williams (2005)), implying that DIC signatures from this study cannot be considered as isolated from the range of data found in the literature. Surprisingly, despite such variation, algal signatures are still often based on the commonly used fractionation of ~20‰ (Schindler & Lubetkin 2004), a possible value for ecosystems with δ^{13}DIC closed to equilibrium with the atmosphere, but unlikely to be valid as DIC signatures decrease. To illustrate this, studies of ecosystems with unusual isotopic composition are therefore particularly useful to assess the reliability of current methods applied in aquatic food-web studies (Cattaneo et al. 2004). In this case, the question concerning whether or not existing tools to assess algal fractionation can be applied in freshwaters is particularly relevant as δ^{13}DIC was far from equilibrium with the atmosphere.

The most probable source of the discrepancy in algal signatures observed in this study comes from the fractionation calculation based on μ/CO_2 as described by Laws et al. (1997). This approach is based on the linear relationship between epsilon and μ/CO_2, observed for cultured marine algae. Minimum fractionation was calculated for the highest μ/CO_2 value based on the assumption that zooplankton signatures depend solely on autochthonous carbon. Although *Daphnia* sp. reliance

on algal carbon was likely valid in this study, as evidenced by concordance between δ^{13}algae-POC and δ^{13}*Daphnia* sp., such an assumption will however fail in ecosystems where terrestrial carbon represents a portion of zooplankton diet (Jones et al. 1999, Pace et al. 2004). In addition, the approach from Karlsson et al. (2003) assumes that μ/CO_2 ratio could be related to zooplankton feeding, which had never been shown in the literature. Further, if experimental studies allowed for the prediction of ε_p, they also clearly indicated that current models are taxa specific. The relationships between ε_p and μ/CO2 obtained for four marine algae were characterized by different slopes and, in the case of *Synechococcus* sp., a different intercept (Laws et al. 1995), as a result of variations in cell geometry (Popp et al. 1998) and potential effects of irradiance cycles, light intensity and nutrient limitation (Burkhardt et al. 1999). Therefore, although our fractionation values followed the same line as in Karlsson (2003) and are in the same range as values obtained for single species (Laws et al. 1995), it is unlikely that a single relationship could be applied to a series of ecosystems characterized by different multiple species assemblages. The unrealistic δ^{13}algal-DIC values obtained in this study suggest that fractionation approach based on μ/CO_2 cannot be applied outside of a taxa-species context. This was supported by our data, since fractionation values obtained to match *Daphnia* sp. and algae-POC (Fig. 2) were not related to those obtained using the μ/CO_2 approach. Further, our results suggest that fractionation could be lower than the commonly applied 20 ‰ and than values obtained from the μ/CO_2 relationship, in agreement with Pace et al. (2004).

Finally, the discrepancy between DIC approach and all other methods can also be related to the source of carbon assimilated by algal. If CO_2 is not the unique source of carbon for algae, the signature of algae will be based on a mixture of carbon forms with an approximately 10‰ difference between CO_2 and bicarbonate signatures (Mook et al. 1974). Bicarbonate uptake is possible in freshwater

ecosystems, given the range of pH typically observed in lakes (6 to 9, (Kalff 2002)) and a number of studies have demonstrated the ability of aquatic plants to incorporate bicarbonate (Talling 1976, Allen & Spence 1981, Maberly & Spence 1983), even in slightly acid mediums with abundant CO_2 (Findenegg 1976). Therefore, potential bicarbonate uptake is plausible in this study, considering the range of pH and that phytoplankton assemblages were mostly composed of diatoms (Marty-unpublished data), which have been reported to have affinities for bicarbonate uptake (Allen & Spence 1981, Tortell et al. 1997, Keller & Morel 1999). However, if bicarbonate could be potentially used as carbon source by algae, it is difficult to estimate its contribution relative to CO_2 uptake because little is known about fractionation occurring during bicarbonate uptake.

1.7 CONCLUSION AND RECOMMENDATIONS

Because of the combined effects of problems with current models to estimate phytoplankton fractionation and possible bicarbonate uptake, the use of inorganic carbon stable isotope to determine algal carbon signature produced unrealistic values in this study. If used to interpret data obtained for other compartments of the food web, such data will lead to erroneous conclusions. Therefore, we caution against application of general rules of isotopic fractionation to all aquatic ecosystems, which, in the case of basal algal signatures, will have tremendous consequences for studies in aquatic food web. Applied to calculations regarding the importance of allochthonous versus autochthonous carbon to organisms, algal signatures obtained via δ^{13}DIC will lead to an overestimation of terrestrial inputs in the composition of organisms and this may partly explain the variation found on this topic in the literature. The discrepancy between DIC approach and other methods highlights the need for further studies on carbon isotope fractionation and the form of carbon taken up by

phytoplankton in freshwaters. A robust fractionation model in freshwater should consider the wide range of δ^{13}DIC found in these systems. Based on our data, fractionation could be lower and far more variable than usually admitted.

Ideally, the best estimate of algal signatures could be obtained on pure algal material separated from POM. Although, simple separation of algae from POM is feasible when large algal organisms and few species are present, such methods will benefit from the development of separation techniques or compound specific analyses. As an alternative, the signature of POC combined to the percentage of algal carbon within particulate organic matter bulk or the signature of a primary consumer such as *Daphnia* sp. could represent a reliable estimate of algal signatures. Considering that δ^{13}POC-algae is derived from a mixing model involving several measurements, the easiest and less expensive approach to determine algal signature remains δ^{13}*Daphnia* sp. However, if a basal signature is required for the determination of carbon sources for higher trophic levels, then the δ^{13}algae-POC approach should be preferred in order to avoid circularity arising from using the signature of organisms to infer zooplankton carbon sources.

1.8 ACKNOWLEDGEMENTS

We are grateful to P. del Giorgio, A. Patoine and C. Vis for their revisions on an earlier version of the manuscript. J. N. Jasmin, M. A. Gagnon, L. Cournoyer and A. Adamowicz provided field and technical support. This research was funded by the Natural Sciences and Engineering Research Council of Canada via a strategic grant (No STP224191-99) in partnership with Hydro-Quebec, by the Chaire en Environnement CRSNG-Hydro Québec- UQAM (DP) and by a fellowship from Fonds de Recherche sur la Nature et les Technologies du Québec (JM). This a

contribution of the Groupe de Recherche Interuniversitaire en Limnologie (GRIL) and Centre de Recherche en Géochimie et en Géodynamique (GÉOTOP).

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CHAPITRE II

AUTOCHTHONOUS CARBON AS THE MAIN SUBSIDY FOR ZOOPLANKTON IN OLIGOTROPHIC LAKES AND RESERVOIRS FROM THE BOREAL ECOREGION.

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2.1 RÉSUMÉ/ ABSTRACT

Une approche isotopique a été appliquée afin de déterminer l'importance des sources de carbone autochtone et allochtone dans la diète du zooplancton d'une série de lacs et réservoirs oligotrophes de la région boréale. Résultant de la minéralisation de la matière organique provenant du bassin versant et de la matière inondée en réservoirs, l'ensemble des écosystèmes était des sources de CO_2 pour l'atmosphère. Les signatures isotopiques du carbone inorganique et organique dissout (moyennes: -29.8 ‰ et -29.4 ‰, respectivement) confirment la dominance des apports d'origine terrestre dans le cycle du carbone. En lacs, le seston était majoritairement d'origine allochtone (57%), alors qu'en réservoirs cette proportion était plus variable (30 à 74%). Les signatures en carbone du zooplancton (moyenne:-32.8 ‰) étaient fortement corrélées à celles des algues (moyenne: -32.0 ‰) et cette relation n'était pas différente d'une relation 1:1. L'importance des algues dans la diète du zooplancton était confirmée expérimentalement à partir des taux d'assimilation qui étaient 6 fois supérieurs dans le cas des algues comparés aux bactéries. Nos résultats démontrent que la production autochtone supporte la communauté du zooplancton dans les écosystèmes dont le pool de carbone est dominé par les apports d'origine allochtone.

A stable isotope approach was applied to assess the importance of autochthonous versus allochthonous carbon in the diet of various zooplankton taxa and species from a series of oligotrophic lakes and large reservoirs situated in the boreal ecoregion. All ecosystems were net sources of CO_2 for the atmosphere resulting from mineralization of organic matter originating from the watershed and from flooded vegetation in reservoirs. The isotopic composition of dissolved inorganic (mean: -29.8 ‰) and organic carbon (mean: -29.4 ‰) confirmed the dominance of respired carbon of terrestrial origin in the overall carbon pool. Seston in lakes was primarily composed of allochthonous carbon (mean: 57%), whereas the relative importance of allochthonous carbon of seston in reservoirs was more variable (30 to 74%). Zooplankton carbon signatures (mean: -32.8 ‰) were strongly related to that of algae (mean: -32.0 ‰), and the relationship did not differ from the 1:1 line. Similarly, results from an experimental labelling of algae and bacteria indicated that assimilation of algal material was on average 6 times higher than that of bacteria. Our results demonstrate that autochthonous production supported zooplankton communities in ecosystems with carbon pools of predominately allochthonous sources.

Key words: zooplankton, autochthonous carbon, allochthonous carbon, carbon stable isotope.

2.2 INTRODUCTION

The quantification of the relative contribution of allochthonous versus autochthonous carbon to aquatic food webs has become a critical issue in limnology (Jones 2005). Field and laboratory evidence indicates that terrestrial subsidies play a major role in carbon cycling of most lakes and explain the excess of CO_2 measured in surface waters (Cole et al. 1994; Algesten et al. 2003; Lennon 2004; Duarte and Prairie 2005). In colored, humic waters, allochthonous carbon inputs are much higher than in clear water lakes (Jones et al. 1998; Salonen et al. 2005), and are responsible for net heterotrophy, where respiration exceeds production (Cole et al. 1994).

Aquatic ecologists recognize the ecological implications of allochthonous carbon in relation to CO_2 production but still lack a comprehensive understanding of the functional dynamics of allochthonous vs. autochthonous carbon cycling in aquatic food webs. In particular, the utilization of allochthonous detrital pathways by higher trophic levels of the planktonic food web has received little attention compared to its role as a subsidy for metabolism (Sobczak et al. 2002). In heterotrophic ecosystems, we may argue that food webs depend primarily on the decomposer pathway. Although validated at a whole-lake scale (Jonsson et al. 2001) and for various levels of the food web (Jones et al. 1999; Grey et al. 2000; Karlsson et al. 2003; Pulido-Villena et al. 2005; Carpenter et al. 2005; Kritzberg et al. 2006), such statements remain unclear because of contradictory results obtained within a wide range of heterotrophic aquatic ecosystems. Autotrophic carbon production was the main energy source for secondary producers in heterotrophic estuaries (Sobczak et al. 2002), rivers (Lewis et al. 2001; Thorp 2002; Martineau et al. 2004; Delong and Thorp 2006), streams (McCutchan and Lewis 2002), large lakes (Gaedke et al. 1996) and waterholes (Bunn et al. 2003). Variations in the utilization of autochthonous versus allochthonous sources by secondary producers are in part related to seasonality

(Zohary et al. 1994; Grey et al. 2001; Pace et al. 2004), trophic state (Cole et al. 2000; Grey et al. 2000) and to the humic content of water (Jones et al. 1999).

In this study, we examine stable isotope patterns in primary producers, primary consumers and terrestrial sources to determine the relative importance of allochthonous carbon versus autochthonous carbon utilization by zooplankton in a number of heterotrophic reservoirs and lakes situated in the Boreal ecoregion. We hypothesized that allochthonous carbon represents the main energy source for zooplankton community in these oligotrophic ecosystems, in which terrestrial inputs dominate the carbon pool. Few studies have quantified the proportion of terrestrial and algal carbon fueling secondary producers in boreal aquatic ecosystems (but see Karlsson et al. (2003) and Jones et al. (1999)). Actual knowledge on the importance of carbon subsidies to consumers is often based on results from small-sized lakes in which metabolism may be well connected to the nearby terrestrial environment (Pulido-Villena et al. 2005; Carpenter et al. 2005; Jones et al. 1999; Grey et al. 2000). Although much less abundant, larger sized ecosystems represent a substantial proportion of the landscape's lake area (Downing et al. 2006). Regarding trophic dynamics, large ecosystems are particularly pertinent to study, as food-chain length is determined by ecosystem size rather than productivity (Post et al. 2000). To our knowledge, no cross-ecosystems studies have assessed the importance of terrestrial versus algal subsidies to secondary producers in a large range of ecosystem sizes and no studies have addressed this question for reservoirs despite their relevance as heterotrophic ecosystems receiving large amount of allochthonous carbon from flooded soil and vegetation.

To discern between allochthonous and autochthonous resources, carbon stable isotope analysis represent a useful tool because of the consistent signatures between a consumer and its diet (Fry and Sherr 1984). Based on the isotopic signatures of dissolved organic and inorganic carbon (DOC and DIC) and particulate organic

carbon (POC), we verified the importance of allochthonous inputs in the carbon pool of these systems. Zooplankton reliance on terrestrial versus autochthonous carbon was assessed based on the signature of algal carbon, derived from that of particulate organic matter (POM). Particular attention was placed to detailed carbon stable isotope signatures of different zooplankton species and taxonomic groups, based on the hypothesis that the contribution of algal versus terrestrial carbon to organisms could differ according to diet. Stable isotope results were further compared to experimental labelling of algae and bacteria, aiming to determine the coupling between zooplankton and the microbial compartment.

2.3 MATERIALS AND METHODS

2.3.1 Study sites

In this study, 6 reservoirs and 16 nearby lakes situated in the Boreal Ecoregion were sampled between 2001 and 2003. The ecosystems were located in 3 regions of northern Quebec (Canada): Sainte-Marguerite (SM) (51°48'N, 50°48'E) (4 lakes and SM-3 reservoir), Manicouagan (M) (51°09'N, 68°39'E,) (3 lakes and MA-5 reservoir) and James Bay territories regions (JB) (54°20'N, 72°13'E) (9 lakes and LG-2, LG-4, LA-1 and LA-2 reservoirs). In SM and M regions, sampling was performed twice (early spring and mid-summer), whereas sites from JB were sampled once in mid-summer. Lakes were sampled at their deepest point and 5 to 11 sites were visited in reservoirs. Because of a rocky landscape in the SM and M regions, SM-3 and MA-5 reservoirs were deep and characterized by a canyon type shape. In contrast, the landscape at JB is rather flat and included a large number of shallow lakes and wetlands. In this region, river damming resulted in the creation of a chain of large and shallow reservoirs with complex dendritic shapes, following the bed of La Grande River. The main characteristics of sampled ecosystems are summarized in Table 1.

Table 2.1: Morphometric and limnological properties of reservoirs (Res.) and lakes situated in James Bay (JB), Manicouagan (M) and Sainte-Marguerite (SM) regions. Mean value (±SE) is indicated when several sites (n) were visited per ecosystem in spring (Sp) and summer (Sm).

Status	Region	Ecosystems	Season	n	Area (km^2)	Mean Depth (m)	Age (years)	CO_2 flux ($mg.m^{-2}.d^{-1}$)	Chl. a ($\mu g.L^{-1}$)	DOC ($mg.L^{-1}$)	POC ($mg.L^{-1}$)	ε_{PAR} (m^{-1})
Res.	JB	LA-1	Sm	5	1143.0	5.7	9	1191.4±302.0	2.5±0.3	5.0±0.2	443.4±47.9	0.8±0.05
Res.	JB	LA-2	Sm	5	286.0	6.3	8	1140.7±387.2	2.2±0.1	3.2±0.1	386.7±17.6	0.6±0.02
Res.	JB	LG-2	Sm	11	2645.0	21.1	23	1232.4±402.4	1.5±0.1	5.4±0.2	221.6±29.4	1.2±0.1
Res.	JB	LG-4	Sm	5	765.0	28.4	20	1891.8±373.9	1.3±0.1	2.7±0.1	317.0±15.9	0.6±0.03
Res.	SM	SM-3	Sp	6	246.1	48.4	1	7622.1±1942.7	0.5±0.2	7.0±0.2	231.6±26.5	1.3±0.04
			Sm	6				8633.7±886.2	2.3±0.7	7.4±0.4	197.8±42.3	1.0±0.08
Res.	M	MA-5	Sp	6	1950.0	61.6	35	3141.4±750.8	0.4±0.1	5.4±0.1	145.9±17.9	0.9±0.14
			Sm	5				1062±122.8	1.0±0.1	6.8±0.5	148.9±21.3	0.7±0.04
Lake	M	Berté	Sp	1	67.4	-	old	929.0	0.5	5.1	90.6	0.6
			Sm	1				784.0	0.6	6.2	86.9	0.6
Lake	JB	Desaulniers	Sm	1	10.6	7.6	old	649.0	1.5	8.5	262.9	1.2
Lake	M	Du Chaunoy	Sm	1	23.2	-	old	203.0	0.8	6.5	116.9	0.3
Lake	SM	Aux cèdres	Sp	1	9.3	-	old	1761.0	1.1	7.8	372.3	1.5
			Sm	1				274.0	1.1	12.0	320.2	1.2
Lake	SM	Houdan	Sp	1	2.7	-	old	1659.0	0.8	7.3	138.6	0.8
			Sm	1				1177.0	0.6	7.0	276.2	1.0
Lake	SM	Germain	Sp	1	24.9	-	old	3186.0	1.1	5.8	210.2	0.8
			Sm	1				1219.0	0.6	10.8	431.2	0.7
Lake	JB	Jean Marie	Sm	1	0.6	2.4	old	613.2	2.1	3.7	453.9	0.8
Lake	JB	Km.12	Sm	1	2.2	4.7	old	104.8	1.3	2.9	214.8	0.6
Lake	JB	Km.17	Sm	1	0.3	1.6	old	500.7	1.9	4.2	430.5	0.9
Lake	JB	Km.380	Sm	1	1.5	3.6	old	193.6	1.2	2.9	320.4	0.5
Lake	M	Matonipi	Sp	1	32.3	-	old	3591.0	1.3	6.9	250.4	1.1
			Sm	1				571.0	0.9	6.8	202.5	1.1
Lake	JB	Patukami	Sm	1	42.5	5.4	old	469.0	1.2	5.5	213.0	0.9
Lake	JB	Polaris	Sm	1	3.1	3.7	old	160.5	1.1	2.2	288.4	0.4
Lake	SM	Rapide	Sp	1	5.8	-	old	5157.7	1.3	7.7	-	-
Lake	JB	Ukau	Sm	1	3.3	4.8	old	725.0	1.6	6.5	320.3	2.3
Lake	JB	Yasinsky	Sm	1	41.0	4.4	old	1390.0	1.8	12.5	102.9	1.3

2.3.2 Sampling procedures and analysis

Vertical temperature, oxygen and pH profiles were measured using a multiprobe (YSI 6600) at each sampling station. The limit of euphotic zone and light coefficient extinction (ε_{par}) were determined with a double quantum sensor (Li-193SA and Li-190SA LI-COR®). For chemical and biological analysis, integrated water samples were collected from the euphotic zone, or from the epilimnion when thermal stratification was observed, using a 4 L Van-Dorn sampler. Samples for DOC concentrations were filtered on 0.45 µm polycarbonate filter (Millipore™) and kept at 4°C before analysis (Shimadzu TOC-5000A™). Chlorophyll a (Chl.a) concentrations were determined spectrophotometrically after overnight extraction of frozen Whatman GF/C filters in 96% hot ethanol and corrected for phaeopigments (Sartory and Groββelaar 1984). With the exception of LG-2 reservoir, CO_2 flux was determined in the field, for each station, using a nondispersive infrared analyzer (LI-7000, LI-COR®) connected to a floating chamber as described in Lambert and Fréchette (2005). At LG-2, gas from the chamber was sampled with a syringe and changes in CO_2 concentration, determined with a gas chromatograph (Varian Star-3400), were integrated over time.

2.3.3 Stable isotopes analysis (SIA)

The signatures of dissolved organic and inorganic carbon ($\delta^{13}C_{DOC}$ and $\delta^{13}C_{DIC}$) were determined for 20 and 29 sites respectively, sampled in spring 2002 and summer 2003. $\delta^{13}C_{DOC}$ was determined on the same samples collected as for DOC concentration analysis. For the isotopic signature of DIC, water samples (15 mL) were collected from the surface (1 m depth) in glass bottles, preserved with $HgCl_2$, sealed and kept at 4°C until analysis. Both $\delta^{13}C_{DOC}$ and $\delta^{13}C_{DIC}$ were determined at G.G. Hatch Isotopes Laboratory-University of Ottawa, using a TIC

TOC analyser (1010 O-I-Analytical) connected to a Finnigan Mat Delta Plus Mass Spectrometer, following the methods described by St-Jean (2003).

POM was collected on pre-combusted and pre-weighted filters (GF/C-Whatman) by filtering lake water (0.5 to 1 L) collected as previously described. Filters were frozen and subsequently dried (45° C), until constant weight. Filters were weighed and analysed for carbon content (%C) and $\delta^{13}C$ at GÉOTOP-UQAM, using a GV Instruments Isoprime™ mass spectrometer coupled to a Carlo Erba Elemental Analyser (NA-1500 series 2).

Zooplankton organisms were sampled from the pelagic zone over the entire water column and to a maximum of 30 m, using a 110 µm mesh sized plankton net (∅ 0.5 m.). Organisms were kept alive in filtered water to allow gut evacuation for 4 to 6 hours. In the laboratory, live zooplankton were narcotized using carbonated water and then sorted manually under a binocular to the genus level (i. e. *Daphnia* sp.; *Epischura* sp.; *Leptodora* sp.), or, in the case of small numbers of individuals, to main taxonomic groups (i.e. Calanoids; Cyclopoids). For copepods, only adult stages were collected and eggs were manually removed from the organism. In order to run SIA, zooplankton sample weight ranged from 0.1 to 0.6 mg. Sorted organisms were directly placed in pre-weighted tin capsules (8x5 D1008-Elemental Microanalysis Ltd.), placed in cryotubes and then shock-frozen in liquid nitrogen to minimize effects of preservation on isotopic signatures (Feuchtmayr and Grey 2003). Prior to SIA, all samples were freeze-dried. SIA were performed on the same equipment as for POC, following a protocol adapted for small-sized samples (Limén and Marty 2004).

Particulate (POM and zooplankton) SIA were performed in triplicates, whereas one sample per station was analysed for the determination of DIC and DOC

signatures. None of the samples were acidified prior to combustion because of low concentrations of inorganic carbonates in Canadian Shield waters. Results are given using the standard δ notation with $\delta=[(R_{sample}/R_{reference})-1] \times 1000$, expressed in units per thousand (‰) and where $R=^{13}C/^{12}C$ (Verardo et al. 1990). Secondary standard (Leucine) of known relation to the international standard of Pee Dee Belemnite was used as reference material. Precision on SI measurements was on average 0.08 ‰.

2.3.4 Calculations and zooplankton carbon source

A partition of variance analysis was performed on zooplankton carbon signatures ($\delta^{13}C_{ZOO}$) considering 3 sources of variance: 1) between ecosystems differences (inter-ecosystems variations), 2) intra-ecosystems differences (inter-sites variations) and, 3) intra-sites differences (due to taxonomic/gender variations). Each reservoir and all the lakes grouped together were considered as individual ecosystems. The number of sites in each reservoir (5 to 11) and the number of lakes (16) were used to determine the variance within each ecosystem and this level was nested within the ecosystem level. Finally, the number of taxonomic groups (3 to 7) was used to assess within sites variance and this level was also nested within sites and ecosystem levels. Additional analyses were performed on the M and SM region data sets alone to determine the effects of seasonality as a potential source of variance on $\delta^{13}C_{ZOO}$.

Phytoplankton carbon signature ($\delta^{13}C_{ALG}$) was estimated considering POM as a mixture of algae and detrital material, according to

$$(1)\ \delta^{13}C_{ALG} = [\delta^{13}C_{POM} - (1-x)(\delta^{13}C_{terr.})]/x$$

$\delta^{13}C_{POM}$ is the carbon signature of particulate organic matter. The proportion of algal carbon in POC (x) was calculated from the ratio between phytoplankton biomass and POC concentration both expressed in $\mu gC.L^{-1}$ (C/Chl.a=80) as described in Marty and Planas (2006). The signature of terrestrial organic carbon ($\delta^{13}C_{terr.}$), adjusted for each region, was determined from ANCOVA, as the intercept of the relationship between $\delta^{13}C_{POM}$ and Chl.a, assuming POC was 100% terrestrial when Chl.a reached zero.

Correlation and regression analyses were used to assess the strength of the relationship between zooplankton and other dietary particulate organic carbon (POM and algal) signatures. The slope and intercept of each relationship were compared to parameters of equality line (1:1) using custom tests. Additional models to predict $\delta^{13}C_{POM}$ and $\delta^{13}C_{ZOO}$ in these systems were constructed based on multiple regressions.

2.3.5 Algae versus bacteria as food source for zooplankton

Zooplankton assimilation rates of algae and bacteria organisms were determined in LA-2 (2 sites), LG-4 (3 sites) reservoirs and in one lake (Lake 380), following the protocol from Bosselmann and Riemann (1986). Bacteria were labeled with ^{3}H-leucine (tritium) (7 $\mu Ci \cdot L^{-1}$) in the dark and algae were labeled with radioactive sodium bicarbonate (10 $\mu Ci \cdot L^{-1}$) in clear bottles. During incubation, temperature was maintained within a 2°C range from in situ conditions. A GE-175W metal-halide lamp, simulating the complete visible light spectrum (400-800 nm) was used as light source during algal labeling. An integrated zooplankton sample (>53 μm) was added to labeled suspensions for 45 min. Assimilation rate was measured after gut evacuation of labeled particles in filtered water (45 min.). After incubations, zooplankton were filtered, rinsed, transferred to scintillation vials and fixed with

formaldehyde (4%). Zooplankton organisms were sorted to main taxonomic groups as previously described for SIA and prepared for counting according to standard protocol (Gulati et al. 1982). Assimilation rates of bacteria and algae ($ml^{-1} \cdot ind^{-1} \cdot h^{-1}$) were calculated by dividing the radioactivity of organisms ($cpm \bullet ind^{-1}$) by that of the filtrate and incubation time ($cpm \cdot ml^{-1} \cdot h^{-1}$).

2.4 RESULTS

Lakes and reservoirs from this study are situated in the boreal region where vegetation is typical of the Taiga in the James Bay region and of the mixed forest in the southern regions. As a result of poor nutrient soils and harsh climate, lichens, bryophytes and slow growing conifers (black spruce and Jack pine) are the most common flora in these regions, with the additional presence of yellow birch and poplar in M and SM regions. The lakes and reservoirs sampled in these regions covered a large range of sizes with surface area varying over 5 orders of magnitude (0.25 to 2645 km^2, Table 1). Deep lakes and reservoirs (SM and M regions) were thermally stratified in summer whereas ecosystems situated on JB region did not stratify because of shallow mean depths and wind mixing. Full oxygen saturation was measured in the entire water column at all sites. Ecosystems were oligotrophic in term of phytoplankton biomass (Chl.a < 2.7 $\mu g \cdot L^{-1}$) and ranged from clear to colored water (DOC: 2.2 to 12.5 $mg \cdot L^{-1}$ and ε_{par}: 0.3 to 2.3 m^{-1}). Reservoir age ranged from 1 year (SM-3) to 35 years (MA-5) (Table 1).

Evasive CO_2 fluxes were measured at water-air interface at all ecosystems. Average CO_2 flux was 2594 $mg \cdot m^{-2} \cdot d^{-1}$, with high variation among ecosystems (105-15209 $mg \cdot m^{-2} \cdot d^{-1}$). The lowest fluxes were measured in the lakes and the old reservoirs LG-2 and MA-5, whereas the highest fluxes were observed in the youngest reservoir SM-3. Based on 2003 data, seasonal variations in CO_2 fluxes consisted in

higher values in spring compared to summer, with the exception of SM-3, where mean fluxes were not significantly different between seasons (t=-0.47, df=12, p=0.32). CO_2 flux was positively related to DOC concentration, indicating the importance of pelagic respiration of terrestrial material, although the relationship was weak (r^2=0.13, df=71, p=0.002) and no significant relationship was found with light extinction coefficient (p>0.05).

2.4.1 Carbon isotopic signature of dissolved inorganic and organic carbon

Average DIC signature was -29.8 ‰ (-15.3 to -43.1 ‰, Table 2). Signatures were generally more variable in lakes compared to reservoirs, but with no significant differences between ecosystem types (p>0.05). $\delta^{13}C_{DIC}$ was significantly lighter in spring (-32.9 ‰) than in summer (-28.4 ‰) (t=-1.95, df=27, p=0.03). Variations in $\delta^{13}C_{DIC}$ were significantly related to pH (r^2=0.39, df=29, p=0.0003), with the lightest signatures measured in the most acidic lake (L. Rapide, -43.1 ‰; pH, 4.8) and the heaviest signatures measured in the most alkaline lake (Km. 12, -15.3 ‰; pH: 7.5). DOC signatures (mean: -29.4 ‰) were similar among sites (C.V.=-6.8) with no significant difference observed between lakes and reservoirs (p=0.98) (Table 2).

2.4.2 Carbon stable isotope of particulate organic matter and phytoplankton

$\delta^{13}C_{POM}$ values varied from -27.5 ‰ to -34.6 ‰ in spring and summer respectively, at SM-3, the recently flooded reservoir. The average POC signature was -29.9 ‰, suggesting that both algal and detrital material were part of the bulk particulate matter, with a possible dominance of terrestrial carbon originating from river inflow and the decomposition of flooded material (Table 2; Fig. 1, A). Overall, POC signatures were generally enriched in lakes compared to reservoirs (t=-4.25,

df=72, $p<0.0001$) and the most depleted values were found in the recently flooded SM-3 reservoir.

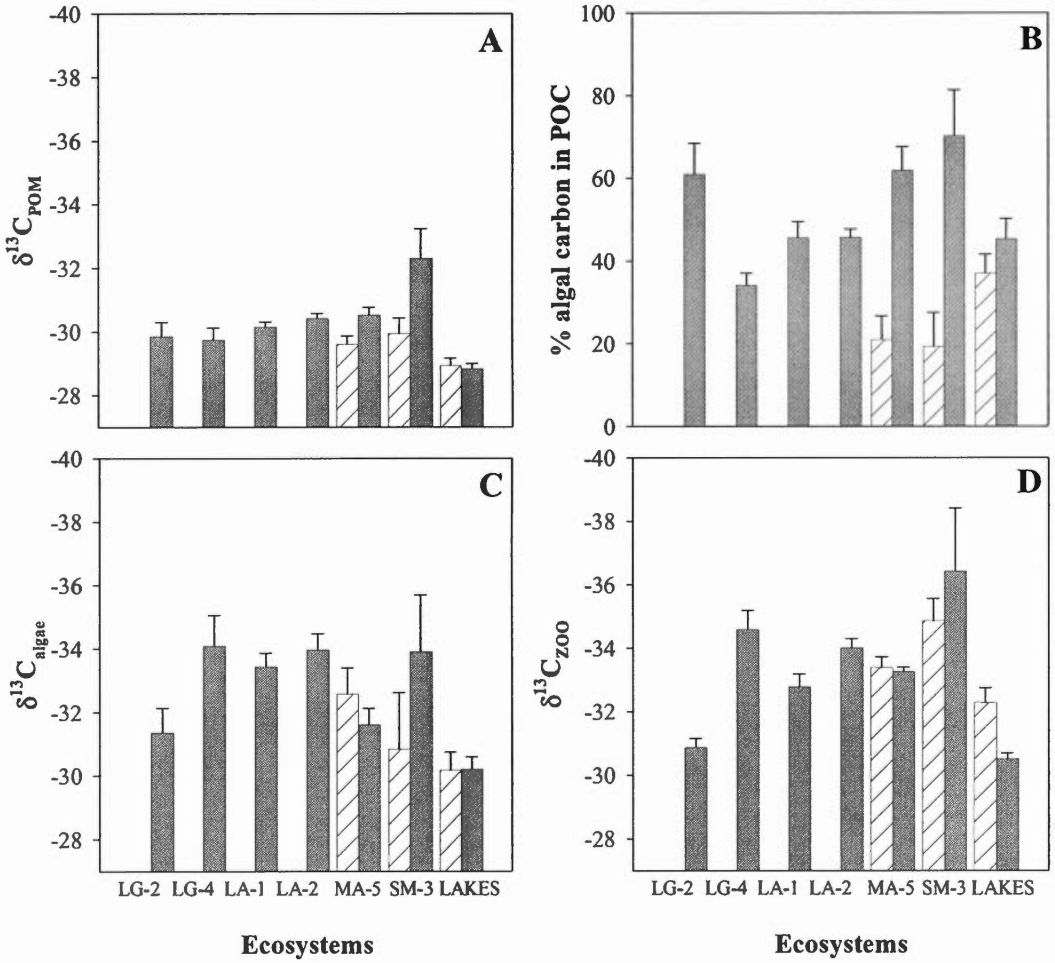

Fig. 2.1: Mean values (± SE) of (A) $\delta^{13}C_{POM}$, (B) percentage of algal carbon in seston, (C) $\delta^{13}C_{ALG}$ and (D) $\delta^{13}C_{ZOO}$ per ecosystem in spring (dashed) and summer (gray).

Table 2.2: Mean (± S E.) $\delta^{13}C$ values per ecosystem (‰) of dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), particulate organic carbon (POC) and zooplankton groups or taxa.

	LA-1	LA-2	LG-2	LG-4	SM-3		MA-5		Lakes	
	Summer	Summer	Summer	Summer	Spring	Summer	Spring	Summer	Spring	Summer
DIC	-26.6 ± 3.2	-29.1 ± 2.0	.	-29.7 ± 1.3	-33.2 ± 1.6	.	-32.4 ± 4.5	.	-33.2 ± 5.4	-28.3 ± 3.4
DOC	-29.1 ± 0.9	-28.4 ± 0.8	.	-30.8 ± 0.8	.	.	.	.	.	-29.4 ± 0.9
POC	-30.1 ± 0.2	-30.4 ± 0.2	-29.9 ± 0.5	-29.7 ± 0.4	-29.9 ± 0.5	-32.3 ± 0.9	-29.6 ± 0.3	-30.5 ± 0.2	-28.9 ± 0.2	-28.8 ± 0.2
Phytoplankton	-33.4 ± 0.4	-34.0 ± 0.5	-31.4 ± 0.8	-34.1 ± 1.0	-30.8 ± 1.8	-33.9 ± 1.8	-32.6 ± 0.8	-31.6 ± 0.5	-30.2 ± 0.6	-30.2 ± 0.4
***Daphnia* sp.**	-33.4 ± 0.3	-34.2 ± 0.3	-31.1 ± 0.3	-34.6 ± 0.7	-36.8 ± 1.1	-36.3 ± 2.3	-33.6 ± 0.6	-33.1 ± 0.2	-30.8 ± 0.5	-30.2 ± 0.2
***Holopedium* sp.**	-33.4 ± 0.3	-33.8 ± 0.4	-30.6 ± 0.3	-34.4 ± 0.6	.	-36.8 ± 1.5	.	-33.4 ± 0.2	-31.1 ± 0.3	-30.5 ± 0.3
***Bosmina* sp.**	-29.1	.	-30.6 ± 0.1	.	.	.	.	.	.	-28.9 ± 0.3
***Leptodora* sp.**	-32.5	-31.9	-32.1	.	.	-32.8	.	.	.	-29.7 ± 0.4
Cyclopoïds	-32.5 ± 0.4	-34.6 ± 0.4	-30.8 ± 0.5	-35.3 ± 0.6	-34.0 ± 0.9	-34.8 ± 1.6	-33.2 ± 0.2	-32.9 ± 0.2	-30.9 ± 0.6	-30.4 ± 0.4
Calanoïds	-33.2 ± 0.8	-34.0 ± 0.1	-31.4 ± 0.7	-34.1 ± 0.8	-33.4 ± 0.5	-36.3 ± 1.7	-33.4 ± 0.3	-33.4 ± 0.2	33.0 ± 0.6	-31.1 ± 0.3
***Epischura* sp.**	-32.6 ± 0.3	-33.0 ± 0.2	.	-33.3 ± 2.3	-36.7	-35.9 ± 2.2		-33.4 ± 0.3		-30.5 ± 0.3
***Chaoborus* sp.**	.	.	.	.	-32.1	.	.	.	-29.8 ± 1	.
Chironomids	.	-30.3	.	.	-32.4 ± 0.3	.	.	.		.

Seasonal variations in $\delta^{13}C_{POM}$ consisted of heavier signatures in spring compared to summer in reservoirs (p=0.001, df=12; p=0.01, df=11, respectively), and no seasonal trends were observed in lakes (p=0.77, df=11). $\delta^{13}C_{POM}$ was negatively related to Chl.a (r^2=0.34, p<0.0001, n=69), in particular in SM-3 reservoir where the highest variation in Chl.a was measured (r^2=0.84, p<0.0001, n=12). Furthermore, parameters of such relationship differed significantly among regions (JB, M and SM) (ANCOVA, r^2=0.54, p<0.0001, n=65) and therefore, region-specific intercept values were used as terrestrial carbon signature in the calculation of algal signatures ($\delta^{13}C_{terr.\ JB}$=-27.5 ‰, $\delta^{13}C_{terr.\ M}$=-29 ‰ and $\delta^{13}C_{terr.\ SM}$=-28.15 ‰). Additional variation in $\delta^{13}C_{POM}$ was explained by a multiple regression model in which log(Chl.a) and log(CO_2 flux) entered with negative coefficients (Table 3).

Table 2.3: Multiple regression models for prediction of $\delta^{13}C_{POM}$ and $\delta^{13}C_{ZOO}$

Predicted variables	Parameters	Estimates	S.E.	p(t)	VIF	Partial r^2
$\delta^{13}C_{POC}$	Intercept	-23.6	0.9	<.0001		
	Log (Chl. a)	-5.8	0.8	<.0001	1	0.28
	Log (CO_2 flux)	-1.3	0.2	<.0001	1	0.24
	n=65, F=34.2, p(F)<.0001, r^2 adj.=0.51					
$\delta^{13}C_{ZOO}$	Intercept	-11.6	3.6	0.002		
	$\delta^{13}C_{POC}$	1	0.1	<.0001	1	0.38
	Log (Surface temperature)	5.7	0.9	<.0001	1	0.15
	Log (zooplankton body weight)	-1.8	0.4	<.0001	1	0.12
	n=65, F=45.2, p(F)<.0001, r^2 adj.=0.67					
	Intercept	-21.8	2	<.0001		
	$\delta^{13}C_{ALG}$	0.6	0.05	<.0001	1	0.58
	Log (Surface temperature)	4.9	0.8	<.0001	1	0.16
	Log (zooplankton body weight)	-0.9	0.3	0.009	1	0.03
	n=56; F=62.7, p(F)<.0001, r^2 adj.=0.77					

Based on a C:Chl.a ratio of 80, algal carbon slightly exceeded POC concentration in 3 sites, and in these cases, POC was considered as 100% algal. Overall, the average proportion of algal carbon in POC was 26 and 51 % in spring (range: 19 to 37 %) and summer (range: 34 to 70%) respectively and no significant seasonal trend was observed in lakes (Fig. 1, B). When averaged seasonally, the proportion of algal carbon was similar in reservoirs and lakes (47 and 43 % respectively). Using Eq. 1, carbon algal signatures ranged from -40.1 to -27.5‰, with a mean value of -32.0‰ (Table 2, Fig. 1, C) and followed similar trend to that of $\delta^{13}C_{POM}$, with most depleted values found in the SM-3 reservoir and enriched $\delta^{13}C_{ALG}$ found in the LG-2 reservoir and lakes. Algal carbon was responsible for the depletion in POC signatures compared to terrestrial signatures: $\delta^{13}C_{ALG}$ was positively correlated to $\delta^{13}C_{POM}$ and was always depleted compared to terrestrial and POC signatures, with the exception of 2 stations from the SM-3 sampled in spring (Fig. 2, A).

2.4.3 Zooplankton carbon signatures

The carbon isotopic composition of 3 to7 zooplankton taxonomic groups was determined at each site. Collected species were generally ubiquitous and consisted of Cladocerans *Daphnia longeremis*, *Holopedium gibberum, Leptodora kindii*; Cyclopoid *Diacyclops thomasi*; Calanoids *Leptodiaptomus minutus* and *Epischura lacustris*. *Bosmina sp.*, *Chironomidae sp.* and *Chaoboridae sp.* were found occasionally. $\delta^{13}C_{ZOO}$ ranged from -39.8 to -28.3‰ (mean value: -32.8‰). Based on summer data, ecosystems, sites and taxonomy effects explained most of total $\delta^{13}C_{ZOO}$ variance (R^2=0.95) (Table 4). Differences among ecosystems accounted for the majority of $\delta^{13}C_{ZOO}$ variance (65 %) (Fig. 1, D), while within ecosystem and within site variations (taxonomy) explained only 19 and 7.9 % of the total variance respectively (Table 4). There was no significant difference in zooplankton carbon

signatures between lakes (p=0.46), hence allowing for these systems to be considered as a single ecosystem type in variance partition analysis. The heaviest signatures were observed in lakes and in the old LG-2 reservoir, whereas lightest signatures were measured in the deeper (LG-4, MA-5) and younger (SM-3) reservoirs (Table 2). Data collected in spring and summer allowed us to consider seasonality as an additional effect in variance partitioning (Table 4). We found no variance associated to seasonal changes in $\delta^{13}C$ of zooplankton (p=0.4, df=30). Similar variance partition to summer data was obtained for the ecosystems, sites and taxonomic effects. Therefore, because of rather homogenous signatures obtained among taxonomic groups, taxa specific $\delta^{13}C_{ZOO}$ values were averaged to explore relationships with other variables.

Table 2.4: Partition of variance of $\delta^{13}C_{ZOO}$ for 2002 (spring/summer) and 2001-2003 (summer) periods. Because of unbalanced data set, Residual maximum likelihood (REML) was used to determine variance components as described in Matthews and Mazumder (2003).

Data set	Random effects	df	SS	F	p	% variance
Spring/Summer 2002, (r^2=0.73, n=359)	Season	1	0.3	0.09	0.76	0
	Ecosystems	2	1095.4	169	<0.0001	55.6
	Sites[ecosystems]	16	224.8	4.3	<0.0001	6.91
	Organisms[ecosystems,sites]	71	452.8	1.96	<0.0001	7.6
	Residuals					29.9
Summer 2001-2003, (r^2=0.95, n=501)	Ecosystems	6	1201.5	386.1	<0.0001	64.95
	Sites[ecosystems]	51	602.6	22.8	<0.0001	19.02
	Organisms[ecosystems,sites]	166	277	3.2	<0.0001	7.86
	Residuals					8.1

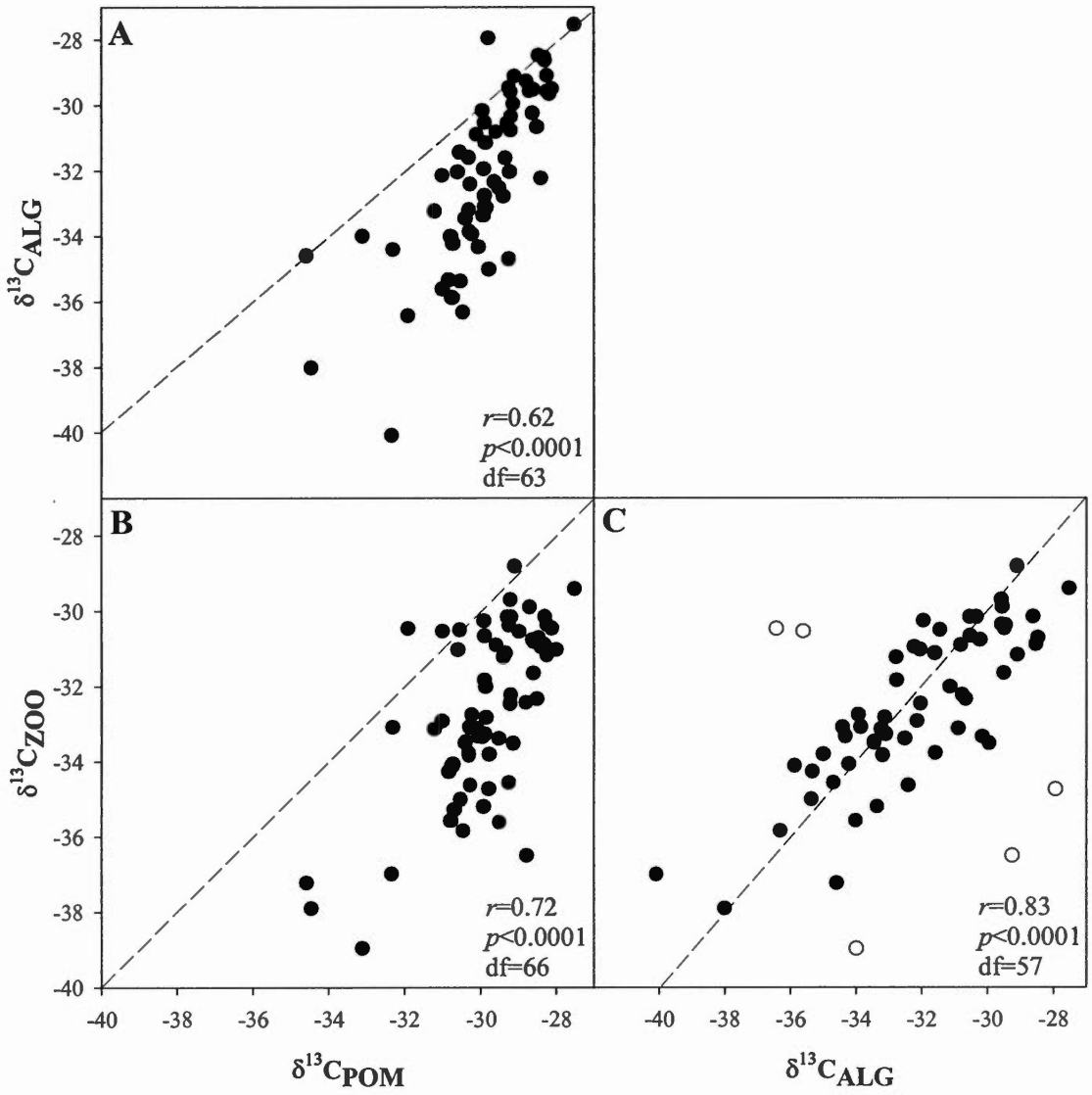

Fig. 2.2: Correlation matrix between $\delta^{13}C_{POM}$, $\delta^{13}C_{ZOO}$ and $\delta^{13}C_{ALG}$. Dashed line indicates equal $\delta^{13}C$ isotopic composition. White points were identified as outliers and excluded from statistical analysis.

Mean zooplankton carbon signatures were positively correlated to POC signatures (r=0.72, p<0.0001, df=66) (Fig. 2, B) and generally depleted relative to POC. Mean depletion between $\delta^{13}C_{ZOO}$ and $\delta^{13}C_{POM}$ was 2.95‰ (range: -9.65 to 2‰). The maximum degree of enrichment was observed in SM-3 reservoir, whereas $\delta^{13}C_{ZOO}$ from lakes and LG-2 reservoirs tended to be similar to that of POC. In cases where zooplankton signature was enriched compared to $\delta^{13}C_{POM}$ (16 cases from LG-2 reservoir and 3 lakes), the degree of enrichment was small (mean: 0.6‰, range: ~0 to 2‰). The difference between the $\delta^{13}C$ values of zooplankton and POM was not related to Chl.a concentration (p=0.15), although the highest depletion values were found in the most productive reservoir (SM-3).

Zooplankton carbon signatures were also strongly related to algal signatures (r=0.83, p<0.0001, df=57) (Fig. 2, C). Parameters from the relationship were not different than 1:1 line (p=0.88), indicating that $\delta^{13}C$ divergence between zooplankton and phytoplankton was not different than zero, regardless of the gradient of signatures. In order to best predict zooplankton carbon signatures, regression models were constructed. $\delta^{13}C_{ZOO}$ was a function of log(surface water temperature), log(Chl.a), log(zooplankton body weight) and $\delta^{13}C_{POM}$ ($R^2_{adj.}$=0.66, p<0.0001, df=60) and a comparable model was obtained with log(surface water temperature) and $\delta^{13}C_{ALG}$ ($R^2_{adj.}$=0.76, p<0.0001, df=55) (Table 3). Morphometric characteristics were also related to zooplankton signatures as a strong positive relationship between $\delta^{13}C_{ZOO}$ and surface area was found in reservoirs, whereas the enriched signatures from lakes aggregated (Fig. 3).

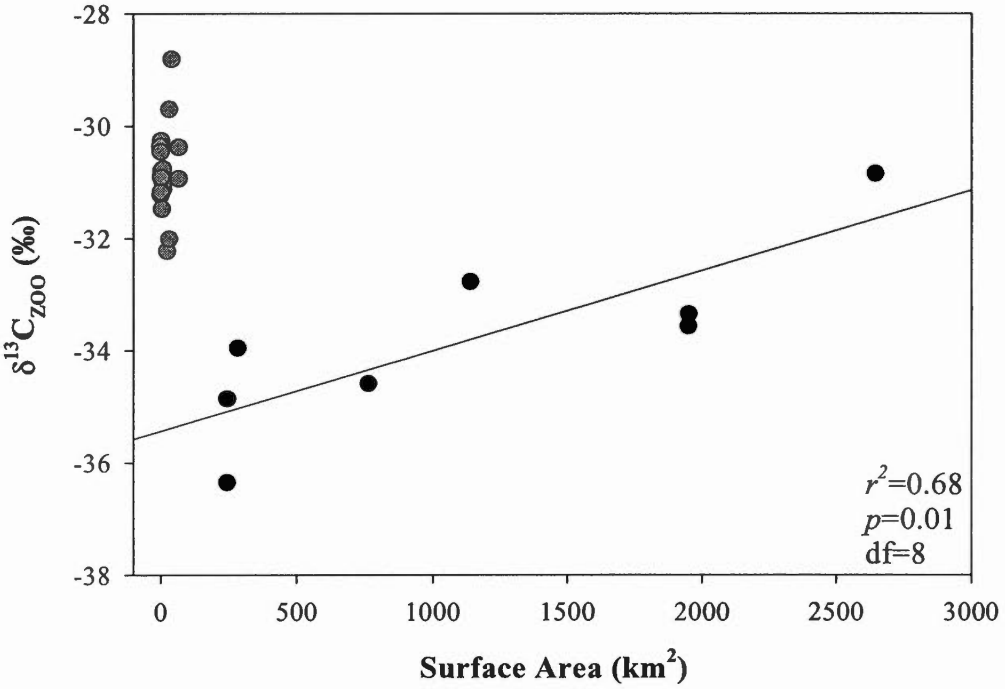

Fig. 2.3: Relationship between mean $\delta^{13}C_{ZOO}$ and ecosystems surface area. Regression line was fitted on reservoirs data only.

2.4.4 Labeling experiment

The labeling experiment tested the hypothesis that bacterial assimilation would be lower in lakes than in reservoirs in which mineralization of flooded organic matter occurs. Further, we hypothesized that assimilation rates would be representative of feeding behaviors, with higher rates for filter feeders compared to detritivorous and carnivorous organisms. Instead, assimilation of algae and bacteria was similar among ecosystems (p=0.6 and p=0.7 respectively). Both algal and bacterial assimilation rates were significantly lower for Cyclopoids, Calanoids and

Holopedium sp. compared to *Daphnia* sp. and *Epischura* sp. (p=0.01 and p=0.002, respectively).

Assimilation of algae was positively related to that of bacteria (Fig. 4), indicating that both organisms were part of zooplankton diet. Further, as shown by the significant intercept of the relationship (p=0.0003), algal assimilation rate was always higher than that of bacteria. Overall, the assimilation of algae accounted for 70 to 100% of total assimilation. In addition, the slope of the relationship was not different than 1 (p=0.2), implying that the proportion of both assimilated food types was similar, regardless of their rate.

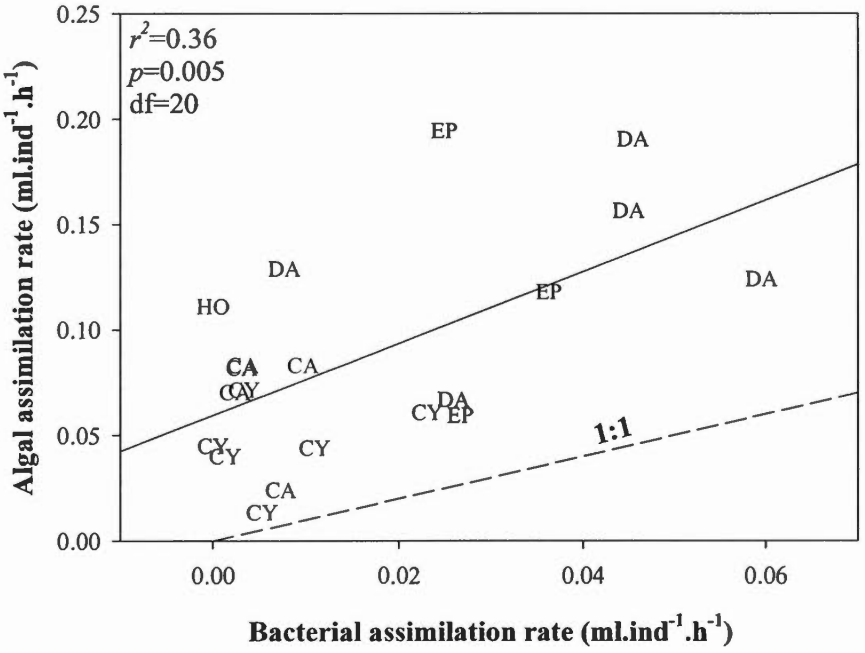

Fig. 2.4: Relationship between algal and bacterial assimilation rates. DA=*Daphnia* sp., CY=Cyclopoids, CA=Calanoids, EP=*Epischura* sp. and HO=*Holopedium* sp.

2.5 DISCUSSION

2.5.1 Sources of carbon available to zooplankton and utilization

According to both stable isotope and experimental approach, autochthonous carbon was the main source of carbon for zooplankton, despite the overall carbon pool allochthony. Therefore, this finding negates our original hypothesis that zooplankton carbon signatures should approach those of POC if allochthonous carbon enters their diet. We also hypothesised that variation in $\delta^{13}C_{ZOO}$ according to taxonomy should be related to differential diet. Instead, our results indicate that algae carbon was the main carbon source driving the overall zooplankton community, as little variations were observed among taxonomic groups. Additionally, algae was the main food source for zooplankton in both spring and summer, and this result suggests that temporal variation in food sources might be limited in these ecosystems because the short ice-free period does not allow for the temporal succession of food sources commonly observed in lakes situated in warmer regions (Zohary et al. 1994; Grey et al. 2001). Therefore, results from this study likely apply over an annual basis.

Crustacean zooplankton can feed on a wide range of particulate organic matter from pelagic and benthic origin, which includes algae, bacteria, protozoans and detritus. The labelling experiments provided evidence that some contributions of organic matter originating from the microbial loop enter metazoan production. However, this amount was minimal, illustrating the poor coupling between microbial and zooplankton organisms. According to production and respiration measurements from the mixed (stratified) or euphotic zone (unstratified) of these systems, gross photosynthesis generally exceeded community respiration (Planas et al. 2005). Such result implies that bacteria production was also supported by autochthonous carbon in the upper layer of the water column. Therefore, although the number of sites was small, results from the labelling experiments were in concordance with those obtained by stable isotopes.

The 1:1 relationship observed between zooplankton and algal signatures, as well as models predicting $\delta^{13}C_{ZOO}$ provide further evidence of the importance of autochthonous carbon for zooplankton. Our results indicate the dominance of pelagic algae in reservoirs but also possibly benthic algae in the case of lakes as the main food source for zooplankton. Lake seston represents a mixture of particulate organic carbon from a wide range of origins such as autotrophs (phytoplankton, macrophytes, re-suspended benthic algae), heterotrophs and detritus of terrestrial origin and the isotopic composition of seston reflects an average of the relative contribution of all of these sources. In this study, algal and terrestrial carbon sources composed most of the POC, allowing for the use of a simple two-sources mixing model to calculate carbon algal signature. Macrophytes were never observed in our ecosystems as the results of poor nutrient availability and the absence of a stable littoral zone due to water level fluctuations in reservoirs. Benthic algal production does not contribute significantly to seston in most reservoirs because the littoral zone is limited by frequent and large changes in water level, often exceeding the depth of the euphotic zone. This does not apply to the shallow LA-1 and LA-2 reservoirs and most lakes from JB region in which mean depth to euphotic zone ratio was lower than 1. At these sites, the sediment surface was exposed to light, and benthic algae could develop in these shallow zones. Carbon signatures of benthic algae are usually enriched compared to that of pelagic algae because of greater diffusion resistance of CO_2 through the biofilm boundary layer (France 1995). Therefore, the carbon signature of seston based on a mixture of benthic and pelagic algae is expected to be enriched compared to non-benthic seston (Hecky and Hesslein 1995). However, $\delta^{13}C_{POC}$ was similar between shallow and deep reservoirs (in which benthic algae contributed little to primary production). The most enriched POC signatures were measured in lakes and in these ecosystems, the resuspension of benthic algal may have influenced seston signature. Observations of seston algal composition confirmed such hypothesis since main algal taxa found in lakes (*Tabellaria*

fenestrata) was meroplanktonic. Therefore, carbon algal proportion in POC and subsequent algal carbon signatures could be either representative of phytoplankton in reservoirs and/or of a mixture of benthic and pelagic autotrophs in lakes.

Our approach to determine carbon algal proportion and algal signature is based on the assumption that all algal carbon contains chlorophyll and ignores non-chlorophyllic autotrophic carbon (detrital autotrophic matter, heterotrophs relying on algal production). Thus, the proportion of algal carbon in seston entering the mixing model could be underestimated. However, such underestimation had little overall influence on carbon algal signatures, as shown previously by sensitivity analyses and by the concordance of signatures obtained from calculation and from a set of physically separated algal material (Marty and Planas 2006). The reliance of mixing models ultimately depends on the difference between the isotopic signatures of end members entering a mixing model. In our study, POC signatures were in general depleted compared to terrestrial signatures and such depletion was due to the presence of algae. Therefore, we were successful to apply a simple mixing model to distinguish an algal signature within POC bulk because of variation in the proportion of algal carbon and also because POC signatures differed from terrestrial signatures.

Zooplankton carbon signatures were generally lighter than those of POM, as commonly reported in freshwater (del Giorgio and France 1996; Grey and Jones 1999; Jones et al. 1999). In this study, such depletion was clearly related to the selective assimilation of light algal compound, although several other explanations have been previously proposed, such as lipid storage, feeding behaviour at a particular depth or selective assimilation of light non-algal material. Our analyses showed that a small proportion of $\delta^{13}C_{ZOO}$ variance was due to taxonomy and seasons, supporting previous results indicating that lipid accumulation is insufficient to account for such depletion (del Giorgio and France 1996; Grey et al. 2000; Zohary

et al. 1994; Matthews and Mazumder 2003). Considering the absence of thermal stratification at most sites, particulate organic signatures are likely to be representative of food sources available for zooplankton at all depths since the seston samples were evenly distributed in the mixed waters. In the summer-stratified ecosystems (M and SM regions), the lack of variation in $\delta^{13}C_{ZOO}$ and $\delta^{13}C_{POM}$ according to seasons also indicates that the signature of food sources for zooplankton remained homogenous even when possibly influenced by stratification. This conclusion was also supported by the comparable signatures obtained among taxonomic groups, including *Daphnia* sp. that has the ability to migrate widely within the water column and therefore the potential to have a different carbon signature than reduced-migratory organisms. Consequently, we expect little effect of vertical feeding behaviour on variation in zooplankton signatures.

Previous studies related zooplankton carbon depletion to the consumption of methanotrophic bacteria, which carbon signature typically ranges between -60 to -80‰ (Bastviken et al. 2003; Jones et al. 1999). We further discuss below the importance of methanotrophy for the isotopic composition of DIC. Regarding their assimilation as food source, methanotrophic bacteria are unlikely available to consumers because oxygen concentration was high in the water column. The labelling experiment provided further evidence of the limited carbon assimilation of bacterial origin compared to algal source. Based on multiple regression models, most of $\delta^{13}C_{ZOO}$ variation was related carbon algal or POC signatures as well as temperature, which controls algal metabolism and therefore carbon assimilation.

The relation between $\delta^{13}C_{ZOO}$ depletion compared to $\delta^{13}C_{POM}$ and selective feeding on $\delta^{13}C$ light phytoplankton was previously reported by studies covering a large gradient of lake trophy and consisted in high depletion between zooplankton and POC signatures in oligotrophic lakes and overlapping signatures in eutrophic

lakes, because of variations in the proportion of algae in POC (del Giorgio and France 1996; Grey et al. 2000). Accordingly, high depletion should be observed for all ecosystems in this study since they are within the oligotrophic status. In contrast, our data demonstrate that a wide range of depletion could be found in oligotrophic ecosystems, comparable to that of lakes covering the full range of trophic states (del Giorgio and France 1996). Consequently, such observations indicate that lake trophy alone cannot account the zooplankton depletion compared to POC, because not only does the proportion of algal carbon in POC vary with productivity, but also the carbon algal signature itself.

Considering the similarity between the algal-zooplankton relationship and the line of equality, others carbon sources than algae are unlikely to be important in zooplankton diet. Departure from this line may reflect carbon fractionation between zooplankton and its food. Fractionation occurring between primary producers and their consumers is usually low (ex: 0.43‰, (Grey et al. 2000)) and therefore will explain little of the residuals. Instead, we believe that residuals are related to zooplankton selective feeding within the algal community. Recent progress in separation techniques has allowed for detailed signatures within phytoplankton community to be determined and showed considerable variation in $\delta^{13}C$ between algal species (Pel et al. 2003; Hamilton et al. 2005; Vuorio et al. 2006). Therefore, we propose that algal signatures obtained from POC represent the average signature of a diverse community in which zooplankton are choosing their preferred food source and thus reflect its signature.

2.5.2 Heterotrophy and isotopic composition of major carbon pools

Zooplankton autochthony was found in a series of oligotrophic lakes and reservoirs characterized by a carbon pool dominated by allochthonous inputs. The

ubiquitous efflux of CO_2 and the isotopic composition of dissolved organic compounds imply that ecosystems from this study were net heterotrophic because of the mineralization of allochthonous carbon. Lakes and reservoirs had similar $\delta^{13}C_{DIC}$ and $\delta^{13}C_{DOC}$ values, indicating comparable processes and sources governed the dynamics of dissolved carbon. Both ecosystem types had efflux of CO_2, and were thus, by definition, net heterotrophic (Kling et al. 1991; Cole et al. 1994; Duarte and Prairie 2005). DOC was of terrestrial origin because its signature was homogenous among ecosystems and similar to that of terrestrial C_3 vegetation (Jones et al. 1999).

The importance of respired carbon in DIC pool was supported by $\delta^{13}C_{DIC}$ data, which were far from equilibrium with atmosphere and similar to terrestrial signatures at most of our sites. Such data indicate that respiration occurring within ecosystems (pelagic and benthic) or externally (peats and ground water) were the primary sources of DIC (Striegl et al. 2001; Jonsson et al. 2003). The light DIC signatures we found in our systems could have been the result of several processes including methanogenesis and photooxidation. Methanogenesis is an important process in the carbon cycling of humic lakes and reservoirs requiring anoxic conditions (Kling et al. 1992). Dissolved oxygen concentrations were always high in the water column of our ecosystems, so methane production could only have occurred during anaerobic decomposition in the sediments, and this methane can be oxidized to CO_2 by methanotrophic bacteria (Rudd et al. 1976). The isotopic signature of CO_2 produced through this process is much lighter than the one produced via the aerobic respiration of organic compounds and influence the overall signature of DIC bulk (Prokopenko and Williams 2005). Additionally, our light $\delta^{13}C_{DIC}$ values in surface waters may be the result of photooxidation of DOC, which occurs commonly in high latitude regions and produces light DIC signatures via photochemically-induced fractionation (Bertilsson and Tranvik 2000; Opsahl and Zepp 2001).

Therefore, zooplankton reliance on algal carbon source did not result from ecosystem autotrophy. Based on similar conclusions in rivers, Thorp (2002) stated a new paradox that is "How can animal biomass within food webs be fuelled primarily by autochthonous autotrophic production if the ecosystem as a whole is heterotrophic?" Such paradox also applied to lakes and reservoirs presented in this study.

2.5.3 Variations in autochthony versus allochthony in oligotrophic ecosystems

Our results contrast with those of most studies from oligotrophic ecosystems in which autochthonous production is often believed to be insufficient to support the food web. This discrepancy could be explained by differences in the characteristics of ecosystems. In addition, as a large number of studies dealing with carbon subsidies for organisms are based on stable isotope approaches, the apparent allochthony versus autochthony of organisms may be related to methodological issues on the calculation of carbon sources signatures.

Most studies looking at carbon subsidies are conducted in small lakes in which terrestrial inputs are subject to little dilution and may be influencing the pelagic food web to a greater extent compared to large systems. Lake colour influences the thickness of the mixed layer and further, the photosynthetically available irradiance (Fee et al. 1992). Low light availability may result in a reduction of algal production and therefore increase the coupling between the microbial loop and metazoans (Jansson et al. 2000). In small lakes, the littoral zone represents a greater proportion of lake surface and its production is key component supporting the food web. As previously discussed, the interpretation of stable isotope data is complicated in these systems because benthic algae and terrestrial carbon could share similar carbon signatures (Hecky and Hesslein 1995). Therefore, the apparent

allochthony of small lakes could result from ignoring the utilization of benthic algae. Our study covered a large range of ecosystem sizes in which autochthonous production is mainly of pelagic origin, thereby eliminating the confounding effects of benthic algae signatures. As a result, zooplankton carbon signatures in the larger ecosystems were depleted compared to terrestrial signatures and variation in such depletion was a function of ecosystem size, underlying variations in algal metabolism (Planas et al. 2005) and therefore carbon fractionation occurring during photosynthesis.

Beside the size effect on carbon sources for zooplankton, we also believe that different conclusions regarding carbon sources to organisms may have been generated because of methodological issues in the calculation of algal signatures. Given the difficulty in separating algae from a bulk sample, different approaches are currently used to infer algal signatures. In this study, the carbon signature of POM with a correction for algal biomass was applied to calculate $\delta^{13}C_{ALG}$. As previously discussed, we recognized the limits of such approach but also its validity when terrestrial and particulate carbon signatures are distinct (Marty and Planas 2006). $\delta^{13}C_{ALG}$ could also be calculated as a function of the signature of carbon assimilated during photosynthesis and algal fractionation (ε_p). The high variation in algal signatures combined to the homogenous $\delta^{13}C$ of DIC among ecosystems indicates the heterogeneity in algal fractionation and therefore implies that no single fractionation value could be applied to calculate algal signatures in ecosystems characterized by different algal communities and high variation in controlling biogeochemical variables (CO_2 concentration, growth rate and $\delta^{13}C_{DIC}$) (Finlay 2004; Marty and Planas 2006). For instance, if the light respired signatures obtained for DIC in this study were applied to calculate algal signatures, we would have concluded that a minimal fraction of zooplankton diet was based on algae and that allochthony dominated the food web. Therefore, we conclude that because of uncertainties

related to fractionation, results based on DIC should be considered with caution until fractionation models are available for freshwaters.

2.6 CONCLUSIONS

Our results do not support the hypothesis that allochthonous subsidies play a significant role as an energy source for zooplankton communities. Instead, we found that allochthony had the weakest nutritional contribution while the algal pool represented the most important source of energy for the food web. Thus, the nutritional range of different organic matter sources must be considered when predicting energy sources for food webs. Although the reliance on autochthonous carbon was only assessed for zooplankton in this study, our conclusion are likely valid for higher trophic levels considering the central position of zooplankton in the food web. Considering the large number and size range of studied ecosystems, our results likely apply to most oligotrophic lakes and reservoirs of northern boreal region, during the ice-free period.

2.7 ACKNOWLEDGEMENTS

We thank M.A. Gagnon and S. Paquet for field assistance; L. Cournoyer, A. Adamowicz and J. McKay for stable isotope laboratory assistance. This research was supported by the National Sciences and Engineering Council of Canada via a strategic grant (No. STP224191-99), in partnership with Hydro-Québec, and by a doctoral fellowship from Fonds de Recherche sur la Nature et les Technologies du Québec (JM). This is a contribution of the Groupe de Recherche Interuniversitaire en Limnologie (GRIL) and Centre de Recherche en Géochimie et en Géodynamique (GÉOTOP).

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CHAPITRE III

SURFACE TEMPERATURE AND TAXONOMY EXPLAIN REGIONAL $\delta^{15}N$ ZOOPLANKTON VARIABILITY IN OLIGOTROPHIC ECOSYSTEMS.

Jérôme Marty; Planas, Dolors, in preparation for submission

3.1 RÉSUMÉ/ ABSTRACT

Les sources de variation de la signature en azote du zooplancton ($\delta^{15}N_{ZOO}$) ont été identifiées pour une série de lacs et réservoirs oligotrophes. En été, 51 % de la variance en $\delta^{15}N_{ZOO}$ étaient associés à des différences taxinomiques et 36.2 % à des variations entre écosystèmes. A partir des données prélevées au printemps et en été, les variations saisonnières expliquaient 35.2 % de la variance total en $\delta^{15}N_{ZOO}$. La signature en carbone et une variable catégorique taxinomique expliquaient 71% de la variance en $\delta^{15}N_{ZOO}$ lorsque les données estivales du réservoir SM-3 étaient exclues de l'analyse. La température de surface et une variable catégorique taxinomique expliquaient 63 % de la variance en $\delta^{15}N_{ZOO}$, pour l'ensemble des données. Nous avons validé ce modèle en l'appliquant avec succès à des données de la littérature. Ces résultats indiquent l'existence d'une signature en azote de base à l'échelle régionale et montrent l'importance de la température comme variable intégrant les variations inter- et intra-écosystèmes en $\delta^{15}N_{ZOO}$.

The sources of variation in the nitrogen signatures of zooplankton ($\delta^{15}N_{ZOO}$) were determined in a set of oligotrophic lakes and reservoirs. In summer, 51 % and 36.2 % of $\delta^{15}N_{ZOO}$ variance was related to differences between taxonomic groups and between ecosystems respectively. Based on spring and summer data, seasonal variations accounted for 35.2% of $\delta^{15}N_{ZOO}$ variance. Stable carbon isotopic signatures and a categorical taxonomic variable explained 71% of the variability in zooplankton $\delta^{15}N$ when a set of data from SM-3 reservoirs characterized by large seasonal $\delta^{15}N$ variations were excluded. Surface temperature and a categorical taxonomic variable explained 63% of $\delta^{15}N_{ZOO}$ in the complete data set. This model successfully explained the range of variation in $\delta^{15}N_{ZOO}$ from literature data but with a significantly different intercept. Such result indicates the existence of a regional baseline $\delta^{15}N$ signature and the importance of surface temperature to account for between and within ecosystems $\delta^{15}N$ sources of variations.

Key words: zooplankton, $\delta^{15}N$ variability, taxonomy, baseline variations.

3.2 INTRODUCTION

Stable isotopes are a common tool in ecology because they provide a continuous measure of the trophic position of organisms within complex food webs involving several pathways of energy sources and mass flow (Peterson and Fry 1987). In aquatic ecosystems, the nitrogen isotopic composition ($\delta^{15}N$) of primary consumers may exhibit wide spatial and temporal variations arising from a number of different sources. Between ecosystem $\delta^{15}N$ variance has been related to the loading of nitrogen compounds with a particular signature originating from anthropogenic sources (i. e. fertilizers, sewage outflows) (Cabana and Rasmussen 1996), and also from nitrogen transformation processes that may occur within a given system (Vander Zanden and Rasmussen 1999; Leggett et al. 2000; Post 2002). The spatial variation in $\delta^{15}N$ within a given lake can also be related to source point effects, but in oligotrophic ecosystems, it more generally reflects habitat variations (Vander Zanden and Rasmussen 1999; Post 2002; Syväranta et al. 2006). Finally, as illustrated for zooplankton communities, a substantial portion of $\delta^{15}N$ variation has been observed within a given site when several trophic levels are present (Matthews and Mazumder 2003; Karlsson et al. 2004; Syväranta et al. 2006). In addition to the spatial variations in $\delta^{15}N$, the signature of consumers varies temporally and this is particularly important for short-lived organisms whose signatures reflect the seasonal variation in diet composition or in the food signature (Zohary et al. 1994; Grey et al. 2001; Matthews and Mazumder 2005).

The aim of this study was to assess the sources of variation in the $\delta^{15}N$ signatures of zooplankton at various levels, including between ecosystems, within a single system, within site variation, among taxonomic groups and seasonal. We collected data from a series of lakes and reservoirs to determine variation in $\delta^{15}N$ between ecosystems. A number of sites within a single ecosystem were used to quantify within-ecosystem variability and the signatures of various taxonomic groups

from the zooplankton community allowed for the quantification of within-site variation. The seasonal variation in $\delta^{15}N$ of zooplankton was examined in a number of these systems sampled in early spring and mid-summer. We identified the sources of such variations by developing predictive models and further determined if similar relationships could successfully predict the variation in zooplankton $\delta^{15}N$ observed in other studies.

3.3 STUDY SITES

A series of 12 lakes and 5 reservoirs situated in the Boreal ecoregion were sampled in 2002 and 2003. The ecosystems were distributed within two areas of Northern Québec: the north shore of the St. Lawrence River (SLR) (SM-3 and MA-5 reservoirs) (51°09'N, 68°39'E) and James Bay territories (JB) (LG-2, LG-4, LA-1 and LA-2) (54°20'N, 72°13'E). Lakes were evenly distributed between these two regions. Mean water depth varied widely among reservoirs and ranged from 62 to 5.7 m for MA-5 and LA-1 reservoirs respectively. Although mean water depth was not available for each lake, lakes were generally shallow, as typically observed in northern lakes. Several sites (see Table 3.1) were sampled within each reservoir whereas one site was sampled per lake, at the deepest point. In the SLR region, sampling was performed in spring, shortly after ice break-up (mid-June) and in mid-summer (end of July). We sampled only once in the JB region, in mid-summer. All these ecosystems were oligotrophic and further details of the morphometry, nutrients and productivity characteristics of each system are available elsewhere (Planas et al. 2005; Marty et al. 2006).

Table. 3.1: $\delta^{15}N$ values (‰) obtained for zooplankton taxonomic groups or genus, in reservoirs (Res.) and lakes. Mean and standard error are indicated when several sites (n) were visited in reservoirs. Spring and summer data are identified as Sp and Sm respectively.

Type	Ecosystem	Season	n	Daphnia sp.	Holopedium sp.	Leptodora sp.	Calanoids	Epischura sp.	Cyclopoids	Chironomidae sp.	Chaoborus sp.
Res.	LA-1	Sm	5	4.9±0.3	5.6±0.3	7.5	7.5±0.1	7.9±0.2	8.2±0.3	14.8±0.9	14.8±0.9
Res.	LA-2	Sm	5	5.3±0.4	5.6±0.2		8.0±0.4	7.5±0.1	9.6±0.2	8.2	
Res.	LG-4	Sm	5	6.3±0.7	5.4±0.6		9.1±0.8	7.8±2.0	11.0±0.4		
Res.	SM-3	Sp	6	9.2±1.3	14.8±0.9		9.9±0.9	11.6±1.3	10.3±1.0	5.2±0.6	7.9
		Sm	6	3.6±1.5	3.3±0.9	5.4	7.0±2.1	5.5±0.1	7.4±0.8	0.2	
Res.	MA-5	Sp	6	8.3±0.6	3.6±1.5	3.6±1.5	9.8±0.3	3.6±1.5	9.1±0.6		
		Sm	5	3.5±0.5	3.5±0.4		7.0±0.4	5.7±0.2	7.9±0.4		
Lake	Berté	Sp	1	4.2	4		6.6		6.5		
		Sm	1	3.2		4.6	2.8	5.4			
Lake	Du Chaunoy	Sm	1	0.1	1.5		3.3	4.2			
Lake	Aux cèdres	Sp	1				4.3		5.3		
		Sm	1	2			5.1	4.4	6		
Lake	Houdan	Sp	1	3.4			4.7		5.3		5.1
		Sm	1	2.3	2.9	5.5		4.8	5.5		
Lake	Germain	Sp	1	2.3			5.8		5.8		
		Sm	1	2.6	3.2	5.3	5.1	4.8	5.7		
Lake	Jean Marie	Sm	1	3.3	3.9		5.1	5.7	5.6		
Lake	Km.12	Sm	1	1.0	1.5	2.9	3.0	4.3	4.9		
Lake	Km.17	Sm	1	3	4.1		5.2	5	5.6		
Lake	Km.380	Sm	1	2.7	2.6		4.4	4.7	5.4		
Lake	Matonipi	Sp	1						7.7		
		Sm	1			3.2	1.9		2.9		
Lake	Polaris	Sm	1								
Lake	Rapide	Sp	1	3.8			5.1		5.5		6

3.4 METHODS

3.4.1 Sampling and chemical analysis

A number of variables were measured in the field or in the laboratory for each station. Oxygen, temperature and pH profiles were measured over the entire water column with a multiprobe (YSI 6600). The limit of the euphotic zone was determined with a double quantum sensor (Li-193SA, Li-190SA, LI-COR®). For chemical and biological analysis, water was sampled from this zone or from the mixed layer when thermal stratification was observed, using a 4-L Van-Dorn sampler. Analytical procedures for chemical and biological variables are described in Marty et al. (2006).

3.4.2 Stable isotope analysis (SIA)

Zooplankton was collected by vertical hauling using a 110µm mesh size plankton net (∅ 50 cm) over the entire water column and to a maximum depth of 30 m. Organisms were kept alive in filtered water for gut evacuation (4 to 6 hours) and then narcotized with club soda prior to sorting according to main species or to main taxonomic groups. Sorted organisms were directly placed in pre-weighted tin capsules (8x5 D1008, Elemental Microanalysis Ltd.) and frozen in the field in liquid nitrogen to minimize potential preservation effects. Samples were not acidified because of the low importance of bicarbonate in Boreal Shield waters. Prior to SIA, all samples were freeze-dried until constant weight and combusted in a Carlo Erba Elemental Analyser connected to a GV Instruments IsoPrime™ mass spectrometer at GÉOTOP-UQAM (Montréal, Canada), following a protocol adapted for small-sized samples (Limén and Marty 2004).

Stable isotope ratios are expressed in delta notation (δ), in parts per thousands (‰), with $\delta=[(R_{sample}/R_{reference})-1] \times 1000$, with $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$. Secondary standard (Leucine) of known relation to the international standard of Pee Dee Belemnite and atmospheric nitrogen were used as reference material for carbon and nitrogen SIA respectively. Samples were analyzed in triplicates. Precision on measurements was calculated as the standard deviation of standards which maximum values were 0.09 and 0.1 ‰ for carbon and nitrogen, respectively.

3.4.3 Sources of variability in zooplankton $\delta^{15}N$

To explore sources of $\delta^{15}N$ variations in zooplankton ($\delta^{15}N_{ZOO}$), a nested residual maximum likelihood (REML) analysis of variance was performed on summer data to distinguish: 1) between system variation, 2) between site variation and 3) within site variation. In this analysis, each reservoir (6) and all lakes grouped together were considered as individual ecosystems. The number of sites within each reservoir (5 to 6) and the number of lakes (12) were used to assess between site variation. Finally, the number of zooplankton taxa or species (3 to 7) was used to identify within site sources of variation. A similar analysis was repeated for the 2002 data alone to quantify the proportion of $\delta^{15}N_{ZOO}$ variance due to seasonality (spring/summer) on $\delta^{15}N_{ZOO}$. Variance partitioning was performed using REML analysis instead of univariate analysis of variance because of unbalanced data sets (see Matthews and Mazumder, (2003)). Relationships between $\delta^{15}N$ of zooplankton and other limnological variables were explored using simple linear regression and ANCOVA analyses were used to determine taxonomic effects on these predictions. Outliers were identified using the outlier distance plot based on jack-knifed distances. Statistical analyses were performed using JMP v5.1.

We used data taken from the literature to test if models from this study could be generally applied to other oligotrophic ecosystems. The data comprised a temporal series from Loch Ness (Grey et al. 2001) and 2 sets of lakes from Sweden (Karlsson et al. 2004) and Southern Québec (Barnett and Beisner, unpublished data). Predicted values obtained from models were compared to observed values. We hypothesized that, in case of 1:1 relationship, the explanatory variables of the models would generally predict both spatial and temporal sources of $\delta^{15}N$ variability.

3.5 RESULTS

The morphometry of ecosystems from this study differed according to region. In SLR region, deep lakes and reservoirs allowed for a strong thermal stratification in summer while ecosystems from JB region were rather shallow and remained unstratified over the summer period. In the summer-stratified systems, the depth of the mixed layer of the water column was similar to that of the euphotic zone, implying that the biological and chemical data are representative of water layer in which primary production occurs. Despite large depth variations, little habitat differentiation according to littoral versus pelagic zone was observed in reservoirs in which the development of a stable littoral community is limited by frequent changes in water level. Therefore, data presented in this study were representative of pelagic conditions in reservoirs and possibly of a combination of pelagic and littoral conditions in the shallowest lakes of JB region.

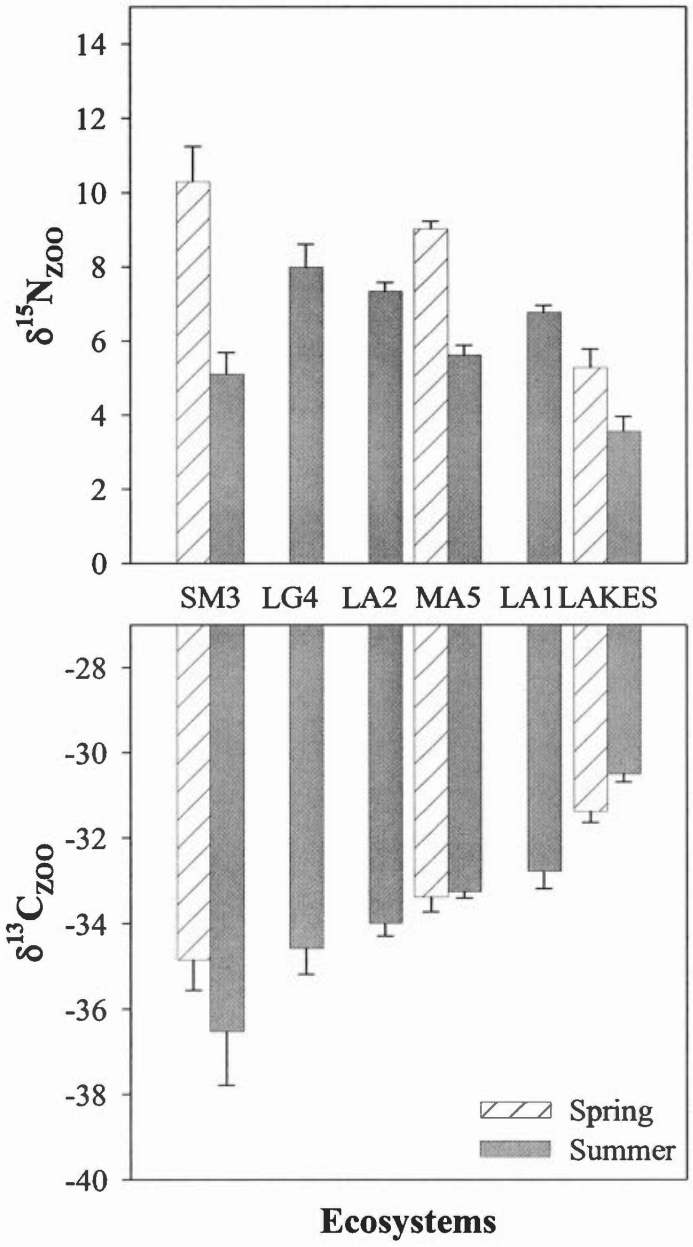
3.5.1 $\delta^{15}N_{ZOO}$ variability

The nitrogen signature of 3 to 7 taxa or genera of zooplankton were determined at each site. Species composition was homogeneous among sites and the dominant species were *Daphnia longeremis*, *Holopedium gibberum* and *Leptodora*

kindii for Cladocerans; *Diacyclops thomasi* for Cyclopoid; *Leptodiaptomus minutus* and *Epischura lacustris* for calanoids. Occasionally, *Chironomidae sp.* and *Chaoboridae sp.* were collected.

In our 12 lakes and 5 reservoirs, $\delta^{15}N_{ZOO}$ was highly variable, ranging from -1.2 to 16.6‰ (mean: 6.5‰, coef. var.: 46.2) (Table 3.1, Fig. 3.1). In summer, most of variance of $\delta^{15}N_{ZOO}$ was explained by taxonomic differences (51%) and between ecosystems variation (36.2%) (Table 3.2). There was little evidence of $\delta^{15}N$ variation according to lake habitat although the lowest $\delta^{15}N$ values was found in lakes in which algal subsidies from the littoral zone seem to be important (Marty et al. 2006) and the highest values were found in the deep SM-3 reservoir. However, lower summer $\delta^{15}N$ values were found in the deepest reservoir (MA-5) compared to the shallow reservoirs (LA-1 and LA-2) and overlapping signatures were found in SM-3 and lakes during the summer. Therefore, there was little evidence of $\delta^{15}N_{ZOO}$ variation according to lake habitat and this was supported by little variance in $\delta^{15}N_{ZOO}$ (6%) associated to between site variation, indicating that nitrogen sources and processes were homogeneous within a given reservoir or between lakes. Within the community ,the $\delta^{15}N$ of Cladocerans (*Daphnia sp.* and *Holopedium sp.*) was the most depleted, whereas Cyclopoids had the most enriched $\delta^{15}N$ values (Table 3.1). The difference between the most depleted and the most enriched $\delta^{15}N$ value obtained at a given site ranged from 0.4 to 10.1‰ (mean: 3.8‰). Assuming a trophic level increment of 3.4 ‰ (Minagawa and Wada 1984), the range of variation in $\delta^{15}N$ according to zooplankton taxa was equivalent to 1 to 3 trophic levels (Fig 3.2).

Fig. 3.1: Mean (± 1 SE) $\delta^{15}N$ and $\delta^{13}C$ of zooplankton (‰) within each ecosystem and season.



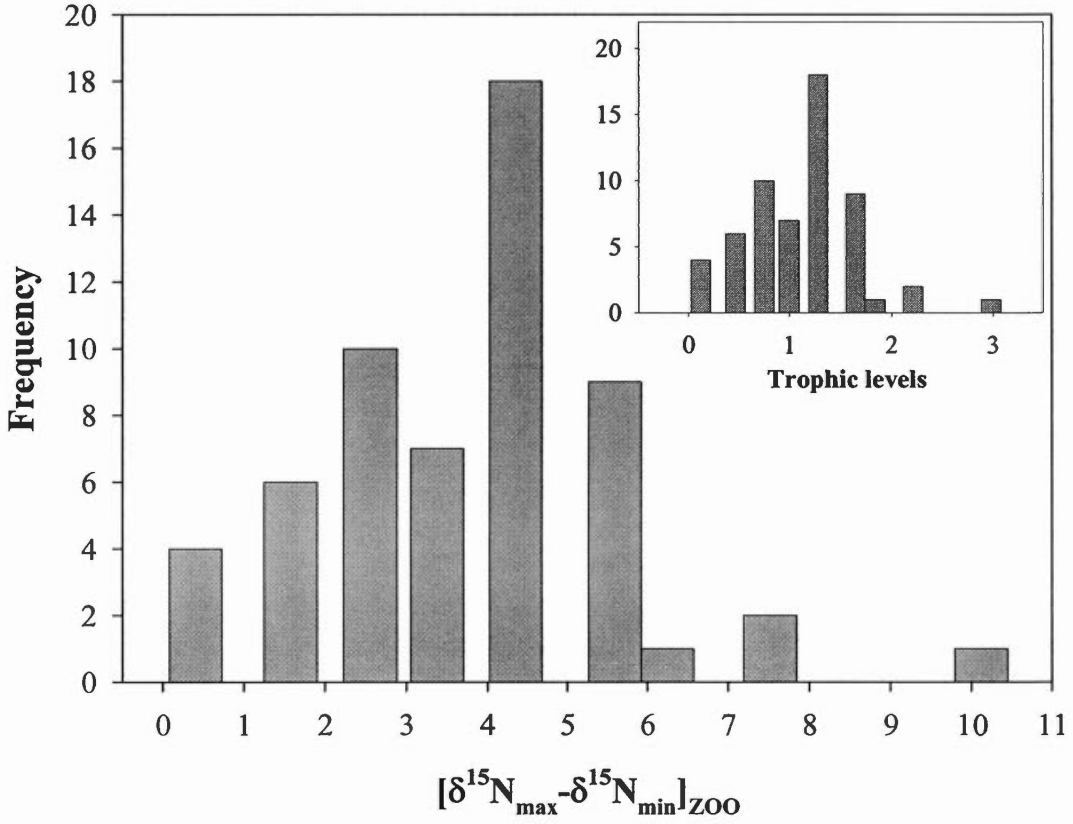


Fig. 3.2: Frequency distributions of length food web (‰) within zooplankton community and corresponding number of trophic levels.

Based on 2002 data, season explained most of $\delta^{15}N_{ZOO}$ variance (35.2%) (Table 3.2) and consisted in significant lower signatures in summer compared to spring (t-test, $p<0.0001$). This trend was particularly strong in the SM-3 reservoir in which $\delta^{15}N$ of zooplankton ranged from 4 to 15 ‰ in summer and spring respectively. The larger range of variation in $\delta^{15}N_{ZOO}$ observed in SM-3 compared to lakes and MA-5 reservoir was responsible for a higher proportion of variance related to inter-ecosystems (22.3%) and inter-sites (12.5 %) effects in the 2002 data set (Table 3.2).

Data sets	Random effects	Variance component	Standard error	% of variance
2001-2003 summer data (r^2=0.93, n=398)	Ecosystems	2.42	1.66	36.2
	Sites[ecosystems]	0.4	0.31	6
	Organisms[ecosystems,sites]	3.4	0.55	51
	Residuals	0.45	.	6.8
	Total	6.67	.	100
2002 spring/summer data (r^2=0.80, n=296)	Season	4.68	8.28	35.2
	Ecosystems[saison]	2.96	2.46	22.3
	Sites[saison,ecosystems]	1.66	0.71	12.5
	Organisms[saison,ecosystems,sites]	1.91	0.54	14.4
	Residuals	2.08	.	15.6
	Total	13.28	.	100

Table 3.2: Variance partition of zooplankton $\delta^{15}N$ for 2002 (spring/summer) and 2001-2003 (summer) data sets.

3.5.2 Ecosystems and sites effect in the $\delta^{15}N$-$\delta^{13}C$ relationship

Zooplankton energy sources were related to trophic position as shown by the negative relationship found between $\delta^{13}C_{ZOO}$ and $\delta^{15}N_{ZOO}$ (r^2=0.44, df=200, $p<0.0001$). In this relationship, 5 sites from SM-3, sampled in summer were identified as outliers and excluded from analysis because of nitrogen signatures lower than predicted for their corresponding carbon signatures. Mean $\delta^{15}N_{ZOO}$ values were also negatively related to surface water temperature and this relationship included the outlier sites from the $\delta^{13}C$-$\delta^{15}N$ relationship (r^2=0.43, df=227, $p<0.0001$). Although significant, the relationship between $\delta^{13}C_{ZOO}$ and temperature was weak (r^2=0.13, df=266, $p<0.0001$).

3.5.3 Taxonomy effect in the $\delta^{15}N$-$\delta^{13}C$ relationship

Analysis of covariance (ANCOVA) was used to test for taxa-specific differences in the general linear relationships between zooplankton $\delta^{13}C$ and $\delta^{15}N$ signatures. For the analysis, only groups or genus with a sufficient number of observations were used. In this prediction of $\delta^{15}N_{ZOO}$ (n=200; r^2=0.71; df=5, 194) (Fig. 3.3, A), we found highly significant effects of $\delta^{13}C_{ZOO}$ (F=260.0; df=1; P<0.0001) and zooplankton taxonomic groups (n=200, F=46.1, df=4, P<0.0001). Taxonomic effect in this model explained an additional 27% of zooplankton variation in $\delta^{15}N$, compared to the simple regression model. The interaction between $\delta^{13}C_{ZOO}$ and taxonomic groups was not significant (n=200; F=1.1; df=4; P=0.3), indicating that the slope of the relationship was the same for all taxa. Thus, taxonomic effect only influenced the intercept of the relationship and was responsible for a shift of the taxa specific $\delta^{15}N$-$\delta^{13}C$ relationship higher or lower relative to general $\delta^{15}N$-$\delta^{13}C$ relationship. The $\delta^{15}N$ value of a given zooplankton taxa or species was predicted by the following equation:

$$\delta^{15}N = \left(-25.3\ (\pm 1.96) + \begin{cases} I_{CYC} = 1.87 \pm 0.20 \\ I_{CAL} = 1.07 \pm 0.21 \\ I_{EPI} = 0.62 \pm 0.26 \\ I_{DAP} = -1.47 \pm 0.22 \\ I_{HOL} = -2.09 \pm 0.25 \end{cases} \right) - 0.96\ (\pm 0.06) \times \delta^{13}C$$

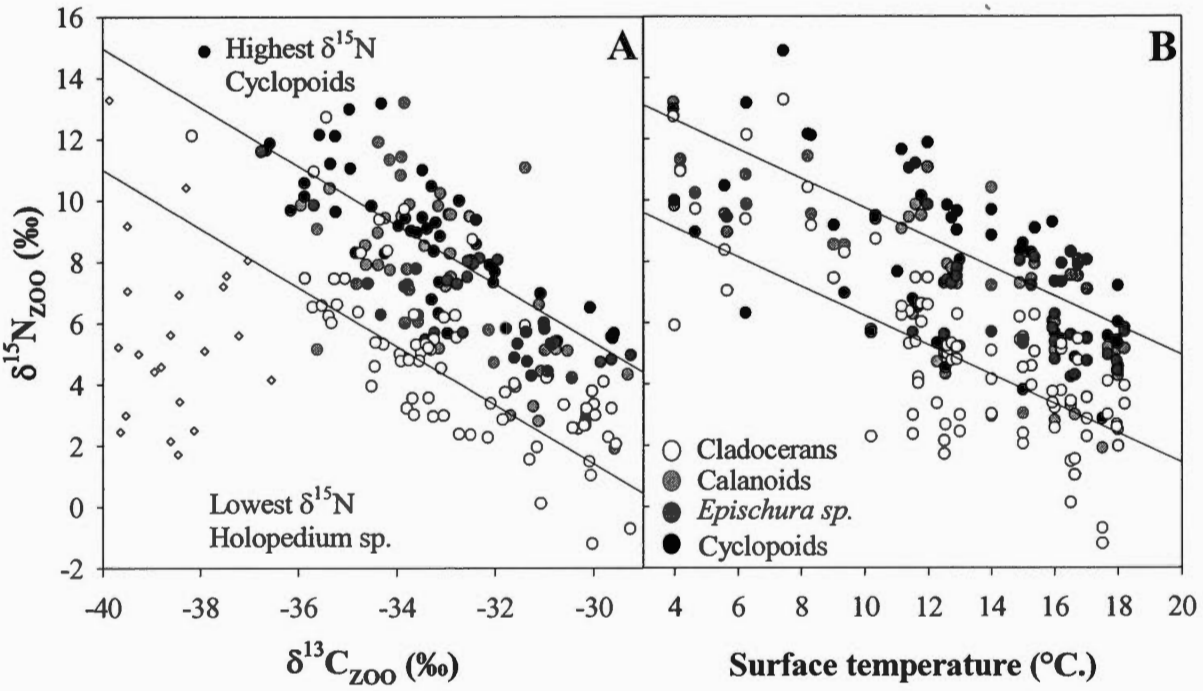


Fig. 3.3: $\delta^{15}N$ of zooplankton taxonomic groups or species as a function of $\delta^{13}C$ (A) and surface temperature (B). Open diamonds in the $\delta^{15}N$-$\delta^{13}C$ relationship indicate outliers. Lines represent relationships for the lowest and highest intercept values obtained for Holopedium sp. and Cyclopoids respectively.

Where I is the intercept correction specific to each taxonomic groups or genus (Cyclopoids (CYC), *Epischura* sp. (EPI), Calanoids (CAL), *Daphnia* sp. (DAP) and *Holopedium* sp. (HOL)).

3.5.4 Taxonomy effect in the $\delta^{15}N$-temperature relationship.

Similar analyses were performed to test for a taxonomic effect in the $\delta^{15}N$-surface water temperature relationship. The general model (n=226; r^2=0.63; df=5,

220) indicated a significant effect of surface temperature (F=220.4; df=1; P<0.0001) and taxonomic groups (F=29.8; df=4; P<0.0001) (Fig. 3.3, B). By considering taxonomic differences, an additional 20% of variance was explained compared to the simple regression model. The interaction between surface temperature and taxonomic groups was not significant (n=226; F=2.0; df=4; P=0.09) and therefore, $\delta^{15}N$ values of a given zooplankton taxonomic group or genus could be predicted as:

$$\delta^{15}N = \left(12.68\ (\pm 0.45) + \begin{cases} I_{CYC} = 1.87 \pm 0.20 \\ I_{CAL} = 1.61 \pm 0.23 \\ I_{EPI} = 0.85 \pm 0.29 \\ I_{DAP} = -1.48 \pm 0.23 \\ I_{HOL} = -1.64 \pm 0.28 \end{cases} \right) - 0.48\ (\pm 0.03) \times \text{Surface temperature}$$

3.5.5 Cross-studies validation

We tested the ability of both models to accurately predict the $\delta^{15}N$ values of zooplankton in other data sets. There was no significant relationship between observed $\delta^{15}N$ and predicted $\delta^{15}N$ generated with the $\delta^{15}N$-$\delta^{13}C$ model (P>0.05). The temperature model significantly predicted $\delta^{15}N_{ZOO}$ values from the literature (r^2=0.34, p<0.0001, df=56). ANCOVA was used to test for study-specific differences in the relationship between observed and predicted $\delta^{15}N$ values and a strong relationship was observed when studies were considered separately (ANCOVA, n=56; r^2=0.81; df=3,52, P<0.0001) (Fig. 3.4). The interaction between predicted values and the source of data was not significant (n=56; F=1.2; P=0.30), indicating that individual relationship shared similar slope value but distinct intercept values. The slope value of the relationship was 0.8, with a 95% confident interval of 0.6 to 1.

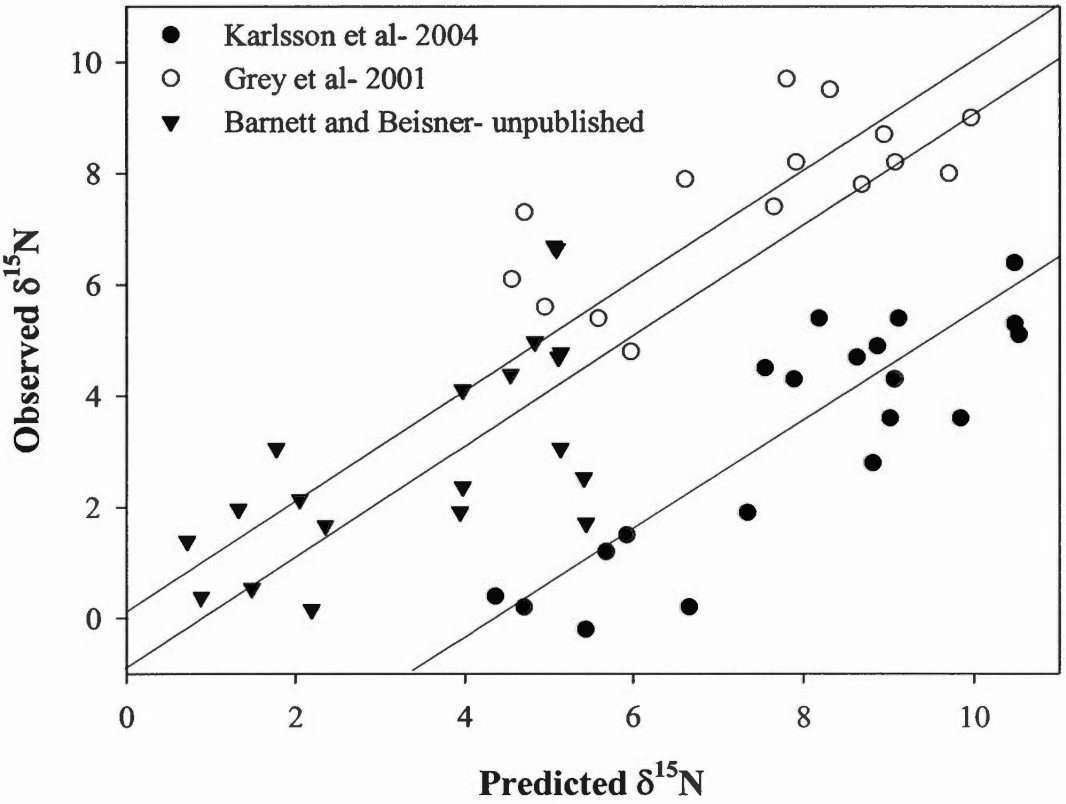


Fig. 3.4: Observed versus predicted $\delta^{15}N$ (‰) values obtained from the surface water temperature model corrected for taxonomic effects based on the temporal series from Loch-Ness (open circles), and two sets of lakes from Estrie (Canada) (black triangles) and Sweden (black circles). See methods for data sources.

3.6 DISCUSSION

Several sources of variation may be involved in explaining the nitrogen isotopic composition of consumers. Spatial variations (between sites and ecosystems variation) in the $\delta^{15}N$ signatures of organisms could either reflect the consumption of diverse food sources with a particular nitrogen signature, or variations in baseline signature. This study adds to other evidences reporting large variations in $\delta^{15}N_{ZOO}$ according to taxonomy (Matthews and Mazumder 2003; 2005). Such variation could

either reflect the utilization of several food sources of different nitrogen signatures or trophic variation. As previously shown in these ecosystems, zooplankton diet relied on algal production as their main food source (Marty et al. 2006) and therefore such results implies that $\delta^{15}N_{ZOO}$ variance relate to trophic variations rather than to feeding behavior.

Our data support the existence of a linear relationship between $\delta^{13}C_{ZOO}$ and $\delta^{15}N_{ZOO}$. A logistic relationship between $\delta^{13}C$ and $\delta^{15}N$ has been previously reported for macro-invertebrates primary consumers and was used as a tool to predict the trophic position of consumers (Vander Zanden and Rasmussen 1999). The existence of such a general relationship has been challenged when a large range of lake productivity and morphology was considered and further criticism was raised because of the difficulty in applying mixing models based on a single isotope to distinct multiple food sources (i.e. profundal, pelagic and littoral) (Post 2002). In this study, most of $\delta^{15}N_{ZOO}$ variance was explained by $\delta^{13}C_{ZOO}$ and this for lakes and reservoirs covering a large range of surface area and depth, representing much of the morphological diversity of boreal lakes. These findings support relationships reported by Vander-Zanden and Rasmussen (1999), indicating that the relationship between $\delta^{13}C_{ZOO}$ and $\delta^{15}N_{ZOO}$ widely applies to oligotrophic ecosystems.

Given the range of morphological characteristics found among ecosystems in this study, we expected $\delta^{15}N$ of primary consumers to increase along a littoral-pelagic-profundal trophic gradient (Vander Zanden and Rasmussen 1999) or with surface area (Post 2002), as the result of nitrogen transformation processes, feeding behavior on light $\delta^{15}N$ compounds in deep ecosystems and differential nitrate sources in pelagic compared to benthic zone. Although lakes had the most depleted $\delta^{15}N_{ZOO}$ values because of possible inputs of light algal material originating from the littoral zone (France 1995), distinction between profundal, pelagic and littoral habitat was

not found in reservoirs. This lack of gradient may result from the characteristics of reservoirs, which do not allow for the development of a stable littoral community, but also possibly from zooplankton mobility, allowing them to feed at all depths. Therefore, macro-invertebrates, as long-lived organisms, may integrate the temporal variations of food source isotopic composition in a particular habitat, whereas zooplankton may better integrate spatial variations in food resources signatures, but over a shorter time span.

We were able to explain most of $\delta^{15}N$ variance with $\delta^{13}C$ but it is worth noting that such analyses did not include a number of sites from SM-3 reservoir, which were identified as outliers and excluded from calculations. For these sites, $\delta^{15}N$ of zooplankton was much lower than predicted by the $\delta^{13}C$- $\delta^{15}N$ relationship and this result was related to seasonal variation, consisting of a higher $\delta^{15}N$ value in spring compared to summer. The temporal variation in $\delta^{15}N$ of zooplankton is a common feature (Zohary et al. 1994; Yoshioka and Wada 1994; Matthews and Mazumder 2005; Syväranta et al. 2006) and seasonality also explained most of $\delta^{15}N_{ZOO}$ variance in this study, when spring and summer sampling were performed.

We found that surface temperature was the most important variable driving nitrogen signatures of zooplankton in both summer and spring seasons. The importance of temperature on the isotopic composition of zooplankton was been previously shown in laboratory experiment, highlighting the control of temperature on the physiology of both food and consumers (Power et al. 2003). Temperature drives the thermal structure of the water and thereby determines the distribution of nutrients in the water column over the seasons. Higher nutrient concentrations were measured in spring as the result of water mixing and inputs from inflowing water after snowmelt and the lower summer concentrations likely related to nutrient incorporation by the biota when the water column is stratified. Variation in nitrate

concentrations in the recently flooded reservoir SM-3 was related to stratification effect and further explained variations in the $\delta^{15}N$ values of zooplankton (Fig. 3.5). Therefore, the seasonal variation in $\delta^{15}N$ values of zooplankton likely reflects the loading of heavier inorganic nitrogen in spring, which becomes less important to primary production in summer when nutrients are limiting in the mixed layer.

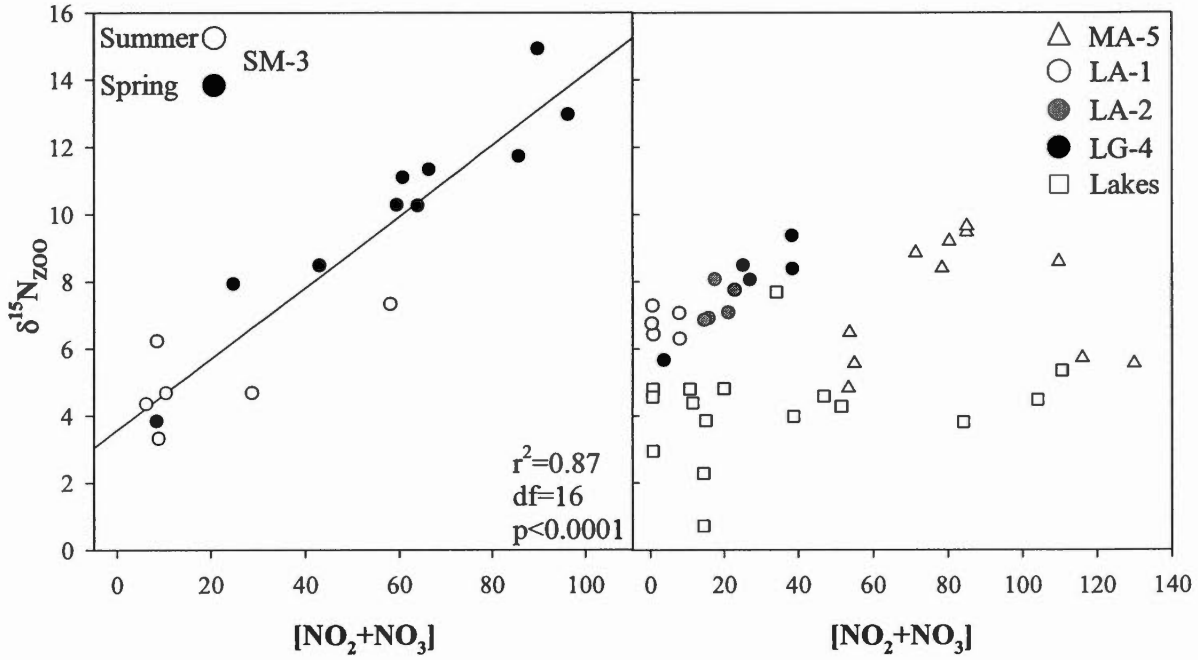


Fig. 3.5: Mean zooplankton $\delta^{15}N$ (‰) as a function of nitrate and nitrite concentration ($\mu g \cdot L^{-1}$) in the SM-3 reservoir (Left) and all other ecosystems (Right).

The source of isotopically heavy nitrogen could originate from nitrogen transformation processes such as denitrification and ammonification, responsible for the production of enriched dissolved inorganic nitrogen because of high N-fractionation. Although anoxia was not observed in the water column of our ecosystems, these processes could occur in the suboxic layers of sediment, in flooded

and forested soils. The importance of nitrogen loading and transformation processes are particularly supported by the higher seasonal variation in $\delta^{15}N$ found in reservoirs compared to lakes because flooded soil decomposition likely plays an important role in the spring nutrient loading prior to stratification.

Our model based on temperature and taxonomy to predict $\delta^{15}N$ has important consequences on the understanding of ecosystem functioning because it highlights the control of a physical variable on the baseline signature of ecosystems. We verified if a similar relationship could predict the nitrogen isotopic composition of organisms in other studies. We successfully predicted the ranged of variation in $\delta^{15}N_{ZOO}$ in the Loch-Ness time series data as well as in 2 sets of multiple lakes data. Therefore, such result indicates that the model successfully accounted for taxonomic and spatial $\delta^{15}N$ sources of variations. In addition, the prediction obtained for the temporal data from Loch-Ness confirms that temperature successfully integrates temporal variation of $\delta^{15}N_{ZOO}$. Based on ANCOVA, we found significant differences in the intercept value of relationship obtained for each study. This result highlights the existence of a baseline signature, specific of each study, responsible for a shift of the predicted $\delta^{15}N_{ZOO}$ values higher or lower, relative to observed values. As $\delta^{15}N_{ZOO}$ variations were well predicted among several ecosystems in which nitrogen transformation processes likely vary, the study-specific baseline likely relate to the signature of inorganic nitrogen entering the aquatic food web in a given region.

3.7 CONCLUSIONS

This study highlights the importance of zooplankton as a community representing several trophic levels within the food web of aquatic ecosystems. Our results demonstrate the need to consider taxonomy when using zooplankton signature as a baseline for trophic levels determination. Considering that a typical zooplankton

community in north temperate lakes is diverse and often includes a substantial proportion of copepods (Rusak et al. 2002), the nitrogen signature of a bulk sample of zooplankton should not be considered as a basal signature. Instead, the lowest $\delta^{15}N$ signatures obtained for cladocerans (*Daphnia sp.* and *Holopedium sp.*) better represents basal nitrogen signature than that of a bulk sample. Between ecosystems $\delta^{15}N_{ZOO}$ variations were related to baseline variations rather than feeding behavior on multiple food sources. When carbon and nitrogen isotopic compositions are related, a correction in the slope of the $\delta^{13}C$-$\delta^{15}N$ relationship could be applied to take into account for the trophic position of organisms. When such relationship is not found, a similar correction could be applied to predict the zooplankton $\delta^{15}N$ signature based on surface water temperature. Although we have not elucidated the factors determining zooplankton $\delta^{15}N$ variation, we have found that surface temperature represents a good indicator of the processes responsible for temporal and spatial variability within a given region. Therefore, this finding highlights the existence of a regional baseline, which must be considered when interpreting stable isotope data from different regions.

3.8 ACKNOLEDGEMENTS

We thank C. Vis for helpful comments on the manuscript. We thank M.A. Gagnon and S. Paquet for field assistance; L. Cournoyer, A. Adamowicz and J. McKay for stable isotope laboratory assistance. The manuscript benefited from discussion with J. Vander Zanden and G. Cabana. This research was supported by the National Sciences and Engineering Council of Canada (NSERC), via a strategic grant (No. STP224191-99), in partnership with Hydro-Québec and a NSERC Discovery Grant (DP), and by a doctoral fellowship from Fonds de Recherche sur la Nature et les Technologies du Québec (JM). This is a contribution of the Groupe de Recherche Interuniversitaire en Limnologie (GRIL) and Centre de Recherche en Géochimie et en Géodynamique (GÉOTOP)

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CHAPITRE IV

PLANKTONIC COMMUNITY DYNAMICS OVER TIME IN A LARGE RESERVOIR AND THEIR INFLUENCE ON CARBON BUDGETS

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Published as Chapter 17 *in* A. Tremblay, L. Varfalvy, C. Roehm, and M. Garneau [eds]. Greenhouse gases Emissions: Fluxes and Processes, Hydroelectric Reservoirs and Natural Environments. Springer, New York.

4.1 RÉSUMÉ/ ABSTRACT

Le but de ce chapitre était de déterminer l'influence des organismes du zooplancton dans le cycle du carbone des lacs et réservoirs du Nord du Québec. Dans une première partie, la dynamique du zooplancton a été suivie dans le réservoir LG-2 durant une période de 7 ans (un an avant la mise en eau et 6 ans après la création du réservoir). En terme de structure de communauté, la création du réservoir a induit une augmentation de la biomasse totale du zooplancton et en particulier celle des Cladocères et des Rotifères. Cette augmentation était expliquée par une combinaison de variables physiques (temps de résidence de l'eau, température et turbidité), chimique (phosphore total) et biologique (Chl. *a*).
La seconde partie de l'étude consiste en une comparaison de la structure du zooplancton exprimée comme limnoplancton (AFDW), pour une série de réservoirs d'âge variable (1 à 35 ans). La taille moyenne des organismes était reliée à la biomasse algale et au flux de carbone mesuré à l'interface eau-atmosphère. Nous avons observé que les effets descendants du zooplancton sur les producteurs primaires pourraient être partiellement responsables du flux de carbone plus élevé dans les jeunes réservoirs comparé aux vieux réservoirs.

The aim of this chapter was to determine the influence of zooplankton organisms on carbon cycling within reservoirs and lakes from Northern Quebec. The first part of the paper presents results from LG-2 reservoir where zooplankton dynamics were followed from 1 year prior to impoundment to 6 years after flooding. In terms of community structure, flooding was associated with an increase in zooplankton biomass with the strongest effects observed for Cladocerans and Rotifers. This increase was related to changes in the physical characteristics of the sampled sites (water residence time, temperature and turbidity), chemical characteristics of the water (total phosphorus) and the abundance of resources (Chl.*a*). The second part of the chapter is a comparison of zooplankton community structure expressed as limnoplankton (AFDW) for several reservoirs of different age (1 to 35 years old). We related the average size of organisms to the algal biomass and finally to the carbon fluxes measured between the water and the atmosphere. We found that part of the larger carbon fluxes observed in young reservoirs compared to older reservoirs may be explained by a top-down control of primary producers by zooplankton.

Key words: zooplankton biomass, reservoirs, trophic upsurge, carbon budget.

4.2 INTRODUCTION

In the past decade, a growing interest has focused on the role of the biota in the global carbon cycle. Freshwater ecosystems represent an important component of the land in northern regions and particularly in Quebec, where they cover about 15% of the land surface (Canadian Center for Remote Sensing, 2001). Consequently, carbon cycling within freshwater ecosystems may contribute to an important part of the total carbon cycling for the north, which has been widely ignored by scientists in the past. Recent studies have shown that the carbon dioxide (CO_2) flux from limnetic habitats to the atmosphere may represent up to 50% of the continental losses of organic plus inorganic carbon to the ocean (Cole *et al.* 1994). Among the factors regulating the carbon balance in freshwater ecosystems, dissolved organic carbon (DOC) plays a major role (Hope *et al.* 1996). Lake and catchment characteristics (Sobek *et al.* 2003), drainage ratio, turnover time (Rasmussen *et al.* 1989) as well as climatic factors (i.e. precipitation, temperature) are also indirectly related to carbon cycling since they regulate dissolved organic carbon inputs to lakes and rivers. Thus, atmospheric CO_2 is regulated by a number of complex physical, chemical and biological processes and in aquatic sciences, an intensive debate over whether aquatics ecosystems are sinks or sources of CO_2 to the atmosphere continues (Cole *et al.* 2000, Carignan *et al.* 2000, del Giorgio & Duarte 2002, Karl *et al.* 2003).

Within the last decade, the issue of whether reservoirs are sinks or sources of CO_2 has been raised with regards to hydroeletric reservoirs, since future trends in the building of dams will depend on their global impact to the environment (Rosenberg 2000). The ability of aquatic ecosystems to buffer atmospheric CO_2 is related to the amount of gross primary production and to the amount of respired carbon (Lyche *et al.* 1996, Planas *et al.* 2005). If we are interested in greenhouse gases (GHG) emissions and in particular, the CO_2 dynamics in aquatic systems, a particular attention should be addressed to determine the relative contribution of algae and

bacterial communities in carbon cycling in freshwaters. However, if we are interested in the mechanisms determining the structure of those communities, many physical, chemical and biological variables must also be considered. One of the biological variables able to influence both algal and bacterial communities is their zooplankton consumers.

Zooplankton communities play a very important role in food-web dynamics because of their central position within the trophic web. They are key to the transfer of carbon from primary producers to higher levels (planktivorous fish) (Galbraith 1967, Hutchinson 1971, Christoffersen *et al.* 1993) and are able to assimilate carbon from a wide range of sources including microbial organisms (bacteria, ciliates and flagellates) (Sherr & Sherr 1984, Sanders & Wickham 1993, Havens *et al.* 2000, Adrian *et al.* 2001, Zöllner *et al.* 2003, Marty *et al.* 2003). Thus, the entire zooplankton community through its impact on food-web structure is able to influence the limnetic carbon cycle and the state of the ecosystem to act as a sink or source of carbon (Schindler *et al.* 1997).

The ecology of reservoirs has been relatively well documented in the literature. Most studies have focused on short-time scale observations, getting a "snap shot" image of mechanisms from reservoirs. However, such an approach may not be relevant in the case of reservoirs since, because of their recent history as a new ecosystem, they behave much more dynamically than natural lakes in many of their limnological variables (Thornton 1990). Thus, long-term data sets are necessary to describe the structure and functioning of communities as well as their resilience within these types of systems (Bonecker *et al.* 2001).

The aims of this chapter are 1-to describe the structure of the zooplankton community in a large reservoir over a long period of time, from one year before impoundment to 6 years after flooding; 2- to determine the most important

environmental variables that have an influence on zooplankton community structure in these systems and 3- to compare the zooplankton community structure between reservoirs of different ages to assess its potential effect on carbon dynamics.

4.3 MATERIALS AND METHODS

4.3.1 Long-term data set (1978-1984)

4.3.1.1 Study area

The James Bay project is the most ambitious hydroelectric project attempted in Canada. The damming of the river La Grande (53° 54'N, 76° 78'W) consisted in the building of a series of 6 dams as well as two 2 major diversions, resulting in the creation of 9 reservoirs covering a total surface of 17 228 km^2 with an installed capability of 15 244 MW. The reservoir LG-2 (or Robert-Bourassa) was first flooded in November 1978 and was filled within a year, covering a surface area of about 2500 km^2. LG-2 reservoir has a mean depth of 22 m, with a maximum depth of 150 m in front of the dam. Water residence time (WRT) is about 6 months.

An intensive monitoring program was performed by the Société d'Énergie de la Baie James (SEBJ) to determine flooding effects on physical, chemical and biological variables. This program started one year before flooding (1978) and last 6 years after impoundment (1979-1984). A series of 6 stations were chosen: 3 stations were originally situated along the La Grande river (LG2400, LG2402 and LG2406) and 3 others over ancient lakes flooded by the reservoir (LG2403, LG2404 and LG2405) (Fig. 4.1). Also, a natural lake (Detcheverry) was sampled to represent an unperturbed ecosystem within the same area. More detailed descriptions of the sites are given in Pinel-Alloul & Méthot (1984), Schetagne & Roy (1985) and Méthot & Pinel-Alloul (1987).

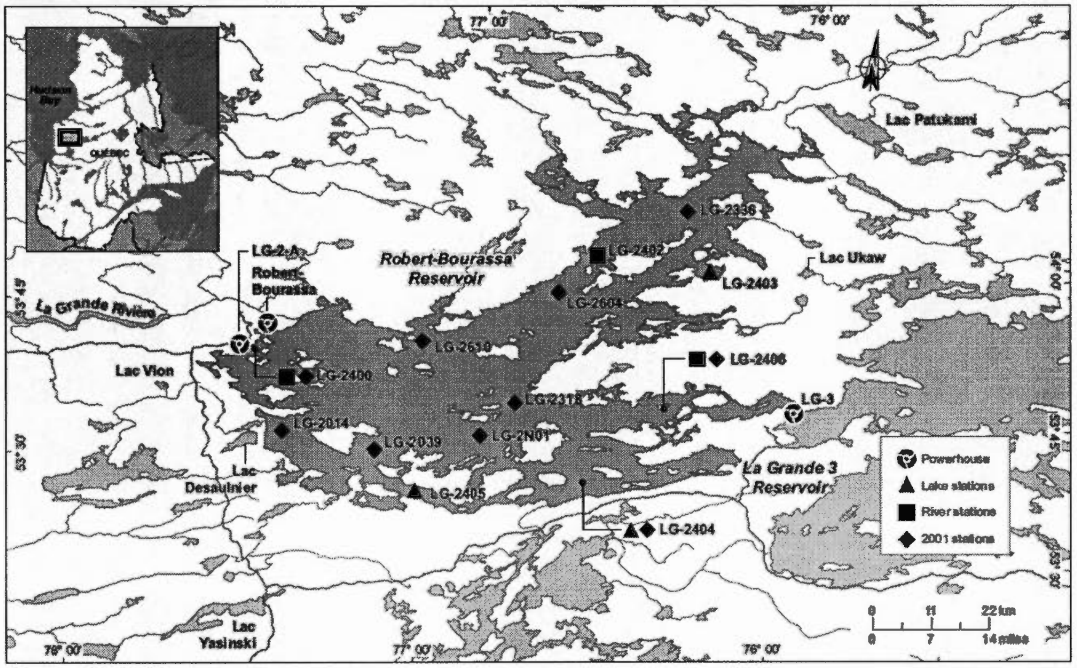

Fig. 4.1 Localisation of the sampling sites in LG-2 reservoir, for the long-term data set and for 2001 sampling.

4.3.1.2 Material and methods

Sampling was performed during the ice-free period (May to October) for 7 years (1978 to 1984) to cover pre-impoundment (1978), impoundment (1979) and post-impoundment (1980-1984) phases. All variables were sampled twice a month for the overall period, for each selected station. A large set of physical and chemical variables were measured in the field (i.e.: temperature, dissolved oxygen, conductivity, water transparency), derived from field measurements such as water residence time or determined in the laboratory from a composite water sample collected in the euphotic zone of the water column (nutrients, chlorophyll *a*, pH, inorganic and organic carbon) (Table 4.1). All chemical analyses were made

following standard procedures (APHA-AWWA-WPCF, 1975) and are described in detail in Schetagne & Roy (1985). Carbonic acid concentration (H_2CO_3) was calculated from bicarbonate concentration using Henry's constant (Kh) corrected for temperature and pH (Sigg *et al.* 1992). The partial pressure of CO_2 (pCO_2) was also estimated from bicarbonate concentration and pH, with appropriate corrections for temperature (Kling *et al.* 1992).

Zooplankton was collected at the same frequency as described above, with a Clarck-Bumpus sampler (75 µm mesh) by oblique tows from 25 m to the surface at deep stations or through the entire water column at shallow stations. All samples were fixed with 5% formalin. Zooplankton abundance ($nb.m^{-3}$) was determined for each species and then converted to biomass ($mg.m^{-3}$) using specific dry weight estimates for cladocerans and copepods (Dumont *et al.* 1975, Pinel-Alloul & Méthot 1979) and volumetric formula for rotifers (Bottrell *et al.* 1976). Biomass estimates were computed for each zooplankton group (cladocerans, calanoids, cyclopoids, nauplii and rotifers) and summed to calculate total zooplankton biomass.

A multiple regression model was constructed which predicts total zooplankton biomass based on environmental variables during the flooding of LG-2. Variables were entered into the model using a mixed stepwise procedure, with probability to enter and leave set to 0.05. Data were log transformed to respect residual homogeneity and normality.

Status	Station	Total phosphorus (µg/l)	Chl.a (µg/l)	pH	Turbidity (utn)	WRT (years)	H_2CO3 (mg/l)	% O_2
Reference Lake	SB400	4.8 (2-18)	1.1 (0.5-2.4)	7 (6.6-7.3)	0.5 (0.1-2.5)	-	4 (2.8-6.5)	95 (85-103)
Lake stations	LG2403	12 (5-18)	2.8 (0-8.5)	6.4 (5.9-6.9)	0.8 (0.4-1.9)	2.5 (2.5-2.5)	11.7 (8.6-16.3)	87.5 (67-100)
	LG2405	16.5 (4-33)	2.9 (0.6-8.4)	6.5 (6.1-7.1)	1.1 (0.5-2.3)	-	12.9 (7-21.2)	80.6 (36-95)
	LG2404	12.8 (5-27)	2.1 (0.6-4.8)	6.3 (6-6.7)	2.3 (0.6-9.8)	0.07 (0-0.2)	11.4 (6.2-16.7)	92 (78-105)
Riverine stations	LG2400	11.1 (5-18)	1.7 (0-5)	6.3 (5.9-6.7)	1 (0.4-5)	1.2 (0-2.5)	17.2 (5.6-23.3)	86 (57-110)
	LG2402	11.7 (0.7-25)	2.1 (0.1-7.2)	6.4 (5.9-6.8)	0.9 (0.2-3.8)	1.1 (0-1.5)	13.2 (4.9-26.6)	87 (64-106)
	LG2406	10.5 (4-18)	1.6 (0.2-4.6)	6.4 (6-6.8)	1 (0.2-5.9)	0.7 (0-2.5)	9.7 (8.6-11.5)	96 (71-115)

Table 4.1: Physical and chemical characteristics in lacustrine and riverine stations of LG-2 and in a reference lake, from 1978 to 1984. Data represents the annual means for each station, with minimal and maximal values.

4.3.2 Recent data set

During the past 3 years (2001-2003), a new research project has been conducted in the major hydroelectric reservoirs of Quebec to assess the contribution of the biological component of the carbon cycle to carbon emissions from reservoirs to the atmosphere. Similar to the long-term data set, environmental variables were measured at several stations within various reservoirs, and in addition, planktonic metabolism (Planas *et al.* 2005) and CO_2 flux arising from the water column to the atmosphere were also measured.

4.3.2.1 Study area

In 2001, sampling was carried out in two reservoirs of La Grande river with 10 sampling sites visited twice on the LG-2 reservoir (Fig. 4.1) and 2 sites on LA-1, a more recently flooded reservoir (7-years), as well as a series of 7 natural lakes situated near the two reservoirs. In 2002, 2 other reservoirs were sampled in the North shore of the St Lawrence region: the Manic-5 reservoir (35 years) and the recently flooded reservoir SM-3 (1 year). Six stations were sampled on each reservoir as well as 6 reference lakes situated in the same region (for station localization, see figure 5 in chapter 18, Planas *et al.* 2005).

4.3.2.2 Zooplankton biomass estimates

Zooplankton was sampled with a 53 μm mesh sized net (diameter: 0.2 m), from 1 m above the sediments to the surface or from a maximum depth of 30 m for the deepest stations. Sampled volumes varied from 15 to 950 L depending on site depth. Zooplankton were first narcotised with carbonated water and then preserved in 4% formaldehyde. In the laboratory, each zooplankton sample (250 ml) was divided into two equal volumes with a Folsom splitter for taxonomic and limnoplankton

analyses. Both sub-samples were then fractionated into 4 size classes by sequential screening through Nitex nets (500 µm, 200 µm, 100 µm and 53 µm) to determine the size spectra of the community for biomass calculations.

To estimate limnoplankton biomass corresponding to sestonic particles, the size fractions from half of the original sample (125 ml), as previously described, were filtered onto on a pre-combusted GF/C (Whatman) glass fiber filter, dried at 40°C for 24 hours and ash-combusted at 500°C for 12 hours. Limnoplankton organic biomass was calculated for each size fraction as the difference between the dry weight and ash weight, expressed in mg of ash-free dry weight (AFDW) of limnoplankton per unit volume. Here, the term limnoplankton is defined as the seston fraction larger than 53 µm, including zooplankton plus algae and detritus (the two latter particularly in size fractions <200 µm). A full description of the limnoplankton analyses has been presented in previous studies (Masson & Pinel-Alloul 1998, Patoine *et al.* 2000).

All limnoplankton data were averaged for each reservoir (all stations within single reservoir) and over time. General differences among sites were tested on log-transformed data using one-way ANOVA and specific differences among sites were determined by comparing means using Tukey-Kramer HSD test.

The specific weight ($mg.ind^{-1}$) of main taxonomic groups was determined using a large 110 um plankton net (0.5 m. diameter) to obtain a large number of organisms. To obtain a precise measurement of weight, organisms were placed in filtered water to allow gut evacuation and then directly placed in a pre-weighted capsule and frozen in liquid nitrogen. In the laboratory, organisms were freeze-dried to avoid loss of volatile organic compounds and then weighed on a Sartorius M2P scale. Weights were averaged for the overall community, per sites.

4.3 RESULTS

4.3.1 Long-term variation in zooplankton community (1978-1984)

Impoundment had a great impact on total zooplankton biomass (TZB) in both inundated rivers and lakes (Fig. 4.2). A gradual increase in zooplankton biomass was observed during the first 5 years of the study. If we consider the first 3 years (pre-impoundment period and one year after flooding: 1978-1980), TZB was higher in lakes stations (19-44 $mg.m^{-3}$), compared to the reference lake (21-17 $mg.m^{-3}$) and river stations (0.18-14 $mg.m^{-3}$). During the following two years (1981-1982), zooplankton biomass was comparable in both types of impounded stations and reached maximum values (42-58 $mg.m^{-3}$) equivalent to 3 to 4 times the biomass from reference lake and 2.7 to 300 times the biomass observed the year previous to impoundment (1978) in the river La Grande. The decrease and stabilization of zooplankton biomass began in 1983. Although double than in the reference lake, zooplankton biomass declined to a level close to the one shown in 1978 in flooded lakes.

The response of each taxonomic group to impoundment is also presented in Fig. 2. The most significant increase in biomass was observed for cladocerans: almost absent before impoundment in river stations (0.04 $mg.m^{-3}$) and low in biomass in lake stations (3.3 $mg.m^{-3}$), they were the most predominant group after 1979 with maximum values reached in 1980, the year following flooding in lake stations (30 $mg.m^{-3}$) and 2 years (1982) after flooding for river stations (23 $mg.m^{-3}$). In lake stations, a decrease in the development of calanoids and cyclopoids copepods at the beginning of the impoundment (1978-79) was concomitant to an increase in rotifers biomass.

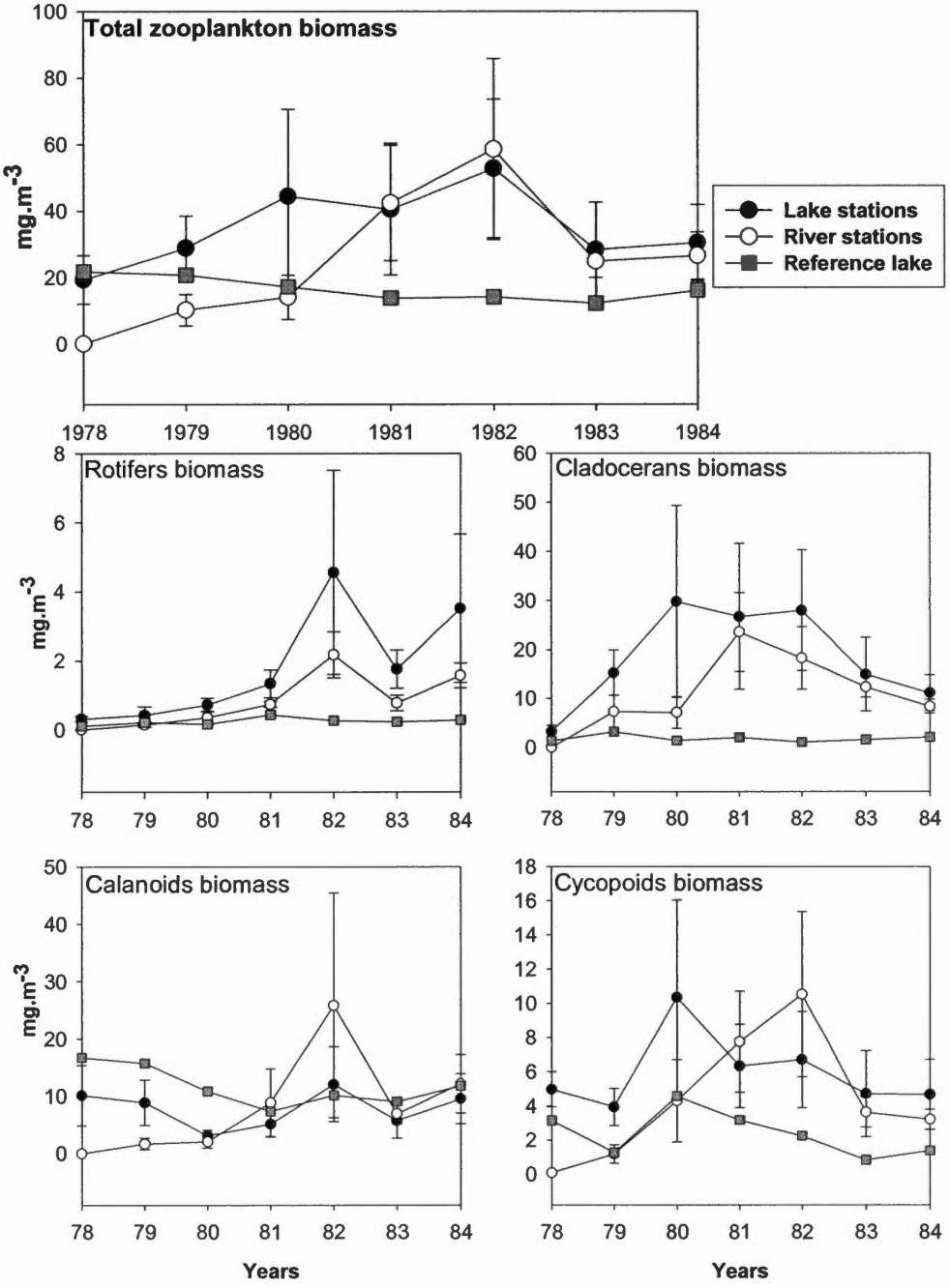

Fig. 4.2: Annual variations (1978-1984) in zooplankton biomass in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE.

Environmental variables that entered in the multiple regressions model to predict total zooplankton biomass are presented in Table 4.2 in the selection order. We observed that zooplankton biomass could be predicted with physical (water residence time, temperature and turbidity), chemical (total phosphorus) and biological variables (chl. *a*), explaining 67% of TZB variance. The order in which variables entered the model indicates how significative was the variable to predict TZB considering the last entered variable. Thus, water residence time was the best predicting variable, followed by temperature, total phosphorus, chl. *a* and turbidity (Table 2).

Variable	Coefficient	S.E.	t	p(t)	r^2	VIF
Zooplankton biomass					0.67	
Intercept	0.19	0.24	0.76	0.44		1.07
Water residence time	0.59	0.04	14.22	<0.0001		1.55
Chl. a	0.45	0.17	2.56	0.011		1.35
Temperature	2.08	0.18	10.99	<0.0001		1.31
Total phosphorus	53.2	16.96	3.14	0.002		1.07
Turbidity	-0.528	0.23	-2.3	0.022		1.44
n=261, F=105.5, p(F)<0.0001, r^2-adj=0.66						

Table 4.2: Multiple regression model for prediction of total zooplankton biomass during flooding period of LG-2 reservoir.

4.3.2 Relation with water quality and trophic status

After impoundment, changes in water quality were observed in most physical and chemical variables (Schetagne & Roy 1985). Change in productivity is illustrated in Fig. 3. Mean total phosphorus (TP) concentration for the 7 years period

was 13.8 $\mu g.L^{-1}$ in the lakes stations, 11.1 $\mu g.L^{-1}$ in the river stations and 4.8 $\mu g.L^{-1}$ in the reference lake. TP increased during the first 3 years after impoundment until 1981 and then decreased slowly the following years (Table 4.1). Nutrient increase was concomitant to that of phytoplankton biomass expressed as chl. *a*: during the three years following flooding, chl. *a* increased from 1 to 3 $\mu g.L^{-1}$. In the reference lake, TP and chl. *a* concentrations remained stable during the 1979-1984 time period with respectively 4.8 $\mu g.L^{-1}$and 1.1 $\mu g.L^{-1}$ on average for the overall period (Fig. 4.3). We noticed an increase in chl. *a*, for all studied sites for the year 1981, suggesting a certain coherence among ecosystems and the potential role of large scale influences in the dynamics of plankton.

Changes in the concentration of carbonic acid (H_2CO_3), pCO_2, the percentage of oxygen and pH are reported in Fig. 4. The pCO_2 values from all sites (reservoir or lake) show that all ecosystems were over-saturated in CO_2, even prior to flooding. In LG-2 reservoir, H_2CO_3 concentration and pCO_2 were 0.083 $mmol.L^{-1}$ and 1500 μatm. respectively, which was about 50% higher than in the reference lake (0.053 $mmol.L^{-1}$ and 1004 μatm.). An increase was observed for 3 years after flooding, followed by a gradual decrease. Over the 7 years, no difference was detected between lake and river stations, but there was a difference between reservoir stations and the natural lake, with values higher in the reservoir, even 5 years after flooding. The percentage of oxygen decreased from 95 to 80% during the first 3 years after flooding and then increased up to a stable value (89%) in 1983 and 1984. However, lower oxygen saturation values persisted in the reservoir compared to the reference lake, even 7 years after flooding. Finally, mean pH tended to be lower in both types of reservoir stations (6.3) compared to the reference lake (7).

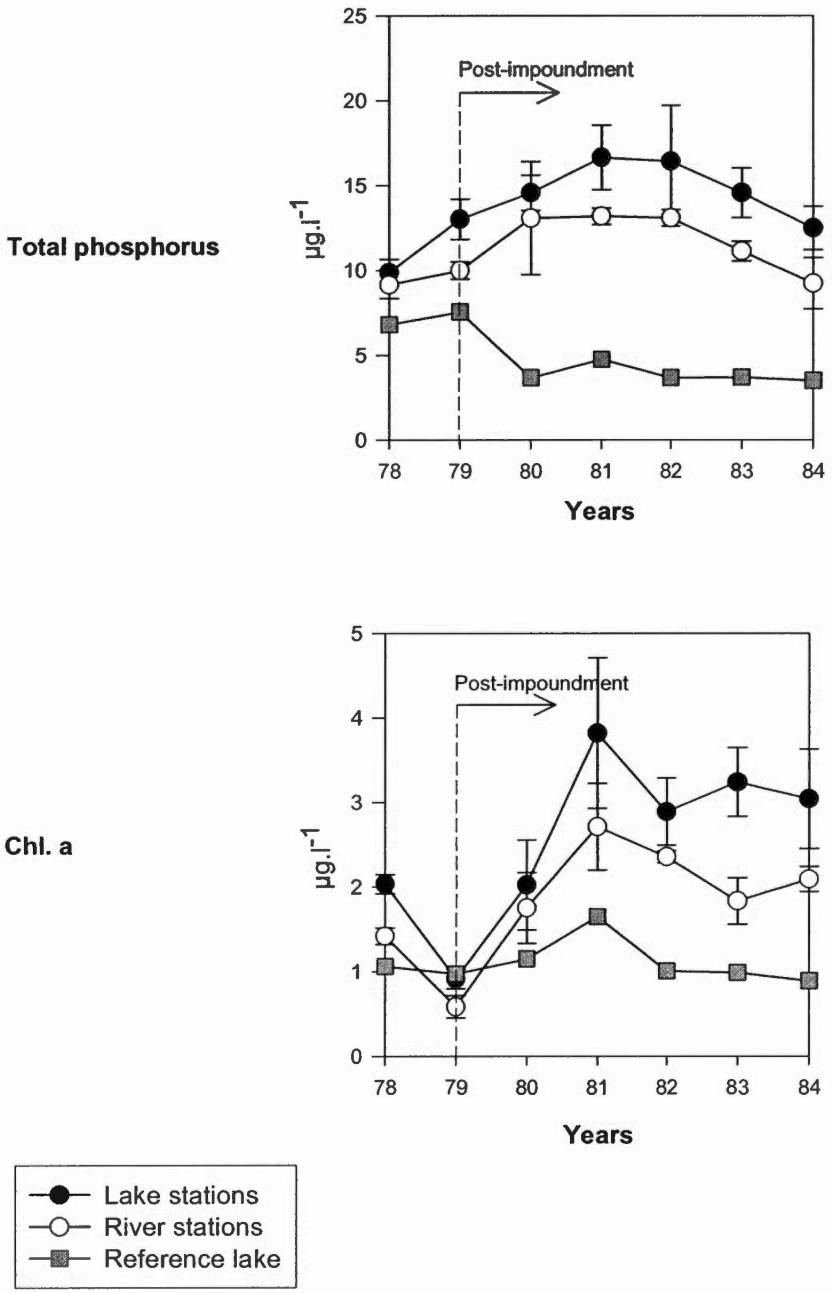

Fig. 4.3: Annual variations (1978-1984) in total phosphorus and chl. *a* in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE

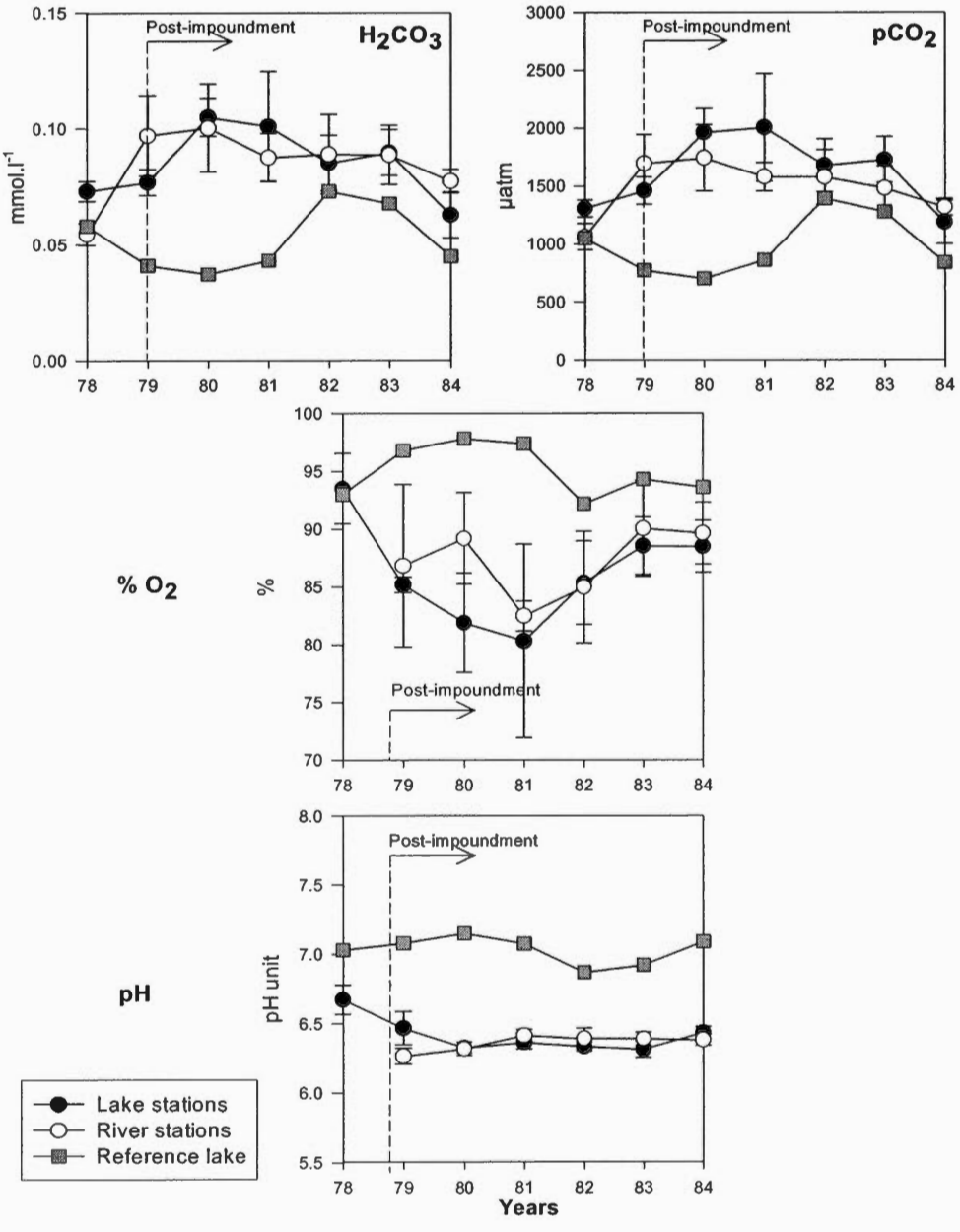

Fig. 4.4: Annual variations (1978-1984) in carbonic acid (H_2CO_3), pCO_2, oxygen and pH in lake and river stations of LG-2 reservoir and in the reference lake. Data are presented as the mean ± SE.

4.3.3 Recent data set: A comparison between reservoirs

4.3.3.1 Limnoplankton

Total limnoplankton biomass expressed in AFDW varied from 7.75 to 50 $mg.m^{-3}$ among sampled reservoirs and lakes with the lowest biomass observed in Manic-5 and maximal values were observed in the LA-1 reservoir. Natural lakes, LG-2 and SM-3 reservoirs had a comparable total limnoplankton biomass (21.9 to 24.5 $mg.m^{-3}$). On average, total limnoplankton biomass (4.7 $mg.m^{-3}$) in the older reservoirs (MA-5, LG-2) was lower than in the young reservoirs (LA-1, SM-3) but didn't differ significantly among reservoirs when considered together (Fig. 4.5).

Within the total limnoplankton, the largest size-fraction (>500 μm) represented 11 to 32 % of total biomass. Taxonomic observations under a binocular stereomicroscope showed that the >500 μm fraction corresponded mostly to cladocerans such as *Daphnia* spp. and *Holopedium* sp., adult calanoids and cyclopoids such as *Epischura* sp., *Leptodiaptomus* sp. and *Mesocyclops* sp. The maximum biomass for this fraction was observed in the recent SM-3 and LA-1 reservoirs with values twice to six times higher compared to the older reservoirs Manic-5 and LG-2 (Table 4.3). AFDW for the >500 μm fraction (4.7 $mg.m^{-3}$) was higher in the lake than in the older reservoirs and lower than in the young reservoirs but did not differ significantly from reservoirs when they were all considered together (Fig. 4.5).

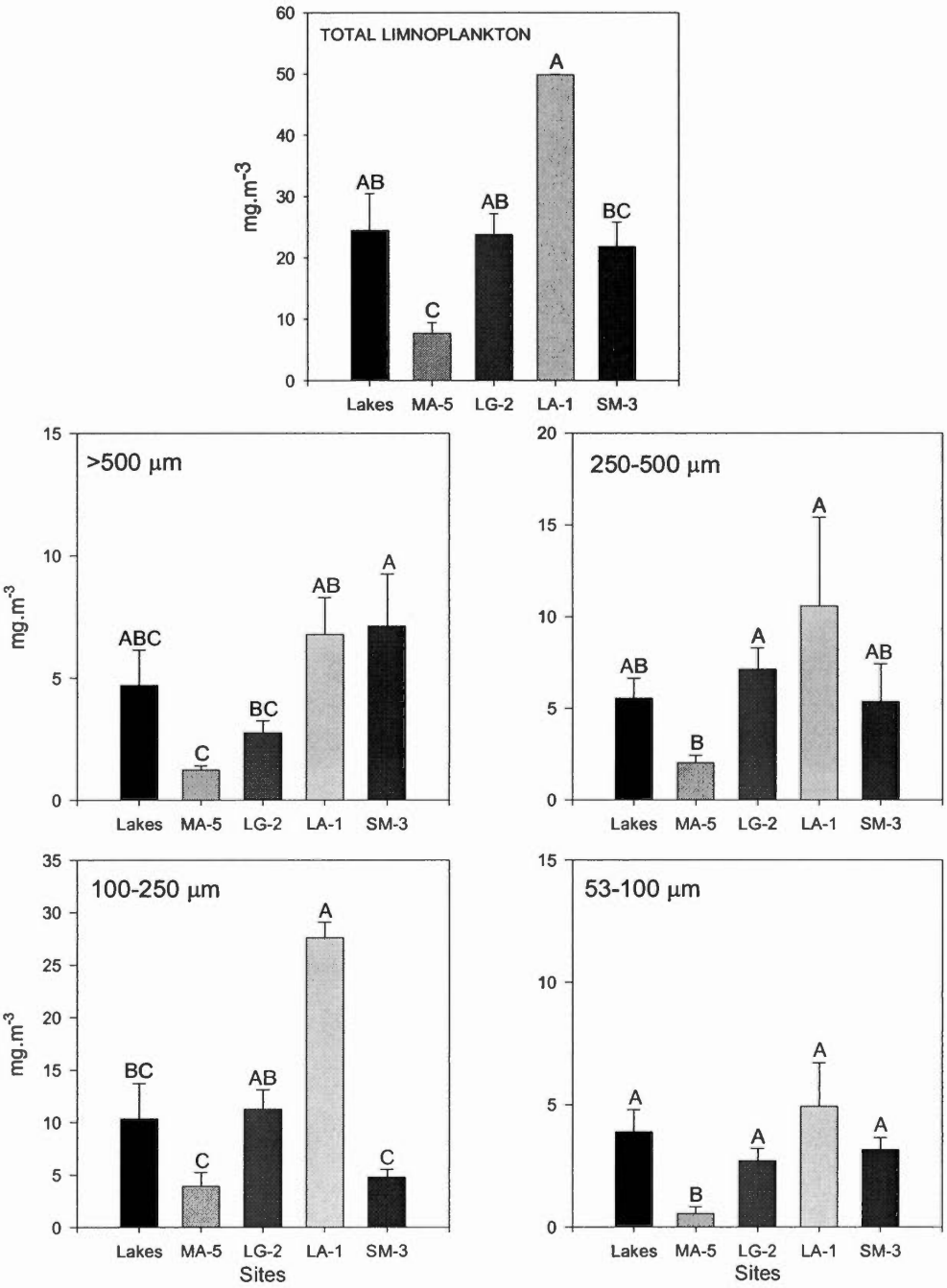

Fig. 4.5: Ash free dry weight (mg. AFDW.L^{-1}) of seston for a group of northern Quebec lakes and reservoirs. Levels not connected by the same letter are significantly different. Data are shown as the mean ± SE.

Table 4.3: Limnoplankton biomass expressed as ash free dry weight (mg. AFDW.L^{-1}) according to size fraction, in a series of 4 reservoirs from Northern Quebec and in natural lakes. Data are presented as the means over sampling period and stations, with minimal and maximal values.

	Lakes	**LA-1**	**LG-2**	**Manic-5**	**SM-3**
N	9	2	12	6	5
Total limnoplankton	24.5 (2.9-63.3)	49.9 (49.8-50)	23.8 (10.7-46.9)	7.7 (4.4-9.7)	21.9 (12.8-34.6)
>500 µm	4.7 (0.4-13.8)	6.8 (5.3-8.3)	2.8 (1-7.3)	1.2 (1-1.5)	7.1 (2.4-16.2)
250-500 µm	5.5 (0.9-9.7)	10.6 (5.7-15.4)	7.1 (1.7-13.4)	2.0 (1.2-2.6)	5.38 (2.9-13.8)
100-250 µm	10.3 (0.9-35)	27.6 (26.1-29.1)	11.2 (4.8-23.2)	3.9 (1.31-5.3)	4.8 (2.3-7.7)
53-100 µm	3.9 (0.7-10.1)	4.9 (3.1-6.7)	2.7 (1.1-6.0)	0.5 (0-0.9)	3.2 (1.6-4.9)

The 200-500 µm fraction accounted for 20 to 30% of total limnoplankton biomass and corresponded to smaller cladocerans such as *Bosmina* sp. and immature copepods. There was no general difference between sites (P=0.07) detected with one-way ANOVA, although a Tukey-Kramer test showed significantly higher values in LA-1 and LG-2 reservoirs than in MA-5 reservoir (Fig. 4.5).

The 100-200 µm fraction was composed of nauplii and large rotifers such as *Kellicottia longispina* as well as a large amount of colonial algae such as *Tabellaria* sp. Because of this large algal contribution, this fraction accounted for 24% (SM-3) to 55% (LA-1) of total limnoplankton biomass. A significant biomass difference of this fraction was observed among sites (P=0.01) with values significantly lower in North shore of the St Laurence region, MA-5 and SM-3 and higher in LA-1. No difference was observed between natural lakes and LG-2 reservoir (Fig. 4.5). Finally, the 53-100 µm fraction was characterized by small rotifers (*Keratella cochlearis, Polyarthra vulgaris*), algae and organic matter; and accounted for a minor proportion to total biomass (7-16%). Only MA-5 was significantly lower in biomass compared to other sites (Fig. 4.5).

4.3.3.2 Zooplankton specific weight

Zooplankton weight variation among sites is presented in Fig. 6. Organisms from the La Grande River region (LG-2, LA-1) were smaller compared to the one in the North shore of the St Lawrence region (MA-5, SM-3). The lowest weights were observed in the LG-2 reservoir (1.7 $\mu g.ind^{-1}$) and then in LA-1 (2.7 $\mu g.ind^{-1}$). The maximum value (26.7 $\mu g.ind^{-1}$) was observed in the recently flooded reservoirs SM-3 with organisms on average 15 times heavier compared to LG-2. Organisms from MA-5 reservoir and natural lakes had a comparable weight (8.5 and 13 $\mu g.ind^{-1}$).

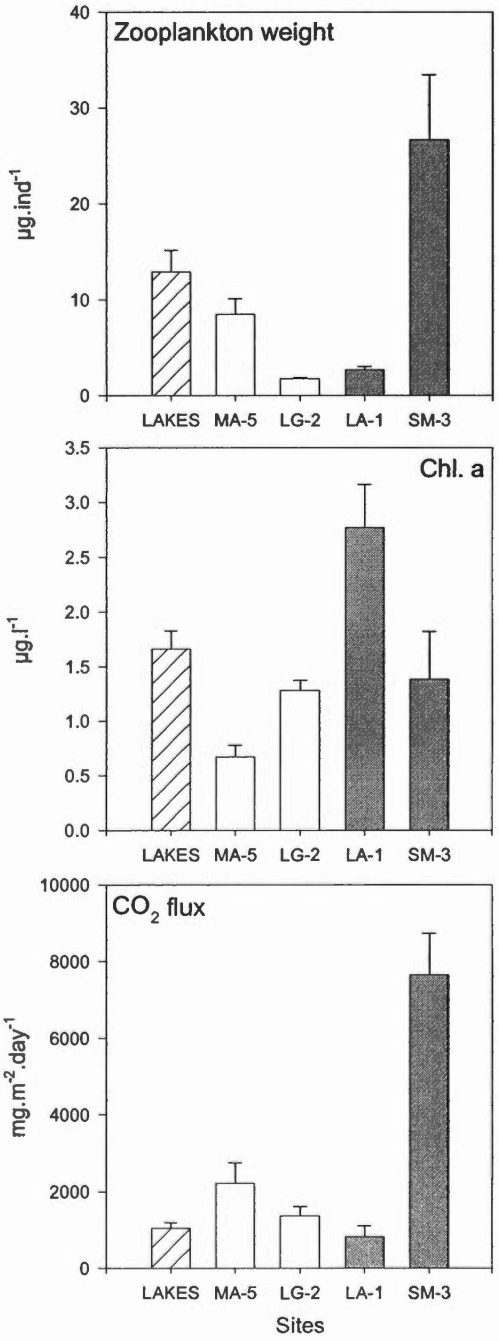

Fig. 4.6: Comparison of zooplankton weight, phytoplankton biomass and evasive CO_2 flux for a group of northern Quebec lakes and reservoirs. Data are presented as the mean ± SE.

4.4 DISCUSSION

One of the most consistent features of new reservoirs is the temporary increase, called "trophic upsurge", of all trophic levels following impoundment. This phenomenon was first observed in Russian reservoirs (Baranov 1962) and also in western and eastern Canada by Duthie & Ostrofsky (1975) and Pinel-Alloul & Méthot (1984). Trophic upsurge is characterized by an initial increase in nutrient concentration (in particular phosphorus), originating from the degradation of labile organic compounds (soils and vegetation compounds) that boosts the overall productivity of the reservoir for about a decade, until nutrients become re-equilibrated to the initial conditions. In LG-2, the trophic upsurge of zooplankton lasted 4 years (1980 to 1984), but no strong depression in productivity was observed the following years, since nutrient concentrations remained stable at least until 5-years after impoundment (Fig 4.3). Variation in the intensity and length of the trophic upsurge may be observed among reservoirs. It depends on the initial trophic state of the flooded ecosystem but also on landscape characteristics such as soil thickness, type of vegetation and its degradability (St Louis *et al.* 2000), watershed slope and human activity (Marzolf 1990a). The recent data on LG-2 (limnoplankton and chl. *a* concentrations), 23 years after impoundment, suggest that the trophic state of the reservoir may even be lower than the one prior to impoundment. This could be explained by the fact that hydrological variables such as water residence time and temperature play a more important role in reservoirs compared to natural lakes, at least for zooplankton community (Naselli-Flores & Barone 1994, 1997, Velho *et al.* 2001).

During LG-2 flooding, all zooplankton taxonomic groups responded to the trophic upsurge but cladocerans and rotifers were the most sensitive to flooding (Fig. 4.2) since they are generally more adapted to variable hydrological characteristics of reservoirs (Branco *et al.* 2002). A comparable result for cladocerans was observed in

the recent data set where the AFDW for the >500 µm. fraction was higher in recent reservoirs compared to older ones. Zooplankton community within reservoirs is usually dominated by fast-growing organisms (r-strategists species) such as cladocerans (Paterson *et al.* 1997) and rotifers (Nogueira 2001). Copepods, because of their longer development time, are often absent in reservoirs where they experience washout if water residence time is shorter than their development time (McLaren 1963).

The difference between river and lake stations illustrates that biological response to impoundment may differ with the type of flooded water body. Considering the type of reservoir or the type of stations within a single reservoir is important in the case of zooplankton since most groups are limited in their development in flowing water. The damming of rivers creates non-flowing or low-flow habitats for organisms in regions where they did not exist previously (Marzolf 1990b), whereas impoundments, which include natural lakes, create flowing habitats in previously non-flowing water bodies. During LG-2 flooding, the impoundment of the La Grande River induced contrasting effects of trophic upsurge on zooplankton since both rivers and lakes were flooded. Lake stations were characterized by higher biomass compared to river stations. This could be explained by the fact that reservoirs differ from lakes in many of their physical and chemical characteristics and the most important of which, in the case of planktonic communities, are variables related to water residence time, temperature, nutrients and turbidity. In LG-2, the minor increment in zooplankton biomass was observed in stations characterized by low water residence time such as LG2400 situated close to the dam and also in stations close to the inflow of water coming from a distinct geologically area, covered by the sediment of the Tyrrel Sea, with a more variable regime (LG2404). The importance of hydrology on zooplankton biomass was confirmed in the multiple regressions model when a majority of the variables that entered the model were related to hydrology.

We hypothesize that food web structure may determined the degree of the ecosystem to act as a sink or source of carbon. In lakes, Schindler *et al.* (1997) observed that the presence of large grazer organisms could be related to a decrease in primary production in turn associated to an increase in the partial pressure of CO_2 at the air-water interface. Considering the long-term data set from the LG-2 reservoir, there was no clear impact from zooplankton on phytoplankton biomass. Instead, the observed trophic upsurge supports a stronger bottom-up impact due to high nutrient availability rather than a top-down control from higher level of the food-web, which is illustrated by the selection of total phosphorus and chl.*a* as variables to predict TZB. This result is consistent with other studies from recently flooded reservoirs (Paterson *et al.* 1997, Holz *et al.* 1997, Thouvenot *et al.* 2000) or natural lakes (Hessen 1989, Jürgens 1994).

It is difficult to link biological variables to the amount of carbon exchanged with the atmosphere since no direct fluxes were measured during LG-2 flooding. To a lower extent, carbonic acid concentration and pCO_2 can be related to the importance of biological processes occurring in the water column and therefore that to the ability of the ecosystem to act as a source or sink of carbon. Photosynthetic activity is usually related to a change in oxygen concentration as well as an increase in pH due to the removal of carbonic acid. On the other hand, when respiration occurs, carbon is released and a decrease in oxygen and pH may be observed in the water column. The higher pCO_2 values in LG-2 after flooding (Fig. 4.4) suggest that this reservoir was probably acting more as a source than a sink of CO_2 to the atmosphere. Both decreases in oxygen and pH validate the fact that respiration might play a major role over photosynthesis during the early years of LG-2. This is not surprising considering that in recently flooded reservoirs, the decomposition of large amount of organic matter implies CO_2 production through respiration process (Duchemin *et al.* 1995, Houel 2003).

When comparing zooplankton specific weight and chl. *a* (Fig. 4.6), reservoirs with large-bodied zooplankton organisms were characterized by a lower phytoplankton biomass. For instance, reservoirs with the heaviest zooplankton organisms (SM-3 and MA-5) had the lowest chl. *a* concentration. Surprisingly, the weight of organisms differed more according to the regions rather than reservoirs age. Regional characteristics such as temperature may explain such difference since ice-free period tend to be shorter for northern ecosystems. Also, reservoirs characteristics such as water residence time may limit the development of organisms and therefore modulate their potential impact on algae.

In the recent data set, direct flux measurements of CO_2 arising from the water column were performed, enabling to relate carbon production to biological processes such as planktonic production and respiration (Planas *et al.* 2005). Data on zooplankton show that carbon flux between the water and the atmosphere may also be influenced by food-web structure. Large organisms were able to suppress phytoplankton more effectively and a greater proportion of carbon was release in the atmosphere. Moreover, we found a negative correlation between zooplankton weight and the ratio between production (AGP) and respiration (R) ($r=-0.98$, $p=0.004$), suggesting the strong effect of food-web structure not only on phytoplankton biomass but also on the metabolic balance of plankton. Finally, we also performed stable isotope analysis on zooplankton, phytoplankton and organic matter to determine the contribution of allochthonous and autochthonous sources of carbon to zooplankton. Preliminary results show that zooplankton organisms rely more on phytoplankton rather than detrital material in reservoirs compared to lakes. Carbon stable analysis confirmed the top-down effect of zooplankton organisms on the algal community as suggested in Fig. 4.6: we found that organisms from younger reservoirs were strongly depleted in $\delta^{13}C$ compared to older reservoirs and natural lakes. Also, we were able to correlate the carbon signatures of organisms with carbon fluxes

measured at the water-atmosphere interface ($r=-0.86$; $p=0.0005$), with the most depleted carbon signatures measured in sites where high carbon fluxes were recorded (Marty et al., in preparation).

4.5 CONCLUSIONS

This study confirms the importance of zooplankton community in the planktonic food webs from reservoirs through bottom-up and top-down control. During the flooding of LG-2, the increase in food resources allowed zooplankton biomass to increase as predicted according the theory of trophic upsurge. However, if zooplankton were not limited by food resource, physical characteristics of reservoirs played a major role in structuring zooplankton communities. Water residence time and temperature accounted the most to predict zooplankton biomass since lower residence time and higher temperature allowed cladocerans and rotifers to respond the most to flooding.

If the long term monitoring of LG-2 suggests a bottom-up effect on zooplankton, data on other studied reservoirs revealed the potential top-down impact from zooplankton on primary producers and in turn, on global carbon cycle. More specifically, we found that zooplankton community structure is able to influence the ability of reservoirs to act as a sink or a source of carbon for the atmosphere.

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CONCLUSION GÉNÉRALE

La signature du carbone algal : un challenge en écologie isotopique

Comment déterminer la composition isotopique du carbon des algues dans les écosystèmes oligotrophes dont la majorité du carbone particulaire est d'origine terrestre ? Cette question est le sujet de nombreuses recherches visant à séparer les algues des détritus (Pel et al. 2003; Hamilton et al. 2005; Vuorio et al. 2006). Cependant, face aux résultats très variables et difficilement interprétables de ces nouvelles techniques, les signatures en carbone algal sont généralement déterminées à partir de méthodes indirectes (Bouillon and Dehairs 2000; Karlsson et al. 2003; Pace et al. 2004). Le premier chapitre de cette thèse représente la première étude visant à comparer les méthodes d'estimation de la signature en carbone des algues.

Nous avons montré la concordance entre les signatures $\delta^{13}C$ des algues obtenues à partir de la signature du carbone particulaire corrigée pour la biomasse algale et à partir de la signature d'un organisme brouteur du zooplancton tel que *Daphnia*. Les signatures de ces deux approches étaient aussi comparables à celle obtenue pour des échantillons concentrés d'algues. Par contre, nous avons obtenu des signatures algales différentes des autres approches en utilisant la signature de carbone inorganique dissout. Ce résultat indique le manque de connaissances permettant de déterminer la forme de carbone fixé durant la photosynthèse et le fractionnement algal. En particulier, nos résultats suggèrent que les modèles développés pour des espèces spécifiques d'algues marines ne peuvent être appliqués pour déterminer le fractionnement des communautés d'algues d'eaux douces. Aussi, en plus d'identifer les méthodes les plus fiables pour estimer la signature du carbone algal, cette étude confirme le besoin de développer des outils prédictifs du fractionnement algal en limnologie (Bade et al. 2006).

Le zooplancton dépend du carbone algal dans les écosystèmes hétérotrophes.

Pour les écosystèmes hétérotrophes, l'utilisation des sources de carbone d'origine autochtone versus allochtone par le zooplancton est un sujet controversé. Il est reconnu que le carbone d'origine terrestre joue un rôle important dans le métabolisme des écosystèmes (Sobczak et al. 2005) et aussi dans la diète des organismes pour des petits lacs (Carpenter et al. 2005). Cependant, un nombre important d'études basées sur plusieurs types d'écosystèmes hétérotrophes ont montré que le carbone autochtone supporte les réseaux trophiques (Thorp 2002; Martineau et al. 2004; Sobczak et al. 2005).

A partir d'une approche isotopique, nous avons évalué l'importance des apports autochtone et allochtone pour une série de lacs et de grands réservoirs hétérotrophes. Cette étude est la première à aborder ce sujet pour un grand nombre de lacs et pour des réservoirs. Nos résultats démontrent que le zooplancton dépend des apports autochtones dans tous ces écosystèmes et ce, malgré la dominance des apports d'origine terrestre. De plus, ce résultat a été validé pour l'ensemble des organismes du zooplancton, quel que soit leur mode alimentaire (herbivore, carnivore, etc...). Cette étude montre l'importance du zooplancton comme communauté faisant le lien entre les producteurs primaires et les organismes situés à des niveaux trophiques supérieurs dans les écosystèmes peu productifs. Nos résultats suggèrent aussi le rôle du zooplancton comme communauté agissant comme un puits de carbone dans le budget total des écosystèmes émetteurs de CO_2 pour l'atmosphère.

La température de surface et la taxinomie expliquent les variations en $\delta^{15}N$ du zooplancton.

Les variations en $\delta^{15}N$ des organismes des écosystèmes aquatiques sont reliées à des variations d'ordre spatial et temporel, dans le cas des organismes à

courte durée de vie (Syväranta et al. 2006). Dans les milieux oligotrophes, le $\delta^{15}N$ des organismes reflète la signature des composés azotés entrant dans le système, ainsi que les processus de transformation de ces composés qui font intervenir un fractionnement important (Vander Zanden and Rasmussen 1999). A ces variations spatiales s'ajoutent des variations temporelles elles-mêmes reliées à des variations dans la diète des organismes ou de la signature de la diète elle-même (Matthews and Mazumder 2005).

Nous avons évalué les sources de variations de la signature $\delta^{15}N$ du zooplancton dans une série de lacs et de réservoirs oligotrophes. En été, les différences taxinomiques représentaient 51 % de la variance en $\delta^{15}N$ du zooplancton. A partir des données de printemps et d'été, nous avons montré que la saisonalité expliquait 35.2 % de la variance en $\delta^{15}N$ du zooplancton. La signature en carbone du zooplancton permet, en tenant compte de la variabilité entre les groupes taxinomiques, de prédire la signature en $\delta^{15}N$. Cependant, dans cette prédiction, plusieurs observations du réservoir SM-3 ayant une signature en $\delta^{15}N$ très basse comparée à leur $\delta^{13}C$ correspondant ont été exclues. Dans ce réservoir, les variations en $\delta^{15}N$ étaient positivement reliées à la concentration en nitrate et nitrite et suivaient un patron saisonnier. Un second modèle basé sur la température de surface a permis, en prenant en compte les effets taxinomiques, de prédire le $\delta^{15}N$ du zooplancton pour l'ensemble des observations. Nous avons vérifié l'existence de la relation température-$\delta^{15}N$ en appliquant le modèle de cette étude à d'autres données provenant de systèmes oligotrophes. Notre modèle prédit généralement les variations en $\delta^{15}N$ du zooplancton mais indique la présence d'une signature de base qui diffère d'une étude à l'autre. Aussi, la température de surface permet d'intégrer les sources de variations en $\delta^{15}N$ inter- et intra-écosystèmes mais ne permet pas de prendre en compte les variations régionales qui influencent la signature de base de la chaîne alimentaire.

La dynamique du zooplancton durant la mise en eau du réservoir LG-2

La création d'un réservoir a des conséquences importantes pour le cycle du carbone de la région innondée. En submergeant des écosystèmes forestiers, la formation d'un réservoir remplace un milieu fixateur de carbone par un écosystème agissant comme une source de carbone pour l'atmosphère. Le chapitre 4 illustre les effets de la mise en eau sur une série de variables chimiques et biologiques du réservoir LG-2. L'augmentation des concentrations en nutriments était accompagnée par une augmentation de productivité de l'écosystème, telle que décrite dans les premières études sur les réservoirs (Baranov 1962). Les organismes du zooplancton dont la reproduction est de type r (cycle de reproduction rapide) ont le plus fortement répondu à l'augmentation des ressources. La durée du pic de productivité a duré 4 ans dans le réservoir LG-2 mais n'est jamais revenu à son niveau de pré-innondation car les concentrations en nutriments sont restées plus élevées. A partir des données provenant de quatre réservoirs et d'une série de lacs, nous avons montré les effets descendants du zooplancton sur les producteurs primaires. La présence d'organismes du zooplancton de plus grande taille était associée à une biomasse algale moindre. Cette réduction des producteurs primaires pouvait être reliée à une augmentation du flux de carbone observé à la surface de l'écosystème. L'influence de la structure des réseaux trophiques sur les échanges en carbone entre l'eau et l'atmosphère a été montré expérimentalement (Schindler et al. 1997) et cette étude est la première observation *in situ* de cette relation.

Pour conclure, cette thèse illustre le rôle du zooplancton dans les réseaux trophiques et le cycle du carbon dans des écosystèmes peu étudiés à ce jour. En plus de leur importance dans le paysage du Nord du Québec, les réservoirs méritent une plus grande attention de la part des écologistes car leurs caractéristiques particulières mettent en lumière des processus plus difficiles à identifier dans des lacs naturels. De

plus, il est urgent de comprendre complètement le fonctionnement de ces écosystèmes dont le nombre suit l'augmentation de la demande en énergie.

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ANNEXE A

STABLE CARBON AND NITROGEN ISOTOPE ANALYSIS ON SMALL-SIZED SAMPLES: A PROTOCOL FOR PREPARATION AND ANALYSING MICROSCOPIC ORGANISMS

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A.1 INTRODUCTION

Stable isotope analysis (SIA) can be a useful tool in food-web research since the specific isotopic signature of a consumer and its diet is predictable.[1] In freshwater and marine ecosystems, most isotopic studies include animals and plants visible to the eye. The main reason for this is that the amount of biomass needed for carbon and nitrogen isotopic composition is easy to obtain with large organisms and studies can be made on individual basis. However, in both pelagic (water column) and benthic (bottom dwelling) foodwebs, small, microscopic organisms are often numerically dominant and can be an important link to higher trophic levels such as fish and crustaceans.

In the pelagic zones of lakes and oceans, zooplankton are able to assimilate carbon from a wide range of sources such as bacteria and unicellular organisms. Similarly, in benthic habitats, microscopic organisms called meiofauna, feed on a wide range of different food sources. The specific signal of food items is reflected in the isotopic signature of the species tissues. It is thus important to divide organisms into species, when possible, to detect its specific feeding strategy and trophic position in the community.

In order to obtain sufficient material for SIA of small sized organisms, a number of individuals have to be pooled together. This can be quite time consuming and probably explains why, so far, only few studies where isotopic techniques have been used include microscopic organisms. Specific techniques for handling and analysing small-sized samples are needed to make isotopic studies of small organisms more accessible to ecologists.

A.2 AIMS

The overall aim is to make stable isotopic analysis of small organisms more accessible to ecologists. We present a protocol for preparing and analysing samples of small sizes. Methodology is described using cases from two types of ecosystems, freshwater and marine because preparations of samples differ in some respects.

A.3 TECHNICAL DESCRIPTION

The isotopic composition was measured by continuous-flow mass spectrometry using a Carlo Erba NA-1500 elemental analyser (EA) inline with a GV Instruments Isoprime mass spectrometer. The carrier gas was ultrahigh purity He (flow rate = 110 ml/min). As an added precaution the carrier gas was passed through a He purifier. Samples were flash-combusted (~1700°C) in the presence of O_2 using the typical combustion column of chromium oxide and silvered cobaltous-cobatic oxide (1000°C). The resulting gases were passed through a Cu reduction furnace (750°C) to reduce oxides of nitrogen to N2 and remove residual oxygen, a Mg perchlorate trap to remove water and a GC column (50°C) to separate the N_2 and CO_2 gases. To analyse both N and C isotopes on the same aliquot of sample it is necessary to dilute the CO_2 with He using a VG-Isochrom Diluter. The extra He was added after the N2 peak had passed into the mass spectrometer but before the CO_2 peak had exited the EA.

For "normal" samples (i.e., >0.3 mg for non-acidified samples and >0.5 mg for acidified samples), the trap current of the source was set to 200 μA. For small samples (<0.3 mg for non-acidified samples and <0.5 mg for acidified samples) the trap current was raised to 600 μA to increase the sensitivity. This increases the N2 background, thus it was critical to insure that the He carrier gas and O2 were pure and that the autosampler was not leaking. For the analysis of carbon isotopes the primary

source of contamination is the tin cups. Cleaning the cups with spectro-grade chloroform followed by a methanol rise and drying in a 65°C oven should be sufficient to remove any contamination. Other steps to decrease N and C backgrounds have recently been described by Carman and Fry.[3]

Results are given using the standard δ notation where $\delta = [(R_{sample}/R_{reference})-1] \times 1{,}000$ expressed in units of per thousand (‰) and where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.[2] The reference materials used were secondary standards (Leucine) of known relation to the international standard of Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen.

A.4) SAMPLE PREPARATION

A.4.1) Marine organisms

Invertebrates from a deep-sea hydrothermal vent at Explorer Ridge were collected with a suction sampler using the remotely-operated vehicle ROPOS. The samples were immediately frozen on board the research vessel at –80°C. In the laboratory, copepods (meiobenthic crustaceans <1mm) were identified under a stereomicroscope and individuals of the same species, *Stygiopontius quadrispinosus,* were grouped together in sufficient numbers for the analyses (Table A.1). The copepods were transferred to a thick glass slide with a 10mm deep concavity. The surplus of water was carefully removed and 20 copepods at a time were clumped together on the border of the slide using a fine needle. The clump of copepods was rolled carefully on the border of the slide in order to remove the remaining water and then carefully picked up with the needle and transferred to a clean pre-weighted tin capsule. The tin capsule contained a droplet of 0.1N HCl in order to remove carbonates. Samples were dried in an oven at 55°C over night. A thick tin capsule ("smooth wall tin capsule" D4057, Elemental Microanalysis Limited) was used for copepods in order to prevent capsule damage by the HCl.

Table A.1. Copepod (*Stygiopontius quadrispinosus*) and polychaete (*Paralvinella sulfincola*) carbon and nitrogen isotopic composition. Sample weight (mg), type of tin capsule, trap current (μA), and standard (C6 sucrose from IAEA for carbon and N-1 from IAEA for nitrogen) precision (±SD) are also presented

sample weight (mg)	tin cap.	species	#	trap	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ precision (‰)	$\delta^{15}N$ precision (‰)
0.23	thick	*S. quadrispinosus*	100	600	-14.5	4.5	0.01 (n=2)	0.06 (n=2)
0.47	thick	*S. quadrispinosus*	100	600	-13.6	4.5	0.01 (n=2)	0.06 (n=2)
0.68	thin	*P. sulfincola*	1	200	-12.8	7.5	0.11 (n=8)	0.13 (n=8)
0.66	thin	*P. sulfincola*	1	200	-13.3	7.2	0.11 (n=8)	0.13 (n=8)
0.53	thin	*P. sulfincola*	1	200	-14.8	8.1	0.11 (n=8)	0.13 (n=8)

Specimens of the polychaete species, *Paralvinella sulfincola* (~ 30mm), were analysed individually. Each specimen was first dissected in order to remove the gut content and then acidified with 0.1N HCl. Polychaetes were acidified in glass vials, rinced once with milli-Q water and dried in an oven at 55°C for 24 hours. The dried animal was ground in the vial with a glass rod and a calculated amount transferred to a tin capsule (8x5 D1008- Elemental Microanalysis Limited). The amount of biomass used was based on the assumption that the organisms contain around 40% carbon and 10% nitrogen. Copepods were analysed with trap current 600 and polychaetes with trap current 200.

A.4.2 Freshwater organisms

Zooplankton (1-2 mm) was collected with a 110μm mesh plankton net from various natural lakes and hydroelectric reservoirs situated in northern Quebec (Canada). They were kept alive 2-4 hours in filtered water to allow gut evacuation and then sorted in the field to species or group depending on abundances; when species were found in low numbers, they were clumped together into a higher taxonomic level in order to obtain a large enough biomass for both carbon and nitrogen SIA (Table A.2). Before sorting, crustacean zooplankton were narcotized with carbonated water to reduce their activity. The number of organisms needed for the analysis was determined by considering C/N composition and length-weight relationships for each species or taxonomic group.

species or group	µg C.ind-1	µg N.ind-1	# ind. needed for trap current 600	
			Min (100µg)	Max (300µg)
Calanoids	2.10±0.77	0.42±0.16	24	73
Cyclopoids	4.29±1.88	0.88±0.39	11	35
Daphnia longeremis	2.84±0.89	0.57±0.18	16	47
Holopedium gibberum	6.92±2.74	1.35±0.53	7	20
Epichura lacustris	9.65±3.95	2.08±0.85	5	15

Table A.2: Carbon and nitrogen content (means±SD, n=44) of freshwater zooplankton organisms and the number of individuals required for dual $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis, using the 600 trap current (µA).

Sorted organisms were placed in pre-washed and pre-weighted tin capsules (8x5 D1008- Elemental Microanalysis Ltd.) and then shock-frozen in cryotubes at -160°C, using liquid nitrogen. Prior to SIA, capsules were freeze-dried and weighed. We tested whether both carbon and nitrogen signatures were influenced by the type of trap used for the analysis. A set of samples were collected as described previously and analyzed with the 200, 400 and 600 trap currents, according to their dry-weight. Specifically, the 600 trap current was used for samples weighing between 0.1 and 0.3 mg; the 400 trap current for samples weighing between 0.3 and 0.4 mg and the 200 trap current for samples weighing over 0.4 mg. The precision for each trap was calculated as the standard deviation of standards (C6 sucrose from IAEA for carbon and N-1 from IAEA for nitrogen) (Table A.3).

Trap current (μA)	$\delta^{13}C$ precision (‰)	$\delta^{15}N$ precision (‰)
200	0.08 (n=4)	0.01 (n=3)
400	0.07 (n=7)	0.07 (n=4)
600	0.09 (n=9)	0.10 (n=4)

Table A.3: Precision of stable carbon and nitrogen isotopic composition of freshwater organisms presented as the variation (SD) of standards used (C6 sucrose from IAEA for carbon and N-1 from IAEA for nitrogen)

A.5 RESULTS AND DISCUSSION

A.5.1 Marine organisms

Animals around deep-sea hydrothermal vents live in an environment of highly fluctuating and often harsh conditions. Still, the animal biomass in these areas is comparable to, or exceeds, the biomass from other highly productive marine areas. Most non-symbiotic organisms are believed to rely directly on the basal trophic level at vents consisting of chemolithautotrophic bacteria. However, the link between these bacteria and large invertebrates may be longer than previously presumed. In this study, the stable isotope results indicate that sulphide worms feed on copepods (Fig. A.1). The copepod, in turn, is most likely grazing on bacteria. This is thus an example where meiofauna can be a link from autotrophic bacteria to higher trophic levels at deep-sea hydrothermal vents.

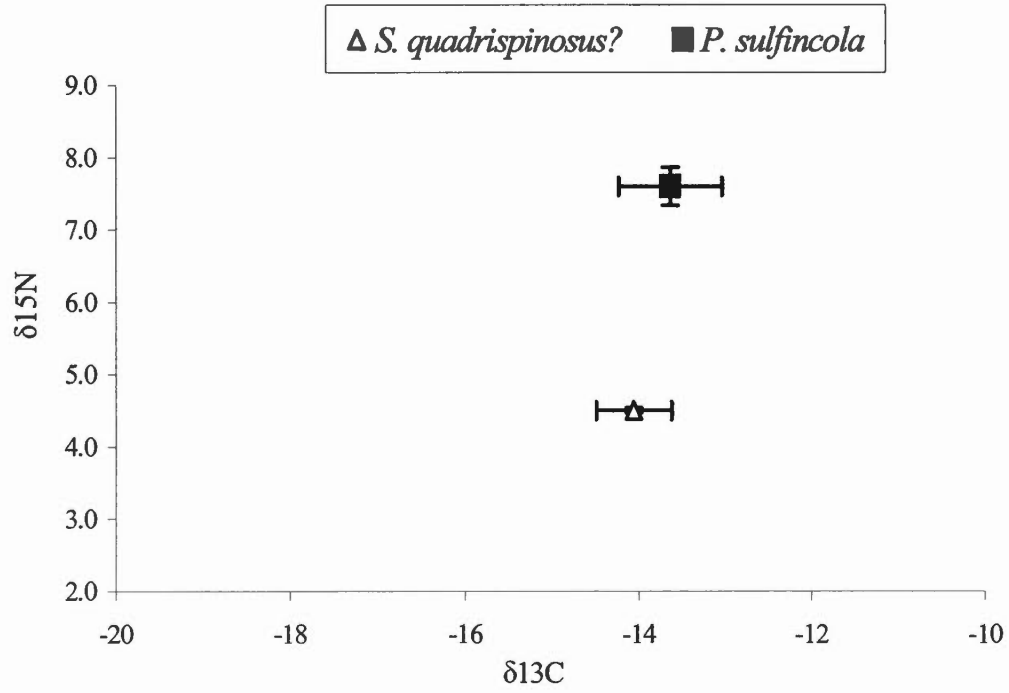

Fig. A.1 Stable carbon and nitrogen signatures of *S. quadrispinosus* and *P. sulfincola* from Explorer ridge. Error bars represent SE.

Each sample of copepods contained 100 individuals, a number which should have been large enough to obtain reliable isotopic results with trap current 600. However, the sample peak heights for both carbon (1.8E-9 A) and nitrogen (2.0E-9 A) isotopes were smaller than expected and on the limit for what can be accepted as a reliable result. One reason for this may be that the carbon and nitrogen content of copepods from hydrothermal vents is different from assumed, 40%, 10% respectively. Another reason may be the acidification treatment that may have caused a loss of organic material. This is supported by the fact that non-acidified pelagic samples, even smaller then above, were successfully analysed with the same trap current (Table A.2).

A.5.2 Freshwater organisms

We were able to get isotopic signatures for samples weighing as little as 100µg (maximum 300µg) by using the 600 trap current. Only 5 to 24 organisms, depending on species or taxa, were needed to obtain 100µg of dry weight with our organisms that contain about 50% carbon and 12% nitrogen (Table A.2). We did not find a significant difference in stable isotopic compositions according to the trap used (1-way ANOVA, $p>0.05$), nor was the utilisation of a more sensitive source associated with a loss of precision (Table 3). Thus, the use of the 600 trap current allowed us to obtain reliable results for specific species in a community that is usually analysed as a bulk sample. We were, for example, able to determine the carbon source and the trophic position of zooplankton species and groups (Fig. A.2) from a northern Quebec lake. The heterogeneity of the nitrogen isotopic compositions reveals that there may be up to 3 distinct trophic levels within the zooplankton community if we consider a typically assumed trophic enrichment of 2-5% between a consumer and its food.

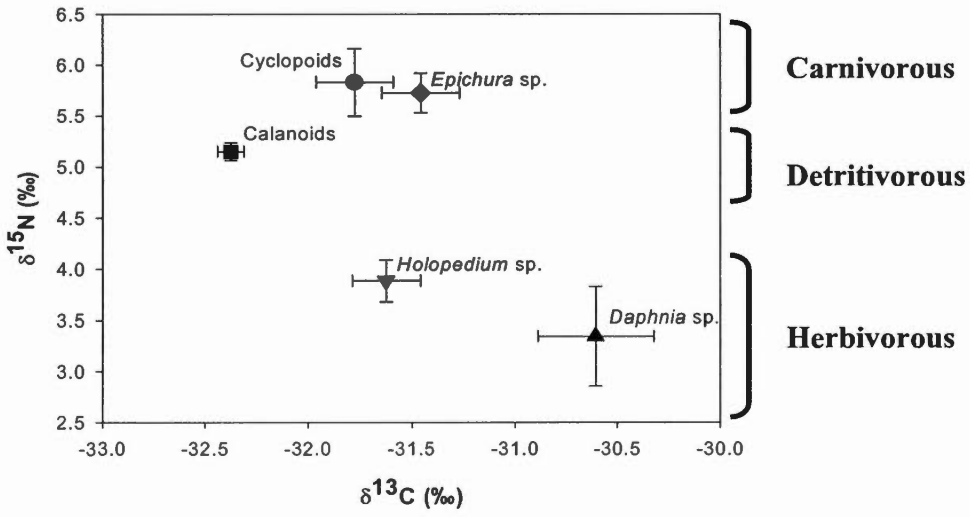

Fig. A.2: Stable carbon and nitrogen isotopic composition of zooplankton organisms (n=3) in lake Jean-Marie (Northern Quebec). Error bars represent standard error

A.6 CONCLUSION

This application note demonstrates the ability of the GV Instruments Isoprime mass spectrometer to perform accurate measurements of stable carbon and nitrogen isotopes for small biological samples. By modifying currently used methods for preparation of samples, we were able to make the handling of small organisms more accessible. We were also able to significantly reduce the number of organisms needed for the analysis by using a more sensitive trap current.

This protocol can be used in other aquatic environments with emphasis on microscopic organisms, but could also easily be adapted for terrestrial communities characterised by small sized organisms.

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ANNEXE B

SOURCE OF CARBON FOR ZOOPLANKTON IN LAKES AND RESERVOIRS FROM NORTHERN QUEBEC (CANADA)

Marty, J. and Planas, D. 2005. Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie. 29:342-344

B.1) INTRODUCTION

The importance of allochthonous versus autochthonous carbon for aquatic food webs is receiving a large attention in aquatic sciences. In the recent years, several studies showed that both autochthonous and allochthonous carbon sources can support secondary productivity in lakes (HESSEN & TRANVIK 1998, KRITZBERG et al. 2004). Specifically, this problematic has been raised for zooplankton since they are key organisms to the transfer of carbon from the base of the food web to higher trophic levels (JONES et al. 1998, GREY et al. 2000, KARLSSON et al. 2003). Carbon stable isotope analyses provide a useful tool to quantify the relative importance of autochthonous and allochthonous carbon to food webs because the stable isotopic composition of a consumer's tissues is related to that of its food (DENIRO & EPSTEIN 1978). Carbon-isotopes ratio ($\delta^{13}C$) change little as carbon moves through food webs (ROUNICK & WINTERBOURN 1986) and thus can be used as a tracer of carbon source (VANDER ZANDEN & RASMUSSEN 2001).

This paper presents the carbon isotopic signatures of zooplankton organisms and its relation to dietary food sources from a series of Canadian northern lakes and reservoirs. Lakes were selected according to a gradient of dissolved organic carbon concentrations. Also, a series of reservoirs were sampled because such ecosystems may be particularly relevant to study the impact of allochthonous carbon since they receive large amounts of organic matter originating from the degradation flooded vegetation (ST LOUIS et al. 2000). Thus, we hypothesised that allochthonous carbon may be a more important food source for zooplankton in reservoirs compared to natural lakes.

B.2) STUDY AREA

A series of 6 large reservoirs (LG-2, Manicouagan, LA-1, LA-2, LG-4 and SM-3) and 14 lakes situated in the northern boreal ecoregion (Quebec, Canada) were sampled once in mid-summer between 2001 and 2003. Several stations were sampled on each reservoir (5 to 10) in order to describe the variability of such large ecosystems (250 to 2600 km^2). A detailed description of sampled sites is presented in PLANAS et al. (2005) and summarized in Table B.1.

Characteristics	**LA1**	**LA2**	**LG2**	**LG4**	**SM3**	**Manic-5**	**Reference lakes**
Area (km^2)	1142.7	286	2645	765	246	1951	15.6 (0.2-42.5)
Mean Depth (m)	5.7	6.3	21	28.4	48.4	61.6	4.6 (1.6-7.56)
Total phosphorus ($\mu g.L^{-1}$)	10.9	7.1	8.8	7.5			7.8 (3.8-14.3)
Chlorophyll a ($\mu g.L^{-1}$)	2.5	2.2	1.5	1.3	2.7	1	1.2 (0.6-2.0)
Dissolved organic carbon ($mg.L^{-1}$)	4	3.2	5.5	2.7	7.4	5.6	6.7 (2.2-12.5)

Table B.1: Main characteristics of sample reservoirs and lakes.

B.3) MATERIALS AND METHODS

Particulate organic matter (POM) was collected on glass-fiber filters (GF/C-Whatman) by filtering a volume of water collected from euphotic zone. The filters were then dried at 45° C and analysed for their carbon signature. Zooplankton organisms were collected from the pelagic zone of each system over the entire water column and to a maximum depth of 30 m, using a 110 µm mesh sized plankton net. Organisms were kept alive in filtered water for at least 2 hours to allow gut evacuation. In the laboratory, organisms were sorted to main genus (i.e. *Daphnia*, *Epichura*, *Holopedium*, *Leptodora*) or, in a small number of cases, to main taxonomic groups (calanoids, cyclopoids). Organisms were placed directly in a pre-weighted

thin capsule and frozen in liquid nitrogen in order to minimize the effects of preservation on isotopic signatures (FEUCHTMAYR & GREY 2003). Sample preparations are presented in detail elsewhere (LIMÉN & MARTY 2004). POM and zooplankton samples were analysed for their carbon signature using a Carlo Erba C/N analyser NA1500 series 2, connected to an Isoprim Mass Spectrometer (Micromass). Results are given using the standard δ notation where:

$$\delta = [(R_{sample}/R_{reference})-1] \times 1{,}000$$

expressed in units of per thousand (‰) and where $R=^{13}C/^{12}C$ or $^{15}N/^{14}N$ (VERARDO et al. 1990). The reference materials used were secondary standards (Leucine) of known relation to the international standard of Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen.

B.4) RESULTS AND DISCUSSION

A Restricted Maximum Likelihood (REML) nested analysis of variance was performed in order to quantify the among- and intra- ecosystem variability. In addition, the determination of the carbon signatures among genus/taxonomic groups of zooplankton allowed for the calculation of intra-site variability, due to possible variations in diet of organisms. The between ecosystems differences explained most of the variance (58%) in zooplankton $\delta^{13}C$, whereas differences within a given and between genus/taxonomic groups only represented 15.6% and 10.4% of the total variance (Table B.2). This results shows that the source of carbon was rather homogenous within a given ecosystem and that all genus/taxonomic groups relied on one principal source of carbon. Using a similar approach, MATTHEWS & MAZUMDER (2003) found that most of the variance was due to taxonomic grouping based on a set of 4 lakes, suggesting that zooplankton taxa had different food sources. However, by increasing the number of sampled ecosystems (14 lakes and 6

reservoirs) and sites (5 to 10), we were able to cover a larger range of $\delta^{13}C$ (-44.2‰ to -26.9‰), with a high number of observations (n=548), allowing for a more robust calculation of the variance partition.

Random effects	**Variance**	**Standard error**	**% of variance**
Ecosystems	4.06	2.44	58.0
Sites[ecosystems]	1.09	0.28	15.6
Organisms[ecosystems,sites]	0.73	0.16	10.4
Residuals	1.11	.	15.9
Total	7.01	.	100

Table B.2: Partition of variance for zooplankton carbon signatures data.

On average per ecosystem, zooplankton carbon signature varied between -30.4‰ in the reference lakes to -36.1‰ in the most recent reservoir SM-3. We also measured the carbon stable isotope of POM since it may be considered as the putative food source for zooplankton (GREY et al. 2000). In the northern boreal region, the allochthonous carbon signature (as terrestrial vegetation signature) was situated around -28‰ (JUNGER & PLANAS 1994) whereas autochthonous carbon (as phytoplankton signature) varied according to temporal and spatial scales and taxonomic composition (FINLAY 2004) but was typically more depleted in $\delta^{13}C$ than POM and DOC derived from terrestrial sources (Peterson & Fry 1987). POM signature varied from -34.6‰ in the most recent reservoir to -27.5‰ in the lakes, with an average of -29.8‰. We observed a positive relationship between POM and zooplankton signatures (r^2=0.83; $p<0.0001$), with a range of divergence between

zooplankton and POM signatures from -2‰ in lakes to -5.2‰ in the recent reservoir SM-3. The degree of divergence between POM and zooplankton signatures could be related to the food selectivity by zooplankton on particulate matter (JONES et al. 1999). The consistent depletion of zooplankton signature compared to POM signature suggests that zooplankton were feeding on isotopically light sources of carbon, especially in reservoirs where the divergence was higher.

Although we don't have the carbon signature of phytoplankton, this study has highlighted the possible importance of autotrophic organisms as the main carbon source for zooplankton in ecosystems such as reservoirs where allochthonous carbon sources predominated. Although it is beyond the scope of this paper, additional stable isotope analysis on dissolved organic carbon, dissolved inorganic carbon and phytoplankton samples will be useful to precisely determine the contribution autochthonous carbon to zooplankton.

B.5 ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from Hydro-Quebec, the Natural Sciences and Engineering Research Council of Canada (Strategic grant to M. Lucotte and D. P.) and Fonds de Recherche sur la Nature et les Technologies (scholarship to J.M.). We also thank the numerous field assistants during the duration of this study as well as people at the GEOTOP stable isotope laboratory.

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