Resources supporting the food web of a naturally productive lake

Ryan D. Batt,a,* Stephen R. Carpenter,a Jonathan J. Cole,b Michael L. Pace,c Timothy J. Cline,a Robert A. Johnson,c and David A. Seekellc

a Center for Limnology, University of Wisconsin, Madison, Wisconsin
b Cary Institute of Ecosystem Studies, Millbrook, New York
c Department of Environmental Science, University of Virginia, Charlottesville, Virginia

Abstract

A Bayesian mixing model and stable isotopes of carbon, nitrogen, and hydrogen were used to evaluate the extent to which six consumers (three fishes, two zooplankton, and a snail) in a naturally productive lake used terrestrial resources, pelagic, and benthic algae, and macrophytes. Resource use varied with consumer habitat use and feeding ability, but allochthony was consistently low (averaging 15% among consumers). The pelagic invertebrates Skistodiaptomus oregonensis and Chaoborus spp. relied on phytoplankton from the epilimnion (59% and 49%, respectively) and to a lesser extent from the metalimnion (28% and 26%, respectively); terrestrial resources comprised 9% and 18% of the diet of these consumers, respectively. The snail Helisoma trivolvis relied mainly on littoral resources (floating-leaved macrophytes; 68% of diet), but terrestrial resources also constituted a substantial portion of its diet (21%). The fishes integrated among habitats more evenly than the other consumers, but pelagic resources formed the largest portion of their diets (Pimephales promelas = 64%, Lepomis gibbosus = 47%, and Perca flavescens = 47%). L. gibbosus was the fish with the most allochthonous diet (23%). The consumers of this productive lake were not highly dependent on allochthonous materials and tended to rely most heavily on local resources, including macrophytes.

The use of terrestrial carbon by consumers is known to be variable among aquatic systems, and past studies have suggested that the magnitude of autotrophic production can explain a portion of this variability in both lotic (Huryn 1996; Nakano and Murakami 2001) and lentic (Cole et al. 2002; Maguire and Grey 2006) systems. However, few studies have examined allochthony in the food webs of productive lakes (Cole in press). An important early effort by Bunn and Boon (1993) used ambient levels of the stable isotopes of carbon and nitrogen to examine the use of terrestrial carbon, phytoplankton, macrophytes, and epiphytes by consumers in several eutrophic billabongs. Although they found that many of the consumers in these productive systems relied primarily on aquatic resources, isotopic analysis was not able to resolve resource use for all consumers, in part because of isotopically indistinct end members.

Other studies of productive lentic systems have either used isotopic labeling experiments or natural abundances of 2H to circumvent the issue of isotopically indistinct aquatic and terrestrial resources. Cole et al. (2002) and Carpenter et al. (2005) enriched aquatic primary producers with 13C in two experimentally fertilized lakes and found that the zooplankton in these systems were highly allochthonous. Carpenter et al. (2005) also found that benthic consumers and fish relied heavily on algal resources. However, the fishes in this study had slow rates of biomass turnover, and because the nutrient fertilization lasted only one season, their long-term allochthony is unclear (Carpenter et al. 2005). Moreover, Babler et al. (2011) used ambient 2H and found that gizzard shad (a detritivorous fish) exhibited a wide range of allochthony among several eutrophic and hypereutrophic impoundments. Together, these studies suggest that while fishes in eutrophic lakes rely
heavily on phytoplankton, the extent of fish allochthony among eutrophic lakes can be highly variable.

Although the importance of allochthonous resources is measured relative to that of aquatic (autochthonous) resources, macrophytes represent a group of autochthonous resources whose contribution to lake food webs is poorly known. The ability of macrophytes to serve as carbon sources to consumers has long been debated (Lodge 1991; Newman 1991), and in several systems their support of food webs appears negligible (Hecky and Hesselein 1995; France 1996). Other studies have found that macrophytes are likely a substantial source of carbon for consumers (Carpenter and Lodge 1986; Sheldon 1987), suggesting that macrophytes could support consumers in macrophyte-rich lakes. In lakes where macrophytes or other resources contribute substantially to a food web but are overlooked during analysis, consumer use of resources, allochthonous or otherwise, may not be accurately evaluated.

The present study uses ambient $^{13}$C, $^{15}$N, and $^{2}$H isotopes to investigate the extent to which allochthonous organic matter supports the food web of a naturally productive lake. Because of the high abundance of aquatic resources in this productive system, we hypothesized that consumer allochthony would be low. With the expectation that resource use would vary among consumers, we assess the extent to which zooplankton, a benthic consumer, and fishes are supported by terrestrial, epilimnetic and metalimnetic phytoplankton, periphyton, and macrophytes. We hypothesized that both allochthony and the use of specific aquatic resources would vary among consumer taxa according to the degree to which consumers use multiple habitats (e.g., fish potentially feed in the littoral, benthic, and pelagic habitats), the availability of the autochthonous resources in those habitats, and consumer feeding capabilities.

Methods

Study site—This study was conducted on Ward Lake, located at the University of Notre Dame Environmental Research Center near the Wisconsin–Michigan border (46° 15′ N, 89° 31′ W) during the summer of 2010. Ward Lake is a small (0.019 km$^2$), shallow (max. depth = 8 m), dimictic and naturally productive lake. In 2010, the summer mean of surface total nitrogen and phosphorus concentrations were 491 $\mu$g L$^{-1}$ and 22.9 $\mu$g L$^{-1}$, respectively. The lake had relatively high light absorbance (color) at 440 nm with a mean G440 of 2.49 m$^{-1}$ and was alkaline with a mean pH of 8.03. The lake also had high chlorophyll $a$ concentrations both at the surface (summer mean = 8.63 $\mu$g L$^{-1}$) and in the metalimnion (46.6 $\mu$g L$^{-1}$), where chlorophyll was at a maximum vertical concentration for most of the summer.

Ward Lake is located in a fen where speckled alder (Alnus incana subsp. rugosa) and sedges (Carex spp.) dominate the riparian plant community. Macrophytes are abundant, with 18% of lake surface area being covered (6% volume infested; percent cover estimated visually from the surface). The macrophyte community is comprised of a number of species, the most abundant of which include a macroalga (Chara sp.), fragrant water lily (Nymphaea odorata), yellow water lily (Nuphar variegata), watershed (Brasenia schreberi), and small pondweed (Potamogeton pusillus). The calanoid copepod Skistodiaptomus oregonensis is abundant, as is the invertebrate predator Chaoborus spp. The fish community is dominated by a number of small fishes, including the pumpkinseed sunfish (Lepomis gibbosus) and the fathead minnow (Pimephales promelas). The top predator in Ward Lake is the yellow perch (Perca flavescens).

Sample collection and isotope analysis—In order to trace the basal resources supporting the lake food web, autochthonic end members and aquatic consumers were collected between May and August 2010 and subsequently analyzed for their $\delta^{13}$C, $\delta^{15}$N, and $\delta^{2}$H (deuterium) signatures. Between June and August, lake water was collected once a month from three sites near the center of the lake in the epilimnion (0.5 m) and in the metalimnion (at the depth of the chlorophyll maximum, which ranged from 2.5 m to 4 m), and filtered through a 25-mm glass-fiber filter (Whatman GF/F), and the filtrate was stored for $\delta^{2}$H$_{2}$O analysis (sample size of $n = 12$). Particulate organic matter (POM) was collected in June and August from lake water taken from three pelagic epilimnion sites ($n = 6$) and two pelagic metalimnion sites ($n = 4$). In June, the liquid portion of the POM filtrate from the epilimnion and metalimnion was evaporated and the dried residue sampled as dissolved organic matter (DOM; $n = 2$). For POM $\delta^{2}$H, the lake water was screened through a 153-µm mesh and POM collected on a MicronSep Cellulosic filter, back-rinsed with a small amount of water, and dried at 60°C. For $\delta^{13}$C and $\delta^{15}$N, the POM was collected on a previously ashed 25-mm GF/F filter and dried.

Terrestrial, benthic, and littoral end members were sampled from around and within Ward Lake in June and August. The terrestrial end members were represented by A. incana subsp. rugosa ($n = 5$) leaves, Carex spp. ($n = 5$), and DOM ($n = 2$). Lake DOM in this region is predominantly allochthonous (Bade et al. 2007). By sampling key tree species in the area and using a Bayesian mixing model, we determined that the DOM in Ward Lake is also largely terrestrial (see below). Therefore, the terrestrial source in this analysis is comprised of shoreline plants and DOM, with DOM being a composite of shoreline and upgradic end members. The littoral end members were characterized by samples of five of the most prevalent macrophyte species in Ward Lake, collected from different locations in the littoral zone on several dates between June and August. The spatial and temporal extent of sampling was constrained by seasonal changes in the presence and relative abundance of each species. Leaves from N. variegata ($n = 5$), N. odorata ($n = 5$), and B. schreberi ($n = 4$) were collected on each date and cleaned of any debris and epiphytic growth. The leaves and stalk of P. pusillus ($n = 5$) and Chara sp. ($n = 5$) were also collected and rinsed several times to remove debris. Periphyton ($n = 6$) served as the benthic end member and was grown on a series of ceramic tiles suspended throughout the littoral zone of the lake at a depth of 0.5 m and collected after
4 weeks of growth in June and in August. Periphyton samples were grown on tiles to facilitate separation of the algae from their substrate in order to obtain samples dominated by algal material. These samples serve as generic physiological and isotopic surrogates for attached algae that may be available to consumers in Ward Lake.

All consumers were sampled in June and again in August, except *P. promelas*, which was sampled in May and June. Zooplankton samples (*n* = 12) were taken from three pelagic epilimnion and three pelagic metalimnion sites by lowering the inlet hose of an open diaphragm bilge pump to the desired depth (0.5 m in the epilimnion and the depth of lowering the inlet hose of an open diaphragm bilge pump to pelagic epilimnion and three pelagic metalimnion sites by August, except that may be available to consumers in Ward Lake. physiological and isotopic surrogates for attached algae from their substrate in order to obtain samples were grown on tiles to facilitate separation of the algae from their substrate in order to obtain samples sufficient for isotopic analysis. *S. oregonensis* was the most abundant zooplankton species (by biomass), and enough *S. oregonensis* individuals were picked from each sample to reach a pooled biomass sufficient for isotopic analysis. Chaoborus spp. (*n* = 6) were collected at night using an oblique net tow through the water column and pooled to reach sufficient biomass for a sample. The snail, *H. trivolvis* (*n* = 6), was sampled from across the littoral zone, and the foot of several individuals was dissected and pooled as a single sample. Fishes (*n* = 6 for each species) were also sampled from the littoral zone, with adult *P. promelas* and juvenile *L. gibbosus* being caught by minnow trap and *P. flavescentis* by hoop net. The intestinal tracts were excised from each fish, and the remainder of an individual treated as one sample.

All solid samples were dried at 60 °C, ground to a powder, and stored in a desiccator pending analysis. Samples were sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University where they were analyzed with isotope ratio mass spectrometers. Analysis of δ^13^H followed the methods of Doucett et al. (2007), and we report nonexchangeable H in this article.

*Model and food web analysis—*This study employed a Bayesian mixing model adapted from Solomon et al. (2011) to describe lake consumers as a mixture of autotrophic resources originating from terrestrial, pelagic epilimnion, pelagic metalimnion, benthic, and littoral habitats. We characterized consumers and end members by their isotopic signatures (δ^13^C, δ^15^N, and δ^2^H), and calculated the end-member composition of consumers using the following system of equations:

\[
\delta^{13}C_{i[j]} = \sum_{j=1}^{4} (\varphi_{S[j]} \times \delta^{13}C_{S[j]}) + \varphi_{C[i]}
\]  \hspace{1cm} (1)

\[
\delta^{15}N_{i[j]} = \sum_{j=1}^{4} (\varphi_{S[j]} \times \delta^{15}N_{S[j]}) + \delta^{15}N_{\text{tot}} + \varphi_{N[i]}
\]  \hspace{1cm} (2)

\[
\delta^{2}H_{i[j]} = (1 - \varphi_{\text{tot}[C]}) \times \sum_{j=1}^{4} (\varphi_{S[j]} \times \delta^{2}H_{S[j]})
\]  \hspace{1cm} (3)

\[\quad + \varphi_{\text{tot}[C]} \times \delta^{2}H_{\text{W}} + \delta^{2}H_{[i]}
\]  \hspace{1cm} (4)

where δ^13^C_{S[j]}, δ^15^N_{S[j]}, and δ^2^H_{S[j]} are the isotopic ratios of sample *i* of consumer species *C*; δ^13^C_{S[j]}, δ^15^N_{S[j]}, and δ^2^H_{S[j]} are the isotopic ratios of the *j*th source *S*; δ^15^N_{\text{tot}} and δ^2^H_{\text{tot}} are residual errors (normally and independently distributed with mean = 0 and variance = σ^2) for each isotopic and consumer sample C_{j[i]}; and \( \varphi_{S[j]} \) is the fraction \( \varphi \) that source *S[j]* contributes to the diet of consumer *C*. The total trophic fraction of δ^15^N (\( \Delta_{\text{tot}} \)) was calculated as

\[
\Delta_{\text{tot}} = \Delta_{\text{Herb}} + \Delta_{\text{Carn}} \times (\tau - 1)
\]  \hspace{1cm} (5)

where \( \Delta_{\text{Herb}} \) is the δ^15^N fractionation of an herbivorous link (mean \( \mu = 2.52 \% \), standard deviation \( \sigma = 2.5 \% \)), \( \Delta_{\text{Carn}} \) is the δ^15^N fractionation of a carnivorous link (\( \mu = 3.4 \% \), \( \sigma = 0.4 \% \)), and \( \tau \) is the trophic level of the consumer as levels above primary producers when primary production equals trophic level 0 (Vander Zanden and Rasmussen 2001). The total proportion of tissue H derived from water \( (W) \) in a given consumer species \( (\varphi_{\text{tot}[C]}) \) was calculated as

\[
\varphi_{\text{tot}[C]} = 1 - (1 - \omega)^{j}
\]  \hspace{1cm} (6)

where \( \omega \) is the fraction of tissue H derived from water by a particular consumer (as opposed to \( \varphi_{\text{tot}[C] \text{P} \}) which also includes water assimilated by the prey of the consumer). For each consumer, the mean and variance of \( \omega \) were selected from the literature values of the most similar taxa available: *S. oregonensis* = 0.20, 0.0016; *Chaoborus* spp. = 0.14, 0.0036; and *H. trivolvis* = 0, 0; fishes = 0.12, 0.0004 (Estep and Dabrowski 1980; Solomon et al. 2009). Based on presumed feeding habits, consumers were assigned a trophic level with a variance of 0.1 and the following means: *S. oregonensis* and *H. trivolvis* were assigned trophic level 1 (primary consumers); *Chaoborus* spp., *L. gibbosus*, and *P. promelas* trophic level 2; and *P. flavescentis* trophic level 3.

The isotopic signatures of phytoplankton end members were estimated in the framework of a Bayesian mixing model by treating POM as a mixture of phytoplankton and terrestrial matter:

\[
\delta X_{\text{POM}[L]} = \theta_{P[L]} \times \delta X_{P[L]} + \theta_{T[L]} \times \delta X_{T} + \epsilon_{X[L]}
\]  \hspace{1cm} (7)

\[\quad 1 = \theta_{T[L]} + \theta_{P[L]}
\]  \hspace{1cm} (8)

where \( \delta X_{\text{POM}[L]} \) and \( \delta X_{P[L]} \) are the respective isotopic signatures for isolate X of POM and phytoplankton in layer L (either the epilimnion or metalimnion), \( \delta X_{T} \) is the isotopic signature of terrestrial matter, \( \theta_{P[L]} \) and \( \theta_{T[L]} \) are the fractions that phytoplankton and terrestrial matter contribute to POM in layer L, and \( \epsilon_{X[L]} \) is the residual error (independently and normally distributed; \( \mu = 0 \), variance = \( \sigma^{2} \)). The values of \( \delta X_{\text{POM}[L]} \) and \( \delta X_{T} \) are known from sampling, whereas \( \delta X_{P[L]} \), \( \theta_{T[L]} \), and \( \theta_{P[L]} \) were not directly measured. However, phytoplankton δ^2^H was calculated from the known Δ^2^H-O photosynthetic fractionation of phytoplankton and from the δ^2^H-O taken from the appropriate layer (Solomon et al. 2009; Cole et al. 2011) and supplied to the model (Eqs. 7 and 8) as an informative prior, permitting the mixing model to estimate the remaining unknown values.
A challenge common among studies employing mixing models is the excess of end members relative to the number equations. In this study, there are 11 end members, and each model has four equations afforded by the three isotopes. The two models described by Eqs. 7 and 8 (one model for each value of L) can estimate the contribution of two sources to the POM mixture from layer L. By assuming that POM was comprised only of terrestrial matter and phytoplankton, the number of end members was limited to three. Some end members were grouped into aggregate sources in order to restrict the number of sources to two. Such grouping is commonly achieved by grouping the end members according to their proximity in isotopic space or by their ecological similarity (Phillips et al. 2005). While the two approaches are not necessarily mutually exclusive, this study employed the latter, and in the case of Eqs. 7 and 8, the terrestrial end members were grouped into one source, while the phytoplankton end member remained a separate source.

In the case of the consumer mixing models (Eqs. 1–4), the number of end members was first limited by excluding Chara sp. and P. pusillus from analysis because their isotopic signatures suggested that they were unlikely to contribute to the diets of any of the consumers (see Results and Discussion). The remaining end members were grouped into sources representing the terrestrial (A. incana subsp. rugosa, Carex spp., and DOM), pelagic epilimnion (phytoplankton), pelagic metalimnion (phytoplankton), benthic (periphyton), and littoral (floating-leafed macrophytes) habitats. Because there are five sources, two mixing models were run for each consumer: the first model grouped the benthic and littoral habitats into a single benthic-littoral source, and the second grouped the pelagic epilimnion and pelagic metalimnion into a single pelagic source while assessing the benthic and littoral habitats separately. Thus, each model examined the contribution of four sources to the diet of a consumer by grouping two similar habitats into a single source.

The composition of DOM was assessed using a Bayesian mixing model similar to that used for the consumers of Ward Lake. In this model, DOM was the mixture, \( \Delta_p^{\text{tot}} \) and \( \omega^{\text{tot}} \) were set equal to zero, and the sources contributing to DOM were pelagic (pooled phytoplankton), benthic (periphyton), littoral (floating-leafed macrophytes), and terrestrial. For this analysis, the terrestrial end member included A. incana subsp. rugosa and Carex spp. as well as several upgradient tree end members. The leaves of these trees were sampled, and their isotopic signatures have been used in previous studies that investigated allochthony in lakes near Ward Lake (Cole et al. 2011; Solomon et al. 2011). The tree species include black spruce (Picea mariana), balsam fir (Abies balsamea), red maple (Acer rubrum), sugar maple (Acer saccharum), white cedar (Thuja occidentalis), and yellow birch (Betula alleghaniensis). For the purposes of this analysis, the isotopic signatures of these tree species were averaged for each species, and the mean of these averages taken to form a single “Tree” end member. This Tree end member was analyzed as a part of the terrestrial source to the DOM mixture. The mode of the posterior distribution of the terrestrial contribution to DOM was 90%, while the modes of the other sources were near 0%. These results suggest that DOM in Ward Lake is predominantly terrestrial. As a result, we treated DOM as a terrestrial end member for the other analyses in this study.

Grouping end members into a single source for use with the Bayesian mixing models required that a composite mean and variance be calculated for each source. The mean of a source comprised of multiple end members was calculated separately for each isotope as the equally weighted average of the isotopic ratios of the end members making up the source. The variance of each source was calculated by one-way analysis of variance using the ratio of a given isotope as the response variable and end members as categorical treatments. If the treatment was not significant \( (p > 0.1) \), the variance of the source was set equal to the residual mean squared error (MSE). If the treatment was significant \( (p < 0.1) \), the variance of the source was calculated as the sum of the residual MSE and the treatment MSE.

All calculations were performed in R (R Development Core Team 2012) and WinBUGS (Lunn et al. 2000) using the contributed R package R2WinBUGS (Sturtz et al. 2005), programs written by the authors, and programs adapted from Solomon et al. (2011). Unless otherwise stated, all parameters were given uninformative priors, and models were fit by running eight Markov chains for 10,000 iterations with a 5000-iteration burn-in period and thinned so as to sample 1000 iterations of the posterior distribution.

Results

Bayesian mixing models were used to estimate the contribution of phytoplankton and terrestrial end members to POM, the isotopic signature of the phytoplankton end member, and the reliance of consumers on basal resources. All estimates are presented here as the mean of the modeled posterior distribution. POM in the epilimnion of Ward Lake was comprised of 43% terrestrial matter and 57% phytoplankton (Fig. 1A,B). Epilimnetic phytoplankton \( \delta^{13}C \) was estimated as \(-33.5\%_o\), \( \delta^{15}N \) as \(-0.14\%_o\) (Fig. 1C,D), and \( \delta^2H \) as \(-230\%_o\). Metalimnetic POM was comprised of slightly less terrestrial matter (38%) and more phytoplankton (62%) than epilimnetic POM (Fig. 2A,B). Metalimnetic phytoplankton \( \delta^{13}C \), \( \delta^{15}N \), and \( \delta^2H \) signatures were \(-36.2\%_o\), 0.16%o (Fig. 2C,D), and \(-232\%_o\), respectively. Phytoplankton \( \delta^2H \) signatures in both habitats were similar to the priors supplied to the model, indicating that additional data had little effect on these distributions.

The isotopic signatures of end members varied between terrestrial, pelagic, benthic, and littoral habitats as well as between consumers (Fig. 3). Phytoplankton were substantially more depleted in \( \delta^{13}C \) and \( \delta^2H \) than the other end members, while P. pusillus was the most enriched in \( \delta^{13}C \) (\(-14.1\%_o\)), followed by Chara sp. (\( \delta^{13}C = -16.7\%_o \)), and Chara sp. had the highest \( \delta^2H \) signature (\(-119\%_o\); Fig. 3A). The separation of end-member \( \delta^{15}N \) was not as strong as for the other isotopes, but phytoplankton were the most enriched, and P. pusillus was the most depleted (Fig. 3B). Pelagic and terrestrial end members were isotopically similar to other end members within the same habitat, but there was more variability within the benthic-littoral habitat, as Chara
sp. and *P. pusillus* were far more enriched in $\delta^{13}C$ than other macrophytes and periphyton, and periphyton was more depleted in $\delta^{2}H$ than the macrophytes.

The signatures of the consumers also varied but less so than the end members (Fig. 3). Among consumers, *S. oregonensis* was the most depleted in $\delta^{13}C$ (−33.5%) and $\delta^{2}H$ (−188%), and *H. trivolvis* was the most depleted in $\delta^{15}N$ (0.17%). *H. trivolvis* had the most enriched signatures for $\delta^{13}C$ (−25.3%) and $\delta^{2}H$ (−151%), and *L. gibbosus* was the most enriched in $\delta^{15}N$ (5.62%).

Pelagic consumers relied heavily on pelagic resources, with the pelagic resource constituting 82% of the diet for *S. oregonensis* and 71% for *Chaoborus* spp. (Fig. 4). Separating the pelagic resource into its constituent end members revealed that epilimnetic phytoplankton contributed more than metalimnetic phytoplankton to the diets of *S. oregonensis* (59% and 28%, respectively) and *Chaoborus* spp. (49% and 26%, respectively; Fig. 4B,C). Terrestrial and benthic-littoral resources contributed minimally to the diets of these pelagic consumers in both groupings of model sources (Fig. 4).

The benthic consumer *H. trivolvis* was supported almost exclusively by benthic-littoral and terrestrial resources (Fig. 4), although the terrestrial component shifted from 43% when the benthic-littoral end members were pooled to 21% when periphyton was modeled as a source separate from the macrophytes (Fig. 4A,E). As a pooled source, the benthic-littoral end members were estimated to make up 54% of the diet (Fig. 4D). As a separate benthic source, periphyton constituted 8% of the diet (Fig. 4G), whereas the littoral source (floating-leaved macrophytes) was estimated to be 68% of the diet (Fig. 4H). Thus, the estimated diet composition of *H. trivolvis* was sensitive to how end members were grouped into sources, with the exception that the importance of pelagic resources remained low.

Fishes were similar to one another in diet composition, consuming mostly terrestrial and pelagic resources, although benthic-littoral resources constituted a nontrivial component of their diet as well (Fig. 4). *P. promelas* relied more heavily on the pooled pelagic resource (64%) than either *L. gibbosus* or *P. flavescens* (47% for both species; Fig. 4F). The terrestrial source was of greatest importance to *L. gibbosus* (27% for the first grouping, 23% for the second) relative to *P. promelas* (11% and 8%) and *P. flavescens* (12% for both groupings; Fig. 4A,E), and the respective benthic-littoral resource use was 20%, 25%, and 38% for these fishes (Fig. 4D). Metalimnetic resources made up less of fish diets than epilimnetic resources, a difference that was larger for *P. promelas* (epilimnetic = 53%, metalimnetic = 17%) than it was for *P. flavescens* (34% and 17%) or *L. gibbosus* (31% and 17%; Fig 4B,C). Although the estimate of resource use from the terrestrial, pelagic, and benthic-littoral habitats varied between the two groupings, the relative importance of these three habitats to fish diets generally remained the same.

Discussion

Ward Lake presents a large number of potential carbon sources to its consumers. Some of these sources appeared to
be unimportant for all consumers—namely, *Chara* sp. and *P. pusillus*. The position of these end members in isotopic space suggested that they were unlikely to be a major carbon source to consumers. These submersed macrophytes had an average $\delta^{13}C$ of $-15.4\%$, and their lack of contribution to consumer diets is consistent with France (1996), who found that macrophytes with a $\delta^{13}C$ signature near $-16\%$ did not support littoral consumers. Consequently, these end members were omitted from mixing models. Despite this initial screening, the number of end members still necessitated their grouping into aggregate sources, and this grouping influenced model estimates. The sensitivity of mixing model estimates to the grouping of end members is a common problem in food web studies that increases the uncertainty surrounding estimates of resource use (Phillips et al. 2005). However, the qualitative importance of resource use was less sensitive to grouping than were the quantitative estimates, and the role of these resources in supporting lake consumers was elucidated.

The pelagic consumers of Ward Lake relied heavily on pelagic resources, which is consistent with the findings of previous studies conducted in experimentally fertilized lakes (Cole et al. 2002; Carpenter et al. 2005). The contrasting terrestrial-phytoplankton composition of pelagic consumers vs. POM is indicative of selective feeding by pelagic herbivores and is consistent with the ability of *S. oregonensis* to feed selectively among phytoplankton (Kerfoot and Kirk 1991). The ratio of color to chlorophyll in a lake can serve as an index of the relative availability of terrestrial and phytoplankton resources, respectively. Past studies of pelagic consumers that were conducted in lakes over a range of color:chlorophyll found that allochthony tends to increase as color:chlorophyll increases (Carpenter et al. 2005; Pace et al. 2007; Cole et al. 2006).

**Fig. 3.** The location of end members (circles) and consumers (gray squares) in isotopic space for (A) $\delta^{2}H$ and $\delta^{13}C$ and for (B) $\delta^{15}N$ and $\delta^{13}C$; all values are given in parts per mil (‰) relative to a standard. Circumscribed numbers designate particular end members and consumers: 1 = speckled alder (*Alnus incana* subsp. rugosa); 2 = watershield (*Brasenia schreberi*); 3 = sedge (*Carex* spp.); 4 = Chaoborus spp.; 5 = *Chara* sp.; 6 = dissolved organic matter; 7 = epilimnetic phytoplankton; 8 = ramshorn snail (*Helisoma trivolvis*); 9 = pumpkinseed sunfish (*Lepomis gibbosus*); 10 = metalimnetic phytoplankton; 11 = yellow water lily (*Nuphar variegata*); 12 = fragrant water lily (*Nymphaea odorata*); 13 = yellow perch (*Perca flavescens*); 14 = periphyton; 15 = fathead minnow (*Pimephales promelas*); 16 = small pondweed (*Potamogeton pusillus*); 17 = calanoid copepod (*Skistodiaptomus oregonensis*); 18 = nearby tree species. Error bars represent ±1 standard deviation; error bars not extending beyond the symbol perimeter are not shown.
The allochthony of *S. oregonensis* relative to the color:chlorophyll in Ward Lake is consistent with the trend found in these past studies, and the relationship suggests that resource use is influenced by the relative availability of resources (Fig. 5). Nonetheless, there is substantial variability around this relationship, and additional data are needed to assess the pattern among systems.

Fig. 4. Box plots of mixing model posterior distributions showing the fraction that each source contributed to the diet of a given consumer. The bottom of the box is the 25th percentile of the posterior, the thick line inside the box is the median, and the top of the box is the 75th percentile. (A–D) Grouping 1 shows the results of a model that pooled benthic and littoral end members into a single source and separated pelagic end members into two sources. (E–H) Grouping 2 pooled pelagic end members and separated benthic and littoral end members. Each source is comprised of one or more end members: terrestrial = *Alnus incana* subsp. *rugosa*, *Carex* spp., and dissolved organic matter; pelagic epilimnion = epilimnetic phytoplankton; pelagic metalimnion = metalimnetic phytoplankton; benthic = periphyton; littoral = *Brasenia schreberi*, *Nuphar variegata*, and *Nymphaea odorata*. Consumer abbreviations are as follows: Calan = Calanoid (*Skistodiaptomus oregonensis*); Chaob = *Chaoborus* spp.; FHM = fathead minnow (*Pimephales promelas*); PKS = pumpkinseed sunfish (*Lepomis gibbosus*); YWP = yellow perch (*Perca flavescens*).
In two other lakes in this region that are less productive and have less pronounced metalimnetic chlorophyll peaks, Cole et al. (2011) demonstrated that zooplankton were supported by a combination of phytoplanktonic and terrestrial sources, with only minor contributions from the metalimnion. Our results point to the need for studying lakes over a broad range of conditions to determine the importance of terrestrial resources to zooplankton and the conditions that enhance or diminish this support.

The benthic consumer *H. trivolvis* fed primarily on a combination of floating-leafed macrophytes and terrestrial matter but fed minimally on periphyton and phytoplankton. While the absence of phytoplankton from the diet of a snail is not surprising, the minimal use of periphyton is more conspicuous because many snails feed heavily on periphyton. However, *H. trivolvis* is known to preferentially consume terrestrial detritus and living macrophytes over periphyton (Lombardo and Cooke 2002). The species of submerged macrophyte consumed by *H. trivolvis* in Lombardo and Cooke (2002) has a far lower phenolic concentration than the floating-leafed macrophytes of this study (specifically, *N. variegata*), making the floating-leafed macrophytes a lower-quality diet item (Smolders et al. 2000). The presence of secondary chemicals such as phenolics is one reason that the consumption of living macrophytes is thought uncommon (Lodge 1991; Newman 1991). However, when macrophytes decompose, their phenolic concentrations quickly decrease, and the alkaline water of Ward Lake accelerates this process (Kok et al. 1992). Furthermore, relatively modest variability surrounded the estimated contribution of littoral (epiphyte-free macrophytes) and terrestrial resources to the diet of the benthic consumer *H. trivolvis*. Therefore, it is likely that *H. trivolvis* was supported mainly by terrestrial and macrophyte (living and/or detrital) resources rather than by periphyton.

The fishes in this study relied on a greater variety of resources than the benthic or pelagic consumers. While all fishes relied on pelagic production for a large portion of their diet, resource use was more evenly distributed among habitats for *L. gibbosus* and *P. flavescens* than it was for other consumers. Although adult *L. gibbosus* are known to feed on mollusks, the individuals sampled in this study were unlikely to feed on mollusks because they were juveniles (mean length = 59.5 mm), permitting the difference in resource use between *L. gibbosus* and *H. trivolvis*. Fishes relied on resources from multiple habitats, a pattern consistent with that observed in other lakes (Vander Zanden and Vadeboncoeur 2002). Resource use by fishes also followed the more generalized expectation that consumers occupying higher trophic levels and with greater mobility will integrate among a greater number of habitats (Lindeman 1942; McCann and Rooney 2009). In this sense, resource use by *P. flavescens* represents the best single-consumer proxy for resource use at the ecosystem level, indicating that resources from all habitats contributed to the lake food web.

The consumers of Ward Lake had consistently low allochthony, but their use of specific autochthonous resources was more variable. Furthermore, just as the food web of Ward Lake tended to use local (autochthonous) resources, consumer resource use tended to reflect their...
habitat use. For example, consumer use of pelagic resources was inversely proportional to littoral resource use, with zooplankton relying heavily on epilimnetic and metalimnetic phytoplankton, the small consuming primarily macrophytes, and the mobile fishes exhibiting intermediate use of pelagic and littoral resources. Although frequently abundant in lakes and important resources in this study, metalimnetic phytoplankton and macrophytes are not commonly considered in the context of the allochthony of lake food webs. We suggest that these resources contributed to the food web because they were abundant and present in habitats used by consumers and that the physiology of the consumers interacted with the characteristics of the resources (e.g., stoichiometry) and the ecosystem (e.g., alkalinity and temperature) in a way that made these resources available and of high quality. Future studies may gain further insight into patterns in resource use by accounting for additional resource characteristics, such as the taxonomic composition of periphyton and phytoplankton. Our findings emphasize the variability in resource use among the consumers of a lake and the importance of considering end members from multiple habitats when assessing which resources support lake food webs.

Resource use is variable among consumers and food webs. However, our findings contribute to a growing body of literature that highlights resource quantity and quality as an important driver of this variability (Brett et al. 2009; Francis et al. 2011; Marcarelli et al. 2011). This literature also suggests that lake trophic status can serve as a measure of the quantity of high-quality autochthonous resources in lakes (Carpenter et al. 2005; Cole et al. 2006; Pace et al. 2007), consistent with the high autochthony of ward Lake consumers. Our study demonstrates that resources such as metalimnetic phytoplankton and macrophytes can be important to lake food webs and should be included in future studies of resource use and allochthony.

Furthermore, the use of deuterium in this study and others (Babler et al. 2011; Cole et al. 2011; Solomon et al. 2011) suggests that considering more end members in allochthony studies is more tractable now than in the past. In conclusion, we encourage future research to build on an emerging understanding of which factors shape patterns in resource use across organisms and ecosystems.

Acknowledgments

We thank Chase Brosseau, Jim Coloso, Emily Kara, Jason Kurtzweil, Laura Smith, and Lee Zinn for assistance in the field and laboratory; Jereme Gaeta for dialogue on study design; Chris Solomon for analytical advice, Gary Belovsky, Michael Cramer, and the University of Notre Dame Environmental Research Center staff for their assistance; and two anonymous referees for their constructive feedback. We are grateful for financial support from the National Science Foundation (Division of Environmental Biology [DEB]-0917696, DEB-0917719, DEB-0917858) and a fellowship from the Wisconsin Alumni Research Foundation.

References


Associate editor: Alexander D. Huryn

Received: 01 March 2012
Accepted: 04 June 2012
Amended: 08 June 2012