

Sympatric variability of isotopic baselines influences modeling of fish trophic patterns

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Abstract Stable isotope signatures of freshwater snails and mussels have been established as a convenient baseline measurement at the primary consumer level for food-web coupling studies. We measured $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of primary consumers, including mussels (*Anodonta woodiana*, *Cristaria plicata*, and *Unio douglasiae*), snails (*Bellamya aeruginosa* and *Hippetis* sp.), and zooplankton from the same habitat within a shallow eutrophic lake. Primary consumers showed positive relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, indicating a linkage between planktonic and benthic habitats in this system. The variation in isotope ratios was higher in short-lived primary consumers (zooplankton) compared with the long-lived primary consumers (mussels and snails), suggesting limited availability of short-lived primary consumers as isotopic baselines in aquatic food-web assessment. Significant differences in isotope ratios were also found among three species of mussels, and when using these mussels separately as pelagic baselines to calculate trophic position and contribution of planktonic and benthic sources of fishes, bias and even misestimates were observed. This finding suggests that caution is needed when multiple primary consumers coexist in the same

habitat, and it is important to assess potential effects of different baselines used.

Keywords Mussel · Snail · Stable isotope · Baseline · Trophic modeling · Trophic interaction · Shallow lake · Eutrophication

Introduction

Stable isotope analysis is commonly used to study food-web structures and material flows in a wide range of aquatic ecosystems (e.g., Cabana and Rasmussen 1996; Vander Zanden et al. 1999; Post 2002; Lee et al. 2002; Gu et al. 2004; Aberle et al. 2005; Uchii et al. 2007; Maki et al. 2009). Nitrogen and carbon are two of the most commonly used elements in isotopic studies of aquatic food webs. Both carbon (C) and nitrogen (N) isotopes show relatively constant fractionation patterns along the food chains, with a mean trophic enrichment of consumer tissue relative to their food source of approximately $3.4 \pm 1\%$ in the $\delta^{15}\text{N}$ values and $0.4 \pm 1.3\%$ in $\delta^{13}\text{C}$ (Post 2002). Moreover, in lakes, $\delta^{13}\text{C}$ is useful for assessing the two major sources of organic matter – littoral production from attached algae and detritus, and pelagic production from phytoplankton – because of depletion (more negative $\delta^{13}\text{C}$) of the base of the littoral food web with regard to that of the pelagic food web (France 1995; Yoshii 1999; Doi et al. 2009). However, considerable variations in the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of the base of the food web have been found within (France 1995; Vander Zanden and Rasmussen 1999; Xu et al. 2005a) and among (Rounick and Winterbourn 1986; Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Xu et al. 2005b, 2008) ecosystems, which baffled ecologists for comparison among multiple

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ecosystems and even multiple sites within a single ecosystem. Producers are highly variable depending on their surrounding physicochemical environments, which has hindered the accurate determination of isotope values (Xu et al. 2005a, b). Therefore, an accurate estimate of baseline values of stable C and N isotopes in interested ecosystems is obviously important to assess the changes in food-web structure and C flow (Post 2002).

Because $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of consumers are a time-integrated reflection of their historic diet assimilation, the direct use of isotopic values of primary producers, which often changed in time and space, as baselines can complicate the interpretation of trophic relations and energy flows in the studied systems (Cabana and Rasmussen 1996). Post (2002) recently provided empirical evidence that low metabolic rate and long-lived primary consumers, mussels and snails, were good temporal integrators of the isotopic variation at the base of the food webs because they integrate highly variable isotope values among primary producers despite considerable temporal variation in the isotopic compositions of the primary producers (Cabana and Rasmussen 1996; McKinney et al. 2001; Post 2002). Therefore, long-lived primary consumers, such as mussels and snails, were suggested as good temporal integrators of the isotopic variation at the base of the food webs, and also effectively reflect spatial differences between different food webs. Meanwhile, the isotopic signature of snails similar to that of periphyton and detritus that forms the base of the littoral or benthic food web, and the isotopic compositions of mussels is similar to that of seston that forms the base of the pelagic or planktonic food web (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). Since stable isotope compositions of these long-lived primary consumers can serve as time-integrated indicators of trophic position (TP) and energy pathways, many aquatic scientists have attempted to answer critical questions in food-web studies using mussels and snails as pelagic and benthic baselines, respectively, especially when conducting comparisons across multiple systems (e.g., Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002; Vander Zanden et al. 2003; Fukumori et al. 2008).

In most previous stable isotope studies, less attention was paid to within- and between-species variations in stable isotope compositions of long-lived primary consumers, which is likely to complicate the explanation of ecological observation in a single ecosystem or across multiple systems when using primary consumers as baselines in the isotopic mixing model. Thus, the aims of this work were to examine the within- and between-species variations in the relative abundance of stable C and N isotopes of mussels and snails sampled from the same habitat in a shallow, eutrophic lake and to evaluate their use as planktonic and

benthic baselines. Furthermore, when differences in isotopic compositions of these long-lived primary consumers are found, the consequence of TP and percentage concentrations of planktonic prey sources of fishes is assessed using different primary consumers as baseline in an isotopic mixing model to provide insights into the future use of these animals as baselines of food-web study.

Materials and methods

Field collection and lab processing

Samples were obtained from the area 1000–1500 m offshore (water depth 1.8–2.3 m and water transparency 30 cm) located at Meiliang Bay ($31^{\circ}26'44''\text{N}$, $120^{\circ}10'25''\text{E}$), Lake Taihu, the third largest freshwater lake in China, on 15 October 2004 (Fig. 1). In our studied system, detritus mainly contains materials from deposit phytoplankton and sedimentary organic matters from anthropogenic input, and organic matter from aquatic macrophyte, terrestrial plant, and benthic algae are expected to be very little because of rare biomass of these plants. Our study area was a hypereutrophic part of Lake Taihu dominated by blue green algae. Sympatric long-lived primary consumers, mussels and snails, were collected by hauling a bottom trawl net $\sim 2\text{--}3$ min with boat velocity of ~ 4 km per h. Mussels and snails were transported to the lab live and were cleaned and dissected. Muscle tissue was then taken for stable isotope analysis because shell material is enriched in ^{13}C and does not reflect what is actually assimilated by consumers (Mitchell et al. 1996).

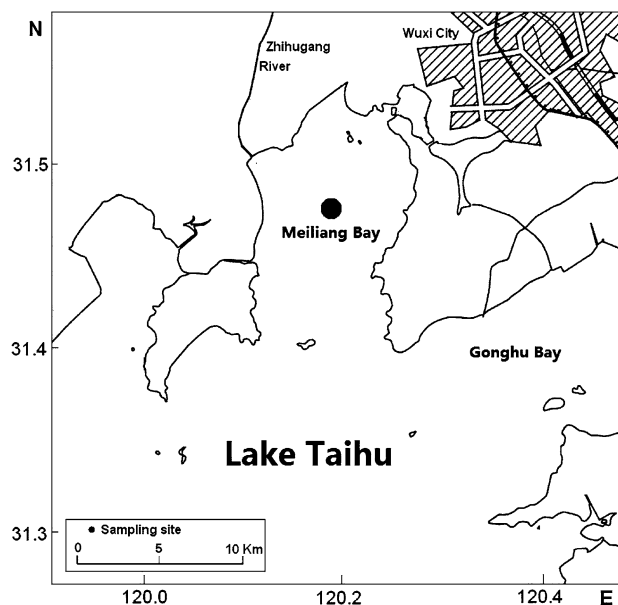


Fig. 1 Sampling site of this study

Zooplankton were collected in the surface water (0–1 m) using a 40 m diameter conical plankton net from the area used for benthic sampling, which avoided any contamination of these samples by resuspended sediment, and were then separated from detritus and phytoplankton using pipes and pins under a stereomicroscope. Zooplankton was then washed and kept in distilled water for about 2 h to allow for gut clearance to eliminate bias in the stable isotope signature of the organism due to items in the recent diet. Fishes were provided by fishermen fishing in our sampling site. A filet of dorsal muscle (white muscle) was removed from each fish. The muscle tissue has been shown to be representative of the overall stable isotope signature in fish (Hesslein et al. 1993).

In the laboratory, samples were either processed immediately or kept frozen for later process. Before analysis, samples were sorted, rinsed with distilled water, and dried in an oven at 60°C to a constant weight. Samples were then ground to a fine powder with a mortar and pestle. The mortar and pestle were washed and dried to prevent cross-contamination between samples. Powder of samples was kept in Eppendorf tubes and sealed in desiccators with silica gel for later analysis.

Studied mussels and snails

Snails, *Bellamya aeruginosa* and *Hippeutis* sp., and mussels, *Anodonta woodiana*, *Cristaria plicata*, and *Unio douglasiae*, are native to the inland waters of China and Southeast Asia (Liu et al. 1979; Han and Shu 1995). Some species have been recorded on the other continents as invasive species. In addition, many folks in this region use these freshwater mussels or snails as a supplemental food source, although these animals concentrate chemicals and other pollutants from the water column and/or surface sediment. Ages of mussel and snail in this study were estimated by growth rings on their shell surface and by age–body weight regressions established based on specimens collected from mid–lower basins of the Yangtze River in previous studies (Chen and Song 1975; Liu et al. 1979; Zhou et al. 1995; Zheng and Wei 1999; Yan et al. 2000). Ages of *B. aeruginosa* and *Hippeutis* sp. ranged from 1 to 7 and from 1 to 5 years, respectively (Chen and Song 1975; Yan et al. 2000), and ages of *A. woodiana*, *C. plicata*, and *U. douglasiae* ranged from 1 to 8, 1 to 4, and 1 to 3 years, respectively (Liu et al. 1979; Zhou et al. 1995; Zheng and Wei 1999). Therefore, compared with short-life-span primary consumers, e.g., zooplankton, mussels and snails in this study could be regarded as long-lived primary consumers. A few investigations of the feeding ecology of these mussels and snails are available. The recent detailed investigations of the food composition of these macroinvertebrates indicated that: (1) snails

(*B. aeruginosa* and *Hippeutis* sp.) consumed a wide range of food items, but mainly detritus (81.2–89.1%) and sand grains (0–8.3%), and algae (including cyanobacteria, diatoms, and green algae) only accounted for 0.3–5.5% of total diet composition; (2) mussels (*A. woodiana*, *C. plicata*, and *U. douglasiae*) fed on primarily algae (54.1–69.1%, mainly cyanobacteria), rotifers (0.7–12.3%), crustaceans (2.2–8.6%), protozoans (0.7–7.9%), and detritus, which accounted for 22.1–29.2% of total diet composition (Liu 2006; Y. Liu, unpublished data). Thus, one can assume that these long-lived primary consumers could be used as benthic and planktonic baselines in trophic studies using stable isotopes (Post 2002).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements

Stable C and N isotope ratios were analyzed with Delta Plus (Finnigan) continuous-flow isotope ratio mass spectrometer (CF-IRMS) directly coupled to an NA2500 elemental analyzer (Carlo Erba). The isotopic compositions of samples were expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ notation using the equations, as follows:

$$\delta^{13}\text{C}(\text{‰}) = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right) \times 10^3$$

$$\delta^{15}\text{N}(\text{‰}) = \left(\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} - 1 \right) \times 10^3$$

where ‰ is parts per thousand and $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ are the atomic ratios of the number of atoms in the sample or standard, and δ is the measure of heavy to light isotope in the sample, whereby higher δ values denote a greater proportion of the heavy isotope. The international standards are Vienna Pee Dee Belemnite (VPDB) and the Earth's atmospheric N for C and N, respectively. International reference materials were carbonatite (IAEA-NBS18) for $\delta^{13}\text{C}$ and ammonium sulfate (IAEA-USGS25) for $\delta^{15}\text{N}$. On a daily basis, an internal working standard, urea ($\delta^{13}\text{C} = -49.44\text{‰}$ and $\delta^{15}\text{N} = -1.53\text{‰}$), was used for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. More than 15% of the samples was analyzed more than three times. The standard deviation (SD) for the replicate samples was 0.2 and 0.3‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Modeling trophic position and dietary contribution of fishes

To compare variations in reliance on benthic versus planktonic prey of fishes (planktivorous *A. nobilis*, omnivorous *C. auratus*, and carnivorous *C. erythropterus*), an isotope-mixing model was used to estimate the contribution of planktonic secondary production to fishes by using the formula:

$$\text{Percent planktonic} = \frac{\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{benthic}}}{\delta^{13}\text{C}_{\text{planktonic}} - \delta^{13}\text{C}_{\text{benthic}}} \times 100\%$$

where $\delta^{13}\text{C}_{\text{fish}}$, $\delta^{13}\text{C}_{\text{planktonic}}$ and $\delta^{13}\text{C}_{\text{benthic}}$ are the mean $\delta^{13}\text{C}$ of the fish, planktonic baseline (zooplankton and each mussel species), and benthic baseline (mean of two snail species), respectively. If the $\delta^{13}\text{C}$ value of the consumers fell beyond either end member in the mixing model (indicating 100% planktonic reliance or 0% planktonic reliance), values were set at either 100% or 0% (Vander Zanden et al. 2003). This occurred in 40% of the estimates (see Table 2). To capture potential spatial heterogeneity in $\delta^{15}\text{N}_{\text{baseline}}$ for fishes that feed on both planktonic and benthic food webs, TP was calculated as follow:

$$\text{Trophic position} = \left\{ \delta^{15}\text{N}_{\text{fish}} - \left[\delta^{15}\text{N}_{\text{planktonic}} \times \alpha + \delta^{15}\text{N}_{\text{benthic}} \times (1 - \alpha) \right] \right\} / 3.4 + \lambda$$

where 3.4 is the generally assumed per trophic level enrichment of $\delta^{15}\text{N}$ (Post 2002); α is the proportion of N in the consumer ultimately derived from the base of planktonic food web and is estimated using C isotopes by the formula mentioned above (Post 2002); $\lambda = 1$ for use of primary producers as planktonic–benthic baseline, $\lambda = 2$ for use of primary consumers as baseline, that is the case of our TP estimation; $\delta^{15}\text{N}_{\text{fish}}$, $\delta^{15}\text{N}_{\text{planktonic}}$ and $\delta^{15}\text{N}_{\text{benthic}}$ represent the mean $\delta^{15}\text{N}$ of the fish, planktonic baseline (mean of zooplankton and each mussel species, respectively), and benthic baseline (mean of two snail species), respectively. Note that these models use primary consumers (rather than primary producers) as isotopic endpoints and thus estimate the contributions of planktonic and benthic secondary production, assuming that there is no trophic enrichment in $\delta^{13}\text{C}$ (trophic fractionation = 0‰) and that mixing is linear (Post 2002; Vander Zanden and Vadeboncoeur 2002).

Statistical analysis

STATISTICA version 6 software (StatSoft Inc., Tulsa, USA) was used for the relative analyses. Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between primary consumers were statistically assessed using Student's *t* test. The relationship between animal weight and stable isotopic values was quantified using linear regression. The variation relative to the mean of each secondary consumer was calculated from the coefficients of variation ($\text{CV} = \text{SD}/\text{mean} \times 100$) (Bunn et al. 1995).

Results

Stable C and N isotope ratios indicated that there was a difference between planktonic and benthic sources in the study area. Primary consumers with planktonic food habit, including zooplankton (mean \pm SD, $\delta^{13}\text{C} = -27.7 \pm 1.1$, $\delta^{15}\text{N} = 13.9 \pm 4.2$, $n = 3$) and mussels *Anodonta woodiana*, *Cristaria plicata*, and *Unio douglasiae*, were more depleted in heavy C and N isotopes than those with benthic food habit, including snails *B. aeruginosa* and *Hippeutis* sp. (Table 1).

Snail $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were significantly higher than those of mussels and zooplankton ($p < 0.001$), whereas no significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were found between the two snails. Significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were also found among mussel species and between mussels and zooplankton ($p < 0.05$). The variation in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was about 2.5–10.4 and 1.1–5.8 times higher in short-lived primary consumer (zooplankton) compared with long-lived primary consumers (mussels and snails). Thus, the CV for $\delta^{13}\text{C}$ was 3.9% for zooplankton compared with 0.7–3.6% for long-lived primary consumers. For $\delta^{15}\text{N}$, the

Table 1 Carbon (C) and nitrogen (N) stable isotopic ratios of primary consumers (mussels, snails, and zooplankton) collected from Lake Taihu, China

Sample name	Number	$\delta^{13}\text{C}$ (‰)				$\delta^{15}\text{N}$ (‰)			
		Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
<i>Bellamyia aeruginosa</i>	21	-22.9	0.8	-24.3	-21.2	15.7	0.7	14.5	17.0
<i>Hippeutis</i> sp.	10	-23.2	0.6	-23.8	-22.0	15.7	0.8	14.4	16.9
<i>Cristaria plicata</i>	8	-24.7	0.2	-24.9	-24.4	12.5	0.4	12.0	12.9
<i>Unio douglasiae</i>	6	-27.4	0.5	-28.2	-27.0	10.5	0.5	9.8	10.9
<i>Anodonta woodiana</i>	22	-29.5	0.8	-31.4	-28.4	5.6	0.7	3.8	6.7
Average of all snails	31	-23.0	0.8	-24.3	-21.2	15.7	0.8	14.4	17.0
Average of all mussels	36	-28.0	2.1	-31.4	-24.4	8.0	3.1	3.8	12.9
Zooplankton	3	-27.7	1.1	-28.8	-26.4	13.9	4.2	9.3	17.6

SD standard deviation, *Min.* minimum, *Max.* maximum

corresponding CVs were 30.2% for zooplankton and from 1.1% to 5.8% for long-lived primary consumers.

Simple linear regression analysis was conducted to examine whether there was any correlation between stable isotope values and the body weight of snails and mussels (Figs. 2 and 3). Muscle $\delta^{13}\text{C}$ of snails *B. limnophila* and *Hippeutis* sp. were negatively correlated with the individual body mass ($r^2 = 0.31$, $p < 0.01$, $n = 21$ and $r^2 = 0.50$, $p = 0.02$, $n = 10$), whereas $\delta^{15}\text{N}$ values of *B. limnophila* showed significant positive relationship with body weight ($r^2 = 0.26$, $p = 0.02$, $n = 21$) and no significant correlation between $\delta^{15}\text{N}$ values of *Hippeutis* sp. and the body weight was found ($r^2 = 0.34$, $p = 0.08$, $n = 10$). Relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of snails was not significant ($r^2 = 0.01$, $p = 0.62$ for *B. limnophila* and $r^2 = 0.28$, $p = 0.12$ for *Hippeutis* sp.). Patterns of changes in stable isotope values with individual body weight of three mussels were variable (Fig. 3), and positive relationship were found between $\delta^{13}\text{C}$ and body weight of *A. woodiana* ($r^2 = 0.45$, $p < 0.001$, $n = 22$) and *C. plicata* ($r^2 = 0.56$, $p < 0.05$,

$n = 8$). Significant relationship was found between muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *A. woodiana* ($r^2 = 0.42$, $p < 0.001$, $n = 22$). Primary consumers showed positive relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ($r^2 = 0.71$, $p < 0.05$, $n = 6$, Fig. 4), indicating a trend of increase in heavy stable isotopes from planktonic to benthic food webs.

Planktivorous *Aristichthys nobilis* (mean \pm SD, $\delta^{13}\text{C} = -27.1 \pm 0.5$, $\delta^{15}\text{N} = 15.6 \pm 0.4$) that relied more on planktonic food sources, such as phytoplankton and zooplankton (Xu and Xie 2004), were relatively depleted in ^{13}C and ^{15}N with regard to benthic omnivorous *Carassius auratus* ($\delta^{13}\text{C} = -23.3 \pm 1.3$, $\delta^{15}\text{N} = 18.7 \pm 0.1$, $n = 3$), which consumed more benthic food sources, such as detritus, chironomid, and shrimp. Carnivorous *Culter erythropterus* had the highest $\delta^{15}\text{N}$ (21.3 ± 0.3 , $n = 3$) among all samples, indicating that this fish is a top predator that also did not rely on planktonic food sources due to its relatively enriched $\delta^{13}\text{C}$ (-22.2 ± 0.4 , $n = 3$) compared with other species.

Discussion

Sympatric mussels and snails showed significant differences in stable isotopic compositions, indicating markedly different food habits of these animals. No difference in isotopic compositions was found between two species of snails, suggesting their similar food source assimilation in the same habitat. Three species of mussels exhibited significantly different stable C and N signatures, suggesting that they selectively ingested and assimilated suspended particulate mixture of benthic–planktonic organic matter throughout the water column. Although no feeding habit studies for these species in the studied area had been conducted, studies on other species of bivalves indicate that they selectively ingest particulate organic matter (POM), such as phytoplankton, microbenthos, detritus, and sedimentary organic matter, from the water column and/or surface sediment (Machas et al. 2003; Page and Lastra

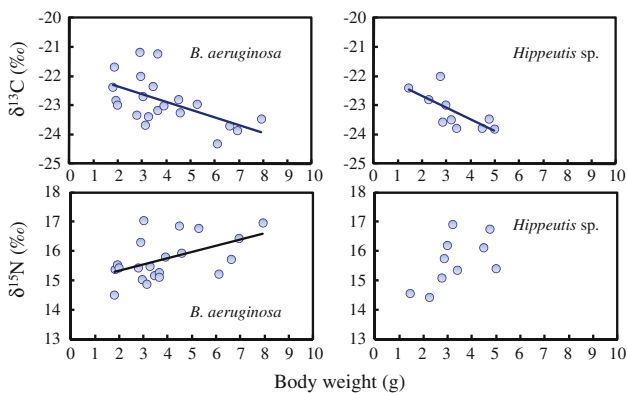
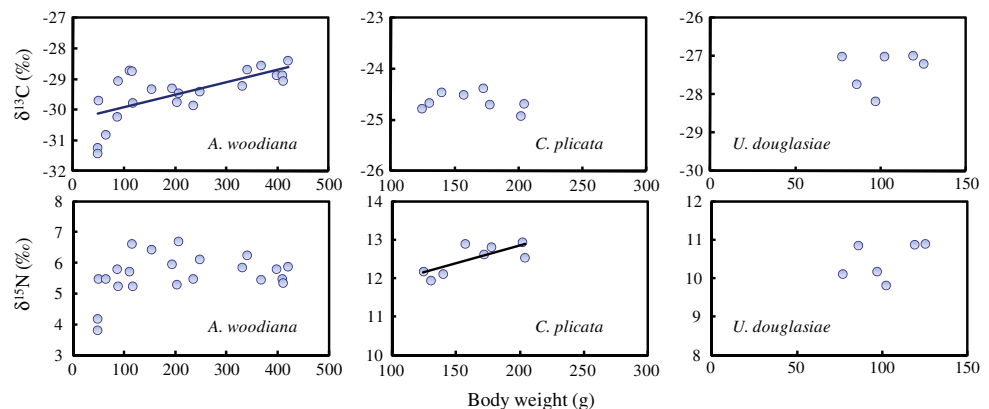


Fig. 2 Relationships between body weight and muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of snails *Bellamyia aeruginosa* and *Hippeutis* sp. $\delta^{13}\text{C}$ of both *B. aeruginosa* and *Hippeutis* sp. negatively correlated with body weight ($r^2 = 0.31$, $p < 0.01$, and $r^2 = 0.50$, $p = 0.02$, respectively), whereas $\delta^{15}\text{N}$ of *Hippeutis* sp. positively correlated with body weight ($r^2 = 0.26$, $p = 0.02$)

Fig. 3 Relationships between body weight and muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of mussels *Anodonta woodiana*, *Cristaria plicata*, and *Unio douglasiae*. Significant correlations were found between muscle $\delta^{13}\text{C}$ and body weight of *A. woodiana* ($r^2 = 0.44$, $p < 0.01$) and between muscle $\delta^{15}\text{N}$ and body weight of *C. plicata* ($r^2 = 0.56$, $p = 0.03$)



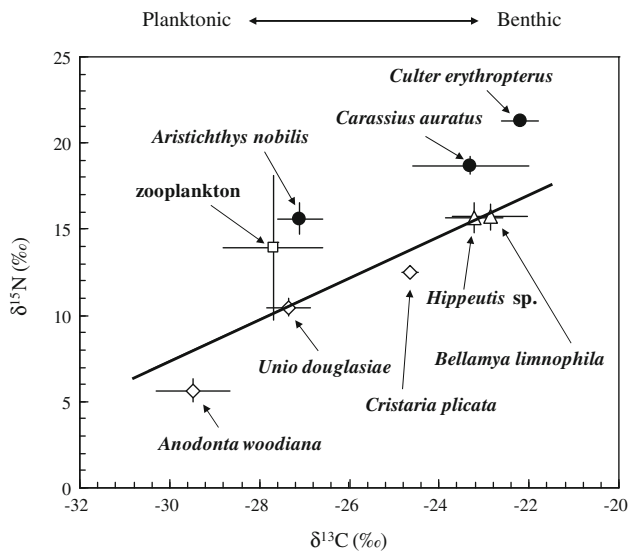


Fig. 4 Relationship between mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of primary consumers with the bars showed the standard deviations (SD). Open diamonds represent mussels, open triangles represent snails, and open square represent zooplankton. Solid line is the trend line that best describes the relationship between mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of primary consumers with a linear equation of $Y = 1.2X + 43.4$ ($r^2 = 0.71$, $p < 0.05$, $n = 6$). Closed circles represent fish species that were used to show the impact of different pelagic baselines in isotopic mixing model on trophic position (TP) and percentage concentration of pelagic prey sources of each fish

2003; Christian et al. 2004; Garton et al. 2005), and the way in which bivalves deal with food quantity and quality appears to be species dependent (Ward et al. 2003). Several challenges persist regarding the extrapolation power of studies relying on stable isotopes signatures, since the enrichment of these isotopes is not only species specific, but also depend on tissue turnover time, environmental conditions, and locations (Gustafson et al. 2007). Spatial variability with regard to species' feeding biology and the trophic plasticity of suspension feeders assessed by stable isotope analyses has been previously demonstrated on a small scale (Dubois et al. 2007). Large-scale spatial patterns in the $\delta^{15}\text{N}$ of a widely distributed bivalve mollusc have been reported (Jennings and Warr 2003). More recently, C and N stable isotopes have shown that the suspension feeder *Cerastoderma edule* was observed to assimilate carbon from plankton, whereas the deposit feeder *Macoma balthica* assimilated carbon from a mixed diet of benthic microalgae and plankton (Rossi et al. 2004). Within a species, the size of the gills and labial palps are known to be flexible (Drent et al. 2004; Piersma and Drent 2003) and related to changing food conditions over time (Honkoop et al. 2003) and space (Drent et al. 2004). In this sense, using one of these species as planktonic baseline in isotopic mass-balance mixing model could bias the TP and percentage concentration of planktonic versus benthic

sources of higher trophic level consumers, e.g., fishes, in this system.

The variation in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was higher in short-lived primary consumers (zooplankton) compared with long-lived primary consumers (mussels and snails), and the CV for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of zooplankton were relatively high compared with those of mussels and snails, which could be attributed to significant temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of phytoplankton, as indicated from the marked deviation of POM isotopic values in this area (Zhou et al. 2009), because zooplankton body size is small and metabolic turnover is high (Grey et al. 2002). Temporal variation of zooplankton $\delta^{15}\text{N}$ is suggested to be substantial and might be largely unrelated to trophic variation (Matthews and Mazumder 2005). For example, O'Reilly et al. (2002) found zooplankton had a $\delta^{15}\text{N}$ signature similar to planktivorous consumers following an upwelling event of isotopically heavy ammonia and demonstrated that $\delta^{15}\text{N}$ signatures of zooplankton could be frequently influenced by changes in N cycling of a pelagic food web, although it is still unknown whether this type of isotopic distortion is a common feature of lakes.

In most situations, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of mussels increased with the increase of mussel weight. Although the mechanism for this relationship is not known, it is unlikely that isotope fractionation would change during growth, as Minagawa and Wada (1984) found that marine mussels fed with the same diet showed consistent isotopic ratios with age. One possible explanation is that mussels are gape limited by the size of their incurrent siphons. Older and larger individuals, with larger inhalant siphons, may eat organisms that feed higher on the food chain, such as drifting invertebrates. This has been documented in other filter-feeder mussels, e.g., the zebra mussel (MacIsaac et al. 1995), and the river-dwelling freshwater mussel *Margaritifera falcata* (Howard et al. 2005). MacIsaac et al. (1995) showed a significant relationship between shell length and diameter of the inhalant siphon and demonstrated that larger zebra mussels were capable of suppressing rotifers abundance. Another possible explanation is that the mussels increased assimilation of benthic food items as they became larger and older, since positive relationship from planktonic to benthic gradient between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was observed in this work (see Fig. 4). For snails, $\delta^{15}\text{N}$ increased with body weight, indicating an increase of assimilating ^{15}N -enriched food sources, as found in mussels. In contrast, $\delta^{13}\text{C}$ of snails negatively correlated with their body weight, which might be attributed to relatively high amounts of lipids in muscle tissue of larger snails, such as that reported in other invertebrates (Schmidt 1999), because lipids are typically depleted in ^{13}C relative to muscle tissue (e.g. Gu et al. 1996a; Sotiropoulos et al. 2004). Differences in food preference between juvenile and

adult snails have been reported (Thomas et al. 1985). Variations in stable isotopes with animal size also have been found in other invertebrates (e.g. Rau et al. 1991; Gu et al. 1997).

Most limnologists study either the benthic or, more often, the pelagic habitat of lake ecosystems (Vadeboncouer et al. 2002). However, in this study, primary consumers showed significant correlation between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Fig. 4). The positioning of these consumers along a planktonic–benthic gradient indicated a linkage between pelagic and benthic habitats in the study site (France 1995; Vadeboncouer et al. 2002; Vander Zanden and Vadeboncouer 2002; Vadeboncouer et al. 2003; Vander Zanden et al. 2003). Our findings contribute to a growing body of evidence that planktonic–benthic habitat coupling is both energetically significant and important in food-web dynamics (Polis and Strong 1996). Division of lacustrine systems into benthic or pelagic habitats will distort the perception of lake food webs (Vadeboncouer et al. 2002), since most of the world's lakes are small and have high ratios of benthic surface area to pelagic volume (Wetzel 1990).

Estimated TP and contribution of pelagic secondary production of carnivorous *C. erythropterus* and omnivorous *C. auratus* remained relatively stable with different pelagic isotopic baselines used in mixing models (Table 2). For *C. erythropterus*, TP and percent pelagic were always 3.6% and 0%, respectively, indicating that this fish fed on a high trophic level of the benthic food web. TP and planktonic pelagic of *C. auratus* ranged from 2.9% to 3.1%

Table 2 Impacts of different planktonic baseline selection on trophic position (TP) and percentage contribution of planktonic prey sources of fishes through simple isotopic mass balance mixing model

Fish species	Planktonic baseline species	Trophic position	Percent planktonic (%)
<i>Carassius auratus</i>	Average of all mussels	3.0	5
	<i>Anodonta woodiana</i>	3.0	5
	<i>Cristaria plicata</i>	3.1	19
	<i>Unio douglasiae</i>	3.0	7
	Zooplankton	2.9	5
<i>Culter erythropterus</i>	Average of all mussels	3.6	0
	<i>Anodonta woodiana</i>	3.6	0
	<i>Cristaria plicata</i>	3.6	0
	<i>Unio douglasiae</i>	3.6	0
	Zooplankton	3.6	0
<i>Aristichthys nobilis</i>	Average of all mussels	3.4	64
	<i>Anodonta woodiana</i>	3.9	64
	<i>Cristaria plicata</i>	2.9	100
	<i>Unio douglasiae</i>	3.4	94
	Zooplankton	2.4	87

and from 5% to 19%, suggesting this fish was a secondary consumer and its production was mainly derived from the benthic food web. *A. nobilis* is a planktivorous fish basically feeding on zooplankton and phytoplankton from the pelagic food web (Xu and Xie 2004). TP and planktonic pelagic of this fish varied markedly from 2.4% to 3.9% and from 64% to 100%, indicating variations in isotopic baselines could cause bias in interpreting the observed characteristics. For example, with the TP of 3.9, *A. nobilis* might feed extensively on higher trophic level consumers, e.g., fish, whereas previous studies demonstrated that this fish fed mainly on zooplankton and phytoplankton, and its TP could only be between 2 and 3 (also Gu et al. 1996b; Xu and Xie 2004). These results highlight the importance of testing the effects of baseline selection on food-web assessment.

Long-lived primary consumers provide a more general baseline for quantitative estimates of TP that simultaneously capture complex trophic interactions and track energy or mass flow through the reticulate pathways of ecological communities within and across multiple ecosystems (e.g., Cabana and Rasmussen 1996; Vander Zanden et al. 1999; Post et al. 2000; Post 2002; Xu et al. 2007, 2008). However, as recent studies suggest, there was nearly one trophic level worth of interpopulation and more than two trophic levels worth of intrapopulation of isotopic baselines within a single sampling site. Previous studies also suggested that there were nearly two trophic levels worth of temporal variation and almost four trophic levels worth of variation in isotopic baselines within a single lake and among lakes, respectively (Post 2002). Obtaining an appropriate baseline to the question of interest is one of the most difficult methodological issues facing the effective application of stable isotopes (Post 2002), and our results, together with previous results, suggest that long-lived primary consumers need to accurately reflect the isotopic signature of the base of the food webs they represent, and it is critical to understand the natural history of these consumers when choosing an isotopic baseline.

Conclusion

As filter feeders and surface grazers, long-lived primary consumers—mussels and snails—are less sensitive to seasonal fluctuations of isotopic compositions of primary producers and are well suited to represent isotopic baselines of planktonic and benthic food webs, respectively. However, within- and among- species variations in isotopic compositions of these species found in this study, although they were sampled from the same habitat, pointed to the importance of testing and evaluating the effects of different baseline selections on calculating TP and contribution of pelagic and

benthic energy of higher-level consumers, especially in planktonic–benthic coupled ecosystems. Meanwhile, it would be worthwhile investigating the ecology and isotopic fractionation of these primary consumers to understand the unusual C and N isotopic signatures and to avoid any misapprehension in future trophic studies.

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References

- Aberle N, Hillebrand H, Grey J, Wiltshire KH (2005) Selectivity and competitive interactions between two benthic invertebrate grazers (*Asellus aquaticus* and *Potamopyrgus antipodarum*): an experimental study using ^{13}C - and ^{15}N -labelled diatoms. *Freshw Biol* 50:369–379
- Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in seagrass and penaeid shrimp: implications for food web studies using multiple stable isotopes. *Limnol Oceanogr* 40:622–625
- Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proc Natl Acad Sci* 93:10844–10847
- Chen Q, Song G (1975) A preliminary study on reproduction and growth of the snail, *Belamya aeruginosa* (Veeve). *Acta Hydrobiol Sin* 5:520–534
- Compton TJ, Kentie R, Storey AW, Veltheim I, Pearson GB, Piersma T (2008) Carbon isotope signatures reveal that diet is related to the relative sizes of the gills and palps in bivalves. *J Exp Mar Biol Ecol* 361:104–110
- Deudero S, Cabanellas M, Blanco A, Tejada S (2009) Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel *Mytilus galloprovincialis*. *J Exp Mar Biol Ecol* 368:181–188
- Doi H, Kikuchi E, Shikano S, Takagi S (2009) Differences in nitrogen and carbon stable isotopes between planktonic and benthic microalgae. *Limnology*. doi:10.1007/s10201-009-0297-1
- Drent J, Luttikhuisen PC, Piersma T (2004) Morphological dynamics in the foraging apparatus of a deposit feeding marine bivalve: phenotypic plasticity and heritable effects. *Funct Ecol* 18:349–356
- Dubois S, Orvain F, Marin-Léal JC, Ropert M, Lefebvre S (2007) Small-scale spatial variability of food partitioning between cultivated oysters and associates suspension feeding species, as revealed by stable isotopes. *Mar Ecol Prog Ser* 336:151–160
- France RL (1995) Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr* 50:1310–1313
- Fukumori K, Oi M, Doi H, Takahashi D, Okuda N, Miller TW, Kuwae M, Miyasaka H, Genkai-Kato M, Koizumi Y, Omori K, Takeoka H (2008) Bivalve tissue as a carbon and nitrogen isotope baseline indicator in coastal ecosystems. *Estuar Coast Shelf Sci* 79:45–50
- Garton DW, Payne CD, Montoya JP (2005) Flexible diet and trophic position of *dreissenid* mussels as inferred from stable isotopes of carbon and nitrogen. *Can J Fish Aquat Sci* 62:1119–1129
- Grey J, Thackeray SJ, Jones RI, Shine A (2002) Ferox trout (*Salmo trutta*) as 'Russian dolls': complementary gut content and stable isotope analyses of the Loch Ness foodweb. *Freshw Biol* 47:1235–1243
- Gu B, Schell DM, Huang X, Yie F (1996a) Stable isotope evidence for dietary overlap between two planktivorous fishes in aquaculture ponds. *Can J Fish Aquat Sci* 53:2814–2818
- Gu B, Schelske CL, Hoyer MV (1996b) Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. *J Fish Biol* 49:1233–1243
- Gu B, Alexander V, Schell DM (1997) Stable isotopes as indicators of carbon flows and trophic structure of the benthic food web in a subarctic lake. *Arch Hydrobiol* 138:329–344
- Gu B, Schelske CL, Hodell DA (2004) Extreme ^{13}C enrichments in a shallow hypereutrophic lake: implications for carbon cycling. *Limnol Oceanogr* 49:1152–1159
- Gustafson L, Showers W, Kwak T, Levine J, Stoskopf M (2007) Temporal and spatial variability in stable isotope compositions of a freshwater mussel—implications for biomonitoring and ecological studies. *Oecologia* 152:140–150
- Han M, Shu Y (1995) Atlas of freshwater biota in China. China Ocean Press, Beijing
- Hesslein RH, Hallard KH, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}\text{S}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. *Can J Fish Aquat Sci* 50:2071–2076
- Honkoop PJC, Bayne BL, Drent J (2003) Flexibility of size of gills and palps in the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). *J Exp Mar Biol Ecol* 282:113–133
- Howard JK, Cuffey KM, Solomon M (2005) Toward using *Margaritifera falcata* as an indicator of base level nitrogen and carbon isotope ratios: insights from two California Coast Range rivers. *Hydrobiologia* 541:229–236
- Kamermans P (1994) Similarity in food source and timing of feeding in deposit- and suspension-feeding bivalves. *Mar Ecol Prog Ser* 104:63–75
- Ke ZX, Xie P, Guo LG (2008) Controlling factors of spring-summer phytoplankton succession in Lake Taihu (Meiliang Bay, China). *Hydrobiologia* 607:41–49
- Lee JY, Yoshioka T, Hanazato T (2002) Faunal trophic interaction in an oligotrophic-dystrophic lake (Shirakoma-ike, Japan). *Limnology* 3:0151–0158
- Liu X (2006) Food composition and food webs of zoobenthos in Yangtze lakes. Ph.D. thesis, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan
- Liu YY, Zhang WZ, Wang YX (eds) (1979) Economic fauna of China: freshwater mollusks. Science Press, Beijing
- Machas R, Santos R, Peterson B (2003) Tracing the flow of organic matter from primary producers to filter feeders in Ria Formosa Lagoon, Southern Portugal. *Estuaries* 26:846–856
- MacIsaac HJ, Lonner CJ, Leach JH (1995) Suppression of microzooplankton by zebra mussels: importance of mussel size. *Freshw Biol* 34:379–387
- Maki K, Kim C, Yoshimizu C, Tayasu I, Miyajima T, Nagata T (2009) Autochthonous origin of semi-labile dissolved organic carbon in a large monomictic lake (Lake Biwa): carbon stable isotope evidence. *Limnology*. doi:10.1007/s10201-009-0299-z
- Minagawa M, Wada E (1984) Step-wise enrichment of ^{15}N along food chains further evidence and the relation between ^{15}N and animal age. *Geochim Cosmochim Acta* 48:1135–1140

- Mitchell MJ, Mills EL, Idrisi N, Michener R (1996) Stable isotopes of nitrogen and carbon in an aquatic food web recently invaded by *Dreissena polymorpha* (Pallas). *Can J Fish Aquat Sci* 53:1445–1450
- Page HM, Lastra M (2003) Diet of intertidal bivalves in the Ria de Arosa (NW Spain): evidence from stable C and N isotope analysis. *Mar Biol* 143:519–532
- Polis G, Strong DR (1996) Food web complexity and community dynamics. *Am Nat* 147:813–846
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83:703–718
- Rau GH, Hopkins TL, Torres JJ (1991) $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in Weddell Sea invertebrates: implications for feeding diversity. *Mar Ecol Prog Ser* 77:1–6
- Rossi F, Herman PMJ, Middelburg JJ (2004) Interspecific and intraspecific variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in deposit- and suspension-feeding bivalves (*Macoma balthica* and *Cerastoderma edule*): evidence of ontogenetic changes in feeding mode of *Macoma balthica*. *Limnol Oceanogr* 49:408–414
- Rounick JS, Winterbourn MJ (1986) Stable carbon isotopes and carbon flow in ecosystems. *Bioscience* 36:171–177
- Smith BN, Berg DJ, Smoot JC, Findlay RH (2004) Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. *J N Am Benthol Soc* 23:101–113
- Sotiropoulos MA, Tonn WM, Wassenaar LI (2004) Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecol Freshw Fish* 13:155–160
- Thomas JD, Nwanko DI, Sterry PR (1985) The feeding strategies of juvenile and adult *Biomphalaria glabrata* (Say) under simulated natural conditions and their relevance to ecological theory and snail control. *Proc R Soc B* 226:177–209
- Uchii K, Okuda N, Yonekura R, Karube Z, Matsui K, Kawabata Z (2007) Trophic polymorphism in bluegill sunfish (*Lepomis macrochirus*) introduced into Lake Biwa: evidence from stable isotope analysis. *Limnology* 8:59–63
- Vadeboncoeur YM, Vander Zanden MJ, Lodge DM (2002) Putting the lake back together: reintegrating benthic pathways into lake food web models. *Bioscience* 52:44–54
- Vander Zanden MJ, Rasmussen JB (1999) Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80:1395–1404
- Vander Zanden MJ, Vadeboncoeur Y (2002) Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* 83:2152–2161
- Vander Zanden MJ, Casselman JM, Rasmussen JB (1999) Stable isotope evidence for food web shifts following species invasions of lakes. *Nature* 401:464–467
- Vander Zanden MJ, Chandra S, Allen BC, Reuter JE, Goldman CR (2003) Historical food web structure and the restoration of native aquatic communities in the Lake Tahoe (California-Nevada) basin. *Ecosystems* 6:274–288
- Ward JE, Levinton JS, Shumway SE (2003) Influence of diet on pre-ingestive particle processing in bivalves I: transport velocities on the ctenidium. *J Exp Mar Biol Ecol* 293:129–149
- Wetzel RG (1990) Land–water interfaces: metabolic and limnological regulators. *Int Ver Theor Angew Limnol Verh* 24:6–24
- Xu J, Xie P (2004) Studies on the food web structure of Lake Donghu using stable carbon and nitrogen isotope ratios. *J Freshw Ecol* 19:645–650
- Xu J, Li S, Xie P (2005a) Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of particulate organic matter from the deep oligotrophic Lake Fuxian connected with the shallow eutrophic Lake Xingyun, China. *Bull Environ Contam Toxicol* 74:281–285
- Xu J, Xie P, Zhang M, Yang H (2005b) Variation in stable isotope signatures of seston and a zooplanktivorous fish in a eutrophic Chinese lake. *Hydrobiologia* 541:215–220
- Xu J, Zhang M, Xie P (2007) Size-related shifts in reliance on benthic and pelagic food webs by lake anchovy. *Ecoscience* 14:170–177
- Xu J, Zhang M, Xie P (2008) Stable isotope changes in freshwater shrimps (*Exopalaemon modestus* and *Macrobrachium nipponensis*): trophic pattern implications. *Hydrobiologia* 605:45–54
- Yan Y, Liang Y, Wang H (2000) Annual production of *Beltamya aeruginosa* in Houhu Lake, Wuhan. *J Lake Sci* 12:68–72
- Yoshii K (1999) Stable isotope analyses of benthic organisms in Lake Baikal. *Hydrobiologia* 411:145–159
- Zheng G, Wei Q (1999) A study on the diet and growth of *Anodonta Woodiana Pacifica* (Heude) in south lake. *Wuhan J Huazhong Agric Univ* 18:48–58
- Zhou A, Qiang J, Wang Q, Meng Y (1995) Enrichment of heavy metals in mussels from Nen and Qiqihaer Rivers. *Arid Environ Monit* 9:44–47