UNIVERSITÉ DU QUÉBEC À CHICOUTIMI

UTILISATION DE LA MATIÈRE ORGANIQUE TERRESTRE PAR LE ZOOPLANCTON DANS LES RÉSEAUX TROPHIQUES AQUATIQUES BORÉAUX

THÈSE
PRÉSENTÉE
COMME EXIGENCE PARTIELLE
DU DOCTORAT EN BIOLOGIE
EXTENSIONNÉE DE
L'UNIVERSITÉ DU QUÉBEC À MONTRÉAL

PAR
GUILLAUME GROSBOIS

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ZOOPLANKTON USE OF TERRESTRIAL ORGANIC MATTER IN BOREAL AQUATIC FOOD WEBS

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« Plus un système vivant est autonome, plus il est dépendant à l'égard de l'écosystème ; en effet, l'autonomie suppose la complexité, laquelle suppose une très grande richesse de relations de toutes sortes avec l'environnement, c'est-à-dire dépend d'interrelations, lesquelles constituent très exactement les dépendances qui sont les conditions de la relative indépendance. » - (Le paradigme perdu, p.32, Points n°109) Edgar Morin

À ma sœur, à mes parents

AVANT-PROPOS

This thesis is structured in 3 chapters, the 1st chapter explores the link between crustacean zooplankton biomass from terrestrial origin (i.e. allochthony) using stable isotopes and the production of crustacean zooplankton community. This chapter is the result of a collaborative work between my supervisor Dr. Milla Rautio, my co-supervisor Dr. Paul del Giorgio, his PhD student Dr. Dominic Vachon and I. DV provided the gross primary production and river flow data. I carried out the sampling, data and statistical analysis as well as zooplankton identification and production calculations. I wrote the article together with MR while DV revised the final version. PdG will provide comments before submitting the paper to a journal.

The second chapter uses fatty acid analyses, a complementary method to stable isotopes to study lipid reserves of the same zooplankton community. It allows a better understanding of how life history strategies influence the building of lipid reserves and how different food sources (phytoplankton, terrestrial organic matter, bacteria) are used in zooplankton life cycle and winter survival. This study has been possible thanks to the collaborative effort of Dr. M. Rautio and her post-doc fellow Dr. Heather Mariash who carried out the survival experiment, as well as MR's PhD student Dr. Tobias Schneider who provided some of the seston fatty acid data. I designed the study with my supervisor's help, carried out the sampling, lab work, data and statistical analysis. I wrote the manuscript with MR. All authors participated to the manuscript revision that is now submitted to Scientific Reports (February 2017).

The third chapter looked at the spatial distribution of the allochthony in the most abundant copepod, *Leptodiaptomus minutus* in Lake Simoncouche and explained it ecologically with the carbon source distribution. This chapter has been published in

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CONTENT

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ABBREVIATIONS

BP	bacterial production
BrFA	branched fatty acid
C	carbon
chl-a	chlorophyll-a
DOM	dissolved organic matter
FA	fatty acids
GPP	gross primary production
LC-SAFA	long chain saturated fatty acids
MO_t	matière organique terrestre
NEP	net ecosystem production
OM	organic matter
PEG	plankton ecological group
POM	particulate organic matter
PUFA	polyunsaturated fatty acids
SAFA	saturated fatty acids
SI	stable isotope
t-DOC	terrestrial dissolved organic carbon
t-DOM	terrestrial dissolved organic matter
t-OC	terrestrial organic carbon
t-OM	terrestrial organic matter
t-POM	terrestrial particulate organic matter
t-POC	terrestrial particulate organic carbon

RÉSUMÉ ET MOTS-CLÉS

La moitié de la matière organique terrestre (MO_t) transportée des bassin-versants vers les océans par les écosystèmes aquatiques est transformée pour être diffusée dans l'atmosphère ou stockée dans les sédiments. Ce rôle de puits ou de source de carbone (C) des écosystèmes aquatiques est grandement influencé par les réseaux trophiques de ces systèmes et notamment le zooplancton tenant une position clé dans ces réseaux. Un nombre croissant de recherches ont démontré une assimilation importante de MOt dans la biomasse zooplanctonique, assimilation que l'on nomme allochthonie. Malgré les conclusions de ces études, le rôle de la MO_t dans les réseaux trophiques aquatiques est encore mal compris surtout lorsque l'on considère que cette source allochtone de l'écosystème qui est largement intégrée dans les tissus zooplanctoniques en milieu naturel ne permet pas une survie ou une reproduction suffisante des organismes en laboratoire. Une augmentation des apports en MO_t est prédite dans un futur proche avec les changements climatiques. Les effets sur les réseaux trophiques aquatiques et notamment le zooplancton sont très peu connus et nécessitent des recherches approfondies.

Ce projet de doctorat vise à quantifier l'importance de la MO_t pour la communauté zooplanctonique afin de mieux comprendre son rôle dans les réseaux trophiques aquatiques. L'intégration de la MO_t par les principaux taxons de la communauté zooplanctonique d'un lac boréal a été étudiée à différentes saisons et dans différents habitats. Premièrement, l'allochtonie basée sur des signatures d'isotopes stables ($\delta^{13}C$) a été mesurée chez les principales espèces zooplanctoniques ainsi que leur production (croissance et reproduction) respective. Pour la première fois, une nouvelle variable représentant le taux de MO_t intégrée dans la biomasse pour chaque semaine a été proposée : l'allotrophie et a été calculée durant une année entière. Deuxièmement, complétant l'approche des isotopes stables, la composition en acides gras biomarqueurs terrestres, algaux et bactériens à été mesurée pour estimer l'allocation de ces différentes sources dans les réserves lipidiques du zooplancton. Le but était d'évaluer si la MO_t était une source alternative à la production primaire autochtone lorsque cette dernière est faible notamment en hiver sous le couvert de glace. Troisièmement la variabilité spatiale intra-lac de l'allochtonie basée sur les isotopes stables $\delta^{13}C$ et $\delta^{2}H$ a été estimée en fonction des bancs de macrophytes aquatiques et des points d'entrée de MO_t via les tributaires du lac. Les hypothèses de recherche prédisaient que l'allochtonie ainsi que les acides gras biomarqueurs terrestres et bactériens seraient plus importants en hiver sous la glace que pendant la période d'eau libre lorsque la production phytoplanctonique est minimale. Il était également supposé que l'allochtonie serait distribuée de manière hétérogène, spatialement, à l'intérieur du lac selon les bancs de macrophytes et les sources de

MO_t. Finalement, il était présumé que les macrophytes diminuent l'allochtonie du zooplancton qui devait au contraire augmenter à proximité des tributaires. Afin de tester ces hypothèses, un échantillonnage de la communauté zooplanctonique et des sources de C a été effectué durant une année entière, de manière hebdomadaire en été et toutes les deux semaines en hiver portant une attention particulière aux processus se passant sous le couvert de glace. Un deuxième échantillonnage s'est intéressé à la distribution spatiale de l'allochtonie de l'espèce principale de la communauté de crustacés zooplanctoniques lors de deux saisons (printemps et été) significativement différentes dans leurs apports de source de C (phytoplancton, apports de MO_t, macrophytes, algues benthiques).

L'allochtonie était importante pour chacune des espèces de zooplancton se stabilisant autour de 60% en automne et durant la majeure partie de l'hiver. Lors de la transition hiver-printemps, l'allochtonie diminua fortement chez toutes les espèces. Lorsque l'allochtonie fut couplée avec la production de zooplancton, la production primaire brute, la production bactérienne ainsi que les nouveaux apports de MO_t ont été identifiés comme les principaux facteurs influençant l'intégration de MO_t dans la biomasse zooplanctonique. Selon l'approche des acides gras, une période critique d'accumulation des réserves lipidiques a été identifiée en automne et se poursuivant en hiver sous la glace, ce qui va à l'encontre du paradigme en matière de disponibilité de nourriture phytoplanctonique en hiver. Cette période a été identifiée comme essentielle à la survie du zooplancton qui reste actif toute l'année y compris en hiver. La distribution spatiale de l'allochtonie dans le zooplancton était hétérogène et influencée par la proximité des bancs de macrophytes ainsi que par les tributaires majeures contribuant à l'hydrologie générale du lac. Pris collectivement, ces résultats ont permis de montrer que l'intégration de la MO_t dans les réseaux trophiques aquatiques est très dynamique saisonnièrement et spatialement même à l'intérieur d'un seul lac. Contrairement à nos hypothèses, le patron saisonnier de l'utilisation de la MO_t par le zooplancton a décrit l'hiver en tant que période critique d'assimilation de C autochtone soulignant l'importance des différentes allocations de la MO_t dans les organismes zooplanctoniques. Ces résultats suggèrent que la MO_t est une source complémentaire mais pas une alternative aux sources autochtones. Pour la première fois, cette étude montre l'hétérogénéité spatiale de l'allochtonie du zooplancton à l'intérieur d'un lac et l'explique écologiquement par la présence de macrophytes et l'hydrologie du lac. Enfin, cette thèse rapporte une quantification unique du taux d'assimilation de la MO_t dans le zooplancton durant une année entière. Appliquer et étendre de nouvelles approches telles que combiner les acides gras et les isotopes stables et mesurer l'allotrophie afin d'estimer les différentes allocations de la MO_t dans d'autres organismes et d'autres écosystèmes, permettra de comprendre comment les écosystèmes aquatiques seront affectés par l'augmentation d'apports en MO_t et leur rôle dans la modification du cycle du C mondial.

Mots-clés : acides gras, allochtonie, hiver, isotopes stables, macrophytes, production du zooplancton

SUMMARY AND KEYWORDS

Half of the global terrestrial organic matter (t-OM) carried from terrestrial ecosystems to the oceans is processed within aquatic ecosystems by being diffused into the atmosphere or stored within sediments. Aquatic food webs play an important role in the processing of t-OM, in particular at the zooplanktonic trophic level. Increasing numbers of studies have demonstrated that a significant share of t-OM is assimilated into the zooplankton biomass, this assimilation being termed "allochthony". However, very little is understood about how this t-OM is used by zooplankton especially given that zooplankton neither survive nor reproduce well when solely utilizing t-OM as a food source. As terrestrial inputs of t-OM are predicted to increase in the coming decades due to climate change, our lack of understanding of the role of t-OM as an allochthonous source makes it difficult to predict possible impacts on aquatic food webs and the consequences for aquatic ecosystems.

This PhD project aims to investigate how zooplankton assemblages, within a boreal lake, use t-OM. Zooplankton use of t-OM is compared between different aquatic habitats and across seasonal and spatial differences in carbon (C) inputs. First, zooplankton allochthony was determined from stable-isotope (SI) measurements. In addition, production (growth and reproduction) of the main zooplankton taxa was measured throughout an entire year including under-ice conditions in winter. The coupling of both measurements permitted, for the first time, an ecosystem-scale quantification of the rate of t-OM assimilated in zooplankton biomass, defined here as "allotrophy". Second, fatty acid (FA) composition was measured throughout the year for the same species of zooplankton to identify algal, terrestrial and bacterial FA biomarkers in the lipid reserves. Third, the spatial distribution of allochthony for the dominant zooplankton species (*Leptodiaptomus minutus*) was measured using the combined $\delta^{13}C$ and $\delta^{2}H$ signatures from different habitats (habitats dominated by t-OM inputs, macrophytes, phytoplankton or benthic algae). Terrestrial inputs were hypothesized to be an alternative source to phytoplankton for zooplankton production and energetic lipid reserves particularly in winter when primary production is low. Aquatic macrophytes were also hypothesized to influence the degree of allochthony representing an alternative autochthonous C source. Sampling occurred weekly during the open-water period and every two weeks under the ice to highlight the oft-neglected winter processes. A second sampling survey measured the within-lake spatial distribution of the allochthony for *L. minutus* at ten sites having variable dominant C sources (t-OM, macrophytes, phytoplankton, benthic algae).

Zooplankton allochthony was high, stabilizing around 60% for all taxa in autumn and for most of the winter. A decrease in allochthony was observed for all species during the transition between winter and spring. FA composition in zooplankton revealed a critical period of phytoplanktonic FA accumulation in autumn and under the ice in the early winter for taxa that remain active throughout the winter. The quantitative estimates of allotrophy demonstrated that t-OM assimilation was relatively efficient during the growth phases of zooplankton and was driven by both gross primary production and fresh t-OM inputs. Finally, a spatial heterogeneity of zooplankton allochthony was detected within the lake influenced by the proximity to macrophyte beds and by lake hydrology. Collectively, these results highlight a seasonal and a spatial variability of t-OM assimilation in zooplankton within a lake. Contrary to our hypothesis, the seasonal pattern of t-OM use by zooplankton shows the winter season to be a critical period of autochthonous C assimilation in lipid reserves and emphasizes the importance of differential allocations of t-OM in zooplankton organisms. These results suggest that terrestrial inputs are complementary C sources, not alternative C sources for zooplankton. Also, this study shows for the first time, a spatial heterogeneity in zooplankton allochthony within a lake explained ecologically by macrophytes and lake hydrology. Finally, this thesis reports a unique quantification of the assimilation rate of t-OM in zooplankton throughout an entire year. Expanding and applying these new approaches of combined SI-FA methods and zooplankton allotrophy for investigating the differential allocation rates of t-OM in other organisms and other ecosystems will greatly contribute to a better understanding of how aquatic food webs will be affected by increasing t-OM inputs and clarify their role in the modification of the global C cycle.

Keywords: allochthony, fatty acids, macrophytes, stable isotopes, winter, zooplankton production

INTRODUCTION

Statement of the problem

For a long time, ecologists have understood that all ecosystems receive considerable amounts of material from outside their boundaries (e.g. Wetzel 1975). It is particularly pronounced in lakes as, due to their convex location in the landscape, waterbodies are linked with their drainage basin through the dissolved and particulate material transported by the downhill flow of water (Jackson and Fisher 1986, Leroux and Loreau 2008, Marcarelli et al. 2011). At global scale, inland waters receive annually three petagrams of carbon (C) namely 3 billion metric tons of C (Lennon et al. 2013). Terrestrial organic C (t-OC) carried by inland waters from terrestrial ecosystems to the oceans is significant in the global C cycle being equivalent to more than half the total C assimilated by the terrestrial biosphere biomass each year, i.e. the net ecosystem production (NEP) of terrestrial world ecosystems (Cole et al. 2007). Aquatic ecosystems are active catalytic environments where terrestrial inputs are diffused into the atmosphere, stored in sediments or used in food webs thereby leaving only 47% to be finally transported into the ocean (Cole et al. 2007). Less conservative studies estimated that 0.4 petagrams were arriving into the oceans from the terrestrial environment implying that freshwater ecosystems process 87% of t-OC (Hedges et al. 1997). Inputs from the terrestrial environment in adjoining freshwater ecosystems are described as “allochthonous” and, for a long time, have been believed to greatly influence the functioning of recipient ecosystems such as lakes (Wetzel 1975), rivers (Jones 1949, 1950), oceans (Polis and Hurd 1996) and estuaries (Teal 1962).

Terrestrial organic matter (t-OM) eventually enters the aquatic food web and is reflected in biomass referred to as the organism's "allochthony". The significance of allochthony in aquatic food webs has been revealed by recent literature (Cole et al. 2011, Karlsson et al. 2012, Berggren et al. 2014). Increasing numbers of studies provide evidence of substantial values of allochthony in bacteria (Berggren et al. 2010a, Guillemette et al. 2016), in zooplankton (Cole et al. 2011, Kelly et al. 2014) and in fish (Glaz et al. 2012, Tanentzap et al. 2014). While all aquatic organisms may play a role in t-OM assimilation, zooplankton occupy a strategic position in aquatic food webs, potentially accessing autochthonous C (i.e. produced within the aquatic ecosystem boundaries) as well as allochthonous C. Support of zooplankton production by autochthonous sources is well established through numerous studies showing direct algal consumption (Fryer 1957, Galloway et al. 2014). Allochthonous sources as support of pelagic zooplankton production is less obvious.

While earlier studies have concluded to a significant support of zooplankton with terrestrial particulate organic matter (t-POM) (Cole et al. 2006), recent studies tend to show that this t-OM supply is rather small (Wenzel et al. 2012, Mehner et al. 2015). However, terrestrial assimilation may occur lower in the food web based on microbial degradation of terrestrial dissolved organic matter (t-DOM) and the subsequent assimilation into the food web at the level characterized by ciliates, flagellates or rotifers (Jansson et al. 2007). Zooplankton make these C sources available for zooplanktivores including numerous fish species (e.g. *Perca flavescens*) or invertebrates (e.g. *Chaoborus obscuripes, Leptodora kindtii*) participating in the control of the higher trophic level allochthony (Tanentzap et al. 2014). Thus, zooplankton has been considered as an indicator of allochthony in aquatic food webs and has been the focus of many studies of allochthony (Perga et al. 2006, Cole et al. 2011, Lee et al. 2013). Allochthony in zooplankton has shown a very wide range estimated to be from less than 5% (Francis et al. 2011) to 100% (Rautio et al. 2011).

Whereas there remains debate about the significance of t-OM contribution for zooplankton in different lake ecosystems (e.g. large clear water lakes or humic lakes), it is now increasingly accepted that allochthony can be often very significant for a large range of zooplankton species (Cole et al. 2011, Wilkinson et al. 2013a, Emery et al. 2015).

Terrestrial OM is comprised of a mix of various molecules that are believed to be either not accessible for zooplankton or lacking essential compounds for zooplankton growth and reproduction. Recent studies tend to show that t-POM flux is relatively small for pelagic crustaceans and reflects poor nutritional conditions (Mehner et al. 2015, Taipale et al. 2015a). Additionally, t-POM has been shown to be poorly available in lipids and protein, mostly composed of lignin that is non-digestible for zooplankton (Taipale et al. 2016). Most importantly, t-POM does not contain polyunsaturated fatty acids (PUFA) essential for organism growth and reproduction (Brett et al. 2009). Terrestrial dissolved organic matter is not directly available for crustacean zooplankton, but is probably utilized by protists via osmotrophy, and preferentially retained in biomass by bacterial communities (Guillemette et al. 2016). However, survival and growth of zooplankton has been evaluated to be lower when organisms are sustained with bacteria rather than with lipid-rich autotrophic phytoplankton cells (Wenzel et al. 2012). Whether t-OM can efficiently sustain zooplankton production remains a point of debate, however as for t-POM, t-DOM is believed to represent a marginal subsidy when both adequate trophic links and upgrading are lacking to make it available for zooplankton.

Recently, it has been suggested that t-OM can have differential allocation by aquatic organisms, such as in bacteria where phytoplankton C is mostly respired and terrestrial C is used for biomass synthesis (Guillemette et al. 2016). Based on this new finding, Taipale et al. (2016) suggested that carbohydrates derived from

terrestrial C were used by zooplankton while phytoplanktonic C were preferentially retained in lipid reserves. As the allochthony in zooplankton is usually measured without any quantification of the respective t-OM used in respiration, energy storage, growth or reproduction, it is very difficult to evaluate the actual role of this t-OM for zooplankton individuals. Simultaneously, several non-exclusive visions of t-OM utilization by aquatic food web emerge. On one hand, t-OM is considered as an alternative food source when autochthonous OM is missing thereby providing the sufficient nutritive conditions for survival while diminishing growth and reproduction (McMeans et al. 2015a, Taipale et al. 2016). On the other hand, t-OM is considered as a complementary resource and assimilated along with autochthonous OM suggesting a synergetic utilization of both sources. The idea behind this concept is that labile autochthonous C sources can induce modifications in the mineralization rate of the more recalcitrant C of terrestrial origin initially present; this being described as the "priming effect" (Guenet et al. 2010). Also, t-OM is often considered by limnologists to be homogeneous, but is, in reality, a mix of material having very different origins and differing states of degradation (Berggren et al. 2010b). Terrestrial dissolved organic matter includes low molecular weight compounds such as carboxylic acids, amino acids and carbohydrates that are easily utilized by bacteria and eventually transferred to higher trophic levels (Berggren et al. 2010a). As mixotroph and heterotroph protists can feed on both aquatic primary producers and bacteria, zooplankton can comprise a mixed C composition (autochthonous versus allochthonous) already present in their prey. Despite a good number of recent studies discussing allochthony, these different mechanisms of how t-OM is assimilated and differentially allocated within the aquatic food web have seldom been addressed and remain uncertain.

Taxonomic differences have been detected in zooplankton allochthony usually reporting that cladocerans followed the C composition in the environment (Rautio et

al. 2011), while calanoid and cyclopoid copepods were less dependent of C sources due to lipid accumulation (McMeans et al. 2015b). Moreover, species have different feeding strategies that determine the degree of allochthony in their biomass with calanoid copepods depending more on phytoplankton cell abundance and becoming more allochthonous when phytoplankton are lacking. However, cyclopoid copepods, usually predators or omnivorous organisms, are more dependent on the degree of allochthony of their prey (Berggren et al. 2014). Cladoceran allochthony is more dependent on what they can filter as well as the size of particles, relying on both phytoplankton availability and the DOM-based food web (Karlsson et al. 2003). To understand the variability of allochthony estimates based on the entire zooplankton community, species composition needs to be accounted for as a major influencing factor.

The vast majority of existing knowledge collected regarding zooplankton allochthony has been acquired from the open-water season starting in the spring and ending in the autumn while the winter season, particularly in ice-covered ecosystems, is usually excluded (Hampton et al. 2015). Winter, however, represents a critical period for zooplankton as many species stay active under the ice and autochthonous production is drastically reduced in a lightless environment (Lizotte 2008). One might naturally think that winter provides the ideal conditions to study zooplankton reliance on terrestrial sources while aquatic primary production is not available. The few available studies of the seasonal patterns of allochthony in zooplankton have shown that food source assimilation was not constant. Rather, zooplankton switched easily from one source to another (Grey et al. 2001, Rautio et al. 2011, Berggren et al. 2015). As these C utilization pathways are highly taxon-dependent (Berggren et al. 2014), they are linked to species' life strategies and particularly to their capacity to store molecules in lipid reserves. Lipid accumulation in winter allows individuals to use organic matter and its associated energy at the time required and not only when

food sources are immediately available in the environment (Schneider et al. 2016). Winter under-ice measurements are thus important for understanding the links to open-water patterns as winter processes are increasingly acknowledged as influencing the full year of seasonal patterns in plankton cycles (Sommer et al. 2012). As the winter season reunites important conditions that influence the degree of allochthony in zooplankton, winter allochthony measurements should help to better understand open-water patterns.

The aim of this project was to better understand the role of the t-OM in aquatic ecosystems focusing on high frequency measurements of seasonal and spatial use of allochthonous C sources by the zooplankton community within a boreal lake. Several innovative aspects were addressed that had been previously ignored or unexplored, such as zooplankton production, winter feeding ecology or within-lake variability. Combining these unique aspects aim, here, to provide a better understanding of the differential use of t-OM in boreal aquatic food webs and identify the driving factors among possible environmental and biological factors such as terrestrial input, macrophyte beds, phytoplankton and bacterial production, water temperature and the lipid composition of seston. This high frequency spatial and seasonal study also aimed to quantify the t-OM role in zooplankton communities and highlight its influence on the complete boreal lake ecosystem.

State of the science

Allochthony in zooplankton biomass

The role of t-OM assimilation in aquatic food webs has been studied for a long time, particularly by stream ecologists who studied the role of allochthonous food sources for invertebrates and fish (Jones 1949, 1950, Teal 1957, Fisher and Likens 1972,

Petersen and Cummins 1974). However, the notion of "allochthony" defined as the terrestrial contribution in aquatic biomass, has been used in literature only very recently. Researching the keyword "allochthony" in the peer reviewed literature of the Scopus® database, within "Agricultural and Biological Sciences" and "Environmental Sciences" disciplines, 140 articles were referenced from 1983 to 2016 (Fig. 1). The number of articles using "allochthony" rose very recently in 2007 and peaked in 2009 (16 references) reflecting a recent and close interest of scientific community in the last years.

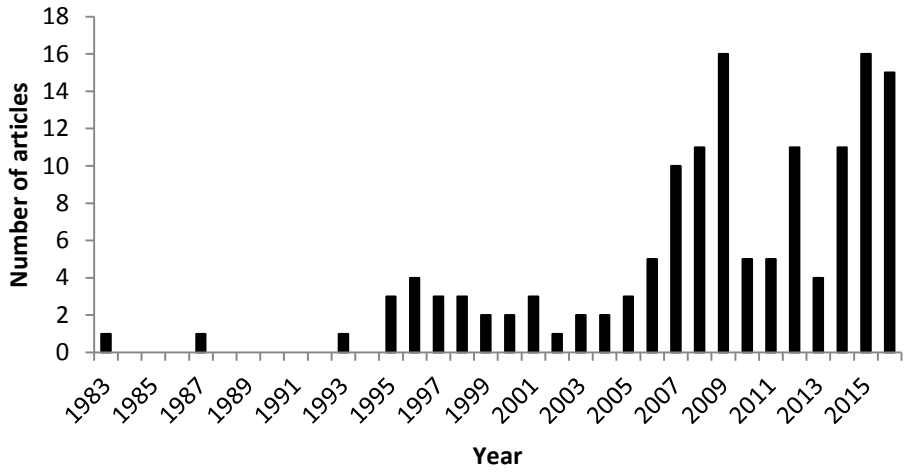

Figure 1 Number of published articles citing "allochthony" (all fields) in the "Agricultural, and Biological Sciences" and "Environmental Sciences" databases from Scopus®.

Allochthony in the biomass of aquatic organisms is almost exclusively calculated from stable-isotope (SI) ratios. The natural occurrence of SI is now widely used in ecology in particular for tracing the fluxes of organic matter such as allochthonous inputs in aquatic biomass within lake ecosystems (del Giorgio and France 1996, Jones et al. 1998, Cole et al. 2002, Carpenter et al. 2005, Rautio and Vincent 2006,

Pace et al. 2007, Taipale et al. 2009, Berggren 2010, Berggren et al. 2015). The most widely used SI ratio in trophic studies is the relative abundance of ^{13}C over ^{12}C due to the C-based compounds that characterize organic matter molecules and the relatively high abundance of ^{13}C compared to ^{12}C in ecosystems. This $^{13}C/^{12}C$ ratio in animal biomass assimilated into their tissues closely reflects the ratio absorbed from their diet when applying a known fractionation (Post 2002, Fry 2006). As ^{13}C occurrence over ^{12}C is much lower (about 1000 times less abundant), a more convenient ratio of SI is applied, symbolized by "δ" through the following eq. [1]:

$$\delta = \left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right) \times 1000 \qquad \text{eq. [1]}$$

where R is the ratio of the less abundant isotope over the more common isotope measured in the sample or in an international standard. The occurrence of the less abundant isotope is usually much lower than the more common isotope for most elements (Fry 2006). Interest in allochthonous inputs was initially raised in Scandinavian small humic lakes (Meili 1992) where bacterial metabolism can be supported by t-OM (Hessen 1992) leading to the theory that allochthony in zooplankton was linked to lake trophy (del Giorgio and France 1996, Grey et al. 2000). However, SI ratios demonstrated that zooplankton can also be significantly (~50%) subsidized by t-OM in a large clear water lake (Jones et al. 1998). The natural occurrence of SI demonstrated that reliance on t-OM can range from < 5% to > 80% (Francis et al. 2011, Wilkinson et al. 2013b). This high variability in t-OM reliance follows a seasonal pattern whether in a clear water Scottish lake (Grey et al. 2001) or in subarctic lakes (Rautio et al. 2011). In lakes from Northern Sweden, allochthony in zooplankton has been linked to pelagic energy mobilization more than t-OM prevalence in the environment, thereby underlying an active role of heterotrophic protists in the assimilation of t-OM within aquatic food webs (Karlsson et al. 2003).

Defining the end-members that play a role in the studied aquatic food web is one of the more difficult tasks. The end-member of allochthonous origin (terrestrial plants) is quite easy to obtain due to the relative stability in bulk $\delta^{13}C$ among C4 plants, while the end-member of autochthonous (phytoplankton) origin is more difficult to estimate and constitutes a strong limitation to SI studies in pelagic ecosystems. For now, no technique allows for physically separating phytoplankton from terrestrial particles in the particulate organic matter (POM) although some estimates can be obtained, e.g. from POM with a correction for algal biomass (Marty and Planas 2008). Pace et al. (2004) circumvented the issue by adding labeled $H^{13}CO_3$ to a small lake enriching the autochthonous C pool of the lake in ^{13}C. It was then possible to trace the C pathway from allochthonous and autochthonous sources to the zooplankton consumer. This method was later used to test the effect of lake trophic status on allochthony demonstrating that allochthony was more pronounced in a dystrophic lake than in a nutrient enriched lake (Carpenter et al. 2005). Both lakes confirmed some substantial allochthonous support of the pelagic food web including POC, zooplankton, the predatory invertebrate *Chaoborus* spp. and fish. Pace et al. (2007), with the same method, showed that in a clear water lake, autochthonous carbon was the dominant source (88–100%) for POC, gram-positive bacteria, copepods and *Chaoborus* spp. Autochthonous carbon would provide a lower fraction (< 70%) of carbon to DOC, gram-negative bacteria and cladocerans leading to the conclusion that a relatively small flux of terrestrial particulate carbon supported ~50% of zooplankton and fish production. France (1995) argued that SI analysis was an irrelevant tool to trace carbon origin because of the global variability found in a compilation of studies. Doucett et al. (1996) maintained that SI analysis was reliable as long as any spatial heterogeneity was taken into account.

Mixing models provided a new perspective on SI studies and added precision in the estimates of allochthony when calculating plausible contributions of each food source

to the consumer biomass to account for uncertainties that were previously neglected (Phillips and Gregg 2001, Phillips and Gregg 2003). Mixing models have the capacity to quantify the share (or fraction) of a food source for a given consumer biomass. As organic matter is composed of C-based compounds, $\delta^{13}C$ has been the first isotope ratio used with mixing models (Phillips 2001). The ratio $\delta^{15}N$ has also been used with a fractionation in animals often considerable and estimated about 3.4 ± 1‰ for each consumer compared to its diet (Post 2002). This high fractionation allows for deduction of the trophic level of the studied organism. Combined $\delta^{13}C$ and $\delta^{15}N$ signatures are powerful due to the complementary food tracers and trophic level information. End-member signatures are sometimes overlapping as with terrestrial and algal $\delta^{13}C$ making it impossible to calculate source contributions. To overcome this issue, deuterium (^{2}H) has been recently used because of its presence in organic matter and a strong separation between aquatic and terrestrial primary production (Doucett et al. 2007). The $\delta^{2}H$ has been shown as a powerful tool in food web studies when the non-exchangeable part of hydrogen in the tissue is considered and "dietary" water is accounted for (Wassenaar and Hobson 2003, Cole et al. 2011). When added to carbon and nitrogen SI, hydrogen can provide a tridimensional stable-isotope signature of consumer and end-member tissues that new mixing models have now the ability to exploit (Batt et al. 2012).

The variability in allochthony has been explored very recently due to these new mixing models and isotopes. For example, a strong reliance on t-OM (20–40%) for pelagic food webs has been confirmed using these isotopes and mixing models (Cole et al. 2011). A multi-lake study measured a strong variability in consumer allochthony (1–76%) that also confirmed the strong reliance of pelagic zooplankton on terrestrial inputs albeit negatively influenced by lake surface area (Wilkinson et al. 2013a). Another multi-lake study sampled a large number of boreal lakes from the Canadian Shield and demonstrated that zooplankton allochthony is highly influenced

by the feeding strategies of species (Berggren et al. 2014). Allochthony in cyclopoid copepods (16%) was linked to predation on lower trophic level organisms, e.g. rotifers, ciliates, flagellates, and to the corresponding microbial food web while calanoid copepod allochthony (18%) was more dependent of the availability of phytoplankton. Finally cladoceran allochthony, such as *Daphnia* (31%), was linked to both pathways (Berggren et al. 2014). The same strong reliance on t-OM (26–94%) has been measured in reservoirs where a large amount of terrestrial C is assimilated into the aquatic environment when the valley is flooded (Emery et al. 2015). Finally, a new approach combining lake metabolism and SI demonstrated that a lake ecosystem can be significantly supported by t-OC including subsidizing 47% of zooplankton biomass (Karlsson et al. 2012).

Fatty acids (FA) are essential molecules for the survival, growth and reproduction of most animals including crustacean zooplankton (Wenzel et al. 2012). These molecules cannot be synthesized *de novo* by animals and need to be acquired from their diet (Arts et al. 2009). Most of the essential FA for survival, growth and reproduction are n-3 polyunsaturated fatty acids (n-3 PUFA) necessary for zooplankton growth and reproduction (Brett et al. 2009). As phytoplankon are recognized as synthesizing most of the n-3 PUFA and terrestrial plants synthesize more n-6 PUFA and saturated fatty acids (SAFA) that zooplankton are unable to directly use, it is usually believed that zooplankton require molecules from algae to survive, grow and reproduce (Taipale et al. 2014). Some lipid species (FA, fatty alcohols, hydrocarbons and sterols) are limited to certain taxa and, being metabolically stable, they are used to trace energy transfers through the food web (Napolitano 1999). For instance, algae are the only organisms that possess the enzyme for producing long chain PUFA such as 20:5n-3 and 22:6n-3 (Iverson 2009). PUFA are related biochemically due to the location of the first double bond such as long chain “n-3” and “n-6” FA, essential for normal functioning of the cell. In the

animal kingdom (consumers), these FA must be acquired from their diet. Heterotroph organisms can elongate and desaturate FA, but they are unable to place a bond between the terminal methyl-end and the n-9 carbon, being incapable of inserting a double bond in the n-3 and n-6 position (Arts and Wainman 1999). FA groups, such as those previously cited, are used as tracers throughout the food web as well as specific FA used as trophic markers of algae, cyanobacteria and bacteria (Taipale et al. 2015b). Studies have shown that lipids from aquatic and terrestrial primary producers have different fatty acid signatures and can therefore be used to characterize the predominant energy source in aquatic systems. Using FA, Brett et al. (2009) showed that *Daphnia* sp. preferentially use algae for their growth and reproduction over terrestrial carbon. It seems then paradoxical that in the allochthony studies, the zooplankton reliance on t-OM is very significant. Combining SI with the FA composition of consumers has provided contradictory results, but this combination is a necessary approach for understanding food source utilization (Perga et al. 2006). Employing only stable isotopes to understand food web functioning and interactions with t-OM may be misleading due to the complex paths that organic matter can follow through to the highest trophic levels (Perga et al. 2008).

Zooplankton reliance on terrestrial organic matter at the ecosystem scale

Allochthony is often presented in zooplankton because of the strategical position that these organisms occupy in the aquatic food web. The ultimate goal is to understand the interactions between terrestrial and aquatic ecosystems or to quantify the aquatic reliance on terrestrial ecosystems and, as such, ecosystem-scale conclusions are often drawn from allochthony in zooplankton. However, the measured allochthony in zooplankton is the result of multiple processes occurring at different levels of the food web that reflects only a part of the extent of the aquatic reliance on the surrounding terrestrial ecosystems which is practically never quantified for the whole

lake ecosystem. This reliance on terrestrial ecosystems depends on: 1) allochthonous and autochthonous fluxes coming into the lake, 2) differential metabolism allocation of t-OM by individuals and 3) the importance of biomass and production at the ecosystem level.

From allochthonous and autochthonous sources to zooplankton

Terrestrial organic matter can overwhelm the environment in aquatic ecosystems and represent more than 90% of the DOC as well as more than half of the POC in boreal and temperate lakes (Meili 1992, Wilkinson et al. 2013b). DOC represents much larger inputs with a usual DOC:POC ratio between 6:1 and 10:1 (Wetzel 1995). This is particularly true when an aquatic ecosystem is surrounded by a coniferous forest producing massive humic inputs from the catchment basin where terrestrial dissolved organic carbon (t-DOC) is dominated by fulvic acids of relatively low molecular weight that can heavily subsidize bacterial production (Hessen 1992, Berggren et al. 2010b). Bacterial biomass can then be composed of a large proportion of t-OC (Guillemette et al. 2016) and be consumed by heterotrophic or mixotrophic protists such as ciliates and flagellates (Martin-Creuzburg et al. 2005). Zooplankton predation of these organisms may cause them to inherit of the same degree of allochthony. This is currently believed to be the major flux of terrestrial organic matter in pelagic aquatic food web.

DOM uptake by aquatic food webs depends very much on the composition, size and age of the molecules. Molecules are referred to as labile, semi-labile or recalcitrant (Kragh and Sondergaard 2004). The terrestrial share of DOM (allochthonous) is composed of molecules that originate from the tissue of terrestrial plants and is often modified by soil microorganism communities before entering inland waters (Solomon et al. 2015). Generally, t-DOM is composed of humic and fulvic acids that contain aromatic hydrocarbons including phenols, carboxylic acids, quinones, catechol and a

non-humic fraction characterized by lipids, carbohydrates, polysaccharides, amino acids, proteins, waxes and resins (McDonald et al. 2004). Among these compounds, low molecular weight carboxylic acids, amino acids and carbohydrates can potentially support all bacterial production in boreal ecosystems (Berggren et al. 2010a). The rest of incoming terrestrial organic matter is highly concentrated in tannins and represents organic compounds non-degraded by microbial fauna in the soil (Daniel 2005). Despite this recalcitrance, the aquatic–terrestrial interface are biogeochemical hotspots for organic matter processing (McClain et al. 2003). Degradation processes preferentially remove oxidized, aromatic compounds, whereas reduced, aliphatic and N-containing compounds are either resistant to degradation or are tightly cycled and thus persist in aquatic systems (Kellerman et al. 2015). The role of allochthonous carbon in aquatic ecosystems is closely related to bacterial abundance, biomass and production (Azam et al. 1983, Roiha et al. 2011) as most of the DOC and POC decomposition is undertaken by planktonic bacteria (Wetzel 1975, Daniel 2005). Depending on the quality of the organic carbon, bacterial productivity might change while organic carbon quantity seems to define community composition (Roiha et al. 2011). Terrestrial OM is thus a mix of molecules in the environment that can directly influence and foster very different bacterial communities and possibly affect higher trophic levels.

Several studies have demonstrated a substantial support of zooplankton via t-POM in temperate lakes (Cole et al. 2006, Pace et al. 2007). However, direct assimilation of t-POM by zooplankton and its uptake by higher trophic levels is now thought to be rather minimal as t-POM is composed of recalcitrant lignin. Recent experiments have demonstrated that zooplankton survives, grows and reproduces poorly with t-POM alone (Brett et al. 2009, Wenzel et al. 2012, Taipale et al. 2014). High degrees of allochthony found in zooplankton are thus most likely the result of DOC assimilation by the microbial community and repackaging by organisms feeding on bacteria.

Differential terrestrial organic matter allocation by zooplankton

Once the food is ingested by zooplankton, including t-OM, it is differentially allocated by the individual in growth, in respiration, in lipid reserve accumulation, in reproduction or in excretion. However, due to technical issues, many studies focusing on allochthony can be considered as presenting the summary of these differential t-OM allocations. For example, a specific degree of allochthony for zooplankton may be the result of the amount of autochthonous versus allochthonous material consumed, the allochthony values of its prey, the allochthonous molecules stored in the lipid reserves, the amount of t-OM used for biomass synthesis and the t-OM respired and excreted by the individual. Zooplankton net production is characterized by biomass synthesis, i.e. individual growth and reproduction (Runge and Roff 2000). Allochthony estimates indicate what is present in consumer biomass *in fine* and do not inform on the t-OM that has been respired by the organisms or transferred to the eggs. These differential allocations have never been tested in zooplankton but have begun to be explored for bacterial communities with a recent study discovering that bacteria preferentially retained t-OM in biomass (Guillemette et al. 2016), while algal C was used for bacterial metabolism and respiration. It is very likely that, as with bacteria, zooplankton utilize molecules from different origins preferentially for growth (biomass synthesis) or respiration (metabolism).

Many zooplankton species can store energetic molecules in lipid reserves (Fig. 2) to be able to fully accomplish their life cycle (Mariash et al. 2011, Varpe 2012). Storing energy is essential for species in environments having very distinct seasons such as those in polar, boreal and temperate ecosystems where resources can be available for only a limited interval during the year. Consumers can then accumulate important food sources while they are available and use them later in the year for the energy-demanding phases of life cycle such as growth or reproduction (Schneider et al. 2016). The latter being described as “capital breeding” as opposed to “income

breeding" referring to organisms that require ingesting resources at the time of growth or reproduction (Stephens et al. 2009). It is generally accepted that high quality food sources, characterized by essential nutrients as PUFA, are preferentially retained in lipid reserves (Koussoroplis et al. 2013). Long-chained SAFA, characteristic of t-OM, are believed to be less interesting than PUFA for growth or reproduction but much less is known regarding their role in cell metabolism.

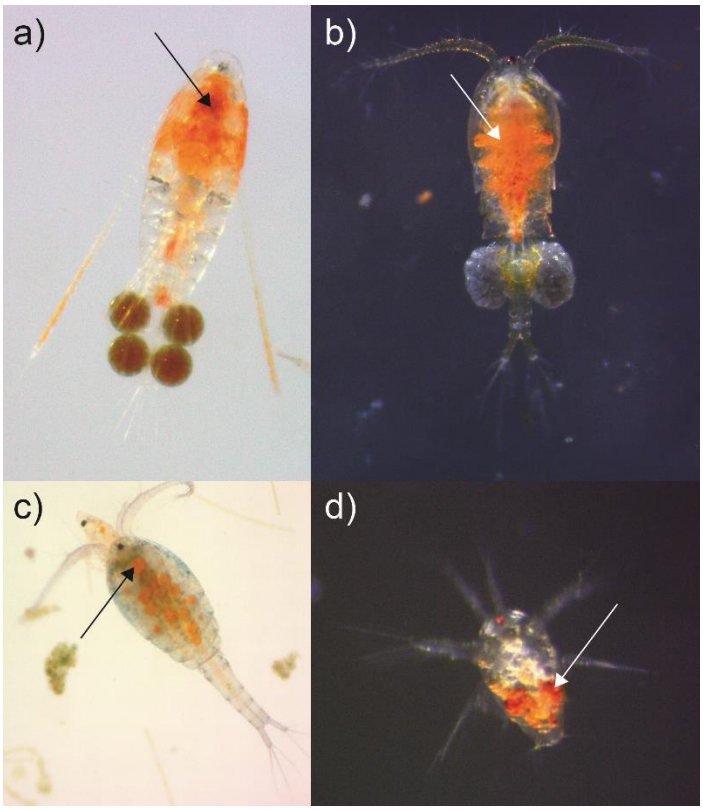

Figure 2 Lipid droplets containing fatty acid reserves of zooplankton (arrow) in a) *Leptodiaptomus minutus*, b) *Cyclops scutifer*, c) *Mesocyclops edax* and d) *cyclopoid larvae* (nauplius).

Upscaling the aquatic reliance on terrestrial environment at the lake ecosystem

Allochthony in aquatic organisms is usually presented as an indicator of the extent of t-OM support for aquatic food webs. However, the degree of allochthony in

zooplankton is usually presented without any quantification of zooplankton biomass, except for a few quantitative examples such as Berggren et al. (2015). This does not allow for estimating the extent of the t-OM influence in the zooplankton community and in the aquatic food web *a fortiori*. From the zooplanktonic community perspective, an estimation of zooplankton biomass from which the degree of allochthony may be calculated is essential to evaluate the role of t-OM in those communities. From the lake ecosystem perspective, assessing zooplankton production with allochthony degree is necessary to estimate whether zooplankton are the key link among C sources as usually depicted and whether zooplankton truly play an important role in the transfer of C within the aquatic food web.

Zooplankton production and biomass are often presented as being synonymous in studies of allochthony, thereby adding to the confusion. However, attempts have been made to clarify this issue (Kelly et al. 2014) with several very recent studies studying the link between allochthony in biomass and consumer production (Karlsson et al. 2012, Lau et al. 2014, Karlsson et al. 2015). In general, the conclusions tend to affirm that allochthony in consumer biomass is not favourable to high production including that of zooplankton production. Quantifying C fluxes is important to know the extent of the aquatic reliance on terrestrial ecosystems and to be able to predict those modifications on the catchment basin that will seriously affect the recipient aquatic ecosystems. Early studies regarding the terrestrial support of aquatic biomass were done from an ecosystem point of view. Indeed, one of the first clues of allochthonous support to aquatic food webs was revealed as aquatic respiration frequently exceeds phytoplankton production in unproductive lakes (Del Giorgio and Peters 1993, 1994). In these lakes, for heterotrophic organisms (bacteria, microzooplankton and macrozooplankton) respiration (R) was higher than primary production (P) implying that heterotroph organisms must have been subsidized by additional external carbon sources. Primary production was only higher than respiration in lakes with

chlorophyll concentrations greater than 17 $mg \cdot m^{-3}$ (Del Giorgio and Peters 1993). In the studied lakes, most of the aquatic respiration measured came from bacterial communities suggesting that heterotrophic processes can be of major importance for the entire food web. Moreover, oligotrophic lakes (total phosphorous < 15 µg L^{-1}) with DOC concentrations > 2.6 mg L^{-1} that represent over 75% of all northern temperate lakes host aquatic communities substantially supported by non phytoplanktonic C sources. This suggests that heterotrophy is likely to be the rule rather than the exception in temperate lakes (Del Giorgio et al. 1999).

Despite an increasing understanding of the t-OM significance in aquatic ecosystems, understand its role in aquatic food webs will require further research. While the spatial variability of allochthony in the landscape has been recently addressed, nothing is known about the spatial within lake variability. Similarly, the seasonal pattern of allochthony and t-OM integration in food webs is largely unknown as winter processes are always overlooked. To report these within lake variations are essential in the quantification of real fluxes of t-OM toward aquatic food webs. Absolute quantifications are still missing in the literature that reports only relative estimations. Furthermore, t-OM is potentially utilized by aquatic organisms for very different purposes as storage, growth, reproduction, respiration or metabolism. Different perspectives using several techniques as stable isotopes or trophic biomarkers allow studying these allocations. Much is thus needed in future research in order to better understand the role of t-OM in aquatic food webs.

Objectives and hypotheses

The overall aim of this thesis is to better understand the role of terrestrial organic matter in zooplankton. Through an extensive study of t-OM contribution to the zooplankton community within a boreal lake, both seasonally and spatially, a greater

understanding has been developed regarding different allocations of t-OM by zooplankton in biomass production or lipid reserves according to seasonally and spatially heterogeneous C inputs. Several specific objectives were defined to structure the project and investigate each aspect of the overall aim (Roman numerals refer to the thesis chapters):

- Assessment of the seasonal and spatial variations of allochthony in the zooplankton community (I, III).
- Identification of the driving factors of variability for zooplankton use of t-OM among the diet carbon sources i.e. phytoplankton, terrestrial organic matter, aquatic macrophytes, benthic algae (I, III).
- Evaluation of the role of t-OM in zooplankton survival during winter and its allocation in zooplankton lipid reserves (II).
- Quantification of t-OM allocation in zooplankton production contextualized in estimates of carbon source fluxes from the lake (I).

Based on the demonstrated link between zooplankton allochthony and the C source dominance in their environment, I hypothesize that the zooplankton allochthony follows the variation of C source availability both spatially and seasonally within a lake (I, III). Zooplankton allochthony is predicted to be low in spring and summer when phytoplankton production is high, but higher in winter when phytoplankton disappear from the lake's water column (I). The spatial pattern of zooplankton allochthony is expected to follow the heterogeneity of C sources with decreasing allochthony in proximity to macrophytes and increasing allochthony near tributary inflows (III). As it has been suggested that t-OM suppresses aquatic biomass production, I also hypothesize that seasonal pattern of zooplankton allochthony is inversely correlated to zooplankton production (I). Finally, as life history strategy influence zooplankton diet and dependence on food sources, I hypothesize that the life history strategies of zooplankton species determines the zooplankton use of t-OM

especially during winter. Zooplankton able to store lipid reserves is expected to accumulate phytoplanktonic FA molecules before winter and then feed on terrestrial or bacterial sources to survive, investing previously accumulated lipid reserves in reproduction during spring. Zooplankton unable to build lipid reserves are predicted to feed much more on t-OM or bacteria in winter when phytoplankton disappear, this being reflected in lipid biomass (structural components) and reserves (II).

Methodological approach and study site

To answer the research question of this project, two very innovative approaches were selected. The first approach consisted in combining two historically separated techniques—SI and FA analyses—to combine the complementary information that each method can provide for the same ecosystem. The second approach was to precisely quantify the amount of t-OM used by the zooplankton community at the ecosystem level as this has never has been done before in such detail. An intensive assessment of the terrestrial contributions to zooplankton in a natural lake ecosystem was thus carried out. To obtain high frequency seasonal and spatial data, two sampling protocols were planned to collect zooplankton individuals (Fig. 3) and establish the plausible driving factors (Fig. 4).

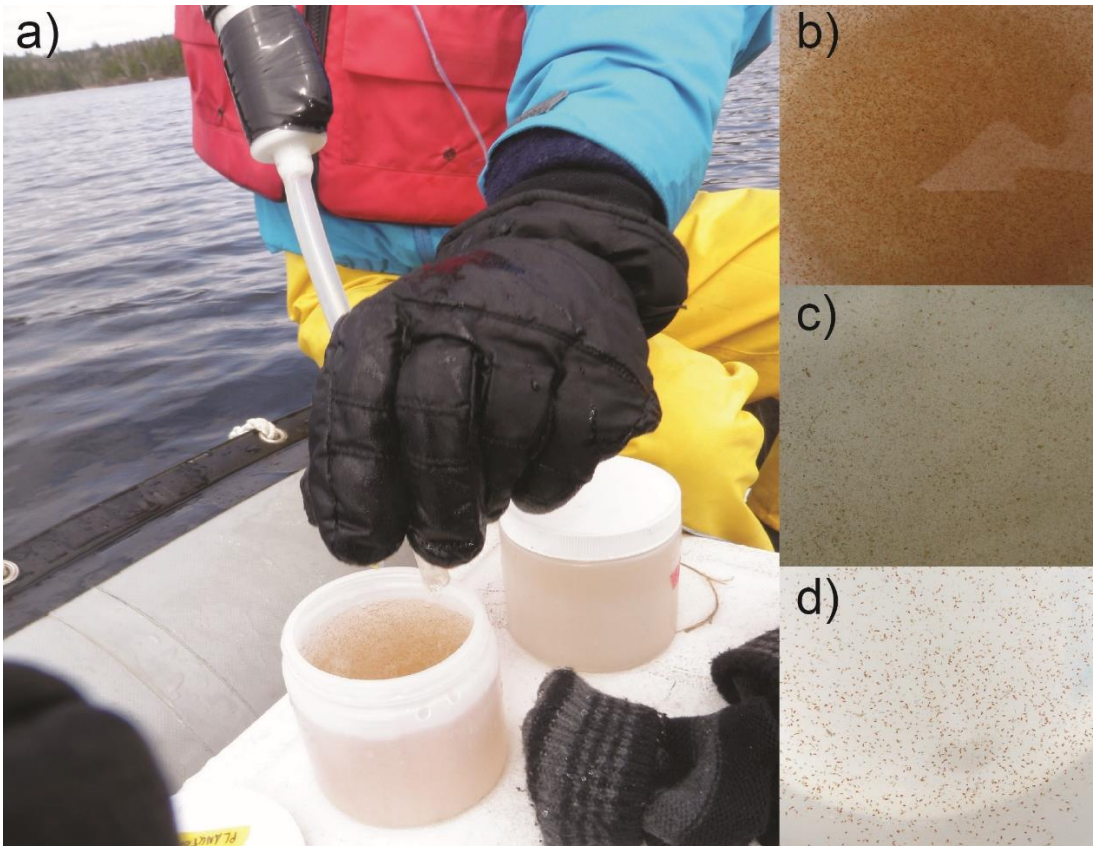

Figure 3 Zooplankton collected with a) a net of 50µm mesh (diameter: 25 cm, length: 70cm) and plastic containers in b) summer, c) spring and d) winter.

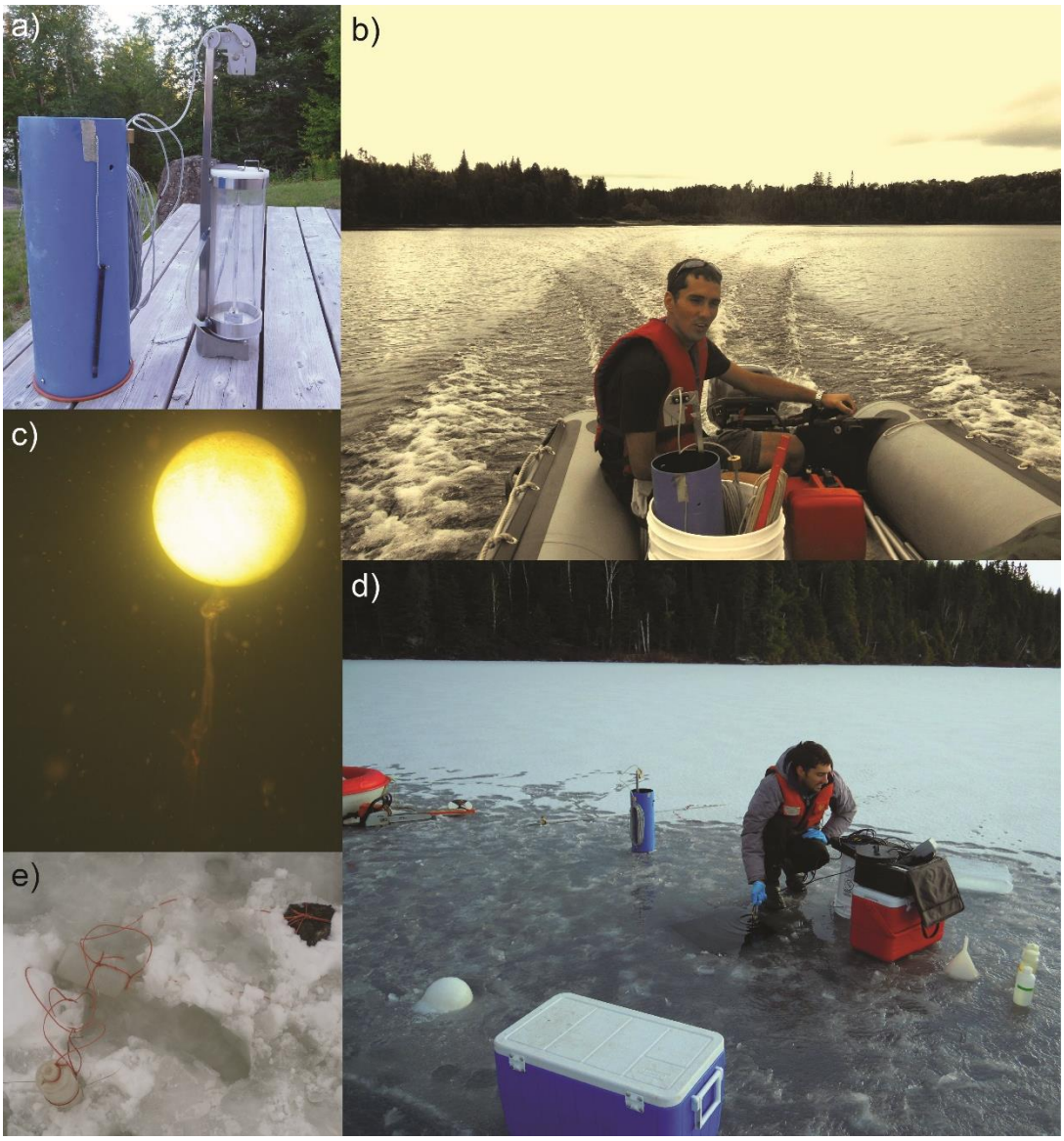

Figure 4 Sampling on Lake Simoncouche with a) a Limnos sampler, b) a zodiac, c) an installed underwater buoy having e) bottles allowing for benthic algae colonization and d) a YSI for physico-chemistry.

The first sampling series was carried out over an entire year covering a complete seasonal pattern including under the ice in winter (Fig. 5), an under-studied period of

the year in limnology (Hampton et al. 2015). The second sampling series covered the entire lake through ten sampling sites representing habitats dominated by different C sources such as phytoplankton, t-OM inputs, macrophytes (Fig. 6) and benthic algae to assess the within-lake spatial pattern of t-OM.

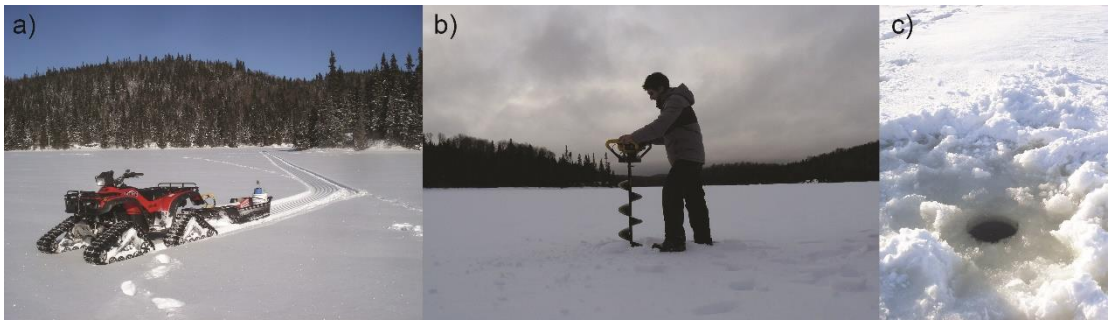

Figure 5 Sampling in winter on Lake Simoncouche: a) using an all-terrain vehicle and b), c) drilling a hole through the ice.

Chapters I and II focused on the most abundant taxa of the zooplankton community: *Leptodiaptomus minutus* (Lilljeborg 1889), *Cyclops scutifer* (Sars 1863), *Mesocyclops edax* (S.A. Forbes 1891) and *Daphnia* spp. (Fig. 7a,b,c,d) that were sampled to estimate the terrestrial contribution to the production and the lipid accumulation of each species. Allochthony was estimated based on the stable-isotope signature of consumers and the plausible sources. Zooplankton production was estimated according to cohort identification and growth rate calculations based on biomass variation over time and developmental time (Runge and Roff 2000, Hirst et al. 2003). Community production was calculated as precisely as possible with the addition of three additional estimates for cladocerans: *Bosmina* spp. (Fig. 7e), *Diaphanosoma* spp. and *Holopedium* sp. The seasonal variability of carbon sources was estimated using the high frequency pelagic gross primary production data from Dr. Dominic Vachon as well as with the incoming water flow. Lipid reserves were

characterized by fatty acid concentrations and were measured with highly precise gas chromatography.

Figure 6 Macrophyte habitats in Lake Simoncouche: a) littoral zone in summer dominated by b) *Brasenia schreberii* and c) *B. schreberii* and *Typha angustifolia*.

The high frequency spatial sampling in Chapter III was designed to collect zooplankton and study within-lake variability of zooplankton using ^{13}C and ^{2}H isotopes coupled with the very recent Bayesian mixing model approach (Wilkinson et al. 2014) to account for consumer and source variability.

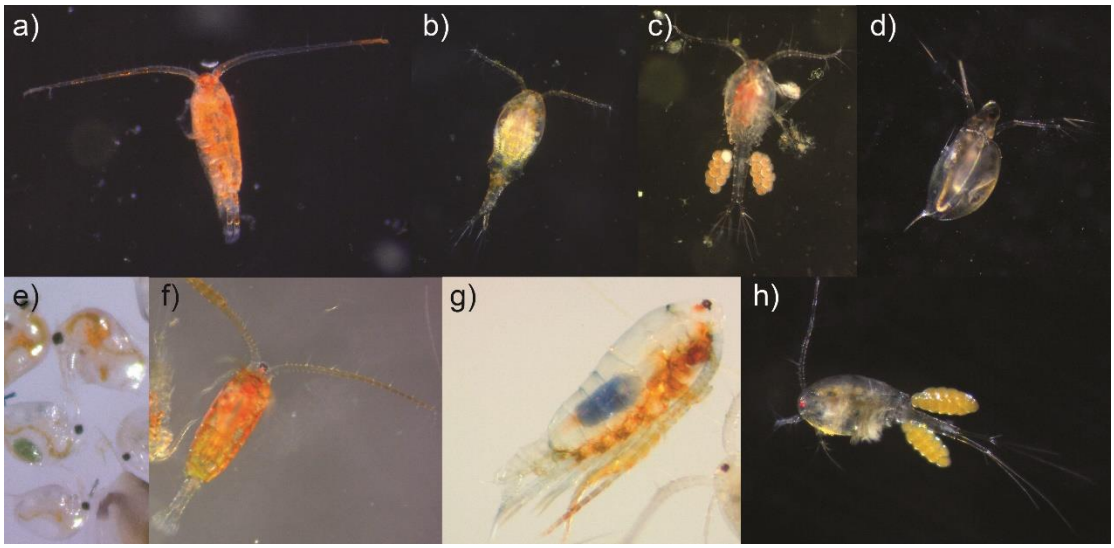

Figure 7 Representative taxa from the zooplankton community of Lake Simoncouche. Main taxa are represented by: a) *Leptodiaptomus minutus*, b) *Cyclops scutifer*, c) *Mesocyclops edax* (with eggs) and *Daphnia* sp. Examples of secondary taxa include: e) *Bosmina* spp., f) *Aglaodiaptomus spatulocrenatus*, g) *Epishura lacustris* and h) *Eucyclops speratus* (with eggs).

The study site is Lake Simoncouche (Fig. 8) from the Forêt d'Enseignement et de Recherche de Simoncouche (FERS) situated in the Laurentide Wildlife Reserve and is easily accessible from the University of Québec in Chicoutimi.

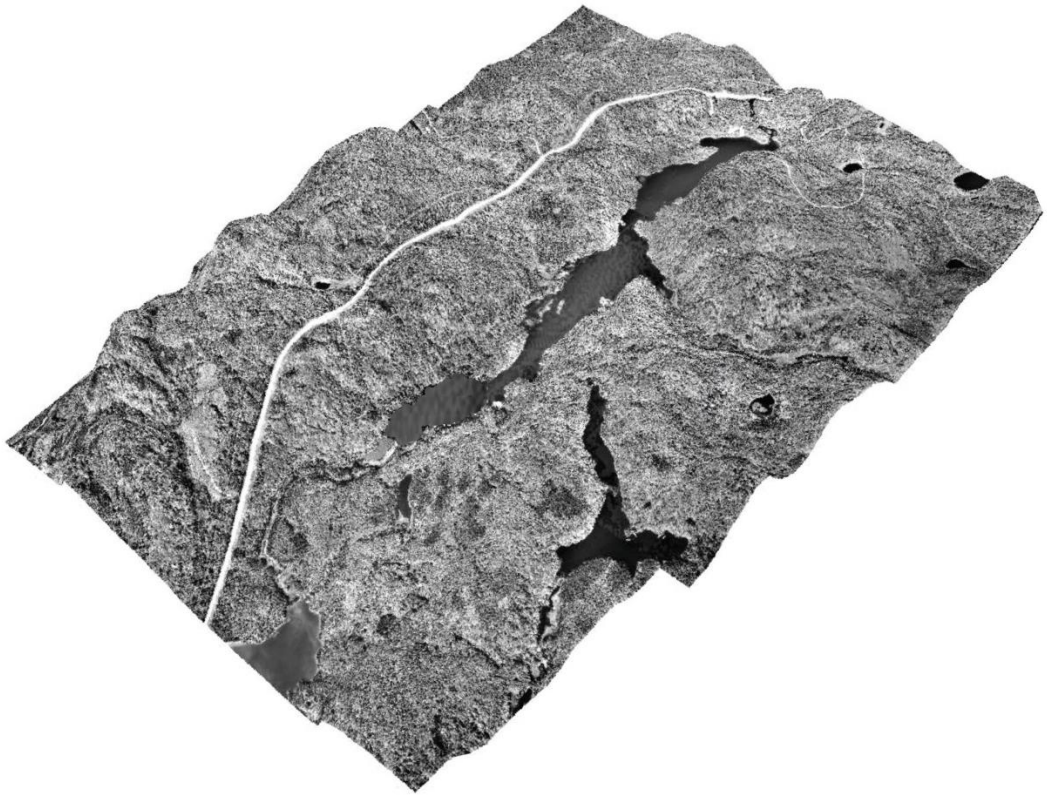

Figure 8 Illustration of a digital surface model developed from aerial photography (1987) and the altimetric curves of Lake Simoncouche and the associated catchment basin using ArcGIS® (M. Montoro-Girona, vegetal and animal laboratory, UQAC).

Lake Simoncouche is a shallow (maximum depth 8 m), mesotrophic and dimictic boreal lake undergoing a strong seasonality including ice cover from the end of November to around late April (Fig. 9). The zooplankton crustacean community is dominated by the previously cited taxa for zooplankton production with a more scarce presence of *Epishura lacustris* (S.A. Forbes 1882), *Aglaodiaptomus spatulocrenatus* (Pearse, 1906), *Mesocyclops leuckarti* (Claus, 1857), *Tropocyclops prasinus* (Fischer, 1860), *Eucyclops speratus* (Lilljeborg, 1901) as well as the invertebrate predators *Leptodora kindtii* (Focke, 1844) and *Chaoborus* sp.

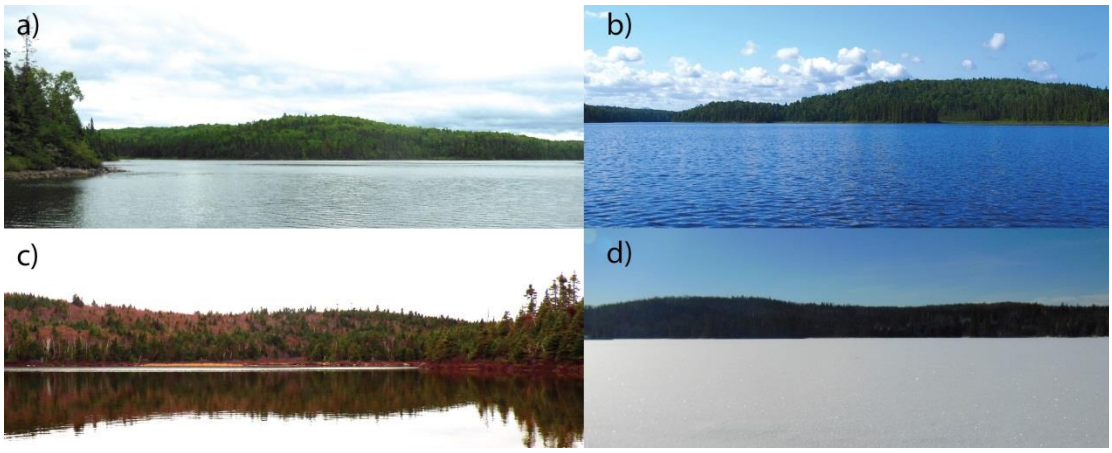

Figure 9 Seasonality of Lake Simoncouche: a) spring, b) summer, c) autumn and d) winter.

Thesis structure

To properly answer the objectives presented above, this project is organized into three chapters reporting the investigation of the specific t-OC use by zooplankton in a boreal aquatic food web.

The first chapter aims to address two main objectives of the thesis: 1) analyze the seasonal variation of allochthony in the main species of the zooplankton community and 2) upscale the t-OC use by zooplankton to the ecosystem scale. A monthly sampling protocol over an entire year (from May 2011 to May 2012) allowed for measuring the t-OC contribution within the zooplankton biomass using stable isotopes to identify when t-OC was most used by the different zooplankton species. Combining this data with some high frequency data from Dr. D. Vachon (see affiliations in chapter I) taken at the same period for the same lake, it was possible to link the seasonal zooplankton allochthony variation with the seasonal variation of C

source inputs in the lake (i.e. gross primary production (GPP) and terrestrial C inputs). Environmental and biological variables were measured with temperature and chlorophyll-a (Chl-a) as a proxy for phytoplankton biomass and the bacterial production (BP) representing the likely uptake of t-OC by the microbial food web. The second objective of this chapter intends to upscale the t-OC use by zooplankton to the entire lake ecosystem contextualizing this uptake within the lake C budget. A very detailed seasonal zooplankton production was calculated for each week (every two weeks during ice cover) to estimate C transfer from potential food sources to aquatic consumer biomass. Combining allochthony and zooplankton production data, we were able to calculate a new variable representing the t-OC contribution in zooplankton productivity rates that we named the "zooplankton allotrophy" measured in mgC m^{-2} d^{-1}. Conclusions were drawn from multiple linear regressions identifying the driving variables among C sources (GPP, terrestrial C inputs), environmental variables (water temperature) and food web variables (Chl-a, BP).

In Chapter II, a complementary view of allochthony based on stable isotopes was developed based on FA accumulation in lipid reserves of the same main zooplankton species as studied in Chapter I over the same period as well as within seston. Identification and quantification of FA in zooplankton and seston allowed the identification of FA biomarkers of terrestrial organic matter, phytoplankton and bacteria. These FA biomarkers revealed the seasonal variation of the availability of the potential sources of lipids for zooplankton and their accumulation in reserves for future utilization by zooplankton. There was a particular focus on the winter period as lipid reserves are known to permit species to survive periods of starvation. Some experimental data from a colleague, Dr. Heather Mariash (see affiliations in chapter II), were tested to determine if zooplankton required some food inputs from the environment or if lipid reserves at the moment of ice formation were enough for zooplankton to remain active during the entire ice covered period. This chapter

provided important observations about terrestrial, algal and bacterial FA allocation in zooplankton lipid reserves and the consequences for the life strategies of the main zooplankton species of a boreal lake.

Chapter III addresses the within-lake zooplankton spatial variability of zooplankton allochthony at two different seasons of the year based on the stable-isotope signature of the main species biomass *Leptodiaptomus minutus*. Sampling was performed during the growing season in spring when phytoplankton and benthic algae were in an exponential growth phase, macrophytes were growing, terrestrial inputs were high due to snow melting and runoff as well as in summer when phytoplankton, benthic algae and macrophyte productions as well as terrestrial inputs were lower. Analyzing this stable-isotope spatial variability with the main C source spatial heterogeneity of four different habitats (tributary inflow, macrophyte beds, pelagic and littoral zones representing respectively terrestrial, macrophyte, phytoplankton and benthic dominance), the natural spatial variability of allochthony was explained based on ecological factors.

The main conclusions of these three chapters are discussed in a final section that highlights and contextualizes the main contributions of the thesis and presents plausible avenues of future research.

CHAPTER I
SEASONAL VARIABILITY OF ZOOPLANKTON PRODUCTION SUPPORTED BY TERRESTRIAL ORGANIC MATTER AND DRIVING FACTORS

RESEARCH ARTICLE

MANUSCRIPT

Title: Terrestrial carbon supports zooplankton production in summer but not in winter in a boreal lake

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Running head: Seasonal zooplankton allotrophy

Key words: allochthony, allotrophy, seasonal pattern, zooplankton, stable isotopes

Abstract

Increasing evidence has demonstrated that zooplankton biomass displays a high share with terrestrial origin (i.e. allochthony) in many lakes. Consequently, although never tested, zooplankton production should also be supplemented by allochthonous terrestrial carbon (i.e. allotrophy). We carried out a detailed seasonal sampling in a medium-sized boreal lake, using stable isotopes and productivity estimates to calculate the degree of zooplankton allotrophy. The sampled lake is characterized with strong temporal patterns in carbon availability to zooplankton, including a period of several months of winter when primary production decreases to very low rates but terrestrial organic matter is abundantly available. Our main objectives were (1) to examine the seasonal changes in the overall zooplankton production and in the zooplankton allochthony to determine if these seasonal patterns were linked, and (2) to estimate zooplankton production supported by terrestrial carbon and relate it to seasonal changes in the potential carbon subsidies. We estimated the production and allochthony of the main zooplankton taxa in the lake (*Leptodiaptomus minutus*, *Cyclops scutifer*, *Mesocyclops edax*, *Daphnia* spp., *Bosmina* spp., *Diaphanosoma* spp, *Holopedium* spp.) along with primary production and terrestrial inputs for one complete year. Community allochthony ranged from 32% to 66% with high values of allochthony (~60%) in all seasons and not correlated with zooplankton production. High terrestrial carbon assimilation i.e. high allotrophy (up to 9 mgC m^{-2} d^{-1}) was found when total zooplankton production was high, stimulated by elevated temperatures and primary productivity in spring and summer or when terrestrial organic carbon inputs were the greatest during spring melt and after storms. The very low zooplankton production under the ice led to a minimum assimilation of terrestrial organic matter in winter (mean 0.7 mgC m^{-2} d^{-1}). These results challenge the common vision of maximum terrestrial carbon assimilated when primary production is low by highly allochthonous communities and rather support the importance of terrestrial carbon in supporting zooplankton and higher trophic levels in boreal inland waters during seasonal productivity peaks

1.1 Introduction

The boreal biome, the second largest terrestrial biome on Earth (Sun et al. 2008), is characterized by high freshwater densities and involves numerous land-water interactions (Lehner and Döll 2004). Freshwater networks are constituted by inter-connected lower points in the landscape and act as collectors of matter and energy fluxes from the drainage basin (Polis et al. 2004). These incoming fluxes including soil leaching (Haaland et al. 2010), nutrient inputs (Mattsson et al. 2005) or pollen harnessing (Masclaux et al. 2013) are materialized by terrestrial organic matter (OM) inputs and prevail over reverse fluxes from aquatic to terrestrial environment (Scharnweber et al. 2014b). This terrestrial OM is increasingly present in these inland waters due to a combination of changes in drainage basin vegetation, precipitation and recovery from industrial acidification (Monteith et al. 2007). In the past, terrestrial carbon was thought unimportant for zooplankton and has not been included in the calculations of energetic flux and material that contribute to the diet of primary and secondary consumers. The increasing evidence that a significant share of zooplankton biomass has a terrestrial origin (i.e. allochthony) (Emery et al. 2015, Mehner et al. 2015) now questions this view. However, as terrestrial OM lacks some essential constituents for growth, the high prevalence of terrestrial C in zooplankton tissues is not considered to promote consumer production but rather to yield organisms with reduced growth capacity (Brett et al. 2009). In this context, the increasing amounts of terrestrial OM in lakes may have crucial impacts to the productivity of lakes and to the sustainability of aquatic food webs.

The secondary production of zooplankton is a key process in aquatic food webs, as both the rate and nutritional quality of produced biomass largely determine the state of lakes and the ecosystem services they provide (Harris 2000). Zooplankton production has been known to link energy and material fluxes from primary

producing phytoplankton (Richardson et al. 2000) and benthic algae (Cazzanelli et al. 2012) to secondary producers such as ciliates, flagellates (Turner et al. 2001) and rotifers (Brandl 2005) to top fish predators (Castonguay et al. 2008). Via this ecosystem-scale flux network, zooplankton can apply a strong top-down pressure on aquatic primary producers (Talling 2003), reducing photosynthetic biomass. At the same time zooplankton also enhances growth of fish larvae (Betsill and Van Den Avyle 1997, Bunnell et al. 2003) and extreme events in low zooplankton abundance can be responsible for collapse of fish populations (Beaugrand et al. 2003) making the understanding of factors that influence zooplankton production of great interest (Karlsen et al. 2015). Zooplankton production which includes both the individual growth of organisms (somatic growth) and the egg production (reproduction) is hence a key to regulating essential processes in an ecosystem (Runge and Roff 2000).

The somatic and reproductive growth of zooplankton in pelagic environment is fueled by bottom-up factors that come from two distinct origins: autochthonous origin materialized by algal production (benthic and pelagic) and allochthonous origin constituted by terrestrial inputs of organic carbon in particulate (t-POC) or dissolved form (t-DOC), the latter being repackaged in biomass of lower trophic level organisms (ciliates, nano-flagellates). Autochthonous algal C sources are essential for zooplankton because they produce polyunsaturated fatty acid (PUFA) that zooplankton require for growth and reproduction but which they are unable to synthesize (Galloway et al. 2014, Taipale et al. 2015a). ω6- and ω3-PUFA produced by high quality phytoplankton are particularly important and preferentially assimilated by zooplankton whether they come from phytoplankton communities (Strandberg et al. 2015) or benthic mats (Mariash et al. 2014).

Allochthonous C in particulate form (t-POC inputs), coming from the drainage basin, on the contrary, is believed to be a poor substrate for zooplankton production, mainly

because it lacks PUFA (Taipale et al. 2014). However, increasing evidence has shown that secondary production of protozoa and metazoa is subsidized by terrestrial OM via the microbial pathway based on t-DOC (Berggren et al. 2010b). Low molecular weight molecules of terrestrial dissolved organic matter (t-DOM) are highly reactive and support a high degree of bacterial metabolism that can be transferred to higher trophic levels, including zooplankton (Guillemette et al. 2013). Recently, t-DOC has been shown to promote zooplankton growth and reproduction when algal C is limited (McMeans et al. 2015a). In fact, many zooplankton have been shown to be highly allochthonous (Emery et al. 2015, Karlsson et al. 2015) and several factors have been identified to influence allochthony e.g. water color (abs_{440nm}), ratio between water color and chlorophyll-a concentration, and POC composition (Carpenter et al. 2005, Batt et al. 2012). Further, different species consume terrestrial OM differently. Berggren et al. (2014) showed with a large scale study across temperate and boreal ecosystems that pathways leading to allochthony are different among the zooplankton communities with cyclopoid copepods characterized by a raptorial predation behaviour and more linked to the DOC pool of the lake, calanoid copepods more linked to the POC pool and cladocerans linked to both C pools.

Recent studies lead to two antagonistic views of terrestrial carbon assimilation in zooplankton. On one hand, terrestrial inputs are considered as an alternative food source when algal production is low (Berggren et al. 2015). In the multi-lake study of Berggren et al. (2014), calanoid allochthony is essentially linked to the phytoplankton dominance in seston rather than terrestrial availability, suggesting that calanoids use terrestrial C as an alternative food source to phytoplankton. On the other hand, evidence of consumer allochthony suppressing their production (Karlsson et al. 2015, Mehner et al. 2015) supports the idea that PUFA-containing algal diet is superior in promoting zooplankton growth. There is some speculation that in order for terrestrial OM to be efficiently available for zooplankton as a C source, the presence of algal C

is required *sensu* priming effect (Guenet et al. 2010) defending another view of t-OC assimilation. In support of priming effect, filter-feeding cladoceran (*Daphnia)* has been shown to be able to better grow and reproduce on terrestrial C if also algal C is available, highlighting a plausible interaction between sources in zooplankton growth and reproduction (McMeans et al. 2015a). Very little is known about if and when zooplankton uses terrestrial C for growth, reproduction or survival giving the contradictory information about beneficial versus disadvantageous effect of terrestrial OM on zooplankton production.

While the feeding link between phytoplankton and zooplankton has been extensively studied since a long time (Durbin and Durbin 1981, Banse 1994, Sommer 2012), feeding habits of zooplankton on components from terrestrial origin i.e. t-POC and microbial food web organisms subsidized by t-DOC raised some interest only recently (Brett et al. 2011, Mehner et al. 2015). Although it is currently well demonstrated that terrestrial OM is assimilated by zooplankton, there is no information how much it contributes to zooplankton growth. In general, zooplankton production is rarely estimated (Nakajima et al. 2014, Sastri et al. 2014) because of the technical challenges and fastidious methods. This study presents an estimation of zooplankton production of seven main taxa of the zooplankton community of a boreal lake along with allochthony calculations permitting a complete year estimation of production based on terrestrial OM i.e. allotrophy. We further measured in detail potential food sources and environmental variables to explore the regulating factors that limit or promote allotrophy. We first hypothesized that zooplankton production and allochthony would be inversely correlated reflecting lower energetic quality of terrestrial OM. It was further expected that the patterns of total zooplankton production and allotrophy are differently influenced by environmental or food source factors. While total production should follow seasonal temperature and primary production, allotrophy should be related to changes in the discharge from the

catchment and to moments when algal-based food is scarce typically during ice-covered seasons. As many zooplankton feed on what is available in the environment such as demonstrated by the $\delta^{13}C$ values of *Daphnia* following the $\delta^{13}C$ POM signature (Rautio et al. 2011), we can reasonably think that during ice-covered periods organisms will display the highest allotrophy. This study shows for the first time a detailed taxa-specific seasonal pattern of allotrophy for a complete year and emphasizes the close interaction between autochthonous and allochthonous sources highlighting the complexity of terrestrial carbon assimilation within a lake. It also underlines the critical role zooplankton play in the transfer of autochthonous and allochthonous compounds to higher trophic levels.

1.2 Methods

1.2.1 Study site

Lake Simoncouche (48°13'N, 71°14'W) is a medium (83 ha), mesotrophic shallow lake (mean depth: 2.2 m) in Quebec, Canada. The lake has been sampled for an entire year from May 2011 to May 2012 from the deepest point of the lake (maximum depth 8 m). The lake is dimictic and typically stratified in epilimnion and metalimnion at its deepest point (no hypolimnion). has one major southern inflow and one main opposite outflow. The drainage basin (2543 ha) is surrounded by boreal forest dominated by *Abies balsamea*, *Picea mariana* and *Betula papyrifera.*

1.2.2 Sampling and continuous measurements

Zooplankton production has been estimated with a weekly sampling of zooplankton community with a Limnos water sampler device (Limnos Oy, Turku, Finland).

Samples were collected from several depths covering the entire water column, and pooled together for production calculations. Zooplankton was collected by sieving the entire sample through a 50 µm net. Formaldehyde was added to the sample to a final concentration of 4% until counting and identification. Additional zooplankton individuals were sampled with tows of a 25 cm diameter net (50 µm) from the complete water column harvesting as much zooplankton as possible for stable-isotope analyses. Potential zooplankton food sources were sampled as well, by collecting terrestrial leaves and branches from litter on the shore all around the lake and in the bottom of the main inflows representing the most likely terrestrial inputs. Litter was selected over DOM as it represents 100% terrestrial material while DOM can be a mix of several sources. Seston samples were collected from the entire water column from several depths once a month, and pooled for one integrated water column sample. Bulk water was sampled in Nalgene bottles to be GF/F-filtered later in the laboratory for seston and chlorophyll-a analyses. Underwater sensor was installed at the depth of 2 m recording epilimnion temperature every three hours. Only epilimnion temperature was considered as about < 5% of the lake volume represents metalimnion. Underwater sensors measured hourly dissolved oxygen (O_2) concentrations 1 m below the surface (Vachon and del Giorgio 2014).

1.2.3 Zooplankton production

Counting and identification

Each individual of the zooplankton community sample was identified using Utermöhl chambers with an inverted microscope (Zeiss Axio Observer A1, x100), according to taxonomy guides from Edmondson (1959) and Czaika (1982). Every zooplankton samples (N_{tot} = 71) were entirely identified except when the density was too high. In such samples, half or quarter of the abundance was counted after dividing the sample with a Folsom's sample divider. A mean of 392 zooplankton individuals were

identified per sample (0.95 confidence limits = 14.8% ; Postel et al. (2000)) assuring a minimum of 100 individuals counted and identified, except for seven samples with very low densities where about 60 individuals have been counted. Nauplii were pooled into two groups according to stages 1 to 3 (NI-III) and stages 4-6 (NIV-VI), whereas copepodites were identified to six stages from C1 to C6. Cladocerans were identified to genus and newly hatched individuals were classified as juveniles. Eggs from all species were counted as well.

Length - dry weight regression

Mean dry weights (DW) for all species and stages were estimated with length-DW regressions. Individuals were measured with an optical camera (AxioCam ERC 5S) and microscope software (AxioVision). Identified species with length-DW equations used are shown in Table 1.1. Individual nauplii, copepodites and adult biomasses from copepod and cladoceran species were calculated from length-DW regressions and were compared to direct adult weight measurements. Weighing every stage of every species was impossible, but we verified the length-weight relationships with measurements for the adult stages of the four most abundant species of the community once a month. Adults were picked, counted (about 200 individuals), freeze-dried and directly weighed using a Mettler Toledo microbalance (XP26 DeltaRange).

Table 1.1 Length-dry weight equations and main species identified with high enough abundances to calculate zooplankton production. Individuals were classified as nauplii (Na), copepodites (Co) and Adult (Ad).

Species	Stage	Equation	Range (mm)	Source
Leptodiaptomus minutus	Na	lnW = 1.2461+2.2650±0.0722*lnL	0.13-0.30	Lawrence et al. 1987
Leptodiaptomus minutus	Co	ln W = 1.0783+2.7879±0.0875*lnL	0.38-1.12	Lawrence et al. 1987
Leptodiaptomus minutus	Ad	Direct weighing		
Cyclops scutifer	Na, Co, Ad	lnW = 1.9526+2.3990±0.0854*lnL	0.14-2.45	Bottrell et al. 1976
Mesocyclops edax	Na, Co, Ad	lnW = 1.9526+2.3990±0.0854*lnL	0.14-2.45	Bottrell et al. 1976
Bosmina spp.		lnW = 3.0896+3.0395±0.2123*lnL	0.28-0.95	Bottrell et al. 1976
Daphnia spp.		lnW = 1.4681+2.8292±0.0723*lnL	0.60-4.00	Bottrell et al. 1976
Diaphanosoma spp.		lnW = 1.6242+3.0468±0.3025*lnL	0.44-1.44	Bottrell et al. 1976
Holopedium spp.		lnW = 2.073+3.19*lnL		Watkins et al. 2011

Production calculations

Zooplankton production has been calculated from several methods using 1) cohort identification when reproduction was well defined in time or 2) population mean weight increment for continuously reproducing species. Typically copepod species showed some well identifiable cohorts while cladoceran reproduction was more diffuse in time. Copepod production P_{cop} $(mgC\ m^{-2}\ d^{-1})$ was calculated according to equation [1] (Runge and Roff 2000).

$$P_{cop} = \sum (g_i * B_i) + g_f * B_f \qquad [1]$$

$$g_f = \frac{E}{F * D_E} * \frac{W_E}{W_F} \qquad [2]$$

$$g_i = \frac{\ln W_i - \ln W_{i-1}}{D_i} \quad [3]$$

with g_f, the rate of female reproduction (d^{-1}), calculated following equation [2] (Hirst et al. 2003) with assumptions that age distribution of eggs is uniform and every egg is viable (egg ratio method), and B_f the female biomass ($mgC\ m^{-2}$). Equation [2] was calculated with E, the number of eggs (*E eggs* m^{-2}), F the number of female (*F females* m^{-2}) and D_E, the time of egg development (*days*). D_E has been calculated from the mean temperature of the water column and literature equations (*Cyclops scutifer* and *Mesocyclops edax*: Taube (1966); *Leptodiaptomus minutus*: McLaren (1966)). W_E and W_F are the mean egg and mean female weight respectively (mgC). g_i represents the growth rate of stage i (d^{-1}, equation [3]), B_i is the biomass of stage i ($mgC\ m^{-2}$). W_i and W_{i-1} are the mean individual weights of stage i and stage $i-1$, respectively (mgC). Also, once cohorts were identified, stage development times (*i.e.* stage duration; D_i) were calculated from the time spent between $T_{50\%(i)}$ and $T_{50\%(i-1)}$ with $T_{50\%(i)}$, the peak median of stage i estimated with 50% of the cohort biomass.

When species were continuously growing and did not show identifiable stages (typically cladoceran species), length measurements have been estimated for each sampling date to calculate the mean weight increment for the entire population and to identify main cohorts (see supplementary information S1). Cladoceran production P_{cla} ($mgC\ m^{-2}\ d^{-1}$) was then calculated using weekly mean weight increment with the equation [4] with g_s, the somatic growth rate ($mgC\ m^{-2}\ d^{-1}$) calculated with equation [5] and g_r, the reproductive rate (d^{-1}) calculated with equation [6].

$$P_{cla} = g_s * B_{tot} + g_r * B_{Ad} \quad [4]$$

$$g_s = \frac{\ln W_t - \ln W_{t-1}}{t - t_0} \quad [5]$$

$$g_r = \frac{E}{I * D} * \frac{W_e}{W_{Ad}} \quad [6]$$

with B_{tot}, the total biomass and B_{Ad}, the adult biomass ($mgC\ m^{-2}$). W_t, the mean individual weight (mgC) for the sampling date t and W_{t-1}, the mean individual weight for the previous sampling date (mgC). I is the abundance of individuals (ind m^{-2}), W_e is the mean egg weight and W_{Ad} is the mean individual adult weight at sampling date t (mgC). D has been calculated as previously for copepods (*Bosmina* spp.: Vijverberg (1980); *Daphnia* spp.: Hanazato and Yasuno (1985); *Diaphanosoma* spp.: Herzig (1984); *Holopedium* spp.: Popadin (2002)). As cladocerans reproduction periods are generally more diffuse along the year than for copepods, the total weight increment approach may under-estimate the growth production of individuals that hatched outside the main population reproduction, i.e. not following the global size increment. In this regard, this method is less precise than when cohort can be identified. However, a high number of measurements of individual cladoceran size [N = 31 (mean) per date] allows well representing the studied population and increase the precision. Biomass DW were converted to carbon content using 0.4 ratio and egg carbon content was calculated from the egg volume following Huntley and Lopez (1992).

1.2.4 Stable-isotope analyses and allochthony

Stable-isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) were carried out on the most abundant zooplankton species (*Cyclops scutifer*, *Mesocyclops edax*, *Leptodiaptomus minutus*, *Daphnia* spp.) of Lake Simoncouche community. Zooplankton individuals were kept

overnight in the fridge in GF/F-filtered lake water to empty their gut content. Three replicates of about 200 individuals of the most abundant species were then individually picked under a dissecting microscope Zeiss Discovery V12 and put into an eppendorf tube at -80°C for each sampling date before freeze-drying and lipid extraction. High lipid content depletes total consumer $\delta^{13}C$ and is seasonally highly variable (Syväranta and Rautio 2010): in the winter season, zooplankton can cope with low food availability by storing high quality nutrient constituents like lipids (Schneider et al. 2016). As we were interested in food source utilization and not storage, individuals were lipid-extracted following Mariash et al. (2011) modified method from Bligh and Dyer (1959) using chloroform/methanol (2:1 v/v) solvent. Individuals were then dried, weighed and analyzed for $\delta^{13}C$ and $\delta^{15}N$ signatures using a FlashEA 1112 element analyzer (Thermo Fisher Scientific Corporation, Whaltham, MA, USA) coupled to a Thermo Finnigan DELTA plus Advantage mass spectrometer.

An algebraic two-sources model was used to calculate zooplankton allochthony ($Allo_{cons}$) following equation [7]:

$$Allo_{cons} = \frac{(\delta^{13}C_{\text{cons}} - \delta^{13}C_{\text{enrich}} - \delta^{13}C_{\text{phyto}})}{\delta^{13}C_{\text{terr}} - \delta^{13}C_{\text{phyto}}} \quad [7]$$

Algebraic model was chosen over Bayesian model as the aim here is to estimate a precise and realistic value of zooplankton allochthony to be able to use it with zooplankton production. We are aware that uncertainties are not accounted with this model. Lipid fractionation between lipid $\delta^{13}C$ and phytoplankton cell $\delta^{13}C$ can vary, the trophic C isotope fractionation include uncertainties as well. However, algebraic model and Bayesian model output (modal) as SIAR are very well correlated (Berggren et al. 2014) confirming that results from this algebraic model reflect the

allochthony calculated by Bayesian mixing models. Zooplankton $\delta^{13}C$ samples ($\delta^{13}C_{cons}$) were analyzed for each sampling date. Terrestrial signal ($\delta^{13}C_{terr}$) showed no seasonal variation (mean: -27.4 ‰; $F_{(3,101)} = 2.04$, $p = 0.11$). As no technique exists to manually separate phytoplankton from terrestrial particles in seston, algae signal ($\delta^{13}C_{phyto}$) was estimated from algal-specific fatty acids (FA) that were recovered from bulk seston samples (Pace et al. 2007, Berggren et al. 2014). We focused on 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3 that are produced by algae (McLeod and Wing 2009, Barberá et al. 2011). Extracted FA methyl esters (FAMEs) were obtained using a methylation procedure and evaporated to dryness. Samples were then shipped to Memorial University of Newfoundland for $\delta^{13}C$ analysis using a gas chromatograph interfaced with an IRMS via a combustion interface. We assumed a lipid fractionation of 3.8 ‰, and all FA $\delta^{13}C$ values were adjusted accordingly (Berggren et al. 2014). $\delta^{13}C$ were different among fatty acids ($F_{(3,8)} = 11.62$, $p < 0.01$) making two groups of essential fatty acids (20:5ω3, 22:6ω3) and (18:3ω3, 18:4ω3). A mean of all dates and FA was included in the model to have a representative signal of algal $\delta^{13}C$ (-40.2 ± 3.3 ‰). Seasonal variability was negligible ($F_{(3,8)} = 1.89$, $p = 0.21$). $\delta^{13}C_{enrich}$ estimate the ^{13}C fractionation at a particular trophic position considering 0.4‰ ^{13}C fractionation per trophic level and estimating trophic level of each species with $\delta^{15}N$ and a ^{15}N fractionation of 3.4 ‰ (Post 2002) with equation [8].

$$\delta^{13}C_{enrich} = \frac{\delta^{15}N_{cons} - \delta^{15}N_{daphnia}}{3.4} + 1 \qquad [8]$$

$\delta^{13}C_{enrich}$ of zooplankton consumers from each sampling date was calculated in relation to $\delta^{15}N_{daphnia}$ considered as the trophic baseline ($\delta^{13}C_{enrich} = 1$; Karlsson et al. (2004)).

1.2.5 Zooplankton production based on terrestrial source i.e. allotrophy

To calculate the allotrophy, the zooplankton production was multiplied by the allochthony ratio. Specific allotrophy for *C. scutifer*, *M. edax*, *L. minutus* and *Daphnia* spp. was calculated with the species-specific production and allochthony. It was not possible to calculate allochthony for all dates for cladocerans *Bosmina* spp., *Diaphanosoma* spp. and *Holopedium* spp. due to the low prevalence of these cladocerans. To estimate their specific allotrophy, we applied the seasonal allochthony pattern of *Daphnia* spp. assuming that all cladocerans displayed similar allochthony degree.

1.2.6 Environmental and food web variables

Lake water inflow was measured from the main and secondary lake inflows (m^3 s^{-1}). Allochthonous C inputs in the lake were estimated from the lake water inflow multiplied with the water concentration of DOC from these two main inflows assuming that riverine DOC was entirely allochthonous. Phytoplankton biomass and production were estimated from chlorophyll-a and O_2 concentration, respectively. Chlorophyll-a concentration was measured by fluorescence following Yentsch and Menzel (1963b). Gross primary production (GPP) was calculated using continuous (hourly) measurements and diurnal changes of dissolved oxygen (O_2) concentrations in surface water as in Vachon and del Giorgio (2014). This method includes GPP measurement from phytoplankton, benthic algae and macrophytes. Bacterial production (BP) was measured following the [^{3}H]-leucine method incorporation of Kirchman (1993). Triplicate aliquots of 1.5 mL water samples were exposed to 40 nM [^{3}H]-leucine during 1 h. Average blank-corrected rates of leucine uptake were converted to rates of C production assuming the standard conversion factor of 1.55 kg

C mol leu^{-1} multiplied with an isotopic dilution factor of 2. Bacteria were incubated at the constant 20°C to exclude the effect of temperature to BP (Adams et al. 2010).

1.2.7 Statistical analyses

ANOVAs and post hoc tests (Tukey's HSD) have been performed using statistical computing environment of R (R Development Core Team 2015). Multiple linear regressions have been carried out on each zooplankton species production (total production and allochthonous production) in JMP v10 software in order to identify which environmental or food web variables were best explaining the seasonal variation of the zooplankton production. Variables were smoothed with centered moving average model (n = 3) removing the sampling variability and log transformed when needed (Table 1.2). Specific lags (Δ) have been identified by cross-correlations between explanatory and response variables for each multiple linear regression. However, as Δ increases, the zone of overlap of the two series shortens; a maximum of Δ = 10 (max: 1/4 of N_{tot} = 42) was set or according to the maximum life span of each species. Lagged and transformed variables were then applied to a stepwise multiple linear regression model selecting the best explicative model according to minimum AICc. To evaluate the probability of variables from the selected model to be included in the best explanatory model, additional coefficient of relative variable importance (RVI) has been calculated. RVIs were calculated as the sum of Akaike weights of all possible models including a given variable (Burnham and Anderson 2002). RVI allows estimating the importance of the tested variable considering every possible model from multiple linear regression analyses. Multiple linear regressions have been done including water temperature (Temp), gross primary production (GPP), chlorophyll-a (Chl-a), bacterial production (BP) and lake water inflow (Flow). If collinearity was detected in selected models i.e. if VIF (Variance Inflation Factor) > 10, correlated explanatory variables were removed from the model. As Temp and

Chl-a were highly correlated ($r = 0.84$, $p < 0.0001$), Chl-a was removed from the plausible explanatory variables. It is indeed very likely that Chl-a reflects more temperature than a potential food source as the link between Chl-a and phytoplankton biomass is controversial (Kruskopf and Flynn 2006). Moreover, GPP indicates a better estimation of autochthonous aquatic primary production (phytoplankton, benthic algae and macrophytes) than Chl-a as it measured a flux of organic matter production contrarily to Chl-a that includes standing biomass with low edibility. GPP was thus included in the multiple linear regressions (MLR) representing autochthonous C production. Further Pearson and Spearman pair-wise correlation coefficients (r) have been calculated in SigmaPlot v12.3 software.

1.3 Results

1.3.1 Total zooplankton production

Total production of zooplankton community reached a maximum of 16.3 mgC m^{-2} d^{-1} in late spring (mid-June) and was lowest (0.1 mgC m^{-2} d^{-1}) in mid-February with an annual mean of 4.6 mgC m^{-2} d^{-1} (Fig. 1.1). Three phases were identifiable ($F_{(2,39)} = 74.7$, $p < 0.001$): 1) April-September with mean ± SD of 6.0 ± 4.0 mgC m^{-2} d^{-1}, 2) October-December with 4.1 ± 3.5 mgC m^{-2} d^{-1}, and 3) January-March with 0.2 ± 0.2 mgC m^{-2} d^{-1}. Four zooplankton taxa (*Cyclops scutifer*, *Mesocyclops edax*, *Leptodiaptomus minutus* and *Daphnia* spp.) represented about 90% of the total annual zooplankton production. The cyclopoids *C. scutifer* and *M. edax* accounted on average for 15% (0.6 mgC m^{-2} d^{-1}) and 8% (0.3 mgC m^{-2} d^{-1}) of the production and reached 3.7 mgC m^{-2} d^{-1} in late May and 3.2 mgC m^{-2} d^{-1} in early July respectively (Fig. 1.2a-1.2b). The calanoid copepod *Leptodiaptomus minutus* contributed 27% to the production (mean 1.1 mgC m^{-2} d^{-1}) with a production maximum of 4.1 mgC m^{-2} d^{-1} in mid-June (Fig. 1.2c). Moreover, this copepod dominated the early spring (late

April) zooplankton production with a rate of 3.2 mgC m^{-2} d^{-1} that was 98% of the total spring production. The most productive taxa, *Daphnia* spp. made on average 39% (1.6 mgC m^{-2} d^{-1}) of total annual zooplankton production (Fig. 1.2d). Its maximum production occurred in late September accounting for 10.6 mgC m^{-2} d^{-1} that made 74% of total community production (28 Sept 2011). The cladoceran *Bosmina* spp. contributed little (0.2 mgC m^{-2} d^{-1}) to the total production (5%) except in mid-December when its production reached 2.8 mgC m^{-2} d^{-1} (Fig. 1.2e). The production of the two other cladoceran taxa (*Diaphanosoma* spp. and *Holopedium* spp.) represented 2% and 4% of the production accounting respectively on average 0.1 mgC m^{-2} d^{-1} and 0.2 mgC m^{-2} d^{-1} (Fig. 1.2f-g). Production of the cladocerans *Diaphanosoma* spp. peaked at 0.8 mgC m^{-2} d^{-1} in August (11%) and *Holopedium* spp. attained 1.2 mgC m^{-2} d^{-1} in mid-June (7%).

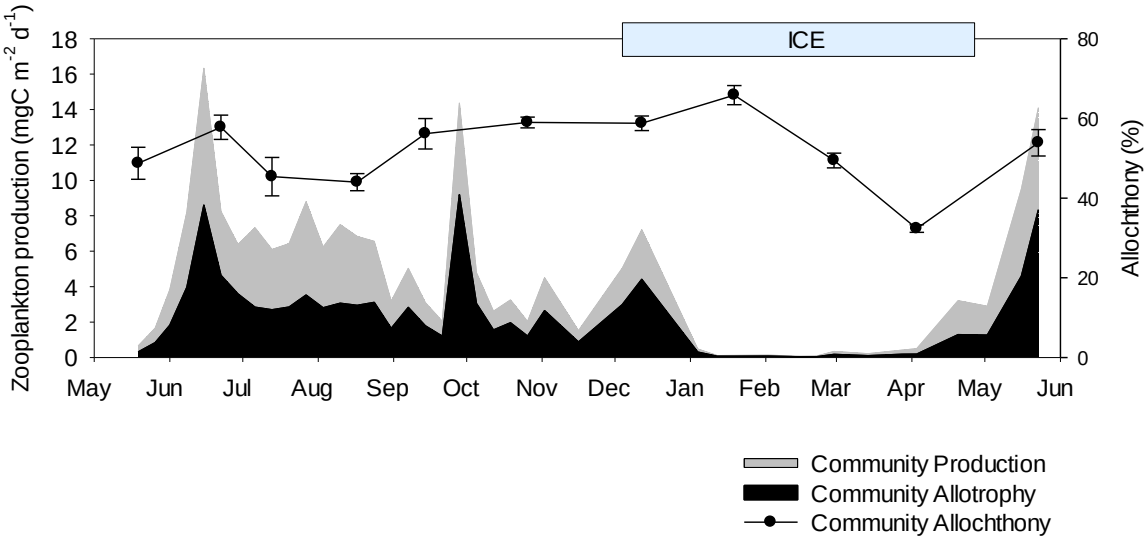

Figure 1.1 Seasonal pattern of zooplankton production (mgC m^{-2} d^{-1}) based on weekly values separating total production and allotrophy. Allochthony ratios are weighed means with species biomass (*C. scutifer*, *M. edax*, *L. minutus*, *Daphnia* spp.) ± weighed SD.

1.3.2 Stable isotopes and allochthony

Wide range of stable-isotope values was measured including ^{13}C enriched values (-26.7 ‰ in mid-May) to much more depleted values (-40.4 ‰) in mid-July, both estimated from *M. edax*. However, mean stable-isotope value for all consumers together (-33.1 ‰) was equidistant from phytoplankton (-40.2‰) and terrestrial values (-27.4‰) (Supporting information S2) providing a mean annual zooplankton allochthony degree of 49%. Additionally, 96% of stable-isotope values (N_{tot} = 77) fell between phytoplankton and terrestrial end-member values. Calculated allochthony for separate sampling dates ranged from 81% for *M. edax* in mid-May to 0% in mid-July, showing that annually the biggest variability was within the same species. No allochthony differences were found among species ($F_{(3,38)}$ = 1.8, p = 0.17) whereas

strong seasonal patterns were detected with significant differences among sampling dates ($F_{(10,38)}$ = 4.6, $p < 0.001$) and with species-date interaction ($F_{(25,38)}$ = 2.2, p = 0.015). Minimal mean annual allochthony 32 ± 1% (weighed mean with biomass ± weighed SD) was measured in early April while maximal values (66 ± 2%) were calculated in mid-January (Fig. 1). Mean allochthony degree of zooplankton community was highly negatively correlated to average GPP (r = -0.83, p <0.01). No direct correlation was detected between average community allochthony and zooplankton production (r = 0.12 p = 0.74) or allotrophy (r = 0.19, p = 0.58).

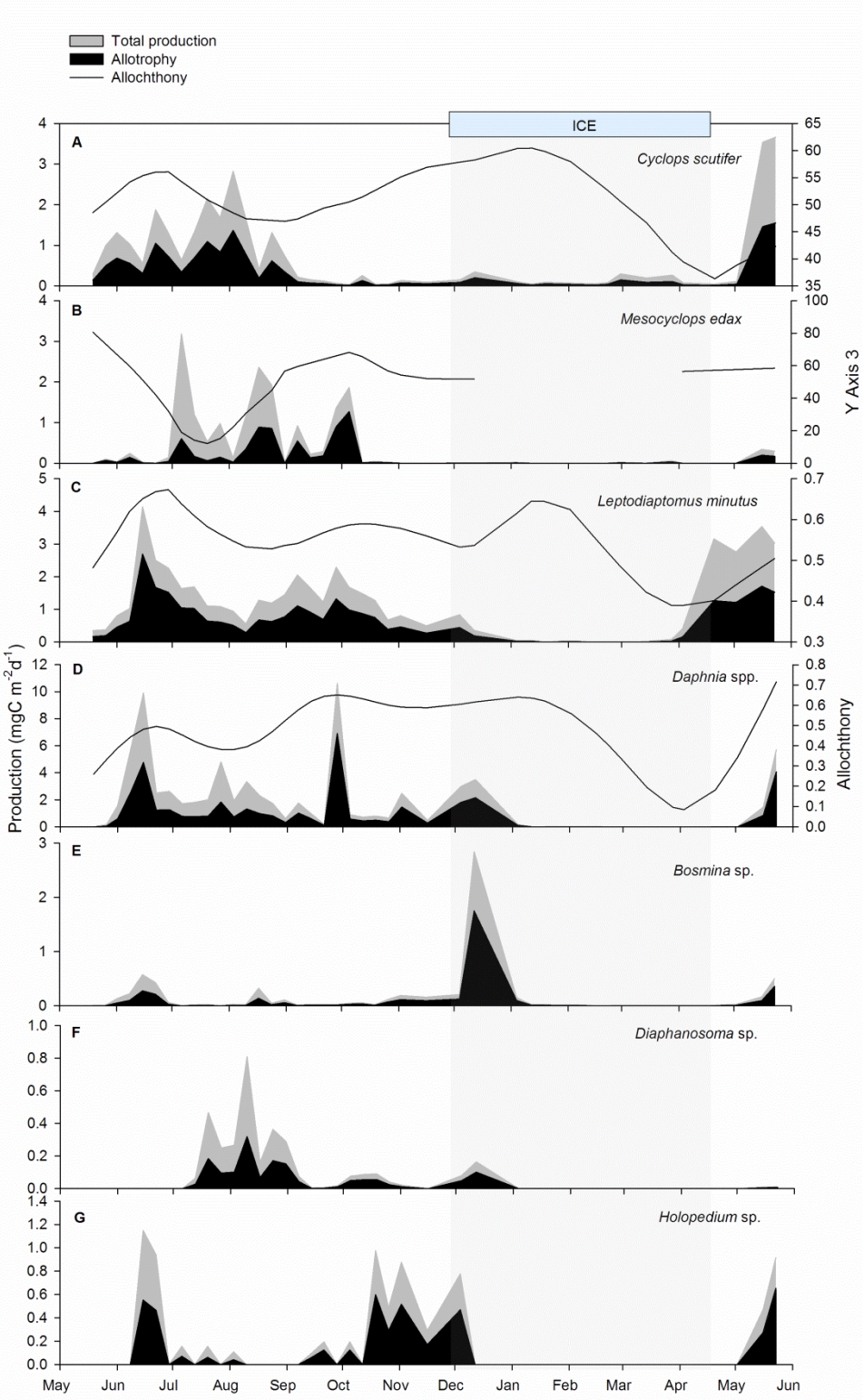
Total production
Allotrophy
Allochthony
ICE
A
Cyclops scutifer
B
Mesocyclops edax
C
Leptodiaptomus minutus
D
Daphnia spp.
E
Bosmina sp.
F
Diaphanosoma sp.
G
Holopedium sp.
Production (mgC m-2d-1)
Y Axis 3
Allochthony
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun

Figure 1.2 Seasonal pattern of zooplanktonic production for the main zooplankton taxa of Lake Simoncouche a) *C. scutifer*, b) *M. edax*, c) *L. minutus*, d) *Daphnia* spp., e) *Bosmina* spp., f) *Diaphanosoma* spp. and g) *Holopedium* spp. Notice the different scales. Black lines are median output from the algebraic model showing the seasonal pattern of allochthony.

1.3.3 Allotrophy

Total allotrophy over the complete year represented 52% of the total mean zooplankton production (annual mean 2.2 mgC m^{-2} d^{-1}), and reached a maximum of 9.2 mgC m^{-2} d^{-1} in autumn (September) when it made 64% of the total production (Fig. 1.1). Similarly to total zooplankton production, allotrophy varied seasonally that allows the identification of three periods significantly different ($F_{(2,39)}$ = 72.0, $p < 0.001$) that was characterized by high values in summer and low in winter. The relative importance of allotrophy followed a different pattern. Allotrophy was 56-64% of the total productivity between September and January while in spring under the ice and in July-August values were lower between 39-47%. The copepods contributed 49%, and cladocerans 51% to the annual mean zooplankton allotrophy. Four most productive taxa represented 90% of allotrophy (*C. scutifer*, *M. edax*, *L. minutus* and *Daphnia* spp.). The mean allotrophy of *C. scutifer* was 0.3 mgC m^{-2} d^{-1} accounting for 14% of community allotrophy. The maximal allotrophy of *C. scutifer* occurred at the time of maximal total production in late May when it was 1.6 mgC m^{-2} d^{-1} (Fig. 1.2a). *M. edax* showed a mean allotrophy of 0.1 mgC m^{-2} d^{-1} that made 6% to the total zooplankton allotrophy and a maximum of 1.3 mgC m^{-2} d^{-1} in early October (Fig. 1.2b). *L. minutus* had a mean annual allotrophy of 0.6 mgC m^{-2} d^{-1} representing on average 28% of total allotrophy and reached a maximal allotrophy of 2.7 mgC m^{-2} d^{-1} in mid-June (Fig. 1.2c). Of all the species, *Daphnia* spp. showed the highest mean allotrophy with 1.0 mgC m^{-2} d^{-1} that accounted on average for 40% of total allotrophy and reached a maximal of 6.9 mgC m^{-2} d^{-1} in late September. The

cladocerans *Bosmina* spp., *Diaphanosoma* spp. and *Holopedium* spp. represented 0.12 (6%), 0.03 (2%) and 0.10 (5%) mgC m^{-2} d^{-1} and a maximum of 1.8, 0.3 and 0.7 mgC m^{-2} d^{-1}, respectively.

1.3.4 Environmental and food web variables

Mean water temperature ranged from 2.8°C in winter to 23.3°C in summer and showed an average of 12.1°C (Fig. 1.3a). Water inflow in the lake showed a mean of about 28,000 m^3 d^{-1} and was relatively stable from mid-September to mid-March. Four high runoff peaks were detected in mid-August (maximum inflow: 61,364 m^3 d^{-1}), late August (159,852 m^3 d^{-1}), late March (107,554 m^3 d^{-1}) and mid-May (88,881 m^3 d^{-1}) (Fig. 1.3a). Maximal Chl-a was measured in late July (31 µg m^{-2}) whereas a minimal occurred in winter (early April: 1 µg m^{-2}; mean December – April: 3 µg m^{-2}). GPP increased sharply in mid-April to reach a maximum (8.1 gC m^{-2} d^{-1}) while ice was still on, it was still high in summer (from May to July) and then decrease slowly in autumn (September – December) to show minimal values in winter (mean January-February: 0.3 gC m^{-2} d^{-1}; Fig. 1.3b). Measured mean bacterial production was 58 mgC m^{-2} d^{-1}, it reached a punctual maximum in early December (573 mgC m^{-2} d^{-1}) and a minimum in late September (2 mgC m^{-2} d^{-1}). Biomass did not follow the same pattern and had a maximum in mid-August (678 mgC m^{-2}) and a minimum in mid-January (163 mgC m^{-2}; Fig. 1.3c). Calculated mean of bacterial biomass was 356 mgC m^{-2}.

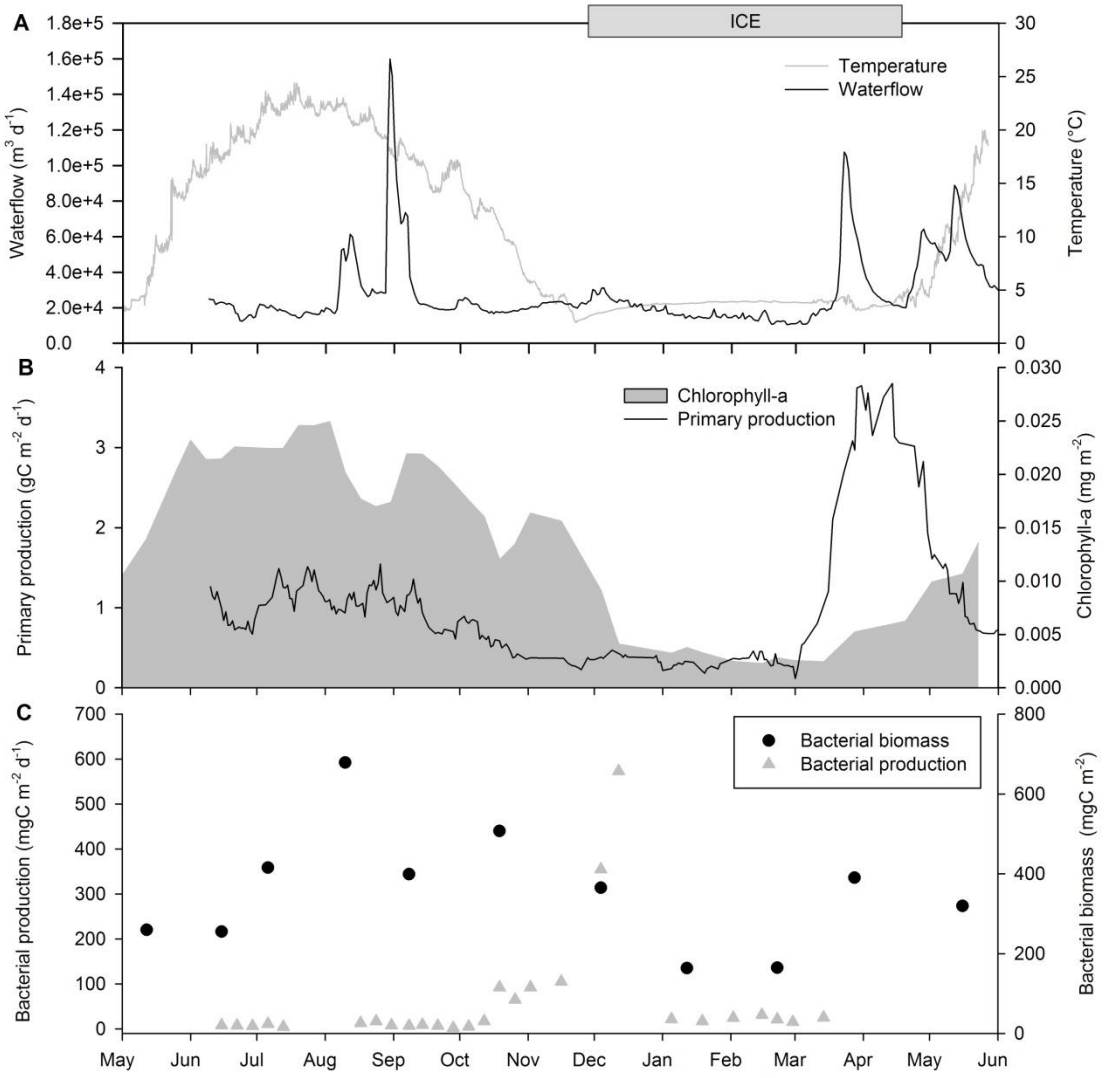

Figure 1.3 Seasonal variation of a) waterflow in the main inlet and epilimnion temperature (2 m), b) chlorophyll-a and primary production, and c) bacteria biomass and production.

1.3.5 Multiple linear regressions

In multiple linear regressions (MLR) analyses, environmental variables temperature (Temp) and water inflow (Flow), and food web variables gross primary production (GPP), chlorophyll-a (Chl-a) and bacterial production (BP) were selected as plausible explanatory variables for the variation in zooplankton production and allotrophy. Total community production was explained by the combination of GPP, BP and Temp (Table 1.2). The relative variable importance (RVI) coefficient of GPP was 1 and the community production correlation with GPP alone was highly significant ($r = 0.81$, $p < 0.001$). Temp and BP were selected in the best model as well, confirmed by the RVI coefficients of 0.88 and 0.93, respectively, while the non-selected Flow variable showed a RVI of 0.38. At the species level, GPP was identified as the only influencing variable for *C. scutifer* ($r = 0.67$, $p < 0.001$) and as the first influencing factor for *Holopedium* spp. (Table 1.2). For *M.* edax, *L. minutus, Daphnia* spp. and *Diaphanosoma* spp. Temp was in the majority of models selected as the first influencing factor. Correlations between Temp and the production of these species were all significant (respectively: $r = 0.90$, $p < 0.001$; $r = 0.59$, $p < 0.001$, $r = 0.68$, $p < 0.001$ and $r = 0.85$, $p < 0.001$). *Bosmina* spp. was the only species that showed BP as the main and only explanatory variable ($r = 0.65$, $p < 0.001$).

Total community allotrophy was explained by the combination of GPP, BP and Flow (Table 1.2) confirmed by the respective RVI coefficients (1.00, 0.99 and 0.95) while Temp was not selected (RVI = 0.45). A significant correlation ($r = 0.55$, $p < 0.001$) was calculated when accounting for a lag of 4 weeks between Flow and community allotrophy. BP was identified to be the first explanatory variable for the seasonal variation of the allotrophy of three species: *M. edax* ($r = 0.70$, $p < 0.001$), *Bosmina* spp. ($r = 0.61$, $p < 0.001$), *Holopedium* spp. ($r = 0.51$, $p = 0.007$). Allotrophy of *C. scutifer* and *L. minutus* were explained firstly by GPP while *Daphnia* spp. and

Diaphanosoma spp. were explained firstly by Temp. Among the selected explanatory variables, identified lags were similar (Table 1.2). Most of the time, Temp was accounted with no lag, GPP with a lag of 3 weeks, Flow with a lag of 4 weeks and BP with a lag of 7 weeks.

Table 1.2 Results of multiple linear regression models (based on lowest AICc) to estimate zooplankton a) total production and b) allotrophy. Temperature (Temp), bacteria production (BP), gross primary production (GPP), chlorophyll-a (Chl-a), and water inflow (Flow) were the variables used in the regression models. Variables are reported with the selected lag applied (Δ in weeks). Explanatory variables were log transformed.

a) Total production	**Regression equations from best model and selected variable(s)(Δ)**	**r^2**	**N**	**p**	**r^2_{adj}**	**RMSE**
Community	**2.0*GPP(3) – 0.5*BP(7) + 1.1*Temp(0) – 10.2**	**0.86**	**27**	**<0.001**	**0.84**	**1.11**
C. scutifer	1.1*GPP(3) – 8.7	0.44	36	<0.001	0.43	0.95
M. edax	0.5*Temp(0) – 0.6	0.77	42	<0.001	0.76	0.20
L. minutus	0.2*Temp(0) + 0.9*GPP(2) – 5.5	0.76	37	<0.001	0.75	0.45
Bosmina spp.	–0.1*BP(8) + 0.8	0.42	26	0.003	0.40	0.18
Daphnia spp.	0.8*Temp(0) – 0.3*BP(4) + 0.6*Flow(5) – 4.9	0.67	28	<0.001	0.64	0.68
Diaphanosoma spp.	0.02*Temp(4) – 0.08	0.72	38	<0.001	0.71	0.11
Holopedium spp.	0.2*GPP(4) + 0.4*Flow(9) – 0.1*Temp(2) – 4.5	0.64	30	<0.001	0.60	0.14
b) Allotrophy						
Community	**1.0*GPP(3) – 0.4*BP(7) + 0.9*Flow(4) – 12.5**	**0.84**	**27**	**<0.001**	**0.82**	**0.62**
C. scutifer	1.0*GPP(3) – 8.8	0.40	36	<0.001	0.39	0.95
M. edax	-0.08*BP(2) + 0.4*GPP(4) – 1.8	0.82	27	<0.001	0.80	0.13
L. minutus	0.3*GPP(3) – 0.09*BP(7) – 1.3	0.80	27	<0.001	0.78	0.18
Bosmina spp.	0.1*BP(8) – 0.1*GPP(0) + 0.2*Flow(4) – 0.2	0.63	26	<0.001	0.58	0.17
Daphnia spp.	0.6*Temp(0) – 0.5	0.49	27	<0.001	0.42	0.19
Diaphanosoma spp.	0.2*Temp(4) – 0.04*Flow(7) + 0.2	0.77	42	<0.001	0.71	0.07
Holopedium spp.	– 0.08*BP(7) + 0.2*Flow(4) +0.03*GPP(4) – 1.9	0.49	27	<0.001	0.42	0.19

1.4 Discussion

Our seasonal results from a boreal lake demonstrate that depending on seasons, terrestrial carbon supported 0.04-9.2 mgC m^{-2} d^{-1} of zooplankton production, which is equivalent to 39-64% of the total production. The amplitude and timing of terrestrial C assimilation by zooplankton were more dependent on the overall zooplankton production than on the allochthony of organisms. High allotrophy took place in spring and summer when the total production peaked and was highly positively correlated with the combined effect of photosynthesis and presence of fresh terrestrial OM (as flow rate). Zooplankton production, including allotrophy was negligible in winter and did not correlate with the allochthony degree of organisms, showing that terrestrial carbon is not an alternative source but rather a supplement to aquatic primary production for zooplankton. These results highlight the importance of terrestrial carbon in sustaining aquatic food webs but also emphasize that in order for the terrestrial carbon to supplement zooplankton (and higher trophic levels) in important quantities, environmental conditions need to be optimum for growth in terms of temperature and availability of higher quality algal diet.

1.4.1 Estimation of zooplankton production and influencing factors

Estimations of zooplankton community production followed the expected seasonal pattern as greatest rates occurred in spring and summer. They were mainly promoted by higher temperatures (Stockwell and Johannsson 1997, Sastri et al. 2014), and primary and bacterial production (Berggren et al. 2015) as was confirmed by the MLR results. Earlier studies have also described a positive log relationship between temperature and zooplankton growth rates (Huntley and Lopez 1992, Shuter and Ing 1997). While temperature was the main driving factor for the majority of zooplankton production, total community production as well as the species-specific productions of *C. scutifer*, *L. minutus* and *Holopedium* spp. were highly correlated with GPP, a more

accurate indicator of primary production than Chl-a and a known variable in fueling zooplankton production (Huot et al. 2007, Berggren et al. 2014, Galloway et al. 2014). While the GPP estimations were relatively high (mean: 949 mgC m^{-2} d^{-1}) for a mesotrophic boreal lake compared to estimations in the USA (GPP_{tot} = 410 – 551 mg C m^{-2} d^{-1} in 3 different lakes) by Cole et al. (2006) or in Norway where Bϕrsheim et al. (1988) measured an average primary production of 222 mgC m^{-2} d^{-1}, the measured seasonal pattern is typical for a boreal lake (Wetzel 2001). The high absolute values result from the high share of benthic primary production (Grosbois et al. 2017) in this shallow lake where light penetrates to the bottom (Schneider et al. 2016). Benthic production was included in the GPP estimations based on daily variation of oxygen concentration in water accounting for the total primary production (i.e. benthic and pelagic). The primary production available for the pelagic zooplankton consumption should then be lower than our estimated GPP. However, as phytoplankton and benthic algae photosynthesis are regulated with similar nutrient and light parameters, both productions should follow a very similar seasonal pattern. As multiple linear regressions are mostly accounting for seasonal patterns of the explanatory variables, phytoplanktonic production should be well represented in our calculated seasonal pattern. Macrophytes should also participate to the oxygen variation in water, however, we show in this thesis (Chapter 3) that macrophyte have only a very small influence on the deepest point of the lake where GPP has been measured. Community production as well as the specific productions of the cladocerans *Daphnia* spp. and *Bosmina* spp were negatively influenced by BP. Seasonal pattern of BP showed an increase in mid-October synchronized with GPP decline. As phytoplankton extracellular carbon release is linked to phytoplankton cell mortality and heterotrophic bacteria are known to feed on these carbon releases (Lasternas and Agustí 2014), BP peak is likely linked to primary producer death. More than a direct link between BP and zooplankton productions, the decline of GPP probably influenced the increase of bacteria production and the decline of zooplankton production. Also, a lag of 7 or 8 weeks between BP and zooplankton production was

included in the best models, this is long when we know that bacteria biomass turnover is from single days to weeks (Billen et al. 1990). A possible explanation is that bacteria provides molecules in early stages of zooplankton as copepod nauplii filter and collect particles of bacterial size range that will negatively influence zooplankton growth and abundances in the following developmental stages as in growth of larval fish that determines fishery recruitment (Jenkins and King 2006).

To calculate zooplankton production using cohort analyses is a time and energy consuming method. Furthermore, no consensus has been made about a standard method for zooplankton production calculations (Sastri 2014), and other publications of new methods (Hirst et al. 2003) have made the review from Runge and Roff (2000) obsolete. When species life cycle allows a clear cohort identification, the stage and egg development time are easily defined, as was here for *C. scutifer*, *M. edax* and *L. minutus*, allowing zooplankton production calculations based on field abundances that give more reliable results than methods from lab estimations (Jiménez-Melero et al. 2013). However, when species are continuously reproducing such as *Daphnia* spp. in Lake Simoncouche for most of the year, development times must be based on literature, as well as on the production/biomass ratio (Johannsson et al. 2000), or deduced from temperature and body size (Geller 1987). All these methods apply coefficients estimated from other systems and have drawbacks and strong probability of biases (Runge and Roff 2000). We estimated cladoceran productions based on the individual size evolution through time ([eq 5], Fig. S1). This method improved estimation reliability as it allowed production calculations for continuously reproducing species that were largely based on field data. To our knowledge, this is the first time that detailed seasonal zooplankton production has been calculated for multiple species of the same community. Previous community production estimations from a geographically nearby lake, based on an enzymatic method (6.50 – 28.88 mgC m^{-2} d^{-1} ; Sastri et al. (2013)) were similar to our community estimations (0.07 – 16.34

mgC m^{-2} d^{-1}). Lower zooplankton productivity in Lake Simoncouche is due to winter values that were not included in Sastri et al. (2013). In general, the total zooplankton production in Lake Simoncouche was high in spring and summer, lower in autumn and absent in winter. This pattern, although never investigated in detail, is well recognized for temperate and boreal lakes (Wetzel 2001). Collectively, the similar range and seasonal pattern with earlier studies suggest that the precision in our productivity estimations is accurate.

1.4.2 Seasonal pattern of zooplankton allochthony

A two source algebraic model was utilized to estimate zooplankton allochthony with terrestrial and phytoplankton as potential food sources, with stable-isotope values that were well separated (Fig. S2). Macrophyte and benthic algae values were not included in the zooplankton allochthony calculations due to their little contribution in organisms in the pelagic environment (Chapter III published as Grosbois et al. 2017). The allochthony ratio measured in the four species studied ranged from 0 to 81% showing large seasonal variability. While generally the same tendencies were found for all species (Fig. 1.3, Fig S2), the different feeding strategies among cladocerans, calanoids and copepods regarding to terrestrial C assimilation may result in different allochthony (Berggren et al. 2014). The calanoid *L. minutus*, cyclopoid *C. scutifer* and the cladocerans showed similar means (respectively 54%, 51% and 47%) and seasonal patterns, yet *M. edax* showed more variability. *M. edax* is known to be a predator (Brandl and Fernando 1979) and thus the tissues are dependent of prey diet and availability. We can hypothesize that the allochthony of *M. edax* followed its prey allochthony and changes in the prey species composition that led to different seasonal variability in allochthony than in the other zooplankton that were suspension feeders.

Zooplankton allochthony was not found to be correlated with zooplankton production nor allotrophy, however some co-occurring patterns can be highlighted in the seasonal variation between allochthony and production (Fig. 1.1). In summer, high productivity values (average 6.5 mgC m^{-2} d^{-1}) from mid-June to September were synchronized with lower allochthony degrees (45%) while the highest allochthony degrees (60%) from mid-September to mid-January were synchronized with low production (average 1.86 mgC m^{-2} d^{-1}). This early winter pattern goes along with recent studies showing that allochthonous zooplankton is less productive (Karlsson et al. 2015, Mehner et al. 2015). Allochthony began to decrease at a high rate in early March when the zooplankton production was null and weeks before the increase of primary production. We propose that zooplankton, that had been lipid-extracted for the stable-isotope analyses, began to use in late winter the previously stored lipids and that these lipid signatures showed up in the zooplankton biomass in the end of the ice cover decreasing the allochthony degree to minimum. The shift in allochthony corresponds with the timing of allocation of lipid storages to the production of spermatophores and gametes in the dominant copepod of the lake, *L. minutus* whose reproduction peaked several weeks later mostly characterized by egg production (Schneider et al. 2016). *L. minutus* is a "capital breeder" meaning that lipid reserves accumulated during the autumn are transferred to eggs production in spring. The accumulated lipids in zooplankton are known to be made of highly ^{13}C depleted algal fatty acids that can be used for zooplankton growth and reproduction (Halvorsen 2015). The low allochthony values in late spring under the ice are additionally influenced by the phytoplankton spring bloom. It is therefore very likely that lipid retention and GPP had strong complementary role in the allochthony degree of zooplankton community in winter.

1.4.3 Zooplankton allotrophy and terrestrial C inputs

Allotrophy is a function of allochthony and zooplankton production, and while high allochthony should increase the allotrophy, a more important precursor to high rate of terrestrial OM assimilation was the high general zooplankton production. Fresh and labile terrestrial OM carried by large volume of inflow water can be rapidly assimilated (Berggren et al. 2010b), increasing allochthony degree. On the opposite, increased terrestrial OM can also inhibit biomass production, as it has been shown for *Daphnia* (Brett et al. 2009) and top consumers (Karlsson et al. 2015). This changes the paradigm that terrestrial C would be more assimilated in allochthonous populations. On the contrary, terrestrial C was more assimilated when populations were highly productive rather than highly allochthonous. Terrestrial inputs are composed with detritus and dissolved molecules including high molecular weight humic substances. Breakdown of these recalcitrant molecules may be facilitated by co-metabolisms using labile carbon from autochthonous origin. This interaction between degradation pathways of recalcitrant and labile carbon, the priming effect, was suggested as a possible mechanism in pelagic environments (Dorado-García et al. 2016). The high availability of labile autochthonous carbon during spring and summer from high GPP suggests that conditions were met to promote terrestrial organic carbon degradation by microbial communities and subsequent assimilation at higher trophic level highlighting a possible priming effect as described in Guenet et al. (2010). On the contrary, our results do not support t-OM being an alternative to phytoplankton like for calanoid copepods in Berggren et al. (2015) nor the negative effect of allochthony on biomass production in Karlsson et al. (2015). Correlation between both variables was not significant ($r = 0.21$, $p = 0.54$) probably because production was primarily influenced by GPP and temperature hiding any possible effect of the allochthony. In Lake Simoncouche, zooplankton biomass that showed lower allochthony degree (40%) accumulated higher amount of terrestrial C (4.9 mg

C m^{-2} d^{-1}) than higher allochthony degree (60 %) that allowed only 3.2 mg C m^{-2} d^{-1} to be assimilated.

Allotrophy is dependent on the inflow of terrestrial carbon to the lake. Here we considered water inflow as a proxy for terrestrial organic matter (OM) inputs. As precipitation would have been an indicator of the terrestrial soil leaching, the correlation with terrestrial OM might be very poor due to soil water retention. Water inflow associated with terrestrial C inputs was positively correlated to higher allotrophy ($r = 0.55$, $p < 0.001$) when accounting for a delay of 4 weeks. Berggren et al. (2010b) demonstrated that fresh labile terrestrial molecules or photodegraded aromatic molecules were substantially assimilated by secondary production, and Grosbois et al. (2017) recently proposed that in Lake Simoncouche there is a lag of several weeks before terrestrial C from DOC is converted to zooplankton biomass. Supporting these studies we demonstrated here that an important share of terrestrial OM is transferred towards higher trophic levels. According to our bacterial production estimations (Fig. 3c) and the high bacteria allochthony degree (76%) calculated by Guillemette et al. (2015), terrestrial C assimilated in the microbial biomass would be on average 44 mg C m^{-2} d^{-1} in Lake Simoncouche. Assuming zooplankton gets the terrestrial C via microbial loop and by feeding on bacteria, zooplankton allotrophy in the lake would represent 7% (3.2 mg C m^{-2} d^{-1}) of the terrestrial C assimilated by bacteria. This demonstrates that it is very likely that from the large pool of bacteria-bound terrestrial C, an important portion was assimilated by zooplankton. Additionally, recalculating zooplankton, C source and bacterial production estimates per month in an annual basis (Fig. 1.4) shows that bacterial production estimation range can supply the zooplankton allotrophy in each month of the year. Zooplankton production estimations are very close as well as previous estimates from northern Sweden lakes of 6.1 mg C m^{-2} d^{-1} and allotrophy of 2.3 mg C m^{-2} d^{-1} (Berggren et al. 2010b).

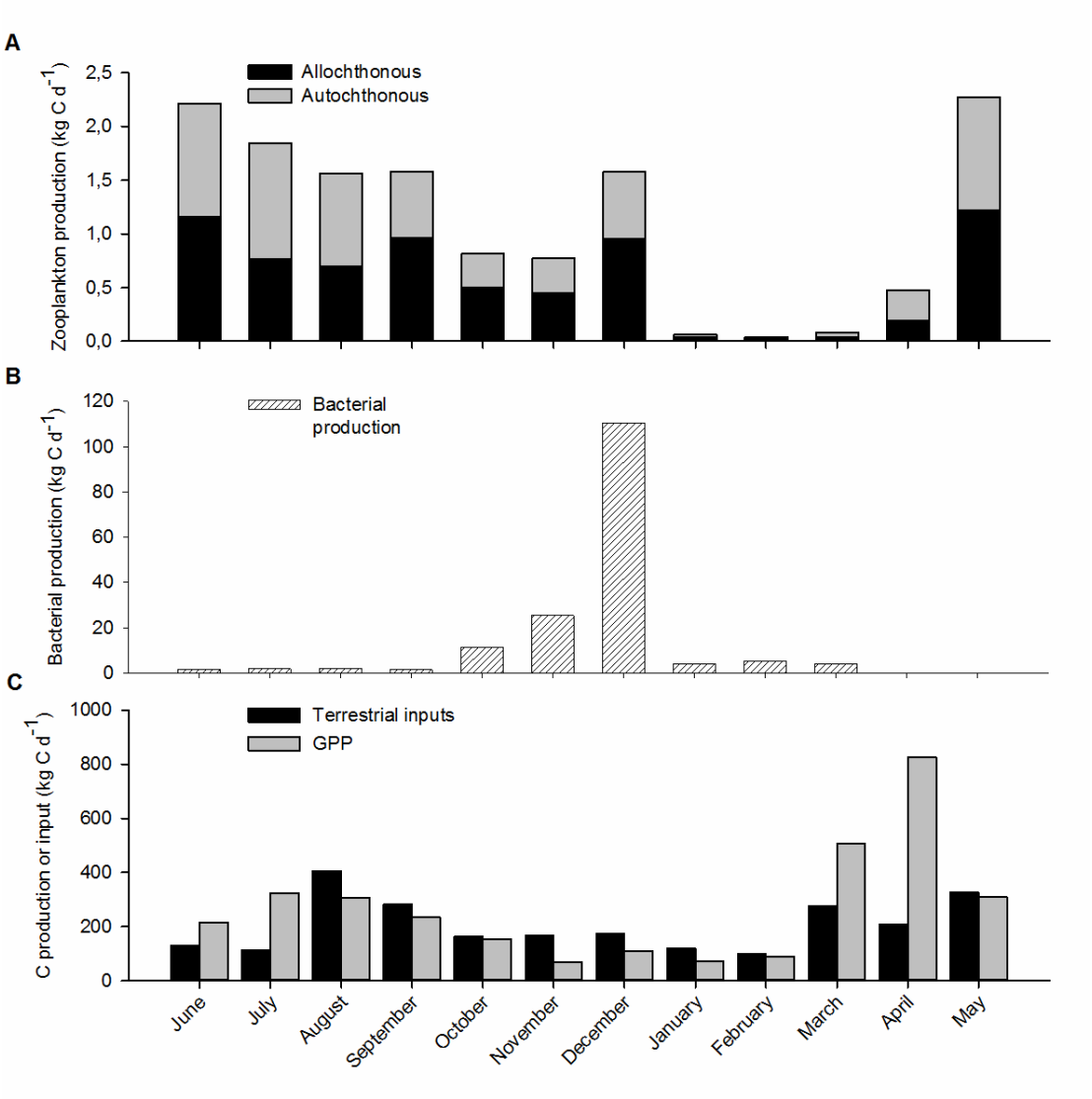
A
Zooplankton production (kg C d-1)
2,5
2,0
1,5
1,0
0,5
0,0
Allochthonous
Autochthonous
B
Bacterial production (kg C d-1)
120
100
80
60
40
20
0
Bacterial production
C
C production or input (kg C d-1)
1000
800
600
400
200
0
Terrestrial inputs
GPP
June
July
August
September
October
November
December
January
February
March
April
May

Figure 1.4 Seasonal pattern of carbon fluxes in Lake Simoncouche food web throughout an entire year with a) production (kg C d^{-1}; cumulated bar) of zooplankton community shared in zooplankton production supported with allochthonous carbon sources i.e. allotrophy (closed bar) and zooplankton production supported with autochthonous carbon sources (grey bar), b) bacterial production (kg C d^{-1}; striped bar) and c) C source inputs as terrestrial inputs calculated from t-DOC and t-POC concentrations multiplied by the waterflow of main tributaries (closed bar; kg C d^{-1}) and gross primary production (GPP) calculated from O_2 concentrations in water (grey bar; kg C d^{-1}).

This study showed in detail the proportion of zooplankton production fueled by terrestrial OM for a complete year in a boreal lake, and demonstrated that periods of substantial terrestrial OM assimilation for zooplankton growth were more linked to high zooplankton production than high allochthony degree. This challenges the binary vision of poorly productive zooplankton communities growing on allochthonous inputs and hyper productive communities fueled by high quality phytoplankton. Rather, a significant contribution of terrestrial OM for growth was observed as long as algal diet was present and temperatures elevated. In such optimal conditions for overall secondary production, fresh inputs of terrestrial OM further increased the prevalence of allotrophy without decreasing the total zooplankton production. Also, the zero growth in winter when terrestrial OM was in excess and algal food absent, evidenced that terrestrial OM alone was not able to sustain zooplankton production. Our observations suggest that increasing rates of terrestrial OM would not affect the aquatic secondary production in boreal inland waters as long as a high quality primary production is maintained.

1.5 Acknowledgements

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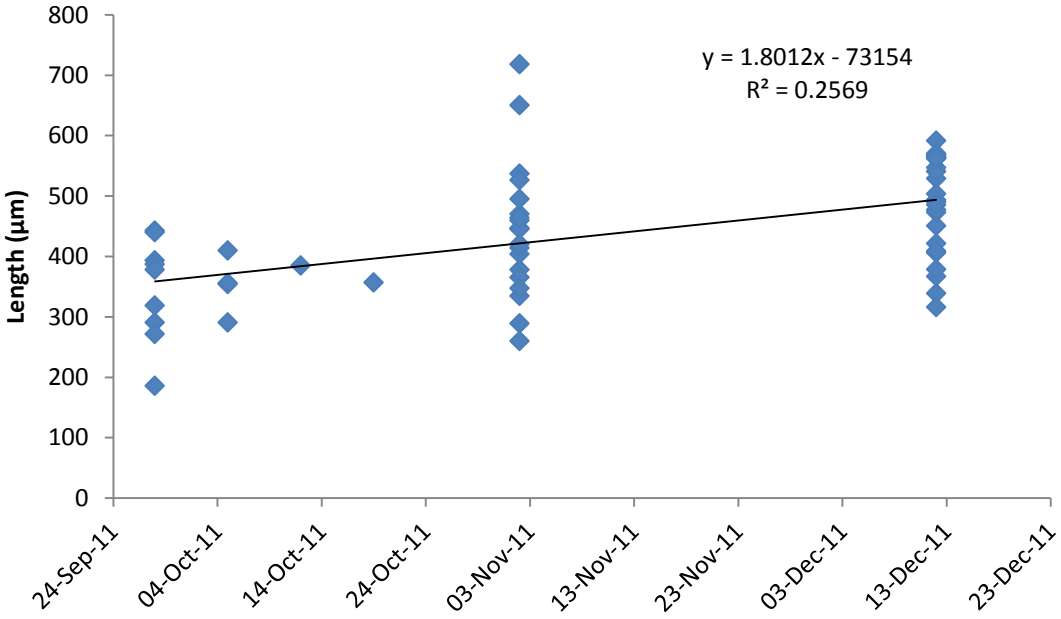
1.6 References

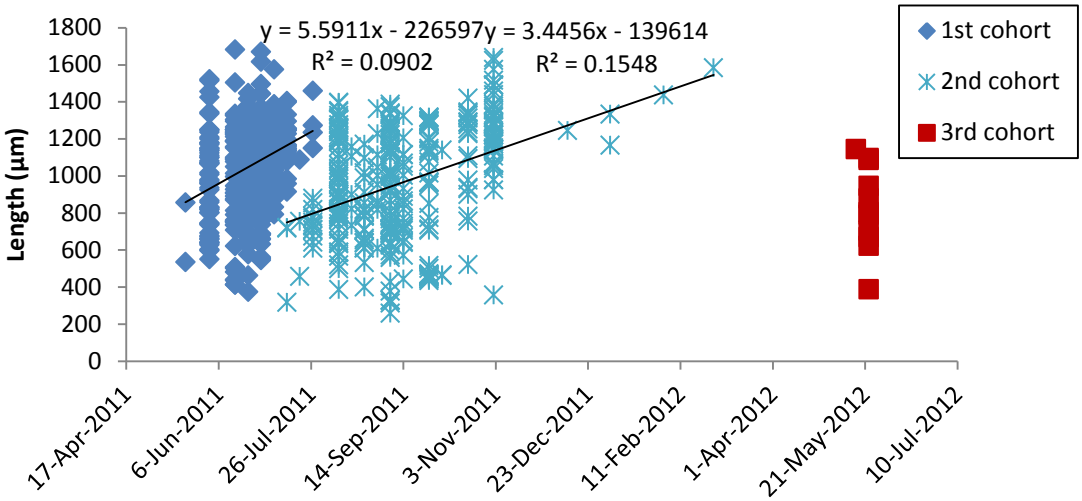
The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

1.7 Supporting information

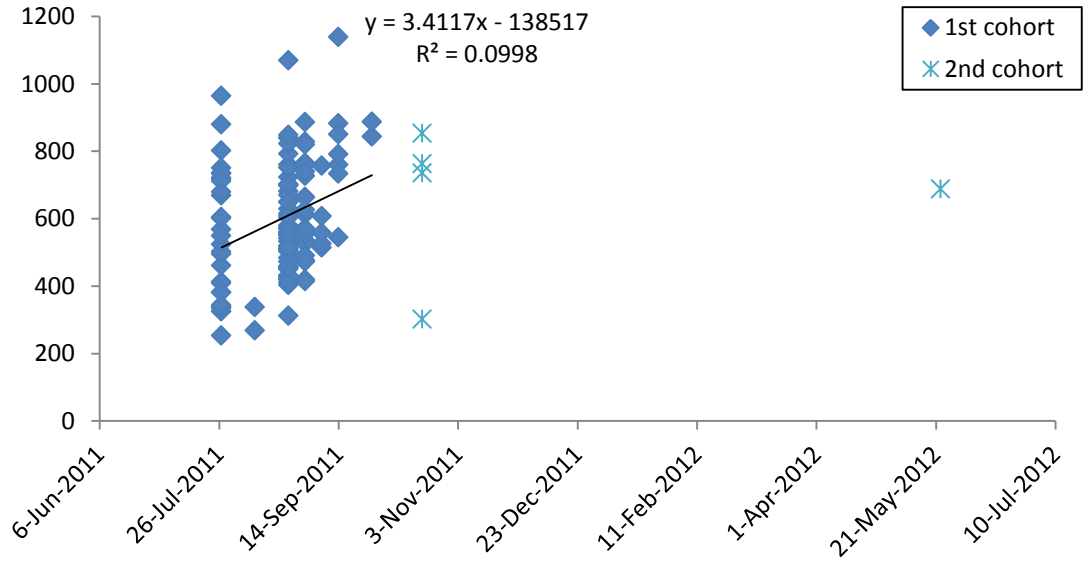
a) *Bosmina* spp.



b) *Daphnia* spp.



c) *Diaphanosoma* spp.



d) Holopedium spp.

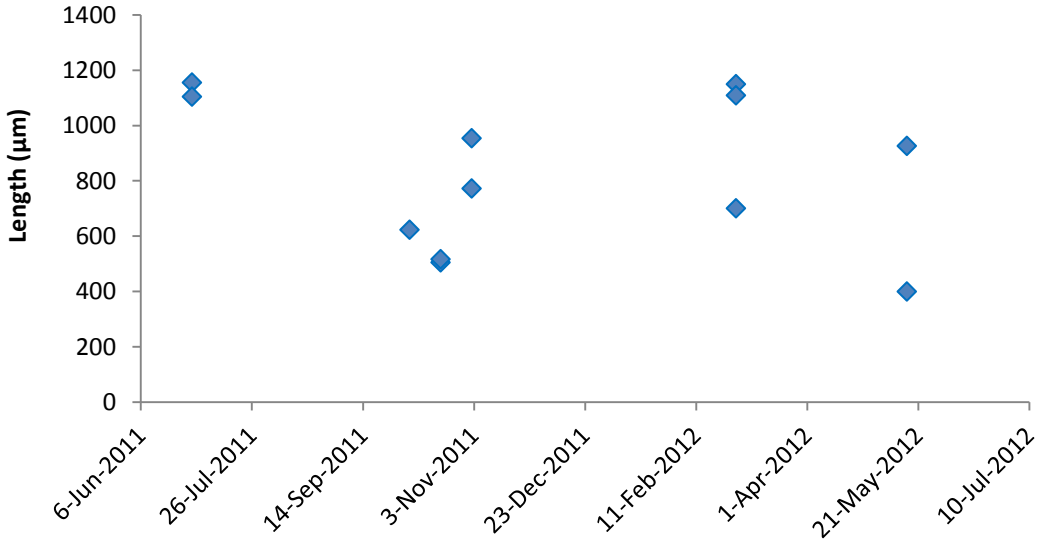


Figure 1.S1 Zooplankton Lengths measured from May 2011 to May 2012 defining cohorts for each species. Only *Holopedium* spp. mean size has been kept and production has been calculated considering the difference between egg weight and mean adult weight.

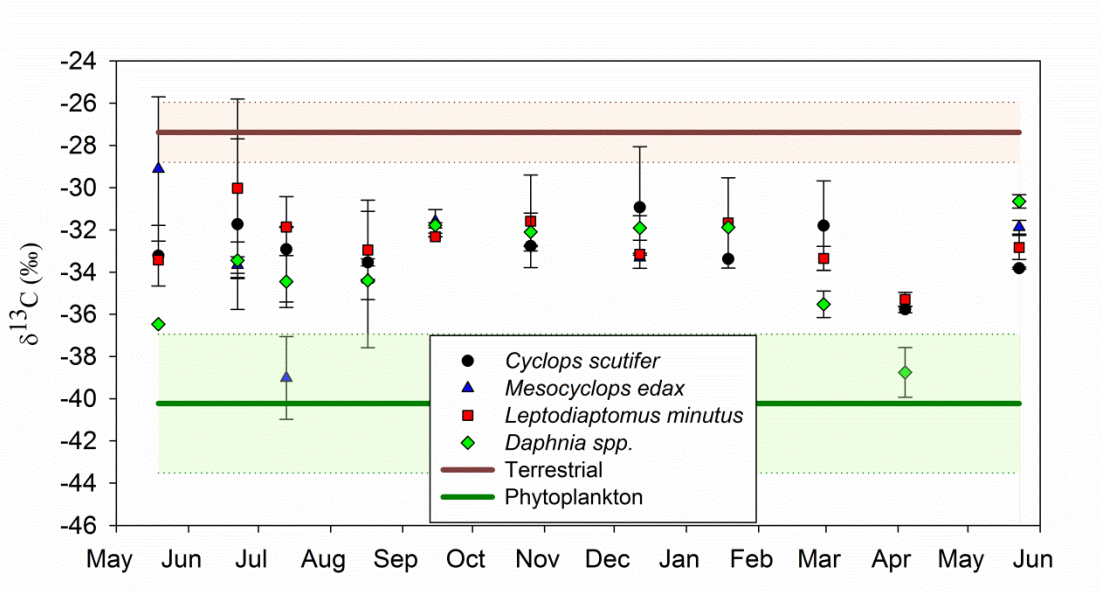


Figure 1.S2 Mean + SD of stable-isotope ($\delta^{13}C$) signatures of zooplankton (*Cyclops scutifer*, *M. edax*, *L. minutus*, *Daphnia* spp.) and potential food sources (terrestrial and phytoplankton) throughout the year 2011–2012.

CHAPTER II
SEASONAL PATTERN OF ZOOPLANKTON LIPID RESERVES AND WINTER LIFE STRATEGIES

RESEARCH ARTICLE

SUBMITTED MANUSCRIPT

Title: Under-ice availability of phytoplankton lipids is key to zooplankton winter survival

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Running head: Zooplankton fatty acids in winter

Key words: boreal lake, *Cyclops scutifer*, *Daphnia*, fatty acid biomarkers, *Leptodiaptomus minutus*, *Mesocyclops edax*, seasonal patterns, terrestrial

Abstract

Shortening winter ice-cover duration in lakes highlights an urgent need for research focused on under-ice ecosystem dynamics and their contributions to whole-ecosystem processes. Low temperature, reduced light and consequent changes in autotrophic and heterotrophic resources alter the diet for long-lived consumers, with consequences on their metabolism in winter. We show in a survival experiment that the copepod *Leptodiaptomus minutus* in a boreal lake does not survive five months under the ice without food. We then report seasonal changes in phytoplankton, terrestrial and bacterial fatty acid (FA) biomarkers in seston and in four zooplankton species for an entire year. Phytoplankton FA were highly available in seston (2.6 µg L^{-1}) throughout the first month under the ice. Zooplankton accumulated them in high quantities (44.8 µg mg dry $weight^{-1}$), building lipid reserves that comprised up to 76% of zooplankton body mass. Terrestrial and bacterial FA were accumulated only in low quantities (< 2.5 µg mg dry $weight^{-1}$). The results highlight the importance of FA reserve accumulation for winter survival as a key ecological process that influences the annual life cycle of the plankton community as well as the overall annual production of aquatic FA for higher trophic levels and ultimately for human consumption.

2.1 Introduction

Winter is the most unexplored season in ecology and has often been portrayed as a dormant period for aquatic organisms especially if the ecosystem is ice-covered (Campbell et al. 2005). However, it is increasingly understood that critical ecological processes do not only take place under the ice but also determine the following summer season (Sommer et al. 2012). Several studies have reported winter-active zooplankton in lakes with evidence from both copepods and cladocerans growing and reproducing under the ice (Rigler et al. 1974, Schneider et al. 2016). However, the phytoplankton resource pool for zooplankton in the winter months preceding the spring bloom is limited (Vincent and Laybourn-Parry 2008) and as a consequence, the challenge for winter-active zooplankton is to cope with the general lack of autotrophic food sources. Therefore, it is believed that zooplankton require alternative energy sources during the ice-covered months.

Because snow and ice covers drastically reduce incoming light (Zdorovennova et al. 2013) for photosynthesis, phytoplankton, benthic algae and macrophytes are thought to be replaced by heterotrophic resources in winter. An increasing number of studies have suggested that allochthonous carbon inputs and microbial loop based bacterial production subsidize zooplankton in winter (Säwström et al. 2009). Indeed, the share of terrestrial carbon in zooplankton biomass, referred to as allochthony, reaches considerable quantities (>50%) in a large number of species and ecosystems and is related to the composition of organic matter sources among lakes (Wilkinson et al. 2013a). It thus appears plausible to assume that on a seasonal scale, zooplankton reliance on heterotrophic sources would be highest during periods when aquatic primary production is low, as is the case in winter (Lizotte 2008).

However, zooplankton also have the ability to efficiently store energy by accumulating lipid reserves in late fall and early winter (Schneider et al. 2016). Lipids are highly energetic molecules as compared to both carbohydrates and proteins (Lee et al. 2006). Lipid reserves in consumers are believed to be mostly derived from autochthonous food sources such as phytoplankton in pelagic environment, which are characterized by their high content of polyunsaturated fatty acids (PUFA). PUFA are a major component of lipids that are considered high quality food (Galloway et al. 2014). However, in a majority of allochthony studies based on stable isotopes, these lipid reserves have not been taken into account (Cole et al. 2011, Mehner et al. 2015) and sometimes have been chemically removed (Rautio et al. 2011), as they are not considered indicators of the recent diet but have potentially been accumulated over a long time period (Syväranta and Rautio 2010). They are thus not considered as part of baseline metabolism, but rather as latent energy reserves to be available for future metabolic requirements, enabling consumers to survive and reproduce during periods of food scarcity (Schneider et al. 2016). In many marine zooplankton, lipids are exhausted over the course of winter, suggesting that they have an important metabolic role under the ice (Hagen and Auel 2001, Lee et al. 2006). Interestingly, the highest zooplankton lipid content also in lakes has been measured during winter (Syväranta and Rautio 2010) precisely when phytoplankton resources are the scarcest in the environment. During this time, dissolved organic carbon (DOC) of terrestrial origin is abundant in boreal lakes (von Wachenfeldt and Tranvik 2008). Although it cannot be directly consumed by zooplankton (Karlsson et al. 2003), DOC may be assimilated in the microbial loop and trophically upgraded by heterotrophic protists (Desvilettes and Bec 2009), thus constituting a potential resource for zooplankton to build lipid reserves or fuel day-to-day metabolism in an environment with low primary production and phytoplankton abundance.

On-going climate warming leads to the mobilization of terrestrial carbon pools, thereby increasing allochthonous carbon inputs from catchments into surface waters worldwide (Solomon et al. 2015). The fate of this terrestrial pool, including its use in sustaining secondary production of plankton and higher trophic levels in lakes is still largely debated (Brett 2014, Mehner et al. 2015). And whether these abundant allochthonous carbon sources contribute to the lipid reserve accumulation of zooplankton in winter, in addition to the well-documented contribution to the biomass (Cole et al. 2011, Wilkinson et al. 2013a), and to subsequent metabolic investment to growth, is not known. This information would contribute to our understanding of the roles, and relative importance of, autochthonous and allochthonous carbon sources in sustaining aquatic food webs.

The composition of lipid reserves differs depending on whether their origin is phytoplanktonic, bacterial or terrestrial. Aquatic food webs are characterized by a high abundance of long-chain n-3 PUFA (Bell and Tocher 2009) while terrestrial ecosystems have a dominance of n-6 PUFA (Taipale et al. 2015a). Moreover, several FA are typically synthesized by terrestrial plants, whereas others are only found in aquatic primary producers (Hixson et al. 2015) and in many cases may be attributed to specific taxa (Napolitano 1999, Taipale et al. 2013). Long-chain saturated fatty acids (LC-SAFA, i.e. C20:0, C22:0, C24:0) are associated with terrestrial plants (Brett et al. 2009) and are abundant in terrestrial leaves e.g. senescent beech leaves contain > 40% of SAFA (Ruess et al. 2007). LC-SAFA are characteristic to various terrestrial plant species from temperate and boreal biomes, including birch, alder, cottonwood, maple and willow but they are common also in reed and peat (Wenzel et al. 2012, Taipale et al. 2015a). Other FA, such as branched and odd numbered FA (iso- and anteiso-C15:0 and C17:0) are biomarkers of bacteria (Haubert et al. 2006). Among PUFA, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) are mainly synthesized by algae

(McLeod and Wing 2009) and are considered essential molecules needed by zooplankton for growth, reproduction and regulation of membrane fluidity. Aquatic organisms have limited capacity to synthesize them *de novo* and thus need to acquire these specific PUFA mainly from their diet (Arts et al. 2009). Decreasing algal production is therefore believed to influence the PUFA availability in the environment. This poses a challenge for winter-reproducing species to produce eggs (Schneider et al. in press) and winter-active species to maintain membrane fluidity (Hiltunen et al. 2016) that needs PUFA when they are at lowest availability.

To better understand how phytoplanktonic, terrestrial and bacterial compounds contribute to zooplankton lipid reserves and how they are used by zooplankton in the course of the year and particularly during winter, we designed a study combining a laboratory survival experiment with a 12-month field survey. First, we experimentally estimated how long accumulated lipid reserves can sustain zooplankton survival. These results were then completed by a one-year lake sampling, where we measured in detail the phytoplanktonic, terrestrial and bacterial fatty acid availability in seston and their accumulation in zooplankton. Since many zooplankton are active in winter under the ice but use lipid reserves mainly for reproduction (Schneider et al. 2016), we hypothesized that the previously accumulated lipids are not used to maintain zooplankton during winter even in the absence of food and that zooplankton requires winter food to survive. To test this hypothesis, we followed the survival of a calanoid copepod *Leptodiaptomus minutus* during winter without food and with winter seston diet, and expected survival to be higher when the copepods had access to winter seston. We also hypothesized that winter seston is dominated by heterotrophic processes and in consequences that terrestrial and bacterial fatty acids contribute to the zooplankton lipid reserves according to their abundance in the seston.

2.2 Methods

2.2.1 Study site and zooplankton community

We sampled Lake Simoncouche (48°13'N, 71°14'W), a medium size mesotrophic shallow lake (mean depth 2.2 m, maximum depth 8 m and surface area 83 ha) situated in Quebec, Canada for one complete year. The lake shows a typical boreal seasonality with ice forming in November and melting in late April to early May. The maximum ice thickness is >50 cm (February), capped with about 40 cm of snow. The catchment basin spreads on 2,543 ha and is covered by a boreal forest dominated by *Abies balsamea*, *Picea mariana* and *Betula papyrifera* (Montoro Girona et al. 2016). Zooplankton crustacean community is dominated by *Leptodiaptomus minutus* (Lilljeborg 1889), *Cyclops scutifer* (Sars 1863), *Mesocyclops edax* (S.A. Forbes 1891) and *Daphnia* spp. with occasional presences of *Epishura lacustris* (S.A. Forbes 1882), *Aglaodiaptomus spatulocrenatus* (Pearse, 1906), *Mesocyclops leuckarti* (Claus, 1857), *Tropocyclops prasinus* (Fischer, 1860), *Eucyclops speratus* (Lilljeborg, 1901) as well as the cladocerans *Bosmina* spp., *Diaphanosoma* spp., *Holopedium* sp. (Zaddach, 1855). Invertebrate predators in the pelagic environment are represented by *Leptodora kindtii* (Focke, 1844) and *Chaoborus* sp.

2.2.2 Survival experiment

We designed a factorial laboratory experiment to estimate zooplankton survival in fed versus starved conditions over time. The experiment lasted 164 days; it started when ice was forming on Lake Simoncouche (20 November 2012) and ended five months later when the ice disappeared (3 May 2013). The experiment was run with the calanoid copepod *Leptodiaptomus minutus*, a common species in eastern North-America that dominates many boreal lakes (Carter et al. 1980). In Lake Simoncouche

it makes up to 93% of the total zooplankton biomass (Grosbois unpublished). The copepods were sampled from the pelagic zone of the lake via vertical net tows and transferred to 1 L plastic containers with lake water and brought to the laboratory. Both treatment modalities were composed of six replicates (N_{tot} = 12) of fifty copepods each, which were selected under a stereomicroscope (Discovery V12, Zeiss, Jena, Germany, x8-x100) and transferred to 250 mL beakers using a pipette. Half of the beakers (6) contained GF/F-filtered lake water and were considered the starved treatment, the other 6 beakers had lake water (<50 µm) providing the copepods with a natural food supply present in the lake (control). The beakers were installed at 4°C in the dark to simulate lake conditions in winter. Water was entirely renewed weekly with fresh Lake Simoncouche water that was either sieved (50 µm) or GF/F-filtered depending on the treatment, and the copepods were counted, survival noted, and dead individuals removed.

2.2.3 Water and zooplankton sampling

Lake water was collected monthly from 12 January 2011 to 16 May 2012 for total dissolved nitrogen (TDN), total dissolved phosphorous (TDP), dissolved organic carbon (DOC), specific UV-absorbance ($SUVA_{254}$), chlorophyll *a* (Chl-a), bacterial biomass (Bact Biom) and from 6 July 2011 to 7 May 2013 for fatty acid (FA) analysis. Samples were taken from the epilimnion at the deepest point of the lake using a 2 L Limnos sampler (Limnos Ltd, Turku, Finland). Water was collected from every meter, pooled to one integrated sample in a bucket and stored in a 4 L Nalgene container. Water for FA analysis was collected from the metalimnion as well (hypolimnion does not form in this shallow lake). In the laboratory, subsamples of water for TDN, TDP, DOC and SUVA were filtered through a cellulose acetate filter (0.2µm) that had been pre-rinsed with Milli-Q water. TDN, TDP, and DOC were analysed using a Lachat Autoanalyser, a ThermoSpectronic spectrophotometer and a

Shimadzu TOC-V, respectively, at the Institut national de la recherche scientifique (INRS), Quebec City, Canada. $SUVA_{254}$, an index of DOC aromaticity associated with allochthonous (terrestrial) carbon sources[43], was measured as absorbance at 254 nm using a Cary 100 UV-Vis spectrophotometer (Agilent, Santa Clara, U.S.A.) and normalized to DOC concentration. Samples for Chl-a (three replicates on each date) were filtered onto GF/F filters that were then folded, wrapped in aluminium foil and stored at -80°C until spectrofluorometric analysis as in Mush (1980) using a Cary Eclipse fluorescence spectrophotometer (Agilent, Santa Clara, U.S.A.). Aliquots for bacterial biomass were first preserved with formaldehyde (2% final concentration), then stained with 4.6-diamido-2-phenylindole (DAPI, final concentration 5 µg ml^{-1}) and filtered onto black cellulose filters (0.2 µm) that were mounted onto microscope slides (three replicates for each date) and stored at -20°C until bacteria cell counting using epifluorescence microscopy with a UV excitation (365 nm) filter and an inverted microscope (Axio Observer A1, Zeiss, Jena, Germany x1000). Seston samples for FA (three replicates for each date) were filtered onto pre-combusted and pre-weighted GF/F filters that were then folded, wrapped in aluminium foil and stored at -80°C until freeze-drying.

Zooplankton was sampled weekly to monthly from 19-May-2011 to 23-May-2012 for identification, abundance, and FA. The samples for FA were collected by vertical net tows with a 50 µm mesh size net (diameter 25 cm) from the entire water column (0-6 m). To estimate zooplankton abundance, water samples (6 to 20 L) were collected weekly with a Limnos sampler from several depths and concentrated to one integrated sample using a 50 µm sieve. Formaldehyde (final concentration: 4%) was added to the abundance samples until identification (Edmondson 1959, Czaika 1982) with an inverted microscope (Axio Observer A1, Zeiss, Jena, Germany, x100) and Utermöhl chambers. Copepod identification was carried out for copepodites and adults further divided into males and females. Cladocerans were counted without

considering developmental stages. When zooplankton density was too high, half or a quarter of the sample was counted after division in Folsom's sample divider. A minimum of 100 individuals were identified per sample except for 7 low density samples (N_{tot} = 71) where about 60 individuals were counted. For zooplankton FA analyses, 100-200 individuals were carefully collected with forceps under stereomicroscope, placed in Eppendorf tubes (1.5 mL) and stored at -80°C until freeze-drying. Fatty acids were analysed from the 4 most abundant taxa of the community: *Leptodiaptomus minutus*, *Cyclops scutifer*, *Mesocyclops edax* and *Daphnia* spp.

2.2.4 Fatty acid analyses

Lipids were extracted from freeze dried seston and zooplankton in chloroform-methanol mixture following Heissenberger et al. (2010). Lipid concentration in zooplankton was calculated from lipid mass measured by gravimetry and from zooplankton mass obtained using a micro-balance (XP26 DeltaRange, Mettler Toledo, Greifensee, Switzerland). Toluene was added to lipid extracts and each sample was trans-esterified at 50°C with 1% methanolic sulfuric acid. The resulting fatty acid methyl esters (FAME) were separated from the rest of the material by adding $KHCO_3$ − water (2% v/v) and hexane. FAME were then identified and quantified by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890A chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 5975C mass spectrometer with triple-axis detector and an Agilent J&W DB-23 column (60 m length, 0.25 mm inner diameter, 0.15 µm film thickness). The resulting retention time and ion composition were used for FAME identification and the peak area of the most abundant FA specific ion (m3z 74, 79, 81 and 87) versus an internal standard (nonadecanoic acid) was used for FAME quantification using calibration curves based on known standard concentrations.

Biomarkers documented only for a unique taxon (phytoplankton, terrestrial plant or bacteria) were selected from freshwater, marine and terrestrial literature (see Supplementary Table S1 online). The unsaturated FA C18:4n3, C20:1n9, C20:4n6, C20:5n3, C22:6n3, C24:1n9 were selected as phytoplankton biomarkers, the long-chained saturated FA C20:0, C22:0, C23:0, C24:0 were selected as terrestrial biomarkers and the branched-chained saturated FA aC15:0, iC15:0, iC17:0 as well as the saturated FA (SAFA) C15:0 and the Cyclic SAFA Cy-C17:0 were selected as bacterial biomarkers.

2.2.5 Statistical analyses

Treatments from the survival experiment were tested with repeated measures ANOVA and refined with contrasts for each date using a non-parametric Wilcoxon test, as the data did not meet the assumptions of homoscedasticity and normality. Kolmogorov-Smirnov and Bartlett's tests were used to test data normality and homoscedasticity respectively. Alpha level was set at 0.05. Principal component analyses (PCA) were applied to normalized FA composition data that was grouped according to FA biomarkers (FAB), polyunsaturated fatty acids (PUFA), n-3 FA, n-6 FA, monounsaturated fatty acids (MUFA) and saturated fatty acids (SAFA). Differences of FA composition among taxa were tested with PermANOVA in PRIMER v.6.1.11 & PERMANOVA+ v.1.0.1. FAB concentrations were compared using student's t-test. All analyses except PERMANOVA were carried out in the R environment v.3.3.1 (R Development Core Team 2015).

2.3 Results

2.3.1 Seasonal abundances in zooplankton community

L. minutus, *C. scutifer* and *Daphnia* spp. were active during the whole year (Fig. 2.1). They were also the most abundant zooplankton in the lake with average annual abundances of 12.9 ± 4.9, 3.3 ± 1.7 and 4.9 ± 2.8 ind L^{-1} (mean ± SD), respectively. They represented 58%, 15% and 22% of the total community abundance. *L. minutus* spent the winter as an adult, but the *C. scutifer* population was entirely made of copepodite (C-IV) individuals from November to April. The *Daphnia* population was comprised of adults under the ice, with relatively high abundances until 12 December and then rather low until mid-May. No other copepods or cladocerans were present in the water column during winter except highly abundant *Bosmina* spp. in early winter and punctual presences of *Eucyclops speratus* and *Tropocyclops prasinus*. The cyclopoid *M. edax* was absent from the water column from November to May although being the fourth most abundant crustacean zooplankton in the lake (1.0 ± 1.1 ind L^{-1} and 5% of the average annual community abundance).

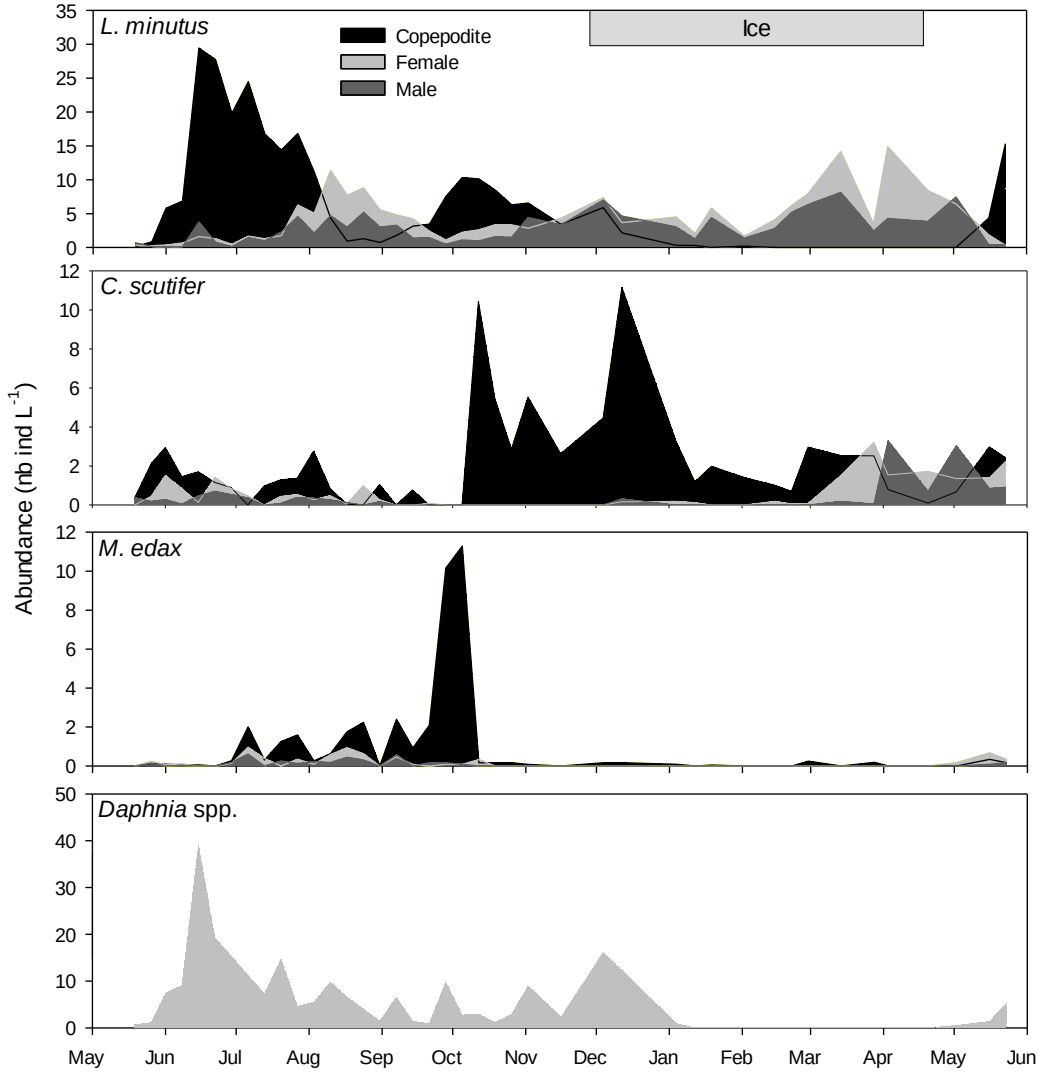

Figure 2.1 Seasonal abundance (nb individuals L^{-1}) of copepodites (copepods), adult females and males of the four main species in the zooplankton community of Lake Simoncouche. *Daphnia* spp. numbers include females and males. Notice the different scales.

2.3.2 Starvation experiment

The survival of *L. minutus* at first followed the same pattern in both treatments but after ten weeks started to differ strongly between starved and fed conditions (Fig. 2.2). Access to food significantly increased zooplankton survival ($F_{(1,245)} = 1682.6$, $p < 0.0001$) which was affected by time ($F_{(24,245)} = 230.3$, $p < 0.0001$) and both factors interacted ($F_{(24,245)} = 48.7$, $p < 0.0001$). During the first 66 days of the experiment, the survival was not significantly different among the treatments ($p > 0.13$; see Supplementary Table 2.S2 online). Only from day 66 (29 January) to day 73 (5 February) the number of individuals alive in the starved condition dropped from 32 ± 6 to 3 ± 2 (mean ± SD; $W = 36$, $p = 0.005$). At the end of the experiment on day 163 (5 May), only 0.2 ± 0.4 starved individuals were still alive (i.e., one individual in one replicate), in contrast to 21 ± 7 surviving individuals with access to food.

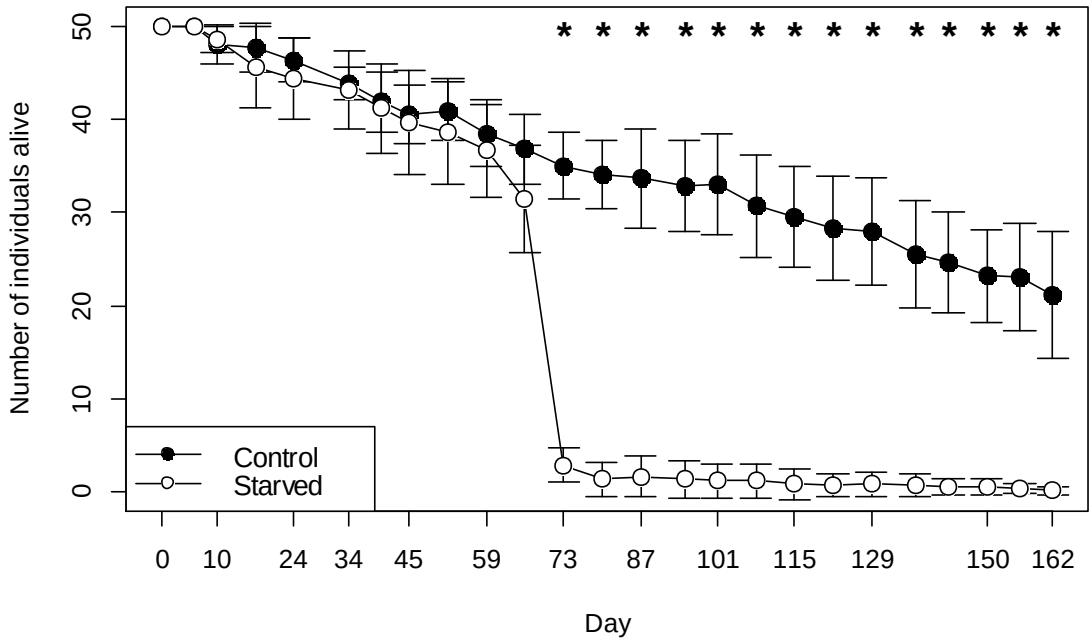

Figure 2.2 Adult L. minutus survival in the starvation experiment for individuals that were collected from the Lake Simoncouche when the lake was freezing in November (20-Nov-2012). Values are means of six replicates and SD. The experiment was terminated when the lake became ice-free in May (3-May-2013). The asterix mark the days when the survival was statistically different between the treatments.

2.3.3 Seasonality in water chemistry and putative food sources

The most pronounced change in water chemistry, followed by shifts in putative food sources, took place in autumn. The most important increase in nutrient concentrations was registered from September to October (Table 2.1) when TDN almost doubled (x 1.8) and TDP more than doubled (x 2.2). DOC increased a month before, reaching its maximum already in September (6.6 mg C L^{-1}). $SUVA_{254}$ was lowest in August (3.4 L mg^{-1} m^{-1}), increased in September (4.1 L mg^{-1} m^{-1}) followed by lower values in winter (3.7 ± 0.1 L mg^{-1} m^{-1}), and then reached its maximum in the end of March (4.5

L mg^{-1} m^{-1}). Chl-a concentration was highest in July (3.5 µg L^{-1}), decreased to a minimum in December (0.2 µg L^{-1}) and remained very low throughout the winter, from December until March (0.4 ± 0.2 µg L^{-1}). Bacterial biomass was highest in October (72.4 µg C L^{-1}) and decreased in winter to the minimum value of 30.2 µg C L^{-1} in January (Table 2.1).

Table 2.1 Environmental and biological variables from the epilimnion of Lake Simoncouche represented by temperature (Temp, °C), total dissolved nitrogen (TDN, mg N L^{-1}), total dissolved phosphorus (TDP, µg P L^{-1}),dissolved organic carbon (DOC, mg C L^{-1}), chlorophyll-a (Chl-a, µg L^{-1}), bacteria biomass (Bact Biom, µg C L^{-1}) and $SUVA_{254nm}$.

Date	Temp	TDN	TDP	DOC	Chl-a	Bact Biom	$SUVA_{254nm}$
12-May-11	5.8	0.20	12.6	4.6	1.9	27.4	4.0
15-Jun-11	17.5	NA	NA	4.6	3.0	39.0	4.1
05-Jul-11	22.8	0.21	5.8	4.9	3.5	62.6	3.6
10-Aug-11	22.0	0.13	3.5	5.3	3.3	48.6	3.4
08-Sep-11	17.3	0.12	4.0	6.6	2.6	58.0	4.1
19-Oct-11	10.8	0.22	8.6	5.8	1.4	72.4	3.9
04-Dec-11	2.9	0.22	5.1	5.6	0.2	52.1	3.6
12-Jan-12	3.7	0.24	4.6	5.4	0.7	30.2	3.8
22-Feb-12	3.9	0.18	5.8	5.3	0.3	35.5	3.7
28-Mar-12	3.2	0.21	5.6	5.1	0.3	57.6	4.5
16-May-12	14.3	0.12	2.7	4.6	1.8	43.3	4.3

The six phytoplankton FA biomarkers (FAB) dominated the seston FAB composition with an annual average of 1.7 ± 1.3 µg L^{-1} (mean ± SD), followed by terrestrial (0.3 ± 0.1 µg L^{-1}) and bacterial FAB (0.2 ± 0.1 µg L^{-1}) (Fig. 2.3). When expressed as a percentage of total FA in seston, the distribution was the following: 8.6% (± 5.6) phytoplankton FAB, 1.5% (± 0.9) terrestrial FAB and 1.2% (± 1.3) bacterial FAB. All FAB of the three potential energy sources were more abundant in summer than in

winter. Phytoplankton FAB had an average concentration of 0.8 µg L^{-1} under the ice and 2.1 µg L^{-1} in the ice-free period (t = -2.25, p = 0.04). The same pattern was observed for terrestrial biomarkers with 0.2 µg L^{-1} in winter and 0.3 µg L^{-1} in summer (t = -2.77, p = 0.02) as well as for bacterial biomarkers with 0.1 µg L^{-1} in winter and 0.2 µg L^{-1} in summer, although this difference was not significant (t = -1.99, p = 0.06).

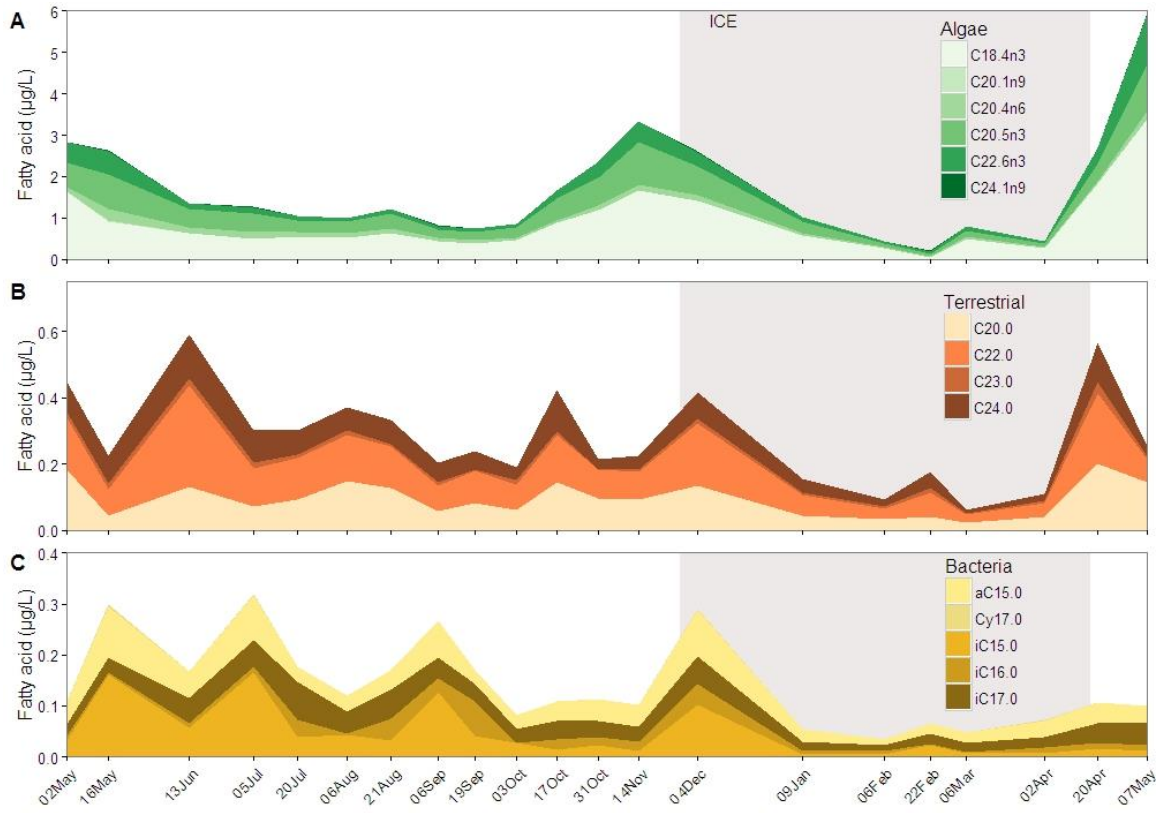

Figure 2.3 Seasonal pattern of seston fatty acids divided by FA biomarkers that represent A) phytoplankton, B) terrestrial and C) bacteria sources. Notice the different scales of y axis. Grey shade represents the period when the lake was ice covered. Dates represent sampling dates.

2.3.4 Total lipids and FA composition in zooplankton

A broad range of lipid content was observed in copepods, especially in *L. minutus*, whose lipid content ranged from 18% (of dry weight) in mid-September to 76% in late January (see Supplementary Fig. 2.S1). *C. scutifer* had its lowest lipid content (21%) in May and highest (50%) in January. The late January maximum of lipids for *L. minutus* and *C. scutifer* revealed that these copepods had accumulated lipids under the ice, while the decrease in their lipid content was measured starting only at the end of February. The lipid content of *M. edax* was stable around 28 ± 9% (mean ± SD) with a maximum of 47% in May. The lipid content of *Daphnia* was highest in winter (33% in February) although it remained relatively stable throughout the year (22 ± 5 %).

The relative ranking of FAB was similar in *L. minutus*, *C. scutifer*, *M. edax* and *Daphnia* sp. (Fig. 2.4).Terrestrial FAB were present in concentrations between 0.05 and 0.5 µg mg dry weight^{-1} (µg mg DW^{-1}) representing 0.5 to 1.3% of total FA, and bacterial FAB in similarly low concentrations between 0.2-1.0 µg mg DW^{-1} (1.2-1.9% of total FA). The most accumulated FAB were from phytoplankton, with an annual mean of 20.9 (40%), 12.4 (33%), 4.7 (19%) and 1.6 µg mg DW^{-1} (10%) for *L. minutus*, *C. scutifer*, *M. edax* and *Daphnia* spp., respectively. *L. minutus* contained more phytoplankton FAB under the ice (38.5 µg mg DW^{-1}) than during the ice-free season (12.2 µg mg DW^{-1}) ($t = 5.1$, $p = 0.004$). The same was true for terrestrial and bacterial FAB ($p < 0.01$). Likewise, *C. scutifer* contained more phytoplankton FAB under the ice (15.9 µg mg DW^{-1}) than during the ice-free season (9.0 µg mg DW^{-1}) ($t = 3.32$, $p = 0.03$), but with no significant difference among seasons for terrestrial and bacterial FAB ($p = 0.05$, $p = 0.15$, respectively). Contrary to the other species, *Daphnia* showed little phytoplankton FAB accumulation over the year (1.85 µg mgDW^{-1}) and no significant difference between ice-covered and ice-free season in

phytoplankton (log transformed; t = -1.25, p = 0.26), terrestrial or bacterial FAB (p > 0.50).

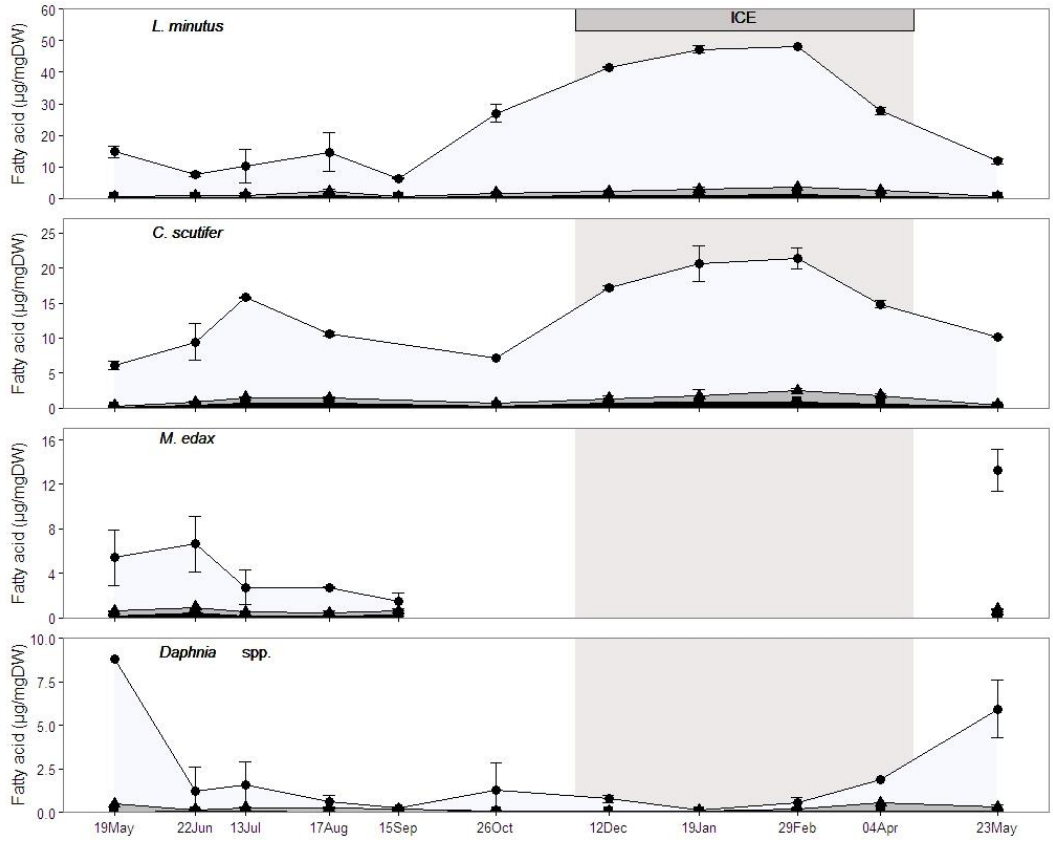

Figure 2.4 Cumulated concentration of fatty acid biomarkers of phytoplankton (filled circle), bacteria (filled triangle) and terrestrial (filled square) in zooplankton. The gap in *M. edax* represents a period of the year when all individuals were absent of the pelagic environment. Notice the different scales in Y axis.

Principal component analysis captured 74% of the total variation in zooplankton FA composition, with 57% to axis 1 and 18% to axis 2 (Fig. 2.5). PUFA, phytoplankton FAB, n-3 FA and SAFA contributed 22%, 21%, 21% and 17%, respectively, to axis 1. Terrestrial FAB and bacterial FAB contributed 49% and 47%, respectively, to axis

2. *L. minutus* and *C. scutifer* were associated with axis 1 corresponding to high PUFA and phytoplankton FAB. The seston samples were characterised mainly by the presence of SAFA as were also the majority of *Daphnia* samples. SAFA composition in seston and *Daphnia* were dominated by C16:0 and C18:0 (data not shown). Some of the seston samples were further associated with bacterial and terrestrial FA. The composition of FA among taxonomic groups (*L. minutus*, *C. scutifer*, *M.* edax, *Daphnia* spp. and seston) was significantly different (PermANOVA, $F_{(4,122)} = 93.2$, $p = 0.001$).

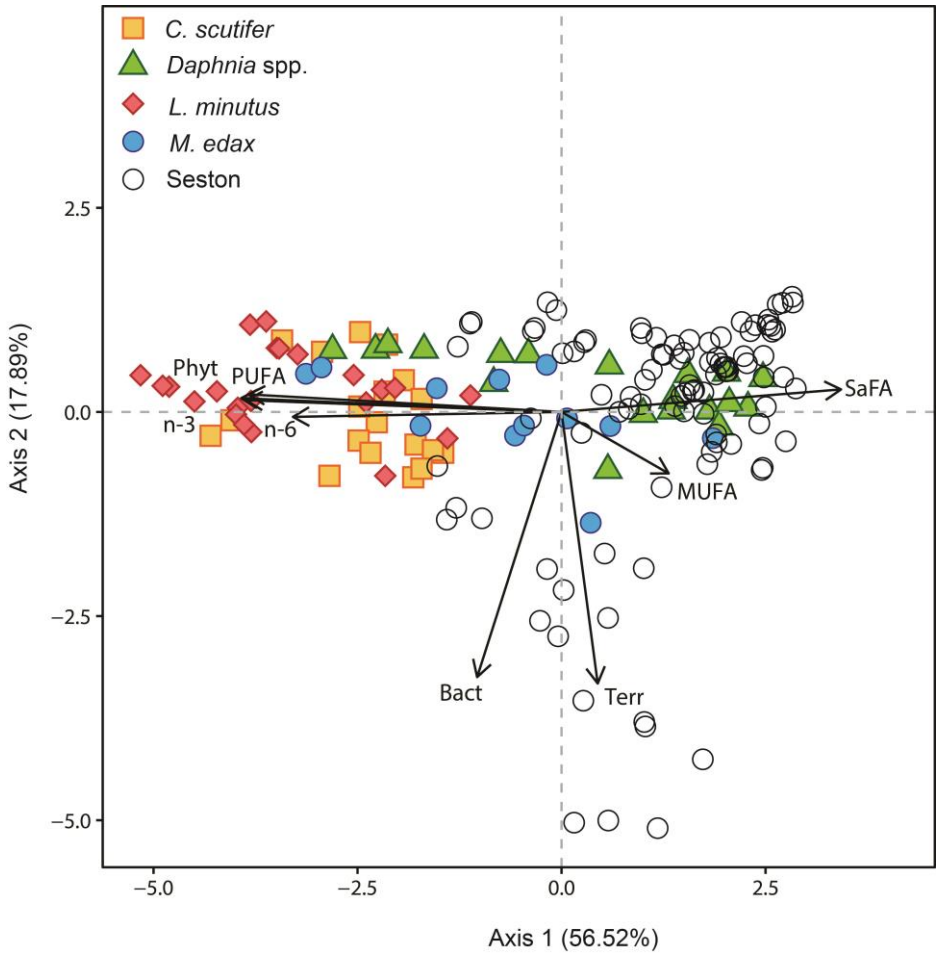

Figure 2.5 Principal component analysis (PCA) on FA composition grouped as polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SAFA), biomarkers of phytoplankton (Phyt), terrestrial (Terr), bacteria (Bact), omega-3 fatty acids (n-3) and omega-6 fatty acids (n-6). The sample scores represent *C. scutifer* (orange), *Daphnia* sp. (green), *L. minutus* (red), *M. edax* (blue) or seston (open). Proportion of explained variance per axes is in parentheses.

2.4 Discussion

Our results demonstrate that the usually overlooked winter season involves key ecosystem processes. The high availability of phytoplankton PUFA in seston and their efficient accumulation by zooplankton establish a critical link between primary producers and higher trophic levels. By use of fatty acid biomarkers (FAB), we showed that during times of low primary production in winter, contrary to our hypotheses, the prevalence of terrestrial organic matter and/or bacteria did not increase in zooplankton lipid reserves. Instead, early winter accumulation of phytoplankton-derived PUFA and their progressive decline in zooplankton lipid reserves in mid- and late-winter suggests that phytoplankton FA are critical for several species of zooplankton to survive and remain in an active stage until spring.

Energy to sustain winter metabolism and survival can be obtained from lipid reserve accumulation that has been considered to take place in autumn before the lake freezes (Mariash et al. 2017). Our experiment on *L. minutus* demonstrated that also early winter is a critical accumulation period; the individuals had not accumulated enough FA at the moment of ice formation to be able to survive the entire winter without external food input. At the ice-on, when the experiment started and the *L. minutus* were collected from the lake, the copepods had accumulated lipid reserves to survive for two months in laboratory, which carried them without additional food to

February. However, the seston FA composition in the lake showed that the phytoplanktonic FA were available until January, i.e. for about one month after the ice had formed (Fig. 2.3). If we extrapolate the survival time without food, obtained from the experiment (66 days), we can calculate that in order to survive until the ice melting (18 April), *L. minutus* could have stopped eating on 12 February. This corresponds well with the moment the lake population of copepods ceased to accumulate FA reserves (19 January, Fig. 2.4), indicating FA in seston were exhausted. Interestingly, they started to use the FA reserves only a month later (29 February), probably corresponding to a minimum metabolic activity in mid-winter. Lake Simoncouche phytoplankton production begins before the ice disappears (Schneider et al. 2016) providing fresh food for the overwintering populations in late winter. We argue that had the *L. minutus* remained in the lake longer before the initiation of the experiment they would have been able to accumulate the reserves needed to carry them over the months when there was no food in the water column. Few studies have estimated the survival of similar species under starved conditions. Elendt and Storch (Elendt and Storch 1990) discussed that *L. minutus* can survive 15.4 days, *M. edax* 24.3 days and *Daphnia magna* only 7.6 days. However, these authors do not report season, lipid content or temperature at which these survival times were estimated, thus limiting a direct comparison with our experiment. Nevertheless, the shorter survival time of *Daphnia* under starved conditions is in accordance with our abundance data that showed that *Daphnia* abundance declined close to zero once the seston PUFA pool was exhausted (Fig. 2.1, Fig. 2.3).

The concentration of phytoplankton FA in seston peaked just before the ice-in and just after the ice-out, and followed the seasonal pattern of phytoplankton FA production in Lake Simoncouche (Schneider et al. in press). The autumn peak decreased gradually but remained at a relatively high level for about a month under the ice (Fig. 2.3). Historically, phytoplankton primary production, like every

biological activity, is assumed negligible in winter, but several recent studies reported under-ice primary production in oceans (Nicol et al. 2008) and in many lakes (Ravet et al. 2010). A seasonal pattern similar to our seston FA composition has also been shown for lakes in the northern USA, with a very high spring bloom associated with phytoplankton FA abundance and a relatively high abundance in autumn (Ravet et al. 2010). Usually eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) content in phytoplankton are limited by nitrogen and phosphorous concentrations in water (Reitan et al. 1994, Rasdi and Qin 2015). Summer TDN and TDP shortage in Lake Simoncouche (Table 1) was followed by a nutrient increase during lake mixing (October). It appears plausible that the autumn nutrient increase in the water column allows the phytoplankton community to synthesize new PUFA that the nutrient depletion in summer does not permit. Also, n-3 FA are synthesized by different phytoplankton species (Taipale et al. 2013), and vertical mixing has been shown to cause major changes in phytoplankton community composition (Lee and Yoo 2016). These phenomena may explain the n-3 FA maximum in autumn.

All FAB increased in quantity when the ice formed, and then decreased to their annual minimum by mid-winter (Fig. 2.3). The winter terrestrial FA minimum coincided with the minimal values of SUVA, indicating decreased input of terrestrial matter from the frozen catchment soils (Weishaar et al. 2003). The minimum concentration of bacterial FA coincided with the minimum in bacterial biomass in winter. The same observation was valid for the autumnal FA peak confirming that seasonal changes in terrestrial and bacterial FAB concentrations followed the presence of terrestrial or bacterial material in seston.

The high variability in FA content and composition among species indicates different adaptations to winter and different life strategies (Mariash et al. 2017) with species that stay active in winter having higher lipid content and FA composition dominated

by PUFA in autumn (Fig. 2.5). Earlier studies have assumed that reserve accumulation would take place in autumn before ice formation (Mariash et al. 2017) and have suggested that zooplankton strongly reduce or even cease their food intake under the ice (Rautio et al. 2011). Here, the copepods *L. minutus* and *C. scutifer* showed a strong ability to accumulate lipids until January, up to 76 % of the biomass, (see Supplementary Fig. 2.S1) emphasizing the importance of early winter in plankton ecology, a season that has been largely ignored in limnological studies. We propose that the autumnal and early winter lipid accumulation allows certain zooplankton to spend the entire winter in an active form either as adults (*L. minutus*) or as C-IV stages (*C. scutifer*), which are the two strategies observed for these species (Elgmork 2004, Schneider et al. 2016). As lack of food and cold temperatures are considered stress factors, *Daphnia* are usually believed to cope with this stress by producing resting eggs (ephippia ; Hiruta and Tochina 2014) . Our observations indicate that the majority of *Daphnia* in Lake Simoncouche followed this strategy (Fig. 2.1), however the individuals that overwintered actively did seemingly well even in late January when they were observed to carry parthenogenetic young (Grosbois, unpublished data, see Supplementary Fig. 2.S2). The cyclopoid *M. edax* also disappeared from the water column to the sediments from November to May as C-V stage copepodites, possibly as a strategy to avoid predators or because they do not have the physiological ability to accumulate lipids.

When consumed, the branched FA are transferred to higher trophic levels without modification and can be measured in the copepod FA composition (Ederington et al. 1995). The branched and saturated FA have been shown to be associated to growth and membrane fluidity regulation in bacteria (Kaneda 1991) but their physiological role in zooplankton is less known. Because of the lack of n-3 and n-6 PUFA, bacteria are commonly considered as a poor food source with negative effects on zooplankton growth and reproduction (Taipale et al. 2014). Similarly, the FA quality of terrestrial

material is considered to be poorer than that of aquatic material (Brett et al. 2009). However, terrestrial organic matter is known to be selectively assimilated in bacteria biomass (Guillemette et al. 2016) and then probably transferred to higher trophic levels via heterotrophic eukaryotic protists and rotifers, therefore making it available to crustacean zooplankton. During this transfer the nutritional quality of terrestrial organic material and bacteria can be trophically upgraded by heterotrophic flagellates (Bec et al. 2003) and ciliates (Martin-Creuzburg et al. 2005). Our results, however, show that despite the potential trophic upgrade of terrestrial and bacterial material, terrestrial and bacterial FAB were not substantially accumulated by zooplankton at any time of the year. The maximal contribution of terrestrial (2.1%) and bacterial FAB (2.9%) in zooplankton was measured for *C. scutifer* in December and February showing that these biomarkers played a small role in the total FA accumulation in the four studied zooplankton species. Our results therefore do not give support to earlier literature that has suggested that zooplankton switch to terrestrial organic carbon sources in lack of phytoplankton production (Berggren et al. 2014, Taipale et al. 2016). It is important to note that our study does not exclude the possibility that zooplankton use terrestrial organic matter for respiration and/or to support cell and tissue renewal. However, the results confirm that terrestrial and bacterial molecules are not preferentially stored in zooplankton lipid reserves, even when terrestrial and bacterial FAB are present and available to zooplankton in seston. This observation is in accordance with the results of Mariash et al. (2011), who showed an absence of SAFA trophic accumulation indicating a lack of terrestrial and bacterial FA accumulation in zooplankton lipid reserves.

Among all the FA accumulated by copepods in winter, PUFA from phytoplankton were the most accumulated demonstrating the predominant role of these FA in the reserve accumulation of organisms that stay active under the ice (Fig. 2.4, Fig. 2.5). These FA are important regulators of membrane fluidity, which is reduced at low

temperatures (Thomas and Dieckmann 2002), and are used by the organisms to cope with winter conditions. They have also been suggested to contribute to organism's metabolic maintenance as well as to investment of n-3 FA into reproduction in late winter and early spring (Schneider et al. 2016). Both *L. minutus* and *C. scutifer* accumulated these FA, especially DHA (data not shown), which is an essential FA for copepod reproduction (Parrish 2009). As *C. scutifer* needed to mature from C-IV stage to adult in winter and then reproduce (Fig. 2.1), it is very likely that the accumulated PUFA were used primarily for growth and then reproduction. FA composition differed among taxa and characterized winter abundant species (*L. minutus* and *C. scutifer* copepods) with high dominance of PUFA from phytoplankton in their accumulated lipids and winter low abundant or absent species (*Daphnia* spp. and *M. edax*) with SAFA dominance (Fig. 2.5). None of these species accumulated bacterial or terrestrial FA substantially. SAFA dominance in *Daphnia* and *M. edax* were characterized by the generalist C16:0 and C18:0 FA rather than terrestrial LC-SAFA or bacterial Br-SAFA. Ephippia-producing *Daphnia* that disappear from the water column in winter do not accumulate FA to biomass but rather invest them to resting eggs (ephippia), in particular EPA(Abrusán et al. 2007) that was largely available in seston before winter. The concentration of phytoplankton FA in *Daphnia* started to increase in April indicating that phytoplankton FA were produced in the seston and available for consumers before the ice melted. *M. edax* predation on *Daphnia* (see Supplementary Fig 2.S3) was confirmed by their similar FA composition (Fig 2.5). Collectively, these results demonstrate that the use and accumulation of FA is closely related to the species life strategy.

To conclude, this study demonstrates that the availability of phytoplankton FA remained high in seston in early winter permitting zooplankton to accumulate and subsequently metabolise n-3 FA throughout winter, thereby providing a mechanism for under ice growth and reproduction (Schneider et al. 2016). Although the

mechanism is known for some marine organisms, no information has earlier been available for freshwaters. To our knowledge, our study also reports for the first time seston and zooplankton bacterial and terrestrial FAB for a complete year. These terrestrial and bacterial FAB were not accumulated by zooplankton in winter, suggesting that terrestrial organic material and bacteria are not used as alternative resources during times of low primary production under the ice. Winter is predicted to be shorter in the future with climate change (Sharma et al. 2016), phytoplankton with low PUFA content such as cyanobacteria are predicted to increase in the next decades (Paerl and Huisman 2009), and the worldwide FA source is predicted not to meet future human needs (Salem Jr and Eggersdorfer 2015). It is therefore essential to better understand how PUFA accumulation by zooplankton is seasonally regulated, how it will be affected by changing ice conditions, and how these changes will drive modifications in FA composition of long-lived consumers such as fish, and ultimately in nutritive inputs for the human population.

2.5 Acknowledgements

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2.6 References

The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

2.7 Supporting information

Table 2.S1 Selected fatty acid biomarkers for phytoplankton, terrestrial organic matter and bacteria. References are listed at the end of the thesis.

Fatty acid	Abbrev-iation	Biomarker	Reference
C18:4n3	SDA	Phytoplankton	Cook et al. (2000), Hughes et al. (2005), Ackman (1989), Sleigh (1987), Mai et al. (1996), McLeod and Wing (2009), Taipale et al. (2013), Taipale et al. (2015b), Stevens et al. (2004).
C20:1n9		Phytoplankton	Taipale et al. (2015b)
C20:4n6	ARA	Phytoplankton	Paradis and Ackman (1977), Cook et al. (2000), Isay and Busarova (1984), Mai et al. (1996), Nelson et al. (2002), Hughes et al. (2005), Kharlamenko et al. (1995), McLeod and Wing (2009), Taipale et al. (2015b)
C20:5n3	EPA	Phytoplankton	Berggren et al. (2014), Ackman et al. (1968), Paradis and Ackman (1977), Isay and Busarova (1984), Ackman (1989), Mai et al. (1996), Cook et al. (2000), Nelson et al. (2002), Hughes et al. (2005), Howell et al. (2003), Boschker et al. (2005), Ratledge and Wilkinson (1988), Dunstan et al. (1993), McLeod and Wing (2009), Kharlamenko et al. (1995), Taipale et al. (2015b)
C22:6n3	DHA	Phytoplankton	Howell et al. (2003), Sleigh (1987), Hamilton (1995), Vazhappilly and Chen (1998), Mansour et al. (1999), Hughes et al. (2005), McLeod and Wing (2009), Simopoulos (1991), Stevens et al. (2004), McMeans et al. (2015b)

C24:1n9	Phytoplankton	Taipale et al. (2015b)
C20:0	Terrestrial	Taipale et al. (2015b)
C22:0	Terrestrial	Taipale et al. (2015b), Wenzel et al. (2012), McMeans et al. (2015b)
C23:0	Terrestrial	Taipale et al. (2015b)
C24:0	Terrestrial	Taipale et al. (2015b), Zelles (1999), Ruess et al. (2007), Bachok et al. (2003), McLeod and Wing (2009), McMeans et al. (2015b)
a-C15:0	Bacteria	Haubert et al. (2006), McLeod and Wing (2009), Taipale et al. (2015b), Pancost and Sinninghe Damsté (2003), McMeans et al. (2015b)
C15:0	Bacteria	Taipale et al. (2015b), McMeans et al. (2015b)
Cy-C17:0	Bacteria	Haubert et al. (2006), (Zelles 1997, 1999)
i-C15:0	Bacteria	Haubert et al. (2006), McLeod and Wing (2009), Taipale et al. (2015b), McMeans et al. (2015b)
i-C17:0	Bacteria	Haubert et al. (2006), Pancost and Sinninghe Damsté (2003), McMeans et al. (2015b)

Table 2.S2 Paired Wilcoxon tests between "Control" and "Starved" treatments per date. p = NA when each replicates had 50 individuals at the beginning of the experiment and no test could be completed.

#	Date	Duration	W	p	N
1	24/11/2012	0	18	NA	12
2	30/11/2012	6	18	NA	12
3	04/12/2012	10	16	0.806	12
4	11/12/2012	17	24	0.372	12
5	18/12/2012	24	23	0.462	12
6	28/12/2012	34	16	0.808	12
7	03/01/2013	40	23	0.462	12
8	08/01/2013	45	23	0.462	12
9	15/01/2013	52	23	0.462	12
10	22/01/2013	59	23	0.462	12
11	29/01/2013	66	28	0.128	12
12	05/02/2013	73	36	0.005 **	12
13	12/02/2013	80	36	0.005 **	12

14	19/02/2013	87	36	0.004 **	12
15	27/02/2013	94	36	0.004 **	12
16	05/03/2013	101	36	0.004 **	12
17	12/03/2013	108	36	0.004 **	12
18	19/03/2013	115	36	0.004 **	12
19	26/03/2013	122	36	0.004 **	12
20	02/04/2013	129	36	0.004 **	12
21	10/04/2013	137	36	0.004 **	12
22	16/04/2013	143	36	0.004 **	12
23	23/04/2013	150	36	0.004 **	12
24	29/04/2013	156	36	0.004 **	12
25	05/05/2013	162	36	0.004 **	12

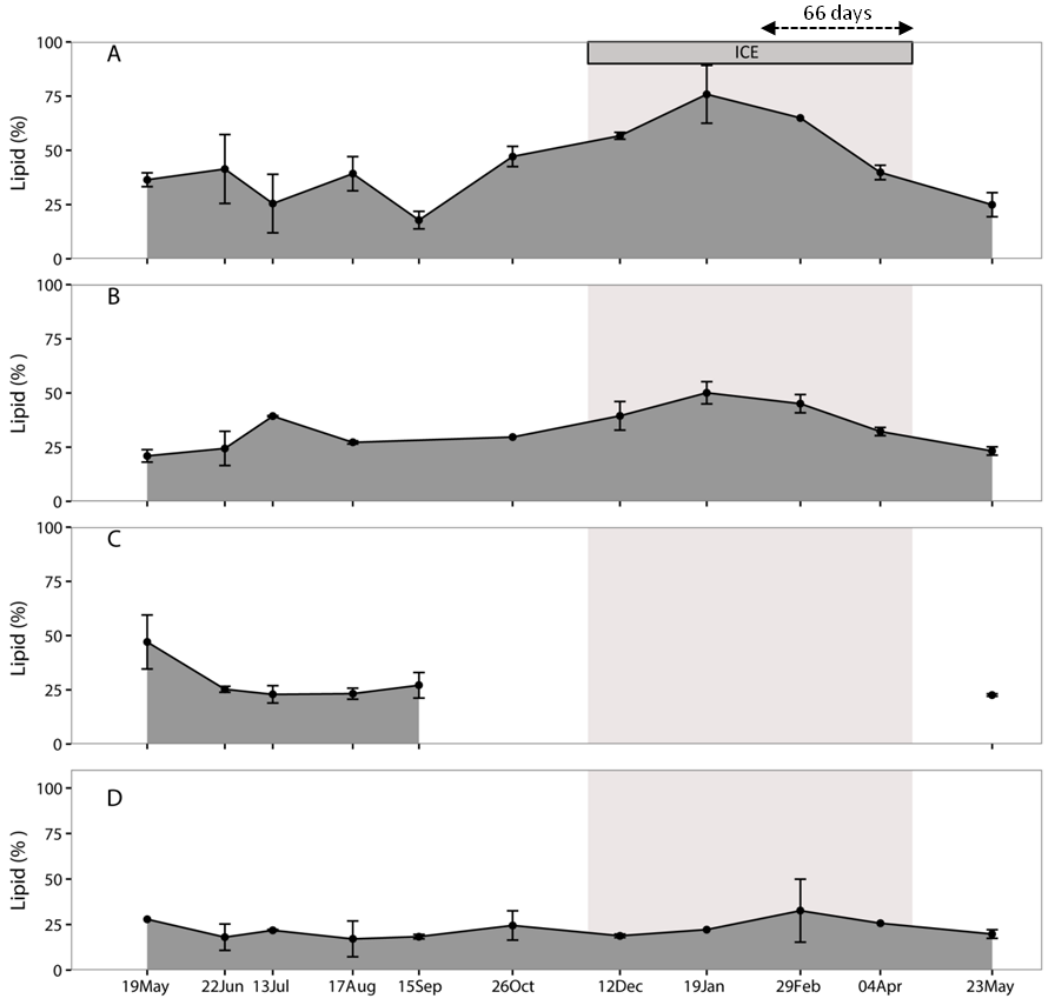

Figure 2.S1 Seasonal pattern of lipid content (%) in 4 different species of zooplankton: A) *Leptodiaptomus minutus*, B) *Cyclops scutifer*, C) *Mesocyclops edax* and D) *Daphnia* spp. Grey shade represents the period when the lake was ice covered. Gaps in certain dates occurred when the species disappeared from the column water.

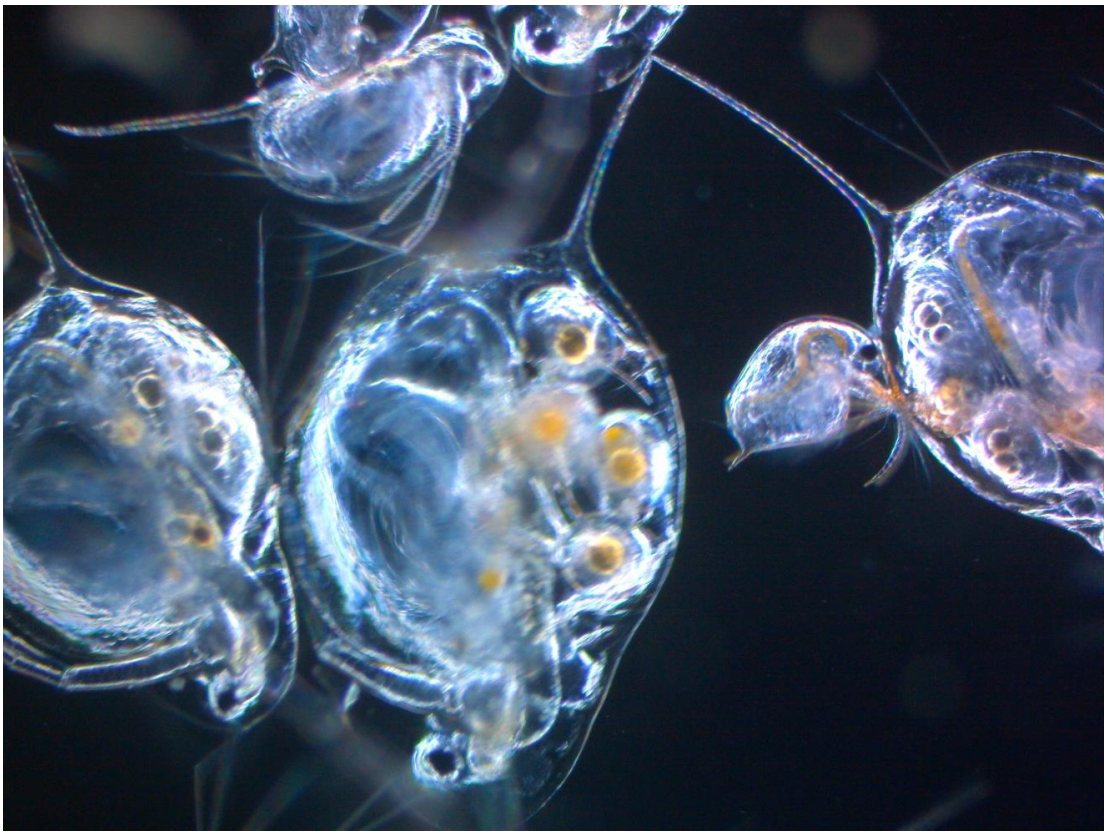

Figure 2.S2 *Daphnia* spp. with parthenogenetic eggs and young (27 January 2017)

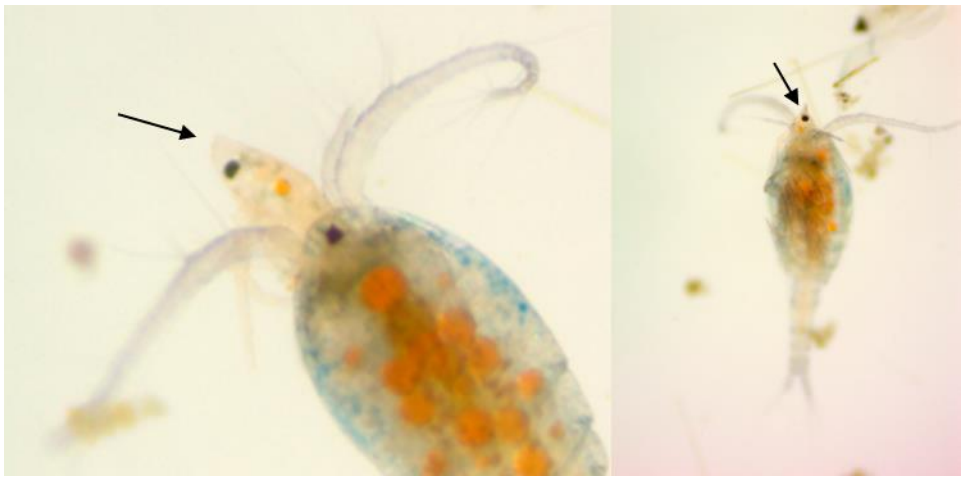

Figure 2.S3 *Mesocyclops edax* eating a *Daphnia* sp. Arrows indicates the prey *Daphnia* sp.

CHAPTER III
SPATIAL DISTRIBUTION OF ZOOPLANKTON ALLOCHTHONY WITHIN A LAKE

PUBLISHED RESEARCH ARTICLE

Title: Zooplankton allochthony is spatially heterogeneous in a boreal lake

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Running head: Spatial heterogeneity of allochthony

Key words: Copepoda, macrophytes, mixing model, stable isotopes, terrestrial carbon

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Abstract

The proportion of consumer biomass from terrestrial origin (i.e. allochthony) has been shown to vary greatly among lakes and also seasonally, but has been assumed to be spatially homogeneous within a lake. Given that the distribution of different organic carbon (C) sources tends to be spatially patchy in most lakes, this assumption may not be warranted. We tested this hypothesis using a spatially-intensive sampling designed to capture the in-lake heterogeneity in terrestrial inputs, phytoplankton, benthic algae and a dominant aquatic macrophyte (*Brasenia schreberi*: Cabombaceae) in a medium-sized boreal lake, and used a dual-isotope Bayesian mixing approach ($\delta^{13}C$, $\delta^{2}H$) to establish the degree of allochthony of the dominant copepod *Leptodiaptomus minutus* (Diaptomididae) across these sites. Samples were collected in spring when tributaries had high flow rates and aquatic primary producers (phytoplankton, macrophytes) had rapid growth rates, and in mid-summer when tributary flows were at the lowest. There was substantial spatial variability in the stable-isotope composition of the copepod and consequently in its levels of allochthony in both seasons. Allochthony in *L. minutus* varied from 34 to 50% in spring and from 45 to 65% in summer, and this range was linked to the spatial variability in the main sources of organic C (terrestrial inputs via tributaries, *B. schreberi* and phytoplankton). Allochthony in *L. minutus* was lowest in areas dominated by macrophytes, and further influenced by the distribution of tributary-derived terrestrial C across the lake. Macrophyte and phytoplankton carbon contributed respectively up to 28% and 38% during growing season (spring) to the diet of the *L. minutus* while benthic algae contribution was negligible. Our results clearly show that the reliance of zooplankton on terrestrial C may be spatially heterogeneous even in a relatively small lake, and in particular that macrophytes, whose distribution is typically patchier than that of phytoplankton, may play a major role in shaping the spatial patterns of zooplankton allochthony in lakes.

3.1 Introduction

One of the major interactions between terrestrial and aquatic ecosystems is mediated by the movement of terrestrial organic carbon to lakes and rivers (Polis et al. 1997, Solomon et al. 2015). At least some of this terrestrial carbon eventually enters aquatic food webs and is selectively allocated to different functions by the aquatic organisms. For example, recent studies have shown that lake bacteria tend to respire algal-derived C, whereas terrestrial carbon is preferentially allocated to biosynthesis (Guillemette et al. 2016). Terrestrial C assimilation leads to variable but often significant proportion of aquatic consumer biomass of terrestrial origin which we refer to as allochthony. The magnitude, variability and regulation of allochthony in freshwaters have received an increasing interest over the past decade, especially in zooplankton (Rautio et al. 2011, Wilkinson et al. 2013a, Berggren et al. 2015). It is now clear that there is a large degree of variability in zooplankton allochthony among lakes (Berggren et al. 2014), from negligible (Pace et al. 2007, Francis et al. 2011) in large, clear water lakes, to > 60% in smaller humic systems (Karlsson et al. 2012, Kelly et al. 2014). Allochthony has been shown to vary as a function of lake productivity (Karlsson et al. 2012), season (Berggren et al., 2015; Rautio et al., 2011), catchment type or size (Babler et al. 2011, Tanentzap et al. 2014) and lake size (Wilkinson et al. 2013a). This high variability in consumer allochthony results from the combination of the terrestrial influence in food sources, the availability of aquatic primary producers and the feeding strategy (Berggren et al. 2014). The main groups of freshwater zooplankton have major functional differences in terms of diet and food preference, and in the retention rates of carbon relative to food source availability and quality (Koussoroplis et al. 2013), which together generate a large degree of variability in allochthony between major zooplankton even within a given lake (Matthews and Mazumder 2003, Brett 2014). While some degree of spatial variability, for example, between profundal and littoral communities (Syväranta et al.

2006) has been addressed, the spatial variability in the relative importance of terrestrial C to lake consumers remains a major uncertainty in our understanding of lake food webs.

Since the variability in allochthony has been shown to be related to organic matter sources among lakes (Wilkinson et al. 2013a), we can reasonably think that within-lake heterogeneity in the various C sources may lead to intra-lake variability in allochthony. For example, although some soil carbon enters the lake by runoff along the land-water interface, most of the terrestrial organic matter arriving to lakes is loaded through the tributaries (Polis et al. 1997). This generates a spatial pattern in the availability of terrestrial C, which has been shown to influence consumer diets (Doi 2009). The spatial patterns of allochthony in aquatic consumers, particularly zooplankton, may be further influenced by the distribution of autochthonous carbon sources i.e. aquatic primary producers (Taipale et al. 2014). Phytoplankton, benthic algae and aquatic macrophytes all contribute to the autochthonous carbon pool, the latter two playing a larger role in shallow depths where light and substrata are not limiting photosynthesis and settlement (Auderset-Joye et al. 2006, Cazzanelli et al. 2012). Currents from tributaries further influence the patterns of macrophyte settlement keeping their biomass low in fast flowing areas (Chambers et al. 1991). Macrophytes and phytoplankton in turn compete for light and nutrients (Scheffer et al. 1993, Vanderstukken et al. 2014) in addition with allelopathic interactions (Erhard and Gross 2006), such that littoral zones with extensive macrophyte development are less likely to have high phytoplankton concentrations. These processes structure the spatial distribution of the different autochthonous organic carbon sources within aquatic ecosystems (Lapierre and Frenette 2009).

The trophic link between terrestrial carbon sources and consumers is mediated by the consumption of dissolved (t-DOC) and particulate terrestrial organic carbon (t-POC)

(Wilkinson et al. 2013a, Berggren et al. 2014). Most of the terrestrial organic matter arriving to lakes is in the form of t-DOC, which itself cannot be taken up by zooplankton and other metazoan consumers. The t-DOC pool can nevertheless be consumed by bacteria and has been shown to support a substantial fraction of the production at the base of the microbial food web in many lakes (Karlsson et al. 2012). Zooplankton acquire allochthonous organic carbon either through the consumption of bacteria or bacterial grazers, or by directly feeding on allochthonous particles (Cole et al. 2006). Although there is still considerable debate as to the importance of these two pathways (Pace et al. 2004, Cole et al. 2006), the current evidence would suggest that the latter is generally minor for zooplankton (Jansson et al. 2007, Berggren et al. 2010b, Mehner et al. 2015), although it may be significant for benthic macroinvertebrates (Gerlach et al. 2014). The autochthonous signature of zooplankton, on the other hand, is acquired through direct feeding on phytoplankton cells, or POC that contains either live or detrital algal C. Benthic algae and associated microbial material and detritus are not available as POC for pelagic suspension feeder zooplankton (Paffenhöfer et al. 1982), although some cladocerans may feed directly on benthic mats (Cazzanelli et al. 2012, Mariash et al. 2014). To our knowledge, there is also no evidence for a direct consumption of macrophyte-derived POC, even when macrophytes contribute to the POC pool (Marinho et al. 2010, Cole and Solomon 2012). However, macrophytes have been shown to release large amounts of DOC to the environment when they are growing (Alber and Valiela 1994, Demarty and Prairie 2009), and are decomposed (Maie et al. 2006). This DOC represents between 1 to 43 % of the total DOC in shallow boreal lakes (Demarty 2009). It is thus likely that the transfer of macrophyte-derived C to zooplankton is also mediated by bacteria, which have been shown to use this organic matter (OM) very efficiently (Findlay et al. 1986, Mann and Wetzel 1996, Wetzel and Sondergaard 1998).

Regardless of its origin, the biologically labile portion of the DOC pool is usually very small and is taken up very rapidly by heterotrophic bacteria upon release to the water (Rosenstock and Simon 2001, Berggren et al. 2010b). Consequently, although the bulk DOC from different sources will move with the water and eventually mix throughout a lake, the labile portion associated to the different DOC sources will most likely be consumed and exhausted locally in the vicinity of the source. Similarly, it is likely that particles originating from the various C sources will sink in the surrounding area of the source and fuel local benthic metabolism. If these various C sources are characterized by different chemical and isotopic properties, then these properties will be transferred to the microbial food web that is utilizing this C locally, and to zooplankton feeding on this food web, in turn potentially generating variability in zooplankton isotopic composition. To our knowledge, there have been no studies to date assessing this potential spatial patchiness in zooplankton allochthony, and the underlying assumption of most previous studies has been that allochthony should be uniform within a given lake.

In this paper we have explicitly tested this assumption by carrying out a high resolution study to quantify the spatial variability in zooplankton allochthony within Lake Simoncouche (Canada), a shallow (2.2 m average depth), medium-sized (0.83 km^2) boreal lake that receives large terrestrially-derived OM inputs from several separate tributaries, but which also has extensive macrophyte development distributed in clumps, and significant algal production in its pelagic and benthic regions. This lake is therefore characterized by a strong spatial heterogeneity in the potential C sources, and we explicitly attempted to link this spatial variability to zooplankton allochthony. In order to do this we first estimated the contributions of different putative carbon sources to Lake Simoncouche by measuring the incoming terrestrial fluxes of DOC and POC, photosynthetic carbon production of phytoplankton and benthic algae, and the DOC release rates of the dominant aquatic macrophyte

Brasenia schreberi (Cabombaceae). We then used DOC aromaticity and biolability as well as ^{13}C isotopic composition of POC to assess how the above mentioned sources contribute to creating spatial heterogeneity across the lake in the putative zooplankton resource pool. Furthermore, we determined the $\delta^{13}C$ and $\delta^{2}H$ of the various sources and of zooplankton biomass to estimate zooplankton allochthony across ten sites within the lake, which covered five habitats dominated by distinct C sources: 1) tributaries with high terrestrial inputs, 2) vicinity of *B. schreberi* beds 3) tributary flowing through macrophyte beds to account for terrestrial-macrophyte interactions, 4) pelagic zones dominated by phytoplankton and 5) near shore control sites far from tributaries or macrophyte clumps to calculate zooplankton allochthony in sites that were not clearly dominated by any of the sources above. Additionally, benthic algae were considered to potentially influence zooplankton allochthony at all sites within Lake Simoncouche given that the mean depth is 2.2 m. Our study was carried out in two seasons to increase the variability range in the relative contribution of different putative zooplankton resources: during the spring, when phytoplankton bloomed, the macrophytes were starting to grow and tributary discharge was in its annual maximum, and in mid-summer, clear water phase, when macrophytes were abundant but growing slowly and discharge from the tributaries was low. We hypothesized that allochthony in zooplankton is spatially structured across the lake driven by the spatial distribution of carbon sources, with the highest allochthony potentially within the plumes of the tributaries. Furthermore, in order to limit the confounding effects of inter-species differences in diet and food preference, we focused our study on one copepod species *Leptodiaptomus minutus* (Calanoida: Diaptomididae), the dominant zooplankton throughout the year in this lake, which is also widespread across the boreal landscape (Carter et al. 1980).

3.2 Material and methods

3.2.1 Study lake and sampling

Lake Simoncouche (48°13'N, 71°14'W, mean depth 2.2 m; maximum depth 8 m; surface area 0.8 km^2, see bathymetric map in supplementary material Fig. 3.S1) is a mesotrophic boreal lake that is surrounded by a dense boreal forest, with its drainage basin dominated by *Abies balsamea* (Pinaceae), *Picea mariana* (Pinaceae) and *Betula papyrifera* (Betulaceae) populations (Montoro Girona et al. 2016). Mean total dissolved phosphorous (TDP) and nitrogen (TDN) concentrations are respectively 8.2 ± 3.1 µgP L^{-1} and 0.3 ± 0.3 mgN L^{-1}, Secchi depth is 3.3 ± 0.3 m, conductivity 115 ± 86 µS cm^{-1}, pH 6.9 ± 0.6, DOC 5.3 ± 0.8 mgC L^{-1} and POC 1.1 ± 0.4 mg L^{-1}. The lake is divided into three basins (Fig. 3.1), with the third basin characterized by an extensive macrophyte bed that can cover more than 25% of total lake surface area, largely dominated by *Brasenia schreberi*, with isolated plants of *Nuphar* sp. (Nymphaeaceae) and *Potamogeton* sp. (Potamogetonaceae). The macrophyte community was also composed to a lesser extent of the submerged *Myriophyllum sibiricum* (Haloragaceae) and the emergent *Typha angustifolia* (Typhaceae). In this study, we focused on the dominant macrophyte *B. schreberi* which has floating leaves. The main tributary represents 70% of the incoming water to the lake and enters in the third basin. The two remaining basins have some isolated areas of macrophytes and the lake is supplied by six permanent and ten intermittent tributaries. From the main tributary in the south to the main outlet in the north, the water crosses the three basins from basin 3 to basin 1. The mean lake water residence time is 50 days, although each of the three major basins likely differs in their own average water residence time, due to their own particular morphometry (see lake morphometry Fig S1). The residence time is also seasonally variable (Vachon & del Giorgio, 2014) amounting to only 30 days in spring but extending to as much as 76

days in winter (D. Vachon, pers. comm.). *Leptodiaptomus minutus* dominates the zooplankton community, representing up to 93% of the total zooplankton biomass in the lake, is found active all year long and is the only species found everywhere in the lake (G. Grosbois, unpubl. data). *Cyclops scutifer* (Cyclopidae), *Mesocyclops edax* (Cyclopidae), *Tropocyclops prasinus* (Cyclopidae), *Aglaodiaptomus spatulocrenatus* (Diaptomidae), *Daphnia* spp. (Daphniidae), *Bosmina* spp. (Bosminidae), *Diaphanosoma* spp. (Sididae) and *Holopedium gibberum* (Holopedidae) constitute the rest of zooplankton community in Lake Simoncouche. The lake was sampled twice in 2013; two weeks after the ice-out (20 and 21 May) when phytoplankton typically produces a spring bloom, macrophytes are in full development, and tributary discharge is high, and again in mid-summer during the clear water phase (5 and 6 August) when phytoplankton is less abundant, macrophytes are widespread but tributary discharge is low.

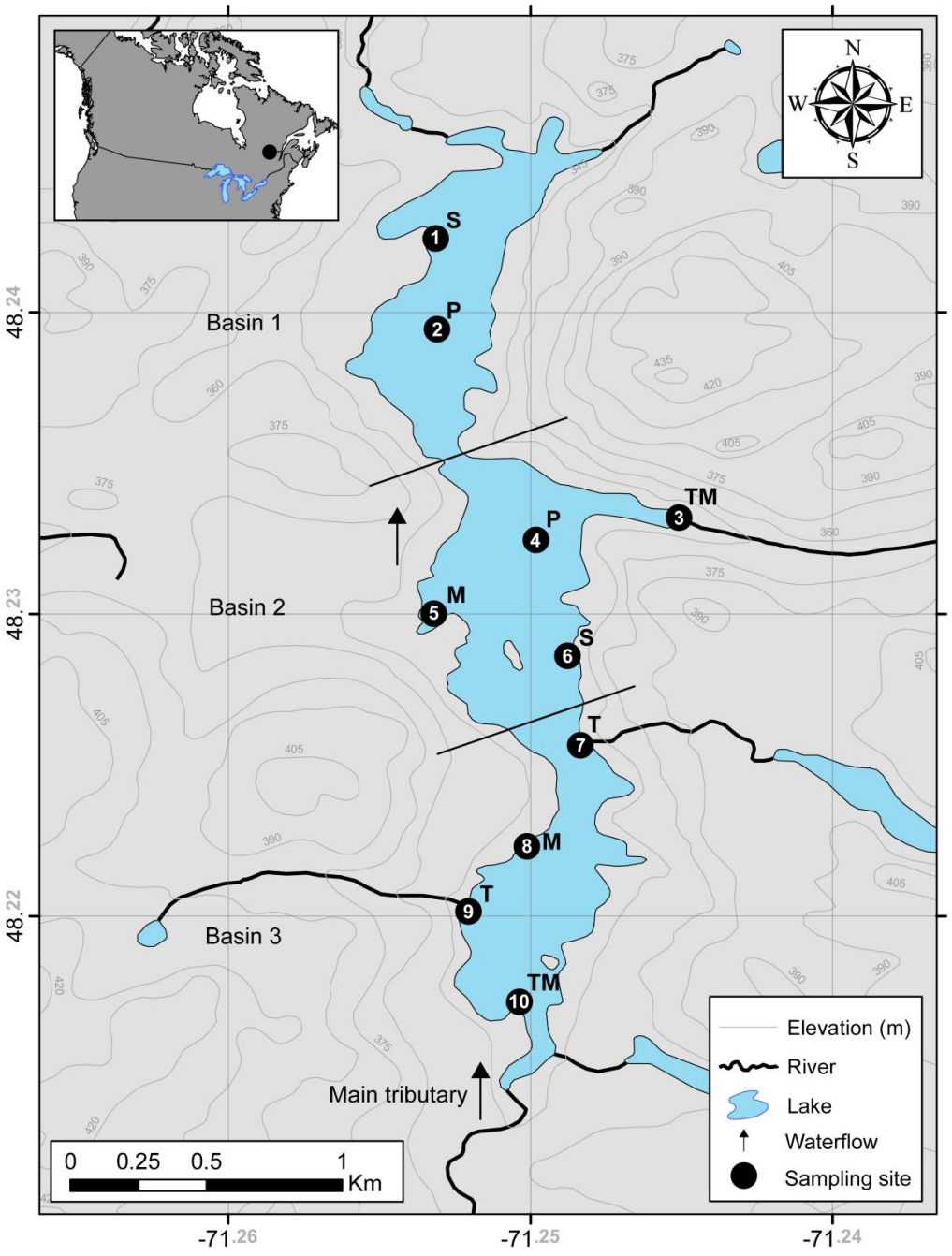
N
W
E
S
Basin 1
Basin 2
Basin 3
S
P
TM
M
T
Main tributary
0 0.25 0.5 1 Km
Elevation (m)
River
Lake
Waterflow
Sampling site
48.24
48.23
48.22
-71.26
-71.25
-71.24

Figure 3.1 Location of Lake Simoncouche (48°13'N, 71°14'O) in the boreal Quebec, Canada. Numbered black dots show the sampling sites in different habitats: tributary (T), macrophytes (M), tributary + macrophytes (TM), pelagic (P) and shore (S). Water flow direction is from basin 3 to basin 1.

3.2.2 Characterization of resource heterogeneity

To characterize the relative contributions of terrestrial carbon inputs, and phytoplankton, benthic algae and macrophyte production to the lake's putative resource pool for zooplankton, we estimated the inputs of t-DOC and t-POC from tributaries, as well as algal production rates and the DOC release from macrophytes. Inputs of t-DOC and t-POC from tributaries were calculated as a function of water discharge, measured with a flowmeter (General Oceanics Inc, 2030R, Miami), and DOC and POC concentrations at the tributary mouths, assuming terrestrial OM dominates the incoming carbon pools (Caraco and Cole 2004). Water for DOC was filtered through combusted GF/D filters and stored in the dark at 4°C for subsequent DOC analyses as in Lapierre and del Giorgio (2014). This type of filter allows the passage of a small portion of the bacterial community, and was chosen because it is the one also used to prepare water for the DOC degradation assays (see below). Previous work in our group has shown that there are no measurable differences in DOC concentration relative to the use of the more conventional 0.45 µm pore size filters (del Giorgio, pers. comm.). Concentrations of POC were estimated only for the size fraction that represents *L. minutus* food source. This was done by passing 20 L of 50 µm sieved lake water (to remove animals) through a 20 µm sieve. Keeping only the > 20 µm fraction allowed collecting the potential food that the selective adult copepods are directly feeding on (Wilson 1973), while very small particles possibly ingested by nauplii, copepodites or rotifers were discarded. Here, this fraction represented about 50% of total POC. The POC samples were kept at -20°C until freeze-drying.

Average macrophyte cover was estimated from aerial photographs taken over several years (1983-2007) and MapInfo professional software v.11.5 (S. Lévesque, unpubl. data). Although the submerged macrophytes cannot be detected using aerial photographs, our field observations showed that the distribution of the latter overlapped almost perfectly with that of *B. schreberi*. Macrophyte DOC release rates were derived on the basis of macrophyte cover following Demarty and Prairie (2009) who measured DOC release rates from similar macrophyte beds also in comparable lakes in Quebec. We used the average rates reported in that study (4.57 mg C m^{-2} h^{-1}) and multiplied these by the total surface covered by macrophytes, and by an average number of daylight hours to estimate the potential whole lake DOC production of *B. schreberi*.

Gross primary production of phytoplankton was calculated with diurnal variations of hourly measurements of dissolved oxygen concentration (O_2) in surface water as in Vachon and del Giorgio (2014). In short, GPP represented by the net O_2 production corrected for respiration rates, was calculated from net ecosystem metabolism (NEM). Daily NEM is defined with the hourly changes of O_2 concentrations over time corrected for gas exchange with the atmosphere and integrated over a period from midnight to 23:00, whereas nighttime changes, NEM_{night}, represents respiration. Assuming that daily and nighttime respiration are similar, GPP is calculated from the difference between integrated night and daily O_2 concentrations (GPP = NEM + NEM_{night}). Photosynthetic rates of benthic algae were measured *in situ* following the ^{14}C-bicarbonate protocol as in Rautio and Vincent (2006). Benthic algae (0.5 cm diameter) suspended in GF/F pre-filtered lake water in replicated 20 mL vials, were spiked with ^{14}C-bicarbonate (specific activity: 80 µCi mL^{-1}) and exposed to eight different light intensities (100%, 75%, 30%, 10%, 4%, 2%, 1%, and 0% of total solar radiation) at the water surface of the lake shore to obtain P-I curves. After 1 h incubation the samples were filtered on GF/F and kept at -20°C until radioactivity

was measured with a scintillation counter (TriCarb 2910TR PerkinElmer, Waltham). PAR intensity was measured at the surface and in the water column during the incubations using a PAR-meter connected to a LiCor Li1000 Data logger, Lincoln. These measurements were used to obtain the vertical light profile, from which the diffuse vertical attenuation coefficient was calculated for the whole lake estimations of phytoplankton and benthic algal productions.

A more detailed spatial characterization of carbon resources to zooplankton was based on POC (>20 µm) and DOC concentrations, ^{13}C carbon isotopic composition of POC (hereafter PO^{13}C), DOC aromaticity and biolability as well as to chlorophyll-a concentration (chl-a). These were measured at ten sites in spring (May) and summer (August) 2013 that were characterized either with macrophyte beds (M), tributaries (T), both macrophyte and tributary influence (TM), pelagic sites (P), and control sites on shore (S) without tributary or macrophytes. Each of these five habitat types was replicated twice resulting in ten sampling sites. The four tributaries selected for this study contributed >95% of the water input to the lake, (D. Vachon, pers. comm.). The specific UV-absorbance ($SUVA_{254}$) was used as an index of DOC aromaticity and the relative proportion of allochthonous (terrestrial) versus autochthonous (algal) carbon sources (Weishaar et al. 2003). It was measured as a DOC normalized absorbance at the wavelength 254 nm using a Cary 100 UV-Vis spectrophotometer (Agilent, Santa Clara). DOC biolability was measured as in Guillemette and del Giorgio (2011). Briefly, water samples (0.5 L) were filtrated through GF/D filter to remove organisms larger than 2.7 µm but to retain the bacteria community. Water was incubated in glass bottles at room temperature in the dark during 14 days. Aliquots were taken every two days, measured for DOC, and lability was estimated from the linear regression of DOC concentration versus time. Chlorophyll-a concentration was measured for each sampling site by filtering 500 mL on GF/F and extracting it on ethanol and measuring by fluorescence following (Yentsch and Menzel 1963a).

3.2.3 Stable-isotope analyses

Terrestrial leaves, macrophytes, benthic algae, phytoplankton, POC and adult *L. minutus* copepods were analyzed for stable isotopes. Once collected, the samples were freeze-dried in the laboratory, grinded and homogenized before encapsulation. The terrestrial signature for $\delta^{13}C$ and $\delta^{2}H$ was obtained either from dead litter collected near each site or live leaves from the main surrounding tree species (n=28). Macrophytes were sampled in sites where they were present (n = 12) and analyzed for the two isotopes. Most samples were from *B. schreberi*, although samples taken from *Nuphar* sp. (n = 3), which was also abundant at site 3, were isotopically indistinguishable from those of *B. schreberi* ($t = 1.47$, $p = 0.19$) and allowed the use of *B. schreberi* as a generic floating-leaf macrophyte indicator. Benthic algae for $\delta^{13}C$ were collected scraping the surface of Nalgene bottles installed in the lake for several months allowing colonization. Due to the lack of material on Nalgene bottles and because it was not possible to physically separate the benthic algal cells from the bulk mat material the benthic algae stable-isotope signature $\delta^{2}H$ was estimated using the Bayesian mixing model with [eq 1] developed by Wilkinson et al. (2013a) using $\delta^{2}H$ of 0.2µm filtered H_2O from each site in the lake and a fractionation distribution ε_H (144.5‰ ± 14.7‰) taken from Berggren et al. (2014). The POC (>20 µm) was collected as described above and was analyzed for $\delta^{13}C$. Zooplankton were sampled from the whole water column with a 50 µm mesh net. The organisms were placed in 500 ml plastic containers with lake water and kept in a cooler until sorting live under a binocular. About 200 adult individuals of *L. minutus* were sorted for each replicate (3) from every site. Samples were then freeze-dried, ground to powder and encapsulated in tin ($\delta^{13}C$ and $\delta^{15}N$) or silver cups ($\delta^{2}H$). The zooplankton $\delta^{15}N$ signature was used to determine zooplankton trophic level, which was then used in the two isotope mixing model. In order to remove storage lipids that might reflect long-term storage diet, lipid extractions were carried out on zooplankton samples

(Syväranta and Rautio 2010). Lipids were removed from zooplankton using 1-mL wash of chloroform/methanol (2:1 v/v) (Bligh and Dyer 1959). Samples were slowly shaken overnight and rinsed three times to remove all the lipids. Lipid-free zooplankton samples were dried in the oven (+60°C) overnight.

Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using a FlashEA 1112 elemental analyzer (Thermo Fisher Scientific Corporation, Waltham) coupled to a Thermo Finnigan DELTA plus Advantage mass spectrometer in the University of Jyväskylä (Jyväskylä, Finland). Deuterium analyses ($\delta^{2}H$) were carried out at Colorado Plateau Stable-Isotope Laboratory in Northern Arizona University. Lake water and solid material $\delta^{2}H$ were measured according to Doucett et al. (2007), using a 1400 C TC/EA coupled to a Thermo-Electron Delta Plus XL mass spectrometer.

To estimate the phytoplankton isotopic composition, $\delta^{13}C$ analyses were carried out on specific algal fatty acids that were recovered from bulk seston samples collected on GF/F filters (Pace et al. 2007, Berggren et al. 2014). We focused on 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3 that are produced by algae (McLeod and Wing 2009, Barberá et al. 2011). Fatty acids (FA) were extracted as in Mariash et al. (2011) using a modified extraction method from Bligh and Dyer (1959). Extracted FA methyl esters (FAMEs) were obtained using a methylation procedure and evaporated to dryness. Samples were then shipped to Memorial University of Newfoundland for $\delta^{13}C$ analysis using a gas chromatograph interfaced with an IRMS via a combustion interface. We assumed a lipid fractionation of 3.8‰, and all FA $\delta^{13}C$ values were adjusted accordingly (Berggren et al. 2014). We analyzed 19 samples from years 2011 to 2013. They showed relatively low seasonal variability (-34.4‰ ─ -45.7‰) in algal FA $\delta^{13}C$, which were always clearly separated from other FA. As for benthic algae, phytoplankton $\delta^{2}H$ signature were estimated using a fractionation distribution (ε_H = 162.8‰ ± 26.1‰) from (Berggren et al. 2014) and [eq 1]:

δ^2H Phytoplankton or Benthic algae(i) = δ^2H H_2O(i) - ε_H [eq 1]

3.2.4 Isotope mixing model

Four potential sources of C were considered to possibly contribute for *L. minutus* diet: terrestrial, phytoplankton, macrophytes (*B. schreberi*) and benthic algae. With two isotopes as tracers (^{13}C and ^{2}H), the mixing model is underdetermined and unique solutions are impossible. We consequently followed a two-step procedure, as recommended by Fry (2013). We initially run a Bayesian SIAR model that took into account the benthic, phytoplankton, terrestrial and macrophyte contributions, to determine which of these four sources likely contributed the least towards the diet of *L. minutus*. SIAR can be run with more sources (n+1) than isotopes (n), and although this greatly increases output uncertainty (Parnell et al. 2010) and number of possible feasible solutions for % source contributions (Fry 2013), it does allow to establish a robust ranking of sources. This procedure allowed us to discard one of the sources (benthic algae), and we were then able to apply a three source, dual-isotope ($\delta^{13}C$ and δ^2H) Bayesian mixing model, adapted from Wilkinson et al. (2014), to more robustly determine the relative contribution of the remaining three major C sources. In this Bayesian mixing model, uncertainties associated to source end-members and consumers but also for the isotopic fractionation between the sources and consumers (carbon fractionation for $\delta^{13}C$ and dietary water contribution for δ^2H) were accounted for. We added a correction to the model for potential trophic carbon fractionation, assuming a per-trophic level enrichment (Δ_C) of 0.4 ± 1.3‰ (Post 2002) adjusted to trophic level (τ). The trophic level of *L. minutus* was estimated on the basis of $\delta^{15}N$ using [eq 2], assuming that the $\delta^{15}N_{Daphnia}$ (see supplementary material Table 3.S2) represent a food web baseline, and $\delta^{15}N$ of *L. minutus* for each site as:

$\tau = (\delta^{15}N_{L.\ minutus} - \delta^{15}N_{Daphnia}) / \Delta_N + 1$ [eq 2]

where Δ_N is the per-trophic-level stable nitrogen isotope fractionation of 3.4 ± 1.0‰ (Post 2002). Trophic enrichment was then calculated accounting for the trophic level using [eq 3] for each site as:

$$\delta^{13}C \text{ trophic enrichment } (\textit{L. minutus}) = \Delta_C * \tau \qquad \text{[eq 3]}$$

where Δ_C is the per-trophic-level stable carbon isotope fractionation of 0.4 ± 1.3‰ (Post 2002). The overall distribution of trophic $\delta^{13}C$ enrichment ± SD was then calculated running equations 2 and 3 in sequence with 50,000 Monte Carlo iterations with random values of Δ_C and Δ_N generated from their assumed mean and SD.

The enrichment in δ^2H across trophic levels is not caused by trophic fractionation *per se* but rather to dietary water. Following Wilkinson et al. (2013a), we assumed that dietary water (ω) contributed 0.07 ± 0.10 per trophic level. The total contribution of water in the organism (ω_{tot} ; [Eq4]) was calculated as:

$$\omega_{tot} = 1 - (1 - \omega)^{\tau} \qquad \text{[eq 4]}$$

where τ is the trophic level. Dietary water enrichment was then calculated with [eq 5] for each sample, as:

$$\delta^2H \text{ enrichment} = \delta^2H_{\textit{L. minutus}} - (\delta^2H_{\textit{L. minutus}} - \omega_{tot} * \delta^2H_{water}) / (1 - \omega_{tot}) \qquad \text{[eq 5]}$$

The overall distribution of δ^2H enrichment ± SD was then calculated with 50,000 Monte Carlo simulations, running [eq 2], [eq 4] and [eq 5] in sequence for each sample. The spatial distribution of allochthony in the lake was visualized in R (R Development Core Team 2015) with kriging interpolation with packages “gstat” and

"maptools", which extrapolate unknown values of zooplankton allochthony for the entire lake surface area from the mixing model outputs (medians) calculated at known locations. The high spatial resolution of this study further allowed us to derive robust average estimates of allochthony for spring and summer for the entire lake with a high number of replicates (n=30) i.e. accounting for all stable-isotope variation in the lake.

3.2.5 Statistical analysis

ANOVAs and Wilcoxon signed-rank tests were performed using the statistical computing environment of R to analyze within-lake differences in DOC, POC, chl-a concentrations and biolability. Pair-wise comparisons were performed using a post hoc test (Tukey's HSD). Pearson correlations were performed using SigmaPlot v.12.3 to test correlations between DOC biolability and chl-a. Food sources, $PO^{13}C$ and zooplankton isotopic composition ($\delta^{13}C$ and $\delta^{2}H$) were normalized subtracting means and dividing by standard deviation when tested using PermANOVA run with PRIMER v 6.1.11 & PERMANOVA+ v1.0.1 (Anderson et al. 2008). PermANOVA analysis was used as a multivariate analysis ($\delta^{13}C$ and $\delta^{2}H$) due to the nature of the stable-isotope data which did not meet the conditions of Gaussian distribution and homoscedasticity.

3.3 Results

3.3.1 Contribution of autochthonous and allochthonous sources to lake resource pool

Terrestrial carbon, phytoplankton, benthic algae and macrophytes all made an important contribution to the Lake Simoncouche carbon pool (Table 3.1). The average spring discharge of the main tributary (site 10) was 689 L s^{-1} while the discharge in the other three tributaries (sites 3, 7 and 9) ranged from less than 67 L s^{-1} to 187 L s^{-1}. Main tributary discharge was about 7-fold lower and the only measurable discharge in summer (97 L s^{-1}). Following the discharge rates, the spring t-DOC input was highest from the main tributary (316 kg d^{-1}) while the smaller tributaries contributed 36 – 94 kg d^{-1}. Summer t-DOC input from the main tributary was almost 6-fold lower (53 kg d^{-1}). Spring input of t-POC was much lower than the t-DOC with the highest inputs coming from the main tributary (9 kg d^{-1}) compared to the other tributaries (2 – 6 kg d^{-1}). Summer t-POC input from the main tributary was about 2-fold lower (4 kg d^{-1}). Mean spring primary production was 330 kgC d^{-1} for the entire lake and decreased in summer to 263 kgC d^{-1}. Benthic production measurements reached 791 kgC d^{-1} in summer accounting for 75% of algal primary production. Macrophytes (*B. schreberi*) were distributed by clump and were totally submersed in spring while leaves reached the surface in summer. Mean cover estimates of macrophytes showed that basins 1, 2 and 3 had macrophyte coverages of 0.54, 2.59 and 11.41 ha respectively, and contained 79% of the total lake macrophyte biomass. Applying the macrophyte DOC releasing rates from Demarty and Prairie (2009), basins 1, 2 and 3 received 0.4, 1.9 and 8.6 kgC d^{-1}, totalling 10.9 kgC d^{-1} DOC generated by macrophytes in the lake as a whole (Table 3.1).

Table 3.1 Contribution of terrestrial, phytoplankton, macrophyte and benthic carbon to Lake Simoncouche in spring and summer. Terrestrial contribution is expressed as t-DOC and t-POC input by the tributaries, phytoplankton and benthic carbon as rate of primary production, and macrophyte carbon contribution as a DOC release from macrophyte beds . NA = not available.

Source	Spring	Summer
t-DOC (kgC d^{-1})	445.8	52.9
t-POC (kgC d^{-1})	17.7	4.4
Phytoplankton (kgC d^{-1})	329.7	262.9
Macrophytes (kgC d^{-1})	NA	10.9
Benthic algae (kgC d^{-1})	NA	790.9

3.3.2 Spatial heterogeneity in the putative zooplankton resource pool

The carbon isotopic composition of POC in the ten different sampling sites (-27.2 ± 1.3‰, Table 3.2) showed similar although slightly less negative values than the measured signature for terrestrial carbon suggesting an overwhelming contribution from terrestrial material with some contribution from an isotopically less depleted source (macrophytes: -24.4‰, benthic algae: -23.0‰). Macrophyte contribution to the resource pool was further evidenced by the spatial distribution of DOC concentrations, which was significantly higher in sites with macrophytes (7.1 mgC L^{-1}, $F_{(1,16)} = 3.40$, $p = 0.01$) than without macrophytes (5.8 mgC L^{-1}) in spring. DOC summer concentrations were less heterogeneous and no spatial distribution was observed ($F_{(9,10)} = 1.655$, $p = 0.22$). The quality of DOC was spatially variable, aromaticity ($SUVA_{254}$) in spring ranged from 2.3 to 4.6, whereas summer values had smaller range (2.7 to 3.2). DOC biolability, which reflects the potential DOC consumption by bacterial communities, varied among the habitats in spring ($F_{(4,6)} = 6.95$, $p = 0.02$) with highest values in the pelagic (Table 3.2), and was correlated with chl-a ($R = 0.80$, $p = 0.02$). Summer DOC biolability showed a different pattern but

still varied across the lake ($F_{(9,10)}$ = 9.00, p = 0.001). Spring concentrations of chlorophyll-a were spatially heterogeneous and significantly different between sites ($F_{(9,18)}$ = 9.29, p < 0.001). This spatial distribution of chl-a followed a gradient with lower concentrations in southern basins 2 (2.3 ± 0.9) and 3 (2.7 ± 1.8) and higher concentration at the opposite of the main tributary in northern basin 1 (3.9 ± 0.7). Summer chl-a concentrations were also spatially distributed and significantly different between sites ($F_{(9,20)}$ = 5.99, p < 0.001). Site 10 near the main tributary had the lowest concentration (0.9 ± 0.4) when compared to the other sites (mean 2.36 ± 0.8).

Table 3.2 Dissolved and particulate organic carbon characteristics of the sampled habitats in spring and summer, including dissolved organic carbon (DOC), specific UV-absorbance (SUVA), rate of DOC degradation (biolability), particulate organic carbon (POC), chlorophyll-a (Chl-a) and 13C of POC (PO13C). Sites as indicated in Fig. 2.1. Values are means ± SD. NA = not available.

		Dissolved organic carbon			Particulate organic carbon		
Habitat	Site	DOC ($mg\ L^{-1}$)	SUVA ($L\ mgC^{-1}\ m^{-1}$)	Biolability ($\mu gC\ L^{-1}\ d^{-1}$)	POC ($mg\ L^{-1}$)	Chl-a ($\mu g\ L^{-1}$)	$PO^{13}C$ (‰)
Spring							
Tributary (T)	7, 9	5.7 ± 0.2	2.5 ± 0.2	18 ± 12	0.4 ± 0.3	3.5 ± 2.2	-26.9 ± 1.2
Macrophytes (M)	5, 8	6.0 ± 0.2	2.6 ± 0.2	26 ± 1	0.4 ± 0.1	1.8 ± 1.1	-27.5 ± 1.0
Tributary + Macrophytes (TM)	3, 10	8.2 ± 2.4	3.6 ± 0.1	53 ± 15	0.6 ± 0.4	3.0 ± 0.8	-25.9 ± 1.3
Pelagic (P)	2, 4	6.0 ± 0.8	4.6 ± NA*	84 ± 22	0.3 ± 0.4	3.4 ± 1.1	-27.2 ± 1.4
Shore (S)	1, 6	5.7 ± 0.2	3.0 ± NA	25 ± NA	0.3 ± 0.2	2.3 ± 1.3	-27.2 ± 1.8
Summer							
Tributary (T)	7, 9	6.6 ± 0.3	2.9 ± 0.3	30 ± 10	0.8 ± 0.2	3.0 ± 0.5	-27.7 ± 1.2
Macrophytes (M)	5, 8	6.3 ± 0.3	2.8 ± 0.2	31 ± 17	0.3 ± 0.1	2.1 ± 0.7	-27.5 ± 0.3
Tributary + Macrophytes (TM)	3, 10	6.5 ± 0.2	3.0 ± 0.0	48 ± 21	0.6 ± 0.3	1.9 ± 1.2	-26.9 ± 0.8
Pelagic (P)	2, 4	6.4 ± 0.1	2.7 ± 0.0	44 ± 9	0.5 ± 0.2	2.4 ± 0.4	-28.2 ± 1.4
Shore (S)	1, 6	6.5 ± 0.1	2.8 ± 0.1	60 ± 17	0.7 ± 0.5	1.7 ± 1.0	-27.9 ± 0.9

*Measured only in Site 2 in Basin 1.

The average $\delta^{13}C$ isotopic compositions of phytoplankton (-40 ± 3‰), terrestrial matter (-28 ± 2‰), macrophyte *B. schreberi* (-24 ± 1‰) and benthic algae (-23 ± 1‰) were significantly different ($F_{(3,53)} = 158.36$, $p = 0.001$) from each other (all data pooled). The mean $\delta^{2}H$ isotopic composition also differed significantly ($F_{(3,85)} = 221.9$, $p = 0.001$) between the sources (phytoplankton -240 ± 7‰, terrestrial organic

matter -154 ± 11‰, macrophytes *B. schreberi* -149 ± 37‰ and benthic algae -224 ± 8‰ ; Fig. 3.2, Table 3.S1).

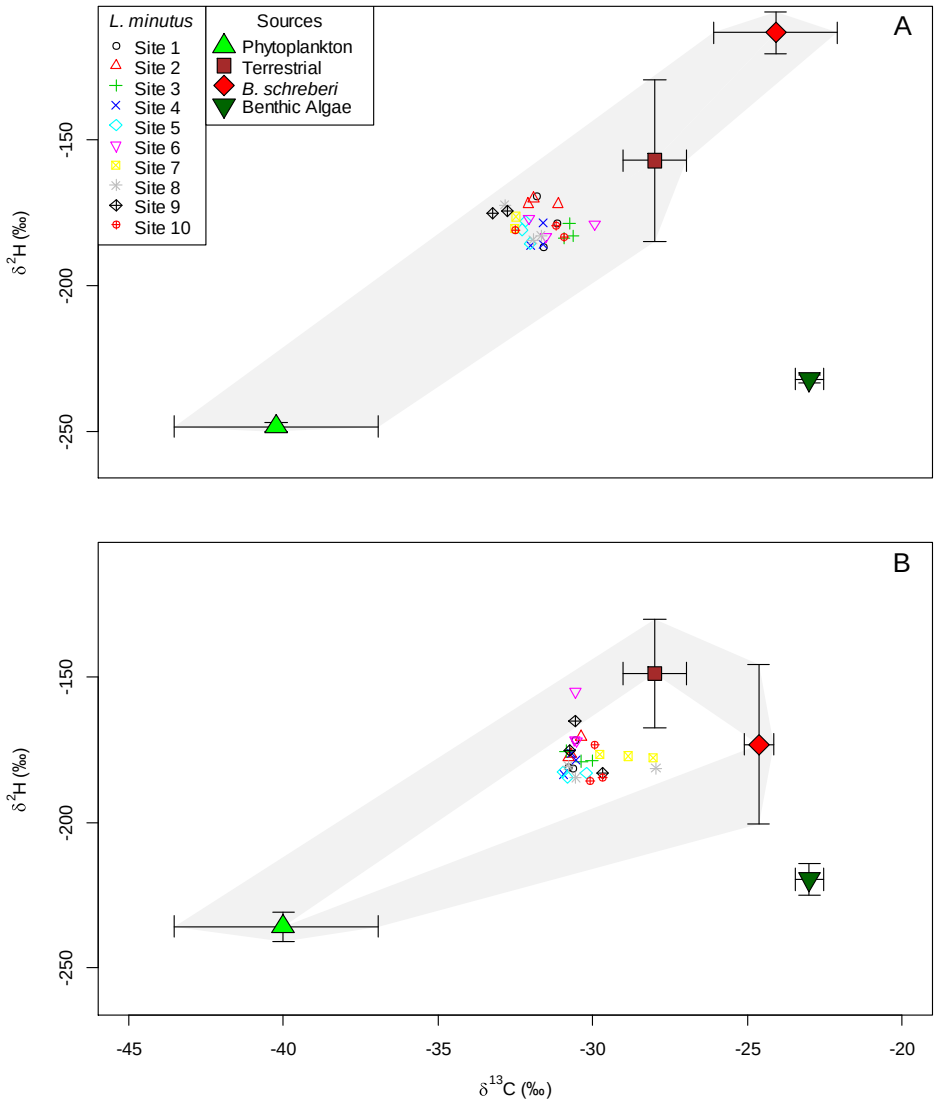

Figure 3.2 The distribution of δ13C and δ2H signatures of *Leptodiaptomus minutus* (corrected for dietary water and carbon fractionation) inside a polygon of the potential food sources + SD: terrestrial, macrophyte (B. schreberi) and phytoplankton. *L. minutus* stable-isotope signatures are represented according to

sampling sites. A) spring (May 2013) and B) summer (August 2013). The isotopic composition of benthic algae that was not included in the Bayesian model is also represented in the figure.

3.3.3 Spatial distribution of allochthony in L. minutus

The $\delta^{13}C$ variability in *L. minutus* among samples showed a range between minimum and maximum value of 3.2‰ in spring (n = 28) and 3.0‰ in summer (n = 30). The spatial range of *L. minutus* $\delta^{2}H$ values was 15.3‰ in spring (n = 26) and 25.7‰ in summer (n = 30) (See supplementary Table 3.S2). There were no significant differences in $\delta^{13}C$ and $\delta^{2}H$ values of *L. minutus* in the sites associated with tributaries in spring (TM and T sites, Fig. 3.1) and all other sites ($F_{(1,24)}$=0.31, p = 0.72). However, *L. minutus* in sites with macrophyte beds had significant differences in $\delta^{13}C$ and $\delta^{2}H$ values compared to those in non-macrophyte sites ($F_{(1,24)}$ = 4.76, p = 0.02). In addition, *L. minutus* collected in sites that had a combination of macrophytes and tributaries (TM) showed $\delta^{13}C$ and $\delta^{2}H$ values that were significantly different from all other sites ($F_{(1,24)}$ = 12.67, p = 0.001). Zooplankton had less depleted $\delta^{13}C$ signatures in sites with both macrophytes and tributary. In summer, no differences were found between the stable-isotope signatures of *L. minutus* sampled at sites with and without tributary influences ($F_{(1,26)}$ = 2.56, p = 0.09), but there were significant differences between sites with and without macrophytes ($F_{(1,26)}$ = 3.21, p = 0.048).

The SIAR outputs showed that the zooplankton diet was expected to be mostly made of phytoplankton, terrestrial and macrophyte originating carbon sources while the benthic organic material was predicted to contribute only 7.2 ± 5‰ (mean ± SD of posterior probability distribution) in spring and 8.2 ± 7‰ in summer to zooplankton diet for all sites (Fig. 3.3a). Given this low apparent contribution of benthic algae, these were excluded from further analyses, which were based on the more robust

Bayesian mass balance model on two isotopes and three food sources (terrestrial, phytoplankton and macrophyte *B. schreberi*). All the isotopic data for *L. minutus* fit well within the source end-member polygons (Fig. 3.2), although in spring the terrestrial and macrophyte end-members were somewhat aligned with the zooplankton (Fig. 3.2a). Consequently, the model did not effectively discriminate the terrestrial and macrophyte contributions in the pooled spring data, as is reflected in the large probability ranges around the mean estimates (Fig. 3.3b). The terrestrial contribution or overall allochthony in spring had a median of 30% but included a high range of the 95 % highest probability densities (0 – 71%), whereas for the summer data, the model output was clearer with a median allochthony of 63 % (41 – 74%) to the *L. minutus* diet. Phytoplankton was the dominant C source in spring (median 42 % with a probability distribution of 28 – 54%, and had a similar but lower contribution in summer (median 34% with a probability distribution of 25 – 42%). Macrophytes appeared to be a significant source in spring, with a median of 28%, but the probability range was large (0 – 51 %), although in summer their contribution to the zooplankton diet was small (Fig. 3.3b).

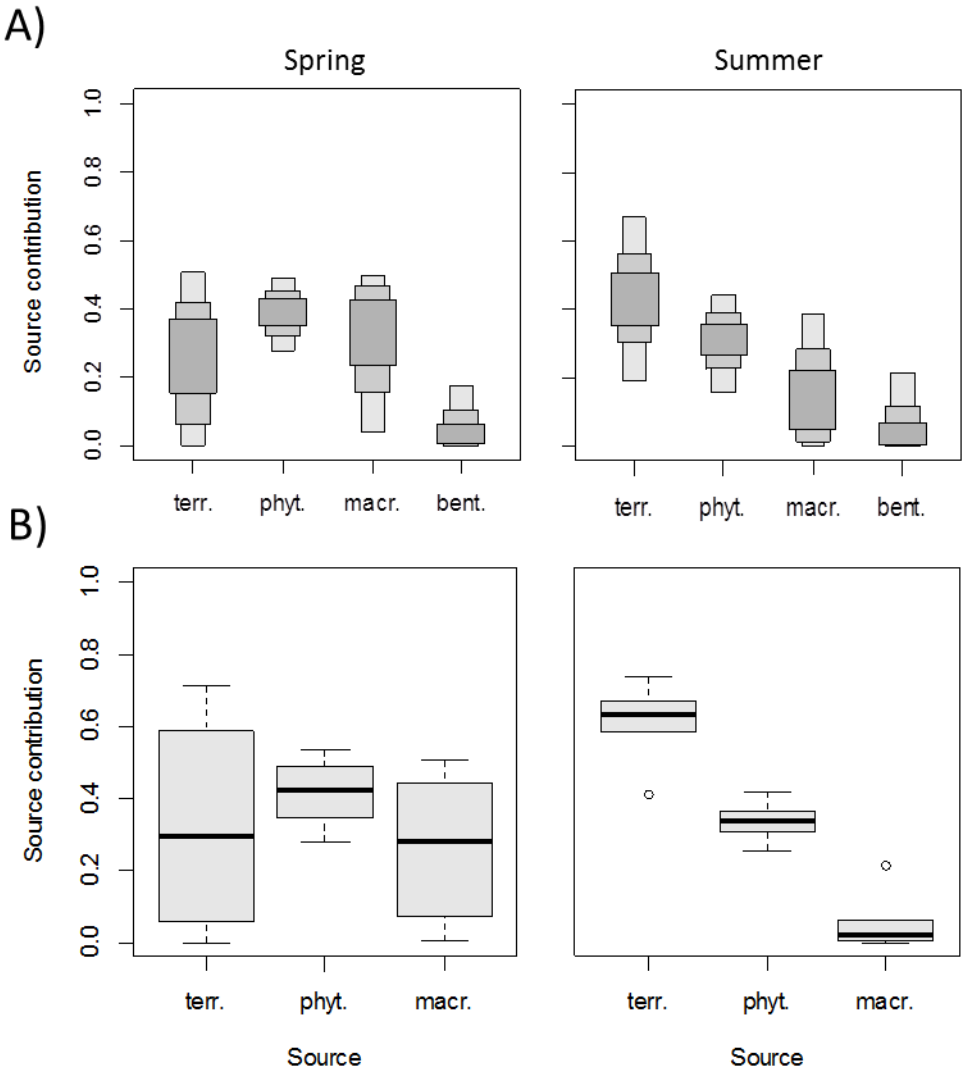

Figure 3.3 a) Spring and summer contributions of terrestrial organic matter, phytoplankton, macrophytes and benthic algae to *L. minutus* tissues calculated with Bayesian SIAR model, and b) fractional spring and summer contribution of phytoplankton, terrestrial organic matter and macrophytes to *L. minutus* tissues, based on Bayesian mixing model. Whisker plots show the distribution of 95% highest densities of contribution probabilities. Open circles are outliers.

At a spatial scale, zooplankton collected from the sites that had no or few macrophytes expressed very low (median 1%) macrophyte-derived C in their tissues. In sites dominated by macrophytes, zooplankton had a much higher putative macrophyte contribution of 29% (0 – 50%; Fig. 3.4). Spatial distribution of allochthony, on the contrary, was not as confined to sites that were in the vicinity of tributaries. In spring when the tributary discharge was at its maximum and water residence time was short (30 days), the incoming terrestrial carbon was assimilated mainly in basin 1, across the lake from the main tributary, where it was reflected as high allochthony values in zooplankton. In summer during the low flow rates, most terrestrial carbon was assimilated close to the main tributary and reflected in zooplankton tissues in the southern part of the lake in basin 3 (Fig. 3.5).

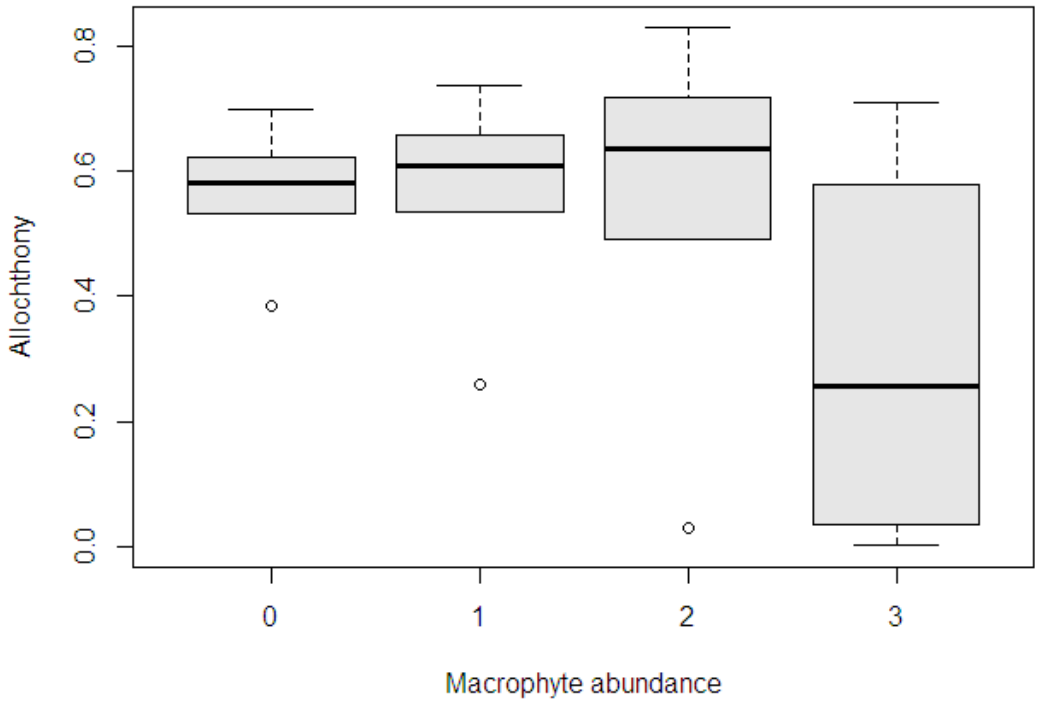

Figure 3.4 *L. minutus* allochthony in relation to the presence of macrophytes. 0 = sites without macrophytes, 1 = low abundance of macrophytes (basins 1 and 2), 2 = high abundance of macrophytes (basin 3), and 3 = macrophyte sites. Whisker plots

show the distribution of 95% highest densities of contribution probabilities. Open circles are outliers.

3.4 Discussion

As expected, we observed a large spatial heterogeneity in the various C sources, both between seasons and spatially across Lake Simoncouche. The pelagic, macrophyte and tributary-dominated sites were characterized by different quantity and quality of organic carbon, as evidenced by varying DOC concentrations and biolability among sites. Our results clearly show that this carbon source heterogeneity was reflected in the zooplankton stable-isotopic composition. The $\delta^{13}C$ variability in *L. minutus* among samples showed a range between minimum and maximum value of 3.2‰ in spring and 3.0‰ in summer, close to the within-lake variability range of 2.7-3.1‰ reported for zooplankton in other lakes (Matthews and Mazumder 2006, Syväranta et al. 2006, Karlsson et al. 2012). The spatial range of *L. minutus* $\delta^{2}H$ values was 15.3‰ in spring and 25.7‰ in summer. To our knowledge, such within-lake $\delta^{2}H$ variability has not been reported for zooplankton in the past, and the variability in zooplankton $\delta^{13}C$ has been either ignored, or attributed to change in community composition or life stages (Grey et al. 2001). Here we show that the isotopic variability in *L. minutus* $\delta^{2}H$ and $\delta^{13}C$ composition was high and attributable to differential use of terrestrial, phytoplankton and macrophyte-based diets (and to a lesser extent, to benthic algae) among different sites. At a fine spatial scale, allochthony in *L. minutus* was most influenced by the presence of macrophytes, which tended to result in decreased proportion of terrestrial C incorporated near macrophyte beds. At a broader spatial scale, the rate of movement of tributary water across the lake and its associated C, which showed strong seasonal patterns, contributed to generating spatial heterogeneity and resulted in zooplankton assimilating different quantities of allochthonous carbon in different lake basins.

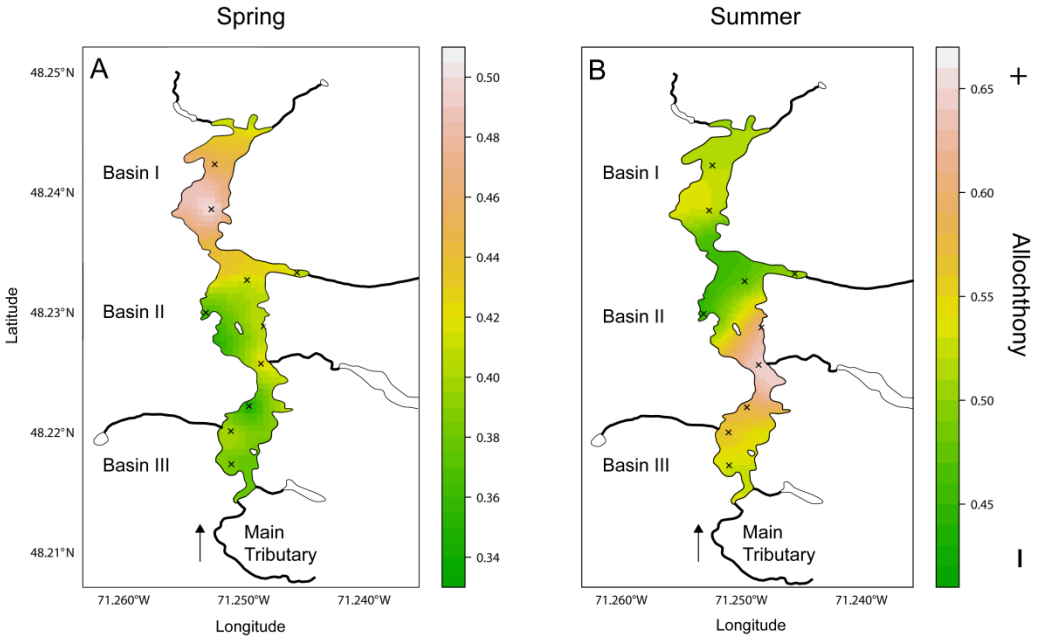

Figure 3.5. Spatial distribution of *L. minutus* allochthony calculated from median output of Bayesian mixing model and extrapolated by kriging in Lake Simoncouche for (a) spring and (b) summer. Notice the different scales.

3.4.1 Spatial heterogeneity of C resources

We have shown that carbon sources were not uniformly distributed across the lake. The majority of terrestrial DOC and POC entered Lake Simoncouche in spatially highly defined location by its main tributary. Carbon inputs (DOC + POC) from this main tributary were highest in spring (325 kgC d^{-1}), about threefold higher than those of the 2nd tributary (100 kgC d^{-1}) and nine fold higher than from the 3rd tributary (38 kgC d^{-1}). This tributary was the only active input of terrestrial C in the summer (57 kgC d^{-1}), clearly demonstrating that t-DOC and t-POC enter the lake unevenly. According to our measurements, basin 3 received around 78% of the tributary C while only 22% entered through basin 2 and very little in basin 1. These tributary C inputs appeared to then dominate the lake C pools: POC $\delta^{13}C$ measured in Lake

Simoncouche (mean -27.3‰) were similar to values reported for terrestrial C3-plants (-28.3‰) indicating allochthonous particles were the dominant component of POC. Whereas we did not measure $\delta^{13}C$ of DOC in this study, previous studies have reported that the isotopic composition of DOC in southern Quebec is also very close to terrestrial C (Berggren et al. 2014). This would suggest that internal sources of DOC and POC are either smaller in magnitude, or yield C that is more labile and therefore is consumed more readily and does not build up in the bulk DOC (Wilkinson et al. 2013b). In the light of our results, the high biolability values associated with macrophyte beds and high seston chl-a content point to the patchy production of labile internal sources, which however were masked with the overwhelming presence of terrestrial $\delta^{13}C$ in the seston C pool.

Lake Simoncouche, like most small and shallow boreal lakes, has a short water retention time (1 to 3 months), and materials brought in by tributaries must be quickly flushed through the lake. There were nevertheless spatial differences in some properties of POC and DOC within the lake. For example, as mentioned above, there were higher lability values in the pelagic (40-89 $\mu gC^{-1}\ d^{-1}$) compared to the inflowing waters (18-39 $\mu gC^{-1}\ L^{-1}$), suggesting that in spite of the high rate of flushing, there may still be local C signatures that reflect local C sources. In this regard, the presence of macrophytes increased the local DOC concentration by up to 20%, and this is probably linked to the release of highly biolabile DOC that bacteria may efficiently convert to new biomass (Findlay et al. 1986). The biolability was lower for the tributary DOC, but passage through macrophyte beds resulted in a 200% increase in biolabile DOC in spring. It is possible that the fresher and more biodegradable carbon from macrophytes (Stets and Cotner 2008) acted as a primer for DOC bacteria degradation. Evidence of such priming effect has been increasingly shown in freshwater literature (Guenet et al. 2010, Danger et al. 2013). Another source of biolabile DOC was revealed with a strong correlation between chl-a and carbon

biolability in spring (R = 0.80, p=0.02) which suggest that the phytoplankton also contributed to a higher carbon consumption by bacteria via high quality DOC release, which is expected for phytoplankton in the exponential growth phase (Myklestad et al. 1989, López-Sandoval et al. 2013).

3.4.2 Spatial variability in putative allochthony

Combining the information of the distribution of the four putative resource pools and their isotopic signatures we were able to make estimates of zooplankton allochthony and its controlling factors across sites within the lake. On average, allochthony of the zooplankton in Lake Simoncouche was moderately high (medians: 34 - 65%) situated in the upper range of the reported allochthony in copepods (3 – 50 %) for North American lakes (Wilkinson et al. 2013a), reflecting the dominance of terrestrial organic material in the lake's resource pool. However, this allochthony was highly variable among different lake habitats and the two seasons considered. A whole lake estimate based on incorporating all samples in the Bayesian mixing model showed a zooplankton allochthony of 30% in spring and 63% in summer. This lower spring allochthony agrees well with previous studies, which showed that calanoids switch to spring herbivory in boreal lakes (Berggren et al. 2015), in subarctic lakes (Rautio et al. 2011) or in marine environment (Gentsch et al. 2008). High zooplankton dependency on spring phytoplankton bloom (Grey et al. 2001) has been attributed to the higher quality of algal food sources and higher diet selectivity from zooplankton. Different physiological demands of spring zooplankton population may also play a role. *L. minutus* demand for highly energetic compounds peaks in spring because individuals are reproducing (Schneider et al. 2016) and require algal-produced polyunsaturated fatty acids that are essential for reproduction (Muller-Navarra et al. 2000, Brett et al. 2009). The allochthony estimates increased in summer. This pattern is in accord with Lake Simoncouche switching from net autotrophy in early summer

to net heterotrophy in August (Vachon and del Giorgio 2014), and supportive of the idea that consumers are more dependent on allochthonous carbon at times when local autochthonous production is reduced.

The spatial variability in allochthony was largely explained by the replacement of allochthonous carbon by macrophyte carbon. When macrophytes were absent or distant from the habitat, *L. minutus* showed high and well constrained allochthony (median 58%). Similarly high allochthony (median 61%) was observed for *L. minutus* in basins 1 and 2 that were located furthest from the main macrophyte bed. However, allochthony in zooplankton sampled next to the growing macrophytes had a median of only 26% (Fig. 3.4). The highly biodegradable carbon leaching out from macrophytes (Findlay et al. 1986) seemed to have reached *L. minutus*, most likely via the microbial loop, but in order to show an influence on the stable-isotope compositions of *L. minutus* the macrophytes needed not only to release large enough quantities of carbon but also to be located in the near vicinity of zooplankton. Such macrophyte influence has also been observed in different basins of a tropical urban lake (de Kluijver et al. 2015).

The spatial variability in *L. minutus* allochthony further indicated that the three lake basins were different in zooplankton assimilation of terrestrial C. Each basin was characterized by very different incoming water inflows and morphometry, with the main tributary in the shallow basin 3 dominating the total inflow. In spring, when the tributary discharge was at its maximum and water residence time was short (30 days), the incoming terrestrial C moved across the lake quickly and terrestrial C appeared to have been carried throughout basins 3 and 2, as was also indicated by the high SUVA values in basin 1, and assimilated mainly in basin 1. Thus, it is interesting to point out that basin 1, which is located furthest from the main tributary, was the basin with the highest degree of allochthony in spring (Fig. 3.5A). The assimilation of this terrestrial

C into zooplankton requires a minimum processing time, because it needs uptake by bacteria and fungi and trophic transfer of the resulting biomass up the microbial food web (Wurzbacher et al. 2010). Our results clearly reflect this process, because at times of high tributary discharge and thus high flushing of the lake water, allochthonous carbon is not necessarily incorporated into zooplankton at the point of entry but rather further downstream, in our case, the distal points such as basin 1. In contrast, in periods of low tributary flow, the residence time of terrestrial C near the point of entry is longer, and this allows for a higher degree of allochthony in the local zooplankton, and we found the highest degree of allochthony (53 – 65 %) in basin 3, close to the main tributary.

Collectively, these results suggest that it is not only the amount and quality of C loaded from land that will determine its influence on aquatic consumer allochthony, but also that the lake morphometry and residence time of this C within the system will play a major role, sensu Grey et al. (2001). Lake Simoncouche is a relatively small, shallow lake that has a short retention time and is thus constantly mixed and flushed, and it could be expected that larger lakes that have longer residence times, and greater spatial decoupling between pelagic, benthic and littoral communities could harbour an even higher degree of spatial variability in zooplankton allochthony. According to our findings, the degree of allochthony in zooplankton appears to be driven mostly by local increases in the availability of autochthonous C derived from phytoplankton and macrophytes. Although phytoplankton is distributed throughout lakes, macrophytes are extremely patchy, and this was here one of the main determinants of the spatial heterogeneity in zooplankton allochthony. Macrophytes are a major feature of the majority of lakes in the boreal biome and elsewhere, but have to our knowledge seldom been considered in food web studies on zooplankton allochthony. It is clear that habitat heterogeneity and alternating states sensu altering phytoplankton dominated vs. macrophyte dominated state in shallow lakes (Janssen

et al. 2014) will have to be taken into account in future studies of allochthony in lakes.

3.5 Acknowledgements

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3.6 References

The reference list of each individual chapter has been included in the final reference section at the end of the thesis.

3.7 Supporting information

Table 3.S1 Raw stable-isotope signatures ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$) of *Leptodiaptomus minutus* and potential food sources in Simoncouche lake. NA = Not available.

Sample	Season	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	N	$\delta^{2}H$ (‰)	N
Water	Spring	NA	NA	NA	-87.6 ± 1.1	9
Terrestrial	Spring	-28.0 ± 1.0	0.1 ± 1.8	8	-157.1 ± 27.8	6
Phytoplankton*	Spring	-40.2 ± 3.3	NA	12	-248.5 ± 1.5	9
B. schreberi macrophyte	Spring	-24.1 ± 2.0	0.6 ± 1.9	6	-113.1 ± 7.1	6
Benthic Algae	Spring	-23.0 ± 0.5	1.2 ± 0.3	3	-232.1 ± 1.5	9
POC >20µm	Spring	-26.9 ± 1.4	2.4 ± 1.3	30	NA	NA
L. minutus	Spring	-32.6 ± 0.8	6.7 ± 0.5	28	-167.0 ± 4.5	26
Daphnia spp.	Spring	NA	3.3 ± 0.4	3	NA	NA
Water	Summer	NA	NA	NA	-74.8 ± 5.3	16
Terrestrial	Summer	-27.9 ± 2.1	-0.9 ± 1.9	15	-148.9 ± 18.6	19
Phytoplankton*	Summer	-40.2 ± 3.3	NA	19	-235.7 ± 5.0	16
B. schreberi macrophytes	Summer	-24.6 ± 0.5	-3.6 ± 3.0	6	-173.2 ± 27.3	9
Benthic Algae	summer	-23.0 ± 0.5	1.2 ± 0.3	3	-219.5 ± 5.3	16
POC >20µm	Summer	-27.6 ± 1.0	2.0 ± 0.8	20	NA	NA
L. minutus	Summer	-31.1 ± 0.8	5.6 ± 0.2	30	-164.6 ± 5.6	30
Daphnia spp.	Summer	NA	2.6 ± 0.1	3	NA	NA

* Phytoplankton values are from the entire year

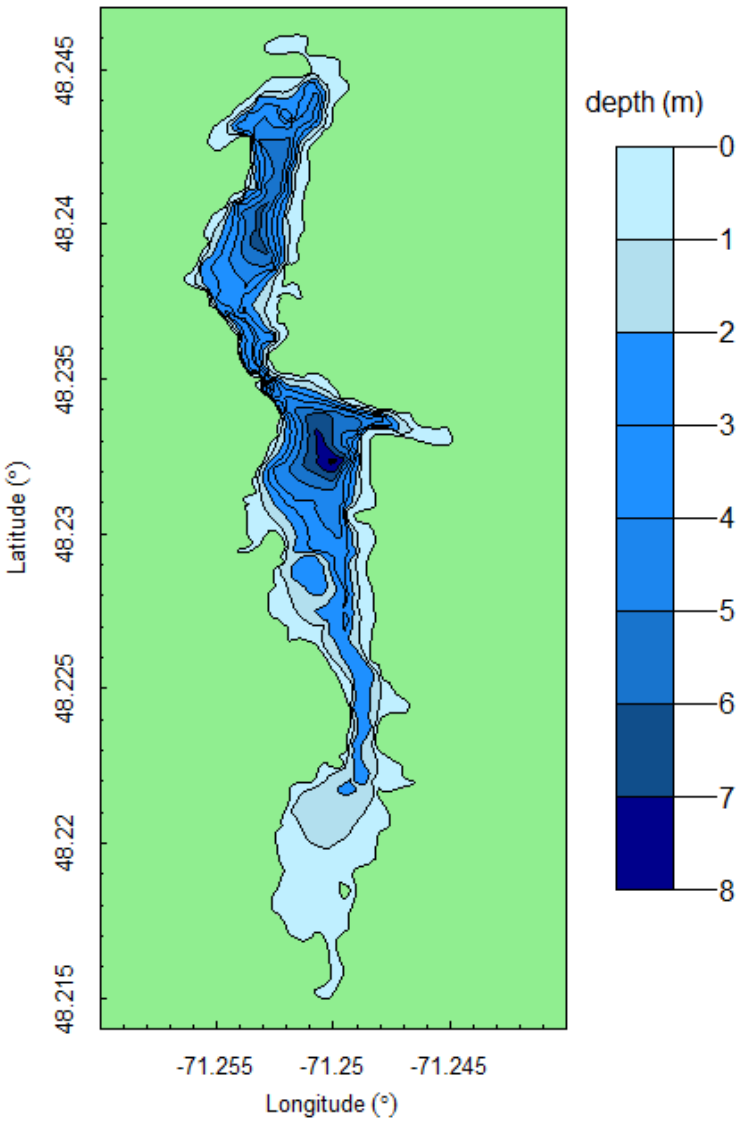

Figure 3.S1 Bathymetric map of Lake Simoncouche.

GENERAL DISCUSSION

Seasonal variability of allochthony

In this study, the terrestrial contribution in biomass, i.e. allochthony, was significant for the zooplankton community. The mean degree of allochthony of the zooplankton community was 52%, ranging from 32% in April to 66% in January and was highly variable across seasons (Fig 1.1). These numbers are very similar to the mean allochthony (56%) calculated for Swedish boreal lakes from late winter to early autumn (Berggren et al. 2015) and are in accordance with the summer allochthony range of 20–40% calculated for zooplankton from North American lakes (Cole et al. 2011). Differences exist among taxa with an annual mean allochthony of 54% in *Leptodiaptomus minutus*, 51% in *Cyclops scutifer* and 47% for both *Mesocycops edax* and *Daphnia* spp. These estimates agree well with a multi-lake study of lakes in North America that estimated cladoceran allochthony to range from 5 to 76% and copepod allochthony from 3 to 50% (Wilkinson et al. 2013a). Mean summer allochthony was lower at 35% and 20%, respectively, for cladocerans and copepods. Our estimates were also higher than for another multi-lake study for boreal lakes that found values of 31%, 18% and 16%, respectively, for cladocerans, cyclopoids and calanoids in Québec (Canada) (Berggren et al. 2014) and allochthony values of 22% for calanoids and cyclopoids and cladocerans both having a mean allochthony of 56% in Swedish lakes (Berggren et al. 2015). Our estimates, being either equal or greater than previous estimates even those coming from humic lakes, are likely due to our lipid extraction. The results from this thesis look separately at the lipid and the non-lipid fractions of zooplankton. This distinction of the considered fraction is one of the major contributions of the study for describing different patterns in t-OM contribution to zooplankton biomass (Fig. 1.1, Fig. 2.4). Phytoplanktonic FA are retained

preferentially in lipid reserves by zooplankton (Galloway et al. (2014); Fig 2.4). Stable-isotope signature of lipids are depleted relative to the entire biomass of the organism and lipid reserves can have important consequences for the stable-isotope signature of organisms (Syväranta and Rautio 2010). In this study, the allochthony ratio of consumers is based on the stable-isotope signature of animals without lipids and consequently does not account for phytoplankton-rich lipid reserves. Lipid reserves would have decreased significantly the general allochthony of the studied consumers and would have accentuated and modified the seasonal pattern of allochthony.

Seasonal patterns of allochthony and the stable-isotope signatures showed very strong similarities among taxa (Fig 1.2). A decrease in allochthony with more depleted stable-isotope signatures in spring were measured for all species (*L. minutus, C. scutifer*, *M. edax* and *Daphnia* spp.) revealing a combined use of lipid reserves and the exploitation of an early phytoplankton bloom starting as soon as the light penetrated the lake through the melting ice. A similar shift of stable-isotope signatures has also been observed in the few published seasonal studies (Grey et al. 2001, Berggren et al. 2015). Allochthony was relatively stable in *C. scutifer* and *L. minutus* in other seasons, especially in the winter when allochthony stabilized around 60%, suggesting that individuals active in the winter were not significantly feeding. A more significant variation was measured for *Daphnia* and the predatory *M. edax*. Low allochthony values slowly increased from the spring to the middle of winter for *Daphnia*. For *M. edax,* allochthony showed a sharp decrease in June to reach the lowest allochthony values for all individuals, increasing again in the autumn. *M. edax* predation on cladocerans, including on *Daphnia* (Fig. 2.S2), is confirmed by the low spring allochthony in cladocerans reflected in the summer values of *M. edax* biomass.

Allocation of terrestrial organic carbon in lipids

Lipid reserves in zooplankton, characterized by FA concentrations, were very variable among species and seasons (Fig. 2.3). Species that stay active throughout the year accumulate lipids and associated FA as water temperatures decrease (Lee et al. 2006). In Lake Simoncouche, zooplankton accumulated FA in the autumn along with decreasing temperatures but also continued after the ice had formed revealing a critical feeding period essential for their survival (Fig 2.1). Contrary to what is commonly believed, algal production is still widely available during the first part of winter despite the very low light conditions (Fig 2.3). Among the accumulated FA, no long-chained saturated FA (LC-SAFA: 20:0, 22:0, 23:0, 24:0), biomarkers of terrestrial organic matter, accumulated in large quantities in any of the studied species (Fig 2.4). In the same way, very few bacterial biomarkers (a15:0, 15:0, i15:0, i17:0) were identified in the accumulated FA. While it does not inform us about the utilisation of t-OM for organism metabolism, the lack of LC-SAFA accumulated in the lipid reserves suggests that t-OM was not directly accumulated in lipid reserves from the environment. These results contradict the idea of t-OM being considered as an alternative to phytoplankton when aquatic primary production is low (McMeans et al. 2015a, Taipale et al. 2016). LC-SAFA are considered as recalcitrant molecules for zooplankton and their role for bacterial growth in not very well understood. Aquatic bacteria can be significantly subsidized by low weight terrestrial molecules (Berggren 2010) but the LC-SAFA effect on aquatic bacteria or other bacteria as endosymbionts is not clear (Maczulak et al. 1981). While LC-SAFA do not seem to represent an appropriate diet for zooplankton, they could be used by bacteria and then transferred to zooplankton. However, according to the low branched FA (BrFA) accumulated in zooplankton, t-OM, even if trophically upgraded in bacteria, appears to have not been assimilated in lipid reserves in any of the studied species. Trophic upgrading by other organisms, such as heterotrophic flagellates, is not well known and the modification

of terrestrial OM in lower trophic levels needs to be investigated (Desvilettes and Bec 2009). However, if massive t-OM inputs would have subsidized bacterivorous heterotrophic flagellates and been assimilated in lipid reserves, significant amounts of unmodified bacterial biomarkers (BrFA) should have been measured in the zooplankton (Ederington et al. 1995).

Combining stable isotopes and fatty acids

The degree of allochthony of the main zooplankton taxa, based on stable isotopes of the non-lipid fraction (Fig 1.2, Fig 3.3) combined with FA analyses of their lipid reserves (Fig 2.5), gave complementary information regarding the differential allocation and the use of t-OM by the zooplankton community. Previous attempts to combine both techniques have provided important contributions to the understanding of t-OM assimilation mechanisms in zooplankton (Perga et al. 2006, Syväranta and Rautio 2010, Yang et al. 2016). Also, one of the best techniques to measure the stable-isotope signature of phytoplankton mixed with terrestrial detritus is to carry out stable-isotope analysis on specific compounds, i.e. PUFA biomarkers of algae (Fig 3.2, Berggren et al. (2014)). Future research looking at allochthony within aquatic organisms will have to account for both approaches; FA analyses provide information regarding the energy stored by consumers while stable isotopes show the origin of the analyzed material. Apart from lipids, other classes of molecules make-up the biomass of aquatic consumers and each class can have a different origin. Taipale et al. (2016) studied the proportions of carbohydrates, lipids, lignin and proteins assimilated in *Daphnia* that grew on a terrestrial, reed or phytoplankton diet and found that carbohydrates can be a substantial source of energy sparing lipids and proteins for structural components of cells. This shows that understanding the different allocation by zooplankton of all C sources including t-OM is fundamental

for understanding the role of each C source for aquatic organisms and aquatic ecosystems.

Terrestrial OM is usually seen as homogeneous among C3 or C4 plants from stable-isotope signatures (Yang et al. 2014). Terrestrial OM is made up of materials having different origins, such as branches, leaves, roots or soil, and is comprised of different types of molecules (e.g. LC-SAFAs, carbohydrates, lignin) that have major differences in lability. While lignin molecules cannot be used by zooplankton because of their resistance properties (Taipale et al. 2016), low molecular weight C (LMWC) compounds can subsidize most of the bacterial production showing high lability (Berggren et al. 2010b). Terrestrial OM in aquatic ecosystems is thus very diverse and its composition can vary tremendously depending on the terrestrial plant communities characterizing the catchment basin. This diversity is not often accounted for in the t-OM measured from the water column but rather ignored with optical and stable-isotope analyses that only record a large terrestrial dominance in the predominant t-DOC (Wilkinson et al. 2013b). In the manner of the LMWC analysis, FA analysis aims to consider the t-OM quality and probability of interest for consumers. Addressing the t-OM composition and quality rather than considering allochthonous input as a uniform mixture would help us to understand when and how t-OM will be used by aquatic organisms.

Driving factors of allochthony

Terrestrial organic matter

In Lake Simoncouche, t-OM inputs have been identified as a driving factor for the seasonal variation of allotrophy as well as for the spatial variation of allochthony via the main tributary and associated hydrology of the lake. Terrestrial OM is under-

dissolved or in particulate form in inland waters and both forms have been identified as driving factors of zooplankton allochthony in the past (Cole et al. 2006, Berggren et al. 2014). Terrestrial DOM can influence cyclopoid allochthony via the microbial food web and bacterivorous micro-zooplankton such as ciliates, flagellates and rotifers (Jansson et al. 2007). As in Berggren et al. (2014), this study found some relation between cyclopoid allochthony (*C. scutifer*) and bacterial production (Chapter I) confirming the same food web links. Terrestrial POM $\delta^{13}C$ is known to influence the allochthony of filtering-feeder cladocerans such as *Daphnia* (Rautio et al. 2011). Calanoid allochthony appears linked to POM food sources as well, but in contrast to filter-feeders, they have the ability to select what they eat and are believed to be more linked to the presence (or lack) of phytoplankton than to the presence of t-POM (Berggren et al. 2015). This is corroborated by the multiple linear regression results presented in Chapter I where gross primary production was the first explaining factor of zooplankton allotrophy. Furthermore, t-POC is now regarded as being a poor subsidy for pelagic zooplankton (Wenzel et al. 2012, Scharnweber et al. 2014a, Mehner et al. 2015). The concentration of t-DOC can rarely explain zooplankton allochthony (Cole et al. 2006, Berggren et al. 2014) in contrast with t-DOC composition and lability that are essential for understanding its integration within aquatic food webs. This study accounted for terrestrial inputs via the tributary inflow rather than the t-DOC concentration in lake water. As this variable represents “fresh” t-OM arriving in the lake—as it did not undergo any metabolic changes in the lake environment—it is very likely that a significant share of this t-OM can be readily assimilated by the aquatic microbial food web and transmitted to higher trophic levels. In this respect, results from this thesis demonstrated that t-OM inputs into the lake have an influence on zooplankton allotrophy. This shows nicely that as t-OM is often non-limiting in aquatic ecosystems, t-OM quality and lability drive t-OM integration in aquatic food webs more than t-OM quantity and availability.

Autochthonous primary production

Gross primary production was the first influencing factor of zooplankton production (Chapter I) and phytoplankton FA were the most accumulated FA in zooplankton lipid reserves from Lake Simoncouche (Chapter II). Furthermore, we know that phytoplankton FA are used by zooplankton, especially by calanoid copepods, for reproduction (Schneider et al. 2016). Phytoplankton compounds are thus extremely important for zooplankton growth and reproduction but might also strongly influence the degree of allochthony in biomass. This is in agreement with the increasing evidence that allochthony in calanoids and cladocerans is defined by the lack of a high quality phytoplankton source more than the presence of t-OM (McMeans et al. 2015a, Taipale et al. 2016). Alternative autotrophic sources can also decrease allochthony, as demonstrated by samples recovered in the vicinity of aquatic macrophyte beds in Lake Simoncouche. Macrophytes are known to release very biolabile DOC that can substantially subsidize the microbial food web (Findlay et al. 1986, Lapierre and Frenette 2009). The lower allochthony in bacteria is consequently reflected in bacterivorous organisms and zooplankton. Macrophyte contribution to zooplankton via particulate organic carbon has never been shown and never specifically tested even if macrophyte contribution to POM has already been measured (Marinho et al. 2010, Cole and Solomon 2012). In arctic aquatic ecosystems, benthic contribution can dominate the primary production of a lake or pond and be a significant source for pelagic consumers (Vadeboncoeur et al. 2002, Mariash et al. 2014) diminishing as well their degree of allochthony. Collectively, these observations demonstrate that autochthonous sources are a major driving factor of allochthony. Whether autochthonous sources represent an alternative source to the allochthonous sources or are synergistically integrated remains uncertain in the literature. The seasonal results obtained in Lake Simoncouche suggest that primary production and terrestrial inputs are assimilated in biomass but not in lipid reserves.

Zooplankton life strategy

Seasonal variation of allochthony and lipid accumulation was taxon-dependent in Lake Simoncouche. This taxon dependence is consistent with species having their own habitat, life strategy and diet. Habitat influences allochthony as some species are associated with littoral, pelagic or benthic environments that can determine the degree of allochthony (Chapter III). Different primary producers dominate each habitat and zooplankton inhabiting each of these habitats are adapted to foraging within these dominant food sources. For example, some very low allochthony ratios have been measured in Pacific western lakes where zooplankton exploit significant primary production taking place below the mixed layer (Francis et al. 2011). It is also well known that littoral organisms, such as macroinvertebrates, are often more allochthonous than that of their pelagic counterparts due to a bigger influence of t-POM inputs from the adjacent terrestrial ecosystem (Scharnweber et al. 2014a). To occupy different ecological niches and avoid strong competition, species from the same habitat have different diets. A species' evolutionary history determines whether the species favours a heterogeneous or homogeneous diet and influences the associated feeding behaviour for each species. Together, they influence the amount of t-OM assimilated. For example, raptorial predation as the dominant feeding behaviour of cyclopoid species will be highly influenced by the allochthony of its prey. On the contrary, filter feeders will be much more dependant of the composition of the suspended particles (Berggren et al. 2014). These allochthony ratios that are dependent of diet are thus driven primarily by evolutionary history. Furthermore, species having one or more well-defined reproduction periods in the year (uni or multi-voltine) have differing degrees of allochthony depending of the moment of their life cycle. Calanoid copepods, such as *L. minutus* for example, accumulate phytoplanktonic FA to be able to transfer them to their offspring via eggs (Schneider et al. 2016). Chapter I and II demonstrated that allochthony in the non-lipidic biomass

can be very high (~60%) in winter even if individuals accumulate phytoplanktonic FA in lipid reserves that represent more than half of their biomass. The species, according to their habitats and their life strategies, can have very different allochthony values that may vary strongly throughout the year. The degree of allochthony of an entire zooplankton community without providing any information about individual species is thus poorly informative.

Upscaling allochthony at the ecosystem level

Combining zooplankton allochthony and production to calculate zooplankton allotrophy for an entire year allows not only estimating when t-OM is used by zooplankton community but also provides a very precise quantification of how much t-OM is assimilated in zooplankton biomass overall. To our knowledge, this is the first time that such precise seasonal estimations have been made for all the main species of a zooplankton community in a lake or in ocean. At the deepest point of the Lake Simoncouche, the estimates of zooplankton production ranged from 16.3 mg C m^{-2} d^{-1} in June to 0.1 mg C m^{-2} d^{-1} in February following known pattern of zooplankton production in aquatic ecosystems with pronounced seasonal variations of physical and biological factors as described by the plankton ecological group (PEG) model (Sommer et al. 2012). Allotrophy ranged from 0.04 mg C m^{-2} d^{-1} to 9.2 mg C m^{-2} d^{-1}. Only two studies have previously tried to link zooplankton production and allochthony through different techniques for calculating zooplankton production estimates using specific equations that deduced zooplankton production from biomass, taxa-specific parameters and water temperature. The first survey (Mehner et al. 2015) calculated crustacean pelagic production from the same period as this study (2011–2012) finding production to range from 3.5 to 12.8 g C m^{-2} y^{-1}. These values from small temperate shallow lakes in northern Germany are higher than those measured for the boreal Lake Simoncouche, having yearly estimates of 1.42 g C m^{-2}

y^{-1} for zooplankton production. The second study (Kelly et al. 2014) focused on ten lakes from the northern Midwest of the United States and estimated that in summer 2011 zooplankton production ranged from 3.7 to 53.1 g dry mass m^{-2} y^{-1} converting into 1.5 to 21.2 g C m^{-2} y^{-1} (C content = 0.4 * dry mass ; Huntley and Lopez (1992)). These values are comparable to those of Lake Simoncouche if we extrapolate the summer mean value to the entire year (16.1 g C m^{-2} y^{-1}), again highlighting the fact that including winter measures is very important for estimating a realistic annual zooplankton production. Comparisons of zooplankton allotrophy are more difficult as this study is the first to provide such estimates. However, raw data from Appendix C of Kelly et al. (2014) allows us to access zooplankton production estimates and the degree of allochthony and thus calculate allotrophy. From eight of the ten lakes studied (allochthony was not available for all the lakes), the zooplankton allotrophy would range from 2.6 to 9.4 g C m^{-2} y^{-1}, very similar to our allotrophy estimates. Other studies aimed to evaluate t-OM effect without performing any specific measurements of zooplankton production (Cole et al. 2002, Francis et al. 2011, Brett et al. 2012, Karlsson et al. 2012, Lau et al. 2014) or fish production (Karlsson et al. 2015). This is not surprising as determining zooplankton production based on cohort analyses is very time consuming and several attempts have tried to circumvent these analyses and standardize the zooplankton production measurements using new enzymatic techniques (Sastri and Roff 2000).

This study presents some quantitative estimates of zooplankton production and allotrophy extrapolated to the entire lake ecosystems. Lake Simoncouche is considered as a typical shallow boreal lake that could permit the first extrapolation to the landscape scale. Estimating the quantitative share of t-OC that is assimilated, stored, processed, respired or transmitted to higher trophic levels by the zooplankton community would provide essential information to the understanding of the role of inland waters in processing t-OC transported from terrestrial to marine ecosystems.

The role of inland waters in the global C cycle has recently been updated (Cole et al. 2007) and is no longer considered to be passive but rather very active, driving CO_2 flux into the atmosphere and C storage within sediments. Both processes are deeply influenced by the aquatic food web including the key zooplanktonic trophic level, thereby highlighting the importance of quantifying zooplankton interactions with t-OM in future research.

CONCLUSIONS

This study is the first to carry out a detailed seasonal and spatial survey of zooplankton allochthony within a lake. It brought important elements to the understanding of the role of zooplankton in t-OM processing at the lake ecosystem scale. This approach is also the first to combine allochthony and zooplankton production to calculate a new variable, defined in this study as zooplankton allotrophy. Finally, this study is among the few that include regular winter measurements of seasonal patterns allowing for a major improvement in the understanding of zooplankton life strategies and the associated role of lipid reserves.

The results of this study showed that, even within a medium-sized lake, the zooplankton use of t-OM was seasonally and spatially variable. From all the environmental and biological factors tested in Lake Simoncouche, conclusions can be drawn about the driving factors of zooplankton assimilation of t-OM. Fresh t-OM inputs were identified as having an influence on the seasonal variability of zooplankton allotrophy at the community level. Primary and bacterial production were also identified as influencing factors via their influence on zooplankton production. Within-lake spatial variability of allochthony was influenced by proximity to aquatic macrophytes at the local scale, whereas at the entire basin scale allochthony was also driven by water currents in the lake. Collectively, these results demonstrate that zooplankton use of t-OM is influenced by both autochthonous C sources, via their availability, and allochthonous C sources, via tributaries and their terrestrial inputs into the lake.

One of the major contributions of this thesis was the exploration of the differential allocation of t-OM in biomass, production and lipid reserves for the dominant taxa of

the zooplankton community. It has been possible to conclude that t-OM was used for zooplankton biomass but not in the lipid fraction of the zooplankton body that is used more for reproduction and by individuals under conditions of starvation. Also, this study demonstrated that under-ice winter conditions are a critical period for zooplankton to complete an essential accumulation of necessary nutritive molecules, such as PUFA, to survive winter while remaining in an active stage. However, t-OM that is always present under the ice when primary production disappears, does not seem to provide an alternative food source for zooplankton as zooplankton production at this time of the year is null and phytoplanktonic PUFA are accumulated predominantly within lipid reserves.

Finally, another important contribution of this study is a first quantitative estimate of the t-OM use by zooplankton at the ecosystem level. Due to the quantitative estimates of C sources, it was possible to identify the driving factors of the seasonal variability of zooplankton allotrophy. These estimates allowed for an accurate evaluation of the actual amount of terrestrial sources used in zooplankton biomass during an entire year within a lake. Contextualized with autochthonous and allochthonous C inputs, these results helped to evaluate the zooplankton contribution in the t-OM processing in aquatic ecosystems.

Future research should follow two main axes: first, the necessity for evaluating the non-studied allocation of t-OM by zooplankton as in respiration or reproduction. The share of t-OM respired by individuals contributes to C mineralization and potentially in the role of aquatic ecosystems as a source of CO_2 diffusion into the atmosphere. The study of t-OM allocation for reproduction follows directly the discoveries in lipid accumulation as both phenomenon are linked (Schneider et al. 2016). The allocation of t-OM for excretion would also contribute to the understanding of zooplankton digestive capacity to select molecules of interest that can counterbalance a non-

selective feeding behaviour. Second, more estimates of zooplankton production should be carried out for different ecosystems and with other species to improve standardized methods for calculating zooplankton production e.g. the existing method for molting crustaceans based on the chitobiase enzyme. This will help to better quantify the t-OM role in aquatic food webs. The next step of this project will be to combine the spatial distribution of zooplankton allochthony with seasonal pattern of the lake in order to calculate a very precise flux of t-OM toward aquatic biomass during an entire year. To be able to then upscale our estimations, different aquatic ecosystems with potentially very different mechanisms for t-OM pathways within their food webs need to be sampled and analyzed. A particular focus should be carried on humic ponds, ecosystems potentially highly influenced by terrestrial inputs that are very numerous in boreal biome (Gutseit 2007) as well as on cyanobacteria-dominated ecosystems that harbour autochthonous primary production of low quality and t-OM that can be a real alternative C source. These studies, in addition to a better understanding of the role of t-OM in aquatic food webs, would contribute to building a real quantitative estimate of the actual role of inland waters in t-OM processing and in the global C cycle.

REFERENCES

Abrusán, G., P. Fink, and W. Lampert. 2007. Biochemical limitation of resting egg production in *Daphnia*. Limnology and Oceanography **52**:1724-1728.

Ackman, R. G. 1989. Marine biogenic lipids, fats and oils. CRC Press.

Ackman, R. G., C. Tocher, and J. McLachlan. 1968. Marine phytoplankter fatty acids. Journal of the Fisheries Board of Canada **25**:1603-1620.

Adams, H. E., B. C. Crump, and G. W. Kling. 2010. Temperature controls on aquatic bacterial production and community dynamics in arctic lakes and streams. Environmental Microbiology **12**:1319-1333.

Alber, M., and I. Valiela. 1994. Production of microbial organic aggregates from macrophyte-derived dissolved organic material. Limnology and Oceanography **39**:37-50.

Anderson, M., R. N. Gorley, and R. K. Clarke. 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E: Plymouth, UK.

Arts, M. T., M. T. Brett, and M. Kainz. 2009. Lipids in aquatic ecosystems. Springer Science & Business Media.

Arts, M. T., and B. Wainman. 1999. Lipids in freshwater ecosystems. Springer Verlag.

Auderset-Joye, D., B. Oertli, A. Lehmann, R. Juge, and J. B. Lachavanne. 2006. The prediction of macrophyte species occurrence in Swiss ponds. Hydrobiologia **570**:175-182.

Azam, F., T. Fenchel, J. Field, J. Gray, L. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Marine ecology progress series. Oldendorf **10**:257-263.

Babler, A. L., A. Pilati, and M. J. Vanni. 2011. Terrestrial support of detritivorous fish populations decreases with watershed size. Ecosphere **2**:art76.

Bachok, Z., P. L. Mfilinge, and M. Tsuchiya. 2003. The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. Journal of Experimental Marine Biology and Ecology **292**:187-197.

Banse, K. 1994. Grazing and zooplankton production as key controls of phytoplankton production in the open ocean. Oceanography **7**:13-20.

Barberá, C., D. Fernández-Jover, J. L. Jiménez, D. G. Silvera, H. Hinz, and J. Moranta. 2011. Trophic ecology of the sea urchin *Spatangus purpureus* elucidated from gonad fatty acids composition analysis. Marine Environmental Research **71**:235-246.

Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, T. J. Cline, R. A. Johnson, and D. A. Seekell. 2012. Resources supporting the food web of a naturally productive lake. Limnology and Oceanography **57**:1443.

Beaugrand, G., K. M. Brander, J. Alistair Lindley, S. Souissi, and P. C. Reid. 2003. Plankton effect on cod recruitment in the North Sea. Nature **426**:661-664.

Bec, A., C. Desvilettes, A. Vera, C. Lemarchand, D. Fontvieille, and G. Bourdier. 2003. Nutritional quality of a freshwater heterotrophic flagellate: trophic upgrading of its microalgal diet for *Daphnia hyalina*. Aquatic Microbial Ecology **32**:203-207.

Bell, M. V., and D. R. Tocher. 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. Pages 211-236 Lipids in aquatic ecosystems. Springer.

Berggren, M. 2010. Bacterial use of allochthonous organic carbon for respiration and growth in boreal freshwater systems. Doctoral Thesis. Umeå University, Umeå.

Berggren, M., A.-K. Bergström, and J. Karlsson. 2015. Intraspecific Autochthonous and Allochthonous Resource Use by Zooplankton in a Humic Lake during the Transitions between Winter, Summer and Fall. PLoS ONE **10**:e0120575.

Berggren, M., H. Laudon, M. Haei, L. Strom, and M. Jansson. 2010a. Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. ISME J **4**:408-416.

Berggren, M., L. Strom, H. Laudon, J. Karlsson, A. Jonsson, R. Giesler, A. K. Bergstrom, and M. Jansson. 2010b. Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. Ecology Letters **13**:870-880.

Berggren, M., S. E. Ziegler, N. F. St-Gelais, B. E. Beisner, and P. A. del Giorgio. 2014. Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. Ecology **95**:1947-1959.

Betsill, R. K., and M. J. Van Den Avyle. 1997. Effect of Temperature and Zooplankton Abundance on Growth and Survival of Larval Threadfin Shad. Transactions of the American Fisheries Society **126**:999-1011.

Billen, G., P. Servais, and S. Becquevort. 1990. Dynamics of bacterioplankton in oligotrophic and eutrophic aquatic environments: bottom-up or top-down control? Hydrobiologia **207**:37-42.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology **37**:911-917.

Boschker, H., J. Kromkamp, and J. Middelburg. 2005. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a turbid, tidal estuary. Limnology and Oceanography **50**:70-80.

Brandl, Z. 2005. Freshwater copepods and rotifers: predators and their prey. Pages 475-489 *in* A. Herzig, R. D. Gulati, C. D. Jersabek, and L. May, editors. Rotifera X: Rotifer Research: Trends, New Tools and Recent Advances, Proceedings of the Xth International Rotifer Symposium, held in Illmitz, Austria, 7–13 June 2003. Springer Netherlands, Dordrecht.

Brandl, Z., and C. Fernando. 1979. The impact of predation by the copepod *Mesocyclops edax* (Forbes) on zooplankton in three lakes in Ontario, Canada. Canadian Journal of Zoology **57**:940-942.

Brett, M. T. 2014. Are phytoplankton in northern Swedish lakes extremely 13C depleted? Limnology and Oceanography **59**:1795-1799.

Brett, M. T., G. B. Arhonditsis, S. Chandra, and M. J. Kainz. 2012. Mass flux calculations show strong allochthonous support of freshwater zooplankton production is unlikely. PLoS One **7**:e39508.

Brett, M. T., S. Chandra, M. J. Kainz, and M. E. Power. 2011. Allochthonous and autochthonous contribution to consumers: Emerging issues workshop report. Limnology and Oceanography bulletin **20**.

Brett, M. T., M. J. Kainz, S. J. Taipale, and H. Seshan. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of the National Academy of Sciences of the United States of America **106**:21197-21201.

Bunnell, D. B., M. J. Gonzalez, and R. A. Stein. 2003. Zooplankton biomass enhances growth, but not survival, of first-feeding *Pomoxis* spp. larvae. Canadian Journal of Fisheries and Aquatic Sciences **60**:1314-1323.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media.

Bϕrsheim, K. Y., S. Andersen, G. H. Johnsen, and S. Norland. 1988. Primary and bacterial production compared to growth and food requirements of *Daphnia longispina* in Lake Kvernavatnet, west Norway. Journal of Plankton Research **10**:921-939.

Campbell, J. L., M. J. Mitchell, P. M. Groffman, L. M. Christenson, and J. P. Hardy. 2005. Winter in northeastern North America: a critical period for ecological processes. Frontiers in Ecology and the Environment **3**:314-322.

Caraco, N., and J. Cole. 2004. When terrestrial organic matter is sent down the river: the importance of allochthonous carbon inputs to the metabolism of lakes and rivers. Food webs at the landscape level:301-316.

Carpenter, S. R., J. J. Cole, M. L. Pace, M. Van de Bogert, D. L. Bade, D. Bastviken, C. M. Gille, J. R. Hodgson, J. F. Kitchell, and E. S. Kritzberg. 2005. Ecosystem subsidies: Terrestrial support of aquatic food webs from C-13 addition to contrasting lakes. Ecology **86**:2737-2750.

Carter, J., M. Dadswell, J. Roff, and W. Sprules. 1980. Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern North America. Canadian Journal of Zoology **58**:1355-1387.

Castonguay, M., S. Plourde, D. Robert, J. A. Runge, and L. Fortier. 2008. Copepod production drives recruitment in a marine fish. Canadian Journal of Fisheries and Aquatic Sciences **65**:1528-1531.

Cazzanelli, M., L. ForsstrÖM, M. Rautio, A. Michelsen, and K. S. Christoffersen. 2012. Benthic resources are the key to *Daphnia middendorffiana* survival in a high arctic pond. Freshwater Biology **57**:541-551.

Chambers, P. A., E. E. Prepas, H. R. Hamilton, and M. L. Bothwell. 1991. Current Velocity and Its Effect on Aquatic Macrophytes in Flowing Waters. Ecological Applications **1**:249-257.

Cole, J. J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proceedings of the National Academy of Sciences of the United States of America **108**:1975-1980.

Cole, J. J., S. R. Carpenter, J. F. Kitchell, and M. L. Pace. 2002. Pathways of organic carbon utilization in small lakes: Results from a whole-lake C-13 addition and coupled model. Limnology and Oceanography **47**:1664-1675.

Cole, J. J., S. R. Carpenter, M. L. Pace, M. C. Van de Bogert, J. L. Kitchell, and J. R. Hodgson. 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecology Letters **9**:558-568.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems **10**:172-185.

Cole, J. J., and C. T. Solomon. 2012. Terrestrial support of zebra mussels and the Hudson River food web: A multi-isotope, Bayesian analysis. Limnology and Oceanography **57**:1802-1815.

Cook, E. J., M. V. Bell, K. D. Black, and M. S. Kelly. 2000. Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: trophic and nutritional implications. Journal of Experimental Marine Biology and Ecology **255**:261-274.

Czaika, S. C. 1982. Identification of Nauplii N1–N6 and Copepodids CI–CVI of the Great Lakes Calanoid and Cyclopoid Copepods (Calanoida, Cyclopoida, Copepoda). Journal of Great Lakes Research **8**:439-469.

Danger, M., J. Cornut, E. Chauvet, P. Chavez, A. Elger, and A. Lecerf. 2013. Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? Ecology **94**:1604-1613.

Daniel, C. B. 2005. The importance of terrestrial carbon in plankton food webs. Limnology.

de Kluijver, A., J. Ning, Z. Liu, E. Jeppesen, R. D. Gulati, and J. J. Middelburg. 2015. Macrophytes and periphyton carbon subsidies to bacterioplankton and zooplankton in a shallow eutrophic lake in tropical China. Limnology and Oceanography **60**:375-385.

Del Giorgio, P. A., J. J. Cole, N. F. Caraco, and R. H. Peters. 1999. Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes. Ecology **80**:1422-1431.

del Giorgio, P. A., and R. L. France. 1996. Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton delta C-13. Limnology and Oceanography **41**:359-365.

Del Giorgio, P. A., and R. H. Peters. 1993. Balance between Phytoplankton Production and Plankton Respiration in Lakes. Canadian Journal of Fisheries and Aquatic Sciences **50**:282-289.

Del Giorgio, P. A., and R. H. Peters. 1994. Patterns in planktonik P-R ratios in lakes - Influence of lake trophy and dissolved organic carbon. Limnology and Oceanography **39**:772-787.

Demarty, M. 2009. Production de carbone organique dissous par le complexe macrophytes-épiphytes: effets de facteurs physico-chimiques, implications sur la productivité des communautés de macrophytes et incidence à l'échelle de l'écosystème. PhD thesis.

Demarty, M., and Y. T. Prairie. 2009. In situ dissolved organic carbon (DOC) release by submerged macrophyte-epiphyte communities in southern Quebec lakes. Canadian Journal of Fisheries and Aquatic Sciences **66**:1522-1531.

Desvilettes, C., and A. Bec. 2009. Formation and transfer of fatty acids in aquatic microbial food webs: role of heterotrophic protists. Pages 25-42 Lipids in Aquatic Ecosystems. Springer.

Doi, H. 2009. Spatial patterns of autochthonous and allochthonous resources in aquatic food webs. Population Ecology **51**:57-64.

Dorado-García, I., J. Syväranta, S. P. Devlin, J. M. Medina-Sánchez, and R. I. Jones. 2016. Experimental assessment of a possible microbial priming effect in a humic boreal lake. Aquatic Sciences **78**:191-202.

Doucett, R. R., D. R. Barton, K. R. A. Guiguer, G. Power, and R. J. Drimmie. 1996. Comment: Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **53**:1913-1915.

Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and B. A. Hungate. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology **88**:1587-1592.

Dunstan, G. A., J. K. Volkman, S. M. Barrett, J.-M. Leroi, and S. Jeffrey. 1993. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry **35**:155-161.

Durbin, A. G., and E. Durbin. 1981. Standing stock and estimated production rates of phytoplankton and zooplankton in Narragansett Bay, Rhode Island. Estuaries **4**:24-41.

Ederington, M. C., G. B. McManus, and H. R. Harvey. 1995. Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnology and Oceanography **40**:860-867.

Edmondson, W. T. 1959. Ward & Whipple's Fresh-Water Biology. Wiley.

Elendt, B.-P., and V. Storch. 1990. Starvation-Induced Alterations of the Ultrastructure of the Midgut of *Daphnia magna* Straus, 1820 (Cladocera). Journal of Crustacean Biology **10**:79-86.

Elgmork, K. 2004. Life cycles of the freshwater, planktonic copepod *Cyclops scutifer* Sars on a north–south gradient in North America. Hydrobiologia **529**:37-48.

Emery, K. A., G. M. Wilkinson, F. G. Ballard, and M. L. Pace. 2015. Use of allochthonous resources by zooplankton in reservoirs. Hydrobiologia **758**:257-269.

Erhard, D., and E. M. Gross. 2006. Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton. Aquatic Botany **85**:203-211.

Findlay, S., L. Carlough, M. T. Crocker, H. K. Gill, J. L. Meyer, and P. J. Smith. 1986. Bacterial Growth on Macrophyte Leachate and Fate of Bacterial Production. Limnology and Oceanography **31**:1335-1341.

Fisher, S. G., and G. E. Likens. 1972. Stream Ecosystem: Organic Energy Budget. Bioscience **22**:33-35.

France, R. 1995. Critical examination of stable isotope analysis as a means for tracing carbon pathways in stream ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **52**:651-656.

Francis, T. B., D. E. Schindler, G. W. Holtgrieve, E. R. Larson, M. D. Scheuerell, B. X. Semmens, and E. J. Ward. 2011. Habitat structure determines resource use by zooplankton in temperate lakes. Ecology Letters **14**:364-372.

Fry, B. 2006. Stable isotope ecology. Springer Verlag.

Fry, B. 2013. Alternative approaches for solving underdetermined isotope mixing problems. Marine Ecology Progress Series **472**:1-13.

Fryer, G. 1957. The food of some freshwater cyclopoid copepods and its ecological significance. Journal of Animal Ecology **26**:263-286.

Galloway, A. W. E., S. J. Taipale, M. Hiltunen, E. Peltomaa, U. Strandberg, M. T. Brett, and P. Kankaala. 2014. Diet-specific biomarkers show that high-quality phytoplankton fuels herbivorous zooplankton in large boreal lakes. Freshwater Biology **59**:1902-1915.

Geller, W. 1987. On estimating the age and the development time of *Daphnia* as a function of body size and temperature. Journal of Plankton Research **9**:1225-1230.

Gentsch, E., T. Kreibich, W. Hagen, and B. Niehoff. 2008. Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments. Journal of Plankton Research **31**:45-60.

Gerlach, A., D. J. Russell, B. Jaeschke, and J. Römbke. 2014. Feeding preferences of native terrestrial isopod species (Oniscoidea, Isopoda) for native and introduced leaf litter. Applied Soil Ecology **83**:95-100.

Glaz, P., P. Sirois, and C. Nozais. 2012. Determination of food sources for benthic invertebrates and brook trout *Salvelinus fontinalis* in Canadian Boreal Shield lakes using stable isotope analysis. Aquatic Biology **17**:107-117.

Grey, J., R. I. Jones, and D. Sleep. 2000. Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. Oecologia **123**:232-240.

Grey, J., R. I. Jones, and D. Sleep. 2001. Seasonal Changes in the Importance of the Source of Organic Matter to the Diet of Zooplankton in Loch Ness, as Indicated by Stable Isotope Analysis. Limnology and Oceanography **46**:505-513.

Grosbois, G., P. A. del Giorgio, and M. Rautio. 2017. Zooplankton allochthony is spatially heterogeneous in a boreal lake. Freshwater Biology **62**:474-490.

Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology **91**:2850-2861.

Guillemette, F., and P. A. del Giorgio. 2011. Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. Limnology and Oceanography **56**:734-748.

Guillemette, F., S. Leigh McCallister, and P. A. del Giorgio. 2016. Selective consumption and metabolic allocation of terrestrial and algal carbon determine allochthony in lake bacteria. ISME J **10**:1373-1382.

Guillemette, F., S. L. McCallister, and P. A. del Giorgio. 2015. Selective consumption and metabolic allocation of terrestrial and algal carbon determine allochthony in lake bacteria. ISME J.

Guillemette, F., S. L. McCallister, and P. A. Giorgio. 2013. Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. Journal of Geophysical Research: Biogeosciences **118**:963-973.

Gutseit, K. 2007. Food Quality for Zooplankton in Humic Lakes. Lund University.

Haaland, S., D. Hongve, H. Laudon, G. Riise, and R. Vogt. 2010. Quantifying the drivers of the increasing colored organic matter in boreal surface waters. Environmental Science & Technology **44**:2975-2980.

Hagen, W., and H. Auel. 2001. Seasonal adaptations and the role of lipids in oceanic zooplankton. Zoology **104**:313-326.

Halvorsen, E. 2015. Significance of lipid storage levels for reproductive output in the Arctic copepod *Calanus hyperboreus*. Marine Ecology Progress Series **540**:259-265.

Hamilton, R. J. 1995. Developments in oils and fats. Springer.

Hampton, S. E., M. V. Moore, T. Ozersky, E. H. Stanley, C. M. Polashenski, and A. W. E. Galloway. 2015. Heating up a cold subject: prospects for under-ice plankton research in lakes. Journal of Plankton Research **37**:277-284.

Hanazato, T., and M. Yasuno. 1985. Effect of Temperature in the Laboratory Studies on Growth, Egg Development and First Parturition of Five Species of Cladocera. Japanese Journal of Limnology (Rikusuigaku Zasshi) **46**:185-191.

Harris, R. P. 2000. Zooplankton methodology manual. Academic Press.

Haubert, D., M. M. Haggblom, R. Langel, S. Scheu, and L. Ruess. 2006. Trophic shift of stable isotopes and fatty acids in *Collembola* on bacterial diets. Soil Biology & Biochemistry **38**:2004-2007.

Hedges, J. I., R. G. Keil, and R. Benner. 1997. What happens to terrestrial organic matter in the ocean? Organic Geochemistry **27**:195-212.

Heissenberger, M., J. Watzke, and M. J. Kainz. 2010. Effect of nutrition on fatty acid profiles of riverine, lacustrine, and aquaculture-raised salmonids of pre-alpine habitats. Hydrobiologia **650**:243-254.

Herzig, A. 1984. Temperature and life cycle strategies of *Diaphanosoma brachyurum*: an experimental study on development, growth, and survival. Archiv für Hydrobiologie **101**:143-178.

Hessen, D. O. 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. Hydrobiologia **229**:115-123.

Hiltunen, M., S. J. Taipale, U. Strandberg, K. K. Kahilainen, and P. Kankaala. 2016. High intraspecific variation in fatty acids of *Eudiaptomus* in boreal and subarctic lakes. Journal of Plankton Research **38**:468-477.

Hirst, A. G., J. C. Roff, and R. S. Lampitt. 2003. A synthesis of growth rates in marine epipelagic invertebrate zooplankton. Advances in Marine Biology, Vol 44 **44**:1-142.

Hixson, S. M., B. Sharma, M. J. Kainz, A. Wacker, and M. T. Arts. 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. Environmental Reviews **23**:414-424.

Howell, K. L., D. W. Pond, D. S. Billett, and P. A. Tyler. 2003. Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. Marine Ecology Progress Series **255**:193-206.

Hughes, A. D., A. I. Catarino, M. S. Kelly, D. K. Barnes, and K. D. Black. 2005. Gonad fatty acids and trophic interactions of the echinoid *Psammechinus miliaris*. Marine Ecology Progress Series **305**:101-111.

Huntley, M. E., and M. D. G. Lopez. 1992. Temperature-Dependent Production of Marine Copepods: A Global Synthesis. The American Naturalist **140**:201-242.

Huot, Y., M. Babin, F. Bruyant, C. Grob, M. Twardowski, and H. Claustre. 2007. Does chlorophyll a provide the best index of phytoplankton biomass for primary productivity studies? Biogeosciences Discussions **4**:707-745.

Isay, S., and N. Busarova. 1984. Study on fatty acid composition of marine organisms—I. Unsaturated fatty acids of Japan Sea invertebrates. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry **77**:803-810.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Lipids in Aquatic Ecosystems:281-308.

Jackson, J. K., and S. G. Fisher. 1986. Secondary Production, Emergence, and Export of Aquatic Insects of a Sonoran Desert Stream. Ecology **67**:629-638.

Janssen, A. B. G., S. Teurlincx, S. Q. An, J. H. Janse, H. W. Paerl, and W. M. Mooij. 2014. Alternative stable states in large shallow lakes? Journal of Great Lakes Research **40**:813-826.

Jansson, M., L. Persson, A. M. De Roos, R. I. Jones, and L. J. Tranvik. 2007. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. Trends in Ecology & Evolution **22**:316-322.

Jenkins, G. P., and D. King. 2006. Variation in larval growth can predict the recruitment of a temperate, seagrass-associated fish. Oecologia **147**:641-649.

Jiménez-Melero, R., J. D. Gilbert, and F. Guerrero. 2013. Secondary production of *Arctodiaptomus salinus* in a shallow saline pond: comparison of methods. Marine Ecology Progress Series **483**:103-116.

Johannsson, O. E., R. Dermott, D. M. Graham, J. A. Dahl, E. Scott Millard, D. D. Myles, and J. LeBlanc. 2000. Benthic and Pelagic Secondary Production in Lake Erie after the Invasion of *Dreissena* spp. with Implications for Fish Production. Journal of Great Lakes Research **26**:31-54.

Jones, J. R. E. 1949. A Further Ecological Study of Calcareous Streams in the `Black Mountain' District of South Wales. Journal of Animal Ecology **18**:142-159.

Jones, J. R. E. 1950. A Further Ecological Study of the River Rheidol: The Food of the Common Insects of the Main-Stream. Journal of Animal Ecology **19**:159-174.

Jones, R. I., J. Grey, D. Sleep, and C. Quarmby. 1998. An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. Proceedings of the Royal Society of London Series B-Biological Sciences **265**:105-111.

Kaneda, T. 1991. Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiological reviews **55**:288-302.

Karlsen, O., T. van der Meeren, I. Ronnestad, A. Mangor-Jensen, T. F. Galloway, E. Kjorsvik, and K. Hamre. 2015. Copepods enhance nutritional status, growth and development in Atlantic cod (*Gadus morhua* L.) larvae - can we identify the underlying factors? Peerj **3**:27.

Karlsson, J., M. Berggren, J. Ask, P. Byström, A. Jonsson, H. Laudon, and M. Jansson. 2012. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. Limnology and Oceanography **57**:1042-1048.

Karlsson, J., A. K. Bergstrom, P. Bystrom, C. Gudasz, P. Rodriguez, and C. Hein. 2015. Terrestrial organic matter input suppresses biomass production in lake ecosystems. Ecology **96**:2870-2876.

Karlsson, J., A. Jonsson, M. Meili, and M. Jansson. 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. Limnology and Oceanography **48**:269-276.

Karlsson, J., A. Jonsson, M. Meili, and M. Jansson. 2004. delta N-15 of zooplankton species subarctic lakes in northern Sweden: effects in s of diet and trophic fractionation. Freshwater Biology **49**:526-534.

Kellerman, A. M., D. N. Kothawala, T. Dittmar, and L. J. Tranvik. 2015. Persistence of dissolved organic matter in lakes related to its molecular characteristics. Nature Geoscience **8**:454-457.

Kelly, P. T., C. T. Solomon, B. C. Weidel, and S. E. Jones. 2014. Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. Ecology **95**:1236-1242.

Kharlamenko, V. I., N. V. Zhukova, S. V. Khotimchenko, V. I. Svetashev, and G. M. Kamenev. 1995. Fatty-Acids as Markers of Food Sources in a Shallow-Water Hydrothermal Ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Marine Ecology Progress Series **120**:231-241.

Kirchman, D. 1993. Leucine incorporation as a measure of biomass production by heterotrophic bacteria. Handbook of methods in aquatic microbial ecology. Lewis:509-512.

Koussoroplis, A.-M., M. J. Kainz, and M. Striebel. 2013. Fatty acid retention under temporally heterogeneous dietary intake in a cladoceran. Oikos **122**:1017-1026.

Kragh, T., and M. Sondergaard. 2004. Production and bioavailability of autochthonous dissolved organic carbon: effects of mesozooplankton. Aquatic Microbial Ecology **36**:61-72.

Kruskopf, M., and K. J. Flynn. 2006. Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. New Phytologist **169**:525-536.

Lapierre, J. F., and P. A. del Giorgio. 2014. Partial coupling and differential regulation of biologically and photochemically labile dissolved organic carbon across boreal aquatic networks. Biogeosciences **11**:5969-5985.

Lapierre, J. F., and J. J. Frenette. 2009. Effects of macrophytes and terrestrial inputs on fluorescent dissolved organic matter in a large river system. Aquatic Sciences **71**:15-24.

Lasternas, S., and S. Agustí. 2014. The percentage of living bacterial cells related to organic carbon release from senescent oceanic phytoplankton. Biogeosciences **11**:6377-6387.

Lau, D. C. P., I. Sundh, T. Vrede, J. Pickova, and W. Goedkoop. 2014. Autochthonous resources are the main driver of consumer production in dystrophic boreal lakes. Ecology **95**:1506-1519.

Lee, J., Y. Lee, C. Jang, J. S. Owen, J.-K. Kim, J. Eum, S. Jung, and B. Kim. 2013. Stable carbon isotope signatures of zooplankton in some reservoirs in Korea. Journal of Ecology and Environment **36**:183-191.

Lee, R. F., W. Hagen, and G. Kattner. 2006. Lipid storage in marine zooplankton. Marine Ecology Progress Series **307**:273-306.

Lee, S., and S. Yoo. 2016. Interannual variability of the phytoplankton community by the changes in vertical mixing and atmospheric deposition in the Ulleung Basin, East Sea: A modelling study. Ecological Modelling **322**:31-47.

Lehner, B., and P. Döll. 2004. Development and validation of a global database of lakes, reservoirs and wetlands. Journal of Hydrology **296**:1-22.

Lennon, J. T., S. K. Hamilton, M. E. Muscarella, A. S. Grandy, K. Wickings, and S. E. Jones. 2013. A source of terrestrial organic carbon to investigate the browning of aquatic ecosystems. PLoS One **8**:e75771.

Leroux, S. J., and M. Loreau. 2008. Subsidy hypothesis and strength of trophic cascades across ecosystems. Ecology Letters **11**:1147-1156.

Lizotte, M. P. 2008. Phytoplankton and primary production. Pages 157-178 *in* W. F. Vincent, and J. Laybourn-Parry, editor. Polar lakes and rivers: limnology of arctic and Antarctic aquatic ecosystems. Oxford University Press, Oxford.

López-Sandoval, D. C., T. Rodríguez-Ramos, P. Cermeño, and E. Marañón. 2013. Exudation of organic carbon by marine phytoplankton: dependence on taxon and cell size. Marine Ecology Progress Series **477**:53-60.

Maczulak, A., B. Dehority, and D. Palmquist. 1981. Effects of long-chain fatty acids on growth of rumen bacteria. Applied and Environmental Microbiology **42**:856-862.

Mai, K., J. P. Mercer, and J. Donlon. 1996. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. V. The role of polyunsaturated fatty acids of macroalgae in abalone nutrition. Aquaculture **139**:77-89.

Maie, N., R. Jaffé, T. Miyoshi, and D. L. Childers. 2006. Quantitative and qualitative aspects of dissolved organic carbon leached from senescent plants in an oligotrophic wetland. Biogeochemistry **78**:285-314.

Mann, C. J., and R. G. Wetzel. 1996. Loading and utilization of dissolved organic carbon from emergent macrophytes. Aquatic Botany **53**:61-72.

Mansour, M. P., J. K. Volkman, A. E. Jackson, and S. I. Blackburn. 1999. The fatty acid and sterol composition of five marine dinoflagellates. Journal of Phycology **35**:710-720.

Marcarelli, A. M., C. V. Baxter, M. M. Mineau, and R. O. Hall. 2011. Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. Ecology **92**:1215-1225.

Mariash, H. L., M. Cazzanelli, M. J. Kainz, and M. Rautio. 2011. Food sources and lipid retention of zooplankton in subarctic ponds. Freshwater Biology **56**:1850-1862.

Mariash, H. L., M. Cusson, and M. Rautio. 2017. Fall composition of storage lipids is associated with the overwintering strategy of *Daphnia*. Lipids **52**:83-91.

Mariash, H. L., S. P. Devlin, L. Forsström, R. I. Jones, and M. Rautio. 2014. Benthic mats offer a potential subsidy to pelagic consumers in tundra pond food webs. Limnology and Oceanography **59**:733-744.

Marinho, C. C., F. Meirelles-Pereira, A. d. R. Gripp, C. d. C. Guimarães, F. d. A. Esteves, and R. L. Bozelli. 2010. Aquatic macrophytes drive sediment stoichiometry and the suspended particulate organic carbon composition of a tropical coastal lagoon. Acta Limnologica Brasiliensia **22**:208-217.

Martin-Creuzburg, D., A. Bec, and E. Elert. 2005. Trophic upgrading of picocyanobacterial carbon by ciliates for nutrition of *Daphnia magna*. Aquatic Microbial Ecology **41**:271-280.

Marty, J., and D. Planas. 2008. Comparison of methods to determine algal delta C-13 in freshwater. Limnology and Oceanography-Methods **6**:51-63.

Masclaux, H., M.-E. Perga, M. Kagami, C. Desvilettes, G. Bourdier, and A. Bec. 2013. How pollen organic matter enters freshwater food webs. Limnology and Oceanography **58**:1185-1195.

Matthews, B., and A. Mazumder. 2003. Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. Limnology and Oceanography **48**:1977-1987.

Matthews, B., and A. Mazumder. 2006. Habitat Specialization and the Exploitation of Allochthonous Carbon by Zooplankton. Ecology **87**:2800-2812.

Mattsson, T., P. Kortelainen, and A. Räike. 2005. Export of DOM from boreal catchments: impacts of land use cover and climate. Biogeochemistry **76**:373-394.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell, and G. Pinay. 2003. Biogeochemical Hot Spots and Hot Moments at the Interface of Terrestrial and Aquatic Ecosystems. Ecosystems **6**:301-312.

McDonald, S., A. G. Bishop, P. D. Prenzler, and K. Robards. 2004. Analytical chemistry of freshwater humic substances. Analytica Chimica Acta **527**:105-124.

McLaren, I. A. 1966. Predicting Development Rate of Copepod Eggs. The Biological Bulletin **131**:457-469.

McLeod, R. J., and S. R. Wing. 2009. Strong pathways for incorporation of terrestrially derived organic matter into benthic communities. Estuarine, Coastal and Shelf Science **82**:645-653.

McMeans, B. C., A. M. Koussoroplis, M. T. Arts, and M. J. Kainz. 2015a. Terrestrial dissolved organic matter supports growth and reproduction of *Daphnia magna* when algae are limiting. Journal of Plankton Research **37**:1201-1209.

McMeans, B. C., A. M. Koussoroplis, and M. J. Kainz. 2015b. Effects of seasonal seston and temperature changes on lake zooplankton fatty acids. Limnology and Oceanography **60**:573-583.

Mehner, T., K. Attermeyer, M. Brauns, S. Brothers, J. Diekmann, U. Gaedke, H. P. Grossart, J. Köhler, B. Lischke, N. Meyer, K. Scharnweber, J. Syväranta, M. J. Vanni, and S. Hilt. 2015. Weak Response of Animal Allochthony and Production to Enhanced Supply of Terrestrial Leaf Litter in Nutrient-Rich Lakes. Ecosystems **19**:311-325.

Meili, M. 1992. Sources, concentrations and characteristics of organic matter in softwater lakes and streams of the swedish forest region. Hydrobiologia **229**:23-41.

Monteith, D. T., J. L. Stoddard, C. D. Evans, H. A. de Wit, M. Forsius, T. Høgåsen, A. Wilander, B. L. Skjelkvåle, D. S. Jeffries, J. Vuorenmaa, B. Keller, J. Kopácek, and J. Vesely. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature **450**:537-540.

Montoro Girona, M., H. Morin, J.-M. Lussier, and D. Walsh. 2016. Radial Growth Response of Black Spruce Stands Ten Years after Experimental Shelterwoods and Seed-Tree Cuttings in Boreal Forest. Forests **7**:240.

Muller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature **403**:74-77.

Mush, E. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Arch. Hydrobiol. Beih **14**:14-36.

Myklestad, S., O. Holmhansen, K. M. Varum, and B. E. Volcani. 1989. Rate of released of extracellular amino-acids and carbohydrates from the marine diatom *Chaetoceros affinis*. Journal of Plankton Research **11**:763-773.

Nakajima, R., T. Yoshida, B. H. R. Othman, and T. Toda. 2014. Biomass and estimated production rates of metazoan zooplankton community in a tropical coral reef of Malaysia. Marine Ecology-an Evolutionary Perspective **35**:112-131.

Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems. Lipids in freshwater ecosystems. Springer:21-44.

Nelson, M., C. Phleger, and P. Nichols. 2002. Seasonal lipid composition in macroalgae of the northeastern Pacific Ocean. Botanica Marina **45**:58-65.

Nicol, S., A. Worby, and R. Leaper. 2008. Changes in the Antarctic sea ice ecosystem: potential effects on krill and baleen whales. Marine and Freshwater Research **59**:361-382.

Pace, M. L., S. R. Carpenter, J. J. Cole, J. J. Coloso, J. F. Kitchell, J. R. Hodgson, J. J. Middelburg, N. D. Preston, C. T. Solomon, and B. C. Weidel. 2007. Does Terrestrial Organic Carbon Subsidize the Planktonic Food Web in a Clear-Water Lake? Limnology and Oceanography **52**:2177-2189.

Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. Van de Bogert, D. L. Bade, E. S. Kritzberg, and D. Bastviken. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature **427**:240-243.

Paerl, H. W., and J. Huisman. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ Microbiol Rep **1**:27-37.

Paffenhöfer, G. A., J. R. Strickler, and M. Alcaraz. 1982. Suspension-feeding by herbivorous calanoid copepods: A cinematographic study. Marine Biology **67**:193-199.

Pancost, R. D., and J. S. Sinninghe Damsté. 2003. Carbon isotopic compositions of prokaryotic lipids as tracers of carbon cycling in diverse settings. Chemical Geology **195**:29-58.

Paradis, M., and R. G. Ackman. 1977. Potential for employing the distribution of anomalous non-methylene-interrupted dienoic fatty acids in several marine invertebrates as part of food web studies. Lipids **12**:170-176.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS One **5**:e9672.

Parrish, C. C. 2009. Essential fatty acids in aquatic food webs. Pages 309-326 Lipids in aquatic ecosystems. Springer.

Perga, M.-E., M. Kainz, and A. Mazumder. 2008. Terrestrial carbon contribution to lake food webs: could the classical stable isotope approach be misleading? Canadian Journal of Fisheries and Aquatic Sciences **65**:2719-2727.

Perga, M. E., M. Kainz, B. Matthews, and A. Mazumder. 2006. Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. Freshwater Biology **51**:2041-2051.

Petersen, R. C., and K. W. Cummins. 1974. Leaf processing in a woodland stream. Freshwater Biology **4**:343-368.

Phillips, D. L. 2001. Mixing Models in Analyses of Diet Using Multiple Stable Isotopes: A Critique. Oecologia **127**:166-170.

Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia **127**:171-179.

Phillips, D. L., and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia **136**:261-269.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Polis, G. A., and S. D. Hurd. 1996. Linking marine and terrestrial food webs: Allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. American Naturalist **147**:396-423.

Polis, G. A., M. E. Power, and G. R. Huxel. 2004. Food webs at the landscape level. University of Chicago Press.

Popadin, K. 2002. Body size of Holopedium gibberum under the influence of fish predation in small subarctic lakes. Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen **28**:204-209.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**:703-718.

Postel, L., H. Fock, and W. Hagen. 2000. 4 Biomass and abundance.

R Development Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retreived from http://www.R-project.org.

Rasdi, N. W., and J. G. Qin. 2015. Effect of N:P ratio on growth and chemical composition of *Nannochloropsis oculata* and *Tisochrysis lutea*. Journal of Applied Phycology **27**:2221-2230.

Ratledge, C., and S. Wilkinson. 1988. Microbial lipids, vol. 1. Academic Presss, London.

Rautio, M., H. Mariash, and L. Forsstrom. 2011. Seasonal shifts between autochthonous and allochthonous carbon contributions to zooplankton diets in a subarctic lake. Limnology and Oceanography **56**:1513-1524.

Rautio, M., and W. F. Vincent. 2006. Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. Freshwater Biology **51**:1038-1052.

Ravet, J. L., M. T. Brett, and G. B. Arhonditsis. 2010. The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA. Ecology **91**:180-190.

Reitan, K. I., J. R. Rainuzzo, and Y. Olsen. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae1. Journal of Phycology **30**:972-979.

Richardson, K., A. Visser, and F. B. Pedersen. 2000. Subsurface phytoplankton blooms fuel pelagic production in the North Sea. Journal of Plankton Research **22**:1663-1671.

Rigler, F. H., M. E. MacCallum, and J. C. Roff. 1974. Production of zooplankton in Char Lake. Journal of the Fisheries Research Board of Canada **31**:637-646.

Roiha, T., M. Tiirola, M. Cazzanelli, and M. Rautio. 2011. Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. Aquatic Sciences.

Rosenstock, B., and M. Simon. 2001. Sources and sinks of dissolved free amino acids and protein in a large and deep mesotrophic lake. Limnology and Oceanography **46**:644-654.

Ruess, L., K. Schütz, S. Migge-Kleian, M. M. Häggblom, E. Kandeler, and S. Scheu. 2007. Lipid composition of *Collembola* and their food resources in deciduous forest stands—implications for feeding strategies. Soil Biology and Biochemistry **39**:1990-2000.

Runge, J. A., and J. C. Roff. 2000. The measurement of growth and reproductive rates.*in* A. Press, editor. Zooplankton Methodology Manual.

Salem Jr, N., and M. Eggersdorfer. 2015. Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? Current Opinion in Clinical Nutrition & Metabolic Care **18**:147-154.

Sastri, A. R. 2014. Proposal for SCOR working group.

Sastri, A. R., J. Gauthier, P. Juneau, and B. E. Beisner. 2014. Biomass and productivity responses of zooplankton communities to experimental thermocline deepening. Limnology and Oceanography **59**:1-16.

Sastri, A. R., P. Juneau, and B. E. Beisner. 2013. Evaluation of chitobiase-based estimates of biomass and production rates for developing freshwater

crustacean zooplankton communities. Journal of Plankton Research **35**:407-420.

Sastri, A. R., and J. C. Roff. 2000. Rate of chitobiase degradation as a measure of development rate in planktonic Crustacea. Canadian Journal of Fisheries and Aquatic Sciences **57**:1965-1968.

Säwström, C., J. Karlsson, J. Laybourn-Parry, and W. Granéli. 2009. Zooplankton feeding on algae and bacteria under ice in Lake Druzhby, East Antarctica. Polar Biology **32**:1195-1202.

Scharnweber, K., J. Syvaranta, S. Hilt, M. Brauns, M. J. Vanni, S. Brothers, J. Kohler, J. Knezevic-Jaric, and T. Mehner. 2014a. Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. Ecology **95**:1496-1505.

Scharnweber, K., M. J. Vanni, S. Hilt, J. Syväranta, and T. Mehner. 2014b. Boomerang ecosystem fluxes: organic carbon inputs from land to lakes are returned to terrestrial food webs via aquatic insects. Oikos **123**:1439-1448.

Scheffer, M., S. Hosper, M. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shallow lakes. Trends in Ecology & Evolution **8**:275-279.

Schneider, T., G. Grosbois, F. W. Vincent, and M. Rautio. in press. Saving for the future: Fall uptake of algal lipids supports copepod egg production in spring. Freshwater Biology.

Schneider, T., G. Grosbois, W. F. Vincent, and M. Rautio. 2016. Carotenoid accumulation in copepods is related to lipid metabolism and reproduction rather than to UV-protection. Limnology and Oceanography **61**:1201-1213.

Sharma, S., J. J. Magnuson, R. D. Batt, L. A. Winslow, J. Korhonen, and Y. Aono. 2016. Direct observations of ice seasonality reveal changes in climate over the past 320-570 years. Sci Rep **6**:25061.

Shuter, B. J., and K. K. Ing. 1997. Factors affecting the production of zooplankton in lakes. Canadian Journal of Fisheries and Aquatic Sciences **54**:359-377.

Simopoulos, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. The American journal of clinical nutrition **54**:438-463.

Sleigh, M. A. 1987. Microbes in the Sea. Halsted Press.

Solomon, C. T., S. E. Jones, B. C. Weidel, I. Buffam, M. L. Fork, J. Karlsson, S. Larsen, J. T. Lennon, J. S. Read, S. Sadro, and J. E. Saros. 2015. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. Ecosystems **18**:376-389.

Sommer, U. 2012. Plankton ecology: succession in plankton communities. Springer Science & Business Media.

Sommer, U., R. Adrian, L. De Senerpont Domis, J. J. Elser, U. Gaedke, B. Ibelings, E. Jeppesen, M. Lürling, J. C. Molinero, W. M. Mooij, E. van Donk, and M. Winder. 2012. Beyond the Plankton Ecology Group (PEG) model: mechanisms driving plankton succession. Annual Review of Ecology, Evolution and Systematics **43**:429-448.

Stephens, P. A., I. L. Boyd, J. M. McNamara, and A. I. Houston. 2009. Capital breeding and income breeding: their meaning, measurement, and worth. Ecology **90**:2057-2067.

Stets, E. G., and J. B. Cotner. 2008. Littoral zones as sources of biodegradable dissolved organic carbon in lakes. Canadian Journal of Fisheries and Aquatic Sciences **65**:2454-2460.

Stevens, C. J., D. Deibel, and C. C. Parrish. 2004. Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov. Journal of Experimental Marine Biology and Ecology **303**:135-156.

Stockwell, J. D., and O. E. Johannsson. 1997. Temperature-dependent allometric models to estimate zooplankton production in temperate freshwater lakes. Canadian Journal of Fisheries and Aquatic Sciences **54**:2350-2360.

Strandberg, U., M. Hiltunen, E. Jelkanen, S. J. Taipale, M. J. Kainz, M. T. Brett, and P. Kankaala. 2015. Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. Science of the Total Environment **536**:858-865.

Sun, J. F., C. H. Peng, H. McCaughey, X. L. Zhou, V. Thomas, F. Berninger, B. St-Onge, and D. Hua. 2008. Simulating carbon exchange of Canadian boreal forests II. Comparing the carbon budgets of a boreal mixedwood stand to a black spruce forest stand. Ecological Modelling **219**:276-286.

Syväranta, J., H. Hamalainen, and R. I. Jones. 2006. Within-lake variability in carbon and nitrogen stable isotope signatures. Freshwater Biology **51**:1090-1102.

Syväranta, J., and M. Rautio. 2010. Zooplankton, lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences. Canadian Journal of Fisheries and Aquatic Sciences **67**:1721-1729.

Taipale, S., P. Kankaala, H. Hamalainen, and R. I. Jones. 2009. Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. Freshwater Biology **54**:90-104.

Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala, and M. T. Brett. 2013. Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology **71**:165-178.

Taipale, S. J., M. T. Brett, M. W. Hahn, D. Martin-Creuzburg, S. Yeung, M. Hiltunen, U. Strandberg, and P. Kankaala. 2014. Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. Ecology **95**:563-576.

Taipale, S. J., A. W. Galloway, S. L. Aalto, K. K. Kahilainen, U. Strandberg, and P. Kankaala. 2016. Terrestrial carbohydrates support freshwater zooplankton during phytoplankton deficiency. Sci Rep **6**:30897.

Taipale, S. J., M. J. Kainz, and M. T. Brett. 2015a. A low -3: -6 ratio in *Daphnia* indicates terrestrial resource utilization and poor nutritional condition. Journal of Plankton Research **37**:596-610.

Taipale, S. J., E. Peltomaa, M. Hiltunen, R. I. Jones, M. W. Hahn, C. Biasi, and M. T. Brett. 2015b. Inferring Phytoplankton, Terrestrial Plant and Bacteria Bulk delta(1)(3)C Values from Compound Specific Analyses of Lipids and Fatty Acids. PLoS One **10**:e0133974.

Talling, J. F. 2003. Phytoplankton–zooplankton seasonal timing and the 'clear-water phase' in some English lakes. Freshwater Biology **48**:39-52.

Tanentzap, A. J., E. J. Szkokan-Emilson, B. W. Kielstra, M. T. Arts, N. D. Yan, and J. M. Gunn. 2014. Forests fuel fish growth in freshwater deltas. Nature communications **5**:4077.

Taube, I. 1966. The temperature dependence of the development of the embryo in *Mesocyclops leuckarti* (Claus) and *Cyclops scutifer* Sars. Thesis. Limnol. Inst. Uppsala. Freshwater biol. Ass. Transl.,(n. ser.) 19.

Teal, J. M. 1957. Community Metabolism in a Temperate Cold Spring. Ecological Monographs **27**:283-302.

Teal, J. M. 1962. Energy-flow in salt-march ecosystem of Georgia. Ecology **43**:614-&.

Thomas, D., and G. Dieckmann. 2002. Antarctic sea ice--a habitat for extremophiles. Science **295**:641-644.

Turner, J. T., H. Levinsen, T. G. Nielsen, and B. W. Hansen. 2001. Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. Marine Ecology Progress Series **221**:209-219.

Vachon, D., and P. A. del Giorgio. 2014. Whole-Lake CO_2 Dynamics in Response to Storm Events in Two Morphologically Different Lakes. Ecosystems **17**:1338-1353.

Vadeboncoeur, Y., M. J. Vander Zanden, and D. M. Lodge. 2002. Putting the Lake Back Together: Reintegrating Benthic Pathways into Lake Food Web Models: Lake ecologists tend to focus their research on pelagic energy pathways, but, from algae to fish, benthic organisms form an integral part of lake food webs. Bioscience **52**:44-54.

Vanderstukken, M., S. A. J. Declerck, E. Decaestecker, and K. Muylaert. 2014. Long-term allelopathic control of phytoplankton by the submerged macrophyte *Elodea nuttallii*. Freshwater Biology **59**:930-941.

Varpe, O. 2012. Fitness and phenology: annual routines and zooplankton adaptations to seasonal cycles. Journal of Plankton Research **34**:267-276.

Vazhappilly, R., and F. Chen. 1998. Heterotrophic production potential of omega-3 polyunsaturated fatty acids by microalgae and algae-like microorganisms. Botanica Marina **41**:553-558.

Vijverberg, J. 1980. Effect of temperature in laboratory studies on development and growth of Cladocera and Copepoda from Tjeukemeer, The Netherlands. Freshwater Biology **10**:317-340.

Vincent, W. F., and J. Laybourn-Parry. 2008. Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems. Oxford University Press.

von Wachenfeldt, E., and L. J. Tranvik. 2008. Sedimentation in boreal lakes — the role of flocculation of allochthonous dissolved organic matter in the water column. Ecosystems **11**:803-814.

Wassenaar, L. I., and K. A. Hobson. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies **39**:211-217.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology **37**:4702-4708.

Wenzel, A., A.-K. Bergström, M. Jansson, and T. Vrede. 2012. Poor direct exploitation of terrestrial particulate organic material from peat layers by *Daphnia galeata*. Canadian Journal of Fisheries and Aquatic Sciences **69**:1870-1880.

Wetzel, R. G. 1975. Limnology: Philadelphia. WB Saunders Company **3**.

Wetzel, R. G. 1995. Death, detritus, and energy-flow in aquatic ecostystems. Freshwater Biology **33**:83-89.

Wetzel, R. G. 2001. Limnology: lake and river ecosystems. Academic press.

Wetzel, R. G., and M. Sondergaard. 1998. Role of submerged macrophytes for the microbial community and dynamics of dissolved organic carbon in aquatic ecosystems. Pages 133-148 *in* E. Jeppesen, M. Sondergaard, M. Sondergaard, and K. Christoffersen, editors. Structuring Role of Subbmerged Macrophytes in Lakes. Springer, New York.

Wilkinson, G. M., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2014. Use of deep autochthonous resources by zooplankton: Results of a metalimnetic addition of ^{13}C to a small lake. Limnology and Oceanography **59**:986-996.

Wilkinson, G. M., S. R. Carpenter, J. J. Cole, M. L. Pace, and C. Yang. 2013a. Terrestrial support of pelagic consumers: patterns and variability revealed by a multilake study. Freshwater Biology **58**:2037-2049.

Wilkinson, G. M., M. L. Pace, and J. J. Cole. 2013b. Terrestrial dominance of organic matter in north temperate lakes. Global Biogeochemical Cycles **27**:43-51.

Wilson, D. S. 1973. Food Size Selection Among Copepods. Ecology **54**:909-914.

Wurzbacher, C. M., F. Bärlocher, and H. P. Grossart. 2010. Fungi in lake ecosystems. Aquatic Microbial Ecology **59**:125-149.

Yang, C., G. M. Wilkinson, J. J. Cole, S. A. Macko, and M. L. Pace. 2014. Assigning hydrogen, carbon, and nitrogen isotope values for phytoplankton and terrestrial detritus in aquatic food web studies. Inland Waters **4**:233-242.

Yang, G., C. Li, K. Guilini, Q. Peng, Y. Wang, Y. Zhang, and Y. Zhang. 2016. Feeding strategies of four dominant copepod species in Prydz Bay, Antarctica: Insights from a combined fatty acid biomarker and stable isotopic approach. Deep Sea Research Part I: Oceanographic Research Papers **114**:55-63.

Yentsch, C. S., and D. W. Menzel. 1963a. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Research **10**:221-231.

Yentsch, C. S., and D. W. Menzel. 1963b. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Pages 221-231 *in* Deep Sea Research and Oceanographic Abstracts. Elsevier.

Zdorovennova, G., R. Zdorovennov, N. Palshin, and A. Terzhevik. 2013. Optical properties of the ice cover on Vendyurskoe lake, Russian Karelia (1995–2012). Annals of Glaciology **54**:121-124.

Zelles, L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere **35**:275-294.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biology and Fertility of Soils **29**:111-129.