Stable isotope patterns in lake food webs reflect productivity gradients

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Abstract. Stable isotopes 13C and 15N are often used in lake ecosystems to assess energy sources and trophic positions, respectively. However, δ13C and δ15N are also influenced by internal biogeochemical processes in epilimnetic and hypolimnetic habitats in lakes, but the extent to which biogeochemical processing mediates isotope values between these two habitats, and whether these patterns are influenced by lake productivity is not known. We sampled δ13C and δ15N in epilimnetic mussels, Chaoborus, cisco (Coregonus artedi), and seston and zooplankton in the epilimnia and hypolimnia of 22 Minnesota (USA) lakes ranging from oligotrophic to eutrophic. We also measured lake temperature–oxygen profiles and light levels to assess factors influencing isotope patterns. Isotope samples were baseline-corrected using epilimnetic mussels in each lake (sample—mussel) to control for watershed-level differences in isotope values. Results showed δ13C in epilimnetic and hypolimnetic zooplankton, hypolimnetic seston, Chaoborus, and cisco became more depleted in δ13C relative to epilimnetic mussels in low-productivity lakes where light penetrated into the hypolimnion, while epilimnetic seston δ13C stayed similar to mussel δ13C in all lakes. This pattern was likely due to hypolimnetic phytoplankton in clearwater lakes incorporating more respired CO2, which is depleted in δ13C, and subsequently passing depleted δ13C values up the food chain. Results also showed habitat differences in δ15N with epilimnetic and hypolimnetic zooplankton, hypolimnetic seston, Chaoborus, and cisco becoming more enriched relative to epilimnetic mussels in low-productivity lakes with higher O2 levels in the hypolimnion, while epilimnetic seston δ15N remained similar to mussel values. The δ15N pattern is consistent with the idea that denitrification and microbial degradation enriched hypolimnetic seston relative to epilimnetic seston in low nutrient lakes, while enhanced epilimnetic primary production enriched epilimnetic δ15N seston relative to hypolimnetic seston in high nutrient lakes. Our results indicate isotopic differences between epilimnetic and hypolimnetic organisms that change along productivity gradients and suggest that microbial processes and the light regime are important drivers.

Key words: δ13C; δ15N; denitrification; eutrophication; food webs; fractionation; microbial processing; primary production; respiration.

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INTRODUCTION

Understanding patterns of trophic relationships and energy flow in food webs is important for understanding ecosystem stability and resilience to natural and anthropogenic disturbances (Rooney et al. 2006, McMeans et al. 2016). Stable isotopes are powerful tools for understanding food web patterns given their ability to capture information on energy flow, as well as their ability to estimate isotopic niche space that reflects resource utilization (Newsome et al. 2007, McMeans et al. 2016). The two most commonly used isotopes in aquatic systems are δ^{13}C and δ^{15}N. Fractionation of δ^{13}C by primary producers varies among suspended phytoplankton vs. sessile periphyton and submerged macrophytes, with the former being more depleted in δ^{13}C values relative to sessile primary producers (France 1995). Moreover, there is little fractionation of δ^{13}C between trophic levels (approximately 0.39‰; Post 2002). Given these patterns, a common use of δ^{13}C is to estimate the relative importance of littoral and benthic (hereafter littoral) vs. pelagic primary production as sources of fixed C for lake food webs (Böecklen et al. 2011). In contrast, δ^{15}N is more fractionated between trophic levels (approximately 3.4‰; Post 2002), making it useful for estimating trophic position and feeding patterns among organisms in food webs. Additionally, when used together, δ^{13}C and δ^{15}N can be coupled with mixing models to generate precise estimates of trophic position and C sources (Post 2002) as well as species-level or community-scale estimates of isotopic niche space (Layman et al. 2007, Jackson et al. 2011) and niche overlap (Swanson et al. 2015).

Differences in δ^{13}C fractionation among primary producers and fractionation of δ^{15}N among trophic levels are well documented, but δ^{13}C and δ^{15}N values can also be influenced by biogeochemical processing and abiotic factors in lakes. For example, microbial fractionation during denitrification can increase δ^{15}N levels in seston, while N fixation can reduce seston δ^{15}N (McCusker et al. 1999, Gu 2009). For δ^{13}C, use of respired C can lead to depleted δ^{13}C in primary producers (Francis et al. 2011), and methane-derived C can strongly deplete δ^{13}C in profundal (Jones et al. 2008) and pelagic (Bastviken et al. 2003) primary consumers. Finally, water temperature can influence both δ^{13}C and δ^{15}N of primary consumers, with δ^{13}C becoming more depleted and δ^{15}N becoming more enriched as temperature declines (Power et al. 2003). Although it is clear that patterns of δ^{13}C and δ^{15}N can be influenced by these biogeochemical processes and abiotic factors, the degree to which isotope patterns vary among lake ecosystems is poorly studied.

Lake productivity and the depth where an organism resides are two factors that influence biogeochemical processes and subsequently influence isotope patterns in lakes. Productivity could influence δ^{15}N patterns as high nutrient levels increase rates of both denitrification (Seitzinger 1988) and N fixation (Howarth et al. 1988). Lake productivity also influences δ^{13}C patterns, as France et al. (1997) showed that seston and zooplankton were more depleted in low-productivity lakes due to enhanced use of respired C. Isotope patterns can also vary between deep- and shallow-water habitats in lakes due to dominance of different biogeochemical processes in the epilimnion vs. hypolimnion (Lehmann et al. 2004, Francis et al. 2011). Several studies have documented differences in δ^{13}C and δ^{15}N between shallow- and deep-water organisms, including zooplankton and aquatic insects (Vander Zanden and Rasmussen 1999), zebra mussels (Yohannes et al. 2014), cisco (Coregonus artedi; Helland et al. 2008), benthic invertebrates (Cummings and Schindler 2013), and seston and zooplankton (Francis et al. 2011). However, many studies have focused on patterns within individual lakes, whereas patterns among lakes are poorly known. Moreover, hypolimnetic characteristics such as the presence of light and dissolved oxygen levels are strongly influenced by lake productivity, suggesting isotopic differences between epilimnetic and hypolimnetic organisms may also be influenced by lake productivity.

Understanding differences between epilimnetic and hypolimnetic organisms is important because comparing isotope relationships among lakes requires baseline corrections to control for natural differences in δ^{13}C and δ^{15}N at the whole-lake scale. Mussels are often recommended for baseline corrections given their reliance on phytoplankton and their long life spans (Post 2002). However, mussels are typically collected from epilimnetic habitats and estimates of pelagic vs. littoral energy flow and estimates of
trophic position could be biased if mussel isotope values differ from hypolimnetic primary consumers in the same lake.

The goal of this study was to test two related questions. First, do δ^{13}C and δ^{15}N differ between epilimnetic and hypolimnetic seston, and, if there are differences, are they related to lake productivity? Second, if there are isotope differences between epilimnetic and hypolimnetic seston, are they passed up the food web, such that deep-water organisms like zooplankton, *Chaoborus*, and cisco have isotope values that differ from epilimnetic organisms? Our results show that relative to epilimnetic mussels and seston, hypolimnetic seston, zooplankton, *Chaoborus*, and cisco become more enriched in δ^{15}N and more depleted in δ^{13}C as lakes become more oligotrophic.

**METHODS**

This study was conducted in 22 lakes dispersed across 38,500 km² and spanning three ecological provinces (based on Omernik 1987) in Minnesota, USA (Fig. 1). The lakes spanned a wide range of physical, chemical, and biological characteristics (Table 1). Lakes in the northeast part of the state are oligotrophic and located in the Laurentian Mixed Forest Province; lakes in the central part of the state are mesotrophic and lie in the Eastern Broadleaf Forest Province, while lakes in the southwest are eutrophic and on the border of the Prairie Parkland Province. Each lake was sampled once during July or August of 2013 through 2015, with similar numbers of lakes sampled each year.

Cisco were sampled using vertical gillnets placed in up to three of the deepest basins in each lake. The nets consisted of seven 61 m deep panels of monofilament webbing (bar-measure mesh size × panel width): 10 × 0.9 m, 13 × 0.9 m, 19 × 1.2 m, 25 × 1.8 m, 32 × 3.0 m, 38 × 3.0 m, 44 × 3.0 m. The 10- and 13-mm panels were sewn together vertically into one net, as were the 19- and 25-mm panels. The 32-, 38-, and 44-mm panels were used individually for a total of five separate vertical nets. Each net was ganged together by a 2-m connecting rope, and the gang (five nets with seven panels) was set as a unit in each basin. Nets were set to span from the lake surface to lake bottom and were fished overnight. Upon collection, a sample of dorsal muscle tissue was collected for stable isotope analyses.

Isotope samples for seston, zooplankton, *Chaoborus*, and mussels were collected during daytime within one week of cisco sampling. Depths of the epilimnion, metalimnion, and hypolimnion were first determined by profiling with a dissolved oxygen and temperature meter at three of the deepest locations in each lake, and at each location, a water sample was collected with a Van Dorn sampler at the midway point of both the epilimnion and the hypolimnion. A subsample of the epilimnion sample was frozen for later analysis of total phosphorus (TP) via persulfate oxidation and ascorbic acid colorimetry (APHA 1989). Another subsample was filtered onto a GF/F filter and frozen, and the filter later was analyzed for chlorophyll a (chl a) via alkaline acetone extraction and fluorometric analysis (Arar and Collins 1997). Samples for seston stable isotopes were collected from both the epilimnion and the hypolimnion at each station by filtering sample water through 80-µm mesh to remove macrozooplankton and then filtering onto a pre-combusted GF/F filter. The particulate matter was wetted with 1% HCl to remove particulate inorganic C, rinsed with nanopure water, and frozen until later processing. Epilimnetic and hypolimnetic zooplankton samples were collected at each location using a Birge closing net with 153-µm mesh, with the net towed from 1 m off the bottom through the entire hypolimnion, and from the bottom of the epilimnion to the lake surface. Sample contents were screened, and carnivorous zooplankton were removed by hand, as were particles of detritus. Samples were then filtered onto pre-combusted GF/C filters, wetted with 1% HCl to remove particulate inorganic C, rinsed with nanopure water, and frozen until analysis. We did not attempt to remove smaller carnivorous individuals, but microscopic analysis indicated the sample was largely herbivorous zooplankton. Several lakes had blooms of large (>153 µm) phytoplankton during the time of sampling, and logistical constraints prevented us from being able to separate seston from zooplankton under these conditions. Thus, we were able to collect epilimnetic zooplankton samples from nine lakes and hypolimnetic zooplankton samples from 14 lakes. There was no discernible
pattern for lakes where we were unable to sample zooplankton, as the issue occurred in both large and small lakes as well as across the productivity gradient. *Chaoborus* were collected at the same three stations with a Ponar grab at the sediment interface, rinsed, and frozen until further analysis. Despite extensive sampling, we were able to collect *Chaoborus* from only 11 study lakes. Up to five mussels (fatmucket, *Lampsilis siliquoidea*) were collected from the epilimnion of each lake by snorkeling and were frozen until further analysis.

We used a light meter to estimate the light extinction coefficient at each sampling station and used the mean value for all subsequent analyses. We also calculated the compensation depth: mixed layer depth ratio for each lake based on the light extinction coefficient and temperature–oxygen profiles. We defined the compensation depth as the depth where light declined to 1% surface light and the mixed layer depth as the deepest depth above the thermocline showing less than a 1°C/m change (Francis et al. 2011). We also determined the temperature where dissolved oxygen equaled 3 mg/L (hereafter TDO3) from temperature–oxygen profiles. The TDO3 value of each lake represents the coldest water temperature where dissolved oxygen stayed at or above 3 mg/L, with colder TDO3 values indicating deeper penetration of dissolved oxygen into the hypolimnion. TDO3 was developed as a metric to assess oxythermal habitat for fish, and it is inversely related to lake depth and positively related to lake surface area and TP (Jacobson et al. 2010). TDO3 may also be useful for understanding isotope patterns in hypolimnetic...
organisms as it reflects the light, temperature, and dissolved oxygen environment in deep-water habitats.

In the laboratory, mussel tissue was extracted by hand, and all isotope samples were dried at 60°C. Mussel, *Chaoborus*, and cisco tissue were subsequently ground, and all isotope samples were analyzed by the University of California Davis Stable Isotope Facility. Each cisco isotope sample was analyzed individually, as were mussel, *Chaoborus*, zooplankton, and seston, unless insufficient material required pooling material across samples (*n* = 13). Pooling samples did not influence our results as all analyses were conducted on lake-wide means of each taxon. Tissue samples were analyzed for δ13C and δ15N using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Analytical precision (SD) was ±0.2‰ for 13C and ±0.3‰ for 15N, respectively. Final δ values were reported as ratios of 13C/12C and 15N/14N relative to the international standards Vienna PeeDee Belemnite and air for C and N, respectively (Peterson and Fry 1987). Recent work has shown that lipids have depleted δ13C values relative to other types of tissues, such that differences in δ13C for a given species could be due to differences in lipid content instead of differences in C source (Smyntek et al. 2007). Thus, we used equation 3 in Post et al. (2007) to correct lipid δ13C values in animal samples with C:N greater than 3.25. Hereafter, all references to δ13C values represent lipid-corrected values. For analysis of isotope data, we used the mean value of cisco, mussels, *Chaoborus*, epilimnetic seston and zooplankton, and hypolimnetic seston and zooplankton for each lake.

**Do δ13C and δ15N differ between epilimnetic and hypolimnetic seston, and are differences related to lake productivity?**

Our first goal was to test whether δ13C and δ15N values differed between epilimnetic and hypolimnetic seston. δ13C and δ15N at the base of food webs vary naturally among lakes, requiring baseline corrections for valid comparisons among lakes (reviewed by Post 2002). Mussels are recommended for making baseline corrections as they are long-lived and consume phytoplankton, so their expected trophic position and carbon source are well known (Post 2002). Thus, we baseline-corrected δ13C and δ15N for all taxa by subtracting the mean value of mussels in each lake from the mean value of each respective taxon, and hereafter, references to baseline-corrected isotope values are denoted as δ13C_{DF} and δ15N_{DF}.

Post (2002) reported organisms on average enrich 0.39‰ for δ13C and 3.4‰ for δ15N relative to the trophic level below. Thus, seston one trophic level below mussels should have δ13C_{DF} and δ15N_{DF} values of ~0.39 and ~3.4, respectively, and be the same in both the epilimnion and the hypolimnion if isotope patterns do not differ between shallow- and deep-water habitats. We used a model-fitting approach to test whether seston δ13C_{DF} and δ15N_{DF} differed between epilimnetic and hypolimnetic habitats, and to test whether any differences were related to lake productivity. We tested five proxy variables for lake productivity (light extinction, chl a, TP, TDO3, and compensation depth:mixed layer depth), and for each variable, we fit a full factorial model with depth (epilimnetic vs. hypolimnetic seston), a lake productivity proxy, and depth × productivity proxy. The most parsimonious model for predicting δ13C_{DF} and δ15N_{DF} in seston was selected based on AICc, Δi (difference in AICc values between top model and other models), and evidence ratios (multiplicative improvement of the best model over other models) (Burnham and Anderson 2004). A lake productivity effect on δ13C_{DF} and δ15N_{DF} in hypolimnetic seston but not in epilimnetic seston would be supported by a significant depth × productivity proxy in the best-supported model.

**Are isotope differences between epilimnetic and hypolimnetic seston passed up the food web?**

Results from our first question showed δ13C_{DF} and δ15N_{DF} differed between epilimnetic and hypolimnetic seston and that hypolimnetic seston δ15N_{DF} was negatively related to compensation depth:mixed layer depth, while δ13C_{DF} was...
negatively related to TDO3 (Fig. 2). Given that zooplankton feed on seston, and *Chaoborus* and cisco feed heavily on zooplankton (K. D. Zimmer et al., unpublished data), an important follow-up question was to test whether isotope patterns in hypolimnetic seston are subsequently passed up the food web to zooplankton, *Chaoborus*, and cisco. Isotope patterns passed up the food web would be shown by $\delta^{13}C_{DF}$ in epilimnetic zooplankton, hypolimnetic zooplankton, *Chaoborus*, and cisco all showing a negative relationship with compensation depth:mixed layer depth, and $\delta^{15}N_{DF}$ being negatively related to TDO3. Thus, we tested for effects of taxa (cisco, *Chaoborus*, epilimnetic zooplankton, hypolimnetic zooplankton), compensation depth:mixed layer depth, and taxa $\times$ compensation depth:mixed layer depth on $\delta^{13}C_{DF}$, and for effects of taxa, TDO3, and taxa $\times$ TDO3 on $\delta^{15}N_{DF}$. Simultaneous analysis of multiple trophic levels could blur the strength of isotope relationships between adjacent trophic levels. Thus, we also tested whether isotope patterns in hypolimnetic zooplankton were passed up to adjacent trophic levels.
levels by regressing $\delta^{13}$C$_{DF}$ and $\delta^{15}$N$_{DF}$ in Chaoborus and cisco against $\delta^{15}$C$_{DF}$ and $\delta^{15}$N$_{DF}$ in hypolimnetic zooplankton. We predicted a positive relationship in $\delta^{13}$C$_{DF}$ and $\delta^{15}$N$_{DF}$ for both Chaoborus and cisco, which would indicate differences between hypolimnetic zooplankton and mussels are passed on to zooplankton predators. Total phosphorus and chl $\alpha$ are commonly used proxies for lake productivity, but compensation depth:mixed layer depth showed the strongest relationship with $\delta^{13}$C$_{DF}$ in hypolimnetic seston, while TDO3 showed the strongest relationship with $\delta^{15}$N$_{DF}$ (Fig. 2). However, algal abundance and nutrient levels may ultimately influence the patterns we observed through direct effects on compensation depth:mixed layer depth and TDO3. Thus, we tested for a significant effect of chl $\alpha$ (due to effects on compensation depth) and lake size (due to effects on mixing depth) on compensation depth:mixed layer depth, and for a significant effect of TP on TDO3 (due to effects on hypolimnetic oxygen depletion rates).

We log-transformed TDO3, TP, chl $\alpha$, and light extinction to increase homoscedasticity of residuals, and all analyses were performed with JMP version 13 (SAS 2012).

**Results**

*Are differences in $\delta^{13}$C and $\delta^{15}$N between epilimnetic and hypolimnetic seston related to lake productivity?*

Results of model selection showed the most parsimonious model for predicting $\delta^{13}$C$_{DF}$ in epilimnetic and hypolimnetic seston was compensation depth:mixed layer depth ($\text{AIC}_c = 203.3, w_i > 0.99$), and evidence ratios indicated it had >2000-fold more support than the second best model (light attenuation, $\text{AIC}_c = 218.8, \Delta_i = 15.5, w_i < 0.01$; Appendix S1: Table S1). Fixed-effect tests for variables in the most parsimonious model indicated effects of depth ($P = 0.030$), compensation depth:mixed layer depth ($P = 0.002$), and depth $\times$ compensation depth:mixed layer depth on $\delta^{13}$C$_{DF}$ ($P < 0.001$; Fig. 2A, B). The slope for epilimnetic seston $\delta^{13}$C$_{DF}$ did not differ from zero ($P = 0.592$), while hypolimnetic seston $\delta^{13}$C$_{DF}$ was negative ($P < 0.001$). This indicates hypolimnetic seston became more depleted in $\delta^{13}$C relative to mussels as light penetrated deeper into the hypolimnion.

For $\delta^{15}$N$_{DF}$ in epilimnetic and hypolimnetic seston, the best-supported model was TDO3 ($\text{AIC}_c = 216.9, w_i > 0.99$), and evidence ratios indicated it had >300-fold more support than the second best model (chl $\alpha$, $\text{AIC}_c = 228.6, \Delta_i = 11.7, w_i < 0.01$; Appendix S1: Table S1). Tests of fixed effects in the best-supported $\delta^{15}$N$_{DF}$ model indicated effects of TDO3 ($P < 0.001$) and a depth $\times$ TDO3 interaction ($P = 0.005$), but no effect of depth on $\delta^{15}$N$_{DF}$ ($P = 0.812$; Fig. 2C, D). Moreover, the slope for hypolimnetic $\delta^{15}$N$_{DF}$ was negative ($P < 0.001$), while the epilimnetic seston slope did not differ from zero ($P = 0.238$). These results show that as TDO3 increased, hypolimnetic seston $\delta^{15}$N$_{DF}$ became more depleted relative to epilimnetic mussels, while $\delta^{15}$N$_{DF}$ in epilimnetic seston remained similar to mussels.

*Are isotope differences between epilimnetic and hypolimnetic seston passed up the food web?*

Significant relationships between compensation depth:mixed layer depth and $\delta^{13}$C$_{DF}$ and between TDO3 and $\delta^{15}$N$_{DF}$ for zooplankton, Chaoborus, and cisco would indicate differences between hypolimnetic seston and epilimnetic seston are passed up through two trophic levels in lake food webs. Full model results for $\delta^{13}$C$_{DF}$ showed a significant effect of compensation depth:mixed layer depth ($P = 0.002$), but no effect of taxa ($P = 0.083$) or taxa $\times$ compensation depth:mixed layer depth ($P = 0.694$). We subsequently dropped the interaction term, and the reduced model showed a significant effect of both taxa ($P = 0.039$) and compensation depth: mixed layer depth on $\delta^{13}$C$_{DF}$ ($P = 0.002$; Fig. 3A, B). Compensation depth:mixed layer depth had a negative effect on $\delta^{13}$C$_{DF}$ of all four taxa, and multiple comparison tests on marginal means indicated $\delta^{13}$C$_{DF}$ in epilimnetic zooplankton was significantly higher than values for hypolimnetic zooplankton and cisco, but did not differ from Chaoborus. In lakes with low compensation depth:mixed layer depth, $\delta^{13}$C$_{DF}$ in hypolimnetic zooplankton ($-0.7$), cisco ($-0.3$), and Chaoborus (0.1) was all slightly depleted relative to the expected values of 0 for zooplankton (same trophic level as mussels) and 0.4 for Chaoborus and cisco (one trophic level above mussels). However, $\delta^{13}$C$_{DF}$ was much more depleted in
hypolimnetic zooplankton (−3.9), cisco (−3.5), and Chaoborus (−3.2) in lakes with high compensation depth:mixed layer depth. Epilimnetic zooplankton δ¹³C_DF averaged 1.3-fold more enriched than cisco, Chaoborus, and hypolimnetic zooplankton across the compensation depth:mixed layer depth gradient, possibly reflecting higher consumption of epilimnetic seston less depleted in δ¹³C.

Results showed that patterns between TDO3 and hypolimnetic seston δ¹⁵N_DF were also passed up the food web, as δ¹⁵N_DF in cisco, Chaoborus, epilimnetic zooplankton, and hypolimnetic zooplankton all showed a negative relationship with TDO3. Full model results showed significant effects of TDO3 (P < 0.001) and taxa (P < 0.001), but no TDO3 × taxa interaction (P = 0.063) on δ¹⁵N_DF. The reduced model
excluding the interaction also showed significant effects of TDO3 \( (P < 0.001) \) and taxa \( (P < 0.001) \) on \( \delta^{15}N_{DF} \) (Fig. 3C, D). Multiple comparison tests on marginal means indicated a gradient of \( \delta^{15}N_{DF} \) values, with cisco greater than both zooplankton types but similar to Chaoborus, and Chaoborus higher than epilimnetic zooplankton but not hypolimnetic zooplankton, and no differences between epilimnetic and hypolimnetic zooplankton. In low TDO3 lakes, all four taxa were more enriched than expected based on mussel isotope values in the same lake, with both epilimnetic (1.7) and hypolimnetic zooplankton (3.1) higher than the expected value of 0, and Chaoborus (4.8) and cisco (6.0) higher than their expected value of 3.4. Thus, in low TDO3 lakes, the \( \delta^{15}N_{DF} \) values of cisco suggest they are 1.8 trophic levels (e.g., 6/3.4) higher than mussels, instead of the expected one trophic level. In contrast, \( \delta^{15}N_{DF} \) values for all four taxa were much closer to expected values in lakes with high TDO3, with \(-0.7\) for epilimnetic zooplankton, \(0.7\) for hypolimnetic zooplankton, \(2.4\) for Chaoborus, and \(3.6\) for cisco.

Analyses of adjacent trophic levels also showed that isotope patterns in hypolimnetic zooplankton were transferred to predators. Hypolimnetic zooplankton \( \delta^{12}C_{DF} \) showed a
positive relationship with both cisco ($P = 0.039$; Fig. 4A) and Chaoborus ($P = 0.003$; Fig. 4B) $\delta^{13}C_{DF}$. Cisco and Chaoborus $\delta^{13}C_{DF}$ were close to the expected value of 0.4 when zooplankton were close to their expected value of 0, but both were more depleted than expected in lakes with the most depleted zooplankton, which occurred in lakes with high compensation depth:mixing layer depth (Fig. 3B). Similarly, hypolimnetic zooplankton $\delta^{15}N_{DF}$ showed a significant relationship with both cisco ($P = 0.001$, Fig. 4C) and Chaoborus ($P = 0.006$, Fig. 4D) $\delta^{15}N_{DF}$. The expected $\delta^{15}N_{DF}$ value of cisco and Chaoborus (3.4) was observed when zooplankton $\delta^{15}N_{DF}$ was close to its expected value of 0, but cisco and Chaoborus both became more enriched than expected as hypolimnetic zooplankton became enriched, which occurred in low TDO3 lakes (Fig. 3D). We also found a significant positive relationship between epilimnetic zooplankton and cisco for $\delta^{13}C_{DF}$ ($P = 0.008$) but not for $\delta^{15}C_{DF}$ ($P = 0.157$; data not shown).

Total phosphorus and chl $a$ are commonly used proxies for lake productivity but did not show the strongest relationship with $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$ in hypolimnetic seston. However, compensation depth:mixed layer depth was negatively related to both chl $a$ ($P = 0.017$) and lake size ($P = 0.004$; Fig. 5A), while there was no relationship between chl $a$ and lake size ($P = 0.663$; data not shown). It is likely that chl $a$ and lake size were both significant because chl $a$ influences compensation depth while lake size influences mixing depth, such that high compensation depth:mixed layer depth values occur in small, clear lakes. TDO3, in turn, showed a positive relationship with TP ($P < 0.001$; Fig. 5B).

Taken together, these results indicate a general pattern where TDO3 increases and compensation depth:mixed layer depth decreases as lakes become more productive, and that hypolimnetic seston $\delta^{15}N_{DF}$ decreases and $\delta^{13}C_{DF}$ increases as lakes become more productive. The isotopic signature of hypolimnetic seston is subsequently passed up the food web to zooplankton, Chaoborus, and cisco (Fig. 6).

**DISCUSSION**

Overall, our results indicate that carbon and nitrogen can be processed differently in the hypolimnion of lakes relative to the epilimnion and that differences tend to be the most extreme between these two regions when these lakes are most unproductive. Specifically, in unproductive systems, the carbon isotopic signature was more depleted and the nitrogen isotopic signature was more enriched in the hypolimnion relative to the epilimnion. These isotopic signatures were manifest in the seston at the base of the hypolimnetic food web and carried through two trophic levels, suggesting that trophic interactions in the surface and hypolimnetic regions are largely separated. This has important implications for our
understanding of biogeochemical processes in lakes, and for use of stable isotopes to understand lake food webs, trophic relationships, and pathways of energy flow.

Our study was not designed to elucidate specific mechanisms driving our results, but there are several possible explanations for the isotope patterns we observed. One explanation is that water temperature differences between the epilimnion and the hypolimnion may drive the pattern we observed, as Power et al. (2003) showed that water temperature was positively related to $\delta^{13}C$ and inversely related to $\delta^{15}N$ in zooplankton fed the same food. For temperature to explain the patterns in our isotope data, the temperature difference between the epilimnion (where mussels were located) and the hypolimnion would have to be inversely related to $\delta^{15}N_{DF}$ and positively related to $\delta^{13}C_{DF}$ in hypolimnetic organisms. However, the difference between the mean epilimnetic and mean hypolimnetic water temperature in each lake showed no relationship with $\delta^{13}C_{DF}$ or $\delta^{15}N_{DF}$ in hypolimnetic seston, hypolimnetic zooplankton, *Chaoborus*, or cisco (all $P > 0.088$). Thus, it seems unlikely that temperature differences between the epilimnion and the hypolimnion in our study sites played a large role in our results.

Changes in consumer diets along the productivity gradient could also explain the isotope patterns. Methane oxidizing bacteria have depleted $\delta^{13}C$ and can contribute to $^{13}C$ depletion in secondary consumers including chironomids (Jones et al. 2008) and zooplankton (Bastviken et al. 2003), so it is possible our $\delta^{13}C_{DF}$ results were driven by increased reliance on methane-derived C in the hypolimnion of low-productivity lakes. However, methane concentrations are positively coupled to lake anoxia (Bastviken et al. 2004) and should therefore be least important in low-productivity lakes. For $\delta^{15}N_{DF}$, it is unlikely that changes in diets caused these patterns, as the entire hypolimnetic food web became more enriched in $\delta^{15}N_{DF}$ in unproductive lakes with low TDO3, not just a single trophic level. Thus, it is unlikely that changes in diets caused these patterns, as the entire hypolimnetic food web became more enriched in $\delta^{15}N_{DF}$ in unproductive lakes with low TDO3, not just a single trophic level. Thus, it is unlikely that changes in diets caused the observed patterns in $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$, and the patterns are instead caused by isotopic changes in hypolimnetic seston at the base of the food web, with the patterns subsequently passed up the food web to zooplankton, *Chaoborus*, and cisco.

Fig. 6. Summarized patterns of isotopes in lake food webs along a productivity gradient. Chlorophyll $a$ and lake size had a negative effect on compensation depth:mixed layer depth, such that light penetrated deepest into the hypolimnion in small, clear lakes. Compensation depth:mixed layer depth, in turn, had a negative effect on $\delta^{13}C_{DF}$ in hypolimnetic seston, epilimnetic and hypolimnetic zooplankton, *Chaoborus*, and cisco. Thus, $\delta^{13}C$ in these organisms became more depleted relative to epilimnetic mussels as compensation depth:mixed layer depth increased. Total phosphorus had a positive effect on TDO3, resulting in moderate oxygen levels deeper in the hypolimnion in lakes with low phosphorus levels. TDO3, in turn, had a negative effect on $\delta^{15}N_{DF}$ in hypolimnetic seston, epilimnetic and hypolimnetic zooplankton, *Chaoborus*, and cisco. Thus, $\delta^{15}N$ in these organisms became more enriched relative to epilimnetic mussels as TDO3 declined.
The most plausible mechanism for depleted $\delta^{13}C_{DF}$ in low-productivity lakes seems to be the use of respired CO$_2$ by hypolimnetic phytoplankton for photosynthesis (Francis et al. 2011). Respired CO$_2$ is depleted in $\delta^{13}C$ (reviewed by Hecky and Hesslein 1995), and adequate hypolimnetic light levels in low-productivity lakes facilitate net photosynthesis, incorporating depleted $\delta^{13}C$ and subsequently passing it on to primary and secondary consumers. Rau (1978) showed respired C could alter ecosystem-scale $\delta^{13}C$ patterns, and Francis et al. (2011) also found that hypolimnetic seston and zooplankton were depleted in $\delta^{13}C$ in high-clarity lakes and attributed it to use of respired CO$_2$ by seston in the hypolimnion.

For $\delta^{15}N$, multiple factors influence seston N isotope ratios, with nitrification, ammonification, and algal assimilation often depleting $\delta^{15}N$ in particulate matter, while denitrification, microbial degradation, and high production rates all enrich particulate matter (reviewed by McCusker et al. 1999). We found a pattern where $\delta^{15}N_{DF}$ was higher in hypolimnetic seston relative to epilimnetic seston in low TDO3 lakes, but then reversed such that $\delta^{15}N_{DF}$ was higher in epilimnetic seston than hypolimnetic seston in high TDO3 lakes. These observations are likely due to two different mechanisms, one occurring in the hypolimnion and the other in the epilimnion. In the hypolimnion, denitrification likely enriches hypolimnetic seston $\delta^{15}N$ in all of our study lakes (McCusker et al. 1999). However, the hypolimnetic seston N pool in unproductive, low TDO3 lakes is probably small relative to rates of denitrification which is likely occurring in anoxic sediments, allowing a net enrichment of the dissolved N pool, with subsequent uptake into seston, that is, phytoplankton. As lakes become more productive, a smaller proportion of the hypolimnetic inorganic N pool is denitrified due to high rates of epilimnetic production and settling of that material into the hypolimnion. In the epilimnion, higher primary production rates in the epilimnion of high TDO3 lakes reduce seston fractionation of N (Seitzinger 1988), enriching epilimnetic seston $\delta^{15}N$ relative to the hypolimnion. Increased enrichment in the epilimnion, combined with less enrichment in the hypolimnion, causes epilimnetic seston to be more enriched in $\delta^{15}N_{DF}$ relative to hypolimnetic seston in high TDO3 lakes. Thus, as TDO3 increases, the net result is a shift from hypolimnetic seston more enriched in unproductive lakes to less enriched in productive lakes. These effects are subsequently passed up the food web, such that $\delta^{15}N$ in hypolimnetic zooplankton, Chaoborus, and cisco is more enriched relative to epilimnetic mussels in low-productivity lakes.

Food web relationships subsequently caused the hypolimnetic seston $\delta^{13}C_{DF}$ and $\delta^{15}N_{DF}$ patterns to be passed up the food web. Zooplankton consumption of hypolimnetic seston altered their isotopic signature relative to non-mobile mussels feeding exclusively on epilimnetic seston, and similar results between our hypolimnetic and epilimnetic zooplankton indicate vertical migration in epilimnetic zooplankton that caused them to also acquire the isotopic pattern of hypolimnetic seston. Subsequent consumption of zooplankton by Chaoborus and cisco caused these secondary consumers to show the same $\delta^{15}C_{DF}$ and $\delta^{15}N_{DF}$ patterns relative to mussel as was found in hypolimnetic seston.

$\delta^{15}N_{DF}$ in hypolimnetic seston demonstrated a stronger relationship with TDO3 than it did with TP, likely due to the fact that oxygen patterns in the hypolimnion had a strong influence on seston $\delta^{15}N$. Microbial denitrification enriches seston $\delta^{15}N$ (Ostrom et al. 1997), and this process may be enhanced in low TDO3 lakes where there would be continuous or extended oxygen exposure times with increased potential for coupled nitrification–denitrification (Kristensen et al. 1995). Moreover, maximum lake depth was also negatively related to TDO3 ($P = 0.028$), indicating settling seston were exposed to microbial degradation in the water column for longer periods of time in low TDO3 lakes.

Clearly, more work needs to be done on the specific mechanisms involved, but our results indicate that $\delta^{13}C$ and $\delta^{15}N$ relationships between hypolimnetic and epilimnetic organisms change along lake productivity gradients, and these relationships appear to be driven by both epilimnetic and hypolimnetic processes. Oxygen (as measured by TDO3) influenced $\delta^{15}N$ and light (as measured by compensation depth: mixed layer depth) influenced $\delta^{13}C$ in the hypolimnion, while primary production rates are likely a key driver of $\delta^{15}N$ in the epilimnion. We also note that sampling each lake once is a
weakness of our study design, as isotope values are temporally variable in lakes, especially in lower trophic levels (Post 2002). However, the patterns we observed were robust enough to be detected despite isotope values likely changing through time and changing at different rates in different trophic levels.

Our results have implications for isotopic analysis of lake food webs, as failure to account for enriched δ15N and depleted δ13C in hypolimnetic organisms will cause biased estimates of trophic position and energy sources in isotope mixing models (sensu Post 2002). For example, in comparable data collected from a lake not used in this study (K. D. Zimmer et al., unpublished data), mean δ15N and δ13C in cisco were 11.6‰ and −31.2‰, respectively, while values of shallow-water fish (largely members of the Cyprinidae and Centrarchidae families) also feeding largely on invertebrate primary consumers were 9.5‰ (δ15N) and −26.3‰ (δ13C). Given a mean enrichment per trophic transfer of 3.4 (Post 2002), a piscivore feeding exclusively on cisco would be estimated as over half a trophic level higher than a piscivore feeding on the shallow-water fish species. For δ13C, importance of pelagic vs. littoral C would be overestimated for piscivores feeding on cisco due to the depleted δ13C in cisco relative to shallow-water fish. Though our study focused on cisco, it seems likely that any deep-water, pelagic species may also show the same pattern, including other coregonid species.

The importance of recognizing δ13C and δ15N change with depth in benthic species has been noted by other authors (Sierszen et al. 2006, Cummings and Schindler 2013), and other studies have shown that δ13C and δ15N differ between shallow- and deep-water pelagic organisms, including zebra mussels (Yohannes et al. 2014), zooplankton (Vander Zanden and Rasmussen 1999), and seston (Francis et al. 2011). However, ours is the first to document depth effects in both δ13C and δ15N in pelagic species, and to show that the relationship between hypolimnetic and epilimnetic organisms is not constant but depends on lake productivity. That similar patterns have been found in a variety of organisms in other studies indicates the pattern observed here is likely widespread. Studies involving multiple lakes that vary in trophic status need to be careful in testing for and quantifying differences in δ13C and δ15N between epilimnetic and hypolimnetic organisms, and potentially treat them as two separate sources for consumers when utilizing mixing models to estimate consumption patterns in food webs.

Stable isotopes are powerful tools for understanding lake food webs, predator–prey relationships, and pathways of energy flow. Our results indicate δ13C and δ15N in hypolimnetic organisms relative to epilimnetic organisms are influenced by lake trophic status. This suggests that differences in the physical and chemical environments, as well as differences in biogeochemical processing, result in enriched δ15N and depleted δ13C in the hypolimnion as lake productivity declines. These results are important for our understanding of how lake productivity influences C and N differently in shallow- and deeper-water habitats and is also important for accurate estimation of both trophic position and energy sources in lake food webs.

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Supporting Information

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