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ECOSPHERE

Altered energy flow in the food web of an experimentally darkened lake

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Citation: Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, R. A. Johnson, J. T. Kurtzweil, and G. M. Wilkinson. 2015. Altered energy flow in the food web of an experimentally darkened lake. Ecosphere 6(3):33. http://dx.doi.org/10.1890/ES14-00241.1

Abstract. Theory suggests that alternative resources may begin to support a food web when highly used resources become less available relative to alternatives. To test the potential for alternative resources to support consumers, we experimentally darkened a lake whose consumers had relied heavily on algal resources (phytoplankton and benthic algae). We estimated the support consumers received from resources before and after darkening using a Bayesian mixing model and stables isotopes of carbon, nitrogen, and hydrogen. Between a prior year and the darkened year, phytoplankton biomass diminished by 60%, and surface dissolved oxygen saturation, pCO_2, and net ecosystem production indicated a shift from autotrophy to heterotrophy. Although a specialist copepod maintained a high reliance on phytoplankton after darkening, a generalist zooplankton predator (*Chaoborus* spp.) derived more support from terrestrial sources. Fishes received less support from benthic algae after darkening, and received greater support from floating-leafed macrophytes or terrestrial resources. Phytoplankton support of fishes increased or was similar between years, resulting in a convergence of the proportion of support that fishes and zooplankton received from phytoplankton. The changes in algal support of fishes suggest that fishes had an increased connection to the pelagic habitat and decreased connection to the benthic habitat after darkening. After darkening, most consumers received more support from resource alternatives like terrestrial material (snail, *Chaoborus* spp., some fishes) or from floating-leafed macrophytes (some fishes). These shifts indicate that resource support of consumers is dynamic, and highlight the potential for increased support of consumer biomass by alternative resources.

Key words: allochthony; Aquashade; automated sensor; dissolved organic carbon; fish; metabolism; snail; stable isotope; whole-ecosystem experiment; zooplankton.

Received 25 July 2014; revised 20 October 2014; accepted 18 December 2014; **published** 23 March 2015. Corresponding Editor: W. Cross.

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INTRODUCTION

Ecosystems across the globe are experiencing rapid shifts in environmental conditions that will alter the availability of the basal sources of carbon that support their food webs. Carbon

fixation by primary producers is the ultimate source of the organic carbon in a food web, and most primary production requires light. In freshwater ecosystems, light availability is closely linked to the concentration of dissolved organic matter (DOM). Lake color and DOM vary around a baseline condition (Pace and Cole 2002), but sustained trends of increasing DOM and color (which reduces clarity) have recently been documented in some freshwaters at northern latitudes (Monteith et al. 2007, Köhler et al. 2013). Furthermore, DOM in many northern lakes is predominantly terrestrial in origin and is strongly correlated with water color (Pace and Cole 2002, Karlsson et al. 2003, Wilkinson et al. 2013*b*), and an influx of DOM could increase the availability of terrestrial carbon resources while decreasing aquatic primary production via light limitation (Jones et al. 2012). Thus changes in DOM can differentially affect the availability of basal resources to lake food webs.

Darkening water could greatly impact animals that rely on resources that are susceptible to light limitation. Fish derive their resources from a variety of habitats, but in many cases benthic algae (periphyton) contribute a larger portion of primary production supporting fish biomass than would be suggested by its relative availability (Vadeboncoeur et al. 2002, Vander Zanden and Vadeboncoeur 2002, Vander Zanden et al. 2011). Periphyton production tends to be higher in clear lakes than in dark lakes (Vadeboncoeur et al. 2008, Ask et al. 2009), and is positively associated with fish biomass per unit area (Karlsson et al. 2009, Finstad et al. 2014). Furthermore, periphyton support of fish is proportionally lower in darker lakes, where a larger portion of fish biomass is derived from alternative resources (Karlsson et al. 2009). If this pattern in lakes spanning a gradient of water clarity were echoed in a lake darkening over time, we would expect alternative resources to be increasingly important to the food web as the lake became darker.

Fish can rely on a variety of other basal resources to complement the decreased support by periphyton. Compared to periphyton, phytoplankton and floating-leafed macrophytes are less prone to light limitation. Evidence for increased fish reliance on phytoplankton in darker lakes stems from isotope studies indicating that fish become more isotopically similar to zooplankton (Karlsson et al. 2009), the primary grazers of phytoplankton. However, terrestrially derived (allochthonous) carbon can support a substantial portion of zooplankton biomass, particularly in dark lakes (Wilkinson et al. 2013*a*). As a result, fish can derive terrestrial resources directly through terrestrial prey, or indirectly through aquatic prey that are comprised of allochthonous material (Weidel et al. 2008, Tanentzap et al. 2014). Floating-leafed macrophytes can also contribute substantially to the biomasses of snails and fish (Batt et al. 2012, Mendonça et al. 2012, Kovalenko and Dibble 2013), although little is known about the controls on the strength of this connection. Therefore, terrestrial material, macrophytes, or phytoplankton could serve as alternative basal resources for fish and other consumers in dark lakes with reduced periphyton abundance.

Theory suggests that allochthonous subsidies and alternative resources can confer stability on food webs (Huxel et al. 2002, Leroux and Loreau 2008, Rooney and McCann 2012, Tunney et al. 2012). However, few field studies have tracked changes in the relative contributions of carbon sources to consumer biomass as primary producers decline. In one experiment, the contribution of terrestrial resources to fish and invertebrates decreased when a lake was eutrophied (Carpenter et al. 2005), and subsequently increased upon cessation of the experimental fertilization (Cole et al. 2011, Solomon et al. 2011). In another system, a zebra mussel invasion led to decreased phytoplankton abundance, and was coincident with enriched zooplankton $\delta^{13}C$, possibly indicating an increase in zooplankton allochthony (Maguire and Grey 2006). By contrast, when phytoplankton production in San Francisco Bay decreased, zooplankton biomass decreased due to dietary recalcitrance of the terrestrial resource in that system (Sobczak et al. 2002). These studies suggest that if local resources are depleted and labile alternatives exist, carbon resources that previously did not contribute substantially to consumer biomass could begin to increase their support of the food web. These shifts in support are reflected in the contributions of sources to consumer biomass, but not necessarily the amount of consumer biomass or production, which might decrease

(Jones et al. 2012, Kelly et al. 2014).

We tested for shifting support of consumer biomass with a whole-ecosystem experiment, whereby we greatly reduced light availability by adding a dye to Ward Lake. The goal was to test the extent to which reducing light transmission in a naturally productive lake would alter ecosystem metabolism and change the basal resources supporting the food web. Prior to manipulation, the animal consumers in Ward Lake were supported by a variety of basal resources, including algae and floating-leafed macrophytes, and to a lesser extent, terrestrial subsidies (Batt et al. 2012). We expected the relative availability of algal resources in a dyed (henceforth darkened) lake to be lower than in the natural state, and that consumer (zooplankton, snail, and fish) support by terrestrial and floating-leafed macrophyte resources would increase relative to algal resources.

Methods

Study site

We estimated resource support of consumers in Ward and Paul lakes in 2010 and 2012. In 2012 we added a blue dye, Aquashade (Applied Biochemists, Germantown, WI), to Ward Lake while using Paul Lake as an unmanipulated reference. Ward and Paul are small (1.9 ha and 1.6 ha, respectively), shallow (maximum depth = 8 m and 12 m, respectively) lakes at the University of Notre Dame Environmental Research Center in northern Wisconsin, USA (46°15′ N, 89°31′ W). These lakes are located less than a kilometer apart and experience similar environmental conditions. Ward Lake is productive relative to nearby lakes (summer surface means in 2010; chlorophyll-*a* [Chl] = 8.6 μg L^{-1}, total phosphorus [TP] = 22.9 μg L^{-1}, total nitrogen [TN] = 491 μg L^{-1}), has high alkalinity compared to other lakes in the region (pH = 8.03, dissolved inorganic carbon [DIC] = 1739 μmol L^{-1}), and is colored with a water absorbance measured at 440 nm (color) of 2.49 m^{-1} (Batt et al. 2012). Paul Lake is less productive (Chl = 6.3 μg L^{-1}, TP = 3.8 μg L^{-1}, TN = 224 μg L^{-1}), has low alkalinity (pH = 6.6, DIC = 113 μmol L^{-1}), and color of 1.34 m^{-1}.

Aquashade manipulation

Aquashade is a commercially available dye marketed for controlling the growth of aquatic plants. It contains the dyes acid blue 9 and acid yellow 23, and is blue in appearance. Aquashade is used to control phytoplankton growth in water bodies through the reduction of photosynthetically available light. Our target concentration for Aquashade in Ward Lake was 1.5 ppm (mL m^{-3}), which was within the range recommended by the manufacturer (0.5–2.0 ppm). We used spectrophotometry to monitor Aquashade concentrations throughout the season because we anticipated that photochemical degradation and vertical mixing of the water column and surface flow into or out of the lake could dilute concentrations after the initial addition. To reach our target concentration of 1.5 ppm at the start of the season, we added 90.8 L of Aquashade to Ward Lake over three days starting 23 April 2012 (day of year 114) to initiate the experiment. Further additions on days 145 (3.8 L), 163 (15.1 L), and 195 (22.7 L) were made to maintain the depth of the photic zone (depth of 1% surface light) and similar Aquashade concentration. In total we added 132.5 L of dye to Ward Lake.

Physical, chemical, and biological sampling

Physical, chemical, and biological characteristics of the lakes were measured weekly in both years (Appendix). These included vertical profiles of light, oxygen and temperature (from the surface into the hypolimnion), profiles of chlorophyll-*a* concentration throughout the photic zone (100% to 1% surface light), and surface measurements of pCO_2. *Chaoborus* spp. and crustacean zooplankton community compositions were estimated from weekly vertical net tows, and their biomasses (dry mass) were estimated by applying standard mass–length regressions to lengths measured under a dissecting microscope (Appendix).

The ecosystem metabolism of each lake was estimated from data gathered by automated sensors. We made high-frequency surface measurements of dissolved oxygen (DO) and vertically distributed measurements of temperature, as well as measurements of the meteorological parameters of air temperature, relative humidity, photosynthetically active radiation, and wind speed (Appendix). It is common in limnological research to estimate metabolism from models involving diel changes in dissolved oxygen and

atmospheric gas exchange (Cole et al. 2000, Stæhr et al. 2010, Hoellein et al. 2013). We used the R package rLakeAnalyzer to calculate high-frequency estimates of mixing depth, which in turn were supplied to models calculating atmospheric gas exchange (Read et al. 2011, 2012). To aid in mitigating potential bias introduced by noisy oxygen time series, we estimated net ecosystem production (NEP), gross primary production (GPP), and respiration (R) using a metabolism model that fits parameters in the framework of a Kalman filter (Batt and Carpenter 2012; Appendix). We use the convention of assigning GPP positive values representing oxygen production and R negative values representing oxygen consumption. NEP can be positive or negative depending on the sign of the sum of GPP and R.

Isotope methods

Resource support of Ward Lake consumers was assessed using stable isotope values of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and hydrogen ($\delta^{2}H$) and a Bayesian mixing model. Our methods follow those of Batt et al. (2012), who measured resource support of consumers in Ward Lake in 2010. Isotope samples were collected in 2010 and 2012, and our 2010 data supplement those data analyzed by Batt et al. (2012). We use the term "resource" to refer to a group of one or more primary producers (individually termed "end members"). Samples were collected monthly between May and August in 2010, and between June and August in 2012. Samples for both consumers and end members were horizontally distributed among 5 sites in 2010, and 3 sites in 2012.

Water samples taken from the epilimnion and metalimnion were analyzed for the $\delta^{2}H$ of H_2O, and $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ of particulate and dissolved organic matter (POM and DOM). In accordance with the methods of previous studies, POM samples formed the basis for calculating the isotope value of the phytoplankton end member (Solomon et al. 2009, Cole et al. 2011, Batt et al. 2012). Terrestrial end members included *Larix laricina*, *Carex* sp., and *Alnus incana* subsp. *rugosa*, and an aggregate "tree" end member taken from previously measured values of *Picea mariana*, *Abies balsamea*, *Acer rubrum*, *Acer saccharum*, *Thuja occidentalis*, and *Betula alleghaniensis* sampled in the watershed surrounding Ward Lake (Cole et al. 2011, Solomon et al. 2011). Macrophytes included floating-leafed morphologies (*Nymphaea odorata*, *Nuphar variegata*, *Brasenia schreberi*, *Potamogeton nodosus*), and submersed morphologies (*Chara* sp., *Najas flexilis*, *Potamogeton pusillus*, *Potamogeton amplifolius*). Benthic algae samples were scraped from ceramic tiles, submerged at a depth of 0.5 m in 2010 and 0.25 m in 2012. Benthic algae samples were taken from tiles so as to avoid contamination by natural substrates. The zooplankton primary consumer *Skistodiaptomus oregonensis* was sampled by bilge pump from the epilimnion and metalimnion, and the zooplankton predator *Chaoborus* spp. was sampled at night using oblique net tows. The snail *Helisoma trivolvis* was collected from the perimeter of the lake, where hoop nets and minnow traps were set to collect the fishes *Pimephales promelas* (fathead minnow), *Phoxinus* spp. (dace), *Umbra limi* (central mud minnow), and young-of-the-year *Ameiurus melas* (black bullhead). The foot of each snail and the non-gut biomass of fishes were analyzed for isotopes, and samples were whole-body for other consumers. All solid samples were dried at 60°C and sent to Colorado Plateau Stable Isotope Laboratory for analysis. A benchtop equilibration procedure was used to correct for the exchange of H atoms between a set of standards including ground algal material and ambient water vapor (Doucett et al. 2007, Appendix).

Isotope mixing model

Resource contributions to consumer biomass were estimated by analyzing consumer isotope values as a mixture of primary producer isotope values. We used a Bayesian mixing model similar to that used by Solomon et al. (2011) and identical to the model presented by Batt et al. (2012). The general form of the mixing model is $\mathbf{Y}_i = \mathbf{X\Phi} + \mathbf{D} + \mathbf{E}_i$. $\mathbf{Y}$ is a 3×1 vector containing the isotope values of sample i of the mixture. $\mathbf{X}$ is a $3 \times S$ matrix of resource isotope values, $\mathbf{\Phi}$ is an $S \times 1$ vector of the proportional contribution of each resource to the organic matter of the consumer, and S is the number of organic matter sources. $\mathbf{D}$ contains discrimination factors, and $\mathbf{E}$ is a vector of error terms. Furthermore, the sum of $\mathbf{\Phi}$ was constrained to equal 1 (resources in $\mathbf{X}$ fully comprise the mixture, $\mathbf{Y}$). Written in long

form, the model is

$$Y = \begin{bmatrix} \delta^{13}C \\ \delta^{15}N \\ \delta^{2}H \end{bmatrix} = \begin{bmatrix} \delta^{13}C_1 & \dots & \delta^{13}C_S \\ \delta^{15}N_1 & \dots & \delta^{15}N_S \\ \delta^{2}H_1(1-\omega) & \dots & \delta^{2}H_S(1-\omega) \end{bmatrix} \begin{bmatrix} \varphi_1 \\ \vdots \\ \varphi_S \end{bmatrix} + \begin{bmatrix} 0 \\ \Delta \\ (\delta^{2}H_2O)\omega \end{bmatrix} + \begin{bmatrix} \varepsilon_C \\ \varepsilon_N \\ \varepsilon_H \end{bmatrix}. \quad (1)$$

The parameters estimated from data are the φ's for each resource (S), and the variances of the ε's, which are normally distributed with means of 0 and variances σ^2_ε. All $\mathbf{\Phi}$ were given minimally informative priors drawn from a uniform distribution in a centered log-ratio space (Solomon et al. 2011). The minimally informative priors have a concave shape: the density is highest near zero, very low in the middle, and is of medium height near one. Posteriors with shape similar to the prior should be recognized as having not been heavily influenced by the data. The total proportion of consumer ^2H derived from ^2H_2O is $\omega = 1 - (1 - \omega_0)^\tau$. The fractional contribution of dietary water (ω_0) is amplified with increasing trophic level, τ, because predators acquire ^2H_2O directly through their environment as well as indirectly through their prey, which incorporate dietary water themselves. The trophic enrichment of $\delta^{15}N$ is $\Delta = \Delta_{herb} + (\tau - 1)\Delta_{carn}$. We used literature values for $\delta^{15}N$ enrichment across herbivorous (Δ_{herb}; mean (μ) = 2.52‰, standard deviation (σ) = 2.5‰) and carnivorous (Δ_{carn}; μ = 3.4‰, σ = 0.4‰) linkages, and for taxon-specific values for dietary water contribution to bulk tissue samples (respective means and standard deviations: *S. oregonensis* = 0.20, 0.04; *Chaoborus* spp. = 0.14, 0.06; *H. trivolvis* = 0.21, 0.03; fishes = 0.12, 0.02) (Estep and Dabrowski 1980, Vander Zanden and Rasmussen 2001, Solomon et al. 2009, Bortolotti et al. 2013). *S. oregonensis* and *H. trivolvis* (the primary consumers) were assigned trophic level 1 (levels above primary producers), and all other consumers were assigned trophic level 2. All trophic levels were assigned a variance of 0.1. Our data do not permit the estimation of isotope turnover rates in consumer tissue, and we assume that the isotopic composition of bulk consumer tissue reflects recently incorporated organic matter. This assumption is met for the consumers born post-manipulation (*S. oregonensis*, *Chaoborus* spp., and young-of-the-year *A. melas*), but is violated if a consumer sampled in 2012 bore the isotopic signature of resources incorporated in 2010. However, not accounting for isotopic disequilibrium in older consumers would result in a conservative bias that underestimates between-year changes in the isotope values of consumers ($\mathbf{Y}$) and in the contributions of resources to those consumers ($\mathbf{\Phi}$).

In the isotope mixing model, the number of resources (S) was limited to four (one plus the number of isotopes measured) because $\mathbf{\Phi}$ was unknown. To reduce resource number, end members can be pooled into ecologically similar groups (Phillips et al. 2005). We pooled end members into the following resources: terrestrial resources, phytoplankton (both depths), floating-leafed macrophytes, submersed macrophytes, all macrophytes, and benthic algae. The isotope values of each resource were taken from the average of its constituent end members. The variances of the resource signatures were determined by performing a one-way analysis of variance for each isotope-resource combination: the variance was set equal to the total mean squared error if the taxa had significantly different signatures ($p < 0.1$), or to the residual mean squared error if they were not significantly different. For each consumer we used known feeding habits and deviance information criterion to select two, three, or four sources from the seven possible groups of pooled end members. Our estimates of resource contribution ($\mathbf{\Phi}$) were robust to resource selection (Appendix).

Resource contributions to POM and DOM were estimated using Eq. 1. In the case of POM, two $\mathbf{\Phi}$ values (terrestrial and phytoplankton) were estimated along with the isotope values of the phytoplankton resource. Phytoplankton δ^2H was given an informative prior based on δ^2H_2O, and $\delta^{13}C$ and $\delta^{15}N$ of phytoplankton were given minimally informative priors (Solomon et al. 2011, Batt et al. 2012). The estimated isotope values for phytoplankton were subsequently used for the phytoplankton end member when estimating the composition of consumers and DOM. The composition of DOM was estimated after algebraically correcting for the influence of

Aquashade on the isotope values of DOM by using measured concentrations, isotope values, and the C:N:H stoichiometry of the dye and of DOM (Appendix). The number of resources (S) was four for DOM, with the model containing terrestrial, macrophyte (both morphologies), phytoplankton, and periphyton resources. For both POM and DOM, trophic fractionation (Δ) and dietary water use (ω) were 0.

The compositions of mixtures (consumers, DOM, POM) from 2010 and 2012 were estimated from independent model runs. Benthic algal and phytoplankton samples used as resources in $\mathbf{X}$ were restricted to the sampling year of the mixture, whereas this distinction was not made for macrophyte or terrestrial resources. The same restriction was applied to POM and δ^2H_2O when modeling phytoplankton isotope values. Analyses were performed in R and JAGS, using the packages R2WinBUGS, rjags, R2jags, and programs written by the authors (Plummer 2003, Sturtz et al. 2005, R Core Team 2014). Each JAGS model run generated 6000 iterations of 8 Markov chains, thinned to 1000 samples of the posterior.

Results

Light attenuation

Aquashade was first added to Ward Lake in the spring of 2012, after which it remained near a concentration of 1.5 ppm. After Aquashade was added, the photic depth of Ward Lake was 2 m—half of the 4 m average in 2010 (Fig. 1). These changes in photic depth equate to a light attenuation coefficient (K_d) of 1.15 m^{-1} in 2010, and 2.30 m^{-1} in 2012. Similarly, the depth of the mixed layer was reduced from 1.8 m in 2010 to 0.9 m in 2012. Meanwhile, Paul Lake, a nearby unmanipulated lake, experienced a slight increase in mixed layer depth (2.4 m in 2010 to 2.8 m in 2012) and in photic zone depth (5.5 m in 2010 to 6.5 m in 2012; $K_d = 0.84$ m^{-1} in 2010, $K_d = 0.71$ m^{-1} in 2012).

Chlorophyll, dissolved gases, metabolism, zooplankton biomass

The areal density of chlorophyll in the photic zone is a measure of the biomass of phytoplankton that could be photosynthesizing. In Paul Lake, areal chlorophyll was similar between years (seasonal average; 54.6 mg m^{-2} in 2010 and 53.6 mg m^{-2} in 2012), but Ward Lake areal chlorophyll decreased from 86.9 mg m^{-2} in 2010 to 34.9 mg m^{-2} in 2012 (Fig. 2). Our model estimates metabolism as epilimnetic volumetric rates (Appendix), but for ease of comparison to areal chlorophyll, we present the product of volumetric metabolism (mmol O_2 m^{-3} d^{-1}) and photic depth (m) as an index of areal metabolism in the photic zone (mmol O_2 m^{-2} d^{-1}). There was a small increase in Paul Lake GPP between years (from 76.8 to 104.9 mmol O_2 m^{-2} d^{-1}). In Ward Lake, volumetric GPP increased between 2010 and 2012 (Appendix), but photic zone GPP was lower in the darkened year (from 136.2 and to 87.3 mmol O_2 m^{-2} d^{-1}). In Paul Lake R was slightly larger in magnitude in the second year:

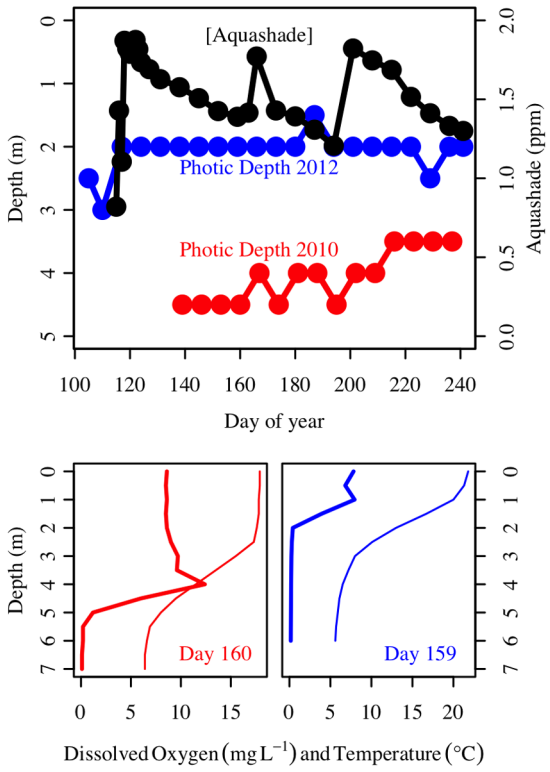

Fig. 1. The top panel shows time series from Ward Lake between April and August: Aquashade concentration in 2012 (black), photic depth in 2010 (red), and photic depth in 2012 (blue). Photic depth was defined as the depth where photosynthetically active radiation (PAR) was equal to 1% of PAR at 0 m. The bottom panels show temperature (thin lines) and dissolved oxygen (thick lines) profiles on a single representative day in each year (day 160 in 2010 and day 159 in 2012).

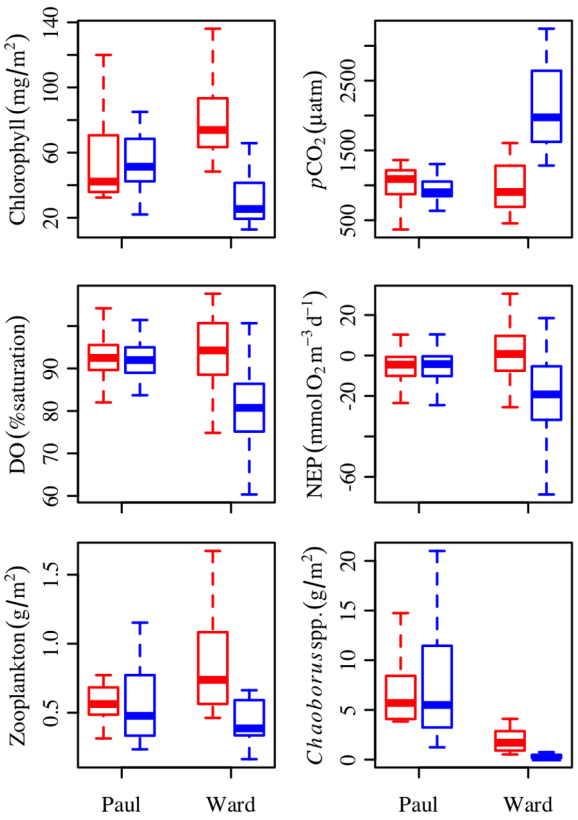

Fig. 2. Box and whisker plots of areal chlorophyll (measured weekly), pCO_2 (measured weekly), dissolved oxygen (DO, percent saturation; daily average of 5-min observations), net ecosystem production (NEP; daily), crustacean zooplankton biomass (weekly), and *Chaoborus* spp. biomass (weekly) in the manipulated lake (Ward Lake) and a nearby reference lake (Paul Lake) in 2010 (red) and in 2012 (blue). Each box bounds the 25–75th percentile of data, with each whisker extending to the most extreme datum that is no more than 1.5 × (interquartile range) beyond the box. The thick line in the center of a box is the median. All data were included in statistical calculations, but to preserve the scaling of axes, outliers are not displayed.

−106.0 mmol O_2 m^{-2} d^{-1} in 2010 and −137.4 mmol O_2 m^{-2} d^{-1} in 2012. In Ward Lake there was a strong increase in the magnitude of volumetric *R* (Appendix), but there was little change in photic zone *R* between years (from −127.5 to −131.7 mmol O_2 m^{-2} d^{-1}). Paul Lake was slightly heterotrophic in both years (NEP = −29.3 O_2 m^{-2} d^{-1} in 2010, −32.5 mmol O_2 m^{-2} d^{-1} in 2012). However, Ward Lake NEP changed drastically, shifting from slightly autotrophic in 2010 (NEP = 8.7 mmol O_2 m^{-2} d^{-1}) to heterotrophic in 2012 (NEP = −43.9 mmol O_2 m^{-2} d^{-1}). Volumetric NEP also shifted toward heterotrophy in Ward Lake (Appendix, Fig. 2). This shift in metabolic balance was also reflected in high-frequency measurements of dissolved oxygen saturation (DO; saturation relative to 1 atm pressure) and in weekly measurements of the partial pressure of CO_2 (pCO_2). Paul Lake DO and pCO_2 were similar between years (DO = 99% and pCO_2 = 987 µatm in 2010, 98% and 941 µatm in 2012), but Ward Lake DO decreased from 101% to 87% and pCO_2 increased from 976 µatm to 2134 µatm (Fig. 2). Summer means of weekly measurements of metalimnetic DO increased slightly in Paul Lake (90% in 2010, 99% in 2012), but decreased drastically in Ward Lake (86% in 2010, 30% in 2012).

In Ward Lake the zooplankton and *Chaoborus* spp. biomass declined between years (zooplankton = 0.9 g/m^2 in 2010 and 0.5 g/m^2 in 2012; *Chaoborus* spp. = 2.1 g/m^2 in 2010 and 0.4 g/m^2 in 2012) (Fig. 2). In Paul Lake, the biomasses of zooplankton (annual mean was 0.6 g/m^2 in 2010 and 0.7 g/m^2 in 2012) and *Chaoborus* spp. (6.8 g/m^2 in 2010 and 9.0 g/m^2 in 2012) were similar between years. The large change in zooplankton biomass in Ward Lake was not attributable to adult *S. oregonensis*, but to decreases in the adults of other copepod taxa and to a large decrease in the biomass of copepod larvae (Appendix).

Isotope values and mixing model results

We summarize mixing model estimates of the composition (**Φ**) of consumers and other pools of organic matter in Ward Lake as means and standard deviations of the posterior distributions of **Φ**, reported as percentages. We refer to mean changes between years that were less than 10% as not being ecologically important.

The sizes of the particulate and dissolved organic matter (POM and DOM) pools were similar between years (Appendix), but their compositions changed. POM in 2010 was 67% algal and 33% terrestrial (both standard deviations [σ] = 8%), and in 2012 algal material comprised 47% and terrestrial material 53% (both σ = 16%) of the POM pool, indicating a decrease in the relative availability of phytoplankton. The DOM analysis included terrestrial material,

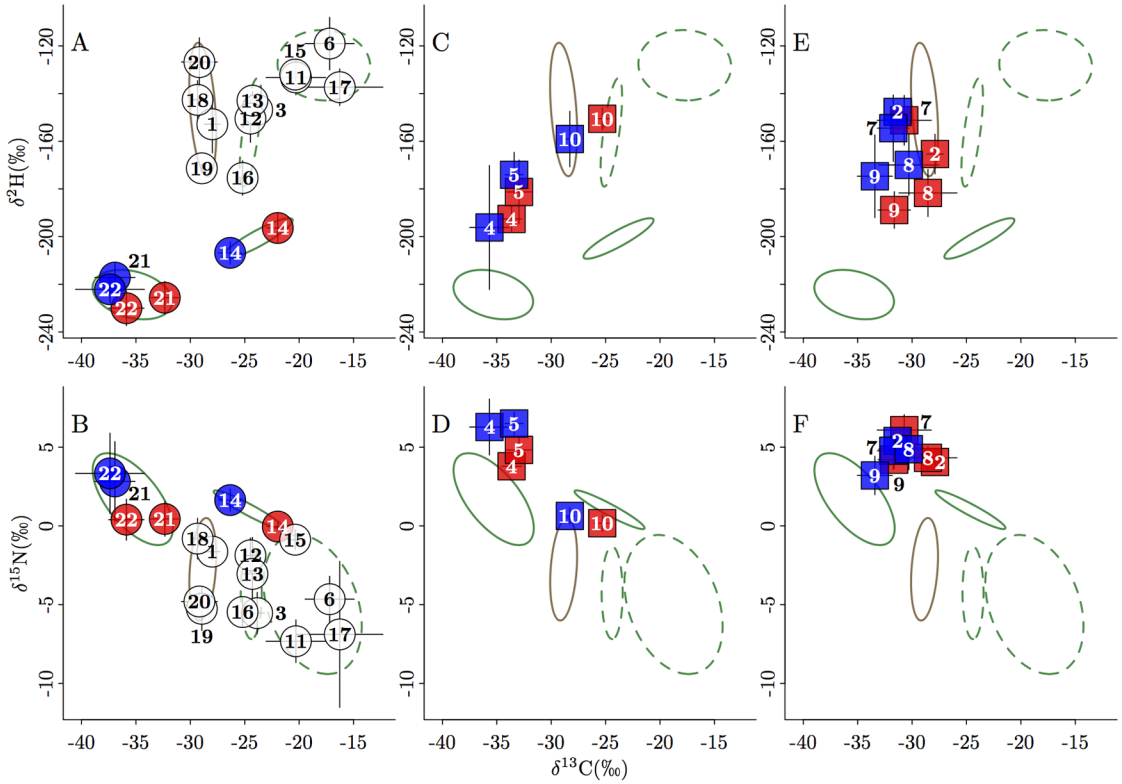

Fig. 3. Biplots of mean end member (circles) and consumer (squares) $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ isotope values and their standard deviations (black error bars). (A, B) End members; brown ellipse indicates terrestrial end members (1 = *A. incana* subsp. *rugosa*, 18 = Carex sp., 19 = *L. laricina*, 20 = nearby tree species), dashed green ellipses indicate macrophytes of floating (3 = *B. schreberi*, 12 = *N. variegata*, 13 = *N. odorata*, 16 = *P. pusillus*) and submersed morphologies (6 = *Chara* sp., 11 = *N. flexilis*, 15 = *P. amplifolius*, 17 = *P. pusillus*), solid green ellipses indicate algal end members (14 = periphyton, 21 = epilimnetic phytoplankton, 22 = metalimnetic phytoplankton). (C, D) Invertebrate consumers; 4 = *S. oregonensis*, 5 = *Chaoborus* spp., 10 = *H. trivolvis*. (E, F) Fish consumers; 2 = *A. melas*, 7 = *U. limi*, 8 = *Phoxinus* spp., 9 = *P. promelas*. Red symbols correspond to 2010 values, blue to 2012, and white to end member values that were pooled between years.

macrophytes (pooled resource of both morphologies), phytoplankton, and periphyton as potential resources. In the baseline year terrestrial material comprised 89% ($\sigma = 8\%$) of the DOM pool, but in 2012 macrophytes (pooled resource of floating-leafed and submersed morphologies) were 56% ($\sigma = 21\%$) of the DOM pool and terrestrial material was only 20% ($\sigma = 23\%$). In 2012, phytoplankton comprised 8% ($\sigma = 7\%$) and periphyton 15% ($\sigma = 9\%$) of DOM.

Groups of end members (phytoplankton, periphyton, terrestrial, floating macrophytes, submersed macrophytes) occupied distinct regions of the three-dimensional (axes of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) isotope space (Fig. 3A, B). Phytoplankton end members had the lowest $\delta^{2}H$ and $\delta^{13}C$ values, and also the highest $\delta^{15}N$ values. By contrast, submersed macrophytes had the highest $\delta^{2}H$ and $\delta^{13}C$ isotope values, and also the lowest $\delta^{15}N$ values. The $\delta^{13}C$ values of the algal end members (periphyton, epilimnetic and metalimnetic phytoplankton) decreased between years; similarly, consumer $\delta^{13}C$ tended to either decrease or be similar between years (Fig. 3C–F). In 2010, $\delta^{2}H$ values for *S. oregonensis* and *Chaoborus* spp. were similar, but in 2012 the $\delta^{2}H$ values for these consumers became more distinct, with mean $\delta^{2}H$ increasing (moving away from phytoplankton in $\delta^{2}H$ space) in *Chaoborus* spp. but decreasing (moving toward phytoplankton)

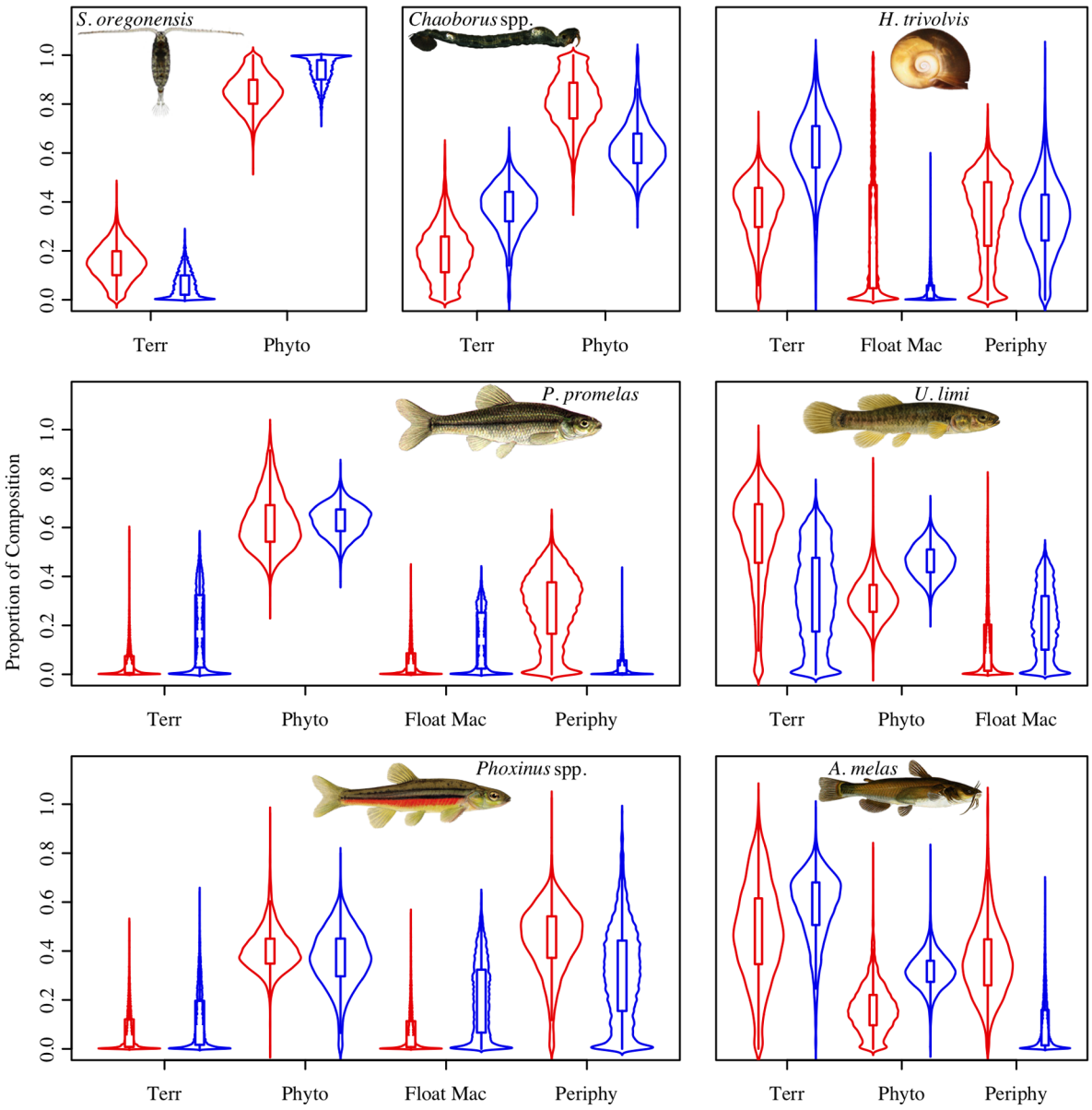

Fig. 4. Violin plots of the proportional contributions of resources to consumers (posterior of **Φ**) in Ward Lake in 2010 (red) and 2012 (blue). Each violin is a mirror image of a nonparametric density estimate of a posterior probability distribution (violins are wider at higher densities), and in the center of the violins are boxplots. Consumers include *S. oregonensis*, *Chaoborus* spp., *H. trivolvis*, *P. promelas*, *U. limi*, *Phoxinus* spp., and young-of-year *A. melas*. Terr = terrestrial; Phyto = phytoplankton; Float Mac = floating-leafed macrophytes; Periphy = periphyton.

in *S. oregonensis* (Fig. 3C). In 2010 the isotope values of the snail *H. trivolvis* were similar to that of the floating leafed macrophytes, but in 2012 its isotope values became similar to the terrestrial end members (Fig. 3C, D). Little change was seen in the isotope values of *U. limi*, but for the three other fishes the largest changes in isotope values involved increases in δ^2H and decreases $\delta^{13}C$ (Fig. 3E, F). These changes in fish isotope values amount to fish shifting away from the region of isotopic space occupied by periphyton.

Periphyton support of fish was lower in 2012

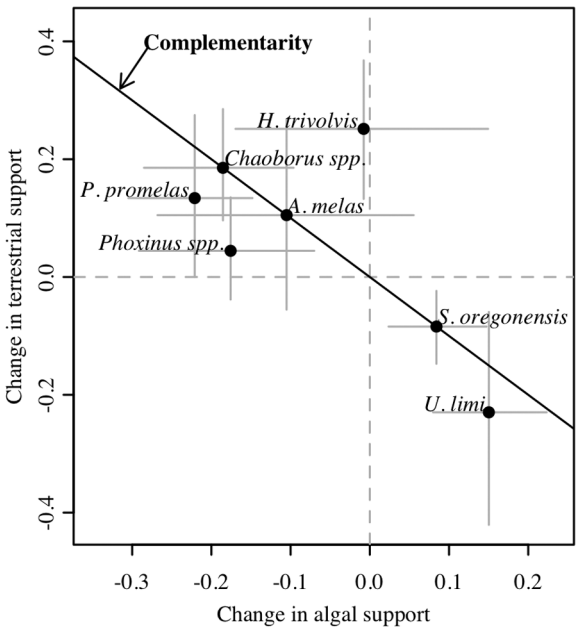

Fig. 5. Changes in resource support between 2010 and 2012. The solid black line indicates where the changes in support by algal and terrestrial resources were perfectly complementary, which was necessitated by model structure for the three consumers that were not supported by macrophytes. Consumers below this line received more support from macrophytes in the second year, and the consumer above the line received less support from macrophytes. Positive values indicate an increase in the median support by the resource in 2012 relative to 2010. Error bars are 25th and 75th percentiles of the differences. The change in the algal resource is the sum of the changes in the phytoplankton and periphyton resources.

than in 2010, but phytoplankton support remained similar between years (Fig. 4). Periphyton was a potential resource for three of the four fishes (not a source for *U. limi*), and support by this resource declined for each—for *P. promelas* periphyton support shifted from a mean posterior of 27% in 2010 to 4% in 2012 (standard deviations (σ) of 14% and 6% in 2010 and 2012, respectively), from 45% to 31% (σ = 14% and 19%) for *Phoxinus spp*., and from 36% to 10% (σ = 15% and 11%) for *A. melas*. However, phytoplankton contribution to fish did not decline, and for two of the fishes, phytoplankton support increased (*U. limi* = 31% in 2010 and 46% in 2012 [σ = 8% and 6%]; *A. melas* = 16% in 2010 and 32% in 2012 [σ = 10% and 7%]). Support by terrestrial and macrophyte resources tended to be similar or increase between years, with the exception that *U. limi* allochthony was lower in 2012 (from 55% down to 32% [σ = 20% and 19%]).

Between the reference and darkened years, allochthony increased for two of the three invertebrate consumers. Allochthony increased for both the snail *H. trivolvis* (from 37% in 2010 to 62% in 2012 [σ = 12% and 14%]) and the predatory zooplankton *Chaoborus* spp. (from 19% to 37% [σ = 11% and 10%]). Increased *Chaoborus* spp. allochthony was complemented by decreased phytoplankton support (from 81% to 63% [σ = 11% and 10%]), whereas the snail received similar support from periphyton (34% in 2010 and in 2012 [σ = 17% and 15%]) but derived less support from floating-leafed macrophytes (from 29% to 4% [σ = 27% and 6%]). In 2010, the zooplankton primary consumer *S. oregeonensis* was 15% (σ = 7%) allochthonous, similar to *Chaoborus* spp. allochthony in the same year. However, unlike *H. trivolvis* and *Chaoborus* spp., *S. oregonensis* did not become more allochthonous in 2012, maintaining a high reliance on phytoplankton in both years (85% in 2010, 93% in 2012 [σ = 7% and 5%]).

Between years, relative resource support (**Φ**) changed for most consumers. The resources (*S*) present in mixing models varied among consumers, and the terrestrial resource was the only resource present in all consumer models. Of the seven consumers, five of them were more allochthonous in 2012 than in 2010, although three of these had the middle 50% of the difference in posteriors overlap with the zero line (Fig. 5). At least one algal resource (phytoplankton or periphyton) was present in each model, and the sum of the change in algal support was negative for four of the consumers (one of these overlapped with the zero line). Note that for the three consumers whose models only contained algal and terrestrial resources, model structure necessitated that any change in support by these resources be complementary. However, the changes in algal and terrestrial support were not perfectly complementary for the four consumers supported by macrophyte resources, and *H. trivolvis* was the only consumer to receive less macrophyte support in 2012 relative to 2010.

In 2010, all fishes derived less support from the

phytoplankton resource than did *Chaoborus* spp. (Fig. 4). Phytoplankton support of *Chaoborus* spp. decreased between years but remained higher than that of the fishes, for which phytoplankton support either increased or was similar between years (Figs. 4, 5). Therefore, compared to 2010, in 2012 the phytoplankton contribution to all fishes was more similar to its contribution to *Chaoborus* spp. (Fig. 6), showing that after darkening the fishes were more closely associated with pelagic energy pathways.

Discussion

Darkening Ward Lake reduced net ecosystem production (NEP), altering the relative availability of resources. NEP provides a broad perspective on food web energy flow because it integrates across processes and habitats. Consistent with patterns of increasingly negative NEP across lakes with increasing DOC concentration, Ward Lake shifted toward heterotrophy after the manipulation. Loss of benthic algal production likely contributed to this shift because in darker lakes benthic algae are consistently less productive per unit area and/or because light sufficient for algal production is constricted to a smaller area of the lake (Vadeboncoeur et al. 2001, Ask et al. 2012). Phytoplankton have a more complicated response to increased DOC and water color (Hanson et al. 2003, Ask et al. 2012), but in Ward Lake, both chlorophyll measurements and the abundance and composition of POM indicate that phytoplankton were less abundant in 2012. Particularly, metalimnetic phytoplankton tend to make a greater contribution to zooplankton biomass in clearer lakes (Francis et al. 2011), and the drastically decreased DO in the metalimnion of Ward Lake after it was darkened suggests that this algal resource was less available in 2012. Therefore, decreased production by both phytoplankton and periphyton likely contributed to heterotrophic NEP in 2012, which suggests that they were less available to consumers in 2012 relative to 2010.

Ward Lake metabolism shifted from autotrophic in the reference year to heterotrophic after the lake was darkened. Decreased (more negative) NEP can result from decreased gross primary production (GPP), increased respiration (R), or both. Both volumetric and photic zone

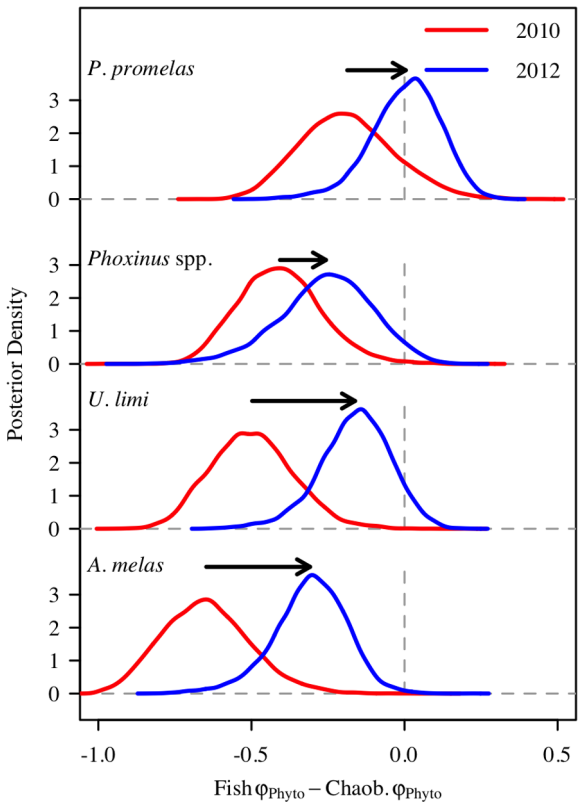

Fig. 6. Phytoplankton support (φ_{Phyto}) of the four fishes, expressed as a deviation from φ_{Phyto} of *Chaoborus* spp. The vertical axis is the density of the difference between the posterior distributions of φ_{Phyto} for the two consumers. Red lines are the posterior densities in 2010, and blue lines are from 2012. Negative values on the horizontal axis (to the left of the vertical dashed line) indicate that phytoplankton comprised a greater proportion of *Chaoborus* spp. than of the fish. The horizontal arrows show the direction and magnitude of the change in Fish φ_{Phyto} − Chaob φ_{Phyto} between years, with the arrow starting at the mean difference in 2010, and ending at the mean difference in 2012. In all cases, phytoplankton support of fish was more similar to support of *Chaoborus* spp. in 2012 than in 2010.

NEP estimates indicate that Ward Lake shifted to heterotrophy in the darkened year. NEP is directly estimated by diel O_2 methods, and NEP is the component of metabolism that is most directly relevant to the inference of heterotrophy. Estimates of GPP and R require additional assumptions. Nonetheless it is instructive to

consider the changes in GPP and *R* after darkening.

Volumetric estimates of metabolism indicate that the shift to heterotrophy was largely attributable to elevated *R*, whereas the index of photic zone metabolism indicates that decreased GPP was responsible for the shift to heterotrophy. Volumetrically, both GPP and *R* increased in Ward Lake (37% increase in GPP, 116% increase in *R*) and in Paul Lake (25% increase in GPP, 20% increase in *R*). The increase in Ward Lake GPP (volumetric) was unexpected. Increased nutrient concentrations can stimulate both GPP and *R*, and relative to 2010, 2012 concentrations of total nitrogen (TN) and total phosphorus (TP) were much higher in both Paul Lake (TN = 223.6 μg/L in 2010, 427.8 μg/L in 2012; TP = 3.9 μg/L in 2010, 14 μg/L in 2012) and in Ward Lake (TN = 491 μg/L in 2010, 726 μg/L in 2012; TP = 23 μg/L in 2010, 58 μg/L in 2012). Thus, it may be that increased nutrients amplified GPP and *R* in both lakes, but that in Ward Lake increased nutrients affected GPP less than *R* because of the massive reduction in light availability.

In contrast to the volumetric estimates, Ward Lake photic zone *R* was similar between years and photic zone GPP decreased by 37% after darkening. Decreased GPP is an intuitive ecosystem response to a massive reduction in light availability, but our estimates of photic zone metabolism assume constant rates of metabolism over depth. In some cases GPP declines and *R* increases over depth (Coloso et al. 2008), which suggests that our index of photic zone metabolism may overestimate GPP and underestimate *R*. However, it is unclear how or if our estimates of photic zone metabolism are biased, because in other cases the metalimnetic portion of the photic zone contains pockets of elevated GPP and/or *R* (Sadro et al. 2011, Staehr et al. 2012, Obrador et al. 2014). For example, in 2010 (but not 2012) Ward Lake had a large peak in chlorophyll and oxygen concentrations near the bottom of the photic zone, which was also the site of intense primary production that contributed to consumer biomass (Batt and Carpenter 2012, Batt et al. 2012). Therefore, photic zone estimates of metabolism introduce uncertainty associated with extrapolation, but unlike volumetric estimates, photic zone estimates may be more holistic in their representation of changes in resource production. Nonetheless, estimates of NEP clearly show that Ward Lake NEP was more negative in the darkened year.

Estimates of decreased floating-leafed macrophyte support and unchanged periphyton support of *H. trivolvis* were surprising because decreased water clarity was expected to impact periphyton more than floating-leafed macrophytes. Posterior estimates of macrophyte support of *H. trivolvis* are diffuse and highly skewed right both years, indicating the relatively high degree of uncertainty in this result. Furthermore, our estimates of macrophyte and periphyton support of *H. trivolvis* differ substantially from those reported by Batt et al. (2012) because here we used a higher value for the ω parameter (the proportion of consumer ^{2}H derived from water). Although we highlight the large degree of uncertainty for floating-leafed macrophyte support of *H. trivolvis*, visual surface surveys indicated that the cover of floating-leafed macrophytes in the littoral zone (within 30 m of shore) of Ward Lake decreased from 27% in 2011 to 16% in 2012. The decline in macrophyte abundance may have also contributed changes in DOM composition: between 2010 and 2012, macrophytes (combined morphologies) supplanted allochthonous material as the dominant constituent of DOM. Thus, possible changes in *H. trivolvis* resource support may be tied to macrophyte organic matter having shifted from living to dissolved forms.

In contrast with previous studies, our hypotheses, and other Ward Lake consumers, the calanoid copepod *S. oregonensis* did not become more allochthonous after light transmission declined and phytoplankton became less abundant. Several studies have documented that zooplankton can use terrestrial resources, especially in high DOC lakes with allochthonous particulate matter, including many lakes that are in the same watershed as Ward Lake (Carpenter et al. 2005, Marcarelli et al. 2011, Wilkinson et al. 2013*a*, Taipale et al. 2014). Rather than increasing in allochthony, *S. oregonensis* maintained high use of phytoplankton, even as the biomasses of other zooplankton and copepod larvae declined considerably. However, because *Chaoborus* spp. is a predator that integrates across the zooplankton community, its moderate increase in allochthony suggests that *Chaoborus* spp. preyed on other taxa

of zooplankton in Ward Lake that were generally more allochthonous than *S. oregonensis* or that increased their allochthony in 2012. Indeed, *S. oregonensis* is a highly selective feeder (Kerfoot and Kirk 1991), and calanoids tend to be less allochthonous than either cyclopoids or cladocerans (Karlsson et al. 2012, Cole 2013, Wilkinson et al. 2013*a*, Berggren et al. 2014). Therefore, our results and those of previous studies suggest that zooplankton may become increasingly allochthonous as phytoplankton become less available, but taxon-specific feeding habits may impose a limit on the extent of change.

The decreased contribution of periphyton to fishes in a darkened Ward Lake is consistent with patterns seen for fish and periphyton across lakes with increasing DOC concentration and decreased benthic primary productivity (Karlsson et al. 2009, Vander Zanden et al. 2011, Ask et al. 2012). Fish in darker lakes tend to be more isotopically similar to zooplankton, but we did not observe consistent complementarity between periphyton and phytoplankton support of fish. However, phytoplankton support of all fishes (including *U. limi*, which did not use periphyton) did become more similar to that of *Chaoborus* spp. In other words, the fishes were more connected to pelagic prey without necessarily relying more on pelagic primary producers, a pattern that was possible because some zooplankton relied less on phytoplankton in 2012. These changes in algal support suggest that, after darkening, fishes had a decreased connection to the benthic habitat and increased connection to the pelagic habitat. Floating-leafed macrophytes were another alternative resource that increased its support of some fishes after Ward Lake was darkened, although for the fishes *P. promelas* and *Phoxinus* spp. the macrophyte posterior was diffuse and highly uncertain. The direction of this response contrasts with the direction of change in macrophyte use observed for *H. trivolvis*, and highlights the diversity of consumer responses to the manipulation and the variety of pathways connecting consumers to basal resources. In fact, the high mobility and trophic position of fishes is thought to facilitate both breadth and flexibility in resource use, which in turn stabilize food web dynamics (Rooney and McCann 2012). Overall, while fish support by periphyton was partially exchanged for support by phytoplankton, their repertoire of compensatory resources was diverse and integrated several habitats and the terrestrial ecosystem.

In this study and in others, posterior estimates of consumer resource support are associated with a high degree of uncertainty. As with any result, posterior estimates of resource support should be interpreted with caution and in the context of ecological mechanisms. However, there may be methodological advances that could aid in reducing this uncertainty. First, increasing the number of food web tracers can reduce potential bias in posteriors (Brett 2014); to this end, increasing the number of stable isotopes measured or using fatty acids as tracers (e.g., Taipale et al. 2014) could be useful in producing more reliable estimates of energy flow in lake ecosystems. Second, as the number of studies estimating resource support of aquatic food webs increases, it may be possible to define properly informed prior expectations about the results of the analysis. These prior expectations could be formally incorporated into estimates of resource support using Bayesian statistics. It is already common for studies of resource support to employ Bayesian statistics, but these studies often use minimally informative priors. Properly incorporating prior information into studies of resource support will require continued development of analytical methods, as well as better characterization of the temporal dynamics of resource support and its drivers.

Future environmental changes may alter the base of food webs, removing preferred resources and forcing consumers to eat less and/or turn to alternative resources. When Ward Lake was darkened, algal resources became less abundant and a higher proportion of consumer biomass was supported by resource alternatives like terrestrial material. This dynamic highlights that the strength of the energetic connection between consumer and resource can change with resource availability and the environment. As climate, watershed vegetation, and lake conditions change, resources available to aquatic consumers are likely to change. Thus ongoing effort is needed to understand other drivers of temporal change in resource support, and how resource support changes over longer time scales. Our study challenges a perspective that holds resources as unimportant if they currently support

a small fraction of consumer biomass, and embraces a view of such resources serving food webs as insurance that may mitigate the adverse effects of constricted energy pathways.

Acknowledgments

R. D. Batt, S. R. Carpenter, J. J. Cole, and M. L. Pace designed the study; R. D. Batt, R. A. Johnson, J. T. Kurtzweil, G. M. Wilkinson collected the data; R. D. Batt analyzed the data; and all authors wrote the manuscript. We thank Emily Read, Jordan Read, Tim Cline, Carol Yang, Cal Buelo, Megan Tomamichel, and Max Kleinhans for help in the field and lab, Luke Winslow, Jordan Read, Tyler Tunney, Tony Ives and Jake Vander Zanden for helpful discussion and comments, two anonymous reviewers for constructive comments, the Notre Dame Environmental Research Center staff, and the National Science Foundation (Division of Environmental Biology [DEB]-0917858, DEB-1144683) and a Bunde Graduate Research Grant from the UW-Madison Department of Zoology for funding.

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Supplemental Material

Appendix

Limnological measurements

During the ice-free periods of 2010 and 2012 we took weekly measurements of a suite of limnological variables in Paul and Ward Lakes using standard methods, which are described briefly below. Detailed descriptions of these methods can be found in previous publications (Carpenter and Kitchell 1993, Carpenter et al. 2001) and in an

online repository (https://cascade.limnology.wisc.edu/public/public_files/methods/CascadeManual1998.pdf).

Depth profiles of temperature and dissolved oxygen (DO) were taken from the surface (0 m) to the hypolimnion at 0.5 m intervals. DO and temperature were measured using a YSI Professional Plus handheld meter. Profiles of photosynthetically active radiation (PAR, 400–700 nm) were taken at 0.25 m intervals from 0 m to 1 m, and at 0.5 m intervals from 1 m until 1% of surface light was reached. PAR was measured with a LI-COR LI-193 underwater sensor for the depth profiles, and with a LI-COR LI-190 Quantum Sensor for surface readings. Depths corresponding to 100%, 50%, 25%, 10%, 5%, and 1% of surface light were obtained in the field by linearly interpolating the ratio of underwater PAR to surface PAR between consecutive depths.

Measurements of chlorophyll (Chl), and dissolved inorganic carbon (DIC) were taken from water samples at depths corresponding to the aforementioned light gradient (100% to 1% surface light), while partial pressure of CO_2 (pCO_2), total nitrogen (TN), total phosphorus (TP) were measured from a pooled sample of three epilimnetic depths. Chl was measured from water samples filtered through a Whatman GF/F glass fiber filter, frozen, extracted in methanol, and analyzed using a fluorometer (Sequoia Turner Model 450). TN and TP were measured from unfiltered water samples (100 mL) that had been preserved using 1 mL of 1N Optima sulfuric acid. DIC was measured on a Shimadzu gas chromatograph (GC-8A) by the syringe gas stripping method of Stainton (1973). *p*CO2 was measured using the large volume equilibration method of Cole et al. (1994).

Weekly daytime vertical net tows were made with a conical net to measure the abundance and composition of the zooplankton biomass and community composition in each lake, and the same was done for *Chaoborus* spp., a zooplankton predator, using nighttime tows. Crustacean zooplankton were sampled using a net with a mesh size of 80 μm, and for *Chaoborus* spp. we sampled with a mesh of 153 μm. Both sets of samples were taken at a central location that was near the deepest point of the lake. In Paul Lake tows were taken from a depth of 8 m, and in Ward Lake from a depth of 6 m. Crustaceans and *Chaoborus* spp. were preserved in a 1% Lugol's solution, subsampled and enumerated. The length of 15 individuals were measured and lengths converted to biomasses following the equations of Downing and Rigler (1984).

Sensor and metabolism methods

We used automated in situ sensors (sondes) to measure dissolved oxygen (DO), which was used to estimate metabolism, which includes gross primary production (GPP), respiration (*R*), and net ecosystem production (NEP). The sonde models were YSI 600XLM (rapid pulse DO probe model 6562) for Ward in 2010, and YSI 6600 V2 (optical DO probe model 6150) for Ward in 2012 and Paul in 2010 and 2012. We used thermistor chains to measure temperature in both lakes. In Paul Lake we used NexSens T-node sensors with thermistors placed every 0.5 m from 0.5 m to 4 m, and an additional thermistor at 5.0 m. In Ward Lake we used HOBO thermistors, which in 2010 were placed every 0.5 m between 0.5 m and 5.5 m, and in 2012 were placed every 0.25 m between 0 m and 2 m, and at 2.5 m and 3.0 m. Before June of 2010 high frequency thermal profiles were not available for Ward Lake, and estimates of mixed layer depth were instead made from manual weekly profiles. We measured photosynthetically active radiation (PAR; W m^{-2} s^{-1}) and wind speed (m s^{-1}) at 2 m above the lake surface with an anemometer on a buoy on nearby Peter Lake (adjacent to Paul Lake, similar in size to both lakes in this study). Hourly measurements of air temperature, relative humidity, barometric pressure were made by a nearby weather station on the UNDEC property, and linearly interpolated to a 5-minute frequency. This weather station also provided supplemental measurements of PAR and wind speed which were used to fill in missing portions of those time series collected by the Peter Lake buoy. Automated sensors for DO and temperature in Ward in 2010 sampled at a 4-minute frequency while sensors in other lakes or years sampled at a 5-minute frequency.

Metabolism was calculated from automated high-frequency measurements of DO using a model that was fit in the framework of a Kalman filter (Batt and Carpenter 2012). This metabolism model uses maximum likelihood to fit parameters, including coefficients that relate PAR to GPP and temperature to *R*, as well as variances of

Table A1. Volumetric and photic zone metabolism estimates for Ward Lake and Paul Lake in 2010 and in 2012.

		Volumetric (mmol O_2 m^{-3} d^{-1})			Photic zone (mmol O_2 m^{-2} d^{-1})		
Lake	Year	GPP	R	NEP	GPP	R	NEP
Ward	2010	32.6	−30.8	1.7	137.8	−129.1	8.7
Ward	2012	44.8	−66.6	−21.8	87.3	−130.6	−43.3
Paul	2010	13.2	−18.1	−5.0	76.7	−105.9	−29.3
Paul	2012	16.4	−21.7	−5.3	103.8	−136.9	−33.1

process and observation error. Process errors propagate throughout a time series but observation errors do not, and can be thought of as noise added to the oxygen data after they are generated. A model that makes this distinction between error types must explicitly consider both the true state of the system (which is unknown) and the observed state of the system (which we measure). Given such a model, the likelihood of the data given a set of parameter estimates can be computed using a Kalman filter (Kalman 1960, Harvey 1989).

The negative log likelihood function involves two key sets of equations that describe the process and observation components of the model:

$$y_t = \alpha_t + \eta_t;\ \eta\ \sim N(0, H) \tag{A.1}$$

$$\alpha_{t|t-1} = \alpha_{t-1} + \iota \times I_{t-1} + \rho \times \log_e T_{t-1} + F_{t-1} + \varepsilon_t;\ \varepsilon\ \sim N(0, Q) \tag{A.2}$$

where y is the observed concentration of oxygen (mmol O_2 m^{-3}), α is the true value of oxygen, η are observation errors, H is the variance of η, ι and ρ are parameters to be estimated, T is water temperature (°C), and F is atmospheric gas exchange (m per 5-minutes) (Read et al. 2012), ε are process errors, and Q is the variance of ε. Written in a form that expands F and solves for gas exchange in continuous time, the process equation becomes:

$$\alpha_{t|t-1} = a_t \times k_{t-1}^{-1} \times z_{t-1} + -\exp\{-k_{t-1} \times z_{t-1}^{-1}\} \times a_t \times k_{t-1}^{-1} \times z_{t-1} + \alpha_{t-1} \tag{A.3}$$

$$a_t = \iota \times I_{t-1} + \rho \times \log_e T_{t-1} + k_{t-1} \times z_{t-1}^{-1} \times O_{s,t-1} \tag{A.4}$$

where z is the depth of the mixed layer in meters (Read et al. 2011), and O_s is the concentration of oxygen at saturation given water temperature and atmospheric pressure. In addition to making predictions for the system state at each time step, the Kalman filter also makes predictions of the error covariance matrix, P:

$$P_{t|t-1} = P_{t-1} \times (k_{t-1} \times z_{t-1}^{-1})^2. \tag{A.5}$$

The subscript $t|t-1$ indicates that the estimates of α and P are only based on observations of y up to y_{t-1}, and have not been updated to reflect information gained by y_t. To incorporate the new information gained from the current observation of dissolved oxygen, y_t, the Kalman filter updates the estimates of the predicted values by accounting for the current observation and the relative uncertainty surrounding the predictions and the observations—this process is akin to a weighted average of the prediction and the observation using precision (inverse of variance) as the weights. The updating equations are as follows:

$$E_t = P_{t|t-1} + H \tag{A.6}$$

$$\alpha_t = \alpha_{t|t-1} + P_{t|t-1} \times (y_t - \alpha_{t|t-1}) \times E_t^{-1} \tag{A.7}$$

$$P_t = P_{t|t-1} - E_t * (P_{t|t-1})^{-2} \tag{A.8}$$

In this implementation of the Kalman filter, we initiate $P_{t=1}$ with Q, and $\alpha_{t=1}$ with $y_{t=1}$. The parameters to be estimated are Q, H, ι, and ρ. The negative log likelihood, L, of the data given the current parameter estimates is

$$L = \sum_{t=1}^{N} 0.5 \times \log_e(2 \times \pi) + 0.5 \times \log_e(E_t) + 0.5 \times (y_t - \alpha_{t|t-1})^2 \times E_t. \tag{A.9}$$

Having fit ι and ρ, they are multiplied by PAR and $\log_e$(temperature) at each time step, and summed up over the course of the day, yielding estimates of GPP and R in mmol O_2 m^{-3} d^{-1},

respectively (Table A1). NEP was calculated as the sum of GPP and R on a given day. These parameters were fit separately for each day of data, and by convention ι is positive and ρ is negative; when these parameters take on the opposite sign, the estimates no longer are ecologically meaningful. On days where either ι or ρ had the wrong sign, that day's estimates of GPP, R, and NEP were discarded from further analysis and did not contribute to seasonal estimates of metabolism.

Light climate

In 2012 (the darkened year) in Ward Lake both the depth of 1% light and the depth of the photic zone decreased to about half of their 2010 values. Because light decays exponentially over depth, if these two values (1% depth and mixing depth) change together, phytoplankton shouldn't experience a brighter light climate. For example, if z_{mix} (depth of mixed layer) = z_{photic} (depth of 1% light) = 4 m, the coefficient of diffuse light attenuation (K_d) between 0 m and 4 m is: $K_d = -\log_e(1\%)/4 = \sim 1.15$ m^{-1}, and the mean light incident upon particles distributed between 0 m (z_1) and 4 m (z_2) when the surface irradiance (I_0) is 1500 W m^{-2} s^{-1} is equal to $(-I_0/K_d) \times (\exp(-K_d \times z_2) - \exp(-K_d \times z_1)) \times (z_2 - z_1)^{-1} = (-1500/1.15) \times (\exp(-1.15 \times 4) - \exp(-1.15 \times 0))/4 = 322.4637$ W m^{-2} s^{-1}, and between 0 m and 2 m when the depth of 1% light is 2 m the average light is 322.4637 W m^{-2} s^{-1}, exactly the same.

However, the importance of both water clarity and mixing depth to average light climate experienced by phytoplankton measured by the sonde raises the interesting question of how changes in mixed layer depth shifted with changes in K_d in Ward Lake between 2010 and 2012. Thus, we calculated average irradiance incident upon particles distributed through the depth layer occupied the sonde (sensor at 0.5 m) in both years, and at a 5 minute frequency using sensor data described in the Appendix, as well as K_d values derived from weekly light profiles linearly interpolated to a 5 minute sampling frequency (estimated as the slope in the regression $\log_e(I_z/I_0) = -K_d \times z$). When sonde depth was less than z_{mix} (z_{mix} calculated as for metabolism calculations), the depth layer occupied by the sonde was between 0 m and z_{mix}; when sonde depth was greater than z_{mix}, the depth layer was said to be between 0.5 m and 1.0 m. We also consider annual averages of light climate where we discard light climates estimated when sonde depth was greater than z_{mix}.

In 2010 in Ward Lake particles in the layer occupied by the sonde experienced a mean light climate of 233 W m^{-2} s^{-1} (median = 43.7 W m^{-2} s^{-1}, standard deviation = 343 W m^{-2} s^{-1}). In 2012, the mean light climate was 135 W m^{-2} s^{-1} (median = 32.3 W m^{-2} s^{-1}, sd = 188 W m^{-2} s^{-1}). This shift equates to the mean light in 2012 being 58% of the mean light in 2010, and the median light in 2012 being 74% of the median light in 2010. If observations are removed when the sonde is not in the mixed layer, the 2010 mean light is 233 W m^{-2} s^{-1} (median = 43.7 W m^{-2} s^{-1}, sd = 343 W m^{-2} s^{-1}) and the 2012 mean light is 97.0 W m^{-2} s^{-1} (median = 11.7 W m^{-2} s^{-1}, sd = 159 W m^{-2} s^{-1}). This second formulation indicates that mean light in 2012 was 42% of the 2010 mean, and that the median light in 2012 was 27% of the 2010 median. Thus, a thorough calculation of the light climate indicates that the phytoplankton in the same layer as the sensors that were used to estimate metabolism experienced a darker light climate in 2012 than in 2010. This result is consistent with the intuition that particles in a darker lake would experience darker conditions.

Sample collection for isotope analysis

Water was sampled from the epilimnion (at 0.5 m) and the metalimnion every month from May through August in 2010, and June through August in 2012. The samples were taken from 5 horizontally distributed locations in 2010, and 3 locations in 2012. The metalimnetic sampling depth was defined as the depth of the maximum saturation of dissolved oxygen (DO) below the mixed layer (Z_{mix}). Sample filtrate (Whatman GF/F filters) was analyzed for water deuterium (δ^2H of H_2O) and the $\delta^{13}C$, $\delta^{15}N$, and δ^2H of dissolved organic matter (DOM). Solid samples of DOM were obtained by evaporating the acidified filtrate (1 mL 1 N H_2SO_4 in 1 L of filtrate) in a glass petri dish. The material collected on the Whatman GF/F filters was used to measure the carbon and nitrogen stable isotopes of particulate organic matter (POM). Material for POM analysis of hydrogen stable isotopes was collected on a MicronSep Cellulosic

filter, washed off of the filter with a small amount of deionized water, and dried at 60°C. DOM, δ^2H_2O, and POM sample sizes were 5, 8, and 33 in 2010, and 6, 18, and 14 in 2012.

Algal end members included phytoplankton and benthic algae. Phytoplankton isotope values were computed from POM and δ^2H_2O, as described in the main text. Benthic algae were sampled from ceramic tiles submerged at 0.5 m and 0.25 m in 2010 and 2012, respectively ($n = 6$, 6; this notation henceforth represents sample sizes in 2010, 2012). Tiles were deployed at horizontally distributed locations in the littoral zone of the lake throughout both summers, and samples were scraped from the tiles on each sampling date. Benthic algae sampled from tiles served as a morphological and isotopic surrogate for attached algae that might be growing in the lake, and the use of tiles avoided the potential for contamination by natural substrates (e.g., wood or sediment).

Macrophytes and terrestrial plants comprised the other end members sampled for isotopes. Macrophytes were sampled throughout the lake and included taxa of floating-leafed (*Nymphaea odorata* [$n = 5$, 2], *Nuphar variegata* [$n = 5$, 2], *Brasenia schreberi* [$n = 4$, 2], *Potamogeton nodosus* [$n = 0$, 2]) and submersed (*Chara* sp. [$n = 5$, 1], *Najas flexilis* [$n = 1$, 3], *Potamogeton pusillus* [$n = 5$, 2], *Potamogeton amplifolius* [$n = 0$, 2]) morphologies. Macrophytes were thoroughly rinsed with tap water, and taxa with broad leaves were also gently scrubbed to remove any remaining epiphytic growth. Terrestrial vegetation (*Larix laricina*, *Carex* sp., *Alnus incana* subsp. *rugosa*) was collected from around the perimeter of the lake (total of 10 samples in 2010, 4 samples in 2012). We used our previously measured isotope values of several tree species sampled from the watershed (*Picea mariana*, *Abies balsamea*, *Acer rubrum*, *Acer saccharum*, *Thuja occidentalis*, *Betula alleghaniensis*) to form an average "tree" isotopic value (Cole et al. 2011, Solomon et al. 2011). Although we sampled macrophytes and terrestrial end members in both years, we pooled samples from both years for use in mixing models. Furthermore, the isotope values of each taxon (e.g., *Chara* sp.) were averaged, then this average was averaged across taxa to form the isotope value of the group (e.g., submersed macrophytes). Therefore, differences in the sample sizes of individual taxa did not result in taxa with larger sample sizes having a larger influence on the group isotope values. Analysis of variance was used to calculate the variance of the groups.

Consumers were sampled on approximately the same dates as end members. All consumers were sampled from locations that were horizontally distributed across the lake. The zooplankton *Skistodiaptomus oregonensis* was collected by pumping water from discrete depths in the epilimnion and metalimnion through a net ($n =$ 34, 18). The zooplankton predator, *Chaoborus* spp., was sampled using nighttime oblique net tows through the epilimnion ($n = 20$, 7). The snail *Helisoma trivolvis* was collected from the littoral zone of the lake, and the foot of each animal saved for isotope analysis ($n = 6$, 6). Fishes were captured using hoop nets and minnow traps placed around the perimeter of the lake. The gut tissue of each fish was excised, and the rest of the body used for isotope analysis. Fish species included *Pimephales promelas* (fathead minnow; $n =$ 12, 14), *Phoxinus* spp. (dace; $n =$ 12, 12), *Umbra limi* (central mud minnow; $n = 9$, 12), and young of the year *Ameiurus melas* (black bullhead; $n = 7$, 5). After sampling, all solid samples were dried at 60°C, ground to a fine powder, and sent to the Colorado Plateau Stable Isotope Laboratory (CPSIL) for isotope analysis (Doucett et al. 2007). At CPSIL, a benchtop equilibration procedure was used to correct for the exchange of H atoms between a set of standards including ground algal material and ambient water vapor. Solid samples were pyrolyzed to H_2 gas and analyzed using a Thermo-Finnigan TC/E and Delta^{PLUS}-XL (Thermo Electron Corporation, Bremen, Germany). The δ^2H of water samples was analyzed using cavity ring-down laser spectroscopy using Los Gatos Research Off-Axis Integrated Cavity Output coupled to a CTC LC-PAL liquid autosampler.

Model selection

We explored models that differed in the sources for which their contribution to consumer biomass (Φ) was to be estimated. Possible sources in the model were terrestrial, phytoplankton, submersed macrophytes, floating macrophytes, all macrophytes, and periphyton. We restricted our sensitivity analysis to models that (1) had at least two but not more than four sources ($2 \leq S$

≤ 4); (2) contained the terrestrial end member (S_1 was always terrestrial); (3) did not contain redundant sources (e.g., could not contain both floating-leafed macrophytes and all macrophytes as separate sources). In total, eighteen models were evaluated for each consumer in each year (252 distinct model runs).

We used known consumer feeding habits and deviance information criterion (DIC) (Spiegelhalter et al. 2002) to choose which end members were included as sources in the mixing models. We only considered models that contained the terrestrial source and at least one other source. For the pelagic invertebrates, the two sources were terrestrial and phytoplankton organic matter. For the snail, DIC was used to select between models that did not contain phytoplankton, and periphyton and floating-leafed macrophytes were selected in addition to terrestrial. Models for the fishes were guided by DIC and contained three or four sources, with model selection always including terrestrial and phytoplankton, and never including submersed macrophytes.

Our estimates of resource support were robust to model selection (Fig. A1). However, comparing across models requires care when the exclusion of a certain group of resources can cause another source to become the only source that bounds consumers in one or more dimensions of isotope space. In this case, the contribution (Φ) of the remaining source would be higher than in models containing other groups that could bound consumer isotope values. For example, phytoplankton and periphyton were the two sources with the lowest values of δ^2H (Fig. 3). The isotope values of several invertebrates (Fig. 3C) and fishes (Fig. 3E) could not easily be explained by a model that contained neither phytoplankton nor periphyton, because these algal end members were the only sources that had lower δ^2H values. In other words, at least one of the algal sources was necessary for bounding consumers in the δ^2H dimension of isotope space. As a result, if a consumer with a low δ^2H value truly relied on phytoplankton, a model that did not contain phytoplankton might erroneously estimate a large contribution by periphyton, even if a model containing both end members estimates low periphyton contribution. For example, the final model for *U. limi* did not contain periphyton, but the average contribution of periphyton to *U. limi* across all models containing periphyton was 30% in 2010 and 34% in 2012. However, averaged across all models containing periphyton *and* phytoplankton, periphyton support of *U. limi* was 8% in 2010 and 7% in 2012. Similarly, for *P. promelas* and *Phoxinus* spp., periphyton support in the selected model was lower than that averaged across all models (Fig. A1, median relative to circles); however, the average support by periphyton in models containing both algal end members was nearly identical to that in the selected model (Fig. A1, median relative to squares).

Size of POC and DOC pools

The sizes of the particulate and dissolved organic carbon (POC and DOC) pools were similar between years in Ward Lake and in Paul Lake. In Paul Lake, POC was 0.42 ± 0.16 mg L^{-1} in 2010 and 0.45 ± 0.17 mg L^{-1} in 2012 (annual mean $\pm$ standard deviation), and in Ward Lake POC was 0.85 ± 0.17 mg L^{-1} in 2010 and 1.0 ± 0.31 mg L^{-1} in 2012. DOC concentrations followed the same pattern, with Paul Lake (4.8 ± 0.81 mg L^{-1} in 2010, 4.5 ± 0.32 mg L^{-1} in 2012) and Ward Lake (9.6 ± 2.7 mg L^{-1} in 2010, 10.6 ± 1.3 mg L^{-1} in 2012) both having similar DOC concentrations between years.

Biomasses of copepod zooplankton

Copepods are the dominant group of zooplankton in Ward Lake. We analyzed one species of calanoid copepod, *S. oregonensis*. The zooplankton biomass in Ward Lake decreased between years, while zooplankton biomass in Paul Lake remained similar (see main text). However, the change in the seasonal average of zooplankton biomass in Ward Lake (from 0.88 g/m^2 in 2010 to 0.52 g/m^2) was not attributable to a decline in *S. oregonensis* adults (Fig. A2, Calanoid), but rather to a 0.20 g/m^2 decrease in copepod larvae (Fig. A2, Nauplii) and a 0.15 g/m^2 decrease in the copepod *Mesocyclops* (Fig. A2).

Aquashade and DOM isotope values

Our goal in analyzing the composition of DOM was to estimate the relative contributions of macrophytes, phytoplankton, periphyton, and terrestrial end members to this pool. However, our experimental manipulation introduced Aquashade to the DOM pool. Therefore, the

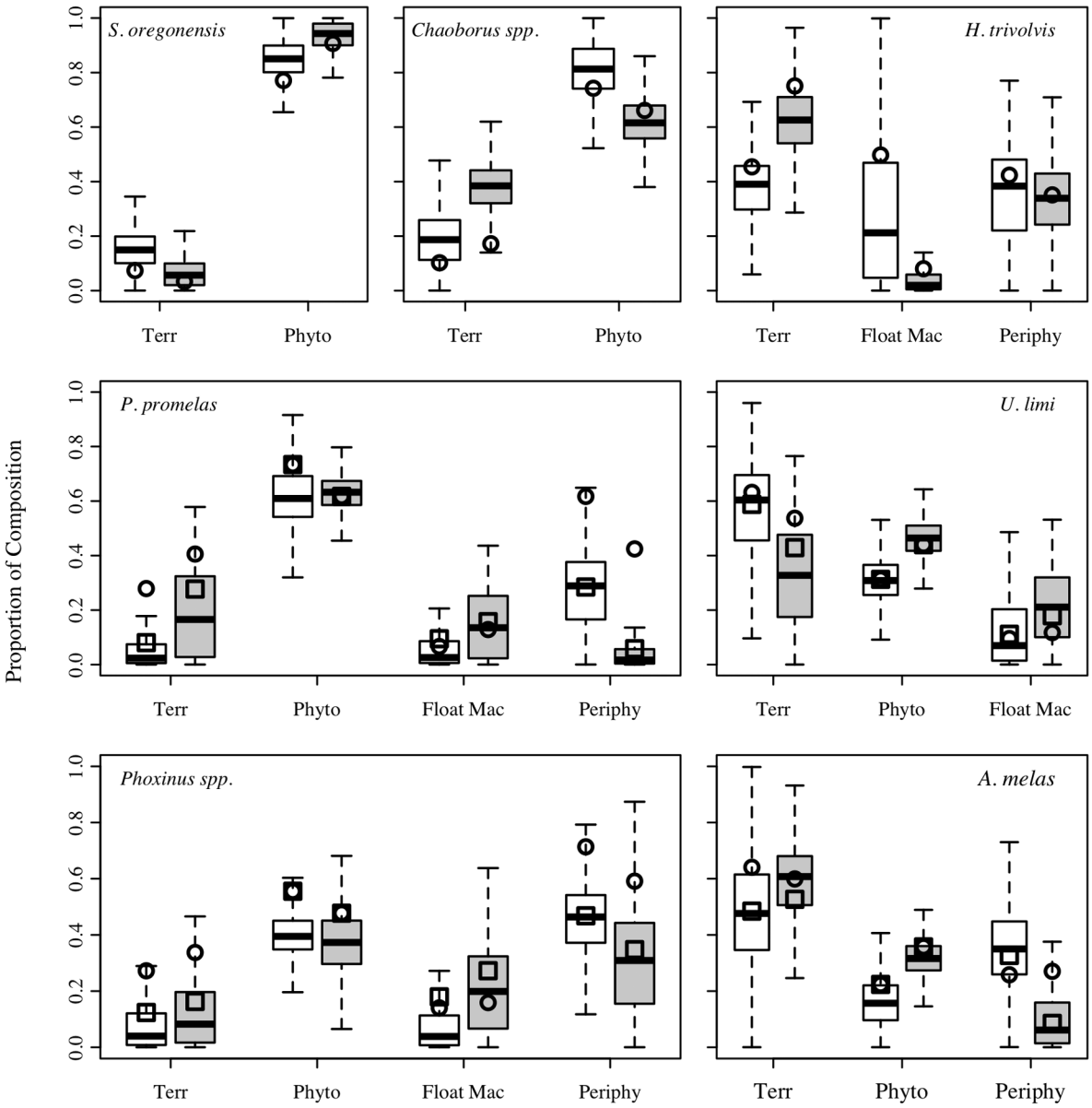

Fig. A1. Boxplots of the proportional contributions of resources to consumers (posterior of Φ) supported by each resource in Ward Lake in 2010 (open) and 2012 (shaded). Boxes bound 25–75 percentiles. Each whisker extends beyond its respective quartile by a factor of 1.5 × (interquartile range), and the dark line designates the median. All data were included in statistical calculations, but outliers are not displayed. The boxes, whiskers, and medians reflect the posterior for the final model. Circles show the average estimate for that resource in any of the 18 models from the sensitivity analysis (which included different combinations of resources) containing that resource. Squares are the average estimate for that resource in any of the 18 models containing that resource *and* the phytoplankton resource.

composition of DOM was estimated after algebraically correcting for the influence of Aquashade on the isotope values of DOM by using measured concentrations, isotope values, and the C:N:H stoichiometry of the dye and of DOM. We estimated the concentration of Aquashade in Ward Lake and corrected for its influence on the isotope value of DOM. Aquashade concentration

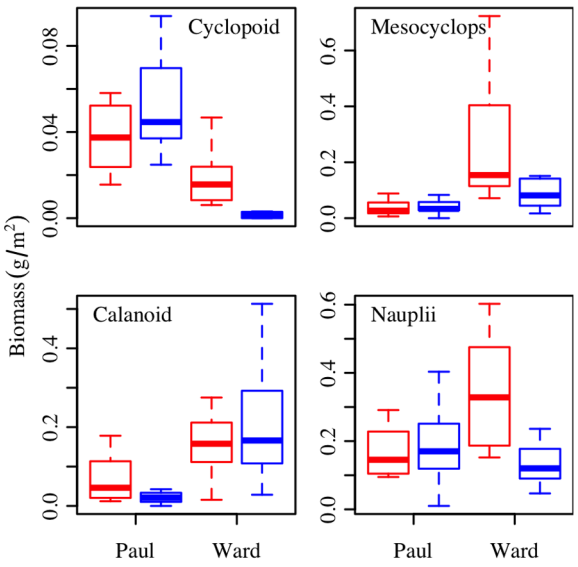

Fig. A2. Biomasses of copepod zooplankton in Ward Lake and Paul Lake in 2010 (red) and 2012 (blue). Boxplot conventions as in Fig. A1.

was estimated using spectrophotometry. Each week we measured the absorbance of lake water between 300 nm and 900 nm, using wavelengths near Aquashade's peak absorbance at 625 nm to estimate dye concentration. Absorbances at longer wavelengths (700–900 nm, far from the peak of Aquashade absorbance) were used to correct the rest of the spectrum for shifts in absorbance that were not caused by a change in Aquashade concentration. We then estimated the volume fraction (ppm) of Aquashade in water by comparing the corrected spectrum to that of a known concentration of Aquashade. To convert the volume fraction of Aquashade to a concentration (mg L^{-1}), we used a density of 1 g mL^{-1}, which is within the range reported in the Material Safety Data Sheet for Aquashade.

To estimate the relative mass contribution of Aquashade to the C, N, and H of total DOM we used stoichiometry measured by the same mass spectrometry analysis that yielded isotope values. The concentration of DOC in Ward Lake in 2012 was 10.65 mg/L. The mass ratios of C:N and C:H in DOM were 25 and 4.347, respectively. The concentration of Aquashade was typically near 1.5 mg/L, and was 49.31% C, 4.39% N and 4.2% H by mass. Therefore, Aquashade was 6.95% of DOC ($0.4931 \times 1.5 \times 10.65^{-1}$), 15.46% of DON ($0.0439 \times 1.5 \times (10.65 \times 25^{-1})^{-1}$), and 2.57% DOH ($0.042 \times 1.5 \times (10.65 \times 4.347^{-1})^{-1}$).

The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ of Aquashade were −26.82‰, −0.02‰, and −65.4‰, respectively. The $\delta^{13}C$, and $\delta^{15}N$ values are similar to those of other end members and consumers in the study. The $\delta^{2}H$ of Aquashade is similar to that of water, which is highly enriched relative to the other pools we analyzed. As a pre-manipulation reference, the isotope values of DOM in 2010 were $\delta^{13}C = -28.2‰$, $\delta^{15}N = -3.7‰$, and $\delta^{2}H = -116.1‰$. In 2012, DOM $\delta^{13}C = -19.5‰$, $\delta^{15}N = 0.1‰$, and $\delta^{2}H = -156.9‰$. After correcting for the contribution of Aquashade to DOM, the DOM isotope values become DOM $\delta^{13}C = -18.9‰$, $\delta^{15}N = 0.1‰$, and $\delta^{2}H = -159.3‰$. The isotope values of DOM changed substantially between years, and this large change was not driven by the influence of Aquashade isotope values. To summarize, Aquashade was a minor portion of the DOM pool and had a minimal impact on our analysis of DOM composition, even if a correction had not been performed.