An isotopic ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) investigation of the composition of particulate organic matter and zooplankton food sources in Lake Superior and across a size-gradient of aquatic systems

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Abstract

Food webs in aquatic systems can be supported both by carbon from recent local primary productivity and by carbon subsidies, such as material from terrestrial ecosystems or past in situ primary productivity. The importance of these subsidies to respiration and biomass production remains a topic of debate. While some studies have reported that terrigenous organic carbon supports disproportionately high zooplankton production, others have suggested that phytoplankton preferentially supports zooplankton production in aquatic ecosystems. Here we apply natural abundance radiocarbon (Δ^{14}C) and stable isotope (δ^{13}C, δ^{15}N) analyses to show that zooplankton in Lake Superior selectively incorporate recently-fixed, locally-produced (autochthonous) organic carbon even though other carbon sources are readily available. Estimates from Bayesian isotopic modeling based on Δ^{14}C and δ^{13}C values show that the average lakewide median contributions of recent in-lake primary production and terrestrial, sedimentary, and bacterial organic carbon to the bulk POM in Lake Superior were 58%, 5%, 33%, and 3%, respectively. However, isotopic modeling estimates also show that recent in situ production contributed a disproportionately large amount (median, 91%) of the carbon in mesozooplankton biomass in Lake Superior. Although terrigenous organic carbon and old organic carbon from resuspended sediments were significant portions (median, 38%) of the available basal food resources, these contributed only a small amount to mesozooplankton biomass (median, 3% from sedimentary organic carbon and 3% from terrigenous organic carbon). Comparison of zooplankton food sources based on their radiocarbon composition showed that terrigenous organic carbon was relatively more important in rivers and small lakes, and the proportion of terrestrially-derived material used by zooplankton correlated with the hydrologic residence time and the ratio of basin area to water surface area.

1. Introduction

The role of terrigenous organic carbon in aquatic food webs is not yet well constrained. Some studies (Pace et al., 2004; Carpenter et al., 2005; Cole et al., 2006) have reported that terrigenous organic carbon supports disproportionately high zooplankton production in lakes. Others have suggested a smaller role for allochthony (the consumption of organic matter produced outside of the system of interest), and have noted that, rather, phytoplankton sustain disproportionately larger and/or most of the zooplankton production in aquatic ecosystems (Brett et al., 2009; Karlsson, 2007; Pace et al., 2007; Karlsson et al., 2012). Still others have reported seasonal shifts in the food resources supporting aquatic food webs such that autochthony (consumption of organic matter produced within the system of interest) is predominant during high within-lake phytoplankton productivity in summer whereas allochthony (as well as heterotrophic bacterial biomass) is most important to zooplankton biomass during winter periods when within-lake primary and secondary production is minimal (Grey et al., 2001; Taipale et al., 2008; Karlsson and Sawstrom, 2009; Rautio et al., 2011).

There has been a realization that terrigenous organic matter exported from catchments is less refractory within aquatic systems than previously recognized, and can fuel microbial metabolism (Jones and Salonen, 1985; Tranvik, 1992), and that even the ancient (old according to radiocarbon measurements) component traditionally thought to be more recalcitrant could support bacterial (Cherrier et al., 1999; Petsch et al., 2001; McCallister et al., 2004), zooplankton (Caraco et al., 2010), and fish production (Schell, 1983). Accordingly, the notion that terrestrial carbon partially sustains food webs in aquatic systems has gained currency in the past few decades (Salonen and Hammar, 1986; Hessen et al., 1990; Meili et al., 1993; Pulido-Villena et al., 2005; Cole et al., 2011). Terrigenous carbon could be introduced and accumulated in aquatic food webs by zooplankton directly feeding on terrestrially-derived detrital particles (Hessen et al., 1990; Cole et al., 2006; Brett et al., 2009), and/or feeding on heterotrophic organisms that consume terrestrially-derived organic carbon (Jones, 1992; Lennon and Pfaff, 2005; Berggren et al., 2010).

Several studies in lakes have concluded that terrigenous food can support aquatic animal consumers (Cole et al., 2006; Karlsson and Sawstrom, 2009), and the relative importance of allochthony in lakes is thought to relate to factors such as lake color (indicating the amount of humic material present), trophic status, and size. Therefore allochthony should be higher in small humic lakes, and lower in eutrophic lakes and/or clear-water lakes with less terrestrial influence on organic matter cycling (Jones, 1992; Pace et al., 2007; Cole et al., 2011). The relative significance of these factors has been difficult to test as neither lab-based studies (Salonen and Hammar, 1986; Brett et al., 2009), small-scale in situ enclosure studies (Hessen et al., 1990) nor whole-lake ^{13}C-labeled bicarbonate addition approaches (Cole et al., 2002; Carpenter et al., 2005; Cole et al., 2006; Pace et al., 2007; Taipale et al., 2008) are easily applied to large-lake or marine systems. Also, the use of whole-lake ^{13}C labeling techniques for estimating the proportion of terrigenous organic carbon supporting zooplankton in lakes is limited or challenged by the fact that unlabeled food particles incorporated by zooplankton could possibly be from metalimnetic phytoplankton or phytoplankton-derived material predating label introduction rather than from terrestrial sources (Brett et al., 2009). Further, quantification of zooplankton food sources using ambient stable carbon isotopic signatures is difficult because of the inherent difficulty in directly measuring the δ^{13}C of phytoplankton, and the narrow and overlapping range of phytoplankton and terrigenous organic matter δ^{13}C signatures, especially in freshwater systems (Hamilton et al., 2005). The dynamic range of Δ^{14}C (-1000 to ~+200‰) is much greater than that of δ^{13}C in organic carbon (-32 to -12‰) (Petsch et al., 2001; McCallister et al., 2004; Wakeham et al., 2006), and provides a more sensitive means for differentiating the sources of organic carbon in the particulate organic matter (POM) matrix and organic carbon sustaining zooplankton secondary production. Also, while both $\delta^{13}C$ and $\Delta^{14}C$ are linear quantities that can be used for isotopic mixing models, $\Delta^{14}C$ has the added advantage of being the same for consumers and their food source in a modern ecosystem (as the $\Delta^{14}C$ calculation corrects for biochemical fractionations) thereby eliminating the need for fractionation correction along trophic levels as is the case for $\delta^{13}C$ (and $\delta^{15}N$).

In this study we examine the possible food sources of mesozooplankton in Lake Superior, the world's largest freshwater lake by surface area (Herdendorf, 1990), using natural abundance radiocarbon distributions. Recent investigations of Lake Superior, an oligotrophic system with low nutrient concentrations and primary productivity and a pronounced deep-chlorophyll maximum (Russ et al., 2004; Barbiero and Tuckman, 2004), have concluded that the lake appears to be net heterotrophic (McManus et al., 2003; Cotner et al., 2004; Russ et al., 2004; Urban et al., 2004; Urban et al., 2005). Terrigenous and resuspended sedimentary organic carbon sources have radiocarbon signatures that are unique and different from those of the lake's dissolved inorganic carbon and recently fixed primary production, hence providing the opportunity for better understanding the role of these possible food sources in mesozooplankton production and food web dynamics in the lake.

We exploit the natural abundance of radiocarbon ($\Delta^{14}C$), stable isotope ($\delta^{13}C$ and $\delta^{15}N$), and elemental compositions (atomic C:N ratio) of mesozooplankton to assess the role of different carbon sources in supporting mesozooplankton production, thereby providing a clearer picture of food web dynamics in Lake Superior. We also assess the putative food sources of zooplankton in a suite of other aquatic systems (riverine, smaller-lakes, and oceanic) for a broader-scale understanding of zooplankton food sources in aquatic food webs.

2. Methods

2.1 Sampling

Cruises were undertaken on the R/V *Blue Heron* to sample Lake Superior in May-June and August-September 2009 during isothermal (mixed) and thermally stratified water conditions, respectively. Site locations, water depths, and sampling depths are given in Fig 1 and Table 1. At each of the eight (8) sampling sites, we first obtained temperature, chlorophyll and depth profiles using a Seabird model 911 plus conductivity, temperature, and depth (CTD) rosette equipped with fluorometer, dissolved oxygen sensor, colored dissolved organic matter (CDOM) sensor, and altimeter (See supplementary Fig 1 A-L). For each of our open-lake stations, where water-column depths ranged from 155 to 388 m, we collected mesozooplankton using 50 m vertical tows through the water column using a 300 μm plankton net. At each of the nearshore stations (ONT and BR) the depth of tow was modified to a maximum depth of 4 to 10 m above the sediment water interface. The biomass was rinsed with lake water into the cod end of the net and duplicate samples were filtered onto glass-fiber filters (precombusted GF/F filters, 0.7 μm pore size), and stored frozen. Although we did not separate mesozooplankton into different groups in this study, a recent survey in the lake shows that copepods are the most dominant zooplankton in the surface waters of offshore Lake Superior (Yurista et al., 2009). In this extensive study at 31 sites over a 3 year period, Yurista et al. (2009) reported ~90% (by biomass) of the crustacean zooplankton in the offshore sites (>100 m water depth region) were copepods, and most of these (~80%) were concentrated in the surface 50 m of the lake water column, which is the depth over which we sampled our zooplankton in the offshore lake. Within the copepods, the taxa calanoids dominated by *Diaptomus copepodites* and *Limnocalanus macrurus* were more abundant in the lake than the cyclopoids which were mostly *Cyclops bicuspidatus thomasi* and *Cyclops* *copepodites* (Yurista et al., 2009). The calanoid copepods contributed ca. 70% of the biomass of crustacean zooplankton in Lake Superior (State of the great lakes report, 2009). For the copepods, the average volumetric concentration by biomass (and by numbers) of adult calanoids, immature calanoids, adult cyclopoids, and immature cyclopoids were 6255 μg m^{-3} (205 m^{-3}), 9682 μg m^{-3} (1864 m^{-3}), 1197 μg m^{-3} (236 m^{-3}), and 1305 μg m^{-3} (864 m^{-3}), respectively (Yurista et al., 2009). For cladocerans, the concentration of bosminids and daphnia were 633 μg m^{-3} (342 m^{-3}) and 4203 μg m^{-3} (309 m^{-3}), respectively (Yurista et al., 2009).

Sediment cores were taken from the open lake sites using an Ocean Instruments multi-corer. Recovered cores were sectioned at 2 cm resolution and kept frozen until further analysis, and the surface sediments (top 0-2 cm inclusive of the flocculant layer) were used in this study.

We collected dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), and particulate organic carbon (POC) samples from surface waters (≤5 m water depth). Water samples were drawn using twelve 8 L Niskin bottles mounted on the CTD rosette. DIC samples were collected directly from the Niskin bottles via pre-cleaned (10% HCl v/v, then ultra pure water [Millipore Milli-Q Plus]) silicone tubing into previously acid-cleaned and combusted (450°C for 4 hours) 500 mL amber Pyrex bottles. The bottles for DIC were rinsed three times with sample and then overflowed with two volumes of the unfiltered water. As quickly as possible after collection a small aliquot of water was removed, and the samples were preserved with saturated mercuric chloride solution, sealed airtight with glass stoppers coated with Apiezon M grease and stored at room temperature in the dark. POC and DOC samples were obtained by filtering lake water through precombusted Whatman GF/F glass fiber filters (450°C for 4 hours; 0.7 μm nominal pore size) via nitrogen pressurized stainless-steel canisters. Approximately 1 L of DOC sample from the resulting filtrate was collected into an acid cleaned and combusted glass bottle, acidified to pH 2 using 6N HCl (American Chemical Society Plus grade) and refrigerated.

After ~10 L of lake water had passed through a GF/F filter, the filter with retained particulate matter (POC sample) was placed in previously-combusted aluminum foil and stored frozen until analysis.

For comparison with our Lake Superior study we collated similar data for a suite of aquatic ecosystems of various sizes and residence times. Data from five northern small lakes sampled between June and September 2004 in Southern Quebec were adapted from McCallister and del Giorgio (2008); these small lakes include Bran-de-Scie, Des Monts, Stukely, Bowker, and Fraser Lakes. Zooplankton biomass and water samples for DOC, DIC, and POC and their isotopic signature were collected at a depth of 0.5 – 1.0 m using a diaphragm pump connected to an acid rinsed (10% HCl) plastic hose (McCallister and del Giorgio, 2008). Zooplankton were collected by passing at least 200 L of water through a 50 μm mesh screen, subsequently washed from the screen and stored overnight in deionized water at 4 ^{o}C to evacuate gut contents prior to isotopic analysis (McCallister and del Giorgio, 2008). The zooplankton samples were dominated by cladocerans and copepods. Cladocerans were primarily comprised of the genus Daphnia, most notably by *Daphnia mendotae* and *Daphnia catawba*, while copepods were dominated by *Diacyclopsbicuspidatus*, *Mesocyclops edax*, and *Letptodiaptomus minutus*. We also collated existing data from the Pacific Ocean (including Pacific coastal ocean, North Central Pacific, and North Eastern Pacific sites) and the Hudson River (eastern New York, USA). Data from the Hudson River was adapted from Caraco et al. (2010). Pacific Ocean zooplankton data included crustaceans and fishes, and were adapted from William et al. (1987), Druffel and William (1990), Druffel and William (1991), and Druffel et al. (1996).

2.2 Radiocarbon ($\Delta^{14}C$) analysis

$\Delta^{14}C$ measurements for Lake Superior samples were performed at the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution (WHOI). POC, zooplankton, and sediments were freeze-dried, acid fumigated (12 N HCl) overnight, and redried, and then converted to CO_2 by combustion in a modified Carlo Erba NA 1500 elemental analyzer (Zigah et al., 2011). DOC and DIC samples were converted to CO_2 by ultraviolet irradiation and phosphoric acid volatilization, respectively. The evolved CO_2 was cryogenically separated and reduced to graphite with H_2 over Fe catalyst (Zigah et al., 2011). The graphite produced was analyzed by accelerator mass spectrometry (AMS) along with primary and secondary standards, and combustion and graphitization process blanks.

Radiocarbon values are reported as $\Delta^{14}C$, the part per thousand deviation of the sample's ^{14}C:^{12}C ratio relative to a nineteenth century wood standard that has been corrected to the activity it would have had in 1950 and a $\delta^{13}C$ of -25‰. $\Delta^{14}C$ was corrected for fractionation using $\delta^{13}C$ of samples according to the convention of Stuiver and Polach (1977). Instrumental precision of the $\Delta^{14}C$ analysis is based on the error of standards or multiple analyses on a target.

2.3 Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and C:N analysis

Carbon and nitrogen contents of bulk POM and zooplankton were measured on a Costech ECS 4010 elemental analyzer (EA) coupled to a Finnigan Delta Plus XP isotope ratio mass spectrometer (IRMS) at the Large Lakes Observatory (LLO). $\delta^{13}C$ of samples (DOC, POC, sediment OC and zooplankton) were determined at NOSAMS using an Optima IRMS on subsamples intended for radiocarbon analyses. Stable nitrogen isotope ratios ($\delta^{15}N$) and a set of samples for $\delta^{13}C$ of POM and $\delta^{13}C$ of zooplankton were measured at LLO using a Finnigan Delta Plus XP IRMS with Conflo III interface (Thermo Fisher Scientific Inc., Waltham, MA) coupled to a Costech ECS 4010 EA. Typical instrumental precisions of $\delta^{15}N$ and $\delta^{13}C$ based on analyses of multiple external standards were 0.17‰ and 0.2‰, respectively. The stable isotope ratios (^{13}C:^{12}C and ^{15}N:^{14}N) are reported as $\delta^{13}C$ and $\delta^{15}N$ respectively, which are the per mil difference relative to Vienna Pee Dee Belemnite carbonate and atmospheric air standards.

2.4 Bayesian MixSIR mixing model for multiple endmembers

The Bayesian isotopic modeling software MixSIR (Version 1.04) (Moore and Semmens 2008; Semmens et al. 2009) was used to partition the proportional contributions of potential OC sources to the bulk POC and to zooplankton diet based on their $\Delta^{14}C$ and $\delta^{13}C$ signatures. The MixSIR model works by determining probability distributions of sources contributing to the observed mixed signal while accounting explicitly for the uncertainty in the isotopic signatures of the sources and fractionation. The uncertainty of $\delta^{13}C$ and $\Delta^{14}C$ values used for modeling here are the analytical uncertainties based on analyses of multiple external standards or multiple analyses of graphite targets in the case of $\Delta^{14}C$. Since isotopic fractionation is already corrected for in the calculation of $\Delta^{14}C$ values, radiocarbon fractionation was not used in the model (thus specified as zero). Isotopic fractionation of +1 was used for $\delta^{13}C$ (Fry and Sherr, 1984). Prior information was not used in the model, hence all possible source combinations were equally possible contributions to the observed mixed signal. The number of iterations used was 10,000,000 (and 100,000,000 when the posterior draws were less than 1000). For each potential source, we report the median and the 5% and 95% confidence percentiles estimates of the proportional contribution of the sources to the measured (observed) value.

2.4.1. Choice of endmembers

To determine carbon sources to POC and food sources supporting mesozooplankton biomass, we chose isotopic end-members based on identifiable unique sources of OC to the POC pool in the lake (Zigah et al., 2011; Zigah et al., 2012). Because our modeling is based upon natural abundance stable carbon and radiocarbon distributions, these end-members vary from those generally used in labeling experiments (e.g., Taipale et al., 2008) or natural abundance stable isotope modeling (e.g., Karlsson et al., 2003). Based upon their unique $\Delta^{14}C$ values, the potential components of POC in the lake include carbon derived from recent photosynthesis (here described as "algal carbon" although it also includes herbivore biomass supported by recent primary production), bacterial OC, terrestrial OC, and resuspended-sediment OC. As this is a novel suite of end-members relative to previous work, we discuss our rationale further below.

Lake-wide primary production in Lake Superior is estimated at 9.73 Tg C per year (Sterner, 2010), although most of the POC input from phytoplankton photosynthesis is thought to be mineralized rapidly (Maier and Swain, 1978; Urban et al., 2005) and does not persist in the lake. The POC pool in the lake is only ~ 1 Tg C, (compared to ~15 Tg DOC and ~122 Tg DIC; Zigah et al., 2012). We assigned a $\delta^{13}C$ value of -30 ± 1‰ as representative of algal C (Sierszen et al. 2006). We used a $\Delta^{14}C$ of DIC as the $\Delta^{14}C$ of algal carbon from recent photosynthesis as DIC-incorporation is the starting point for algal biomass production (McNichol and Lihini, 2007; McCarthy et al., 2011). For calculating $\Delta^{14}C$ values, measured radiocarbon values are normalized to remove mass-dependent isotopic fractionation such that $\Delta^{14}C$ values reflect only time (age) or mixing (variably aged components). To normalize the sample, fractionation between ^{14}C and ^{12}C is assumed to be approximately twice that between ^{13}C and ^{12}C since the mass difference between ^{14}C and ^{12}C is twice that between ^{13}C and ^{12}C (Donahue et al., 1990; McNichol and Lihini, 2007). Therefore, in a modern system, the $\Delta^{14}C$ of algal carbon tracks that of DIC that was incorporated.

Bacterial carbon is another identifiable component of POC in the lake (Cotner et al., 2004). For $\Delta^{14}C$ and $\delta^{13}C$ of bacterial carbon, we used the $\Delta^{14}C$ and $\delta^{13}C$ of DOC, the main microbial food source, as we do not have direct measurement of bacterial biomass $\Delta^{14}C$ and $\delta^{13}C$. We acknowledge that this is only a first order approximation of the $\Delta^{14}C$ and $\delta^{13}C$ of bacteria in Lake Superior, and look forward to further refining this model endpoint when better data become available.

Radiocarbon values of bulk POC in the lake suggest that they contain a pre-aged carbon source that may result from sediment resuspension and further that this resuspension can impact surface water samples as well as deeper samples (Zigah et al., 2011; Zigah et al., 2012). This finding is consistent with previous studies showing the importance of sediment resuspension in Lake Superior (Urban et al., 2005; Churchill and Williams, 2004; Flood, 1989; Flood and Johnson, 1984). In our Lake Superior work (see below), the OC in the surface sediments (0-2 cm) at the various study stations across the lake have $\Delta^{14}C$ values that are older (^{14}C-depleted) than recent algal OC from lake photosynthesis. The physical mechanisms transporting such old OC from the lake sediments into the lake surface water are not well known. However, Lake Superior is dimictic, thus, there is density driven vertical mixing of the water column twice each year. Hence, organic materials resuspended into the deep waters due to strong bottom currents could be introduced into the surface waters during the lake overturn. In our lake surface (top 0-2 cm) sediment samples, the $\delta^{13}C$ values of OC were -27.3‰, -26.9‰, -27.0‰, -26.2‰, and -27.0‰, and the $\Delta^{14}C$ values were -20 ± 3‰, -37 ± 2‰, -23 ± 2‰, -117 ± 2‰, and -36 ± 2‰ at sites CM, SM, NM, WM, and EM respectively, and the corresponding site-specific values were used as the sediment OC end-member for each site in the lake. We note that this end member assignment is a first order approximation as lateral advection of old OC from shallower depths, especially at the WM site, is also possible.

The choice of end member for terrestrial OC was challenging because there are both ‘old’ and ‘recent’ terrestrial OC components. The radiocarbon value of recently synthesized terrestrial OC reflects the radiocarbon value of atmospheric CO_2. We determined the radiocarbon value of atmospheric CO_2 using the radiocarbon content of corn leaves (annual plant) collected in the western watershed of the lake ($\Delta^{14}C = 38 \pm 2‰$, Zigah et al., 2011). While this approach does not cover the entire watershed of the lake, we do not think there would be considerable differences across the basins because most variations in atmospheric ^{14}C occur at a larger spatial scale. The remoteness of the lake from big industrial plants or big cities, the uniformity of surface $\Delta^{14}C$-DIC across the lake, and the absence of considerable soot (black) carbon in the POC pools across the lake (Zigah et al., 2012) suggest little regional variation in atmospheric ^{14}C around Lake Superior. To account for the fact that terrestrially produced OC could spend some time in the soil before delivery to the lake, we performed additional model runs replacing the corn $\Delta^{14}C$ value with that of POC collected during high flow conditions in June 2008 from Amity Creek ($\Delta^{14}C = 11 \pm 2‰$; $\delta^{13}C = -27.3‰$, Zigah et al., 2011), a primarily forested watershed north shore stream which drains into western Lake Superior. The choice of highflow data was because most terrestrial influx in streams and rivers occurs during storm flows. While we note that using data from one stream within the watershed might not be representative, the similarity of high flow Amity Creek POC $\Delta^{14}C$ to nearshore POC $\Delta^{14}C$ ($\Delta^{14}C$ range of 7-17‰) from both the southern and northern nearshore regions of the lake that we sampled implies that our terrestrial end-member POC-$\Delta^{14}C$ is a good first approximation.

2.5 Zooplankton allochthony based on $\Delta^{14}C$

Zooplankton allochthony in Lake Superior was estimated using a binary (terrigenous and autochthonous) mixing model as follows:

$$\Delta^{14}C_{\text{Zoop}} = f \bullet \Delta^{14}C_{\text{Terr}} + (1 - f) \bullet \Delta^{14}C_{\text{Algal}} \qquad (1)$$

where f is the fraction of terrestrial OC in the zooplankton biomass, (1 – f) is the fraction of algal-derived carbon in the zooplankton biomass, and the subscripts 'Terr' and 'Algal' refer to terrestrial and algal-derived, respectively. We used $\Delta^{14}C$ of DIC as the algal-derived OC endmember. For the terrestrial endmember, we used the atmospheric CO_2 $\Delta^{14}C$ and $\Delta^{14}C$ of POC from high flow Amity Creek in separate model runs for sensitivity analysis.

2.6 Statistical analyses

We used SigmaPlot 9.0 (Systat Software Inc., San Jose, California, USA) for all statistical analyses. Relationships among samples were tested via correlation analyses in which case we report the Pearson's correlation coefficient (r), probability (*p*), and number of samples (n). The difference between isotopic composition of zooplankton and that of DIC, POC and DOC was determined using paired t-tests, and for these we reported the two-tailed probability value (*p*), and the number of samples (n). Significance difference or correlation was tested at 95% confidence level ($\alpha = 0.05$).

3. Results

3.1 Lake Superior isotopic distributions

The bulk POC in the lake (including both stratified and isothermal surface samples) had a mean $\Delta^{14}C$ value of 10 ± 29‰ (range -55‰ to 39‰, n = 14) (Fig. 2A and 2B; Table 2), and the $\Delta^{14}C$ of DOC in the lake was 38 ± 21‰ (range -10‰ to 74‰, n = 13) (Fig. 2A and 2B). $\Delta^{14}C$ of mesozooplankton varied from 36 to 38‰ at NB and ONT sites (both nearshore regions) to 62‰ at CM and SM (both offshore regions) (Fig. 2A and 2B). At each site $\Delta^{14}C$ of mesozooplankton and $\Delta^{14}C$ of DIC were similar (Fig. 2A and 2B) and a paired t-test showed no significant difference in their values ($p = 0.96$, n = 13). In contrast, $\Delta^{14}C$ of mesozooplankton was significantly more positive (thus, ^{14}C-enriched) than $\Delta^{14}C$ of either POC (t-test, $p < 0.0001$, n = 13) (Fig. 2A and 2B) or DOC (t-test, p = 0.03, n = 13) (Fig. 2A and 2B).

The $\delta^{13}C$ and $\delta^{15}N$ of POM in Lake Superior exhibited seasonal shifts. The bulk POM was more ^{13}C-enriched ($\delta^{13}C$, mean = -28.2 ± 0.6‰, range -27.1‰ to -28.9‰, n = 7) and ^{15}N-depleted ($\delta^{15}N$, mean = 0.5 ± 0.8‰, range -3.9‰ to -2.0‰, n = 7; Table 2) during stratification in August (excluding $\delta^{13}C$ of POM at EM) compared to the bulk POM in the isothermal lake in June ($\delta^{13}C$, mean = -29.9 ± 0.4‰, range -29.5‰ to -30.4‰, n = 7; $\delta^{15}N$, mean = -2.9 ± 0.6‰, range -0.6‰ to 1.7‰, n = 7; Table 2). In contrast, the stable isotopic composition of mesozooplankton in Lake Superior exhibited no seasonal shift for carbon and a smaller shift for nitrogen (Table 2). The $\delta^{13}C$ of mesozooplankton in Lake Superior was -30.0 ± 0.6‰ (range -29.5‰ to -31.2‰, n = 7) during isothermal conditions in June, and -30.0 ± 1.0‰ (range -28.2‰ to -31.3‰, n = 6) during stratification in August (excluding $\delta^{13}C_{POM}$ from ONT; Table 2). The average $\delta^{15}N$ of mesozooplankton in the isothermal lake in June was 5.0 ± 0.5‰ (range 4.2‰ to 5.4‰, n = 6), and shifted to 3.4 ± 0.8‰ (range 2.4‰ to 4.4‰, n = 6) in August (excluding ONT data in both seasons) (Table 2).

The $\delta^{15}N$ and $\delta^{13}C$ values of consumers reflect both the isotopic composition of the incorporated food plus biochemical fractionations. Movement across trophic levels imposes additional fractionation on the resulting biomass, with consumers exhibiting ^{13}C-enriched values of ~0.5-1‰ (Fry and Sherr, 1984) and ^{15}N-enriched values of 2-3‰ (Fry, 1991) relative to their food source. In Lake Superior, the mesozooplankton were ^{15}N-enriched by an average of ~4‰ relative to bulk POM during isothermal condition, and ^{15}N-enriched by ~6‰ relative to bulk POM during stratification (Table 2). In contrast to $\delta^{15}N$ values, mesozooplankton were ^{13}C-depleted by an average of ~1‰ relative to bulk POM during stratification in August (Table 2).

The C:N values of mesozooplankton ranged from 6.2 to 8.7 (mean 7.1, n = 14), and were consistently and significantly lower than the C:N values (C:N, mean 8.2, range 7.0 to 9.5) of bulk POM (t test, *p* = 0.001, n = 14) (Table 2).

3.2. Modeling sources of POC and Mesozooplankton diet in Lake Superior

3.2.1. POC sources

The contribution of potential source materials to the bulk POC was estimated using the Bayesian MixSIR model based on source $\Delta^{14}C$ and $\delta^{13}C$ signatures. Based on the model results, the median (and 5% and 95% confidence percentiles) contribution of algal carbon to the bulk POM varied from 10% (5 – 14%) at the EM site during isothermal condition to 85% (77 – 93%) at the NM site during isothermal condition (Table 3). The median contribution of terrestrial carbon to bulk POM ranged from 1% (0.1 – 5%) at EM site during isothermal condition to 19% (2 – 47%) at the WM site during stratification (Table 3). Sedimentary OC influence on bulk POM varied from a median of 10% (2 – 15%) during stratification at WM site to 87% (84-91%) during isothermal condition at EM site (Table 3). The average lakewide (including both seasons) median contributions of algal, terrestrial, sedimentary, and bacterial OC to the bulk POM were 58%, 4%, 34%, and 2% (Table 3), and the corresponding values with creek POC as terrestrial endmember were 58%, 6%, 32%, and 3%, respectively (Table 3).

3.2.2. Sources of mesozooplankton diet

After estimating the relative contributions of potential basal food resources to the bulk POM, we used the Bayesian MixSIR mixing model to evaluate the relative contributions of these basal foods to mesozooplankton production in Lake Superior. Algal-based food contributed a disproportionately large amount to the mesozooplankton biomass in Lake Superior, with a median contribution (average of both isothermal and stratified season at all sites) of 91% (range:

85 - 95%). Mesozooplankton in Lake Superior also appear to gain a lakewide median of 3% (2-4%) of their biomass carbon from consumption of bacterial biomass (Table 4). Although making up a considerable portion of the bulk POC in the lake, OC from the sediment and terrestrial POC contributed minimally (median: 3% each) to mesozooplankton carbon (Table 4).

Based on a two-endmember mixing model using recent in-lake primary production and terrestrial inputs as the endmembers, mesozooplankton allochthony varied across the lake sites, ranging from 0-54% (with corn leaves used as the terrestrial endmember) or 0-25% (with creek POC as the terrestrial endmember) and was in most cases much lower than mesozooplankton autochthony (Table 5). The mesozooplankton autochthony estimates from both multiple endmember (Table 4) and binary endmember (Table 5) models were comparable with a lakewide average offset of ~8% or 3% if the Isothermal SM site with large difference is excluded. The offset is only ≤ 1% when the creek POC is used as the terrestrial endmember. The mesozooplankton allochthony estimate from the binary model varied from the terrestrial contribution from the multiple endmember Bayesian model with a lakewide average offset of ~18% or 11% (if Isothermal SM is excluded). The offset, however, is ≤6% when creek POC is used as terrestrial endmember.

3.3 Cross-system comparisons of isotopic distributions (Hudson River, Small Lakes, Lake Superior, and the Pacific Ocean)

Zooplankton in the Hudson River had pre-aged radiocarbon content ($\Delta^{14}C$ = -236‰; Caraco et al., 2010), and were ^{14}C-depleted relative to recent terrestrial OC, algae (based on $\Delta^{14}C_{DIC}$), POC, and DOC (Table 6). The $\Delta^{14}C$ of zooplankton in small lakes (Bran-de-Scie, Des Monts, Stukely, Bowker, and Fraser Lakes) ranged from -2‰ in Bowker Lake to 40‰ in Des Monts Lake (Fig. 3A), and was consistently ^{14}C-depleted relative to concurrent POC, DOC, and

DIC (with the exception of Fraser Lake with a DIC Δ^{14}C of -91‰ vs. a zooplankton Δ^{14}C of 10‰, Table 6; Fig. 3A). It is evident from $\Delta\Delta^{14}C_{DIC-Zoop}$ ($\Delta^{14}C_{DIC}$ - $\Delta^{14}C_{Zoop}$) vs. $\Delta\Delta^{14}C_{POC-Zoop}$ ($\Delta^{14}C_{POC}$ - $\Delta^{14}C_{Zoop}$) that the Δ^{14}C values of zooplankton in the small lakes are more similar to Δ^{14}C of DIC than Δ^{14}C of POC, with the exception of Fraser Lake where the absolute values of $\Delta\Delta^{14}C_{DIC-Zoop}$ and $\Delta\Delta^{14}C_{POC-Zoop}$ were almost the same (Table 6).

In the coastal Pacific Ocean, $\Delta^{14}C_{DIC}$ of 100‰ and $\Delta^{14}C_{POC}$ of 100‰ were identical and slightly ^{14}C-enriched compared to $\Delta^{14}C_{zoop}$ of 76‰ (Fig. 3B; Table 6). A similar trend was observed in the north central Pacific Ocean where $\Delta^{14}C_{zoop}$ of 124‰ was slightly ^{14}C-depleted relative to the $\Delta^{14}C_{DIC}$ of 132‰ and $\Delta^{14}C_{POC}$ of 139‰ (Fig. 3B; Table 6). In contrast, the zooplankton in the northeast Pacific Ocean had Δ^{14}C values identical to Δ^{14}C of POC, but different from the Δ^{14}C of DIC as evident in the $\Delta\Delta^{14}C_{DIC-Zoop}$ and $\Delta\Delta^{14}C_{POC-Zoop}$ of 69‰ and -4‰, respectively (Table 6).

The entire dataset was pooled to assess inter-system trends (thus small-to-large water body ecosystems) in $\Delta^{14}C_{zoops}$ vs. $\Delta^{14}C_{DIC}$, and $\Delta^{14}C_{zoops}$ vs. $\Delta^{14}C_{POC}$. There was a positive correlation between Δ^{14}C values of zooplankton and DIC (excluding Hudson River) in the pooled Δ^{14}C data (r = 0.82, $p < 0.0001$, n = 14) (Fig. 4A), with ~67% of the variation in Δ^{14}C of zooplankton accounted for by the changes in Δ^{14}C of phytoplankton utilizing in situ DIC (based on correlation coefficient of 0.67, Table 4A). In contrast, Δ^{14}C of zooplankton was not correlated to Δ^{14}C of bulk POC (r = 0.03, $p = 0.92$, n = 14) (Fig. 4B).

There was a positive correlation between $\Delta\Delta^{14}C_{DIC-Zoop}$ and the ratio of basin area to lake surface area (correlation, r = 0.88, $p = 0.047$) (Fig. 5A). Although marginally significant (correlation, r = -0.84, $p = 0.078$), there was a negative correlation between the hydrological residence time of the lakes and $\Delta\Delta^{14}C_{DIC-Zoop,}$ (Fig. 5B).

4. Discussion

4.1 Composition of bulk POM, and putative food sources for consumers in Lake Superior

Isotopic signatures of baseline food resources can be used to assess their relative importance in the diet of their animal consumers. Food-source tracking using isotopic signatures works if a measurable contrast exists between the potential food resources. Mesozooplankton in Lake Superior could obtain their diet from recent primary production (perhaps cycled through an additional small herbivore first), bacterial biomass, terrestrial OC, or OC from resuspended sediments.

Our results indicate that in Lake Superior, the proportional median contribution of recent primary production to bulk POC was 58% (Table 3). This is not surprising for a large cold temperate oligotrophic lake with low levels of autochthonous primary production (Cotner et al., 2004; Sterner, 2010). Although the estimated annual lake-wide primary production is 9.73 Tg C (Sterner, 2010), the OC input from phytoplankton photosynthesis is thought to be mineralized rapidly (Maier and Swain, 1978; Urban et al., 2005) and does not persist in the lake. Consequently, the POC pool in the lake is estimated at only ~ 1 Tg C (compared to ~15 Tg DOC and ~122 Tg DIC; Urban et al., 2005; Zigah et al., 2012).

Our model estimates show that the combined proportions of terrestrial OC and resuspended-sediment OC can constitute a considerable fraction of the basal food available to consumers in the lake (Table 3). These estimates are consistent with published values from previous studies in the lake. Urban et al. (2004) reported that resuspended sedimentary OC contributed 10-35% of OC in sinking POC off the Keweenaw Peninsula at the depth of 25-35 m in the lake.

4.2 Stable isotopes and C:N ratios

Although bulk POM was ^{13}C-enriched and ^{15}N-depleted during stratification in August relative to isothermal conditions in June, the mesozooplankton in the lake did not exhibit such seasonal changes in their $\delta^{13}C$ and $\delta^{15}N$ signatures (Table 2). Mesozooplankton in the lake were generally ^{13}C-depleted relative to the bulk POM, especially in the productive surface waters during stratification in August. Such ^{13}C-depletion of mesozooplankton compared to bulk POM has been reported by several researchers (del Giorgio and France, 1996; Karlsson et al., 2003; Pulido-Villena et al., 2005; Mathews and Mazumder, 2006; McCallister and del Giorgio, 2008), and suggests that the mesozooplankton were primarily supported by a subsurface algal food with ^{13}C-depleted values, or a baseline algal food source within the surface POM with ^{13}C-depleted values as typical trophic-level enrichments for $\delta^{13}C$ are +0.5 to +1‰ (Fry and Sherr, 1984). Another possibility is the accumulation and/or storage of lipids by the mesozooplankton from their food, thus making their entire biomass or whole body more ^{13}C-depleted than their food source as lipids are more ^{13}C-depleted than other biochemicals in their biomass (DeNiro and Epstein, 1978; McConnaughey and McRoy, 1979; Kling et al., 1992; Matthew and Mazumder, 2005; Smyntek et al., 2007). Mesozooplankton in Lake Superior do exhibit an increase in C:N values during stratification in August relative to isothermal conditions in June, which is consistent with increasing accumulation and storage of lipids during the more productive and warmer season (McConnaughey and McRoy, 1979; Kiljunen et al., 2006; Smyntek et al., 2007; Syvaranta and Rautio, 2010).

4.3 Radiocarbon-based estimation of mesozooplankton food sources in Lake Superior

Taken together, and without seasonal comparison, the stable C and N isotope values do not distinguish mesozooplankton from the bulk POM pool from which they feed, especially during isothermal conditions in the lake. Adding radiocarbon information allows for the estimation of mesozooplankton dependence on food sources other than that year's in situ primary production (and its immediate consumers), and also significantly refines the relationship between POM and mesozooplankton.

Mesozooplankton in Lake Superior in both isothermal and stratified conditions have $\Delta^{14}C$ values that track those of co-occurring DIC rather than bulk POM (Fig. 1), indicating that the mesozooplankton in this system are preferentially feeding on food resources resulting from contemporary photosynthesis rather than indiscriminately upon bulk POM. Bayesian MixSIR modeling results generally show that most of the mesozooplankton biomass in the entire lake, and in both seasons (medians 85-95%; Table 4) came from incorporation of recent primary production. These results are generally consistent with mesozooplankton autochthony estimates from binary isotopic mixing modeling with the exception of SM site during isothermal conditions (range 61-100% or 75-100% depending on choice of terrestrial endmember as shown in Table 5). Both approaches show considerable enrichment in mesozooplankton biomass relative to the proportion of "algae" in bulk POC (median, 58%; Table 3). That algal carbon dominantly supports mesozooplankton biomass production was not surprising as algal-derived food is generally known to be labile and the most preferred food option for secondary producers (Brett et al., 2009). Our results agree with previous studies in other lakes (del Giorgio and France, 1996; Cole et al., 2002; McCallister and del Giorgio, 2008; Mohammed and Taylor, 2009; Karlsson et al., 2012) and rivers (Sobczak et al., 2002; Thorp and Delong, 2002; Meersche et al., 2009) that reported that zooplankton were sustained disproportionately and/or largely by phytoplankton biomass.

Mesozooplankton dependence on organic carbon subsidies (sedimentary and terrestrial OC) in Lake Superior was small (Table 4), although these organic carbon resources make up a considerable fraction of the bulk POC in the lake (Table 3). Contrary to our results, other studies have reported larger use of non-algal food by zooplankton in some aquatic systems based on either natural abundances of $\Delta^{14}C$ (Schell, 1983; Caraco et al., 2010), $\delta^{13}C$ and $\delta^{15}N$ (Meili et al., 1996; Jones et al., 1998; Karlsson et al., 2004; Matthews and Mazumber, 2006), or whole lake addition of ^{13}C-labeled bicarbonates (Carpenter et al., 2005; Pace et al., 2007; Taipale et al., 2008).

Differently aged components (modern vs. ancient) of organic carbon subsidies may have different fates in aquatic ecosystems. The relative ages of the non-algal OC that support heterotrophic microbial communities and the upper trophic levels of food webs are not well known although this knowledge is essential in understanding food web dynamics. In Lake Superior, although pre-aged organic carbon from the sediment was a putative food option in the lake, and constituted a median proportion of as much as 87% (84-91%) of the available food carbon (POC) during isothermal (mixed-lake water) condition at EM site and 84% (78-87%) during stratified condition at SM site (Table 3), mesozooplankton in the lake only incorporated trace amounts (median: 3% [1-7%]) of this old carbon into their biomass (Table 4). This observation could be due to a general decrease in palatability of considerably aged organic carbon or could be due to the extensive amount of reworking this material has experienced in Lake Superior. Some studies have suggested that modern terrestrial organic carbon supports heterotrophic respiration (Mayorga et al., 2005) whereas ancient terrestrial components could be important food sources for heterotrophic microbes (McCallister et al., 2004) and animal consumers (Ishikawa et al., 2010) in certain aquatic systems. In contrast to Lake Superior, studies of the Hudson River food-web (Caraco et al., 2010) and bacterial biomass production in the Hudson and York River systems (McCallister et al., 2004) have shown that both mesozooplankton and bacteria can use considerably aged reduced carbon as a food source. Also, in the open ocean in eastern North Pacific, radiocarbon studies show that bacteria assimilate both modern and ancient organic carbon (Cherrier et al., 1999). Schell (1983) in a study of the Colville River and coastal Alaskan Beaufort Sea reported that old carbon from peat in the catchment introduced primarily into food webs in the freshwater portions of the system, i.e., anadromous fish and ducks feeding in these areas. While it is still not clear which aquatic variables drive the relative utilization of ancient vs. modern food sources in these systems, some studies have indicated that terrestrial materials from the catchment are less refractory than previously thought (Hessen, 1992; Tranvik, 1992), and others have suggested addition of new synthesized algal food could act as co-metabolic primer facilitating the use of the aged (potentially refractory) organic material (Horvath, 1972; McCallister et al., 2004; Goni et al., 2006; Aller et al., 2008).

It should be noted that, while this study is one of the most extensive isotopic (particularly radiocarbon) investigations of the ecosystem of any of the great lakes in the world, our results represent a general large-scale view of the ecosystem functioning of Lake Superior since the spatial and temporal coverage of this study is limited to 8 sampling sites covering nearshore and offshore locations, and visited twice during thermal stratification and mixed-lake condition. A high resolution spatial and temporal sampling scheme would be needed for a more detailed understanding of the feeding habits and/ ecology of the mesozooplankton in the lake.

4.4 Comparison of zooplankton food sources in small-to-large aquatic systems

To gain cross-system insight into the food sources supporting animal consumers in aquatic systems, we compared the food sources of zooplankton in the Hudson River, five separate small northern lakes, and different sites in the North Pacific Ocean, to the food resources supporting zooplankton in a large lake (Lake Superior). This cross-system dataset is not representative of global lake diversity and/or variability and are only from North America as we cannot find radiocarbon composition of zooplankton in aquatic systems in other parts of the world. Trends observed and discussed here give a broad picture of ecosystem functioning across lake size gradient in the US and Canada. A more globally distributed dataset is needed to ascertain whether the trends observed in this study are consistent with the global view of the relationship between lake-size and zooplankton ecology.

In the Hudson River, and Bran-de-Scie, Des Monts, Stukely, and Bowker Lakes, the zooplankton biomass was generally largely supported by in-situ primary production (and its immediate consumers) as evidenced by smaller values of $\Delta\Delta^{14}C_{DIC\text{-}Zoop}$ relative to $\Delta\Delta^{14}C_{POC\text{-}Zoop}$ (Table 6). However, the ^{14}C-depletion of zooplankton biomass relative to the putative autochthonous food sources (Table 6) indicates the use of some aged allochthonous food source by the zooplankton for their dietary needs. Zooplankton incorporation of aged allochthonous food in these small lake systems contrasts with observations in Lake Superior, where the mesozooplankton preferentially and heavily depended on in situ primary production. Conservative estimates based on $\Delta\Delta^{14}C_{DIC\text{-}Zoop}$ indicate that the proportion of allochthonous food supporting zooplankton in the small lakes (except Fraser Lake) was larger than that in open Lake Superior (Table 6). This is also consistent with the observed relationship between zooplankton dependence on allochthonous food resources and variables such as ratio of catchment area to lake surface area (Fig. 5A), and water residence time (Fig. 5B). The ratio of basin area to surface area of a lake gives an indication of potential terrestrial subsidy to the lake's ecosystem. As the basin area-to-surface area ratio increases, suggesting potentially higher terrestrial influence, the difference between $\Delta^{14}C_{Zoop}$ and $\Delta^{14}C_{DIC}$ also increases as reflected in the positive correlation between $\Delta\Delta^{14}C_{DIC\text{-}Zoop}$ and the ratio of basin area to lake surface area (Fig. 5A). Hydrologic residence time is a variable that is related to lake size. Small lakes tend to have shorter water residence times whereas large lakes usually hold water for longer time periods (Table 1). There was generally a negative relationship between the hydrological residence time of the lakes and $\Delta\Delta^{14}C_{DIC\text{-}Zoop}$ (Fig. 5B), implying the difference between $\Delta^{14}C$ of zooplankton and $\Delta^{14}C$ of DIC decreases with an increase in lake water residence time, and by extension, with lake size.

In the oceanic sites, $\Delta^{14}C$ values of zooplankton and bulk POM were similar at all sites (Fig. 3B), suggesting that either the bulk POM was almost entirely derived from that year's primary production, or that the zooplankton were indiscriminately feeding on the bulk POM. It is worth noting however that estimating zooplankton food sources in the oceanic sites is complicated by the considerable differences in $\Delta^{14}C$ values of DIC with depth and laterally, such that water mass movements, and migratory feeding of zooplankton (and upper trophic organisms) could significantly mask the actual radiocarbon relationships between zooplankton, DIC and POC. The pooled data from the small lakes, Lake Superior and the Pacific Ocean show strong correlation between $\Delta^{14}C$ values of zooplankton and DIC, but poor correlation between $\Delta^{14}C$ of zooplankton and bulk POM (Fig. 4A, 4B) indicating that in most aquatic ecosystems, recent in-situ primary production is the most preferred food resource for zooplankton.

It is worth stating that different zooplankton groups do have different feeding and/or ecological strategies, and the observed zooplankton food preferences and the relationship between lake size and allochthony of zooplankton discussed above could be influenced by this. While the higher mesozooplankton autochthony seen in Lake superior relative to the smaller lakes could be attributed to the specific filter feeding style of the predominant calanoid copepods (~70% of crustacean zooplankton) in the lake, the small offset between $\Delta^{14}C$ of algae (based on $\Delta^{14}C$-DIC) and $\Delta^{14}C$ of the bulk mesozooplankton suggest the remaining zooplankton groups in Lake Superior (~30%) including cyclopoid copepods and daphnids were also largely feeding on algae, although these zooplankton groups are adapted to utilize other food options such as detritus, protists, bacteria and other zooplankton. Since cladocerans such as daphnids, and cyclopoid copepods are typically more abundant in small nutrient enriched aquatic systems (Gannon and Stember, 1978; Balcer et al., 1984), it could be argued that their adaptation for feeding on non-algal food options is responsible for the relatively high zooplankton allochthony seen in the smaller lakes. However, the zooplankton composition itself could be coupled to nutrient (N and P) availability (Demott and Gulati, 1999; Schulz and Sterner, 1999; Conde-Porcuna et al., 2002) and hence terrestrial influence, consistent with the observed pattern of increasing zooplankton allochthony with terrestrial influence as seen in this study.

5. Conclusions

Our isotopic investigation shows that intermediate trophic-level mesozooplankton in Lake Superior prefer to incorporate fresh autochthonous food, despite the availability of other organic carbon sources, and that upper trophic levels are likely not supported by terrestrial and/or resuspended-sediment OC subsidies to the carbon cycle. A similar trend is apparent in our oceanic sites, and selected small lakes, although the small lakes do exhibit a higher degree of zooplankton dependence on allochthonous food resources. This provides real-world support to lab studies showing preferential incorporation of phytoplankton fatty acids and POC into herbivorous zooplankton offered mixed diets of terrestrially-derived and phytoplankton-derived particulate organic matter (Brett et al., 2009) and confirms in a large lake, and similar clear-water systems such as the open oceans, the observation from unproductive small lakes that zooplankton selectively incorporate fresh autochthonous organic carbon (Karlsson, 2007; Karlsson et al., 2012). Our results suggest that if spatial or temporal subsidies of organic carbon fuel the net heterotrophy seen in Lake Superior, their effects are limited to the microbial loop and lower trophic levels, and do not extend to mesozooplankton and higher trophic levels. Further research should focus upon catabolic metabolism of mesozooplankton and both anabolic and catabolic metabolism in the microbial loop to further our understanding of such subsidies in the carbon cycle and energy transfer.

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References

Aller, R. C., Blair, N. E., and Brunskill, G. J.: Early diagenetic cycling, incineration, and burial of sedimentary organic carbon in the central Gulf of Papua (Papua New Guinea), J. Geophys. Res. 113, F01S09, doi:10.1029/2006JF000689, 2008.

Balcer, M. D., Korda, N. L., and Dodson, S. I.: Zooplankton of the great lakes: A guide to identification and ecology of the common crustacean species, The University of Wisconsin press, pp. 6-10, 1984.

Barbiero, R. P. and Tuchman, M. L.: The deep chlorophyll maximum in Lake Superior. J. Great Lakes Res, 30, 256-268, 2004.

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

Brett, M. T., Kainz, M. J., Taipale, S. J., and Seshan, H.: Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production, PNAS 106, 21197-21201, 2009.

Caraco, N., Bauer, J. E., Cole, J. J., Petsch, S., and Raymond, P.: Millenial-aged organic carbon subsidies to a modern river food web, Ecology 91, 2385-2393, 2010.

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

Cherrier, J., Bauer, J. E., Druffel, E. R. M., Coffin, R. B., and Chanton, J. P.: Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon, Limnol. Oceanogr. 44, 730-736, 1999.

Churchill, J. H., and Williams, A. J.: Bottom stress generation and sediment transport over the shelf and slope off of Lake Superior's Keweenaw peninsula, *J. Geophys. Res*., 109, C10S04, doi:10.1029/2003JC001997, 2004.

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

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

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

Conde-Porcuna, J. M., Ramos-Rodriguez, E., and Perez-Martinez, C.: Correlation between nutrient concentrations and zooplankton populations in a mesotrophic reservoir. Freshwater Biology 47, 1463-1473, 2002.

Cotner, J. B., Biddanda, B. A., Makino, W., and Stets, E.: Organic carbon biogeochemistry of Lake Superior, Aquat. Ecosyst. Health Manag.7, 451-464, 2004.

Del Giorgio, P. A., and France, R. L.: Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton δ^{13}C, Limnol. Oceanogr. 41, 359-365, 1996.

DeMott, W. R., and Gulati, R. D.: Phosphorus limitation in Daphnia: evidence from a long term study of three hypereutrophic Dutch lakes. Limnol. Oceanogr. 44, 1557–1564, 1999.

Deniro, M. J., and Epstein, S.: Influence of diet on the distribution of carbon isotopes in animals, Geochim. Cosmochim. Acta 42, 495-506, 1978.

Donahue, D. J., Linick, T. W., and Jull, A. J. T.: Isotope ratio and background corrections for accelerator mass spectrometry radiocarbon measurements. *Radiocarbon* 32, 135-142, 1990.

Druffel, E. R. M., Bauer, J. E., Williams , P. M., Griffin, S., and Wolgast, D.: Seasonal variability of particulate organic radiocarbon in the Northeast Pacific Ocean, J. Geophys. Res. 101, 543-552, 1996.

Druffel, E. R. M., and Williams, P. M.: Identification of a deep marine source of particulate organic carbon using bomb ^{14}C. Nature 347, 172-174, 1990.

Druffel, E. R. M., and Williams, P. M.: Radiocarbon in seawater and organisms from the Pacific coast of Baja California. Radiocarbon 33, 291-296, 1991.

Flood, R. D.: Submersible studies of current-modified bottom topography in Lake Superior, *J. Great Lakes Res*., 15, 3-14, 1989.

Flood, R. D., and Johnson, T. C.: Side-scan sonar targets in Lake Superior-evidence for bedforms and sediment transport, *Sedimentology, 31*, 311-333, 1984.

Fry, B.: Stable isotopes diagrams of freshwater food webs. Ecology 72, 2293-2297, 1991.

Fry, B., and Sherr, E.: δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems, Contributions in Marine Science 27, 13-47, 1984.

Gannon, J. E., and Stemberger, R. S.: Zooplankton (especially crustacean communities and rotifiers) as indicators of water quality. Trans. Amer. Micros. Soc. 97, 16-35, 1978.

Goni, M. A., Monacci, N., Gisewhite, R., Ogston, A., Crockett, J., and Nittrouer, C. A.: Distribution and sources of particulate organic matter in the water column and sediments of the Fly River Delta, Gulf of Papua (Papua New Guinea). Estuarine Coastal and Shelf Science 69, 225-245, 2006.

Grey, J. R., Jones, R. I., and Sleep, D.: Seasonal changes in the importance of the source of the organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. Limnol. Oceanogr. 46, 505-513, 2001.

Hamilton, S. K., Sippel, S. J., and Bunn, S. E.: Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica, Limnol. Oceanogr. Methods 3, 149-157, 2005.

Herdendorf, C. E.: Distribution of the world's large lakes, p. 3-38. In M. M. Tilzer, and C. Serruya [ed.], Large Lakes. Springer-Verlag, 1990.

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

Hessen, D. O., Anderson, T., and Lyche, A.: Carbon metabolism in a humic lake: pool sizes and cycling through zooplankton, Limnol. Oceanogr. 35, 84-99, 1990.

Horvath, R. S.: Microbial co-metabolism and the degradation of organic compounds in nature. Bacteriol Rev. 36, 146-155, 1972.

Ishikawa, N. F., Uchida, M., Shibata, Y., and Tayasu, I.: A new application of radiocarbon (^{14}C) concentrations to stream food web analysis, Nuclear Instruments and Methods in Physics Research B 268, 1175–1178, 2010.

Jones, R. I.: The influence of humic substances on lacustrine planktonic food chains, Hydrobiologia 229, 73-91, 1992.

Jones, R. I., Grey, J., Sleep, D., and Quarmby, C.: An assessment, using stable isotopes, of the importance of allochthonous organic carbon sources to the pelagic food web in Loch Ness. Proc. R. Soc. Lond. B. 265, 105-111, 1998.

Jones, R. I., and Salonen, K.: The importance of bacteria utilization of released phytoplankton photosynthate in two humic forest lakes in southern Finland, Holarctic Ecology 8, 133-140, 1985.

Karlsson, J.: Different carbon support for respiration and secondary production in unproductive lakes, Oikos 116, 1691-1696, 2007.

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

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

Karlsson, J., and Sawstrom, C.: Benthic algae support zooplankton growth during winter in a clear-water lake, Oikos 118, 539-544, 2009.

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

Keough, M., Sierszen, E., and Hagley, C. A.: Analysis of a Lake Superior coastal food web with stable isotope techniques, Limnol. Oceanogr. 41, 136-146, 1996.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., and Jones, R. I.: A revised model for lipid-normalizing $\delta^{13}C$ values from aquatic organisms, with implications for isotopic mixing models, J. Appl. Ecol. 43, 1213-1222, 2006.

Kling, G. W., Fry, B., and O'Brien, W. J.: Stable isotopes and planktonic trophic structure in arctic lakes, Ecology 73, 561-566, 1992.

Lennon J. T., and Pfaff, L. E.: Source and supply of terrestrial organic matter affects aquatic microbial metabolism. Aquat. Microb. Ecol. 39, 107-119, 2005.

Maier, W. J., and Swain, W. R.: Lake Superior organic carbon budget, *Water Res.*, *12*, 403-412, 1978.

Matthews, B., and Mazumder, A.: Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}C$. Freshwater Biology 50, 502-515, 2005.

Matthews, B., and Mazumder, A.: Habitat specialization and the exploitation of allochthonous carbon by zooplankton, Ecology 87, 2800-2812, 2006.

Mayorga, E., Aufdenkampe, A. K., Masiello, C. A., Krusche, A. V., Hedges, J. L., Quay, P. D., Richey, J. E., and Brown, T. A.: Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers, Nature 436, 538-541, 2005.

McCallister, S. L., Bauer, J. E., Cherrier, J., and Ducklow, H. W.: Assessing the sources and ages of organic matter supporting estuarine bacterial production: A novel multiple isotopes (δ^{13}C, δ^{15}N, Δ^{14}C) approach. Limnol. Oceanogr. 49, 1687-1702, 2004.

McCallister, S. L., and del Giorgio, P. A.: Direct measurement of the δ^{13}C signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO_2 fluxes, Limnol. Oceanogr. 53, 1204-1216, 2008.

McCarthy, M. D., Beaupre, S. R., Walker, B. D., Voparil, I., Guilderson , T. P., and Druffel, E. R. M.: Chemosynthetic origin of ^{14}C-depleted dissolved organic matter in a ridge-flank hydrothermal system, *Nature Geoscience*, *4*, 32-36, 2011.

McConnaughey, T., and McRoy, C. P.: Food-web structure and the fractionation of carbon isotopes in the Bering Sea, Marine Biology 53, 257-262, 1979.

McNichol, A. P., and Aluwihare, L. I.: The Power of Radiocarbon in Biogeochemical Studies of the Marine Carbon Cycle: Insights from studies of Dissolved and Particulate Organic Carbon (DOC and POC), *Chem. Rev*. 107, 443-466, 2007.

McManus, J., Heinen , E. A., and Baehr , M. M.: Hypolimnetic oxidation rates in Lake Superior: Role of dissolved organic material on the lake’s carbon budget, Limnol. Oceanogr. 48, 1624-1632, 2003.

Meersche, K. V. D., Rijswijk, P. V., Soetaert, K., and Middelburg, J. J.: Autochthonous and allochthonous contributions to mesozooplankton diet in a tidal river and estuary: Integrating carbon isotope and fatty acid constraints, Limnol. Oceanogr. 54, 62-74, 2009.

Meili, M., Fry, B., and Kling, G. W.: Fractionation of stable isotopes (^{13}C, ^{15}N) in the food web of a humic lake. Verh. Int. Ver. Limnol. 25, 501-505, 1993.

Meili, M., Kling, G. W., Fry, B., Bell, R. T., and Ahlgren, I.: Sources and Partitioning of organic matter in a pelagic microbial food web inferred from the isotopic composition (δ^{13}C and δ^{15}N) of zooplankton species. Arch Hydrobiol. Spec. Issues Adv. Limnol. 48, 53-61, 1996.

Mohamed, M. N., and Taylor, W. D.: Relative contribution of autochthonous and allochthonous carbon to limnetic zooplankton: A new cross-system approach. Fund Appl. Limnol. 175, 113-124, 2009.

Moore, J. W. and Semmens, B. X.: Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett., 11, 470-480, 2008.

Pace, M. L., Carpenter, S. R., Cole , J. J., Coloso, J., Kitchell, J. F., Hodgson, J. R., Middelburgg, J. J., Preston, N. D., Solomon, C. T., and Weidel, B.: Does terrestrial organic carbon subsidize the planktonic food webs in a clear-water lake?, Limnol. Oceanogr., 52, 2177–2189, 2007.

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

Petsch, S. T., Eglinton , T. I., and Edwards, K. J.: ^{14}C-Dead living Biomass: Evidence for microbial assimilation of ancient organic carbon during shale weathering, Science 292, 1127-1131, 2001.

Pulido-Villena, E., Reche, I., Morales-Baquero, R.: Food web reliance on allochthonous carbon in two high mountain lakes with contrasting catchments: a stable isotope approach, Can. J. Fish. Aquat. Sci. 62, 2640-2648, 2005.

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

Russ, M. E., Ostrom, N. E., Gandhi, H., Ostrom, P. H., and Urban, N. R.: Temporal and spatial variations in R : P ratios in Lake Superior, an oligotrophic freshwater environment, J. Geophys. Res., 109, C10S12, doi: 10.1029/2003JC001890, 2004.

Salonen, K., and Hammar, T.: On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters, Oecologia 68, 246-253, 1986.

Schell, D. L.: Carbon-13 and Carbon-14 Abundances in Alaskan Aquatic Organisms: Delayed Production from Peat in Arctic Food Webs, Science 219, 1068-1071, 1983.

Schulz, K. L., and Sterner, R. W.: Phytoplankton phosphorus limitation and food quality for Bosmina. Limnol. Oceanogr. 44, 1549–1556, 1999.

Semmens, B. X., Moore, J. W., and Ward, E. J.: Improving Bayesian isotope mixing models: a response to Jackson et al. (2009). Ecol. Lett., 12, E6-E8, 2009.

Smyntek, P. M., Teece, A. M., Schultz, K. L., Thackeray, S. J.: A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models, Limnol. Oceanogr. 52, 2135-2146, 2007.

Sobczak, W. V., Cloern, J. E., Jassby, A. D., and Muller-Solger, A. B.: Bioavailability of organic matter in a highly disturbed estuary: the role of detrital and algal resources, PNAS 99, 8101-8105, 2002.

Environment Canada and The US EPA, State of the great lakes 2009, Zooplankton populations, pp. 97-101, 2009.

Sterner, R. W.: In situ-measured primary production in Lake Superior, J. Gt. Lakes Res. 36, 139-149, 2010.

Stuiver, M., and Polach, H. A.: Discussion: Reporting of ^{14}C data, Radiocarbon 19, 355-363, 1977.

Syvaranta, J., and Rautio, M.: Zooplankton, Lipids and stable isotopes: importance of seasonal, latitudinal, and taxonomic differences, Can. J. Fish. Aquat. Sci. 67, 1721-1729, 2010.

Taipale, S., Kankaala , P., Tiirola, M., and Jones, R. I.: Whole-Lake dissolved inorganic ^{13}C additions reveal seasonal shifts in zooplankton diet, Ecology 89, 463-474, 2008.

Thorp, J. H., and Delong, M. D.: Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers? Oikos 96, 543-550, 2002.

Tranvik, L. J.: Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop, Hydrobiologia 229, 107-114, 1992.

Urban, N. R., Auer, M. T., Green, S. A., Lu, X., Apul, D. S., Powell, K. D., and Bub, L.: Carbon cycling in Lake Superior, J. Geophys. Res. 110, C06S90, doi:10.1029/2003JC002230, 2005.

Urban, N. R., Lu, X., Chai, Y., and Apul, D. S.: Sediment trap studies in Lake Superior: Insights into resuspension, cross-margin transport, and carbon cycling, J. Gt. Lakes Res. 30, 147-161, 2004.

Wakeham, S. G., McNichol, A. P., Kostka, J. E., and Pease, T. K.: Natural-abundance radiocarbon as a tracer of assimilation of petroleum carbon by bacteria in salt marsh sediments, Geochim. Cosmochim. Acta 70, 1761-1771, 2006.

Williams, P. M., Druffel, E. R. M., and Smith Jr , K. L.: Dietary carbon sources for deep-sea organisms as inferred from their organic radiocarbon activities, Deep-Sea Research 34, 253-266, 1987.

Yurista, P. M., Kelly, J. R., and Miller, S. E.: Lake Superior zooplankton biomass: alternative estimate from a probability-based net survey and spatially extensive LOPC surveys, J. Great Lakes Res. 35, 337-346, 2009.

Zigah, P. K., Minor, E. C., Werne, J. P., and McCallister , S. L.: Radiocarbon and stable carbon isotopic insights into provenance and cycling of carbon in Lake Superior, Limnol. Oceanogr. 56, 867-886, 2011.

Zigah, P. K., Minor, E. C., and Werne, J. P.: Radiocarbon and stable- isotope geochemistry of organic and inorganic carbon in Lake Superior, Global Biogeochem. Cycles. 26, GB1023; doi:10.1029/2011GB004132, 2012.

Table 1. Coordinates, sampling depths, hydrologic residence, basin area, and surface areas of the rivers, small lakes, Lake Superior and the Pacific Ocean. nd = not determined or not known.

Aquatic system	Water column condition	Date of sampling	Latitude	Longitude	Water depth (m)	Sampling depth (m)	Hydrologic residence time (years)	Surface area (km^2)	Basin area (km^2)	Basin area / Surface area
Hudson river	Isothermal (mixed)	2004-2005	40°.42 N- 44°.06 N	73 °56 W- 74 °01 W	7	0.2	0.3	760.41	34628.1	45.54*
Bran-de-Scie	Stratified (thermally)	1 Sep 2004	45°.41 N	72 °20 W	8.4	0.5-1	0.026	0.13	26.3	202.31[++]
Des Monts	Stratified	8 Sep 2004	45° 40 N	72 °18 W	5.5	0.5-1	0.013	0.26	46.5	178.85[++]
Stukely	Stratified	15 Sep 2004	45°38 N	72° 25 W	33.1	0.5-1	4.03	4	20.8	5.20[++]
Bowker	Stratified	15 Sep 2004	45 °41 N	72° 22 W	60.4	0.5-1	8.96	2.5	10.9	4.36[++]
Fraser	Stratified	28 Sep 2004	45° 39 N	72 °18 W	18.7	0.5-1	0.36	1.6	61.8	38.63[++]
Baptism river mouth (BR)	Isothermal	21 Jun 2009	47^{o}33 N	91^{o}19 W	20	0-15	191	82170	127700	1.55
Baptism river mouth (BR)	Stratified	24 Aug 2009	47^{o}33 N	91^{o}19 W	20	0-15	191	82170	127700	1.55
Ontonagon river mouth (ONT)	Isothermal	19 Jun 2009	46^{o}9 N	89^{o}34 W	20	0-10	191	82170	127700	1.55
Nipigon Bay (NB)	Stratified	16 Aug 2009	48^{o}86 N	87^{o}76 W	62	0-50	191	82170	127700	1.55
Western Lake Superior (WM)	Isothermal	20 Jun 2009	47^{o}31 N	89^{o}85 W	171	0-50	191	82170	127700	1.55
Western Lake Superior (WM)	Stratified	23 Aug 2009	47^{o}31 N	89^{o}85 W	171	0-50	191	82170	127700	1.55
Central Lake Superior (CM)	Isothermal	15 Jun 2009	48^{o}03 N	87^{o}74 W	257	0-50	191	82170	127700	1.55
Eastern Lake Superior (EM)	Isothermal	17 Jun 2009	47^{o}56 N	86^{o}65 W	242	0-50	191	82170	127700	1.55
Eastern Lake Superior (EM)	Stratified	15 Aug 2009	47^{o}56 N	86^{o}65 W	242	0-50	191	82170	127700	1.55
Southern Lake Superior (SM)	Isothermal	14 Jun 2009	46^{o}91 N	86^{o}6 W	398	0-50	191	82170	127700	1.55
Southern Lake Superior (SM)	Stratified	19 Aug 2009	46^{o}91 N	86^{o}6 W	398	0-50	191	82170	127700	1.55
Northern Lake Superior (NM)	Isothermal	16 Jun 2009	48^{o}49 N	87^{o}06 W	216	0-50	191	82170	127700	1.55
Northern Lake Superior (NM)	Stratified	16 Aug 2009	48^{o}49 N	87^{o}06 W	216	0-50	191	82170	127700	1.55
Pacific coastal ocean	Stratified	1980,1986, 1987	27^{o}33.0 N; 33^{o}50 N	114^{o}52.3 W; 118^{o}50. W	~900	0-20	37000	165200000	20300000	0.12**
North Central Pacific (NCP)	Stratified	1972-1983	~30^{o}39'N; 31^{o}00'N	155^{o}23W; 159^{o}00W	~5800	0-1700	37000	165200000	20300000	0.12[#]
Northeast Pacific (NEP)	Stratified	1975-1977	32^{o}34 N; 34^{o}00 N	~120^{o}45 W; 123^{o}00 W	~4100	0-1500	37000	165200000	20300000	0.12[+]

* Caraco et al., 2010,

** Druffel and William 1990 and Druffel and William, 1991,

[#] William et al., 1987 and Druffel and William, 1990

[+] William et al., 1987 and Druffel et al., 1996

[++] McCallister and del Giorgio, 2008

Table 2. Radiocarbon, stable isotope and atomic C:N ratio of zooplankton (Zoop) and organic and inorganic carbon pools in the surface waters of Lake Superior. Precision of radiocarbon values is based on analyses of multiple external standards, and those of stable isotopes are based on replicate sample analyses.

	$\Delta^{14}C$ (‰)				$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		Molar C/N	
Station and date	DIC	Zoop	DOC	POC	Zoop	DOC	POC	POC	Zoop	POC	Zoop
Jun-09 Isothermal											
WM	56 ± 4	57 ± 4	49 ± 4	21 ± 4	-29.5 ± 0.3	-26.5	-29.5 ± 0.4	1.7	5.4	7.2	6.4
CM	61 ± 4	52 ± 4	58 ± 4	-55 ± 4	-29.9 ± 1.1	-25.9	-29.9 ± 0.1	0.1	4.6	7.9	6.2
EM	59 ± 5	63 ± 3	42 ± 4	-24 ± 3	-30.0 ± 1.0	-26.3	-30.2 ± 0.2	0.1	4.9	7.9	6.7
SM	62 ± 3	49 ± 3	25 ± 5	34 ± 5	-29.7 ± 0.0	-26.0	-29.8 ± 0.0	0.4	4.2	8	7.1
NM	52 ± 2	65 ± 4	22 ± 4	34 ± 3	-30.0 ± 1.1	-26.5	-30.4 ± 0.7	0.7	5.4	7	6.2
ONT	38 ± 2	54 ± 3	-10 ± 3	16 ± 3	-31.2 ± 0.0	nd	-29.5 ± 0.3	-0.6	2.8	9.1	7.7
BR	54 ± 4	47 ± 4	38 ± 4	14 ± 4	-29.5 ± 1.8	nd	-30.4 ± 0.4	1.1	5.4	9.5	6.5
Aug-09 Stratified											
WM	61 ± 3	62 ± 4	51 ± 3	33 ± 4	-30.3 ± 0.9	-26.1	-27.8 ± 0.0	-2.8	3.6	8.4	7.6
CM	62 ± 3	nd	nd	39 ± 3	nd	-26.0	-28.8 ± 0.5	-2.3	Nd	8.3	nd
EM	59 ± 4	56 ± 3	54 ± 3	38 ± 4	-30.1 ± 0.5	-26.0	-30.2 ± 1.4	-3.9	3.9	7.6	7.3
SM	54 ± 4	54 ± 3	27 ± 4	-24 ± 3	-29.4 ± 0.5	-26.0	-28.1 ± 0.0	-2.9	3.4	8.4	6.5
NM	50 ± 3	61 ± 4	21 ± 3	22 ± 3	-30.4 ± 0.5	-26.1	-28.9 ± 0.0	-2.9	4.4	8.5	8.2
ONT	56 ± 4	nd	nd	nd	-25.9 ± 0.4	-28.3	-27.2 ± 0.0	-3.7	0.1	8.3	8.1
BR	60 ± 4	44 ± 3	74 ± 4	7 ± 3	-28.2 ± 0.2	-26.5	-28.4 ± 0.1	-2.7	2.6	8.4	5.8
NB	36 ± 3	36 ± 4	39 ± 4	-19 ± 4	-31.3 ± 0.8	-26.5	-28.1 ± 0.2	-2	2.4	7.9	8.7

Table 3. Proportional median contribution of various food resources to the bulk POM in the water column of Lake Superior. The Bayesian MixSIR model was used for these calculations with algal carbon from recent photosynthesis, terrestrial POC, sedimentary carbon, and bacterial biomass as end-members (as described in section 2.4.1). The values in parentheses are the 5% and 95% confidence percentiles. The regular values are model estimates when corn leaves is used as the terrestrial end member; bold values are when POC from high flow Amity creek is used as the terrestrial endmember.

Station	Condition	Algal Carbon	Terrestrial POC	Sedimentary OC	Bacterial carbon
CM	Stratified	0.67 (0.59 - 0.75)	0.04 (0.003 - 0.16)	0.24 (0.17 - 0.30)	0.02 (0.002 - 0.09)
		0.67 (0.59 - 0.75)	**0.08 (0.01 - 0.30)**	**0.20 (0.06 - 0.28)**	**0.02 (0.002 - 0.09)**
EM	Isothermal	0.10 (0.05 - 0.14)	0.01 (0.001 - 0.05)	0.87 (0.84 - 0.91)	0.01 (0.001 - 0.04)
		0.11 (0.06 - 0.14)	**0.01 (0.001 - 0.04)**	**0.87 (0.84 - 0.91)**	**0.01 (0.00 - 0.04)**
EM	Stratified	0.81 (0.75 - 0.88)	0.02 (0.001 - 0.07)	0.15 (0.09 - 0.21)	0.01 (0.001 - 0.04)
		0.81 (0.75 - 0.88)	**0.03 (0.002 - 0.12)**	**0.14 (0.06 - 0.20)**	**0.01 (0.001 - 0.04)**
NM	Isothermal	0.85 (0.77 - 0.93)	0.02 (0.001 - 0.06)	0.11 (0.03 - 0.18)	0.02 (0.001 - 0.07)
		0.84 (0.77 - 0.92)	**0.03 (0.002 - 0.11)**	**0.10 (0.02 - 0.18)**	**0.02 (0.001 - 0.07)**
NM	Stratified	0.55 (0.45 - 0.63)	0.05 (0.004 - 0.17)	0.34 (0.27 - 0.40)	0.04 (0.004 - 0.15)
		0.55 (0.42 - 0.63)	**0.10 (0.01 - 0.40)**	**0.28 (0.13 - 0.37)**	**0.04 (0.003 - 0.15)**
SM	Isothermal	0.74 (0.68 - 0.79)	0.02 (0.001 - 0.07)	0.22 (0.16 - 0.28)	0.01 (0.001 - 0.06)
		0.74 (0.68 - 0.79)	**0.03 (0.002 - 0.11)**	**0.21 (0.14 - 0.27)**	**0.01 (0.001 - 0.06)**
SM	Stratified	0.11 (0.03 - 0.15)	0.03 (0.002 - 0.10)	0.84 (0.80 - 0.87)	0.02 (0.001 - 0.07)
		0.11 (0.05 - 0.16)	**0.03 (0.002 - 0.12)**	**0.83 (0.78 - 0.87)**	**0.02 (0.001 - 0.07)**
WM	Isothermal	0.77(0.71 - 0.82)	0.02 (0.001 - 0.08)	0.18 (0.15 - 0.21)	0.02 (0.001 - 0.06)
		0.77 (0.71- 0.81)	**0.03 (0.002 - 0.10)**	**0.18 (0.15 - 0.21)**	**0.02 (0.001 - 0.06)**
WM	Stratified	0.60 (0.43 - 0.75)	0.15 (0.01 - 0.42)	0.13 (0.09 - 0.16)	0.09 (0.01 - 0.24)
		0.59 (0.44 - 0.74)	**0.19 (0.02 - 0.47)**	**0.10 (0.02 - 0.15)**	**0.09 (0.01 - 0.24)**

Table 4. Proportional median contribution of putative food sources to zooplankton biomass in Lake Superior. The Bayesian MixSIR model was used in computing these values using algal carbon from recent photosynthesis, terrestrial POC, sedimentary carbon, and bacterial biomass as food options (as described in section 2.4.1). The values in parentheses are the 5% and 95% confidence percentiles. As in Table 3, the regular values are model estimates when corn leaves is used as the terrestrial end member; bold values are when POC from high flow Amity creek is used as the terrestrial endmember.

Station	Condition	Algal Carbon	Terrestrial POC	Sedimentary OC	Bacterial carbon
CM	Isothermal	0.88 (0.79 - 0.95)	0.03 (0.002 - 0.12)	0.06 (0.01 - 0.12)	0.02 (0.002 - 0.07)
		0.88 (0.80 - 0.95)	**0.04 (0.003 - 0.12)**	**0.05 (0.01 - 0.11)**	**0.02 (0.002 - 0.07)**
EM	Isothermal	0.94 (0.88 - 0.98)	0.02 (0.002 - 0.08)	0.01 (0.001 - 0.04)	0.02 (0.001 - 0.07)
		0.95 (0.89 - 0.98)	**0.02 (0.001 - 0.06)**	**0.01 (0.001 - 0.04)**	**0.02 (0.001 - 0.07)**
EM	Stratified	0.92 (0.85 - 0.97)	0.02 (0.001 - 0.10)	0.02 (0.002 - 0.06)	0.02 (0.001 - 0.08)
		0.92 (0.86 - 0.97)	**0.02 (0.002 - 0.08)**	**0.02 (0.002 - 0.06)**	**0.02 (0.001 - 0.08)**
SM	Isothermal	0.85 (0.77 - 0.92)	0.03 (0.003 - 0.12)	0.07 (0.02 - 0.13)	0.03 (0.002 - 0.10)
		0.85 (0.78 - 0.92)	**0.05 (0.003 - 0.15)**	**0.06 (0.01 - 0.12)**	**0.03 (0.002 - 0.10)**
SM	Stratified	0.90 (0.81 - 0.96)	0.04 (0.003 - 0.14)	0.02 (0.001 - 0.06)	0.03 (0.002 - 0.09)
		0.92 (0.84 - 0.97)	**0.03 (0.002 - 0.09)**	**0.02 (0.001 - 0.05)**	**0.03 (0.002 - 0.10)**
WM	Isothermal	0.91 (0.81 - 0.97)	0.03 (0.003 - 0.12)	0.01 (0.001 - 0.03)	0.03 (0.003 - 0.12)
		0.92 (0.84 - 0.97)	**0.02 (0.002 - 0.08)**	**0.01 (0.001 - 0.03)**	**0.04 (0.003 - 0.13)**
WM	Stratified	0.95 (0.88 - 0.98)	0.02 (0.002 - 0.07)	0.01 (0.001 - 0.03)	0.02 (0.001 - 0.07)
		0.95 (0.89 - 0.98)	**0.02 (0.001 - 0.06)**	**0.01 (0.001 - 0.03)**	**0.02 (0.001 - 0.07)**

Table 5. Zooplankton allochthony vs. autochthony in Lake Superior based on radiocarbon values of algae (based on DIC) and terrestrial organic carbon (with radiocarbon of atmospheric CO_2 as the terrestrial endmember). The values in parenthesis are estimates when the radiocarbon value of POC from high flow Amity creek is used as the terrestrial endmember; nd is not determined due to the absence of data (not measured or lost during sample processing) or no feasible solution from the model.

Site	Condition	Zooplankton autochthony (%)	Zooplankton allochthony (%)
WM	Isothermal	100 (100)	0 (0)
WM	Stratified	nd	nd
CM	Isothermal	61 (82)	39 (18)
CM	Stratified	nd	nd
EM	Isothermal	84 (92)	16 (8)
EM	Stratified	86 (94)	14 (6)
SM	Isothermal	46 (75)	54 (25)
SM	Stratified	100 (100)	0 (0)

Table 6. Comparison of the radiocarbon signatures of DIC, DOC, POC, and zooplankton in small-to-large surface area aquatic systems. The Open Lake Superior values are the averages of all the open lake sites during isothermal condition, and during stratification.

Aquatic system	Δ^{14}C-DIC (‰)	Δ^{14}C- DOC (‰)	Δ^{14}C-POC (‰)	Δ^{14}C-Zoop (‰)	$\Delta\Delta^{14}$C (DIC-Zoop) (‰)	$\Delta\Delta^{14}$C (POC-Zoop) (‰)	Reference
Hudson river	-57 ± 14	40 ± 9	-145 ± 27	-236 ± 25	179 ± 29	91 ± 37	Caraco et al., 2010
Bran-de-Scie	37 ± 3	14 ± 5	115 ± 3	11 ± 4	26 ± 5	104 ± 5	McCallister and del Giorgio, 2008
Des Monts	52 ± 3	73 ± 3	111 ± 4	40 ± 3	13 ± 4	71 ± 5	McCallister and del Giorgio, 2008
Stukely	37 ± 4	93 ± 4	179 ± 4	29 ± 4	8 ± 6	150 ± 6	McCallister and del Giorgio, 2008
Bowker	-0.4 ± 3	76 ± 4	74 ± 4	-2 ± 4	1 ± 5	76 ± 6	McCallister and del Giorgio, 2008
Fraser	-90.9 ± 3	101 ± 4	106 ± 4	10 ± 6	-101 ± 7	96 ± 7	McCallister and del Giorgio, 2008
Baptism river mouth (BR)	54 ± 4	38 ± 4	14 ± 4	47 ± 4	7 ± 6	-33 ± 6	This study
Baptism river mouth (BR)	60 ± 4	74 ± 4	7 ± 3	44 ± 3	16 ± 5	-37 ± 4	This study
Ontonagon river mouth (ONT)	38 ± 2	-10 ± 3	16 ± 3	54 ± 3	-16 ± 4	-38 ± 4	This study
Nipigon Bay (NB)	36 ± 3	39 ± 4	-19 ± 4	36 ± 4	0 ± 5	-55 ± 6	This study
Open Lake Superior (isothermal)	58 ± 4	39 ± 4	2 ± 3	57 ± 4	-1 ± 5	-49 ± 5	This study
Open Lake Superior (stratified)	56 ± 4	38 ± 3	17 ± 4	58 ± 4	-2 ± 5	-41 ± 5	This study
Pacific coastal ocean	100 ± 4	-200	100	76 ± 5	24 ± 6	24 ± 5	Druffel and Williams, 1991; Druffel and Williams, 1990
North Central Pacific (NCP)	132	-200	139 ± 9	124 ± 46	8	15	Williams et al., 1987; Druffel and Williams, 1990
Northeast Pacific (NEP)	155	-200	82	86 ± 40	69	-4	Williams et al., 1987; Druffel et al., 1996

FIGURE LEGENDS

Figure 1. Map of Lake Superior showing sampling sites across the lake. The open lake sites include western station/mooring (WM), central mooring (CM), northern mooring (NM), eastern mooring (EM), and southern mooring (SM). The nearshore sites are off Baptism River (BR), off Ontonagon River (ONT), and off Nipigon Bay (NB).

Figure 2. $\Delta^{14}C$ values for DIC, Mesozooplankton, DOC, and POC samples in (A) the isothermal lake in June, and (B) the stratified lake in August 2009.

Figure 3. $\Delta^{14}C$ values for DIC, Zooplankton, DOC, and POC samples in (A) selected small lakes that includes Bran-de-Scie (Br), Des Monts (De), Stukely (St), Bowker (Bo), and Fraser (Fr) Lakes, and (B) sites in the Pacific Ocean that includes Pacific coastal ocean (PC), North Central Pacific (NCP), and North Eastern Pacific (NEP). Data adapted from William et al., 1987, Druffel and William, 1990, Druffel and William, 1991, Druffel et al., 1996.

Figure 4. The relationship between $\Delta^{14}C$ of zooplankton and (A) $\Delta^{14}C$ of DIC, and (B) $\Delta^{14}C$ of POC. These comparisons show that the radiocarbon signatures of zooplankton are largely determined by those of DIC, and hence algal carbon from recent photosynthesis within the lake. For Lake Superior, two points, the average isothermal values and average stratification values, were used in order to not bias the trends.

Figure 5. Relationship between the difference of $\Delta^{14}C$ values of DIC and Zooplankton, and (A) ratio of basin area to lake surface area, and (B) hydrologic residence time of the various lakes. Although marginally significant correlation ($p = 0.08$) between $\Delta\Delta^{14}C_{\text{DIC-Zoop}}$ and

hydrologic residence time, these two plots generally illustrate that zooplankton support by allochthonous organic carbon is related to variables that indicate terrestrial influence.

Supplementary Figure 1: CTD data of sampling sites in Lake Superior showing the depth profiles of temperature, beam transmission (Wetlab CStar,%), fluorescence (Wetlab Wetstar, mg/m^3), dissolved oxygen, and wet CDOM (Wetlab CDOM, mg/m^3) in the mixed water column in June and stratified condition in August.

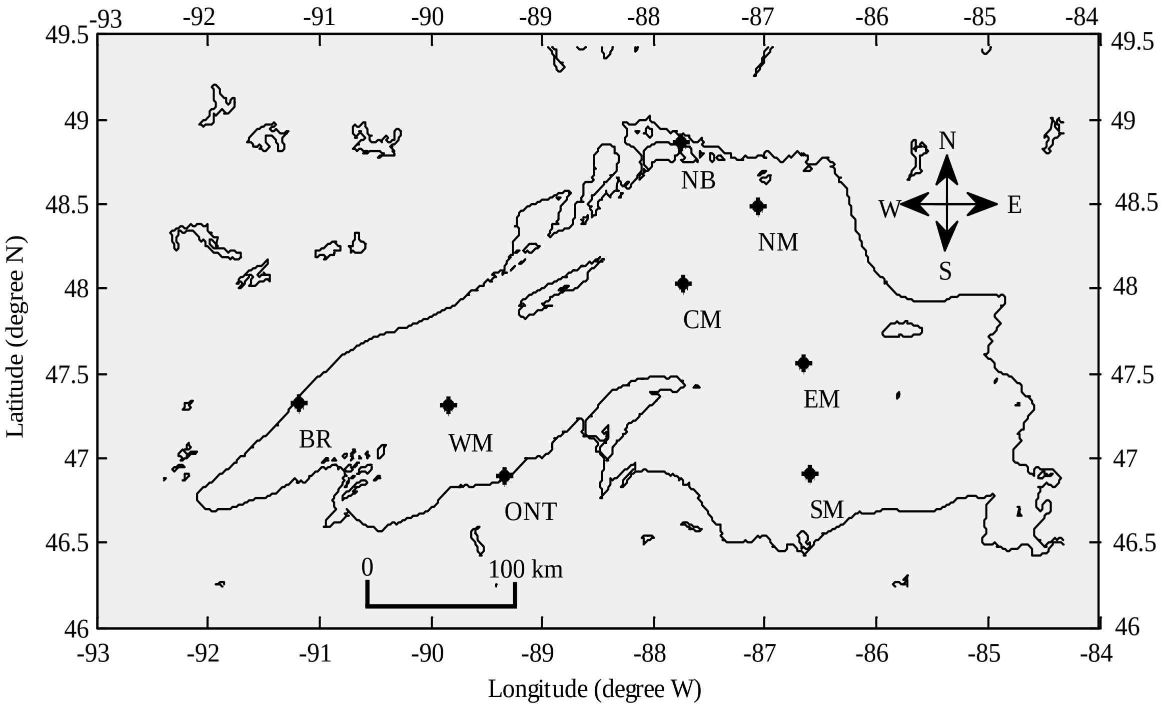
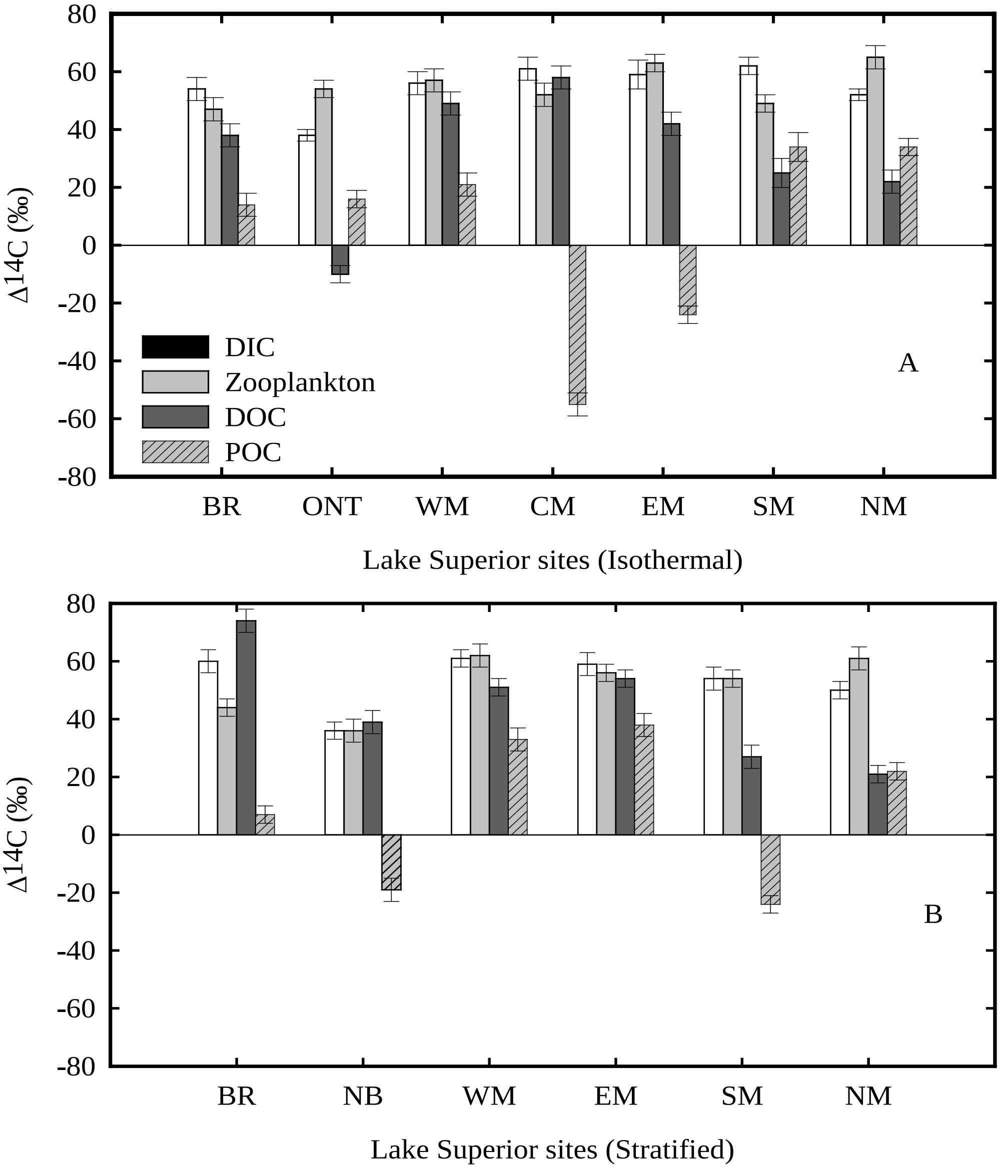

-93
-92
-91
-90
-89
-88
-87
-86
-85
-84
49.5
49
48.5
48
47.5
47
46.5
46
Latitude (degree N)
Longitude (degree W)
NB
NM
CM
EM
BR
WM
ONT
SM
N
S
W
E
0
100 km

Figure 1.



80
60
40
20
0
-20
-40
-60
-80
Δ14C (‰)
DIC
Zooplankton
DOC
POC
A
BR
ONT
WM
CM
EM
SM
NM
Lake Superior sites (Isothermal)
80
60
40
20
0
-20
-40
-60
-80
Δ14C (‰)
B
BR
NB
WM
EM
SM
NM
Lake Superior sites (Stratified)

Figure 2.

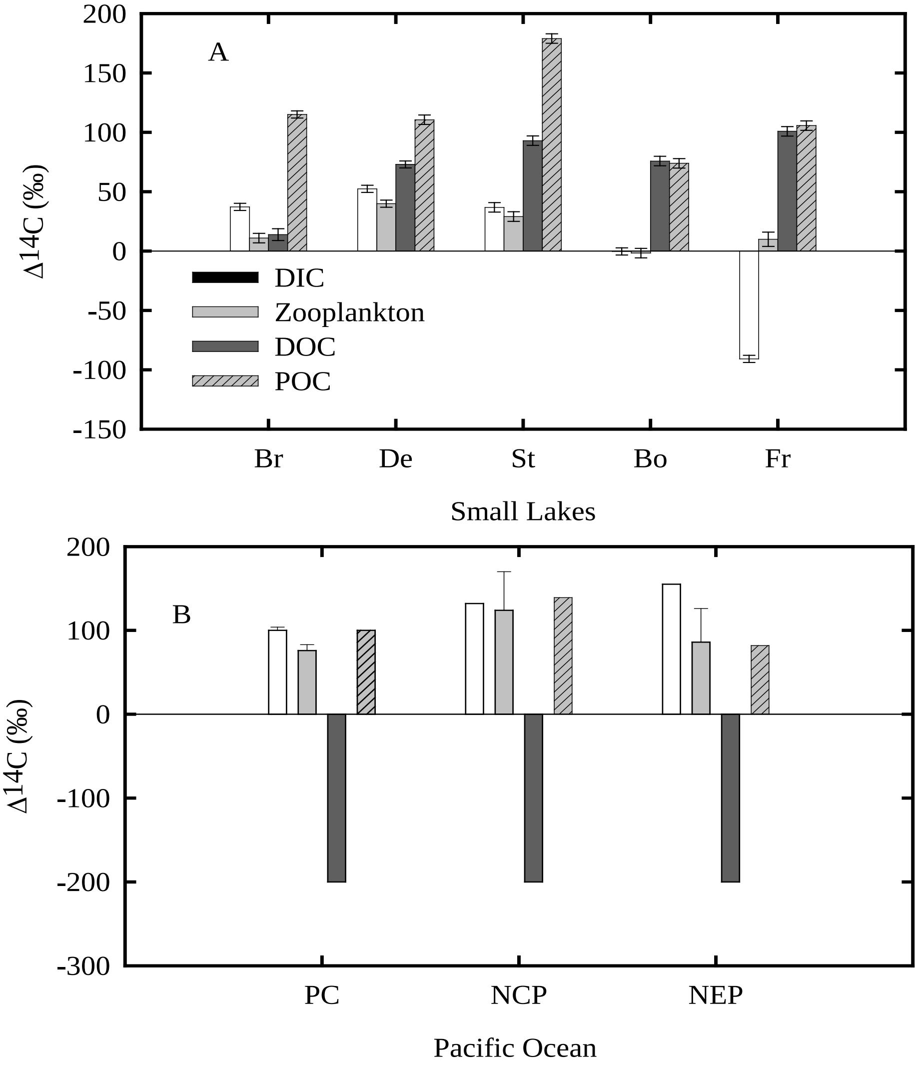

A
Δ14C (‰)
200
150
100
50
0
-50
-100
-150
DIC
Zooplankton
DOC
POC
Br
De
St
Bo
Fr
Small Lakes
B
Δ14C (‰)
200
100
0
-100
-200
-300
PC
NCP
NEP
Pacific Ocean

Figure 3.

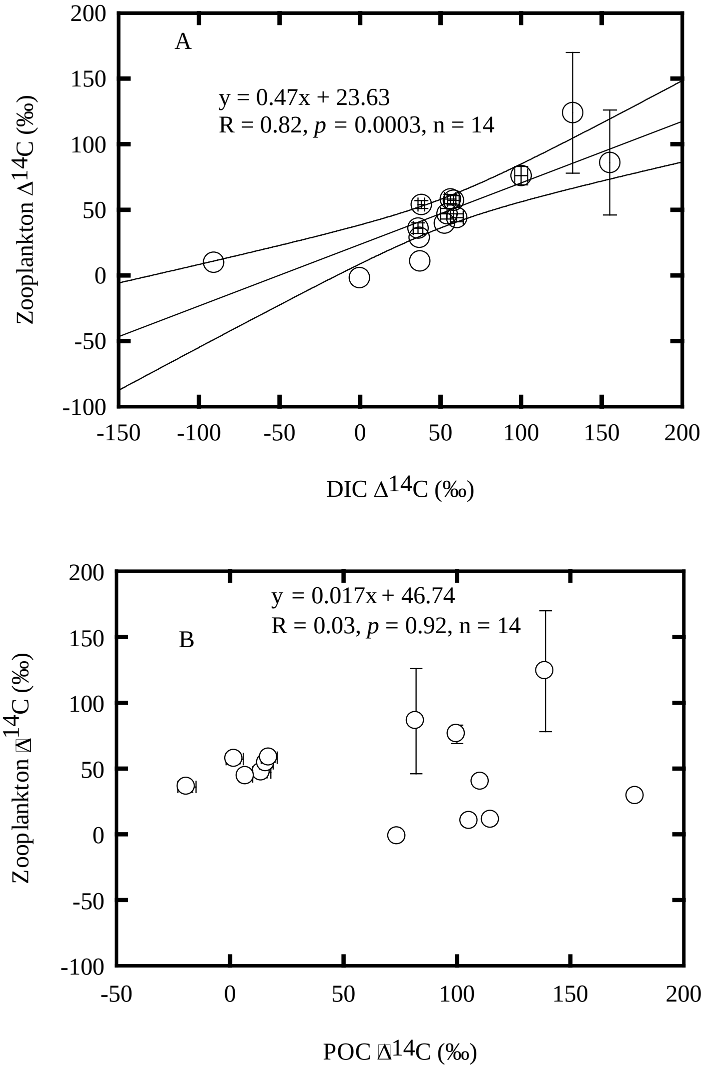
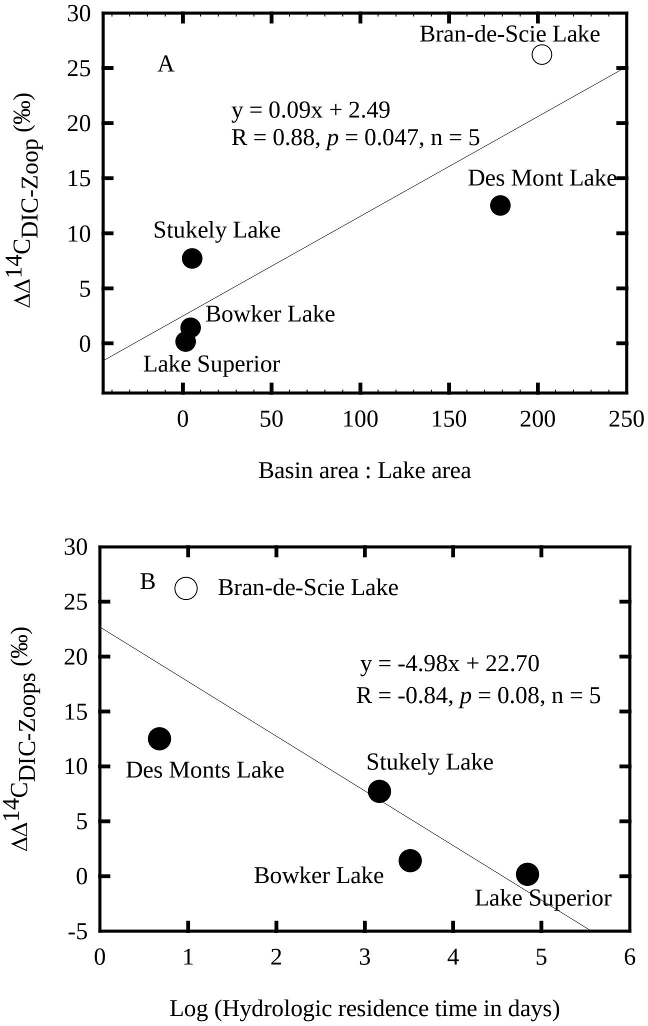

A
y = 0.47x + 23.63
R = 0.82, p = 0.0003, n = 14
Zooplankton Δ14C (‰)
200
150
100
50
0
-50
-100
-150
-100
-50
0
50
100
150
200
DIC Δ14C (‰)
y = 0.017x + 46.74
R = 0.03, p = 0.92, n = 14
B
Zooplankton Δ14C (‰)
200
150
100
50
0
-50
-100
-50
0
50
100
150
200
POC Δ14C (‰)

Figure 4.



A
30
25
20
15
10
5
0
ΔΔ14CDIC-Zoop (‰)
Bran-de-Scie Lake
y = 0.09x + 2.49
R = 0.88, p = 0.047, n = 5
Des Mont Lake
Stukely Lake
Bowker Lake
Lake Superior
0
50
100
150
200
250
Basin area : Lake area
B
30
25
20
15
10
5
0
-5
ΔΔ14CDIC-Zoops (‰)
Bran-de-Scie Lake
y = -4.98x + 22.70
R = -0.84, p = 0.08, n = 5
Des Monts Lake
Stukely Lake
Bowker Lake
Lake Superior
0
1
2
3
4
5
6
Log (Hydrologic residence time in days)

Figure 5.