Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen

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Edited* by David M. Karl, University of Hawaii, Honolulu, HI, and approved December 27, 2010 (received for review September 1, 2010)

Cross-ecosystem subsidies to food webs can alter metabolic balances in the receiving (subsidized) system and free the food web, or particular consumers, from the energetic constraints of local primary production. Although cross-ecosystem subsidies between terrestrial and aquatic systems have been well recognized for benthic organisms in streams, rivers, and the littoral zones of lakes, terrestrial subsidies to pelagic consumers are more difficult to demonstrate and remain controversial. Here, we adopt a unique approach by using stable isotopes of H, C, and N to estimate terrestrial support to zooplankton in two contrasting lakes. Zooplankton (*Holopedium, Daphnia, and Leptodiaptomus*) are comprised of ≈20–40% of organic material of terrestrial origin. These estimates are as high as, or higher than, prior measures obtained by experimentally manipulating the inorganic ^{13}C content of these lakes to augment the small, natural contrast in ^{13}C between terrestrial and algal photosynthesis. Our study gives credence to a growing literature, which we review here, suggesting that significant terrestrial support of pelagic crustaceans (zooplankton) is widespread.

allochthony | terrestrial subsidy

External inputs strongly influence ecosystems. Alterations of inputs, such as limiting nutrients or dispersing organisms, can lead to major transformations. Controlling excessive inputs or, in some cases, restoring ancestral inputs are often focal concerns of ecosystem management (1). Nonetheless, ecological theory is just beginning to account for inputs of materials and organisms and the ways in which they subsidize food webs (2–4). Theory is limited in part by the difficulty of measuring the utilization by consumers of allochthonous (or exogenous) inputs. In this context, the open waters of lakes and oceans present special challenges, yet understanding the support of pelagic ecosystems is crucial for understanding their functioning.

Aquatic systems receive organic material from two fundamentally different sources: primary production that occurred within the system's boundaries (autochthonous sources) and primary production imported from the terrestrial watershed (allochthonous sources). In lakes, streams, and rivers, the loading of allochthonous organic material is usually as large to much larger than autochthonous primary production (5), and dissolved compounds of terrestrial origin dominate the standing stock of organic matter in these waters (6, 7). In the past, it was generally assumed that this terrestrial organic matter was mostly refractory and was either buried in sediments or exported. Work on the metabolic balances of aquatic systems has reversed this view. In many aquatic systems, respiration (the degradation of organic C to CO_2 by all organisms combined) exceeds gross primary production (the formation of organic matter by photosynthesis; ref. 8). This simple balance demonstrates that at least some portion of the terrestrial input must be actively catabolized in the receiving aquatic system.

That terrestrial material is catabolized suggests that some secondary production of microbes, invertebrates, or fish may be supported directly or indirectly by terrestrial inputs. Using multiple approaches (litter exclusion, gut contents, biomarkers, and stable isotopes), a number of authors have reported that some fishes and benthic invertebrates in streams and the littoral zones of rivers and lakes are indeed supported in part by terrestrial organic matter (e.g., refs. 9–16). Demonstrating a terrestrial influence on pelagic food webs in lakes had been both more difficult and more controversial. Terrestrial organic matter could become available to pelagic organisms by several mechanisms: microbial uptake of terrestrial dissolved organic carbon (DOC) followed by consumption of these microbes by protozoans or zooplankton (17, 18), direct consumption of terrestrial DOC by zooplankton via osmotrophy (19), or by consumption of terrestrially derived particles by zooplankton (20, 21). Terrestrial contributions to zooplankton have been estimated with different methods, predominantly by using ambient ^{13}C and ^{15}N (11, 17, 20, 22–34). Although the majority of these studies suggest significant terrestrial support of zooplankton (Table S1), this interpretation is debatable for three reasons: (*i*) The pathways outlined above are hard to quantify and gut contents are difficult to determine in zooplankton (35); (*ii*) approaches using stable isotopes can be problematic because it is difficult to directly measure the isotopic signature of phytoplankton. Suspended particulate organic matter [seston, or particulate organic matter (POM)] is only partially comprised of phytoplankton, and physically isolating the phytoplankton is only possible under certain conditions (36); and (*iii*) even where measurement or estimation is possible, phytoplankton can be isotopically similar to terrestrial organic matter, especially for carbon (37).

In an attempt to overcome some of these difficulties, we conducted a series of experiments in which we greatly elevated the ^{13}C of dissolved inorganic carbon (DIC) in the surface mixed layer of several small lakes, creating strong contrasts in $\delta^{13}C$ between autochthonous primary production and allochthonous inputs. From these experiments and associated models, we calculated that zooplankton could be subsidized from 30 to 70% by terrestrial C in systems that had low phytoplankton biomass (chlorophyll) and high DOC (29, 38). Experimentally elevating primary production with nutrient additions greatly reduced this terrestrial contribution to <10% (20, 27, 29). In a clear water lake low in both phytoplankton and DOC, we found low ter-

Author contributions: J.J.C., S.R.C., and M.L.P. performed research; J.J.C., S.R.C., M.L.P., and C.T.S. analyzed data; and J.J.C., S.R.C., J.K., M.L.P., C.T.S., and B.W. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1012807108/-/DCSupplemental.

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restrial subsidies to a calanoid copepod (2%) and a modest subsidy to a cladoceran (30%; ref. 31 and Table S1).

Whole-lake ^{13}C experiments have several potential biases that may lead to an overestimation of allochthony. Because the ^{13}C was added only to the surface mixed layer, photosynthesis below the mixed layer is not labeled and might be confused with terrestrial C in the analysis. Second, autochthonous primary production that occurred before the ^{13}C addition would not have been labeled, and the resulting autochthonous detritus again could be counted as allochthonous by the analysis. Although these issues were partially addressed (15, 20, 29), Brett et al. (21) recently argued that these ^{13}C addition experiments greatly overestimated allochthony to zooplankton. Here, we estimate allochthony to zooplankton with an independent and unique approach. We used ambient stable isotopes of C, H, and N over depth and time to independently assess terrestrial contributions to zooplankton in two contrasting lakes in which ^{13}C addition experiments had been performed. Further, we present a unique method, based on deuterium (δ^2H) to estimate the isotopic signature of phytoplankton without having to separate them physically from seston, and present a comprehensive review of the literature on allochthony to zooplankton.

Results

Study Sites. We sampled Paul and Crampton lakes monthly (May–August 2009) to measure isotopic pools and background conditions (Table S2). Paul Lake (L.) is small (1.7 ha) and has a brown color because of significant concentrations of chromophoric dissolved organic matter. The lake is mesotrophic (chlorophyll-a 2–4 $\mu g \cdot liter^{-1}$ in the oxic zone), sharply stratified with a steep thermocline beginning at ≈3.5 m, and anoxic <5.5 m; Crampton is larger (25 ha), clear, oligotrophic (chlorophyll a 1–2 $\mu g \cdot liter^{-1}$), oxic throughout its water column, with a broad thermocline. Both lakes had slight chlorophyll maxima in oxic waters. The dominant crustacean zooplankton in Paul L. were cladocerans including *Daphnia* (*D. rosea* and *D. pulex*) and *Holopedium gibberum* as well as cyclopoid copepods. Crampton L. had *Holopedium gibberum*, small cyclopoid copepods, and a calanoid copepod, *Leptodiaptomus minutus*.

Isotopic Composition of Endmembers. The isotopic composition of benthic and pelagic primary producers in the lake and in the watershed was distinct for some of the isotopes (Fig. 1 and Table S3). Dilution-regrowth cultures of phytoplankton (*Methods*) from these lakes confirmed the large fractionation for δ^2H between water and algae reported (34, 39), and there was little difference between deep and surface water phytoplankton. In the surface waters of both lakes, phytoplankton had a δ^2H of near −200‰ with low variance (Table S3). In contrast, terrestrial vegetation was much heavier than phytoplankton (by 65‰) averaging −129‰. Suspended POM in both lakes had $\delta^{13}C$ midway between that of phytoplankton and terrestrial vegetation (Table S3). Assuming that POM is a mixture of terrestrial vegetation and phytoplankton, we calculated the C and N isotopic signatures of phytoplankton at each depth (Table S3 and *Methods*). In Paul L., the calculated $\delta^{13}C$ of phytoplankton was lower compared with terrestrial sources, averaging −31.3 ± 2.2 in surface water and ≈1‰ lower at depth (Fig. 1 and Table S3) In Crampton L., surface phytoplankton (−28.8 ± 0.08) was very close to that of terrestrial organic C and was lower by ≈4‰ at depth (Fig. 1 and Table S3). In both lakes, the calculated $\delta^{15}N$ of phytoplankton increased with depth and was higher than terrestrial values (Fig. 1 and Table S3).

Isotopic Signatures of Zooplankton and Seston. In both lakes, POM and zooplankton had isotopic signatures that varied little over depth and were intermediate between potential aquatic and terrestrial sources (Fig. S1). In both lakes, there were some

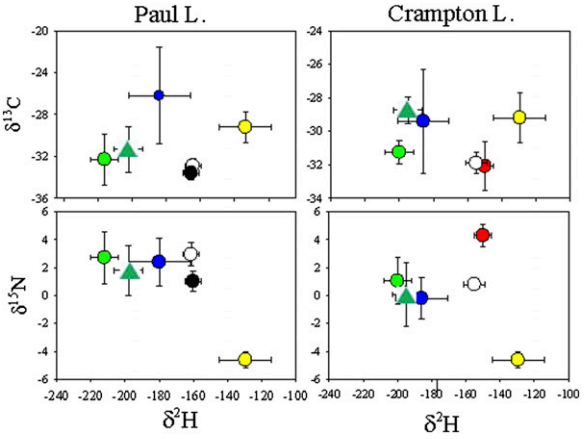

Fig. 1. Isotope biplots for Paul (*Left*) and Crampton lakes (*Right*) for 2009. Zooplankton [*Daphnia* (Paul; filled black circles); *Holopedium* (both lakes, open circles); and *Leptodiaptomus* (Crampton, filled red circle] are shown in relation to possible food sources: phytoplankton in the upper mixed layer (green filled circle); deep water phytoplankton (dark green triangle); littoral benthic algae (filled blue circle), and terrestrial vegetation (filled yellow circle). Means and SDs are shown; in some cases, the symbols are larger than the SDs. Zooplankton are averages for all depths and dates taken. For surface water phytoplankton, SDs combine variance over time and the depths within the upper mixed layer; for periphyton and deep water phytoplankton, the SDs are temporal only. POM, not shown here, is shown in Fig. S1.

significant differences among taxa in isotopic composition. In Paul L., *Daphnia* and *Holopedium* differed significantly in δ^2H and $\delta^{15}N$ but not in $\delta^{13}C$. In Crampton L., *Leptodiaptomus* and *Holopedium* differed significantly in all three isotopes. For Paul L., there was no significant difference in the zooplankton isotopes over depth (each pair, *t* test). In Crampton, only $\delta^{15}N$ was significantly (*t* test, $P < 0.05$) higher at 7 m relative to the other depths. Because the differences among depths were small and inconsistent, and because zooplankton can move throughout the water column, we kept the taxa separate in the following analyses but combined data over depth and date to increase the sample size.

Zooplankton were distinct from their basal food resources (i.e., terrestrial and algal end members) for δ^2H in all cases (Fig. 1). Littoral benthic algae and both surface and deep phytoplankton were significantly lower in δ^2H compared with zooplankton (Fig. 1). The cladoceran zooplankton in both Paul and Crampton lakes were higher in $\delta^{15}N$ compared with terrestrial sources by 4–6‰ suggesting that, with the expected trophic enrichment, terrestrial N was a likely partial source of food for zooplankton. In contrast, cladocerans were similar to or slightly lower than algal $\delta^{15}N$ sources, suggesting algae are not sole N sources for these zooplankton (Fig. 1). *Leptodiaptomus* in Crampton L. was higher by 2–3‰ in $\delta^{15}N$ compared with phytoplankton and by as much as 8‰ compared with terrestrial sources. Depending on the extent to which *Leptodiaptomus* is a primary consumer or an omnivore (i.e., feeding partially on other zooplankton), either terrestrial or algal N are possible food sources. The $\delta^{13}C$ of zooplankton in Paul L. was lower than benthic algal sources and close to, but slightly lower than, either deep or surface phytoplankton sources. It is likely that some lower $\delta^{13}C$ source (e.g., methanotrophic bacteria; ref. 40) is used by zooplankton in this lake (see below).

In our modeling analysis, we treat all zooplankton as primary consumers that feed on some mixture of algal and terrestrial resources. We use 15% as an estimate of dietary water (*Methods*) for consumers. We calculated the δ^2H in food (e.g., corrected for dietary water) of each zooplankton taxa and used these corrected values for the isotope modeling with δ^2H alone and for δ^2H in

combination of $\delta^{13}C$ and $\delta^{15}N$. Using $\delta^{2}H$ alone, all models suggest zooplankton are comprised in part of terrestrial organic matter with median estimates ranging from 10 to 30% depending on the model (Fig. 2). However, the $\delta^{2}H$-alone models are not well constrained with 5 and 95 percentiles ranges from near 0 to >40% (Fig. 2). Adding $\delta^{13}C$ and $\delta^{15}N$ to the models greatly reduced the range of possible values of the percentage of terrestrial organic matter (ϕT) in zooplankton biomass (Fig. 3). With the three isotope models, none include zero ϕT at the extremes, and the medians range from ≈25 to 39% (Fig. 3). Further, no models that excluded terrestrial sources fit within a tolerance of 3‰ (*Methods*). Collectively, these models provide strong evidence that zooplankton are significantly supported by terrestrial organic matter.

Methanotrophic bacteria are a possible food source to zooplankton, and even modest consumption of these bacteria results in significant ^{13}C depletion (41). We do not have direct measurements of the isotopes of methanotrophs but provide an estimate based on assumptions from the literature for ^{13}C and $\delta^{2}H$ (ref. 42 and Table S3), with the additional assumption that methanotrophic bacteria, as primary autototrophs, have the same $\delta^{15}N$ as phytoplankton. Adding methanotrophs has little effect on the estimate of ϕT in the models (Fig. 3).

Discussion

Terrestrial Support of Zooplankton. Based on analysis of ambient stable isotopes, zooplankton in the two study lakes are comprised in part of allochthonous organic matter. Although the estimates of the magnitude are uncertain and vary with lake, taxon, and the sources used in each model, ϕT exceeded 10% in all cases and was between 20 and 40% for most of the source combinations we tried. In no case using multiple isotopes could we explain the composition of zooplankton from any combination of surface and deep water phytoplankton in the absence of some terrestrial material. The mean values of multiple models are in agreement with those obtained from the whole lake ^{13}C additions for the same lakes for cladocerans and substantially higher for the copepod *Leptodiaptomus* (ref. 31 and Table S1). The ^{13}C addition experiments have two potential biases that could result in overestimates of allochthony: deep feeding by vertically migrating zooplankton and consumption of detritus of algal origin that was produced before the addition of the label (15, 21). However, the ambient isotope approach used here did not give substantially lower estimates of ϕT than the ^{13}C additions, indicating that neither of these potential biases were of major importance for zooplankton in these lakes.

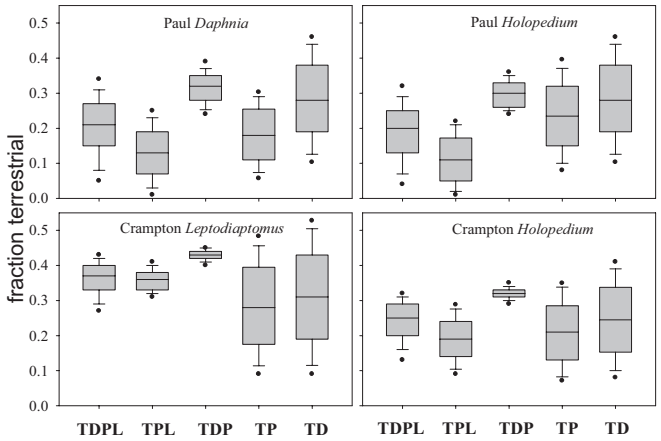

Fig. 2. Box-whisker plots of the fraction of zooplankton biomass from terrestrial organic matter estimated from IsoSource models by using only one stable isotope ratio ($\delta^{2}H$) in Paul and Crampton lakes. The box boundaries represent the 25th and 75th percentile, the horizontal line is the median, and the whiskers mark the 10th and 90th percentiles. The dots denote the 5 and 95% values of the distribution. The models include combinations of possible sources: T, terrestrial; P, phytoplankton in the upper mixed layer; D, phytoplankton at the chlorophyll max in oxic water; L, benthic algae.

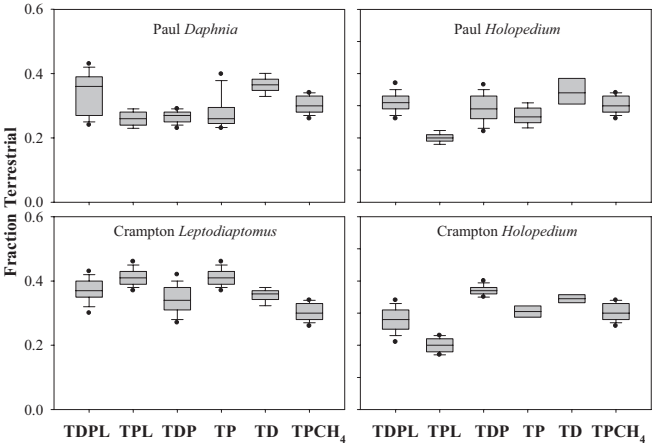

Fig. 3. Box-whisker plots of the fraction of zooplankton biomass from terrestrial organic matter estimated from IsoSource models by using three stable isotopes ($\delta^{2}H$, $\delta^{15}N$, and $\delta^{13}C$) in Paul and Crampton lakes. The box boundaries represent the 25th and 75th percentile, the horizontal line is the median, and the whiskers mark the 10th and 90th percentiles. The dots denote the 5 and 95% values of the distribution. The models include combinations of possible sources: T, terrestrial; P, phytoplankton in the upper mixed layer; D, phytoplankton at the chlorophyll maximum in oxic water; L, benthic algae; CH_4, metanotrophic bacteria.

The isotopic modeling indicates zooplankton are comprised of multiple sources in these lakes, but distinguishing among some of these sources is difficult. For example, the autotrophic components are not well separated from each other isotopically. Thus, models often cannot distinguish among two or more of these sources in terms of their support of zooplankton. In Paul L., although there was not strong isotopic separation between surface and deep phytoplankton, ^{13}C in periphyton was lower than in phytoplankton. In models that included phytoplankton and periphyton, periphyton was a minor possible source to zooplankton, <10% for either *Daphnia* or *Holopedium*. In Crampton L., none of the autochthonous components were well separated and all sources were likely in some models. If we sum the three possible autochthonous sources in each model, we can compare ϕT to support by total autochthonous production, and this comparison revealed a consistent pattern. Autotrophic production (some combination of benthic algae plus surface and deep phytoplankton) accounted for >60% (at the median of the IsoSource distributions) of zooplankton biomass in all cases.

That zooplankton consume more algae (probably phytoplankton) than terrestrial detritus agrees with our assessments of ϕT from whole lake ^{13}C additions and with most estimates from the literature (Table S1). That zooplankton are formed in part from terrestrial detritus also agrees with the whole lake ^{13}C additions in these lakes and with most prior studies that have used a variety of methods. The ^{13}C additions suggest that ϕT for cladocerans was ≈30% in Crampton L. (31) and 20–37% in Paul (20, 38). This result agrees well with the estimates developed here by using only ambient isotope measurements. The ^{13}C addition analysis for Crampton L. suggested that *Leptodiaptomus* was almost entirely supported (98%) by autochthonous primary production, whereas the ambient isotope approach leads to a higher estimate of support by allochthonous sources (30–40%). The ambient isotope approach treats all zooplankton as primary consumers. This assumption is reasonable for cladocerans but

may not apply to *Leptodiaptomus*, which consumes small zooplankton, heterotrophic protists (e.g., ciliates), phytoplankton, and detritus (30, 35, 43, 44). The higher $\delta^{15}N$ of *Leptodiaptomus* is the result of feeding on a higher trophic level than *Holopedium*, feeding on different basal food sources, or both (Fig. 1). Thus, our simple analysis may overestimate ϕT for *Leptodiaptomus*, and a more sophisticated approach may be needed for secondary consumers or those, like *Leptodiaptomus*, which probably feed at multiple tropic levels (13).

Literature Review. At least 15 prior studies have attempted, using a variety of methods, to estimate allochthony in zooplankton (Table S1). Of these, four provided only qualitative assessments of which two (22, 23) describe allochthony as being low while two others (11, 24) describe allochthony as large or very large. All of the studies that provide quantitative estimates suggest that allochthony for some zooplankton taxa in the systems studied is >20%, and many of the studies estimate much higher values (50–70%; e.g., refs. 26 and 28). Most of the published studies used ambient stable isotopes (largely ^{13}C, some in combination with ^{15}N) as the basis of the estimate. One intriguing study used specific fatty acid biomarkers and traced the C supporting whitefish (*Coregonus* 11) to terrestrial material that transferred to fish via copepods. Another study that used ambient ^{14}C, which can help distinguish materials based on their age found high allochthony for zooplankton, in the Hudson River where old allochthonous carbon is important (34).

Isotopic Signatures of Phytoplankton. We used δ^2H both as a food web tracer and, because of its high contrast between terrestrial and algal photosynthesis, as a way to estimate the isotopic signature of phytoplankton (*Methods* and Table S3). The calculated values of $\delta^{13}C$ for phytoplankton using the δ^2H approach returned values of −31.3‰ in Paul L. and −29‰ in Crampton, which are comparable to estimates we obtained from the ^{13}C addition experiments (31, 38) and imply phytosynthetic fractionation (^{13}C-CO_2 minus ^{13}C-phytoplankton) values in the range previously observed in freshwaters (−12 to −20‰; refs. 31 and 45). Using δ^2H, we estimate that POM is highly allochthonous, >80% in both lakes. This estimate is somewhat higher than we estimated from the whole-lake ^{13}C additions for Paul L. and much higher than that for Crampton L; this discrepancy may reflect differences among years, methods, or both. However, if we assumed a lower ^{13}C value for phytoplankton, terrestrial organic C would have to be an even larger fraction of the POM in both lakes. The δ^2H approach estimated $\delta^{15}N$ values for phytoplankton of 1.8‰ in Paul and 0.08‰ in Crampton. These values are higher than the measured terrestrial ^{15}N by 4–6‰. Similar contrasts were reported by France (46) for benthic algae in forested lakes. Our estimated values of $\delta^{15}N$ in phytoplankton were slightly lower (by 0.3–0.6‰) compared with our measured values in benthic algae. Thus, δ^2H may be a promising tool in estimating the ^{13}C and ^{15}N isotopic signature of phytoplankton, and more work needs to be done to refine its use.

Role of Methane. Many studies, including this one, find that zooplankton have lower ^{13}C than measurable sources (22, 47). Hypotheses advanced to explain this discrepancy include the following: a lower ^{13}C signature in the phytoplankton consumed by zooplankton (30, 48); feeding deep in the water column where ^{13}C is sometimes lower than in surface waters (22, 30) and the consumption of methanotrophic bacteria, which can be have extremely low ^{13}C signatures (42). For our study, neither the seston nor our estimates of the phytoplankton ^{13}C signature showed marked ^{13}C depletion over depth in oxic water (Table S3), and we have accounted for the possibility of feeding deep in the water column in the mixing models. In Paul L., but not Crampton L., it is conceivable that there is a source of food in anoxic water that we did not measure (e.g., purple or green sulfur bacteria). Using this source would require zooplankton to feed in anoxic water. If the zooplankton are restricted to oxic water, the consumption of methanotrophic bacteria is quite likely. These lakes have measureable CH_4 concentrations at all depths including surface waters (49) and measurable but low rates of CH_4 oxidation in oxic waters (50). Including methanotrophic bacteria in the mixing, models provided a better fit for ^{13}C, but has little effect on the estimate of ϕT for any of the taxa-lake combinations.

How Do Zooplankton Access Terrestrial Organic Matter. The standing stock of DOM in most lakes in this region is heavily dominated by terrestrial sources (7, 20). The importance of terrestrial organic matter to POM is more variable. In experimentally fertilized lakes, POM was almost entirely derived from autochthonous primary production (27, 29). Based on the ^{13}C addition to Crampton L., Pace et al. (31) estimated that POM was highly autocthonous (31). In contrast in this study, consistent with the *Leptodiaptomus* results, we calculate that POM was highly allochthonous. Although methodological differences cannot be ruled out, year-to-year variability is a possibility. Because Crampton L. is dilute, small differences in either terrestrial loading or primary production could account for this discrepancy. Given the allochthonous nature of combined dissolved and particulate organic matter in these lakes, it is striking that terrestrial organic matter comprises only 25–30% of zooplankton biomass. The relatively high reliance on algal material demonstrates that zooplankton are quite selective in keeping with physiological studies (51, 52) and some field studies (22, 23).

This study does not address the pathways that provide zooplankton with terrestrial organic matter; some of our prior work does. Bacterial uptake of DOM, and subsequent consumption by zooplankton, is likely only part of the story. From the ^{13}C experiments, we estimate for Paul L. that this pathway provides <10% of the terrestrial C that zooplankton consume (20). This low supply is the result of low rates of bacterial production compared with zooplankton demand and that bacteria assimilate DOM of both algal and terrestrial origin in about equal proportion (53). There are a number of reports of invertebrates that take up DOC directly (54, 55) but very few for crustacean zooplankton. Speas and Duffy (19) suggest the process occurs in *Daphnia* but is not significant to its C balance. It is likely, therefore, that zooplankton are consuming particles that either entered the lake from land or formed by flocculation of DOM. Because direct aeolian inputs appear to provide only a small fraction (<10%) of the total zooplankton demand (56), flocculation of DOM is the most likely mechanism for a large source of terrestrial particles (57).

There is ample evidence that some zooplankton, especially cladocerans, will ingest numerous kinds of particles (52). Our isotopic evidence suggests that particles of terrestrial origin must also be assimilated by zooplankton. Recently, Brett et al. (21) measured assimilation of terrestrial organic matter by *Daphnia magna* in laboratory experiments. *D. magna* grew and reproduced poorly on diets of terrestrial particles alone (red alder leaves), but growth and reproduction were positive on mixtures of algae and alder leaves even when the algal component was as low as 20% of the total (21). On mixtures approaching what we estimate here for cladocerans (30% terrestrial, 70% algae), growth and reproduction were not different from diets using nutritious laboratory algae that produced maximal growth (21). Hence, despite the arguments of Brett et al. (21) that zooplankton are not supported by terrestrial organic matter, their laboratory results are consistent with the field analyses reported here and elsewhere (Table S1). Zooplankton do not grow simply on nutritious algae but subsist on algae of variable quality (58) and on organic matter derived from terrestrial sources.

Conclusions. Using a unique method that can be applied to a wide range of ecosystems, we found significant terrestrial support of pelagic zooplankton. Our findings support previous findings of allochthony in pelagic systems and counter arguments that allochthony is an artifact of methods. The literature reports a range in ϕT for zooplankton among systems and taxa (Table S1), and some patterns are consistent with the feeding ecology of zooplankton. Zooplankton are selective feeders, some taxa more than others, and phytoplankton is usually a preferred food. It is only when the concentration of particles of terrestrial origin (or bacteria that consumed terrestrial DOM) of an appropriate particle-size is considerably larger than the concentration of edible phytoplankton that we would expect significant ϕT. Accordingly, ϕT should be highest in humic lakes with low phytoplankton biomass, and lowest in either eutrophic lakes, or clear-water lakes with limited terrestrial inputs. This pattern is supported by our findings and existing literature (Table S1). As methods improve and more studies are conducted, we expect considerable variation in support of consumers by allochthonous resources, which should lead to the development of models that explain this variation among ecosystems. Further, improved isotope mixing models that better incorporate uncertainty in sources (48, 59) will likely aid in producing better models.

Methods

Sample Collection. Samples were taken at four depths in each lake four to six times during the ice-free season of 2009. Zooplankton were collected with an open diaphragm bilge pump where the inlet hose was set at the desired sampling depth, and the outlet hose pumped water through an 80-μm mesh net. Samples for seston were collected by the same method without filtering the water (i.e., whole water samples). Zooplankton samples were sorted by taxa under a dissecting microscope, dried (40 °C), and desiccated pending isotope analysis. Seston samples were filtered in the laboratory shortly after collection. For ^{13}C and ^{15}N samples, seston was collected on 25-mm glass fiber filters (Whatman GF/F) and dried. For δ^2H, samples were filtered through 47-mm MicronSep Cellulosic. The accumulated seston was back-rinsed into a small volume of water and then dried. This separate procedure for δ^2H was used because glass fiber filters interfere with the δ^2H analysis. From the same samples as the isotopes, aliquots were taken for the analysis of chlorophyll-a by fluorometry. Because the filters clog as particles accumulate, bacteria are included in the seston but we cannot separately estimate the isotopic signature of bacteria. Profiles of dissolved oxygen and temperature were taken by using a model YSI Professional Plus meter.

Isotope Analysis. Stable isotope ratios of organic samples were measured on isotope ratio mass spectrometers at the University of Alaska ($\delta^{13}C$ and $\delta^{15}N$) and the University of Northern Arizona (δ^2H). Methods for δ^2H analysis followed those of Doucett et al. (39) and Finlay et al. (60), including a benchtop equilibration to correct for exchange of H atoms between samples and ambient water vapor (61–63). Water samples were analyzed for δ^2H via cavity-ring-down laser spectroscopy. The δ^2H is the nonexchangeable fraction (39).

Isotopic Signatures of Phytoplankton. We obtained the δ^2H values of phytoplankton by performing dilution-regrowth experiments in the surface waters of each lake. Water was collected and filtered through Whatman GF/F filters (4 liters) and mixed with small inocula of unfiltered water. The samples were incubated under ambient light at 22 °C with aeration. By taking frequent samples for chlorophyll-a, we could assess when enough growth (≈5 μg of chl-a·liter^{-1}) had occurred so that enough new particulate material could be easily collected, which took from 4 to 10 d depending on lake and time. The δ^2H of phytoplankton was estimated from the δ^2H of the new material. These experiments provide ε_H (the contrast between δ^2H in phytoplankton and water), which allows the calculation of phytoplankton δ^2H at any time and depth. We assumed that POM is comprised of a mixture of phytoplankton and terrestrial material and solved for the ^{13}C and ^{15}N of phytoplankton algebraically (Table S2 for details). The consistency of ε_H in these lakes and in the literature (5, 15, 39, 60) justifies this approach.

Isotope Modeling. We used the multiple polygon model of Phillips and Gregg (ref. 64; IsoSource) to analyze source contributions to zooplankton. We chose IsoSource, which solves iteratively for feasible mixing solutions, for several reasons. The model is designed to handle situations where there are more possible sources than isotopes, which is the case for some of our model runs. More importantly, IsoSource is a well-tested model, available to all (www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm), and widely cited in the literature.

IsoSource addresses variability in source isotope signatures by using a tolerance parameter that allows models to fit within a certain range of the mean (64). We used tolerance parameters of 1–3‰, which are similar to the range among replicate field samples and reflect the uncertainty in sources (Table S3). For each source, IsoSource computes a frequency distribution of the proportion of organic matter that the source contributes to the consumer. In most cases, this distribution has a single well-defined peak. We express the IsoSource results as box-and-whisker plots of these distributions.

Dietary Water and Trophic Fractionation. To model food sources to zooplankton, we needed to estimate trophic fractionation in ^{15}N and for dietary water for δ^2H. Solomon et al. (65) showed that trophic fractionation for δ^2H was negligible. For ^{15}N, we made the standard assumption that consumers are higher by 3‰ than their food sources, recognizing that there is variability around this mean (66). A fraction of an organism's nonexchangeable H comes from water rather than assimilated food. Because we are interested in the food web, we need to estimate the fraction of dietary water and correct for it. We created a series of models in IsoSource (64) in which we used one isotope (^2H), the δ^2H of water in each lake, and the possible food sources (phytoplankton, deep phytoplankton, benthic algae, and terrestrial vegetation). We fit a range of possibilities for dietary water in 20 models with various combinations of these sources (Fig. S2). The medians of these models ranged from 17 to 12%, and the box-whisker plots of the full distribution are reasonably narrow. None of the models fit with dietary water <10%; a large majority (13 of 20) fit with dietary water between 10 and 15%; and only 1 model fit >25% (Fig. S2). In the analysis presented in the text, we used 15% for dietary water, which is in agreement with both these model runs and the recent review of the literature by Solomon et al. (65).

We tested the effect of different values of dietary water on the outcome in models that included multiple isotopes and sources. Decreasing the estimate of dietary water to 10% increased ϕT and increasing it to 20% decreased ϕT; models with dietary water >25% had no solution. For example, for *Daphnia* in Paul L., the three isotope models that included all sources (terrestrial, surface phytoplankton, deep phytoplankton, and benthic algae) with diet water at 10, 15, and 20% had decreasing means (± SD) for ϕT of 0.4 (±0.036); 0.324 (±0.036), and 0.146 (±0.008), respectively. At diet water of 22% or above, no solution was obtained within a tolerance of 3‰. Clearly, uncertainty in dietary water leads to uncertainty in the magnitude of ϕT. A final caveat concerns the possible alteration δ^2H (or the other isotopes) in organic matter as it decomposes. Large differences in the isotopic signatures of living phytoplankton and detritus derived from phytoplankton, for which there is no evidence, could complicate this analysis. Because the residence time for particles in these water columns is short (days), it is unlikely to see a large diagenetic effect.

ACKNOWLEDGMENTS. We thank Jim Coloso and Laura Smith who collected much of the data shown here and Jim Hodgson, Jon Frum for inspiration in writing this paper. The work took place at the University of Notre Dame Environmental Research Center, and we are grateful for the assistance from Dr. Gary Belovsky and his staff. We thank the National Science Foundation for funding.

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