Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes

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Abstract. The importance of terrestrial-derived organic matter for lake zooplankton communities remains debated, partly because little is known about the basic pathways by which allochthonous carbon is transferred to zooplankton, and whether these vary among the major taxonomic and functional groups. We quantified allochthony of three zooplankton groups (*Cladocera*, *Calanoida*, and *Cyclopoida*) across 18 lakes in Québec, spanning broad gradients of dissolved organic matter (DOM) and lake trophy, using a multi-isotope ($\delta^2H + \delta^{13}C$), multi-source (terrestrial, phytoplanktonic, benthic) approach. All three zooplankton groups had significant levels of allochthony, but differed greatly in their respective patterns across lakes. Allochthony in *Calanoida* and *Cyclopoida* was linked to detrital food chains based on particulate organic matter (POM) and on DOM, respectively, whereas in *Cladocera* it appeared related to both pathways; not surprisingly this latter group had the highest mean allochthony (0.31; compared to 0.18 in *Cyclopoida* and 0.16 in *Calanoida*). This study highlights the complexity of the pathways of delivery and transfer of terrestrial organic matter in freshwaters, and underscores the role that microbial food webs play in this transfer.

Key words: allochthony; bacterial production; boreal lakes; Calanoida; Cladocera; Cyclopoida; dissolved organic matter; particulate organic matter; temperate lakes; zooplankton.

Introduction

Microcrustacean zooplankton composed of cladocerans, cyclopoids, and calanoids represent a critical food chain link between small microorganisms (microalgae, rotifers, protozoa, bacteria) and larger organisms (e.g., fish) in lakes. Classically, it is assumed that freshwater zooplankton use food sources originating from aquatic primary production, but accumulating evidence suggests that calanoids and cladocerans, in particular (Cole et al. 2011), but also cyclopoids (Rautio et al. 2011, Karlsson et al. 2012), systematically show traces of organic matter produced outside of the aquatic environment, in the surrounding terrestrial landscape.

The portion of a zooplankter's body carbon content that is of terrestrial origin is referred to as "allochthony." Published estimates of the allochthony of freshwater crustacean zooplankton are extremely variable, showing the full range from 0.00 to 1.00 (Appendix C: Table C1), but often the highest values are reported for unproductive lakes (Karlsson et al. 2003, Carpenter et al. 2005) or brown-water lakes characterized by low zooplankton production (Kelly et al. 2014). Many of the early allochthony assessments, based on $\delta^{13}C$ analyses only, have not sufficiently considered temporal, spatial,

and interspecies variation in $\delta^{13}C$ of aquatic primary producers (Brett et al. 2009, Francis et al. 2011). Nonetheless, recent comparisons between multi-element isotopic profiles (involving combinations of δ^2H, $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$) of zooplankton and their food sources provide strong support for significant allochthony (Caraco et al. 2010, Kankaala et al. 2010, Cole et al. 2011, Solomon et al. 2011). Allochthony has also been experimentally demonstrated where phytoplankton or allochthonous particles have been labelled with ^{13}C, ^{14}C, or ^3H, directly assessing the assimilation of different sources by zooplankton. Such studies have ranged from laboratory bottle experiments (Salonen and Hammar 1986) to in situ mesocosm studies (Hessen et al. 1990, Taipale et al. 2007) and whole-lake phytoplankton $\delta^{13}C$ manipulations (Pace et al. 2004, Cole et al. 2006, Taipale et al. 2008).

Despite considerable efforts to establish the significance of zooplankton allochthony, surprisingly little is known about the basic mechanisms by which allochthonous organic matter is incorporated into aquatic food webs. It has been suggested that uptake of allochthonous dissolved organic matter (DOM) by bacteria represents a key point of entry for terrestrial carbon, with spinoff effects on the allochthony of organisms at all trophic levels (Karlsson et al. 2003, Jansson et al. 2007). This hypothesis agrees with observations of bacterial carbon being channeled to higher trophic levels through bacterivorous protozoa and other microzooplankton (Jansson et al. 1999, Berggren et al. 2010).

However, other studies have found this pathway unlikely because the observed rates of bacterial production are often too low, particularly in unproductive lakes (Pace et al. 2004, Cole et al. 2006). An alternative hypothesis, supported by whole-lake $\delta^{13}C$ manipulations, is that zooplankters are able to directly assimilate allochthonous particulate organic matter (POM) in the form of leaf fragments or flocculated DOM (Pace et al. 2004, Cole et al. 2006). However, in laboratory studies, the efficiency by which such detrital POM is assimilated and used for growth by zooplankton tends to be low (Brett et al. 2009, Taipale et al. 2014). No study has yet addressed the relative importance of the POM- vs. DOM-based pathways of allochthony across zooplankton groups and lake ecosystem types.

The discussion about zooplankton allochthony has mainly focused on the entire microcrustacean community as one functional unit; a conceptual shortcoming. Considering the distinct feeding strategies that are expressed both within and between cladoceran, cyclopoid, and calanoid zooplankton communities (Barnett et al. 2007), the presumption that the mechanisms behind allochthony should be the same for all microcrustaceans lacks a theoretical basis. The hypothesis of allochthonous POM assimilation may fit well with nonselective filtering feeding behavior typical of cladocerans (Barnett et al. 2007), but strongly selective raptorial feeders, e.g., among cyclopoids, are not known to use allochthonous POM (Dobberfuhl et al. 1997, Barnett et al. 2007). Further, it could also be expected that for selective suspension feeders, such as most calanoids, allochthony is regulated differently compared to nonselective filter feeders.

We here present the results of a multi-isotope ($\delta^2H + \delta^{13}C$), multi-source (terrestrial, phytoplanktonic, benthic), stable isotope mixing model, applied to quantify allochthony in cladoceran, cyclopoid, and calanoid zooplankton assemblages across a range of boreal and temperate lakes, spanning broad ranges in productivity and terrestrial influence. We have further measured a wide range of geographical, limnological, biogeochemical, and microbial features of these systems, which we use to explore patterns of regulation of zooplankton allochthony. We hypothesize that DOM characteristics and availability, as well as the associated bacterial activity are positive regulators of allochthony of all zooplankton groups in oligotrophic and brown-water lakes. We further hypothesize that the allochthony of filter/suspension feeding zooplankton (cladocerans and calanoids) should also be linked to characteristics of the POM pool while not for selective raptorial feeders (cyclopoids). Our results show widespread zooplankton allochthony across lake types, but strongly contrasting patterns of allochthony regulation among major zooplankton groups. This study highlights the complexity of the pathways of delivery and transfer of terrestrial C in freshwaters, and calls for a diversified discussion about the magnitude and regulation of allochthony in zooplankton of different taxonomic and functional groups.

MATERIALS AND METHODS

Sites.—Eleven clear-water lakes (Color$_{abs440}$ [standard light absorption coefficient per meter of water depth at a wavelength of 440 nm] 0.2–1.0 m^{-1}) and seven brown-water lakes (Color$_{abs440}$ 4.7–8.2 m^{-1}) were selected across southeastern and western Québec, Canada (Table 1; see Plate 1). Clear-water lakes were generally oligotrophic in terms of total phosphorus (TP; mean = 6 µg P/L) and chlorophyll *a* (chl *a*; mean = 1.8 µg/L), while brown-water lakes were mesotrophic (mean TP = 24 µg P/L; mean chl *a* = 3.5 µg/L). Collectively, the lakes span large ranges in DOM (3–18 mg C/L), area (0.01–47 km^2), depth (1.4–21 m), and dominant zooplankton taxa (Table 1).

Sampling.—Microcrustaceans were sampled by vertically hauling a plankton net (mesh size 110 µm) from 1 m above the sediments to the surface, at the center or the deepest point of each lake. In addition, a 50-L water sample was taken for analytical purposes at 0.5 m depth using a peristaltic pump. After 12–24 h gut evacuation in filtered lake water, zooplankton were hand-picked and separated into calanoid copepods, cyclopoid copepods, and cladocerans.

Terrestrial source material was collected from randomly selected sites within the study catchments. The samples consisted of forest soils (*n* = 5 samples, different depths including litter), peat soils (*n* = 3 samples), *Sphagnum* plants (*n* = 4 samples), fresh leaf and needle mixes (*n* = 4 samples, several tree species), and DOM from small shaded headwater streams that were sampled as far upstream as possible (*n* = 6 samples). In four lakes with contrasting DOM and chl *a* concentrations (Hébécourt, John, Quasion and des Frères; Table 1), benthic algae were collected in mid-summer by scraping periphyton off of acid-washed pre-deployed (three weeks earlier) ceramic tiles in the upper, mid, and lower parts of the euphotic zone. From the same four lakes, occasional samples were collected for rotifers (net haul; *n* = 1, >3000 hand-picked individuals per sample), dipteran larvae (by net haul or collected from tiles; *n* = 2, 10–100 individuals per sample), gastropod and bivalve muscle tissue (hand-collected; *n* = 5, 3–5 individuals per sample), and fish (rod fishing; *n* = 3, dorsal muscle tissue from 1 individual per sample).

POM samples for basic analyses were obtained by filtering a known volume of 1000–2000 mL of lake water through 0.7-µm glass fiber filters (Whatman GF/F; Whatman International, Maidstone, UK). POM for fatty acid analyses were obtained by filtering 25–40 L water through 1.0-µm glass fiber filters (PALL A/E; PALL Life Sciences, Port Washington, New York, USA).

Laboratory analyses.—Stable isotopic ratios of animals, POM, DOM, dissolved inorganic carbon (DIC), water, and terrestrial and benthic source material were

TABLE 1.   Basic characteristics of the 18 study lakes.

No.	Site name	Latitude (°N)	Longitude (°W)	Area (km²)	Color$_{abs440}$ (m⁻¹)	DOM (mg C/L)	TP (µg/L)	TN (mg/L)	Chl a (µg/L)	Depth (m)	pH	δ²H-H₂O (‰)	n
Brown-water lakes													
1	Duparquet	48.48519	79.26385	46.96	4.66	16.1	33	0.40	2.6	6.7	7.77	−86.7	2
2	Hébécourt	48.51212	79.37740	7.99	4.99	13.4	25	0.43	5.8	3.4	8.09	−82.1	3
3	Matissard	48.43141	78.68851	0.59	5.18	12.6	30	0.40	2.6	2.7	7.16	−86.6	2
4	Voisin	48.49000	79.34333	1.84	5.58	17.0	26	0.48	5.6	3.4	7.42	−83.8	1
5	Dances	48.52325	79.42607	0.25	5.64	17.1	18	0.56	2.8	1.5	7.29	−81.6	1
6	Quasion	49.97888	79.50583	0.30	6.68	18.0	9	0.38	1.8	1.4	7.43	−85.0	3
7	St-Hélène	49.81472	78.32139	1.15	8.18	16.2	27	0.33	3.5	1.5	7.06	−72.2	2
Clear-water lakes													
8	Achigan	45.94435	73.96678	5.37	0.58	6.3	5	0.17	1.4	16.4	7.55	−74.1	1
9	du Camping Hanté	48.82679	79.17287	0.01	1.04	6.6	8	0.25	2.6	7.3	8.89	−76.7	1
10	Connelly	45.89380	73.96678	1.16	1.04	6.7	8	0.17	2.5	20.5	7.81	−71.7	1
11	Cristal	45.97370	73.89020	0.28	0.23	8.1	5	0.19	1.1	14.8	8.08	−69.5	1
12	Croche	45.99210	74.00514	0.18	0.58	5.5	4	0.14	3.7	11.8	7.11	−68.4	1
13	Deauville	45.99197	74.09027	0.19	0.35	9.0	7	0.16	1.6†	10.0	8.03	−61.7	1
14	des Frères	48.93593	79.17992	0.02	0.46	5.3	8	0.11	1.8	10.0	5.08	−72.6	1
15	Fortune	48.19083	79.30194	0.75	1.04	9.4	7	0.23	1.1	12.6	8.19	−70.5	2
16	John	49.34604	79.20164	0.20	0.54	5.7	6	0.14	1.3	16.8	6.68	−71.2	2
17	Morency	45.92860	74.03520	0.23	0.58	3.5	7	0.22	1.0†	12.0	8.33	−65.7	1
18	Saint-Pé	50.11536	78.81511	0.42	0.69	10.7	6	0.12	1.2	15.2	8.07	−76.7	1

Notes: For lakes that were sampled more than once ($n > 1$), mean values are shown. See text for explanation of variables. Dominant zooplankton taxa in the different lakes are indicated in Fig. 3. Variables are Color$_{abs440}$, standard light absorption coefficient per meter water at a wavelength of 440 nm; DOM, dissolved organic material; TP, total phosphorus; TN, total nitrogen; chl a, chlorophyll a; δ²H-H₂O, stable hydrogen isotope ratio of water; and n, number of observations.

† Chl a data from sampling date are missing. The value represents an alternative sampling date in the same lake during the same season.

analyzed at the Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Flagstaff, Arizona, USA. The results are expressed using the δ notation in per mil (‰) as $\delta = (R_{samp}/R_{std} - 1) \times 1000$, where R_{std} is the ratio of $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^2H/^1H$ of standards Vienna Pee Dee Belemnite, atmospheric air, and Vienna Standard Mean Ocean Water, respectively, and R_{samp} is the ratio of these in the sample. Solid material for isotopic analysis was freeze dried, homogenized, transferred to tin (δ¹³C and δ¹⁵N) or silver (δ²H) capsules, and stored in a desiccator until analysis. Samples for δ¹³C of DOM and DIC, and δ²H of water, were stored refrigerated in gas tight containers without air bubbles until analysis.

The concentrations and δ¹³C of DOM and DIC were measured on 0.45-µm filtered lake water using an OI Analytical (College Station, Texas, USA) TIC/TOC Analyzer coupled to a DELTA plus XL (Thermo Finnigan, Bremen, Germany) isotope ratio mass spectrometer (IRMS) with a ConfloII system. The δ¹³C of CO_2 was calculated from the δ¹³C of DIC, pH, and temperature according to Mook et al. (1974). Solid samples in tin capsules were analyzed for carbon content, nitrogen content, δ¹³C, and δ¹⁵N on a ECS4010 (Costech, Valencia, California, USA) or a NC2100 (Carlo Erba, Milan, Italy) Elemental Analyzer interfaced with a DELTA V Advantage or a DELTA plus Advantage using ConfloIII (Thermo Finnigan, Bremen, Germany). It was assumed that pure samples of plant and animal biomass contained no inorganic carbon. Samples of POM and other non-pure materials

were acid fumigated to remove inorganic carbon. The δ²H of water and of solid material in silver capsules (including POM and freeze-dried DOM powder) was measured according to Doucett et al. (2007), using a DELTA plus XL IRMS with ConfloII. Organic matter samples and standards were equilibrated with local water vapor to correct for exchangeable H. Solid samples were analyzed by means of pyrolysis, and the isotopic composition of H_2 gas was measured using the IRMS. The δ²H of water samples was analyzed using headspace equilibration with H_2 gas and a platinum catalyst.

Carbon stable isotope ratios of phytoplankton phospholipid fatty acids (PLFAs), extracted from bulk POM, were used as an approach to determine the phytoplankton end member (Pace et al. 2007, Kankaala et al. 2010). Polyunsaturated fatty acids were extracted from frozen filters using a modified Bligh-Dyer extraction, followed by solid phase extraction with silicic acid to isolate the PLFAs from the neutral and glycolipids according to White and Ringelberg (1998). The resulting fatty acid methyl esters (FAMEs), obtained using a single-step saponification and methylation procedure (Findlay 2004), were analyzed for their carbon stable isotope ratios using a gas chromatograph interfaced with an IRMS via a combustion interface. The FAMEs were purified using reverse-phase C18 resin solid phase extraction, and each sample was then spiked with a known quantity of ethyl arachidate and ethyl tetracosanoate as quantification standards. A small aliquot of the methanol used in the methylation of each sample

batch was analyzed for $\delta^{13}C$ on an Aurora 1020 TOC Analyzer (OI Analytical, College Station, Texas, USA) coupled to a DELTA V isotope ratio mass spectrometer and used to correct for the change in the fatty acid $\delta^{13}C$ imparted by the added methyl C. The FAMEs were identified using an Agilent 6890 GC (Agilent Technologies, Santa Clara, California, USA) interfaced with an Agilent 5973 inert mass selective detector in conjunction with known standards and the NIST mass spectral library. The $\delta^{13}C$ of the FAMEs were determined using an Agilent 6890 GC interfaced with a MAT 252 (Thermo Finnigan, Bremen, Germany) interfaced with a GC/C III. We assessed the precision and accuracy of $\delta^{13}C_{PLFA}$ analyses using repeated injections (≥ 2 per sample) and comparison to a commercially available bacterial acid methyl ester standard mixture (ester mixture "F8") supplied by Indiana University. We conducted all GC analyses using the same operating parameters, capillary column (BPX-70 [70% cyanopropyl polysilphenylene-siloxane]; 50 m, 0.32 mm internal diameter, 0.32 μm film thickness; SGE Analytical Science, Austin, Texas, USA), and each GC employed a split/splitless injector operated in splitless mode.

Bacterial production (BP) was measured in the ambient samples, following the [³H]-leucine incorporation method by Smith and Azam (1992). Triplicate aliquots of 1.5-mL water samples were exposed to 40 nmol/L [³H]-leucine for 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 leucine multiplied with an isotopic dilution factor of 2. Total community respiration (R) was measured as O_2 consumption rates in unfiltered water from all lakes using a "Fibox 3" system (PreSens, Regensburg, Germany). Within 2–6 h from sampling, duplicate 500-mL Erlenmeyer flasks, equipped with O_2 optodes, were filled for each sample, sealed with silicone stoppers and incubated for 48 h at ambient epilimnetic temperatures using dark circulating water baths. During the incubations, O_2 was measured three to five times and R was calculated as the absolute slope of linear regression models for O_2 (μg O_2/L) vs. time (d). Bioavailability of DOM ($BDOM_{14d}$; mg C/L) was assessed on 1.0 μm filtered water during 14-d dark incubations at 20°C (eight time points). $BDOM_{14d}$ was calculated as the absolute slope of linear regression models for dissolved organic carbon (measured on an OI Analytical TIC/TOC Analyzer) vs. time, multiplied by total incubation time. TP was measured using the molybdenum-blue method after persulfate digestion and total nitrogen (TN) as nitrates after alkaline persulphate digestion. Water color was analyzed at 440 nm using a UV/Vis UltroSpec 2100 spectrophotometer (Biochrom, Cambridge, UK) and chl a was measured spectrophotometrically in ethanol extracts.

The in situ partial pressure of CO_2 (pCO_2) was measured at 0.5 m depth at lake center, using an EGM-4 infrared gas analyzer (PP Systems, Amesbury, MA,

USA) receiving air, equilibrated with the water by pumping (100 L/h) through a Liqui-Cel MiniModule (Membrana, Charlotte, North Carolina, USA).

Allochthony assessment.—We first used an algebraic two-source stable hydrogen mixing model (Eq. 1) (Karlsson et al. 2012, Wilkinson et al. 2013) to obtain a rough estimate of allochthony (ΦT), based on the δ^2H of samples (δ^2H_{sam}), the terrestrial source (δ^2H_{terr}) the phytoplankton source (δ^2H_{phyt}), and on δ^2H enrichment due to dietary water

$$\Phi T = (\delta^2H_{sam} - \delta^2H \text{ enrichment} - \delta^2H_{phyt})$$
$$\div (\delta^2H_{terr} - \delta^2H_{phyt}). \qquad (1)$$

However, as this model can only handle two sources and one isotope ratio, we also used the software package SIAR 4.0 for R to examine more advanced models of allochthony, in which $\delta^{13}C$ was added as an additional isotope ratio and benthic algae was added as an additional source (software *available online*).[4] By using multiple stable isotopes and taking into account the uncertainty (SD) of all input parameters, stable isotope analysis in R (SIAR) generates distributions of probable source contributions to an organism (or organic matter pool). SIAR uses three different data matrices: (1) a "consumer" matrix containing observed isotope ratios in consumers or in organic matter pools; (2) a "source" matrix with means ± SD of the isotope ratios in different sources; (3) a "trophic enrichment factor" matrix with the means ± SD of the increase in each isotope ratio from source to consumer level. All input data used are shown in Appendix A. The model was run using the siarsolomcmcv4 command, which is adapted for single-organism-sample models, with default parameters (iterations = 500 000, burn in = 50 000, thinby = 15; SIAR's built-in diagnostic for convergence was used and we found no apparent need for longer Monte Carlo Markov chain analyses [MCMCs]).

The SIAR model was also used to assess allochthony in a few cases where δ^2H was missing owing to a lack of sufficient sample material: in the rotifer sample (mass 11 μg) and in 11 out of 27 DOM samples where we underestimated the amount of water required to freeze dry. In these cases, the SIAR model was run with only one isotope ($\delta^{13}C$) and two sources (terrestrial and phytoplankton). Allochthony of the total crustacean zooplankton community was calculated as biomass-weighted mean allochthony of the different zooplankton groups. Biomass of zooplankton was determined using standard length–dry-mass regressions (Culver et al. 1985) applied to microscopically assessed length measurements of each species.

Sources.—To represent the terrestrial source in the SIAR, we used the mean ± SD of the isotope ratios in all ($n = 22$; see *Sampling*) terrestrial organic matter samples ($-27.28‰ ± 1.51‰$ for $\delta^{13}C$; $-129.8‰ ±$

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[4] http://cran.r-project.org/web/packages/siar/index.html

13.7‰ for δ^2H), which overlapped with the isotope ratios of lignin in most vascular plants (DeBond et al. 2013). There were no internal patterns in this data.

Phytoplankton was represented by the mean ± SD of $\delta^{13}C$ of three PLFA biomarkers: the diatom marker 20:5ω3 and the green algal markers 18:2ω6 and 18:3ω3 (Wood 1988, Napolitano 1999). The means were adjusted with +3.8‰ (see rationale in Appendix C) to correct for the difference between $\delta^{13}C$ in PLFAs and in whole organisms, resulting in a range of phytoplankton $\delta^{13}C$ from −42.9‰ to −31.0‰. The mean PLFA-inferred phytoplankton $\delta^{13}C$ was −36.8‰, which is 21.8 units per mil lower than the mean $\delta^{13}C$ of CO_2 (−15.0‰) in the same samples. This difference is close to reported values of photosynthetic stable carbon isotope fractionation in unproductive and clear freshwaters (18–20‰; Jones et al. 2001, Pace et al. 2007), to which the study lakes should be compared with, but in the upper range for freshwaters in general (see Appendix C: Table C1). When PLFA data were missing (6 out of 27 sampling occasions), the phytoplankton source was considered as $\delta^{13}C$ of CO_2 minus 21.8‰ ± 3.6‰, which is the mean ± SD of the paired differences in $\delta^{13}C$ between phytoplankton and CO_2 found in the remaining 21 samples for which PLFA-based phytoplankton $\delta^{13}C$ estimates were available. Phytoplankton δ^2H was calculated as δ^2H of H_2O minus the estimated mean (±SD) difference in δ^2H between phytoplankton and H_2O (162.8‰ ± 26.1‰). This photosynthetic ^2H fractionation, which shows a close agreement with published values (Appendix C), was derived based on using $\delta^{13}C$ to estimate the proportions of POM that were of terrestrial and phytoplankton origin, respectively, and calculating phytoplankton δ^2H through a mass balance. The calculation was only performed in deeper stratified lakes where benthic contributions to epilimnetic POM can be assumed to be negligible. See Appendix C for procedures, justification, and uncertainty analysis.

Benthic photosynthetic stable C isotope fractionation is strongly coupled to the degree of benthic CO_2 limitation (Hecky and Hesslein 1995). Therefore, to predict this fractionation in our lakes, a benthic CO_2 limitation index was calculated from the partial pressure of CO_2 (pCO_2) and proxy variables for benthic productivity. This calculation followed Eq. 2, where Z_{sec} is secchi depth (m), A_{lake} is lake area (km²), and Z_{max} is maximum depth at the lake center

$$CO_2 \text{ limitation index} = \left(Z_{sec} \times \log(A_{lake})/Z_{max}\right)/pCO_2. \tag{2}$$

In theory, limitation by CO_2 is high when benthic productivity is high, but pCO_2 is low (Hecky and Hesslein 1995). The benthic productivity, in turn, is high in clear lakes (high Z_{sec}) with a flat shape, i.e., a low littoral slope (Duarte and Kalff 1986). Because we were missing direct measurements of the littoral slope, we
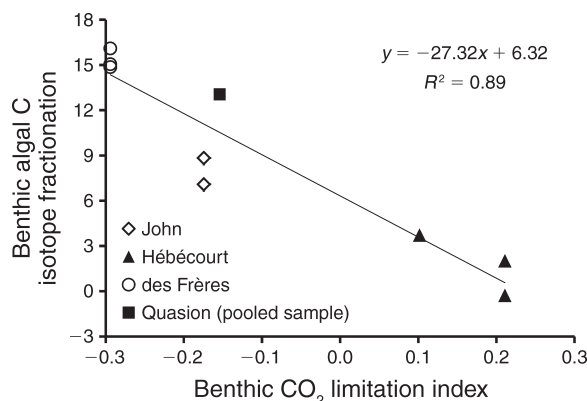

FIG. 1. The linear relationship between benthic algal stable C isotope fractionation ($\delta^{13}C$ of algae growing on ceramic tiles minus $\delta^{13}C$ of CO_2 in water at 0.5 m depth on sampling date) and the benthic CO_2 limitation index (benthic production relative to CO_2 concentration) ($r^2 = 0.89$, $n = 9$, $P < 0.001$). Note that only two of the tiles in John had enough material for analysis and material from all tiles in Quasion needed to be pooled for complete isotopic analysis. The Hébécourt samples were collected on two dates, which explains their slightly different values on the x-axis.

used $\log(A_{lake})/Z_{max}$ as a general measure of the "flatness" of the lakes. In agreement with theory (Hecky and Hesslein 1995), our index showed a strong relationship with observed photosynthetic stable carbon isotope fractionation of algae from the ceramic tiles ($r^2 = 0.89$, $P < 0.001$, $n = 9$; Fig. 1). For each sampling occasion, we estimated the $\delta^{13}C$ of the benthic algal source from the $\delta^{13}C$ of CO_2 minus the benthic algal stable carbon isotope fractionation (regressed from relationship in Fig. 1). The average magnitude of the residuals in the relationship in Fig. 1 (mean = 1.1‰) was used to represent SD. Finally, we used the δ^2H of H_2O minus the mean (±SD) difference in δ^2H between algae on the tiles and ambient H_2O (144.5‰ ± 14.7‰, $n = 9$) to estimate the δ^2H of benthic algae in each lake and sampling occasion. Table C1 in Appendix C compares our methods to the approach applied in previous allochthony studies that include a benthic end member.

As the study lakes range from moderately productive to highly oligotrophic, emerging macrophytes were rare and did not cover a significant portion of any lake areas. Thus, these were not considered to be a potential source. Submerged macrophyte-derived organic matter was considered to be too close to the benthic algal source in its isotopic composition to be considered as a separate source. For other possible sources of importance, see *Discussion*.

Trophic stable isotope enrichment.—For DOM and POM, the trophic stable isotope enrichment was set to 0‰ ± 0‰. For organismal samples, the $\delta^{13}C$ enrichment was calculated based on an assumed per-trophic-level carbon isotope fractionation (Δ_C) of 0.4‰ ± 1.3‰ (Post 2002) multiplied with τ, i.e., the number of trophic levels between the source and consumer level (Eq. 3). We

PLATE 1. Quebec, Canada, boreal landscape seen by floatplane. Photo credit: Monique Ariane Rezende.

assumed $\tau = 1$ for all cladoceran samples (Karlsson et al. 2004) and estimated τ for other samples from their $\delta^{15}N$ values in relation to the $\delta^{15}N$ of cladocerans by applying a per-trophic-level stable nitrogen isotope fractionation (Δ_N) of 3.4‰ ± 1.0‰ (Post 2002), according to Eq. 4. In a few samples containing no cladocerans ($n = 5$; Fig. 3), we applied fictive $\delta^{15}N_{clad}$ values that were 2.7‰ lower than those of calanoids (mean difference between calanoids and cladocerans)

$$\delta^{13}C \text{ enrichment} = \Delta c \times \tau \qquad (3)$$

$$\tau = (\delta^{15}N_{samp} - \delta^{15}N_{clad})/\Delta_N + 1. \qquad (4)$$

The overall trophic $\delta^{13}C$ enrichment ± SD for each sample was computed from 50 000 Monte Carlo simulations, each representing a calculation according to Eqs. 3–4, with random values of Δ_C and Δ_N, generated from their assumed means and SD.

The enrichment in δ^2H across trophic levels is not caused by trophic fractionation in its true definition, but is due to dietary water, which leads to incorporation of an isotopically heavier hydrogen source (Solomon et al. 2009). For each trophic level, we assumed that dietary water contributed to a fraction of consumer H (ω) of 0.15 ± 0.10, based on mean and SD of the data compiled by Solomon et al. (2009), excluding marine jellyfish. The applied distribution of ω was validated by

algebraic solutions of ω in six zooplankton samples that could be assumed to be completely autochthonous (Appendix B). Total contribution of water-derived H (ω_{tot}) was calculated as

$$\omega_{tot} = 1 - (1 - \omega)^\tau. \qquad (5)$$

The overall δ^2H enrichment ± SD (Eq. 6) for each sample was calculated from the output of 50 000 Monte Carlo simulations, each following Eqs. 4–6 in sequence. The simulations used randomized ω and Δ_N values, generated from their normal distributions

$$\delta^2H \text{ enrichment} = \delta^2H_{samp} - (\delta^2H_{samp} - \omega_{tot} \times \delta^2H_{water})$$
$$\div (1 - \omega_{tot}). \qquad (6)$$

Sensitivity analyses.—We tested the sensitivity of the mixing model results to assumptions regarding critical model parameters. In short, the SIAR model was run under alternative scenarios, where different fixed values of ω were applied (Appendix B), and the full potential difference in $\delta^{13}C$ between biomarker PLFAs and phytoplankton was explored (Appendix C). We also tested the effects of potential processing (repackaging) of the terrestrial organic matter, before it reached the

crustacean zooplankton, through a detrital food chain with two extra steps of microbial consumers (Appendix C). Setting ω to extreme values (0.05 and 0.25, respectively) resulted in probability distributions that showed offsets in the peak (modal allochthony) values by roughly 0.01 units in low allochthony samples, and 0.10 units in high allochthony samples. Nonetheless, these alternative distributions largely overlapped with the probability distributions in the basic scenario where ω is normally distributed with a mean of 0.15 and a standard deviation of 0.10 (Appendix B). Therefore, we consider that the basic scenario by itself gives a sufficient representation of the uncertainties with regard to ω. Modeling based on the other alternative scenarios (Appendix C) generated allochthony values of animals that were on average only 0.02–0.03 units different than the allochthony values from the basic models.

Multivariate statistical analyses.—To explore the links between the zooplankton allochthony values and environmental variables, we used redundancy analysis (RDA; Vegan 2.0 for R). Mean data values were used for sites sampled more than once to avoid disproportionate influences. Strongly skewed variables (skew > 2) were log-transformed prior to analysis. For sites with missing taxonomic groups, each missing allochthony value was replaced with the mean allochthony value for the taxon in question, before generating a first RDA model. Resulting site scores (WA scores) on the two first RDA axes were then used to regress (multiple linear regression) new allochthony values to fill the gaps of the missing values. A second RDA model was generated and the procedure was repeated iteratively until allochthony values with a perfect fit were obtained. This procedure minimized the qualitative influence of missing values. However, it did lead to slightly elevated R^2 values for the overall model. To compensate for this, we used a 1% significance level instead of 5% when assessing the significance of the RDA axes, using the built-in permutation tests in Vegan 2.0.

Results

There was a very strong ($r^2 = 0.95$, $n = 67$, $P < 0.001$, slope = 0.76) linear relationship between the modal values of the SIAR allochthony probability distributions (ΦT; most likely terrestrial contribution) and the degree of allochthony obtained from the two-source algebraic stable hydrogen mixing model (Fig. 2a). However, the slope of this relationship was lower than 1, implying that SIAR tends to provide more conservative estimates of allochthony, especially at high allochthony levels (Fig. 2a). Yet because of the strength of this relationship, and its consistency across taxa and sample types, we hereafter only report ΦT from the SIAR models.

SIAR modeling resulted in ΦT values that spanned from 0.02 to 0.49 in zooplankton samples and from 0.66 to 0.98 in DOM samples (Figs. 2 and 3). In 85% of the cases, samples appeared within the isotope "mixing polygons," taking 1 SD of source isotope ratios into
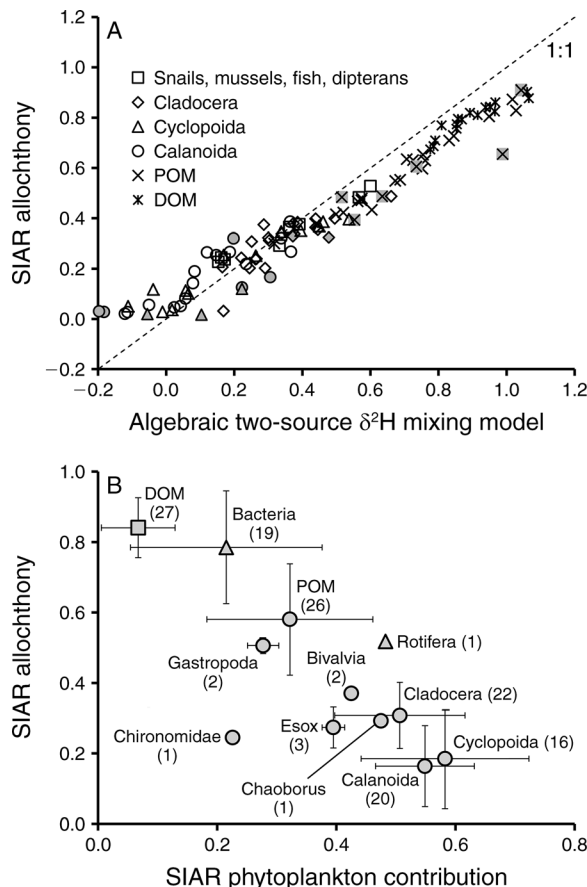

FIG. 2. (A) Modeled most likely (modal) allochthony from three-source, two-isotope, SIAR models, plotted against allochthony results from an algebraic two-source stable hydrogen mixing model. Gray symbols represent potentially problematic stable isotope analysis in R (SIAR) models with samples falling slightly outside of the mixing polygons as shown in Appendix C: Fig. C1. (B) Allochthonous and phytoplanktonic contributions (mean ± SD) to dissolved organic matter (DOM), particulate organic matter (POM), and organisms of various taxa. Number of samples (n) is shown in parentheses. Circles show three-source, two-isotope, SIAR models. Triangles show two-source (benthic excluded), one-isotope (δ^2H excluded), SIAR models. The square shows mean result from varying model type used for DOM (see Fig. 3). Bacterial allochthony data from the study lakes is based on mean δ^{13}C of bacterial biomarker fatty acids i15:0 and a15:0, corrected +3.8 for whole-cell vs. biomarker fractionation, and analyzed in relation to δ^{13}C source values, assuming trophic ^{13}C enrichment of 0.4‰ (S. E. Ziegler, *unpublished data*).

account (Appendix C: Fig. C1). The range of possible allochthony values was well constrained in each two-isotope (δ^{13}C and δ^2H) SIAR model (Fig. 3; solid bars), including all models for zooplankton, but in some cases where δ^2H data was missing, there was considerable scatter in the output from the one-isotope (δ^{13}C only) models (Fig. 3; open bars). In most samples, phytoplankton dominated the source contribution from autochthonous end members. Thus the samples were typically aligned in a scatter between the terrestrial and
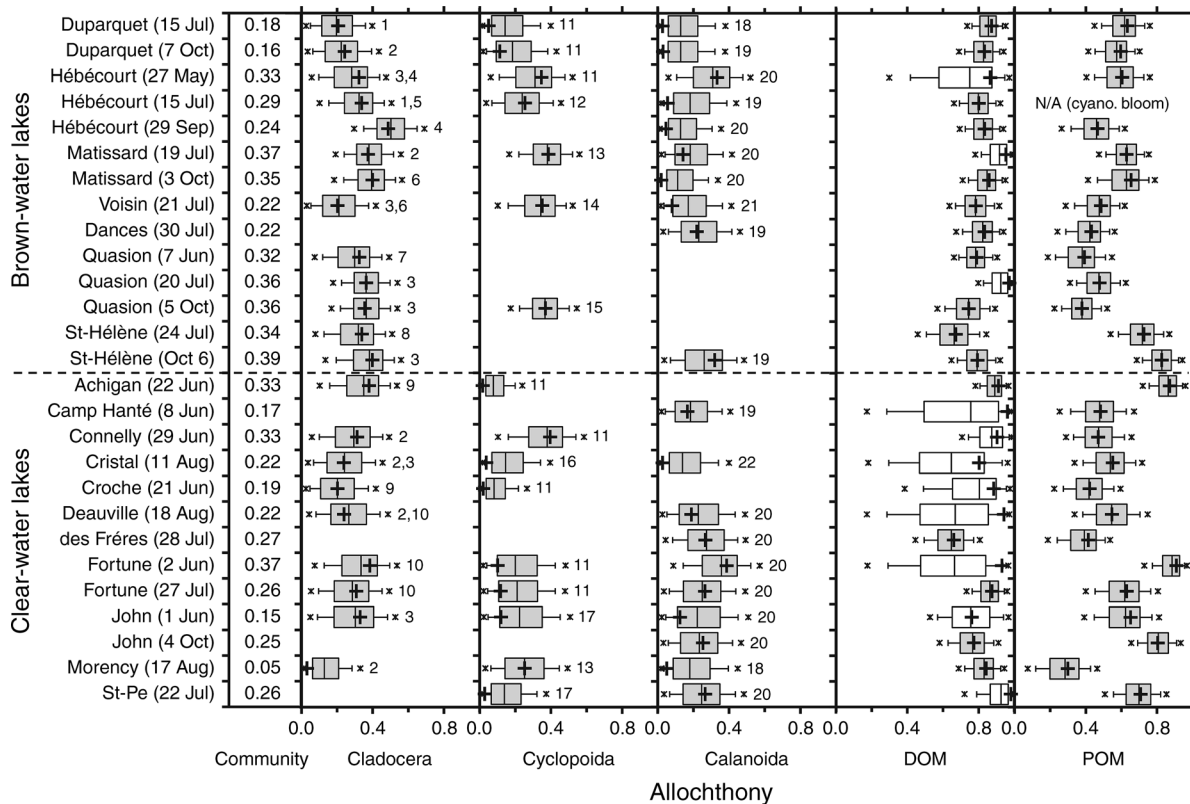
FIG. 3. Most likely values of allochthony (plus symbols) and allochthony probability distributions (box and whisker plots) shown for zooplankton, DOM, and POM. Whiskers show the 10th and 90th percentiles and asterisks the 5th and 95th percentiles. The "community" column shows biomass-weighted average allochthony of the three zooplankton groups. Solid gray bars show three-source (terrestrial, phytoplanktonic, benthic), two-source ($\delta^{13}C$, δ^2H), SIAR models. Open bars show two-source (terrestrial, phytoplanktonic), one-isotope ($\delta^{13}C$) models. Numbers beside bars indicate dominating species: 1, *Daphnia ambigua*; 2, *Daphnia galeata*; 3, *Holopedium gibberum*; 4, *Sinobosmina liederi*; 5, *Daphnia retrocurva*; 6, *Bosmina longispina*; 7, *Daphnia pulex*; 8, *Leptodora kindtii*; 9, *Daphnia dubia*; 10, *Diaphanosoma birgei*; 11, *Diacyclops bicuspidatus*; 12, *Mesocyclops edax*; 13, *Tropocyclops prasinus mexicanus*; 14, *Paracyclops affinis*; 15, *Diacyclops navus*; 16, *Cyclops scutifer*; 17, unknown; 18, *Epischura lacustris*; 19, *Skistodiaptomus oregonensis*; 20, *Leptodiaptomus minutus*; 21, unknown; and 22, *Leptodiaptomus sicilis*.

phytoplankton corners of the isotope mixing polygons (Appendix C: Fig. C1). In some individual samples, especially the chironomid sample from lake John, the benthic contribution (not shown) was well constrained and showed probability distributions clearly separated from 0.

DOM and bacteria showed the highest ΦT, on average about 0.8, followed by POM, rotifers (*Kellicottia* sp., Hébécourt) and gastropods (*Viviparidae*, Hébécourt) around 0.5–0.6, bivalves (*Unionidae*, Hébécourt) around 0.4, and fish (*Esox* sp., Hébécourt) plus dipterans (*Chaoborus*, des Frères; *Chironomidae*, John) around 0.25–0.30 (Fig. 2b). The mean ± SD ΦT of the total crustacean community was 0.27 ± 0.08 ($n = 27$), but within this total community, the allochthony of cladocerans (ΦT_{clad}; $n = 22$), cyclopoids (ΦT_{cycl}; $n = 16$), and calanoids (ΦT_{cal}; $n = 20$) was 0.28 ± 0.13, 0.18 ± 0.15, and 0.16 ± 0.12, respectively. Notably, there were no significant correlations between ΦT of the different zooplankton groups. Mean ΦT_{clad} was significantly higher than ΦT_{cycl} ($t = 3.12$, df = 36, $P < 0.01$) and of

ΦT_{cal} ($t = 4.33$, df = 40, $P < 0.001$; independent t tests), but ΦT did not differ between the copepods. Cyclopoids showed significantly higher mean ΦT ($t = 2.22$, df = 14, $P < 0.05$) in brown-water lakes (0.27) than in clear-water lakes (0.12). For cladocerans and the total crustacean community, mean ΦT was similar in brown-water lakes (0.33 and 0.30, respectively) and in clear-water lakes (0.27 and 0.24, respectively). ΦT_{cal} in brown-water systems was surprisingly low (mean = 0.13), but not significantly different from that in clear-water systems (mean = 0.20).

The allochthony of particulate organic matter (ΦT_{POM}) showed a strong gradient with values ranging from 0.30 to 0.91 (Figs. 3–4). ΦT_{POM} was positively correlated to ΦT_{cal} ($r^2 = 0.24$, $n = 19$, $P < 0.05$), to ΦT_{clad} ($r^2 = 0.22$, $n = 21$, $P < 0.05$) and to ΦT of the whole crustacean community ($r^2 = 0.17$, $n = 26$, $P < 0.05$; Fig. 4a). A relatively strong correlation with ΦT_{POM} was found in the subset of *Daphnia*-dominated cladoceran samples ($r^2 = 0.40$, $n = 10$, $P < 0.05$; Fig. 4a).
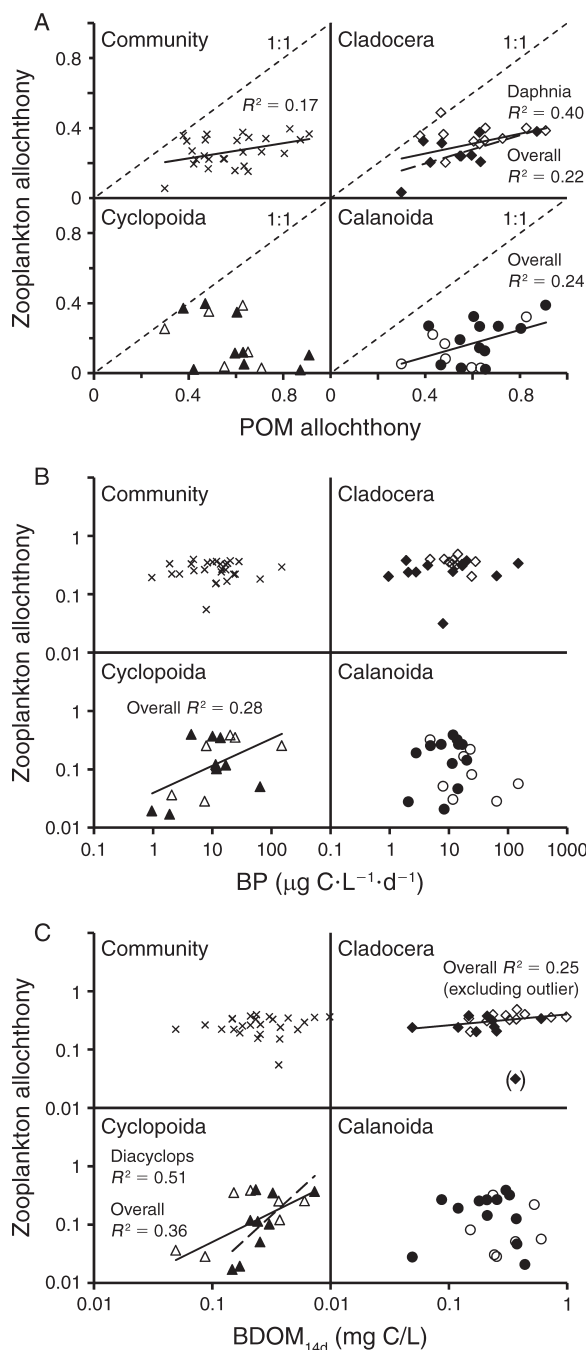
FIG. 4. Allochthony of crustacean zooplankton in relation to (A) the allochthony of particulate organic matter, (B) bacterial production (BP), and (C) bioavailable dissolved organic matter (BDOM) measured in 14-d dark incubations. Note the logarithmic scales in panels B and C. Solid symbols represent samples dominated by *Daphnia* sp. in the case of cladocerans, *Diacyclops* sp. in the case of cyclopoids, and *Leptodiatomus* sp. in the case of calanoids. Open symbols represent other dominant taxa. "Community" shows biomass-weighted average allochthony of the three zooplankton groups. Solid lines show significant ($P <$ 0.05) relationships based on all plotted observations. Dashed lines show significant relationships for the subsets of *Daphnia*-, *Diacyclops*-, and *Leptodiatomus*-dominated samples.

There were no relationships between ΦT of cyclopoids and ΦT_{POM}.

Different zooplankton groups did not consistently show correlations with bioavailable DOM ($BDOM_{14d}$) concentrations and BP rates. Only ΦT_{cycl} showed significant positive log-log correlations with BP ($r^2 = 0.28$, $n = 16$, $P < 0.06$; Fig. 4b) and $BDOM_{14d}$ ($r^2 = 0.36$, $n = 16$, $P < 0.01$; Fig. 4c). There was also a significant correlation between ΦT_{clad} and $BDOM_{14d}$ ($r^2 = 0.25$, $n = 20$, $P < 0.01$, excluding one apparent outlier; Fig. 4c). A particularly strong correlation between correlation between ΦT and $BDOM_{14d}$ was found in the subset of *Diacyclops*-dominated cyclopoid samples ($r^2 = 0.51$, $n = 9$, $P < 0.05$; Fig. 4c). Cyclopoids were the only crustacean group with a positive correlation between ΦT and $Color_{abs440}$ ($r^2 = 0.31$, $n = 16$, $P < 0.05$; not shown), although a significant positive correlation also existed between ΦT of the whole community and $Color_{abs440}$ ($r^2 = 0.21$, $n = 27$, $P < 0.05$; not shown).

Redundancy analyses resulted in a model with two significant axes (overall $R^2 = 0.84$, $R^2_{adj} = 0.51$; Fig. 5). The first axis (RDA1; $R^2 = 0.59$, $P < 0.001$) was characterized by a strong positive loading for ΦT_{POM} and negative loadings for $\delta^{15}N$ of POM, basin to lake area (drainage ratio) and for resource levels in the dissolved and particulate carbon and nutrient fractions.
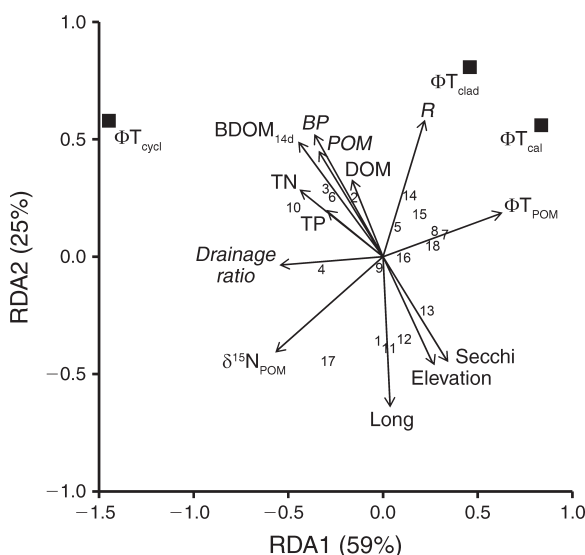

FIG. 5. Results from redundancy analyses (RDA) on the relationships between the allochthony of metazoan zooplankton taxa and environmental variables. Environmental variables are shown by vectors, taxa scores by solid squares, and site scores by site numbers. Log-transformed variables are in italic font. For graphical purposes, taxa scores have been scaled with factor 2.5. Environmental variables are TN, total nitrogen; TP, total phosphorus; drainage ratio, ratio of watershed area to lake area; $\delta_{15}N_{POM}$, stable nitrogen isotope ratio of particulate organic matter; Long, longitude; Elevation, elevation above sea level; and Secchi, Secchi disk sight depth. ΦT_{cycl}, ΦT_{clad}, ΦT_{cal}, and ΦT_{POM} represent the allochthony of cyclopoids, cladocerans, calanoids, and particulate organic matter, respectively.

The second axis (RDA2; $R^2 = 0.25$, $P < 0.001$) was positively related to the resource variables, but in particular, to high rates of community respiration, bacterial production (BP), and to concentrations of $BDOM_{14d}$ (Fig. 5). Variables that scored negatively on RDA2 were Secchi depth, elevation, longitude, and $\delta^{15}N$ of POM.

While ΦT of all zooplankton groups scored positively on RDA2, especially cladocerans, there were strong contrasts between the groups with regard to RDA1. ΦT_{cycl} scored negatively on RDA1, while ΦT_{clad} scored moderately positive and ΦT_{cal} strongly positive (Fig. 5). The allochthony of the DOM pool and of bacteria, which were high in all samples (Fig. 2b), showed very little variability, and these variables were therefore not included as a predictors in the RDA. Thus, DOM was synonymous with allochthonous DOM and BP was synonymous with BP based on allochthonous sources.

DISCUSSION

There is a growing volume of literature that converges to suggest that allochthony is a widespread feature of zooplankton communities in temperate and boreal lakes, but that it is also extremely variable both across lakes and temporally, ranging from undetectable to dominant (see Appendix C: Table C1, for a synthesis of studies on the topic). Some of the variability observed between studies is no doubt related to methodological issues, especially since there is still much uncertainty in the determination of the signature of end members and their spatial and temporal variation. In this regard, we here used a multi-isotope approach, with the aim of combining the strengths of different isotope ratios while explicitly incorporating their respective uncertainties in order to better constrain allochthony. We detected allochthony in zooplankton throughout our diverse set of clear-water and brown-water lakes, in a range ($\Phi T = 0.02$–0.49) that is in agreement with previous studies in northern lakes (Table C1). Terrestrially derived organic C is thus not only degraded by photochemical and biological pathways in these northern lakes (McCallister and del Giorgio 2008, Lapierre et al. 2013), but also appears to enter pelagic food webs in amounts that are high enough to be discerned using isotopic approaches.

The magnitude of allochthony not only varied greatly among lakes, but also significantly among zooplankton groups within lakes. Allochthony in cladocerans, cyclopoids, and calanoids was clearly linked to different carbon pathways and regulated by contrasting mechanisms (Figs. 4–5). Cyclopoids, which tend to be selective raptorial feeders (Barnett et al. 2007), had allochthony values (ΦT_{cycl}) uncoupled from those of the particulate organic matter (POM) pool (Fig. 4a) making it unlikely that cyclopoids assimilated terrestrial-derived organic matter by ingesting POM. Rather, ΦT_{cycl} values were positively correlated to water color ($Color_{abs440}$), concentrations of bioavailable dissolved organic matter ($BDOM_{14d}$) and to bacterial production (BP), indicating

that the cyclopoids obtained their allochthony from a microbial food chain based on terrestrial-derived DOM (Jansson et al. 2007, Berggren et al. 2010). This interpretation agrees with previous findings of cyclopoids in temperate lakes showing an increasingly terrestrial-like $\delta^{13}C$ signature along a gradient of increasing DOM (Persaud et al. 2009). Counterintuitively, ΦT_{cycl} in our study was positively related to TP and TN as the brown-water lakes were relatively nutrient rich. These brown lakes also had the highest concentrations of allochthonous DOM and the highest rates of BP (predominately based on allochthonous C; Fig. 2), allowing for cyclopoid utilization of the resource niche formed by allochthonous organic matter that is mobilized via DOM-based detrital food chains. Although cyclopoids do not tend to feed directly on bacteria, they are key consumers of large bacterivorous microzooplankton such as ciliates and rotifers (Dobberfuhl et al. 1997, Nakamura and Turner 1997). In fact, cyclopoids of the genus *Diacyclops* sp., in our study dominating in lakes with a particularly tight coupling between ΦT_{cycl} and $BDOM_{14d}$, are especially known for their feeding on smaller zooplankton (Dobberfuhl et al. 1997). The importance of microzooplankon as a potential mediator of allochthonous organic matter was further highlighted by the high allochthony ($\Phi T = 0.52$) of *Kellicottia* found in Hébécourt (Fig. 2), which agrees with reports of effective bacterivory in this rotifer (Bogdan et al. 1980, Walz 1995), given the dominance of allochthonous organic carbon in the bacterial biomass of the study lakes. Collectively, our results suggest that the DOM–bacteria–microzooplankton pathway is a primary route for allochthony in cyclopoids.

In sharp contrast, the allochthony of calanoids (ΦT_{cal}) was not related to $Color_{abs440}$, $BDOM_{14d}$, or to BP, suggesting minor relevance of the DOM–bacteria–microzooplankton pathway of allochthony. The same lack of correlations to $Color_{abs440}$, $BDOM_{14d}$, and BP was observed for the subset of the calanoid samples ($n = 12$) that were dominated by *Leptodiatomus* sp. Instead, ΦT_{cal} was best predicted by ΦT_{POM}, suggesting a reliance on POM of terrestrial origin (Pace et al. 2004, Cole et al. 2006, 2011). It is notable that the lakes with the highest ΦT_{POM} were not generally the lakes with the highest overall terrestrial influence. For example, the clear ultra-oligotrophic (chl $a = 1.1$–1.3 μg/L) lakes Fortune, John, and St-Pe, all with very small catchment areas (drainage ratio 1.3–2.2), showed relatively high values of ΦT_{POM} (0.63–0.91) and of ΦT_{cal} (0.13–0.39). This indicates that it is not the terrestrial influence per se that causes calanoid allochthony, but rather the lack of phytoplankton in relation to the abundance of terrestrial particles. The relationship between ΦT_{cal} and ΦT_{POM} might indicate that calanoids directly ingest terrestrial particulate C, but it could also reflect the consumption of particle-associated microbes that are linked to the POM pool (Simon et al. 2002).

The regulation of cladoceran allochthony (ΦT_{clad}) overlapped with the regulation of both ΦT_{cycl} and ΦT_{cal}, suggesting that allochthony in this group is linked to a POM-based and a DOM-based pathway, both likely terrestrial but differing in nature and origin. Moreover, cladocerans are much more effective as direct grazers of bacteria in comparison with the other two groups (Persaud and Dillon 2011). Multiple pathways of allochthony in this group might also explain why the average ΦT_{clad} was significantly higher (0.31) than that of ΦT_{cycl} (0.18) or ΦT_{cal} (0.16). Notably, the ΦT_{clad} was strongly positively coupled to lake community respiration, a general indicator of overall ecosystem heterotrophy in unproductive and humic lakes. It also showed the highest values in low-altitude western systems. Especially in Nord-du-Québec, south of James Bay, the ΦT_{clad} values were consistently high ($n = 6$; see Quasion, Sainte-Hélène, and John in Fig. 3), between 0.33 and 0.40, possibly reflecting the low primary production and high inputs of terrestrial organic matter typical for northern boreal lakes (Karlsson et al. 2012).

Previous studies have shown that the allochthony of lake zooplankton communities can be positively related to the amount of colored DOC (Color$_{abs440}$) and, especially, to the ratio between Color$_{abs440}$ and chl a (Carpenter et al. 2005, Batt et al. 2012). Based on the relationship between ΦT and color compiled for temperate lakes by Batt et al. (2012), it could be expected that the zooplankton in our boreal brown-water lakes, that have 10-fold higher Color$_{abs440}$: chl a ratios (compare Table 1), would be completely based an allochthonous organic matter. Yet, our results show that there rather appears to be an upper limit to the allochthony in these lakes somewhere around 0.5, which is similar to the zooplankton allochthony reported by Karlsson et al. (2012) for an extremely brown and unproductive lake in northern Sweden. This study shows that the relationship between community allochthony and Color$_{abs440}$ is weak in boreal and northern temperate lakes, and expressed mainly for the cyclopoid part of the community.

By using a multi-isotope ($\delta^2 H + \delta^{13} C$), multi-source (terrestrial, phytoplanktonic, benthic), stable isotope mixing model, we generated allochthony estimates that were not biased by organic matter produced by epilimnetic phytoplankton or benthic algae. It is, however, possible that they were biased by ^{13}C and ^2H depleted methane-derived organic matter (Kankaala et al. 2010) or by organic matter produced by deep hypolimnetic phytoplankton (Francis et al. 2011). Because of the systematically lower $\delta^{13}C$ and δ^2H that may occur in below-thermocline CO_2 and H_2O pools, respectively, deep phytoplankton can potentially become depleted in ^{13}C and ^2H relative to superficial phytoplankton. Thus, both methane-derived organic matter and deep phytoplankton could lead to lowered $\delta^{13}C$ and δ^2H consumer values, which in our case would imply that some of the zooplankton captured in the whole water column hauls appeared more phytoplankton-like than they actually were. Our allochthony estimates can therefore be considered to be conservative, i.e., lower bound estimates, with regard to the possible impact of these alternative sources. However, in a typical boreal lake with minor importance of vertical water δ^2H variability, inclusion of the δ^2H in the model should dampen the effect of this possible error source and lead to reasonable allochthony estimates (Karlsson et al. 2012).

While our SIAR models produced generally meaningful solutions, 15% of the samples appeared outside the mixing polygons (based on values from sources ± 1 SD as shown in Appendix C), which is slightly more than what is expected by chance. The outliers could be caused by influence of alternative sources discussed above, but also by overestimations of water contributions to consumer H, again possibly leading lower bound estimates of allochthony. This highlights the need of further refinements of the assumptions invoked in isotope-based allochthony models in general (Erhardt and Bedrick 2013), but also to the benefit of using multiple isotopes to compensate weaknesses in interpretations from single isotope ratios (Soto et al. 2013). See Appendix C for uncertainty analyses and further discussion of our modeling approach.

In summary, we show that calanoids and cyclopoids exhibit different patterns of allochthony regulation, pointing to the importance of detrital food chains linked to POM and DOM, respectively. *Cladocera* seemingly obtain allochthony from both pathways, thereby maximizing the utilization of allochthonous organic matter sources relative to the other groups. While our study focused on three broad taxonomic zooplankton groups, it is likely that the heterogeneity that we observed in the patterns of allochthony exists at finer taxonomic scales, both within and among lakes. These results highlight the diversity and complexity of pathways of transfer and delivery of terrestrial C from the base of the food web to higher trophic levels, and the resulting niche heterogeneity may be one of the key components of zooplankton functional diversity in these northern landscapes. While the general importance of terrestrial-derived organic matter for zooplankton remains debated (Francis et al. 2011, Kelly et al. 2014), our study suggests that the discussion should not be whether allochthony exists or not, but rather how and why it varies across lakes, and further points to the need for a nuanced discussion about the mechanisms leading to allochthony of different zooplankton groups in different types of lake ecosystems.

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Supplemental Material

Appendix A

Input data used for allochthony modeling in SIAR 4.0 for R (*Ecological Archives* E095-171-A1).

Appendix B

Validation of the assumed distribution of ω (dietary water contribution per trophic level) in zooplankton, and coupled sensitivity analysis (*Ecological Archives* E095-171-A2).

Appendix C

Assumptions involved in the determination of stable isotope composition of phytoplankton: justification and literature comparison (*Ecological Archives* E095-171-A3).

ERRATUM

Martin Berggren and colleagues have discovered an error in their paper in the July 2014 issue (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). The authors intended to use hectares as the input unit for lake area in Eq. 2, which calculates a benthic algal CO_2 limitation index from secchi depth, lake morphometry and partial pressure of CO_2. However, in error, lake area in square kilometers was used. This generated negative and potentially invalid index values for cases in which lake areas were smaller than 1 km^2, because of the logarithm transformation used in the equation.

If lake area in hectares is used, the problem of negative log values is avoided, but this correction causes a change in the relationship between the index and the benthic algal photosynthetic ^{13}C fractionation in Fig. 1 (see corrected figure below). Further, it changes (by $-2.0‰ \pm 4.3‰$, mean $\pm$ SD) the calculated benthic algal $\delta^{13}C$ source values used in the isotope mixing model to assess allochthony. However, the correction affects the allochthony only marginally, as seen in the below comparison between all corrected model allochthony and the corresponding published values.

There are no important changes in magnitude, variability, or patterns in the corrected allochthony values when compared to the published data, and no conclusions from the published paper are altered. The sole change in terms of the statistical significance is that the weak relationship between allochthony in the total crustacean community and in POM changes from significant ($P < 0.05$, $r^2 = 0.17$) to marginally significant ($P < 0.10$, $r^2 = 0.11$).

Finally, the authors also note that no units are provided for pCO_2 in Eq. 2 and wish to clarify that log-transformed units of ppmv (parts per million by volume) were used as pCO_2 input data.