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Mussel isotope signature as indicator of nutrient pollution in a freshwater eutrophic lake: species, spatial, and seasonal variability

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Abstract Stable nitrogen isotope ratios of five mussel species from littoral and pelagic areas were investigated with different trophic states in the eutrophic Lake Taihu, the third largest lake in China. Interpopulation variability for these mussels was relatively small in foot tissues because of the slow turnover time. Seasonal and spatial variations among the δ^{15}N values of mussels might be due in part to the natural variation in δ^{15}N values of potential food sources and the variation in the amount of human pollutions discharged into various locations of the lake. Although the increase of mussel δ^{15}N values was accompanied by the increase of nutrient concentrations in most situations in this study, statistically significant correlations were only 22% of the total correlations in this survey, which might be attributed to the different time-scale variations in nutrient concentrations and isotope signatures and the unknown details of the trophic pathways and metabolism for incorporation of these nutrients.

Keywords Freshwater mussel · Stable nitrogen isotope · Nutrient pollution · Bioindicator · Biomonitoring

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Introduction

The anthropogenic load of nitrogen and phosphorus compounds is a persistent problem in aquatic ecosystems throughout the world (Smith et al. 1999). Increased nutrient pollutions can lead to eutrophication and further results in harmful algal blooms and less dissolved oxygen, associated with fish kills and health risks in drinking water use, and decreases in the abundance and diversity of resident biota and the lose of ecosystem functions (Nixon 1995; Paerl 1988, 1997). In order to understand the detrimental effects of nutrient inputs, it is important to track the sources of nutrient loadings into aquatic ecosystems, and stable isotope techniques is demonstrated to be competent for identifying sources of surface water nutrient pollution (McClelland and Valiela 1998; Showers et al. 1990, 1999). For example, nitrate derived from commercial fertilizers has typical δ^{15}N values that range from about −2‰ to +4‰;

δ^{15}N values of +3‰to +9‰have been reported for soil organic nitrogen-nitrate; human and animal waste nitrate δ^{15}N values vary from approximately +10 to +22‰(Heaton 1986). Many factors may be involved in establishing the δ^{15}N value at a site, but studies have related increases in δ^{15}N values in aquatic systems to the extent of anthropogenic activity in watersheds and the input of wastewater (Cabana and Rasmussen 1996), since the lighter nitrogen isotope volatilizes more readily, and the heavier isotope remains in the animal and human sewages (Heaton 1986; Macko and Ostrom 1994; Showers et al. 1999).

Constant nitrogen isotope fractionation has been found during uptake and assimilation of nitrogen by phytoplankton (Pennock et al. 1996) and during trophic transfer along food chains (Post 2002). For example, the constant enrichment of δ^{15}N in consumers relative to their diet means that the trophic level can be calculated as $\lambda + (\delta^{15}N_{consumer} + \delta^{15}N_{baseline})/\Delta$, where λ is the trophic position of the organism used to estimate $\delta^{15}N_{baseline}$ (for example, $\lambda = 1$ for primary producers), $\delta^{15}N_{consumer}$ is measured directly, $\delta^{15}N_{baseline}$ is the corresponding value at the base of the food web, and Δ is the enrichment of δ^{15}N per trophic level (Post 2002; Xu et al. 2005b). Arguably, compared with the more ephemeral isotopic signatures of short-lived producer organisms, long-lived consumers with low trophic position can be used as more suitable indicators of anthropogenic contamination because they integrate waste inputs over long time periods and the nitrogen isotope signature reflects movement of waste through the food chain.

As filter-feeders, mussels remove and assimilate algae, detritus, and microorganisms from the water column and can be regarded as primary consumer that occupies a position near the base of the food chain in aquatic ecosystems (Gosner 1971). They are also important in nutrient flow and cycling (Jordan and Valiela 1982). By processing large amounts of nitrogen, and because of their relatively low tissue turnover rates (McMahon 1991), the δ^{15}N of mussels can be a good indicator of nutrient pollution to an ecosystem. By retaining nitrogen from primary producers in their tissues, mussels reflect the stable isotopic composition of source nitrogen. However, something must be known of the variability in mussel ^{15}N values within ecosystems. Interspecies, spatial, and seasonal variability within ecosystem is important because the precision of nutrient pollution estimates calculated using near-sessile mussels would be limited in part by variability in mussel ^{15}N values due to the nonhomogeneity of interested system.

In light of these facts, we investigated the variability in δ^{15}N of five mussel species sampled from four sites with different trophic states in the eutrophic Lake Taihu, the third largest lake in China (Fig. 1). Our principal objectives were to examine

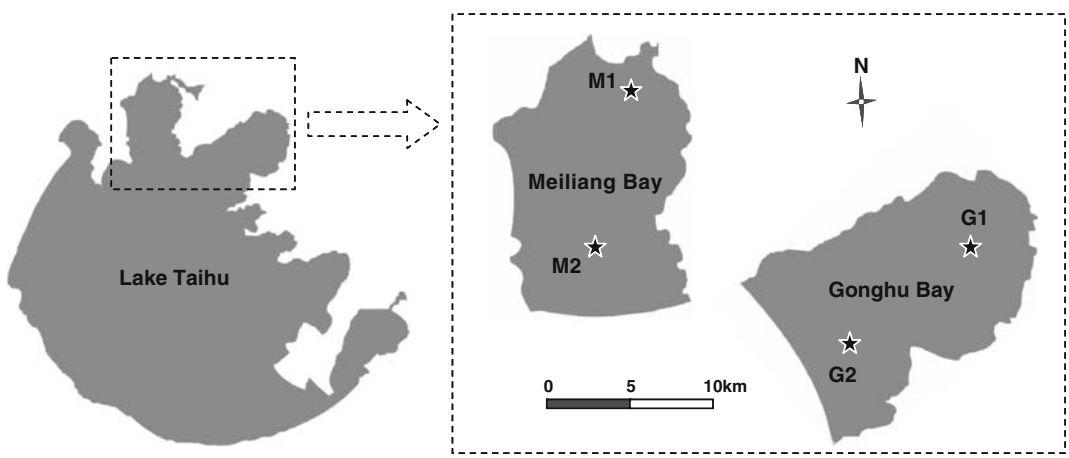

Fig. 1 Map of Lake Taihu in China showing Meiliang and Gonghu Bays and the locations of this investigation

between-species, seasonal, and spatial variability in δ^{15}N of mussels and the relationships between δ^{15}N of mussels and nutrient pollution, and to assess how well the mussel perform as bioindicators of anthropogenic pollution. Since no direct measures of wastewater input were available for sites in the present study, we used measured nitrogen and phosphorus species concentrations to indicate anthropogenic pollution and compared these values with the δ^{15}N values of mussel muscle.

Materials and methods

Study site

Lake Taihu (30°5′–32°8′ N and 119°8′–121°55′ E) is located in the east part of China. It is the third largest freshwater lake in China and has a surface area of 2,338 km^2 with a mean water depth of 1.9 m and a maximum depth of about 2.6 m. This lake is of historical importance in trade, politics, agriculture, and culture. About 35 million people inhabit the 36,500-km^2 watershed of Taihu Lake (Xu et al. 2008; Pu et al. 1998). During the past decades, the lake has undergone a steady increase in eutrophication with a regular occurrence of cyanobacterial surface blooms in the warm seasons of each year (Gao et al. 2007; Pu et al. 1998). Meiliang Bay, with water surface area of 125 km^2, accommodates municipal and industry wastewater from Wuxi City and is the most eutrophic part of the lake with extremely dense accumulation of *Microcystis* blooms by wind in the warm season (Fan et al. 2005). Gonghu Bay is located at the northeast portion of Taihu Lake with abundant submerged plants present during our sampling period. Four sampling sites, named M1, M2, G1, and G2, were set-up based on the eutrophic status in these areas (Fig. 1).

Sample collection

Mussel samples were obtained from sediment surface at four sites in Meiliang and Gonghu Bays (Fig. 1), in spring (April) and summer (July) 2005. Five individuals of each mussel species, including *Hyriopsis cumingii*, *Arconaia lanceolata*, *Anodonta woodiana woodiana*, *Cristaria plicata*, *Lamprotula rochechouarti* were sampled depending on the availability. At each site, water samples were also collected for chemical analysis, including total nitrogen (TN) and phosphorus (TP), nitrate (NO_3–N) and soluble reactive phosphorus (SRP) concentrations.

Sample preparation

Mussel adductor muscle tissue was removed, rinsed in distilled water and oven dried (60°C,

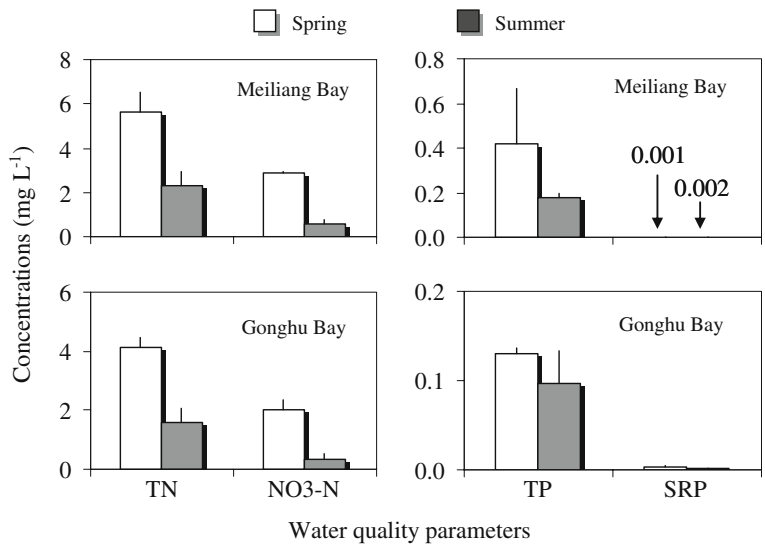

Fig. 2 Variations of total nitrogen and phosphorus concentrations (TN and TP) and nitrate (NO_3–N) and soluble reactive phosphorus (SRP) concentrations in spring and summer in Meiliang and Gonghu Bays during the sampling period. *Error bars* represented the standard deviations

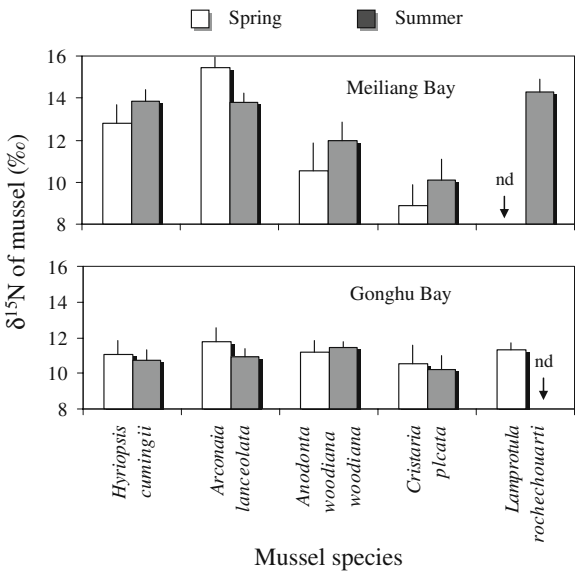

Fig. 3 Variations of nitrogen stable isotopes in mussel species in spring and summer in Meiliang and Gonghu Bays. *Error bars* represented the standard deviations

48 h). Adductor muscle tissue was used in this study as muscle tissue has low turnover rates (Gorokhova and Hansson 1999) and is therefore more representative of a time-integrated diet. The tissues were then ground to a fine powder with a mortar and pestle and then stored in a desiccator with a silica gel desiccant for subsequent stable isotope analysis.

Chemical analysis

Total nitrogen was measured by alkaline potassium persulfate digestion-UV spectrophotometric method, and nitrate was determined by the Nessler method (APHA 1995). Total phosphorus was determined by digesting sample with potassium persulfate and measured by molybdenum blue colorimetric method with soluble reactive phosphorus measured using Murphy and Riley procedure (Murphy and Riley 1962). Stable nitrogen isotope ratios were analyzed with Delta Plus (Finnigan) continuous flow isotope ratio mass spectrometer (CF-IRMS) directly coupled to an NC2500 elemental analyzer (Carlo Erba). The isotopic compositions of samples were expressed as parts-per-thousand (‰) differences from a standard reference material using the equation: $\delta^{15}\mathrm{N}\ (‰) = \left(R_{\mathrm{sample}} / R_{\mathrm{standard}} - 1\right) \times 1,000$, where R is $^{15}N/^{14}N$. The standard reference

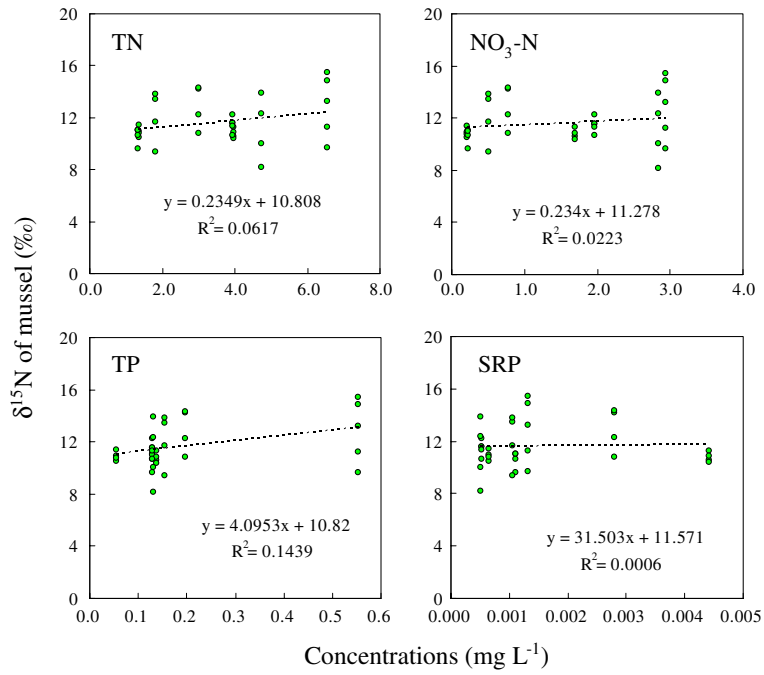

Fig. 4 Relationship between pooled δ^{15}N values of mussel and nutrient concentrations of lake water

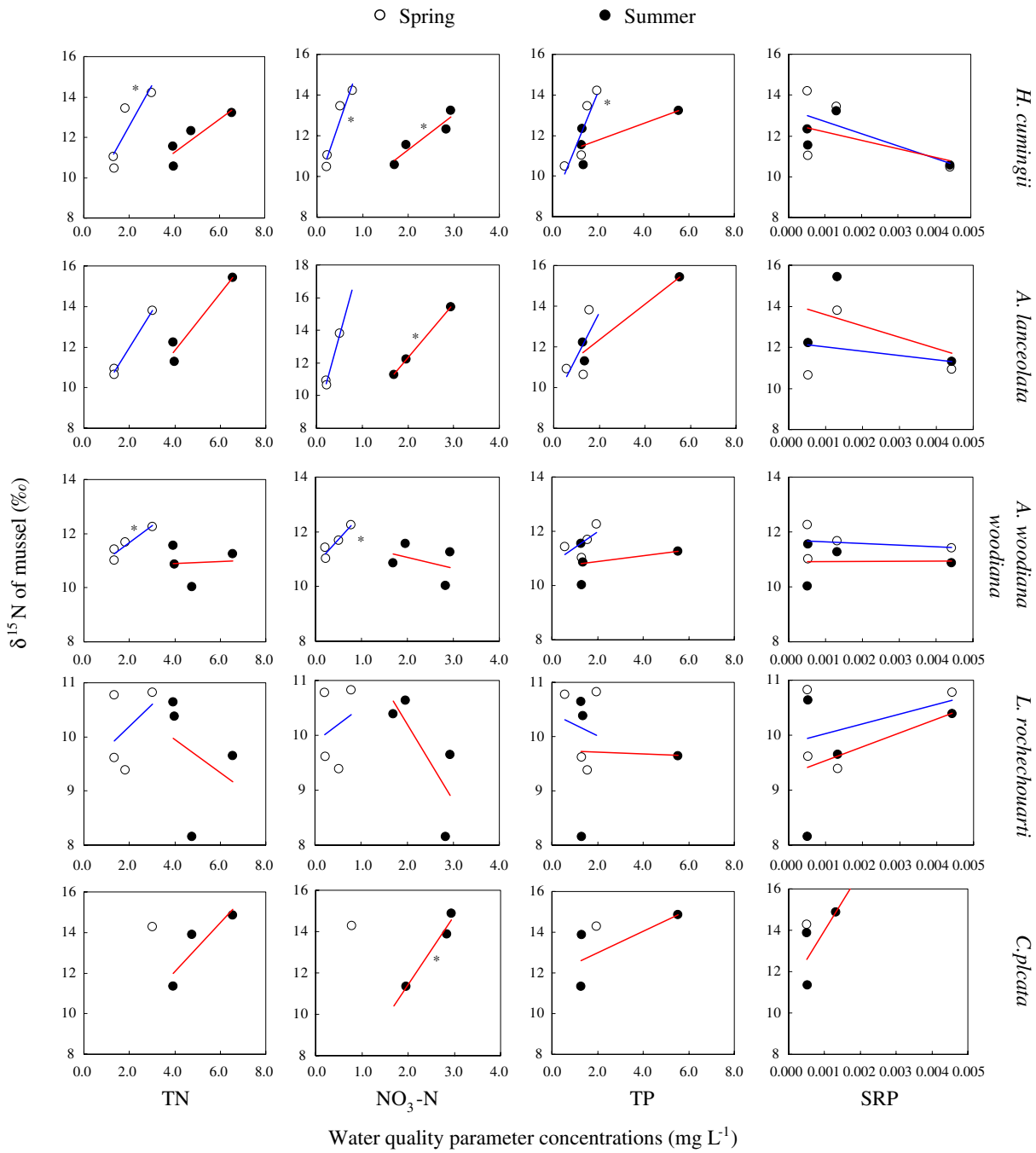

Fig. 5 Relationship between δ^{15}N values of each mussel species and nutrient concentrations of lake water. Significant correlations ($p < 0.05$) were marked with *asterisks beside the regression lines*, which occupied only 22% of the total correlations, among which 63% was found with NO_3–N, 25% with TN and 12% with TP

material was atmospheric nitrogen. The international reference material used was ammonium sulfate (IAEA-USGS25). More than 20% of the samples were analyzed two or more times and the standard errors of replicate analyses were approximately ±0.3‰.

Statistical analyses

To examine the correlations between δ^{15}N values of mussels and total nitrogen and phosphorus concentrations, STATISTICA for Windows statistical software (version 6.0) was used for the relative analyses.

Results

During our sampling periods, total nitrogen and phosphorus concentrations ranged from 1.32 to 6.54 mg L^{-1} and from 0.05 to 0.57 mg L^{-1}, respectively; nitrate and soluble reactive phosphorus concentrations ranged from 0.20 to 2.94 mg L^{-1} and from 0.0005 to 0.0045 mg L^{-1}, respectively. Seasonal variation of TN and NO_3–N were significant with higher concentrations in spring (t test, $p < 0.01$), while TP and SRP showed no significant seasonal variations (t test, $p > 0.05$) in both Meiliang and Gonghu Bays (Fig. 2). Spatial variations were also observed in this study with significantly higher concentrations of TN, NO_3–N, and TP in spring (t test, $p < 0.01$) and with significantly higher concentrations of TP, NO_3–N, and SRP in summer (t test, $p < 0.01$) in Meiliang Bay than Gonghu Bay (Fig. 2).

Overall, δ^{15}N of mussels varied from 8.1‰ to 15.8‰, with an average of 11.5‰, and varied spatially and temporally (Fig. 3). Because different mussel species co-occurred at our sampling sites (*H. cumingii*, *A. lanceolata*, *A. woodiana woodiana*, *C. plcata*, *L. rochechouarti*), they were analyzed first to test for interspecific differences in δ^{15}N signatures. T tests showed significant differences in isotopic nitrogen values ($p < 0.05$) between species (power of test was 0.99), except for *H. cumingii* and *A. lanceolata* ($p > 0.1$) in summer in Meiliang Bay and in spring and summer in Gonghu Bay. It was then analyzed to test for spatial and seasonal differences in δ^{15}N signatures. T tests showed no significant differences in isotopic nitrogen values of mussels ($p > 0.1$) between sites M1 and M2 in Meiliang Bay and between sites G1 and G2 in Gonghu Bay, but significant differences were found in mussel δ^{15}N signatures between Meiliang Bay and Gonghu Bay where different trophic status are underwent. Seasonal differences in δ^{15}N signatures were found significantly for mussels in Meiliang Bay except for *L. rochechouarti* ($p > 0.1$) and for only one species *A. lanceolata* in Gonghu Bay.

The increase of mussel δ^{15}N values was accompanied by the increase of nutrient concentrations in most situations (Figs. 4 and 5). However, no significant correlations were observed between δ^{15}N of mussels and nutrient concentrations (Fig. 4). δ^{15}N values of each mussel species were then analyzed separately with the nutrient concentrations to find whether there were significant correlations. Significant correlations were observed between δ^{15}N of mussels and nutrient concentrations ($p < 0.05$, Fig. 5), but occupied only 22% of the total correlations, among which 63% was found with NO^3–N, 25% with TN, and 12% with TP (see detail in Fig. 5).

Discussion

Assessment of anthropogenic pollution in aquatic ecosystems with δ^{15}N isotopic method has mostly been concentrated on plants, sediment, invertebrates, and fishes as biological indicators (McKinney et al. 2002; Cole et al. 2004; Xu et al. 2005a, b; Lake et al. 2001; Schlacher et al. 2005). Generally, tissue turnover rates of mussels are slower than algae, macrophytes, and small invertebrates, which suggests that δ^{15}N of mussels are less fluctuating over time than small primary producers and consumers and thus integrate waste inputs over long time periods and provide assessment of better precision (Cabana and Rasmussen 1996).

In this study, interpopulation variability for these mussels was relatively small in foot tissues because muscle tissue turnover time was reported as slow compared with other tissues such as hemolymph, stomach gland, and gut contents for the freshwater (Raikow and Hamilton 2001) and marine mussel species (Hawkins 1985). Therefore, relatively small sample size of each mussel species could reasonably provide estimates of a population mean (Lancaster and Waldron 2001). There were variations among the δ^{15}N values of mussels obtained from sites located in Meiliang and Gonghu Bays, Taihu Lake, both seasonally and spatially. The variation between spring and

summer may be due in part to natural variation in δ^{15}N values of potential food sources and subsequent mussel growth, such as phytoplankton (Xu et al. 2007a) and suspended particulate organic matter (Correll et al. 2001). The spatial variation in δ^{15}N signatures generally reflect variation in the amount of human pollutions discharged into aquatic ecosystems at various locations and elevated δ^{15}N signatures in aquatic biota are known to correlate with increased exposure to human septic or sewage waste (Costanzo et al. 2001, 2003; Cole et al. 2004; Bode et al. 2006; Steffy and Kilham 2004; Kaushal et al. 2006). Thus, it is likely that mussels with high δ^{15}N signatures collected in Meiliang Bay were exposed to more human pollutions than those with low δ^{15}N signatures in Gonghu Bay, and this spatial distribution of δ^{15}N values, together with the nutrient concentrations, might also suggest that Meiliang Bay received more human waste than Gonghu Bay in Lake Taihu.

As the relative contribution of wastewater to the total loading to an aquatic ecosystem increases, δ^{15}N signatures of primary production and consumers higher up the food chain increase, as well. Therefore, trends between δ^{15}N and water quality parameters taken on the same day from different locations within an aquatic ecosystem would most likely reflect spatial variation in human septic or sewage input (McClelland et al. 1997; Udy and Dennison 1997; McClelland and Valiela 1998; Costanzo et al. 2001; Wayland and Hobson 2001; Costanzo et al. 2003; Cole et al. 2004; Steffy and Kilham 2004; Kaushal et al. 2006). Although the increase of mussel δ^{15}N values was accompanied by the increase of nutrient concentrations in most situations in this study, statistically significant correlations were only 22% of the total correlations in this survey, which might be attributed to the fact that mussel tissues responded slowly to habitat change and the corresponding isotope signatures. On the isotopic point of view, a tissue with a higher turnover rate might best detect short-time change, whereas lower turnover rate tissues might be preferred for the estimation of long-term changes in the environment. Therefore, the lack of tight correlations between water quality and mussel isotopic compositions was probably due to the different time-scale variations in nutrient concentrations and isotope signatures and the unknown details of the trophic pathways and metabolism for incorporation of these nutrients. Consequently, tandem evaluation of water (or a short-lived primary producer) and bivalve isotopic signatures would offer, rather than redundancy, a selection of complementary vantage points for evaluation of both long- and short-term effects of nutrients.

In conclusion, our research helps to clarify interspecies, temporal, and spatial variability in stable isotopic compositions of freshwater mussels and, consequently, their efficiency as isotopic bioindicator to assess the anthropologic pollution in freshwater systems. Variability in species, time, and space need to be considered for providing assessment of better precision. Although the shifts in δ^{15}N of mussels were not always significantly consistent with different trophic states as indicated by nutrient concentrations, this technique, as it has been suggested, might be less susceptible to the pitfalls of measuring nutrient concentrations in the water column, e.g., subsampling error or occasional nutrient spikes.

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