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Efficiency of crustacean zooplankton in transferring allochthonous carbon in a boreal lake

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Abstract. Increased incorporation of terrestrial organic matter (t-OM) into consumer biomass (allochthony) is believed to reduce growth capacity. In this study, we examined the relationship between crustacean zooplankton allochthony and production in a boreal lake that displays strong seasonal variability in t-OM inputs. Contrary to our hypotheses, we found no effect of allochthony on production at the community and the species levels. The high-frequency seasonal sampling (time-for-space) allowed for estimating the efficiency of zooplankton in converting this external carbon source to growth. From the daily t-OM inputs in the lake (57–3,027 kg C/d), the zooplankton community transferred 0.2% into biomass (0.01–2.36 kg C/d); this level was of the same magnitude as the carbon transfer efficiency for algal-derived carbon (0.4%). In the context of the boundless carbon cycle, which integrates inland waters as a biologically active component of the terrestrial landscape, the use of the time-for-space approach for the quantifying of t-OM trophic transfer efficiency by zooplankton is a critical step toward a better understanding of the effects of increasing external carbon fluxes on pelagic food webs.

Key words: allochthony; allochtrophy; carbon transfer efficiency; Cyclops scutifer; Daphnia; Leptodiaptomus minutus; *seasonal pattern; secondary production; stable isotopes.*

Introduction

The high abundance of lakes in the boreal landscape creates dynamic land–water interactions that enhance matter and energy fluxes from the drainage basin toward these waterbodies (Lehner and Döll 2004, Polis et al. 2004). The incoming fluxes are materialized by terrestrial organic matter (t-OM) inputs into lakes, which have increased during the last decades—a process called browning (Monteith et al. 2007, Creed et al. 2018, Wauthy et al. 2018). Terrestrial OM has long been considered an unimportant resource for pelagic aquatic food webs and has been often excluded from the calculations of carbon flux supporting primary and secondary consumers. Increased evidence of a significant share of zooplankton biomass having a terrestrial origin (i.e., allochthony; Emery et al. 2015, Cole et al. 2011, Pace et al. 2004, Berggren et al. 2018), questions this view of t-OM as an unimportant food source. However, as t-OM lacks essential elements for growth (Taipale et al. 2014), the high abundance of terrestrial carbon in zooplankton tissues is not expected to promote the production of consumer biomass, but rather to result in organisms having a reduced growth capacity (Brett et al. 2009). In this context, the increasing amount of t-OM being transported into lakes has become a key concern, as this t-OM may have crucial impacts on the productivity of lakes and the sustainability of aquatic food webs.

Although a complete understanding of lake carbon cycles is highly dependent on all seasons, all other seasons but summer remain little explored in limnology, and direct evidence regarding terrestrial carbon impacts on aquatic food webs at an annual scale have remained elusive. Inputs of t-OM in lakes are highly variable in time and strongly dependent on seasonal or weather-related events (Lambert et al. 2013). For example, precipitations and snow melting create surface runoff in the watershed that increases t-OM loads in lakes by carrying and dissolving soil and litter OM (Sebestyen et al. 2008, Caverly et al. 2013). Extreme storm events can have a major impact on the amount of t-OM entering the lake and on the entire lake carbon cycle (Dhillon and

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Inamdar 2013, Vachon and del Giorgio 2014). They also influence aquatic food webs, because fresh t-OM from recent weather-related events is rapidly assimilated by aquatic bacteria and can fuel the microbial loop more efficiently than recalcitrant older t-OM (Berggren et al. 2009). Algal primary production in lakes is also strongly dependent on season, and maximum of growth (algal bloom) is often observed during the spring and autumn seasons when nutrients are made available from the sediments to the pelagic with lake mixing. However, very little information exists about winter algal ecology even if freshwater ecologists are increasingly recognizing that winter biota can play a larger role in the lake annual cycle (Salonen et al. 2009, Sommer et al. 2012, Hampton et al. 2015). As only 2% of peer-reviewed freshwater literature has included under-ice processes (Hampton et al. 2015), the contribution of winter algae and t-OM inputs to the whole-lake productivity stays largely unknown. Only high-frequency temporal studies can therefore measure the importance of these seasonal events on food web productivity and, via a time-for-space approach, increase our spatially based understanding of aquatic reliance to t-OM inputs.

Zooplankton production, which includes both the individual growth of organisms (somatic growth) and egg production (reproduction), is key in regulating the essential processes of lacustrine ecosystems (Runge and Roff 2000), such as preventing the formation of algal blooms (Talling 2003) or enhancing the growth of fish larvae (Bunnell et al. 2003). The resulting biomass of zooplankton can be fueled by terrestrially produced organic carbon of two distinct forms: organic carbon in dissolved (t-DOC) or particulate (t-POC) form. The former represents the dominant fraction of t-OM in lakes (Brett et al. 2012, Koehler et al. 2012) and is made available to protozoa and metazoa via microbial pathways (Berggren et al. 2010, Jones et al. 2017). Low-molecular-weight t-DOC is highly reactive and supports a high degree of bacterial biomass that can be transferred to higher trophic levels, including zooplankton (Guillemette et al. 2013). This terrestrial organic carbon, repackaged in bacterial biomass from t-DOC and transferred to upper trophic levels, can be upgraded trophically and thereby provide essential elements for zooplankton growth (Tang et al. 2019). In support of this pathway, t-DOC can explain a portion of zooplankton growth and reproduction when algal carbon is limited (McMeans et al. 2015). In a large-scale study across temperate and boreal ecosystems, Berggren et al. (2014) showed that cyclopoid copepods were linked to the lake DOC pool through their raptorial predation on bacterial feeders. Calanoids, on the other hand, assimilate t-OM from particles or from particle-associated microbes that are linked to the POC pool (Simon et al. 2002). Although the mechanisms behind the trophic link from t-OM to zooplankton are increasingly understood, it is still not fully comprehended how the different content of terrestrial organic carbon in zooplankton biomass, that is, allochthony, influences the secondary production of pelagic zooplankton.

The increasing amount of terrestrial carbon in inland waters raises concerns as to the future efficiency of lake biota in converting this externally sourced C to growth. The efficiency of C transfer from t-OM to zooplankton is believed to be low based on laboratory experiments with *Daphnia* (Brett et al. 2009, Taipale et al. 2014). Terrestrial OM transfer efficiencies in the natural environment largely remain elusive, as their estimations require quantification of the amount of terrestrial C that enters the lake and the amount of t-OM assimilated by the various species. Thus, calculations of carbon transfer efficiency have been made among within-lake trophic levels from phytoplankton and benthic algal producers to fish (Kemp et al. 2001, Vander Zanden et al. 2006, Lischke et al. 2017), but have never been done for terrestrial inputs to the aquatic food web. Including t-OM to transfer efficiency calculations for lake food webs would contribute to quantifying their importance in lakes and to building a new ecological concept of the boundless C cycle that integrates inland waters as biologically active components of the terrestrial landscape that contribute to the processing of large amounts of organic carbon at the global scale (Battin et al. 2009).

This study is the first attempt to test how allochthony influences direct field measurements of zooplankton production and to quantify the C transfer efficiency from terrestrial organic matter to zooplankton in their natural lake environment. We did this by (1) measuring the quantity of t-OM in the crustacean zooplankton biomass—using $\delta^{13}C$ isotopes and mass balance modeling—and comparing this t-OM quantity with crustacean zooplankton production; and (2) quantifying t-OM input rates into a boreal lake, t-OM uptake by crustacean zooplankton, and therefore t-OM transfer efficiency to crustacean zooplankton. To provide a perspective for our estimates of t-OM transfer efficiency, we also estimated the C transfer efficiency from phytoplankton to crustacean zooplankton by estimating whole-lake gross primary production (GPP). We performed these calculations for seven crustacean zooplankton species over a full year, including the much-less-studied spring, winter, and fall seasons. The use of the time-for-space approach aims to advance the understanding of t-OM contribution to the whole-lake productivity, which is currently mostly based on the summer data of spatial studies. We hypothesized that zooplankton allochthony and production would be inversely correlated, reflecting the lower energetic quality of t-OM. We also expected that the C transfer efficiency from t-OM to zooplankton would be lower than that of algal-OM.

Methods

Study site and sampling

Lake Simoncouche (48°23′ N, 71°25′ W) is a medium-sized (83 ha), mesotrophic shallow lake (mean depth:

2.2 m) in Quebec, Canada. The lake was sampled for one complete year, from May 2011 to May 2012, at the deepest point (maximum depth 8 m). Annual mean of DOC concentration in the lake was 5.3 ± 0.8 mg C/L, and Secchi depth was 3.4 ± 0.3 m. The lake has one major inflow and one main outflow on the opposite shore to the inflow. The drainage basin (2,543 ha) consists of boreal forest dominated by *Abies balsamea*, *Picea mariana*, and *Betula papyrifera* (Montoro Girona et al. 2016).

Crustacean zooplankton were sampled weekly in the open-water season and twice a month under the ice. Ten to twenty liters of lake water were collected at different depths (between 0 and 7 m), concentrated using a 50-m plankton net and stored in Nalgene bottles. Formaldehyde was added to the sample to a final concentration of 4% until counting and identification. Additional zooplankton were sampled monthly for stable isotope analyses (SIA) by towing a 50-m plankton net up through the water column. These zooplankton were kept alive in the fridge in GF/F-filtered lake water to empty their gut contents until further processing for SIA within 24 h.

Terrestrial leaves and branches from litter along the shore around the lake and along the bottom of the main inflows were collected for SIA to measure the $\delta^{13}C$ value of the allochthonous food source for zooplankton. The $\delta^{13}C$ of DOM was measured on 0.45 m filtered lake water using a TIC/TOC Analyzer (OI Analytical, College Station, Texas, USA) coupled to a DELTA plus XL isotope ratio mass spectrometer (IRMS) with a ConfloII system (Thermo Finnigan, Bremen, Germany). As terrestrial ^{13}C values were very similar to dissolved organic carbon (DOC) ^{13}C values (summer mean ± SD = −27.52 ± 0.04‰, Vachon, *unpublished*), we assume that terrestrial leaf ^{13}C signatures were representative of both particulate and dissolved allochthonous food source. The $\delta^{13}C$ signature of the autochthonous diet was obtained from the $\delta^{13}C$ signature of algal fatty acids (FA) following Grosbois et al. (2017*a*). A 2-L sample was collected from the water column once a month and filtered on a precombusted GF/F filter to collect seston. Algal-specific fatty acids were extracted from each seston sample and sent to Memorial University of Newfoundland to estimate their $\delta^{13}C$ stable isotope signature. SIA relied on the use of a gas chromatograph interfaced with an isotope ratio mass spectrometer (IRMS) via a combustion interface. We assumed a lipid fractionation of 3.8‰, and all FA $\delta^{13}C$ values were adjusted accordingly to calculate the phytoplankton ^{13}C signature (Berggren et al. 2014). Environmental variables that could potentially drive zooplankton production were also measured. Water temperature was recorded at 2-m depth every 3 h (Starmon Mini, Star-Oddi, Iceland). Chlorophyll-a and bacterial production were measured weekly in the summer and twice a month in winter; bacterial biomass was measured monthly. Sampling was performed with a 2-L Limnos water sampler device (Limnos Oy, Turku, Finland) from the integrated water column; water samples recovered from various depths were mixed to produce a single sample to represent the water column.

Calculating crustacean zooplankton production

Crustacean zooplankton production was based on changes in biomass that were estimated weekly in summer and twice a month in winter. This production was calculated using (1) cohort identification when the reproduction of a species was well defined in time, and (2) the population mean weight increment for a continuously reproducing species. Members of the zooplankton community were identified and sexed using Utermöhl chambers and an inverted microscope (Axio Observer A1, Zeiss, Jena, Germany, ×50–100), based on taxonomy guides from Edmondson (1959) and Czaika (1982). Mean dry weight (DW) for all species and stages was estimated using length–DW regressions. Individuals were measured using an optical camera (AxioCam ERC 5S, Zeiss, Germany) and microscope software (AxioVision, Zeiss). Identified species and the length–DW equations are presented in Appendix S1: Table S1. Length–weight relationships were verified by weighing directly the four most abundant species once a month using an XP 26 DeltaRange microbalance (Mettler-Toledo, Greifensee, Switzerland). Seasonal changes in species biomass were calculated and combined to estimate crustacean zooplankton community production rates (mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) that were normalized to the mean depth of the lake (2.2 m). Copepod production P_{cop} (mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) was calculated according to Eq. 1 :

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

$$g_f = \frac{E}{F * D_E} * \frac{W_E}{W_F} \quad (2)$$

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

with g_f the rate of female reproduction (per day), calculated following Eq. 2 (Hirst et al. 2003) with assumptions that age distribution of eggs is uniform and every egg is viable (egg ratio method), and *Bf* the female biomass (mg C/m^2). Equation 2 was calculated with E, the number of eggs (E eggs per square meter), F the number of female (F females per square meter) 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 (mg C). g_i represents the growth rate of stage i (per day, Eq. 3), B_i is the biomass of stage i (mg C/m^2). W_i and W_{i-1} are the mean individual weights of stage i and stage $i-1$, respectively (mg C). 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 were estimated for each sampling date to calculate the mean weight increment for the entire population and to identify main cohorts. Cladoceran production P_{cla} (mg C·m^{-2}·d^{-1}) was calculated using weekly mean weight increment with Eq. 4 with g_s, the somatic growth rate (mg C·m^{-2}·d^{-1}) calculated with Eq. 5 and g_r, the reproductive rate (per day), calculated with Eq. 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 (mg C/m^2). W_t, the mean individual weight (mg C) for the sampling date t and W_{t-1}, the mean individual weight for the previous sampling date (mg C). I is the abundance of individuals (individuals per square meter), W_e is the mean egg weight, and W_{Ad} is the mean individual adult weight at sampling date t (mg C). 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). 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).

Stable isotope analyses and allochthony

Stable isotope analyses (^{13}C and ^{15}N) were carried out on the four most abundant crustacean zooplankton taxa in the lake: *C. scutifer*, *M. edax*, *L. minutus*, and *Daphnia* spp. Three replicates, each having about 200 individually picked specimens (Discovery V12 dissecting microscope, Zeiss, Jena, Germany), were stored in Eppendorf tubes and kept at −80°C before being freeze-dried. In the winter season, crustacean zooplankton can cope with low food availability by storing lipids (Schneider et al. 2016). Because we were interested in the direct food source use and not storage, the samples were lipid-extracted using chloroform/methanol (2:1 v/v) solvent following Grosbois et al. (2017*b*), which is a modified method of Bligh and Dyer (1959). Lipid-free crustacean zooplankton were then dried, weighed, and analyzed for ^{13}C and ^{15}N signatures using a FlashEA 1112 element analyzer (Thermo Fisher Scientific Corporation, Waltham, Massachusetts, USA) coupled to a Thermo Finnigan DELTA plus Advantage mass spectrometer at University of Jyväskylä.

An algebraic two-source model was used to calculate crustacean zooplankton allochthony ($Allo_{cons}$) following Eq. 7 where $^{13}C_{cons}$ is the stable isotope signal of zooplankton, $^{13}C_{enrich}$ is the ^{13}C fractionation estimate based on ^{15}N and trophic level for a given zooplankton taxon (see Appendix S2 for more details). Finally, $^{13}C_{phyto}$ and $^{13}C_{terr}$ are the stable isotope signals of phytoplankton and t-OM, respectively:

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

We did not consider methane as a possible end-member in the zooplankton allochthony calculations because methanogenesis mainly occurs in anoxic freshwaters (Mattson and Likens 1992). As Lake Simoncouche is shallow and well oxygenated, anaerobic metabolism is likely limited compared to aerobic metabolism. Moreover, Crevecoeur et al. (2019) recently showed that methanotrophs on the surface waters only constitute for about 0.1% of the bacterial community in Quebec lakes. Because of the depleted ^{13}C values of methane (about −70‰), zooplankton biomass found with methane contribution in a boreal lake has consequently very depleted ^{13}C values (about −50‰; Kankaala et al. 2006). Such depleted values were not measured in Lake Simoncouche (lowest zooplankton ^{13}C values = −40‰), further confirming the insignificant contribution of methane to zooplankton biomass. To calculate the allochthony of the entire crustacean zooplankton community, allochthony values from each species was weighed with respective biomass and summed.

To estimate how constrained the allochthony results were from the algebraic model, we also ran a Bayesian mixing model that accounts for the uncertainties of source, fractionation, and consumer ^{13}C measurements. The Bayesian model used here is a modified model from Wilkinson et al. (2013) adapted for ^{13}C measurements. 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 consumers (*C. scutifer*, *L. minutus*, *M. edax*, *Daphnia* spp.) was estimated on the basis of δ^{15}N using Eq. 8, assuming that the $\delta^{15}N_{Daphnia}$ represents a food-web baseline; that is, *Daphnia* spp. is considered as primary consumers, and ^{15}N of consumer for each date as

$$\tau = \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{Daphnia})}{\Delta_N + 1} \quad (8)$$

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. 9 as

$$\delta^{13}\text{C trophic enrichment (consumer)} = \Delta_C * \tau \quad (9)$$

where Δ_C is the per-trophic-level stable carbon isotope fractionation of $0.4 \pm 1.3‰$ (Post 2002). The overall distribution of trophic δ^{13}C enrichment $\pm$ SD was then calculated running Eqs. 8 and 9 in sequence with 50,000 Monte Carlo iterations with random values of Δ_C and Δ_N generated from their assumed mean and SD. Bayesian output medians were consistent with the outputs from the algebraic model (see Appendix S3: Fig. S1) The results from the algebraic model were chosen over the results from the Bayesian model as we needed a unique output value for the allochtrophy calculations that the Bayesian model does not provide.

Crustacean zooplankton allochtrophy

To calculate the proportion of crustacean zooplankton production that was based solely on t-OM, crustacean zooplankton production was multiplied by the allochthony ratio. We called this product allochtrophy. Specific allochtrophy ratios for *C. scutifer*, *M. edax*, *L. minutus*, and *Daphnia* spp. were calculated using species-specific production and allochthony. It was not possible to calculate allochthony for the cladocerans *Bosmina* spp., *Diaphanosoma* spp., and *Holopedium* spp. because of the low abundance of these cladocerans. To estimate their specific allochtrophy, we used the seasonal allochthony pattern of *Daphnia* spp. assuming that all cladocerans, as filter-feeders, displayed a similar degree of allochthony.

Lake carbon inputs and zooplankton carbon transfer efficiency

Inputs of total terrestrial organic carbon (t-OC) to the lake were estimated from estimations of terrestrial dissolved organic carbon (t-DOC) and terrestrial particulate organic carbon (t-POC) inputs to the lake. Inputs of t-DOC to the lake were estimated from measurements of river and soil water DOC concentration, water inflow to the lake from the main and secondary rivers and a water mass balance that estimated lateral water inflow (Vachon et al. 2017*a*; see details in Appendix S4: Section S1). Input of t-POC from Lake Simoncouche main tributary was estimated multiplying t-POC water content (mg C/L) by water inflow (m^3/s). The relative proportion of t-POC inputs compared to t-DOC inputs from the main tributary was used to correct the total t-DOC inputs (main tributary and lateral inputs) to obtain t-OC inputs to the lake (Appendix S4: Table S1, Fig. S3). Production rates of in-lake C, that is, GPP, were estimated from high-frequency surface O_2 concentration measurements and modeled based on light availability (Vachon and del Giorgio 2014, Vachon et al. 2017*b*). Every hour, underwater sensors measured dissolved oxygen (O_2) concentrations 1 m below the surface. This free water method not only captures pelagic GPP, but also includes to some extent benthic algae production (see details in Appendix S4: Section S2). The t-OM transfer efficiency ($t-$TE, %) in crustacean zooplankton was calculated as a function of t-OC flow to the lake ($t-\text{OC}_\text{i}$, mg C·m^{-2}·d^{-1}), crustacean zooplankton allochthony from the algebraic model (Zoo_{allo}, %) and crustacean zooplankton production (Zoo_{prod}, mg C·m^{-2}·d^{-1}) following Eq. 10:

$$t-\text{TE} = (\text{t}-\text{OC}_\text{i}) * \text{Zoo}_\text{allo} * \text{Zoo}_\text{prod} \quad (10)$$

The C transfer efficiency was calculated similarly for phytoplankton, replacing $t-\text{OC}_\text{i}$ with algal production (mg C·m^{-2}·d^{-1}) and zooplankton allochthony with zooplankton autochthony, that is, portion of zooplankton biomass of phytoplankton origin.

Limnological analyses

Chl-a concentration (Chl-a), which is a proxy of phytoplankton biomass, was extracted in ethanol and measured by fluorescence following Yentsch and Menzel (1963). Bacterial production (BP) was measured using the [^{3}H]-leucine incorporation method (Kirchman 1993). Triplicate aliquots of 1.5-mL water samples were spiked with 40 nM of [^{3}H]-leucine and were incubated for 1 h. Average blank-corrected rates of leucine uptake were converted to C production assuming the standard conversion factor of 1.55 kg C mol per leu multiplied by an isotopic dilution factor of 2. Bacteria were incubated at a constant 20°C to exclude the effect of temperature on BP (Adams et al. 2010). Bacterial biomass was estimated from bacterial cell abundance counted via epifluorescence microscopy (Axio Observer A1, Zeiss, Jena, Germany, 1000) using a UV excitation (365 nm) and 4.6-diamido-2-phenylindole (DAPI) –stained cells. Each counted bacterial cell was assigned a 0.1-m^3 volume and converted to C content with a factor of 0.308 pg C/m^3 (Fry 1990).

Statistical analyses

ANOVAs and post hoc tests (Tukey's honestly significant difference [HSD]) were performed to detect differences in allochthony, productivity, and allochtrophy among species and seasons. Homoscedasticity of variances and data normality were respectively verified with Bartlett's and Kolmogorov–Smirnov tests. The relationship between crustacean zooplankton allochthony and productivity was tested via a linear regression at the community level and linear mixed models for individual species. In the linear mixed models, production and allochthony variables were normalized by dividing them by their respective root mean squares. All potential models that described the relationship between allochthony and productivity with and without temperature effects

were compared using Akaike's information criterion (AIC_c), and the best model was selected. All foregoing analyses were done using statistical computing environment of R; linear mixed models have been performed with the "lme4" and "AICcmodavg" packages (R Development Core Team 2015).

Multiple linear regression (MLR) models were used to identify the environmental or food-web variables that best explain the seasonal variation in total production and the allochtrophy of the crustacean zooplankton community and individual species. The explanatory variables included water temperature (Temp), gross primary production (GPP), bacterial production (BP), and t-DOC inputs ($t\text{-}DOC_I$). Different time lags (Δ) were tested for each variable, selected accordingly to the best specific model fit and included in explanatory variables to account for time that biological production requires to respond to a change in the environment. The explanatory variables were smoothed with a centered moving average model ($n = 3$) to remove sampling variability and were log-transformed when autocorrelated or not meeting criteria of distribution normality. The best models were selected according to minimum AIC_c. The analyses were run using JMP v10 software.

Results

Total crustacean zooplankton production

Total production of the crustacean zooplankton community reached a maximum of 5.6 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ in mid-June and was lowest (0.02 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) in mid-February, having an annual mean ± SD of 1.3 ± 1.0 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ (Fig. 1). We identified three seasonal phases in zooplankton production ($F_{(2,39)} = 74.7$, $P < 0.001$): (1) April–September with a mean of 1.9 ± 1.4 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$, (2) October–December with a mean of 1.5 ± 0.6 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$, and (3) January–March with a mean of 0.07 ± 0.05 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$. Four crustacean zooplankton taxa (*C. scutifer*, *M. edax*, *L. minutus*, and *Daphnia* spp.) represented 90% of the total annual crustacean zooplankton production. The cyclopoids *C. scutifer* and *M. edax* accounted on average for 15% (0.2 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) and 8% (0.1 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) of the total production and reached 1.1 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ in late May and 1.0 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ in early July, respectively (Fig. 2A, B). The calanoid copepod *L. minutus* contributed 27% to the production total (mean 0.4 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) with a maximum production rate of 1.3 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ in mid-June (Fig. 2C). The most productive taxa, *Daphnia* spp., represented 39% (0.7 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) of the total annual crustacean zooplankton production (Fig. 2D). Its maximum production rate occurred in late September at 3.3 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$. The cladoceran *Bosmina* spp. contributed little (0.05 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) to total production (5%) except in mid-December, when its production rate reached 0.9 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ (Fig. 2E). The production rates of the two other cladoceran taxa (*Diaphanosoma* spp. and *Holopedium* spp.) represented 2% and 4% of the total production, respectively (0.05 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$for *Diaphanosoma* spp. and 0.4 mg $C{\cdot}m^{-2}{\cdot}d^{-1}$ for *Holopedium* spp. (Fig. 2F, G)).

Stable isotopes and allochthony

Zooplankton stable-isotope values ranged widely from ^{13}C enriched values (−26.7‰ in mid-May) to much more depleted values (−40.4‰) in mid-July, both

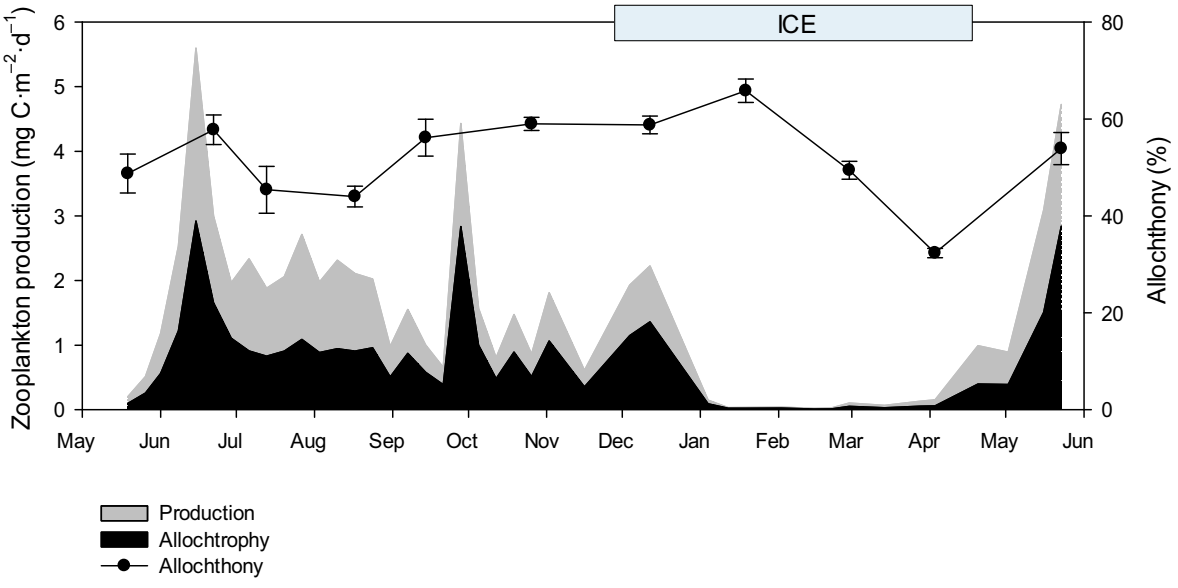

Fig. 1. Seasonal pattern of crustacean zooplankton production (mg $C{\cdot}m^{-2}{\cdot}d^{-1}$) based on weekly and biweekly values separating total production and allochtrophy. Allochthony is the weighted mean ± SD accounting for the biomass of *Cyclops scutifer*, *Mesocyclops edax*, *Leptodiaptomus minutus*, and *Daphnia* spp.

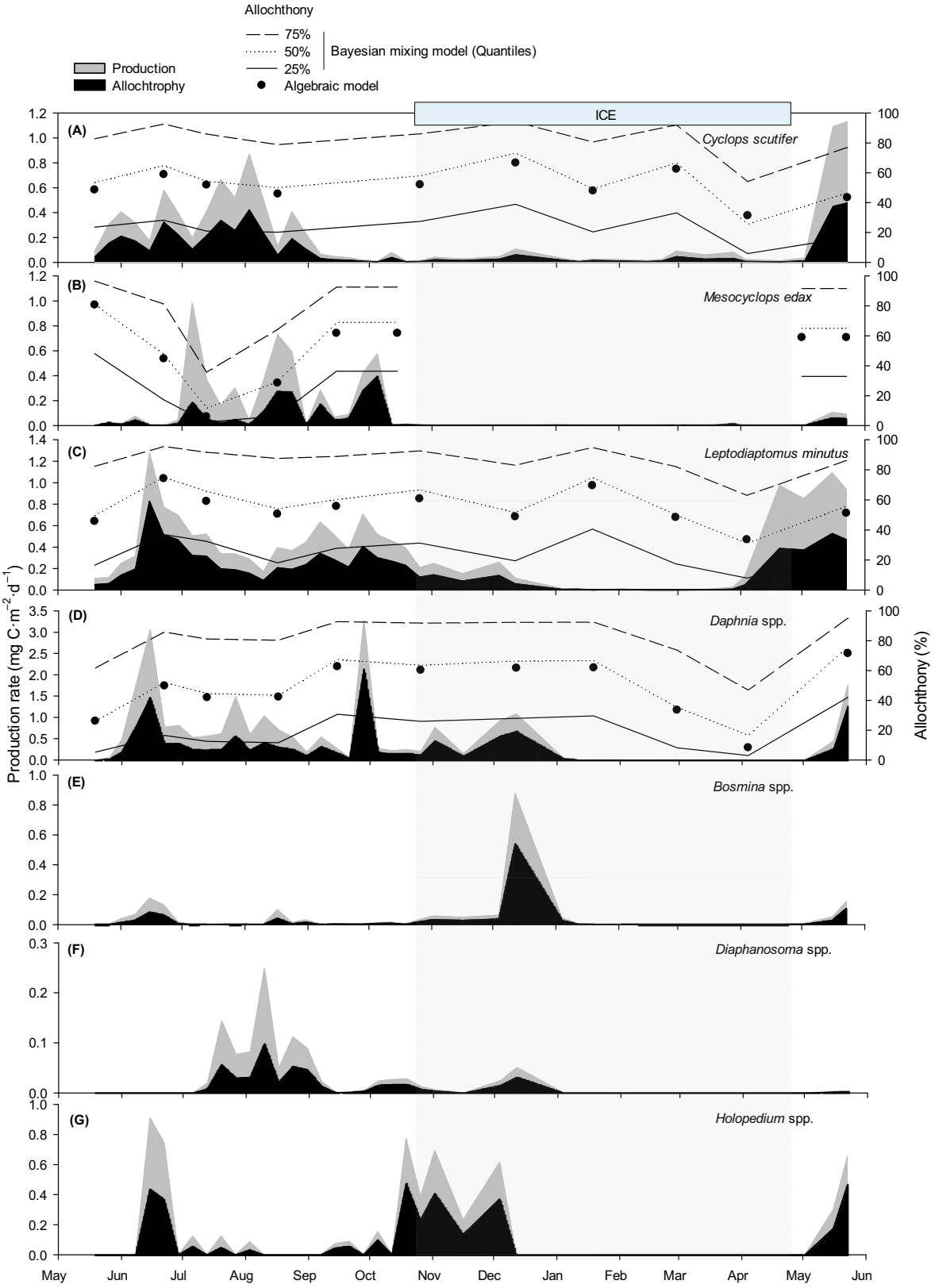

FIG. 2. Seasonal pattern of zooplankton production for the main crustacean zooplankton taxa of Lake Simoncouche; (A) *Cyclops scutifer*, (B) *Mesocyclops edax*, (C) *Leptodiaptomus minutus*, (D) *Daphnia* spp., (E) *Bosmina* spp., (F) *Diaphanosoma* spp.,

(Fig. 2. *Continued*)
and (G) *Holopedium* spp. Note the different scales. Black lines represent the posterior distribution of each consumer allochthony with 25% (solid), 50% (dotted), and 75% (dashed) quantiles. Closed circles represent the output from the algebraic model showing the seasonal pattern of allochthony.

estimated from *M. edax*. However, mean (±SD) stable-isotope values for all consumers combined (−33.1 ± 2.4‰) was equidistant from phytoplankton (−40.2 ± 3.3‰) and terrestrial values (−27.4 ± 1.4‰); this gave a mean annual degree of crustacean zooplankton allochthony of 52 ± 9%. 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% in mid-May to 0% in mid-July for *M. edax*, showing that over the year, variability was highest within the same species. No differences in allochthony were found between species ($F_{(3,38)}$ = 1.8, P = 0.17), whereas strong seasonal patterns were detected ($F_{(10,38)}$ = 4.6, P < 0.001) with species–date interactions ($F_{(25,38)}$ = 2.2, P = 0.015). Minimum values of mean annual allochthony (32 ± 1%; weighted mean with biomass ± SD) for all zooplankton taxa were recorded in early April, and maximum values (66 ± 2%) were observed in mid-January (Fig. 1).

Allochtrophy

Allochtrophy varied seasonally, similarly to total zooplankton production ($F_{(2,39)}$ = 56.0, P < 0.001). The annual mean allochtrophy was 0.7 mg C·m^{-2}·d^{-1}, and was characterized by high values in the summer (maximum of 2.9 mg C·m^{-2}·d^{-1} in June) and by low values in winter (minimum of 0.01 mg C·m^{-2}·d^{-1} in February; Fig. 1). The four most productive taxa represented 90% of the community allochtrophy (*C. scutifer*, *M. edax*, *L. minutus*, and *Daphnia* spp.). The mean annual allochtrophy for *C. scutifer* was 0.1 mg C·m^{-2}·d^{-1}, accounting for 14% of the community allochtrophy (Fig. 2A). *Mesocyclops edax* had a mean annual allochtrophy of 0.05 mg C·m^{-2}·d^{-1}, representing 6% of the community allochtrophy (Fig. 2B). *Leptodiaptomus minutus* had a mean annual allochtrophy of 0.2 mg C·m^{-2}·d^{-1} representing on average 28% of community allochtrophy and reached a peak for allochtrophy of 0.8 mg C·m^{-2}·d^{-1} in mid-June (Fig. 2C). Of all crustacean zooplankton species, *Daphnia* spp. had the highest mean allochtrophy at 0.3 mg C·m^{-2}·d^{-1} that accounted, on average, for 40% of community allochtrophy and peaked at 2.1 mg C·m^{-2}·d^{-1} in late September. The cladocerans *Bosmina* spp., *Diaphanosoma* spp., and *Holopedium* spp. assimilated on average 0.03 (6%), 0.01 (2%), and 0.08 (5%) mg C·m^{-2}·d^{-1} of t-OM over the year, respectively.

Lack of a relationship between zooplankton allochthony and production

The degree of allochthony was not related to crustacean zooplankton production in any of the tested models. At the community level, allochthony and production rates were random and not significantly related (r^2 = 0.02, P = 0.67, Fig. 3A). Results of linear mixed models, which included water temperature and zooplankton allochthony and production at the species level, confirmed the lack of a relationship (P > 0.05, Fig. 3B). See Appendix S5 for details of the model results.

Carbon transfer efficiency

Total t-OC$_I$ to the lake had an annual mean of 543 ± 509 mg C·m^{-2}·d^{-1} (Fig. 4A). Four high input peaks were detected: mid-August (2,276 mg C·m^{-2}·d^{-1}), late August (3,633 mg C·m^{-2}·d^{-1}), late March (3,472 mg C·m^{-2}·d^{-1}), and mid-May (2,131 mg C·m^{-2}·d^{-1}). GPP had an annual mean of 178 ± 190 mg C·m^{-2}·d^{-1}, increasing sharply in mid-April to reach a maximum of 618 mg C·m^{-2}·d^{-1} during the summer. GPP then decreased slowly through the autumn (August–December) to minimal values in winter (mean December–March: 4.3 mg C·m^{-2}·d^{-1}; Fig. 4A). From the 454 kg of t-OC that entered Lake Simoncouche per day (min–max: 57–3,028 kg t-OC/d), an annual mean of 0.61 kg C/d (0.01–2.36 kg t-OC/d) was assimilated into crustacean zooplankton biomass. An annual mean of 153 kg of aquatic carbon was produced per day (0.1–623.7 kg C/d) by algae from which 0.56 kg C/d (0.01–1.99 kg C/d) was assimilated into crustacean zooplankton biomass. The assimilation rate, i.e., the t-TE, ranged from 0.01% in February–March to 0.8% in June (Fig. 5). The C transfer efficiency for algae ranged from 0.1% in July to 1.1% in November. We excluded the GPP under-ice values, as C transfer efficiency for algae was greater than 100% in December; this suggests an underestimation of under-ice GPP values. The mean annual algal C transfer efficiency of 0.4% was higher than the mean annual t-OM transfer efficiency of 0.2%.

The control of crustacean zooplankton production by the environment

Similar to t-DOC$_I$ and GPP, other potentially important environmental variables for crustacean zooplankton production demonstrated a large seasonal variability (Fig. 4). Mean water temperature ranged from 2.8°C in winter to 23.3°C in summer. Chl-a ranged from 0.3 μg·m^{-2}·d^{-1} in midwinter to a maximum of 10.7 μg·m^{-2}·d^{-1} at the end of July. Measured mean bacterial production was 18 mg C·m^{-2}·d^{-1}; it reached a punctual maximum in early December (177 mg C·m^{-2}·d^{-1}) and a minimum in late September (1.5 mg C·m^{-2}·d^{-1}). Bacterial biomass ranged from 46 mg C/m^2 in January to 201 mg C/m^2 in August (Fig. 4C).

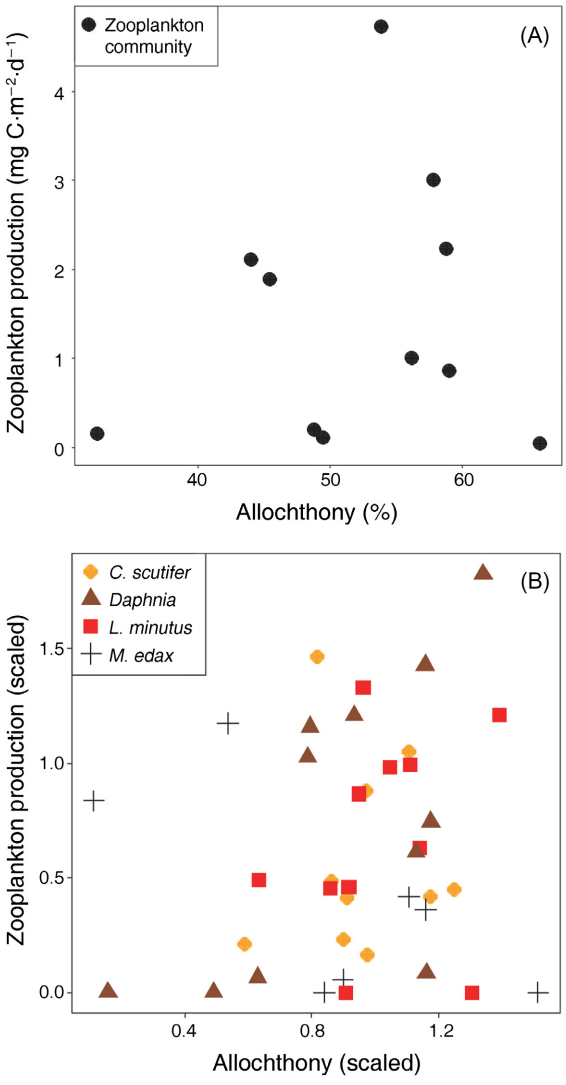

FIG. 3. The absence of relationship between crustacean zooplankton allochthony and production at the (A) community and (B) species level. The community allochthony was weighted according to species biomass. Allochthony and production variables at the species level were scaled to fit the linear mixed model assumptions. See Appendix S5 for details related to linear mixing model results.

Seasonal variation of total community production was best explained by the seasonal pattern of GPP alone (Table 1 a; $r^2_{adj} = 0.74$). At the species level, GPP was also identified as the only influencing variable on *C. scutifer* ($r^2_{adj} = 0.76$) and as an influencing factor on *Daphnia* spp. and *Holopedium* spp. (Table 1a). Temperature was the only influencing factor on *M. edax* and *L. minutus* productivity and also influenced *Diaphanosoma* spp. production rates when combined with t-DOC$_I$. *Bosmina* spp. were the only taxa that had BP as the sole major explanatory variable of the seasonal pattern of its production rates ($r^2_{adj} = 0.71$).

Total crustacean zooplankton community allochtrophy was best explained by the combination of temperature, BP and t-DOC$_I$ (Table 1b). A significant correlation ($r = 0.48$, $P = 0.003$) was calculated when accounting for a lag of four weeks between log(t-DOC$_I$) and the community allochtrophy. The allochtrophy of three species (*M. edax*, *Daphnia* spp., and *Diaphanosoma* spp.) was influenced by t-DOC$_I$ inputs when accounting for a time lag of 4, 5, and 2 weeks, respectively. BP was identified as an explanatory variable for the variation in seasonal allochtrophy of every species (Table 1b). Allochtrophy of all species, except for *Bosmina* spp., was influenced by GPP.

Discussion

This study presents a detailed seasonal pattern of crustacean zooplankton use of terrestrial organic matter from which two main results emerge. First, zooplankton production was not suppressed when allochthony increased; these variables were not related in any way to each other both at the community and the species level. Second, at the ecosystem scale, we found that zooplankton were able to transfer the t-OM into their biomass with a transfer efficiency that was in the same range of magnitude (annual mean = 0.2%) as their efficiency of transferring algal carbon (annual mean = 0.4%). To estimate those trophic transfer efficiencies, we quantified for the first time the amount of C assimilated in zooplankton biomass coming from the watershed, that is, the total amount of C coming from the watershed going into the lake (annual mean: 454 kg C/d) and the amount of C that is assimilated in zooplankton from these C inputs (annual mean: 0.6 kg/d). This is a major step forward compared to previous studies that are only considering the allochthony proportions (Cole et al. 2011, Wilkinson et al. 2013, Berggren et al. 2014, Grosbois et al. 2017*a*).

Our results highlight that once t-OM is assimilated by crustacean zooplankton, it does not suppress production. This is the first time that this relationship is tested seasonally with empirical data of zooplankton production and allochthony. Kelly et al. (2014) have tested this relationship previously in a spatial study and according to their results, the residual variation in zooplankton production that was not explained by t-OM inputs was negatively related to allochthony. The allochthony effect on zooplankton production might differ between lakes, but differences in the results can likely be attributed to differences in methodology. Kelly et al. (2014) calculated zooplankton production using a regression model based on standing biomass (Plante and Downing 1989), whereas we calculated zooplankton production using direct estimates of the rates of change in biomass among cohorts. Zooplankton production rates based on biomass can be biased as biomass and production are sometimes uncoupled, such as observed in Lake Simoncouche where the highest seasonal biomass is found in winter (Grosbois and Rautio 2018), even if this

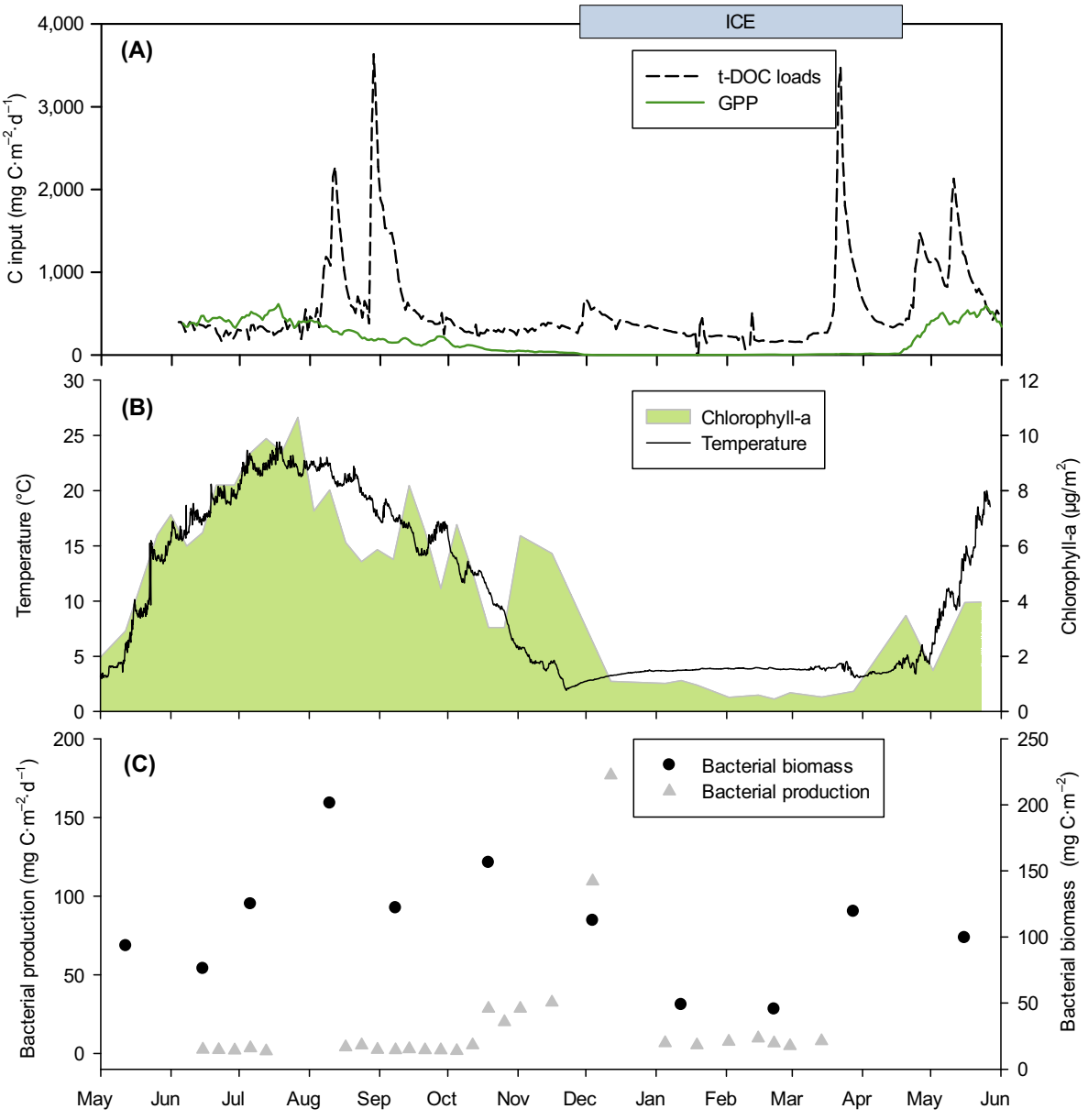

FIG. 4. Seasonal variation of (A) daily terrestrial dissolved organic carbon (t-DOC) loads and lake gross primary production (GPP, 7 d moving average), (B) chlorophyll-a concentration and epilimnion temperature (2 m) measured every 3 h and (C) daily bacterial biomass and production for Lake Simoncouche.

period represents a stagnant phase without growth for months on end. Regression models based on standing biomass are therefore not adapted for seasonal studies, but direct estimates of zooplankton production can circumvent this bias. Meanwhile, our allochthony estimations (annual mean ± SD: 52 ± 9 %) are in accordance with previous studies such as Pace et al. (2004): 22–50%; Carpenter et al. (2005): 22–73%; Cole et al. (2006): 33–73%; Solomon et al. (2011): 20–80%. They are also comparable with Kelly et al. (2014): 29–52% who used the median of the posterior distribution for the terrestrial contribution from a Bayesian mixing model as the point estimate for zooplankton allochthony in regression analyses. Although we used the terrestrial contribution output of an algebraic model, we also used Bayesian mixing model to estimate output uncertainties. The medians of the posterior distribution for terrestrial contributions from the Bayesian model in this study were very close to our algebraic estimates of zooplankton allochthony (Pearson correlation $r = 0.96$; see Appendix S3). Algebraic estimates can therefore be utilized in place of the posterior distribution median from Bayesian mixing

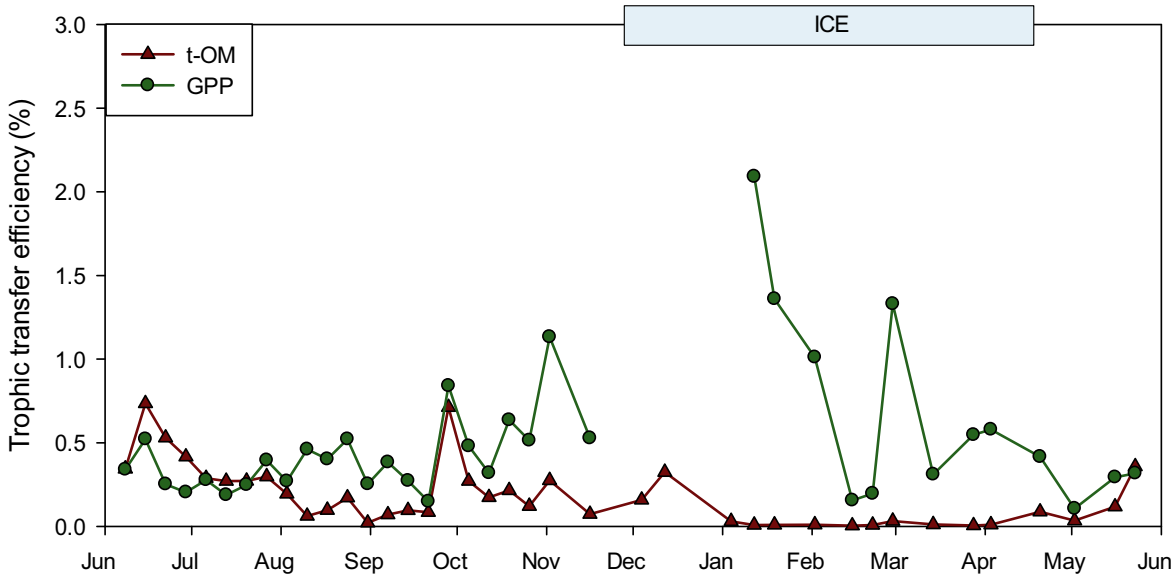

FIG. 5. The efficiency of crustacean zooplankton (trophic transfer efficiency, %) in converting organic carbon from terrestrial origin (t-OM) and algal production (GPP) to biomass. December values for GPP are not shown, as they were overestimated (>100 %). Under-ice primary production was modeled using a 5% light exposure under the ice that did not account for the specificity of early winter thin and often snow-free ice that has optical properties close to that of water.

model as it has been used in many of the recent allochthony studies (Wilkinson et al. 2013, Kelly et al. 2014).

This study brings an important temporal perspective on the terrestrial use by crustacean zooplankton as our understanding of the terrestrial role in aquatic food web is mostly built on comparative studies across space. Spatial studies usually test the impact of t-OM quantity on the proportion of terrestrial C in aquatic biomass (i.e., allochthony). For example, Berggren et al. (2014) and Wilkinson et al. (2013) use a set of lakes with different DOM concentrations (respectively from 4 to 18 and from 3 to 27 mg C/L) to test the influence of t-OM on zooplankton allochthony. With this study, we quantified the seasonal variability of dynamic t-OM inputs (57–3027 kg C/d) in a lake and their integration by zooplankton (0.01–2.36 kg C/d). Seasonal study permits therefore to estimate dynamic fluxes and not only variables relative to standing biomass. Contrary to the commonly accepted paradigm that terrestrial C is assimilated more in winter zooplankton populations because of the low algal availability (Rautio et al. 2011), our quantitative results demonstrate that crustacean zooplankton assimilate terrestrial C mostly during the seasonal production peaks in the summer (winter: 0.03 kg C/d; summer: 0.76 kg C/d). The explanation for this pattern may reside in the presence of phytoplankton in summer. Breakdown of terrestrial detritus and recalcitrant dissolved high-molecular-weight humic substances may be facilitated by co-metabolism using carbon from algal origin (Guenet et al. 2010). This interaction between degradation pathways of recalcitrant and labile carbon, the priming effect, has been suggested as a possible mechanism for terrestrial C consumption in pelagic environments (Dorado-García et al. 2016). The high GPP during spring and summer suggests that labile algal exudates were available to promote terrestrial organic carbon degradation by microbial communities and its subsequent assimilation at higher trophic levels. It can also come from the assimilation of fresh t-OM coming from recent runoff as it is less degraded than old recalcitrant t-OM and easily assimilated by bacterial communities making t-OM available for higher trophic levels (Berggren et al. 2010).

Support for the idea that t-DOM does not suppress zooplankton comes from lability studies of t-DOM. Fresh and labile terrestrial OM carried by large volumes of inflowing water can be assimilated rapidly by bacteria (Berggren et al. 2010), thereby increasing allochthony. Guillemette et al. (2013) estimated that the labile portion of fresh t-DOC ($1.0 \pm 0.3\%$) was only two times lower than in the DOC of algal origin ($2.1 \pm 0.8\%$) by tracking the production and isotopic signature of bacterial respiratory CO_2 of temperate lakes. But a more interesting difference between terrestrial and algal DOC is their different use by bacteria. Terrestrial C is allocated preferentially to bacterial biomass, whereas algal C is used in bacterial respiration (Guillemette et al. 2016). This suggests that although algal C is more labile, the transfer efficiency of terrestrial C to higher trophic levels should be higher. However, this needs to be counterbalanced by the fact that zooplankton access algal C via the direct consumption of phytoplankton cells; this increases algal uptake and explains the observed higher trophic transfer efficiency (TTE) for phytoplankton C than t-OM. Taken

TABLE 1. Results of the multiple linear regression models (based on lowest Akaike information criterion [AIC_c]) to estimate crustacean zooplankton (a) total production and (b) allochtrophy. Temperature (Temp), bacteria production (BP), gross primary production (GPP), and terrestrial dissolved organic carbon inputs (t-DOC) were included as explanatory variables in the models. The Δ values indicate the time lag in weeks for each variable based on the best model selection.

	Variables and (Δ)	*N*	*P*	r^2_{adj}	Root mean-square error
(a) Total production					
Community	GPP(+1)	39	<0.001	0.74	1.50
Cyclops scutifer	GPP	40	<0.001	0.76	0.35
Mesocyclops edax	Temp(+1)	41	<0.001	0.80	0.21
Leptodiaptomus minutus	Temp	42	0.0006	0.24	0.76
Bosmina spp.	BP	28	<0.001	0.71	0.15
Daphnia spp.	BP, GPP(+1)	28	0.0236	0.60	0.85
Diaphanosoma spp.	log(t-DOC), Temp(+2)	40	0.0271	0.57	0.08
Holopedium spp.	log(BP), GPP(+2)	28	0.0111	0.30	0.18
(b) Allochtrophy					
Community	Temp, log(BP), log(t-DOC + 4)	26	0.0408	0.67	0.80
C. scutifer	GPP, log(BP)	28	0.0200	0.88	0.09
M. edax	Temp, GPP, log(t-DOC + 4), log(BP + 1)	28	<0.001	0.86	0.08
L. minutus	Log(GPP + 3), log(BP + 2)	28	0.0059	0.83	0.18
Bosmina spp.	log(BP)	28	0.0002	0.40	0.14
Daphnia spp.	log(BP), log(t-DOC + 5), GPP(+1)	25	0.0059	0.53	0.44
Diaphanosoma spp.	Temp(+3), log(t-DOC + 2), GPP, log(BP)	28	<0.001	0.75	0.02
Holopedium spp.	log(BP), GPP(+1)	28	0.0313	0.28	0.11

together, zooplankton can be highly efficient in accessing t-DOM when it is repackaged within microbes and protozoa and in accessing algal carbon when phytoplankton cells are grazed directly.

Trophic transfer efficiency is difficult to estimate as it requires the assessment of production from adjacent trophic level productions. The efficiency of t-DOM uptake by zooplankton is even more challenging as mechanisms are complex and can include several trophic levels. So that terrestrial transfer efficiency in zooplankton biomass can be estimated, zooplankton allochthony, zooplankton production and the quantification of t-OM inputs into the lake need to be measured. Our study is the first to compile all the necessary parameters to estimate TTE at the terrestrial– (and phytoplankton–) zooplankton interfaces. The estimated t-OM inputs and GPP represent the upper range of potentially available algal and terrestrial material for zooplankton, as part of this OC will never be accessed by zooplankton due, for example, to resources competition with bacterial respiration or because OC is recalcitrant. This OM inputs overestimation thus results in under-estimating the real TTE (i.e., the transfer efficiency of truly available OM). However, Mehner et al. (2018) calculated that in both pelagic and benthic food webs TTE was substantially lower than the traditionally assumed rate of 10% estimated by Lindeman (1942). They estimated mean TTE to be from 1% to 3.6% (mean = 1.9%) in two temperate lakes accounting for all trophic levels from aquatic primary producers to top consumers. This TTE estimation is very close to the TTE estimations presented here from the boreal Lake Simoncouche. Lower terrestrial TTE (compared to aquatic only TTE) into zooplankton biomass may find an explanation in the fact that several trophic levels (e.g., bacteria, ciliates, rotifers) can be included between t-DOM and zooplankton (Jones et al. 2017).

We defined the new concept of "allochtrophy" to refer at the flux of t-OM assimilated in aquatic biomass. As no earlier study had previously measured this dynamic variable but was more focused on the static allochthony values, this concept was not required earlier. With new methods making estimation of zooplankton production cheaper and more accessible (Yebra et al. 2017), such a concept will be increasingly needed in future studies referring to assimilated t-OM in aquatic biomass. In Lake Simoncouche, allochtrophy was dependent on the inflow of terrestrial carbon to the lake. However, t-DOM loads were best correlated with allochtrophy when accounting for a delay of 4 weeks ($r = 0.48$, $P = 0.003$). Grosbois et al. (2017*a*) also proposed a lag of several weeks before terrestrial C from DOC was converted to zooplankton biomass in Lake Simoncouche. Although fresh labile terrestrial molecules can be assimilated rapidly in bacteria (Berggren et al. 2010), the observed lag suggests the main pathway for a terrestrial C transfer to zooplankton includes photochemical degradation of the aromatic terrestrial molecules with high molecular weight (Lapierre and del Giorgio 2014) as well as a multiple trophic level food web (e.g., ciliates, flagellates, rotifers) based on the microbial loop. The time lag between changes in terrestrial inputs and effects on aquatic food webs is a critical aspect that has only been rarely addressed on allochthony studies (but see Berggren et al. 2015) as only temporal sampling can address and

identify those important parameters. This study shows valuable novel results that address one of the main needs identified in Solomon et al. (2015): the inclusion of temporal studies that, thanks to the time-for-space approach, increase our understanding of t-DOM effects on aquatic food webs..

This study tests for the first time the relationship between zooplankton allochthony and production and demonstrates that those variables are not correlated at both species and community level in Lake Simoncouche. This challenges the binary vision of poorly productive zooplankton communities growing on terrestrial inputs and hyperproductive communities fueled by high-quality phytoplankton. Rather, both carbon sources contribute to zooplankton growth throughout the year and mostly during the warmer temperatures from spring to autumn. More importantly, this study quantifies for the first time the amount of t-OM assimilated in zooplankton in a typical boreal lake and the transfer efficiency from both t-OM and algal inputs of the lake. Based on temporal measurements that cover a complete year in the boreal Lake Simoncouche (time-for-space approach), we demonstrate that crustacean zooplankton converted both terrestrial and algal C to growth at a similar transfer efficiency (<1% of available carbon flux). The estimations of those dynamic fluxes allowed to define the new concept of allochtrophy as the proportion of crustacean zooplankton production fuelled by t-OM. These empirical estimations will participate in building more accurate models integrating lakes in the global terrestrial C cycle such as in Cole et al. (2007) and Battin et al. (2009).

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