

Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model

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Abstract. Fluxes of organic matter across habitat boundaries are common in food webs. These fluxes may strongly influence community dynamics, depending on the extent to which they are used by consumers. Yet understanding of basal resource use by consumers is limited, because describing trophic pathways in complex food webs is difficult. We quantified resource use for zooplankton, zoobenthos, and fishes in four low-productivity lakes, using a Bayesian mixing model and measurements of hydrogen, carbon, and nitrogen stable isotope ratios. Multiple sources of uncertainty were explicitly incorporated into the model. As a result, posterior estimates of resource use were often broad distributions; nevertheless, clear patterns were evident. Zooplankton relied on terrestrial and pelagic primary production, while zoobenthos and fishes relied on terrestrial and benthic primary production. Across all consumer groups terrestrial reliance tended to be higher, and benthic reliance lower, in lakes where light penetration was low due to inputs of terrestrial dissolved organic carbon. These results support and refine an emerging consensus that terrestrial and benthic support of lake food webs can be substantial, and they imply that changes in the relative availability of basal resources drive the strength of cross-habitat trophic connections.

Key words: allochthonous; autochthonous; cross-habitat linkages; deuterium; dissolved organic carbon; ecosystem; light extinction; stable isotope; subsidy.

INTRODUCTION

Movements of organic matter and organisms across apparently distinct habitat boundaries link consumers in one habitat to primary producers in another. These cross-habitat connections are a widespread feature of food webs (Polis et al. 1997). Theory suggests that the effects of cross-habitat linkages, which may be strongly stabilizing or destabilizing to consumer dynamics, ultimately depend not on input or availability, but rather on the degree to which consumers use organic matter from the different habitats (Huxel and McCann 1998, Post et al. 2000, Rooney et al. 2006). Yet because the trophic pathways that separate consumers from basal sources of primary production in various habitats are often complex, estimates of resource use in the field are difficult, scarce, and subject to considerable uncertainty. Therefore, while existing conceptual models describe the controls on cross-habitat inputs (e.g., Polis and Hurd 1996, Witman et al. 2004), similar models are

lacking to describe the controls on how consumers use those resources.

Basal resources in lake ecosystems originate from three distinct habitats: as autochthonous primary production in pelagic (open-water) and benthic (bottom) habitats, and as allochthonous primary production in adjacent terrestrial habitats. In the low-productivity lakes that dominate many regions, the relative availability of these three resources is controlled by terrestrial inputs and their effects on light attenuation (Ask et al. 2009a, Karlsson et al. 2009). Low nutrient levels in these systems limit pelagic phytoplankton production. Benthic algae, in contrast, can access nutrients from sediment pore waters (at least on soft substrates) and are thus light, rather than nutrient, limited. Under these conditions, benthic primary production substantially contributes to, and may even dominate, whole-lake autochthonous production (Vadeboncoeur et al. 2003, 2008, Ask et al. 2009a). Terrestrial dissolved organic matter provides a resource for heterotrophs, but also reduces light penetration and thereby benthic algal production. Thus as loading of terrestrial dissolved organic carbon (DOC) increases in low-productivity lakes, the relative availability of terrestrial, pelagic, and benthic basal resources changes.

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A variety of processes at multiple trophic levels create linkages among terrestrial, pelagic, and benthic energy pathways in lake food webs (Schindler and Scheuerell 2002, Vadeboncoeur et al. 2002). For instance, benthic consumers may utilize pelagic production that settles on the bottom, pelagic consumers may utilize dissolved or particulate terrestrial detritus, and fishes may consume benthic, pelagic, or terrestrial prey. While traditional models emphasized pelagic primary production as the most important basal resource in lakes, recent studies have demonstrated significant use of terrestrial (Grey et al. 2001, Carpenter et al. 2005, Matthews and Mazumder 2006, Taipale et al. 2008) or benthic (Vander Zanden and Vadeboncoeur 2002, Karlsson and Byström 2005) resources by a variety of consumers. Therefore, while it is clear that cross-habitat linkages are important in lake food webs, as yet our understanding of these linkages has remained incomplete. Due to methodological constraints, no study has uniquely quantified terrestrial, pelagic, and benthic basal resource use, nor explored how use of these three resources varies among taxa or systems.

In this study we used a novel approach to quantify resource use for a suite of consumers in four lakes in which the relative availability of terrestrial, pelagic, and benthic resources differed strongly. Previous whole-lake ^{13}C -addition experiments in these lakes estimated allochthonous and autochthonous resource use (Carpenter et al. 2005, Pace et al. 2007, Solomon et al. 2008, Weidel et al. 2008). However, such experiments have three important limitations. First, they label all autochthonous primary production, and so have little power to distinguish between benthic and pelagic resource use. Second, the expense of the tracer makes it impractical to conduct such experiments in large systems or a large number of systems, limiting the usefulness of this technique for understanding the controls on resource use. Third, autochthonous primary production that occurs below the mixed layer or prior to the beginning of the ^{13}C addition is not labeled and may be isotopically similar to allochthonous resources; thus estimates of allochthonous resource use from such experiments likely represent upper bounds, and true allochthonous resource use could be lower for consumers that feed below the mixed layer or on old, detrital, autochthonous material that was produced before the start of the experiment (Carpenter et al. 2005, Solomon et al. 2008). In the present study we sought to overcome those potential limitations by using natural-abundance stable isotope ratios of hydrogen, carbon, and nitrogen, together with a Bayesian stable isotope mixing model, to estimate resource use. Adding the H isotope axis to the mixing model in addition to the more commonly used C and N provides additional power to resolve resource use, particularly due to the strong separation in H stable isotope ratios between aquatic and terrestrial primary production (Doucett et al. 2007). This approach allowed us to quantify consumer reliance on primary

production from each habitat, to incorporate substantial existing knowledge about some parameters such as trophic fractionation, and to account for multiple sources of uncertainty inherent in isotope mixing models. We hypothesized that HCN-derived estimates of allochthonous resource use would be lower than those derived from the ^{13}C -additions for zoobenthos and fishes, which are strongly linked to sediment detrital pools of autochthonous production, but not for zooplankton. More importantly, we hypothesized that terrestrial, pelagic, and benthic resource use would track among-lake differences in relative availability, as indicated by water clarity. Specifically, we expected to observe low use of pelagic resources (particularly for zoobenthos and fishes) in all lakes, and increases in terrestrial use coupled to decreases in benthic use in lakes with higher DOC concentrations and lower light penetration.

METHODS

Study system

Crampton, Paul, Peter, and Tuesday lakes are located in a lake district on the Wisconsin–Michigan border (89°32' W, 46°13' N). All four lakes are dimictic, softwater systems with negligible macrophyte growth. They differ considerably in area, DOC concentrations, light penetration, and other characteristics (Table 1).

Bayesian mixing model

We used a Bayesian mixing model to estimate use of terrestrial, pelagic, and benthic resources by consumer populations while accounting for multiple sources of mixing model uncertainty. For a population of consumers of a given taxon in a given lake, the H, C, and N stable isotope ratios of an individual i (or an aggregated sample of several individuals) are given by:

$$\begin{aligned} \delta D_{\text{cons},i} &= \omega_{\text{tot}} \times \delta D_{\text{W}} \\ &+ (1 - \omega_{\text{tot}}) \times (\phi_{\text{T}} \times \delta D_{\text{T}} + \phi_{\text{P}} \times \delta D_{\text{P}} \\ &+ \phi_{\text{B}} \times \delta D_{\text{B}}) + \varepsilon_{\text{D},i} \\ \delta^{13}\text{C}_{\text{cons},i} &= \phi_{\text{T}} \times \delta^{13}\text{C}_{\text{T}} + \phi_{\text{P}} \times \delta^{13}\text{C}_{\text{P}} + \phi_{\text{B}} \times \delta^{13}\text{C}_{\text{B}} \\ &+ \varepsilon_{\text{C},i} \\ \delta^{15}\text{N}_{\text{cons},i} &= \phi_{\text{T}} \times \delta^{15}\text{N}_{\text{T}} + \phi_{\text{P}} \times \delta^{15}\text{N}_{\text{P}} + \phi_{\text{B}} \times \delta^{15}\text{N}_{\text{B}} \\ &+ \Delta_{\text{tot}} + \varepsilon_{\text{N},i}. \end{aligned} \quad (1)$$

In Eq. 1, $\delta D_{\text{cons},i}$, δD_{W} , δD_{T} , δD_{P} , and δD_{B} are the H stable isotope ratios for, respectively, consumer i , mean lake water, and mean terrestrial, pelagic, and benthic sources of primary production; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are the C and N stable isotope ratios, with similar subscripts; ϕ is the proportional reliance of a consumer on the terrestrial, pelagic, or benthic resource ($\phi_{\text{T}} + \phi_{\text{P}} + \phi_{\text{B}} = 1$); ω_{tot} is the proportion of the H in consumer tissues

that is derived from environmental water; Δ_{tot} is the total trophic enrichment of N isotopes in the consumer relative to its basal resources, which is equivalent to the trophic position of the consumer multiplied by the mean per-trophic level fractionation of N. The residual variation in each isotope X for individual i , $\epsilon_{X,i}$, is assumed to be independent among individuals and isotopes, and is normally distributed with mean 0 and variance σ_X^2 .

The constraint that the terrestrial, pelagic, and benthic resource proportions ϕ must sum to one requires special care in model fitting (Moore and Semmens 2008, Jackson et al. 2009, Semmens et al. 2009a). Two options that yield similar results are to model ϕ using the Dirichlet distribution, or to fit using a transformed version of the resource proportions (Semmens et al. 2009b). We chose the latter approach, and used the centered log-ratio (CLR) transform of ϕ , which centers proportions on their geometric mean (Semmens et al. 2009b). We put uninformative uniform(-3,5) priors in CLR-transformed space on each of the three ϕ parameters.

Informative priors for the remaining parameters of Eq. 1 were derived from the literature and our own observations. We calculated a prior mean ω_{tot} for each consumer taxon based on an equation for the trophic compounding of water:

$$\omega_{\text{tot}} = 1 - (1 - \omega)^\tau \quad (2)$$

where ω is the per-trophic-level contribution of environmental water to consumer H and τ is the trophic position of the consumer as trophic levels above primary producers (Solomon et al. 2009). We used $\omega = 0.25 \pm 0.10$ (mean \pm SD, $n = 5$) based on published estimates from controlled experiments with zooplankton, zoobenthos, and fishes (Solomon et al. 2009, Wang et al. 2009). We estimated trophic levels based on the literature and our own observations (Appendix A), with a prior variance of 0.1^2 on each trophic position estimate. The prior variance for ω_{tot} was calculated by first-order Gaussian error propagation of Eq. 2 (Meyer 1975). We then chose a beta prior on ω_{tot} such that it had the calculated prior mean and variance. The mean and variance of a normal prior for the total trophic fractionation of N (Δ_{tot}) were calculated similarly:

$$\Delta_{\text{tot}} = \Delta_N \times \tau \quad (3)$$

where Δ_N is the per-trophic-level isotopic enrichment of N. We used $\Delta_N = 2.52 \pm 1.46$ (mean \pm sd, $n = 40$) based on the data for ammonotelic organisms in Vanderklift and Ponsard (2003), calculated the variance of Δ_{tot} by error propagation, and truncated the prior distribution at 0. Finally, the residual variances σ_D^2 , σ_C^2 , and σ_N^2 depended on the observed variances of the stable isotope ratios of the terrestrial, pelagic, benthic, and water sources (by error propagation of the appropriate line of Eq. 1) plus a uniform(0, 100) prior estimate of unexplained variation. We fit the model using the WinBUGS

TABLE 1. Physical and chemical characteristics of the four study lakes.

Characteristic	Crampton	Paul	Peter	Tuesday
Area (ha)	25.7	1.7	2.7	0.9
Maximum depth (m)	18.5	12.0	19.0	18.0
Mean depth (m)	4.9	3.7	5.7	6.9
Light extinction (k_D , m^{-1})	0.58	0.96	0.86	1.37
Total P ($\mu\text{g/L}$)	8.5	9.0	9.2	12.4
DOC (mg/L)	3.7	4.3	5.0	8.4
Color (m^{-1})	0.6	1.5	1.3	3.5
Chlorophyll ($\mu\text{g/L}$)	3.1	4.5	4.4	6.8

Notes: Light extinction is the mean value calculated from light profiles taken throughout the growing season ($n = 15\text{--}45$ per lake) using LI-COR PAR sensors (LI-190 and LI-193) and data logger (LI-1000; LI-COR, Lincoln, Nebraska, USA). Chemical analyses were measured on pooled mixed layer samples collected throughout the growing season ($n = 4\text{--}14$). Total P samples were digested with sodium persulfate and analyzed with a Lachat auto-analyzer (Loveland, Colorado, USA). Dissolved organic C (DOC) was measured on a Shimadzu organic carbon analyzer (Kyoto, Japan). Color was measured with a spectrophotometer as absorbance at 440 nm. Chlorophyll was measured with a fluorometer after methanol extraction. Complete methods are available online (<http://ecostudies.org/cascade>).

software (Lunn et al. 2000), running each of five Markov chains for 10 000 iterations, discarding a 500-iteration burn-in period from each chain, and thinning the remaining iterations so that we retained ~ 1000 samples from the posterior distributions of the parameters. Convergence was assessed by ensuring that the scale reduction factor R_{hat} was < 1.1 (Gelman et al. 2004).

Posterior distributions for the parameters of Eq. 1 were sometimes skewed, so we use medians as point estimates unless otherwise noted. Point estimates of allochthony (ϕ_T) from the HCN-Bayes approach were compared to estimates derived from previous ^{13}C -addition experiments in these lakes. Two or three separate modeling approaches were used to estimate allochthony for each consumer from the ^{13}C additions in Peter, Paul, and Tuesday lakes (Carpenter et al. 2005). Because all three models gave similar results, only the simplest was used to estimate allochthony in Crampton Lake (Pace et al. 2007, Solomon et al. 2008, Weidel et al. 2008). When multiple estimates were available, we took the mean as the point estimate to compare to the HCN-derived estimate.

Stable isotope ratios

For all three sources of basal production, and for water as well, sampling was spread over the 2007 growing season from shortly after leaf-out until leaf fall. Water was collected with a Van Dorn bottle from 0.5 m depth on four dates (10 dates for Paul Lake), filtered (GF/F 0.7 μm), and stored at 4°C until analysis. Samples of leaf material ($n = 81$) were collected on four dates from the dominant deciduous (*Acer saccharum*, *Acer rubrum*, and *Betula alleghaniensis*) and coniferous (*Abies balsamea*, *Picea mariana*, *Tsuga canadensis*, and *Thuja*

occidentalis) tree species, from each of the four watersheds. There were no differences in stable isotope ratios among watersheds (results not shown), so we pooled samples to estimate δD_T , $\delta^{13}C_T$, and $\delta^{15}N_T$. Furthermore, the stable isotope ratios of the terrestrial vegetation samples were indistinguishable from those derived from more limited sampling of dissolved organic matter from surface and inflowing groundwater from the four lakes (results not shown), suggesting that terrestrial vegetation was an appropriate representation of the terrestrial end member. Benthic periphyton samples were scraped from natural wood substrates and ceramic tiles in the epilimnion ($n = 5-9$ per lake, five dates). Indirect methods were used to estimate H, C, and N stable isotope ratios of phytoplankton due to the difficulty of isolating pure phytoplankton samples from lake water in quantities sufficient for isotopic analysis. Phytoplankton δD was calculated by multiplying measured water δD by experimentally derived estimates of phyto-water α , the photosynthetic fractionation factor for H isotopes (Appendix B). Phytoplankton $\delta^{13}C$ and $\delta^{15}N$ were calculated from measurements of particulate organic matter (POM) $\delta^{13}C$ ($n = 5-14$ per lake) and $\delta^{15}N$ ($n = 26-39$ per lake). We assumed that POM includes phytoplankton and terrestrial constituents, and solved for the $\delta^{13}C$ and $\delta^{15}N$ of the phytoplankton constituent by using our measured $\delta^{13}C$ and $\delta^{15}N$ of POM and terrestrial vegetation, along with previously published estimates of the proportion of POM that is terrestrially-derived in these four lakes (Carpenter et al. 2005, Pace et al. 2007). We used first-order Gaussian error propagation to calculate the variance of δD_P (which was estimated as a function of two random variables) as well as the variances of $\delta^{13}C_P$ and $\delta^{15}N_P$ (functions of three random variables).

Zooplankton, zoobenthos, and fishes were sampled from each lake on four occasions (seven occasions for Paul zooplankton) between late May and early October 2007. We sampled the dominant taxa from each group in each lake (Appendix A). Odonate and chironomid larvae were collected at 1 m depth by Ekman dredge and D-net at three sites in each lake. Zooplankton were collected at night by oblique net tows (80 μm for crustacean zooplankton, 153 μm for *Chaoborus*) through the mixed layer. Both zooplankton and zoobenthos were held overnight to allow for gut clearance. Fishes were collected by minnow trap, electrofishing, or angling, and a small sample of dorsal muscle tissue was removed from each individual. To integrate over individual variation in stable isotope ratios, and to obtain sufficient mass for analysis for zooplankton and zoobenthos, we ran pooled samples of multiple individuals for a given taxon/lake/date. The number of individuals in a pooled sample was 20-60 chironomids; 1-7 odonates; 100-4000 crustacean zooplankton; 100-200 *Chaoborus*; 2-5 fishes. Pooled samples were dried at 60°C, ground to a fine powder, and prepared for determination of stable isotope ratios. Because it is currently unclear whether samples for δD

analysis should be lipid extracted (Jardine et al. 2009), we conducted a preliminary analysis in which 36 samples (including producers and consumers) were analyzed for both bulk and lipid-free δD . Because the effects of lipid extraction on δD were similar for producers and consumers and lipid extraction therefore had little effect on mixing model results, we did not extract lipids from the samples used in the analyses reported here.

Stable isotope ratios of organic samples were measured on isotope ratio mass spectrometers (IRMS) at the University of Alaska ($\delta^{13}C$ and $\delta^{15}N$) and the University of Northern Arizona (δD). Methods for δD analysis followed those of Doucett et al. (2007), including a bench-top equilibration to correct for exchange of H atoms between samples and ambient water vapor (DeNiro and Epstein 1981, Wassenaar and Hobson 2000, Wassenaar and Hobson 2003). Water samples were analyzed for δD via cavity-ring-down laser spectroscopy.

RESULTS

Stable isotope ratios of terrestrial, pelagic, and benthic sources of primary production varied within and among lakes (Fig. 1, Appendix C). There was strong isotopic separation relative to noise between allochthonous and autochthonous (benthic and pelagic) sources in δD , and fairly strong separation between allochthonous and autochthonous sources in $\delta^{15}N$. Isotopic separations between the two autochthonous sources were generally weak relative to noise, although mean periphyton $\delta^{13}C$ was slightly higher (by 2-8‰) than mean phytoplankton $\delta^{13}C$ in all four lakes. Among-lake variation in $\delta^{13}C$ of both of these sources tracked differences in the $\delta^{13}C$ of dissolved inorganic carbon (data not shown).

Consumer H, C, and N stable isotope ratios differed among lakes, taxa, and individual samples, reflecting differences in resource use, resource stable isotope ratios, and other factors (Fig. 1, Appendix D). We fit the mixing model of Eq. 1 to these data in order to estimate resource use for each consumer in each lake. Tests of the model on simulated data demonstrated that posterior distributions captured the "true" values of the resource use parameters ϕ even when prior information about the physiological parameters ω_{tot} and Δ_{tot} was biased; weak isotopic separation between benthic and pelagic resources sometimes led to biased point estimates for these resources, although 95% Bayesian credible intervals included the true values even in these cases (Appendix E).

Posterior estimates of the total contribution of environmental water to tissue H (ω_{tot}) were between 0.18 and 0.56, with lower values for crustacean zooplankton and chironomids, and higher values for the higher trophic position zoobenthos, zooplankton, and fishes (Appendix F). Relative to their priors, posterior distributions of ω_{tot} were generally narrow and were shifted towards lower values. Posterior

distributions of Δ_{tot} also tended to be narrower, but not consistently shifted up or down, relative to their priors (Appendix F). Estimated medians of Δ_{tot} were between 2‰ and 5‰ for low trophic position zooplankton and zoobenthos, between 4.5‰ and 8.5‰ for high-trophic-position zooplankton and zoobenthos, and between 6‰ and 10‰ for fishes. The width of 95% Bayesian credible intervals (95% CIs) for ω_{tot} and Δ_{tot} increased from low trophic position groups to high trophic position groups, reflecting the propagation across multiple trophic levels of the uncertainty about water contributions and N fractionation associated with a single trophic transfer.

Posterior distributions for the resource proportions ϕ were broad, reflecting their uninformative priors, the multiple sources of uncertainty inherent in isotope mixing models, and the relatively small sample sizes of consumers available in this study (Fig. 2). This was particularly true for the fishes, probably due to greater uncertainty about ω_{tot} and Δ_{tot} at higher trophic positions. Nonetheless, the data were informative for some fishes and for most of the zooplankton and zoobenthos, such that the probability masses of posterior distributions differed appreciably from those of the priors. Allochthonous inputs were an important resource for a wide variety of consumer groups across contrasting lake types; terrestrial use was greater than use of autochthonous (benthic + pelagic) sources for over half of the 23 consumer–lake groups that we considered. Benthic use was often substantial for zoobenthos and fishes in the clearer lakes, but was generally low (<0.20) for zooplankton. Pelagic use was low for all taxa in all four lakes, with median values <0.45 even for zooplankton, and usually <0.20 for zoobenthos and fishes (Fig. 2).

Among-lake variation in resource use was consistent with the availability predictions of the light-limitation hypothesis, with some interesting exceptions (Fig. 3). For simplicity, we describe among-lake patterns using the estimated medians, but emphasize that these are only point estimates of the resource use parameters ϕ . The wide confidence intervals on ϕ indicate that none of these patterns can be interpreted as significant in a strict hypothesis-testing framework. Terrestrial use increased with light extinction rates (k_D) for crustacean zooplankton, chironomids, odonates, cyprinids, and largemouth bass (*Micropterus salmoides*). There was a concomitant decrease in either benthic or pelagic use with k_D for all of these groups. No clear trends in resource use across lakes were apparent for *Chaoborus*, nor for *Lepomis* across the two relatively clear lakes where this taxon occurred.

The relationship between allochthony estimates derived from the HCN approach in the present study and those derived from our previous ^{13}C -addition experiments varied among taxonomic groups. HCN-derived estimates tended to be higher than ^{13}C -addition estimates for zooplankton, similar to ^{13}C -addition estimates for zoobenthos, and lower than ^{13}C -addition estimates

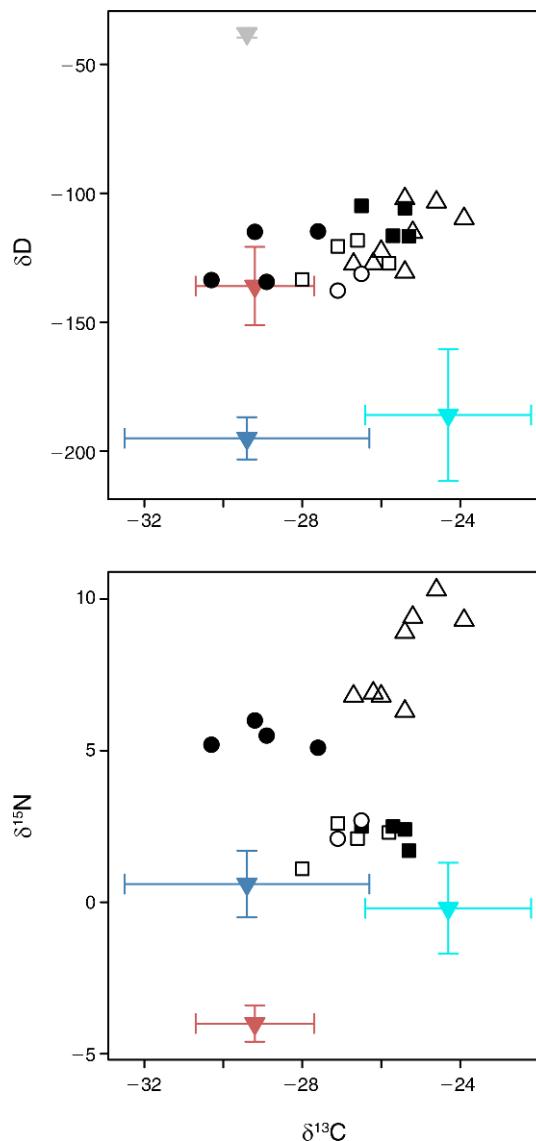


FIG. 1. Data used in mixing models for Crampton Lake. Inverted triangles show mean (\pm SD) H (δD), C, and N stable isotope ratios of terrestrial (red), pelagic (blue), and benthic (cyan) basal resources, as well as H stable isotope ratio of lake water (gray; plotted at arbitrary $\delta^{13}\text{C}$). Remaining points show stable isotope ratios of zooplankton (open circles, Crustacea; solid circles, *Chaoborus* spp.), zoobenthos (open squares, Chironomidae; solid squares, Odonata), and fishes (open triangles, *Lepomis macrochirus*; solid triangles, *Micropterus salmoides*). See Appendices A, C, and D for additional taxonomic details and data for other lakes.

for fishes (Fig. 4). Uncertainty about allochthony is substantial using either approach (e.g., Fig. 2), so these comparisons of point estimates should be interpreted with caution.

DISCUSSION

Our results demonstrate that terrestrial, benthic, and pelagic primary production all contribute to supporting



PLATE 1. Terrestrial organic matter on the surface of Crampton Lake, Wisconsin–Michigan (USA). Photo credit: B. C. Weidel.

lake food webs, and that their relative importance varies with both organismal- and ecosystem-level properties. While traditional models of lake food webs emphasize the importance of pelagic primary production, we found that terrestrial and benthic primary production were equally or even more important than pelagic primary production for consumers in the lakes that we studied. Our results thus support an emerging consensus that terrestrial and benthic support of lake food webs can be substantial (Vadeboncoeur et al. 2002, Jansson et al. 2007). In all four lakes, for the taxa that we sampled, terrestrial and benthic resources together supported at least 60–70% of the biomass of zoobenthos and fishes, with the terrestrial resources particularly important for zoobenthos and the benthic resources particularly important for the fishes. Similarly, terrestrial resources supported 20–80% of the biomass of zooplankton across the four lakes (see Plate 1). We sampled only a few representatives of the diverse species assemblages of these lakes, and it is possible that pelagic primary production might be more important for other taxa. For instance, profundal zoobenthos probably rely more heavily on pelagic primary production than do littoral zoobenthos, because phytoplankton detritus accumulates in profundal regions. Our study was also limited to relatively small and unproductive lakes; pelagic primary production might support more consumer production in

larger or more productive systems. Nonetheless, the taxa that we considered were the dominant zooplankton, littoral zoobenthos, and fishes in each lake. Furthermore, while our study lakes are small, they represent the dominant size class of lakes globally, and our largest, while <30 ha, is larger than ~99% of all the lakes in the world (Downing et al. 2006). It seems likely, therefore, that terrestrial and benthic primary production support a substantial portion of consumer production in many lakes around the world.

Bayesian methods inherently emphasize parameter uncertainty, and we have accentuated that emphasis by explicitly incorporating multiple sources uncertainty into our mixing model. We see this as a strength of our modeling approach, because it allows conclusions to be interpreted appropriately. For instance, we are fairly confident, despite the multiple sources of uncertainty, that terrestrial resources are important for odonate larvae in Tuesday Lake; the posterior probability that they derive more than half of their energy from this resource is >95% (Fig. 2). In contrast, we are much less confident about the importance of terrestrial resources for odonates in Crampton Lake, for which the posterior estimate of terrestrial use was fairly diffuse (Fig. 2). While mixing models have become more sophisticated in their treatment of uncertainty as stable isotope food web techniques have matured (Phillips and Gregg 2003, Moore and Semmens 2008), very few studies account for uncertainty in resource stable isotopes, consumer stable isotopes, and physiological parameters, as we have here. Future studies might be able to reduce uncertainty about consumer resource use by measuring consumer and resource stable isotope ratios more precisely or by considering alternate model structures such as varying ϕ_x across isotopes. Furthermore, there is a clear need for controlled experiments to improve prior information about the water contribution, ω ; existing estimates are scarce, and simulations indicate that better prior constraints on ω yield substantial improvements in posterior credible intervals for consumer resource use (Appendix E). With or without these steps to reduce uncertainty, we recommend our modeling approach—incorporating multiple sources of uncertainty in a Bayesian framework—as one way to more firmly ground conclusions drawn from stable isotope data.

As we had hypothesized, among-lake variation in resource use was associated with the among-lake differences in resource availability predicted by the DOC–light hypothesis (Ask et al. 2009a). In particular, for many taxa there was a tendency toward less reliance on benthic primary production and greater reliance on terrestrial resources with increasing DOC and light extinction (Fig. 3). There are at least two explanations for this observation: terrestrial C might represent a true subsidy, supporting consumer production that would not have occurred otherwise; alternatively, it could be that consumers substitute one resource for another as resource availability changes. Karlsson et al. (2009)

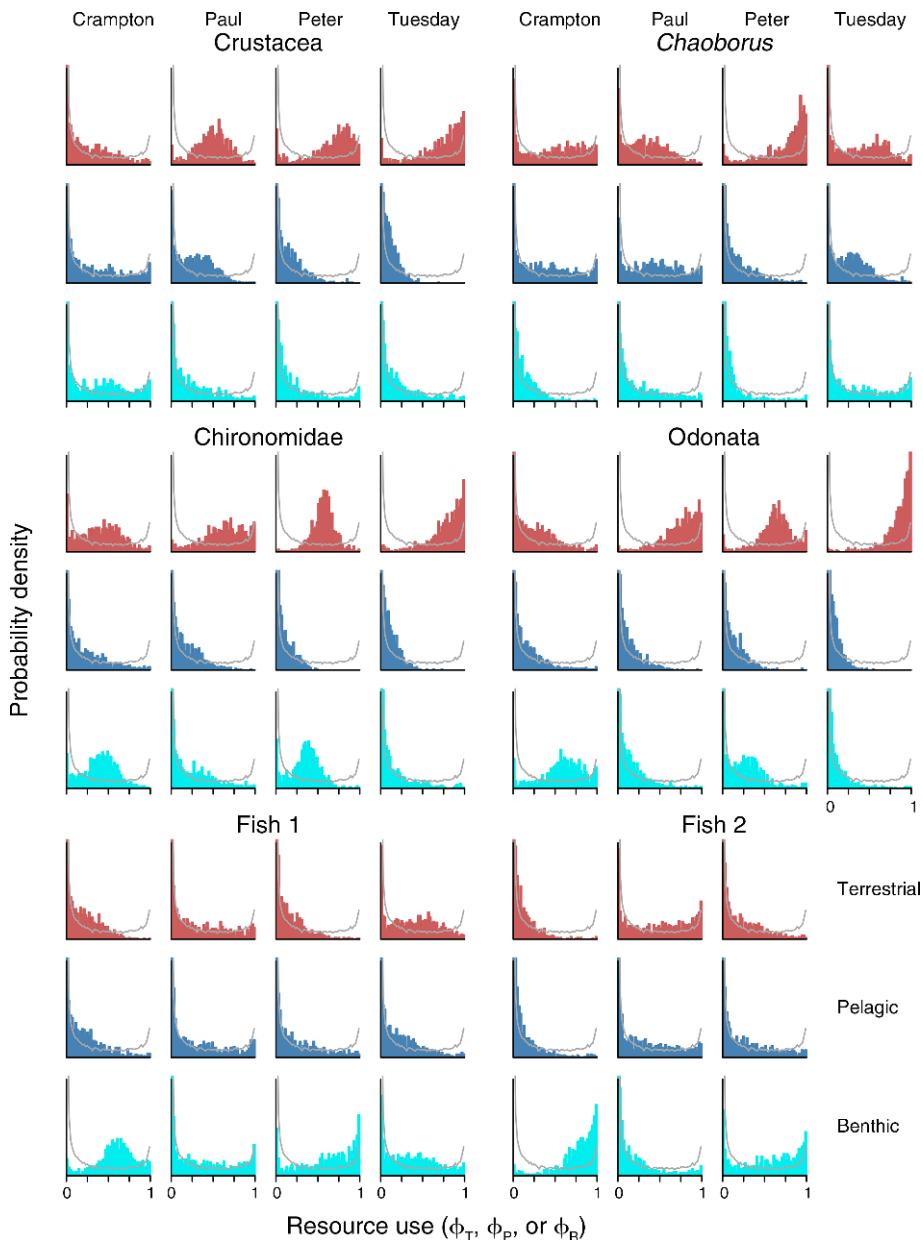


FIG. 2. Posterior estimates of use of terrestrial (red), pelagic (blue), and benthic (cyan) basal resources ($\phi_T + \phi_P + \phi_B = 1$) for six consumer groups in four lakes. Within each consumer group, the columns are (from left to right) Crampton, Paul, Peter, and Tuesday lakes. Gray lines show the uninformative prior distributions. Within each lake, “Fish 1” and “Fish 2” indicate, respectively, lower and higher trophic position fishes; see Appendix A for taxonomic details.

showed lower fish catches in stained lakes (where we observe high terrestrial use) than in clear-water lakes (where we observe low terrestrial use). This pattern suggests that fish may substitute terrestrially derived energy for autochthonously derived energy as terrestrial inputs increase, rather than subsidizing growth with previously unavailable terrestrial resources. Better estimates of fish production, coupled to estimates of energy mobilization (as in Ask et al. 2009b) and of resource use (as in this study) will improve our ability to address

these questions. It is also clear that measurements of resource use, while they show which energy pathways support consumer production, do not necessarily describe the importance of a resource to consumer dynamics (Paine 1980). For instance, lab experiments indicate that while cladoceran zooplankton can survive, grow, and reproduce even on unconditioned leaf detritus from a single terrestrial tree species, their performance is enhanced if they can also consume even a small amount of phytoplankton (Brett et al. 2009). Distinguishing

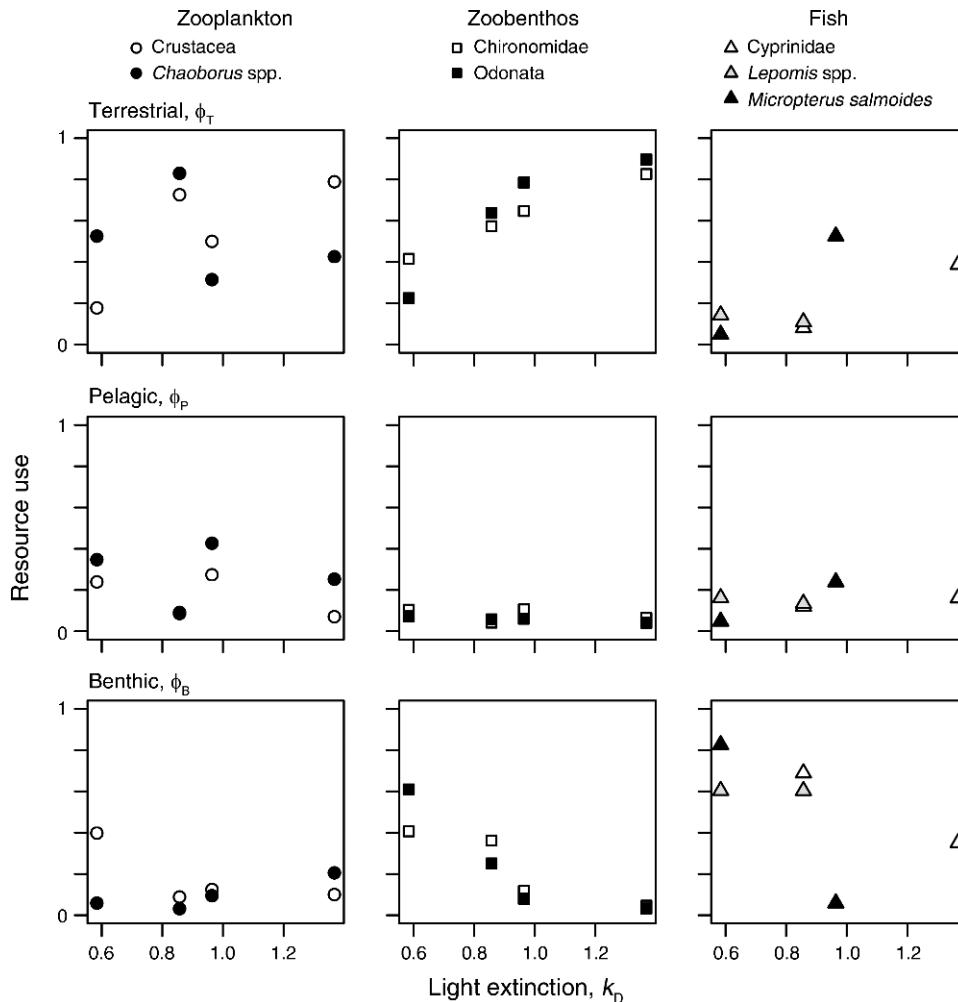


FIG. 3. Median posterior estimates of terrestrial, pelagic, and benthic basal resource use for each consumer group in each lake, plotted against the rate of light extinction.

between resource subsidies and resource substitutions, and linking energetic fluxes to dynamic consequences, remain important challenges for food web research.

Previous ^{13}C -addition experiments, like the results of the present study, indicated substantial allochthonous support of many consumers in these lakes. Yet it was unclear to what extent those ^{13}C additions overestimated allochthony, because of the potential for unlabeled autochthonous production in such experiments. Brett et al. (2009) recently suggested that, for this reason, the actual allochthony of zooplankton in Paul, Peter, and Tuesday lakes may have been much lower than the 30–70% that we reported (Carpenter et al. 2005). Results from the present study refute that suggestion and support the ^{13}C -addition estimates; HCN-derived point estimates of zooplankton allochthony were actually higher than those derived from the ^{13}C additions (Fig. 4), suggesting that neither detrital autochthonous production nor autochthonous production from below

the mixed layer significantly support zooplankton production in these lakes. This conclusion is consistent with our understanding of these ecosystems. Detrital particles sink quickly, such that most unlabeled autochthonous detritus probably settles out within a few days of the beginning of a ^{13}C addition experiment. And autochthonous production below the mixed layer is limited in our lakes; even in Crampton Lake, which has by far the deepest light penetration of the four, <30% of whole-lake autochthonous production occurs below the mixed layer (Coloso et al. 2008). Both the HCN and ^{13}C -addition approaches appear to be acceptable for estimating zooplankton resource use in our lakes. In other systems, however, zooplankton do utilize substantial deep autochthonous production (Matthews and Mazumder 2006); natural abundance tracer studies such as we employed here are likely to be better tools for measuring resource use in such systems than epilimnetic tracer additions. Given these uncertainties and among-

lake differences, more work is clearly needed to understand the vertical structure of trophic linkages in lakes.

The fishes that we considered prey heavily on zoobenthos, yet had higher benthic resource use than did chironomids or odonates from the same lake (Figs. 2 and 3), as well as lower terrestrial resource use than indicated by the ^{13}C -addition experiments (Fig. 4). There are at least two possible explanations for these patterns. First, fishes might prey selectively on zoobenthos that have higher benthic resource use than do chironomids or odonates. This could explain both the high benthic reliance of fishes relative to the zoobenthos that we considered, and the low terrestrial reliance of fishes relative to our previous estimates, assuming that the selected taxa incorporate some detrital benthic production that would have gone unlabeled by the ^{13}C additions. In Crampton Lake, for instance, focal fishes in the present study were adult largemouth bass and bluegill. Much of the growth of largemouth bass in Crampton is attributable to consumption of bluegill and young-of-year yellow perch, which in turn rely heavily on Trichoptera larvae for their growth, which in turn use autochthonous (presumably benthic) resources to a greater extent than either chironomids or odonates (Weidel et al. 2008). Thus previous work provides some support for this first explanation. A second possibility is that our prior estimates of ω_{tot} were too high for fishes, due to overestimating either the per-trophic-level water contribution, ω , or consumer trophic position, τ (Eq. 2). Overestimating ω_{tot} tends to force lower estimates of terrestrial resource use; because the resource proportions must sum to one, this in turn can force higher estimates of benthic resource use.

The prevalence of zoobenthos in the diets of many fishes has usually been interpreted as evidence that fishes rely heavily on benthic primary production. Our results suggest that this may not necessarily be the case. We observed that terrestrial resources were as or more important than benthic resources for chironomids and odonates even in relatively large and clear Crampton Lake, and were far more important than benthic resources in the other three lakes. We had previously estimated high terrestrial reliance for chironomids and odonates based on ^{13}C -addition experiments, but had hypothesized that much of their apparent terrestrial reliance was due to consumption of unlabeled autochthonous production (Carpenter et al. 2005, Solomon et al. 2008, Weidel et al. 2008). That hypothesis is not supported by the results of the present study, which yield allochthony estimates for zoobenthos that are very similar to the ^{13}C -addition estimates (Fig. 4). Some other taxa of zoobenthos probably do rely more heavily on benthic primary production. Nonetheless, chironomids and odonates often comprise a large proportion of benthic secondary production; in Crampton Lake, for instance, they account for $\sim 60\%$ of total zoobenthic production in the littoral zone (Babler et al. 2008). Most

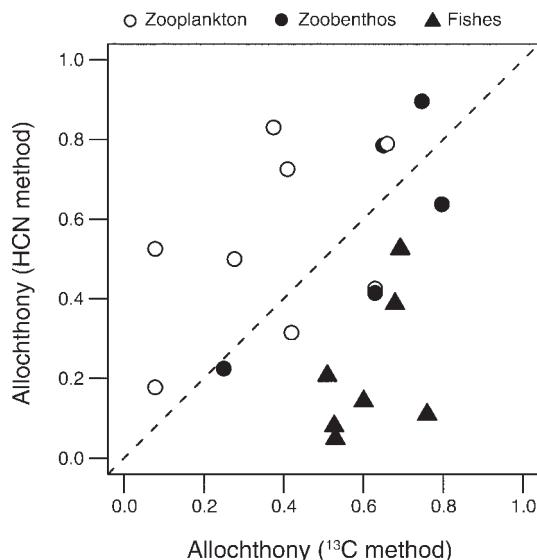


FIG. 4. Comparison of allochthony point estimates from HCN-Bayes approach and previous ^{13}C -addition experiments. Dashed line is the 1:1 line. HCN-Bayes values are the median of the posterior distribution from this study; ^{13}C values are the mean of estimates from between one and three independent models (Carpenter et al. 2005, Pace et al. 2007, Solomon et al. 2008, Weidel et al. 2008).

of the production of much of the littoral zoobenthic assemblage may therefore be supported by terrestrial resources, not by “current” nor “old” benthic primary production, particularly in high-DOC lakes. The prevalence of zoobenthic prey in fish diets may sometimes indicate that fishes are linked to terrestrial or terrestrial and benthic resources, rather than to benthic resources alone.

Quantitative estimates of cross-habitat trophic connections have been scarce in the literature despite the ubiquity and potential importance of such connections in food webs. By using a Bayesian mixing model to integrate measurements of three different stable isotope ratios, we were able to provide the first simultaneous, unique estimates benthic, pelagic, and terrestrial basal resource use. Furthermore, our estimates provide perhaps the most complete accounting to date of the many sources of uncertainty that enter into mixing model calculations. This novel approach is a powerful way to elucidate trophic pathways in aquatic and terrestrial food webs. In aquatic systems in particular, measuring H isotope ratios along with those of C and N may greatly improve our ability to distinguish the source of primary production that support consumers. Such data are essential if we are to understand how, why, and with what consequence basal resource use varies in ecosystems.

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APPENDIX A

Taxa sampled in each lake, and their assumed mean trophic positions (*Ecological Archives* E092-090-A1).

APPENDIX B

Description of laboratory experiments to estimate phyto-water α , the photosynthetic fractionation of H isotopes by algae (*Ecological Archives* E092-090-A2).

APPENDIX C

Hydrogen (δ D), carbon (δ^{13} C), and nitrogen (δ^{15} N) stable isotope ratios of basal resources and water in each lake. (*Ecological Archives* E092-090-A3).

APPENDIX D

Hydrogen (δ D), carbon (δ^{13} C), and nitrogen (δ^{15} N) stable isotope ratios of consumers from each lake (*Ecological Archives* E092-090-A4).

APPENDIX E

Description of the methods and results for simulations that we ran in order to validate the mixing model that we use in this paper, by testing the ability of the model to recover the true parameters from simulated data (*Ecological Archives* E092-090-A5).

APPENDIX F

Prior distributions and posterior distributions of the total water contribution to tissue H and the total trophic fractionation of N between sources and consumers (*Ecological Archives* E092-090-A6).