Zebra mussel $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as a proxy for depth-specific pelagic isotope profiles and lake temperature

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Abstract Zebra mussels (*Dreissena polymorpha*) can be used to provide a baseline stable isotope signature, time-integrated with primary production. However, since zebra mussels are uncommon in pelagic zones, their potential as reference species in pelagic water columns has not been fully explored. By investigating mussels growing suspended on a single vertical cable in Lake Constance, we were able to document seasonal (April, May, and August) and depth-dependent (0–22 m) variation in mussel $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. We found a strong correlation between temperature and mussel $\delta^{15}\text{N}$ from differing depths, and a strong relationship between temperature and $\Delta^{15}\text{N}$ (estimated as $\delta^{15}\text{N}_{\text{mussel}} - \delta^{15}\text{N}_{\text{POM}}$) and $\Delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{mussel}} - \delta^{13}\text{C}_{\text{POM}}$). In a pattern that remained consistent over all months, $\Delta^{15}\text{N}$ decreased with temperature, to the extent that negative values were recorded at temperatures $>13^\circ\text{C}$. Utilizing cable-dwelling pelagic mussels as indicators of variation in isotope and temperature for pelagic water could be

used as a novel field approach, comparable to in situ experimentation. We suggest that the pelagic mussel approach can be employed in tandem with benthic or littoral mussel isotope values, to develop mussel-based lake isoscapes. Such isoscapes may be pertinent to the study of seasonal trophic limnoecology and in tracking the movements of animals.

Keywords Stable isotopes · Particulate organic matter (POM) · Lake Constance · Temperature

Introduction

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes are used to describe ecological, physiological, and trophic relationships in a wide range of biological communities (e.g., Gannes et al., 1997; O'Reilly et al., 2002). Usually, the isotopic composition of organic compounds at the base of a given food web affects the isotopic values of organisms at higher trophic levels. Hence the isotopic composition of consumers can be expected to vary over space and time according to variations in basal isotopic values (Cabana & Rasmussen, 1996; Post, 2002). Isotopic studies take this potential difference into account by “calibrating” results against baseline isotopic values, obtained from primary producers or consumers deemed likely to reflect the isotopic variations observed (Post, 2002).

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The potential of a primary consumer to precisely and accurately reflect baseline isotopic values for a community depends on several factors, including the temporal and spatial extent of nutrient integration. Freshwater bivalves, such as zebra mussels (*Dreissena polymorpha*), are useful model organisms due to their ability to exhibit time-integrated isotopic values relative to primary producers. As filter feeders, mussels rely on detritus, algae, and other microorganisms; their relatively long life span, sessile nature, and ease of collection make them a good source of baseline isotopic data (e.g., Cabana & Rasmussen, 1996; Fry et al., 1999; Post, 2002). Zebra mussel stable isotope values have been used as references for studies on trophic status and as indicators of baseline isotope values (Haynes et al., 2005; Maguire & Grey, 2006; Vuorio et al., 2006).

However, as zebra mussels are invasive species and naturally scarce in pelagic zones, their usefulness in stable isotope studies is never extended to investigations of pelagic water columns. Our aim was to test the potential of zebra mussels growing suspended on a vertical cable in the pelagic zone of Lake Constance to provide a proxy for the baseline isotopic values of primary pelagic dietary sources. We conducted stable isotope analysis of pelagic zebra mussels ($\delta^{13}\text{C}_{\text{mussel}}$ and $\delta^{15}\text{N}_{\text{mussel}}$) growing at multiple depths (0–22 m) in the pelagic zone of the western basin of Upper Lake Constance (Überlinger-See 47°76'N 9°13'E) over a 3-month investigation period (April, May, and August 2012). In May, we used samples of particulate organic matter (POM) collected from multiple depths (0–22 m) to explore depth-based POM isotopic patterns for a single month. However, as a result of wave-induced turbulence with horizontal or vertical relocation of organic matter mean resident mussel isotope values may not always correspond with fluctuating in-point POM isotope values. To reduce such errors, we applied monthly POM isotope values, representing isotopic integration over time. For this purpose, we analyzed POM isotope values using mixed water samples collected from the 0–20 m water column (for each of the 3-month investigations: April, May, and August 2012). We also related depth-specific mussel $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles to lake temperatures recorded during each sampling month and a month earlier.

Materials and methods

Sample collection

Zebra mussels

Zebra mussels were collected from the pelagic zone of Lake Constance, where they had naturally recruited to a single cable installed on June 2010 and lowered from a buoy to a depth of 25 m. Cable was lifted up and mussels of random size were sampled at 2 m intervals from the surface (0 m) down to their maximum depths of 0–19 m in April, 22 m in late May, and 0–12 m in August 2012. Up to 15 individuals were collected from each depth, each month.

Particulate organic matter

In April, May, and August, we obtained POM from mixed water samples collected every 2 weeks from the upper 0–20 m water column. These POM isotopic values ($\delta^{15}\text{N}_{\text{POM}}$ or $\delta^{13}\text{C}_{\text{POM}}$) were averaged over a given month (three sampling months). To obtain POM isotopic values, from each water sample, a sub-sample of ca. 2 l was filtered through a pre-combusted Whatman GF/F filter on the day of collection. For the three study months, monthly average POM isotope values derived from mixed water samples (0–20 m water depth) were used to estimate isotopic differences between zebra mussel and POM by subtracting POM values from that of the zebra mussels:

$$\Delta^{15}\text{N} = \delta^{15}\text{N}_{\text{mussel}} - \delta^{15}\text{N}_{\text{POM}},$$

$$\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{mussel}} - \delta^{13}\text{C}_{\text{POM}}.$$

Additionally in May, POM isotope values were assessed using water collected from depth-specific water samples that correspond to depths from which zebra mussel samples were obtained. Briefly, for May only, depth-specific POM isotopic values (derived from depth-specific water samples) were used to estimate isotopic differences between zebra mussel and POM ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$).

Depth-specific ambient lake temperature

Ambient lake temperatures were profiled every week in April, May, and July using data collected from

multiple depths by calibrated conductivity temperature depth (CTD) probes. These data were used to examine the relationship between depth-specific monthly zebra mussel isotope values and average lake temperatures for the same month, the previous month, and the average over 2 months.

Stable isotope analysis

Dried and pulverized whole body samples (except mussel shell) were weighed (ca. 0.7 mg) in tin cups to the nearest 0.001 mg, using a micro analytical balance. Samples were then combusted in a vario Micro cube elemental analyzer (Elementar, Analysensysteme, Germany). The resulting CO₂ and N₂ were separated by gas chromatography and passed into a Micromass (Isoprime Ltd., Manchester, UK) isotope ratio mass spectrometer (IRMS) for determination of ¹³C/¹²C or ¹⁵N/¹⁴N ratios.

All stable isotope values ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) are reported in the δ notation (per mill) where $\delta = (1,000 \times [R_{\text{sample}}/R_{\text{standard}}] - 1)\text{‰}$; relative to the Pee Dee Belemnite (PDB) for carbon and atmospheric N₂ for nitrogen in parts per thousand deviations (‰). Two sulfanilamides (Iso-prime internal standard) and two caseins were used as laboratory standards for every 10 unknowns in sequence. Replicate assays of internal laboratory standards indicate measurement errors (SD) of ± 0.05 and 0.12‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Lipids are depleted in ¹³C compared to whole organisms (or other tissues) and the lipid content of individual animal tissue samples can be variable (Peterson & Fry, 1987; Kling et al., 1992). Thus, measurements of $\delta^{13}\text{C}$ were normalized for lipid content following the equation for aquatic organisms described in Post et al. (2007).

Statistical analysis

A Kolmogorov Smirnov test (and Levene's test of homogeneity of variance) showed that all variables were normally distributed (each element tested separately). Analysis of variance (ANOVA) followed by a Scheffe's post-hoc test were performed to detect differences between the 3 months. For each month and each element separately, a regression model was used to investigate the relationship between stable isotope values and depth. Isotopic differences across depths and months were tested applying analysis of variance

with a model of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ taking month, depth, and the interaction term month $\times$ depth as factors. The relationships between zebra mussel stable isotope values and average temperature were examined using Pearson correlation.

Results

Time-based and depth-specific $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

Mean ($\pm$ SE) of mussel $\delta^{15}\text{N}$ values for April, May, and August were $7.8 \pm 0.1\text{‰}$ ($N = 45$), $6.0 \pm 0.1\text{‰}$ ($N = 40$), and $6.7 \pm 0.1\text{‰}$ ($N = 24$), respectively. Mean ($\pm$ SE) monthly mussel $\delta^{13}\text{C}$ values were $-34.3 \pm 0.1\text{‰}$, $-34.7 \pm 0.04\text{‰}$, and $-31.4 \pm 0.2\text{‰}$, with a ca. 3‰ increase in August compared to the other 2 months. ANOVA revealed significant differences between months for mussel $\delta^{15}\text{N}$ ($F_{2,108} = 86$, $P < 0.001$) and mussel $\delta^{13}\text{C}$ ($F_{2,108} = 151$, $P < 0.001$). Post-hoc tests showed that, while the difference in $\delta^{13}\text{C}_{\text{mussel}}$ between April and May was marginally significant ($P = 0.04$), all other months showed significant differences ($P < 0.001$, for both elements).

For all the three sampling times, mussel $\delta^{15}\text{N}$ showed an increase with depth (Fig. 1). ANOVA (mussel $\delta^{13}\text{C}$: $F_{1,110} = 28.57$, $r^2 = 0.91$, $P < 0.001$) indicated a significant effect of month ($F_{1,2} = 215.86$, $P < 0.001$), depth ($F_{1,12} = 7.89$, $P < 0.001$), and the interaction of month $\times$ depth ($F_{1,14} = 4.61$, $P < 0.001$). Significant effects were also recorded for month ($F_{1,2} = 135.08$, $P < 0.001$), depth ($F_{1,12} = 11.11$, $P < 0.001$), and for month $\times$ depth ($F_{1,14} = 1.89$, $P = 0.04$) for mussel $\delta^{15}\text{N}$ ($F_{1,109} = 19.20$, $r^2 = 0.87$, $P < 0.001$), Table 1.

April, May, and August monthly values of POM $\delta^{15}\text{N}$ (mean $\pm$ SE) averaged from mixed water samples were 4.9 ± 0.3 , 9.7 ± 0.8 , and $5.9 \pm 0.4\text{‰}$, respectively. Mean ($\pm$ SE) POM $\delta^{13}\text{C}$ values in April, May, and August were -33.2 ± 0.4 , -30.3 ± 1.0 , and $-27.8 \pm 0.2\text{‰}$, respectively.

The relationships of $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ to depth are shown in Fig. 2. May $\Delta^{15}\text{N}$ was strongly correlated to depth (Pearson correlation coefficients $\Delta^{15}\text{N}$: $r = 0.94$, $P < 0.001$; $\Delta^{13}\text{C}$: $r = 0.38$, $P = 0.31$).

Isotopic patterns and lake temperature

As shown in Fig. 3, the stratification of Lake Constance at our study site started in April with a

Fig. 1 Depth related stable isotope values and patterns in zebra mussels and POM (May) from three sampling months (mean $\pm$ SE)

Table 1 Relationship between zebra mussel stable isotopes and depth for the 3 months (* $P < 0.05$)

Depth	$\delta^{13}\text{C}_{\text{zebra mussels}}$	$\delta^{15}\text{N}_{\text{zebra mussels}}$
April		
r^2	0.05	0.66
P	0.63	<0.001*
n	45	45
May		
r^2	0.02	0.27
P	0.76	0.01*
n	40	40
August		
r^2	0.91	0.79
P	<0.001*	<0.001*
n	24	24

temperature gradient developing from the surface down to 22 m depth. Surface temperatures increased and temperature gradient became more pronounced from April to May and into August. For each sampling date, mussel isotope values were strongly correlated with lake temperature values (particularly in August, Fig. 4). Interestingly, mussel $\delta^{15}\text{N}$ exhibited a stronger relationship with ambient lake temperature in all study months and at all depths (Tables 1, 2). These results provide evidence that the nitrogen isotope composition of bivalves responds to changes in depth-specific lake temperature profiles. The lack of tight

coupling (in April and May but not in August) between mussel $\delta^{13}\text{C}$ and depth might also point to temperature differences between August and April or May; and hence to lake's pattern of seasonal thermal gradient.

Mussel $\delta^{15}\text{N}$ values were strongly correlated with depth-specific temperature values for the three sampling dates, while for $\delta^{13}\text{C}$ values this correlation only held in August (Table 2; Fig. 4).

Interestingly, the differences between mussel and POM $\delta^{15}\text{N}$ values ($\Delta^{15}\text{N}$ estimated from integrated monthly POM) appeared to be clearly related to ambient temperature. In a consistent pattern over all months, $\Delta^{15}\text{N}$ decreased with temperature, even reaching negative values at temperatures $>13^\circ\text{C}$.

The relationships of $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ (based on depth-integrated POM samples) to temperature are shown in Fig. 5. Temperature was strongly correlated to $\Delta^{15}\text{N}$ (Pearson correlation coefficients $\Delta^{15}\text{N}$ $r = -0.94$, $P < 0.001$).

Discussion

Spatio-temporal variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

To assess depth-based mussel $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ variability between months, we sampled pelagic mussels growing in Lake Constance between April and August. We found both time-based and depth-specific

Fig. 2 Relationship between mean $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$, and depth during May. $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were calculated using POM isotopic values derived from depth specific water samples

Fig. 3 Water temperature from surface to 22 m depth, measured with a calibrated conductivity temperature depth (CTD) probe

variation in pelagic mussel $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Within-lake seasonal fluctuation and depth-related variation in isotopic values of pelagic zooplankton have been reported for several other lakes (e.g., Matthews & Mazumder, 2003; Santer et al., 2006; Syväranta et al., 2006). Additionally, the spatial and temporal variations observed here are in accordance with earlier studies conducted on zebra mussels in other lakes (e.g., Guzzo et al., 2011).

Our April and May $\delta^{13}\text{C}_{\text{mussel}}$ values were almost identical. This temporal similarity could be a function of minimal variation in baseline $\delta^{13}\text{C}_{\text{POM}}$ during over a 2-month period. This is further supported by POM

Fig. 4 Relationship between water depth, lake temperature (gray marker, geometric mean of July and August), and stable isotope values (black marker) in mussels (August)

$\delta^{13}\text{C}$ values for May, wherein POM $\delta^{13}\text{C}$ values (range -34.2 to -35.2‰) are highly consistent over the 10 sampling depths (Fig. 1b). Comparable $\delta^{13}\text{C}$ values [ca. -35 and -33‰ , respectively (without lipid correction)] have previously been observed in benthic zebra mussels collected from an area of Upper Lake Constance very close to our study at one meter depth and below mean low water depth (October 2005 and 2006, Gergs et al., 2011).

Zebra mussels from a littoral zone [Lower Lake Constance; Iznang, $47^{\circ}42'47.7''\text{N}$, $8^{\circ}58'24.6''\text{E}$,

Table 2 Pearson correlation between zebra mussel stable isotopes and lake temperatures for the same month, the previous month, and the average over 2 months (* $P < 0.05$)

		Temperature (°C)			
		April $\delta^{15}\text{N}$ (‰), $n = 12$		April $\delta^{13}\text{C}$ (‰), $n = 12$	
March	r	0.95, $P < 0.001^*$		r	0.07, $P = 0.83$
April	r	0.91, $P < 0.001^*$		r	0.58, $P = 0.04^*$
Mean	r	0.93, $P < 0.001^*$		r	0.14, $P = 0.66$
		May $\delta^{15}\text{N}$ (‰), $n = 10$		May $\delta^{13}\text{C}$ (‰), $n = 10$	
April	r	0.27, $P = 0.46$		r	0.52, $P = 0.12$
May	r	0.81, $P < 0.01^*$		r	0.23, $P = 0.53$
Mean	r	0.80, $P < 0.001^*$		r	0.42, $P = 0.22$
		August $\delta^{15}\text{N}$ (‰), $n = 7$		August $\delta^{13}\text{C}$ (‰), $n = 7$	
July	r	0.99, $P < 0.001^*$		r	0.99, $P < 0.001^*$
August	r	0.98, $P < 0.001^*$		r	0.98, $P < 0.001^*$
Mean	r	0.99, $P < 0.001^*$		r	0.99, $P < 0.001^*$

Matuszak (2012)] collected at 2 m depth between June and October 2008 and 2009 exhibited $\delta^{15}\text{N}$ ca. 6‰ and $\delta^{13}\text{C}$ of ca. -28 ‰ (after lipid normalization), indicating that littoral mussels are relatively more enriched in ^{13}C than our pelagic specimens (range -34.7 to -31.4 ‰). In contrast to our findings, Matuszak (2012) reported a lack of depth-specific (0–8 m) variation in littoral mussel $\delta^{15}\text{N}$ collected between June and August 2009.

The depth-specific variation in pelagic mussel $\delta^{13}\text{C}$ reported in this study for August has an identical pattern to that reported for littoral mussels in June and July by Matuszak (2012). Two previous studies (Gergs et al., 2011; Matuszak et al., 2011) and our present assessment allow comparison of benthic, littoral, and pelagic zebra mussels from similar and differing geographic locations within the same lake. The results are in accordance with the generally expected variation in isotopes between benthic, littoral, and pelagic habitats, between years and in different seasons, and point to the suitability of zebra mussels as faithful indicators of isotopic conditions of different lake zones.

Water depth, lake temperature, and mussel stable isotope

Fresh water mussels living in higher temperature might have relatively elevated metabolic rate; and a

Fig. 5 Relationship between $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ and temperature. $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were calculated using mean monthly POM isotopic values derived from mixed water samples (0–20 m water depth)

relatively significant increase in carbon and nitrogen turnover rate (MacMahon, 1983). Thus, isotopic turnover rates are expected to vary between animals living at different temperatures (and, therefore, water depths or seasons). Our results fit with this hypothesis in that mussel $\delta^{13}\text{C}$ value was much more strongly associated with depth in August, when the lake water temperature had a highly pronounced gradient, than in April and May.

Assuming that POM represents the major dietary source for zebra mussels in May (and assuming that no other eco-physiological variables alter the temporal equilibrium in isotope turnover), average May $\Delta^{15}\text{N} = (\delta^{15}\text{N}_{\text{mussel}} - \delta^{15}\text{N}_{\text{POM}})$ is ca. 1.2‰ greater at depth than at the surface. Variations in stable isotope turnover rate associated with temperature could translate into ecologically significant spatio-temporal variation in isotopes values. Generally, $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ were seen to be more positive during cooler seasons. In

a recent study, Guzzo et al. (2011) report variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for seston and zebra mussels collected in Lake Erie. Mussel $\delta^{15}\text{N}$ was not always more enriched than seston $\delta^{15}\text{N}$ values. In the warmer months of July and August, $\Delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{mussel}} - \delta^{15}\text{N}_{\text{seston}}$) values were more negative (ca. -1.5 and -3‰ in July and August, respectively) and more positive in cooler months [e.g., in September $\Delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{mussel}} - \delta^{13}\text{C}_{\text{seston}}$) ca. $+3\text{‰}$]. Several studies that deal with tropical aquatic food webs note that $\Delta^{15}\text{N}$ is generally much smaller than in typical temperate systems (ca. 1.6‰ compared to the expected ca. 3.4‰ per trophic transfer) while $\Delta^{13}\text{C}$ is rather high (ca. 1.4‰ per trophic level compared to the typical ca. 0.5‰ per trophic level) (Kilham & Pringle, 2000; Verburg et al., 2007; Kilham et al., 2009). These observations highlight the need for further investigation of the effects of temperature on stable isotope turnover rates and diet tissue discrimination patterns.

Alternatively, since mussels are selective feeders, it may be that isotope values observed for bulk POM are not always accurately representative of the isotope values of actual food, particularly during warm and productive seasons. Seasonal dietary shifts toward other food sources might result in more negative $\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$ during warmer seasons. In pelagic oligotrophic lakes, however, POM is likely to constitute a major part of the diet of mussels in deeper water columns and in cooler seasons.

Finally, May POM isotopic value obtained from depth-specific and column-integrated water samples showed considerable difference. This could be related to rapid temporal variation in POM isotopes. Column-integrated water samplings were conducted on 8th and 22nd of May, while depth-specific water and zebra mussel sampling was conducted on the 31st of May. POM stable isotope analysis conducted as part of our routine lake-monitoring activity, using mixed water samples, show that for almost all months there exist a considerable variation in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values within a period of a week or less (Yohannes et al. unpublished data). The mean isotopic values from depth-specific POM collected at the end of May have a mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ comparable to the isotope values of POM in early June.

To summarize, this short-term field study identifies temperature as a key parameter in isotopic studies of secondary consumers. Being sessile, relatively slow to respond to lake isotope conditions, but reliable in

reflecting depth-specific temperatures (for at least the 3 months that we studied), pelagic mussel isotope values may usefully serve as models for an “isotopic temperature clock.” Differences in temperature between depths, estimates of turnover rate of mussel isotopes, and the magnitude of mussel isotopic shift at particular temperatures might be used to estimate time elapsed since a temperature change took place.

Overall, utilizing sessile, cable-dwelling zebra mussels as indicators of variation in isotopic values in pelagic water could be used as a novel field approach, allowing for in situ experimentation. The method is potentially suited to long- or short-term monitoring of isotope values in pelagic waters, and sampling is much less costly than for mobile zooplankton. Developing this potential will require well-designed experimental frameworks from which isotopic turnover rates and trophic discrimination patterns can be accurately estimated. Without empirical data on isotopic tissue turnover rates on different temperatures, depths, and dietary conditions, the method remains limited and there remain difficulties in interpreting the results. Conclusion on zebra mussels as indicators of spatio-temporal isotopic variation in isotopic values of primary producers in pelagic water must be taken with cautions.

Yet, zebra mussels can also provide a reasonable comparison of pelagic, littoral, and benthic systems within the same lake, as they are often present in these habitats, unlike other secondary consumers. Therefore, in pelagic zones and in lakes already harboring this invasive species, zebra mussels can provide a reliable reflection of depth-specific isotopic values and seasonal variability between and within habitats of the same lake. Tandem evaluation of season- and depth-related isotopic signatures would provide a useful approach to identifying both long- and short-term changes in lake isoscapes. Lake isoscapes based on this species might also be beneficial to isotope studies attempting to track movements of other animals (e.g., fishes and daphnia).

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