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Icefish (*Salangidae*) as an Indicator of Anthropogenic Pollution in Freshwater Systems Using Nitrogen Isotope Analysis

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Abstract We investigated differences in $\delta^{15}N$ of seston and icefishes from seven freshwater ecosystems with different trophic states in China. An increase of seston $\delta^{15}N$ values was accompanied by an increase of total nitrogen and phosphorus concentrations. Significantly positive correlations were observed between $\delta^{15}N$ of icefishes and $\delta^{15}N$ of seston, total nitrogen and phosphorus concentrations. This study demonstrated that icefishes could be preferred indicators of anthropogenic contamination in test systems because they integrated waste inputs over long time periods and reflected the movement of waste through the pelagic food chain.

Keywords Icefish · $\delta^{15}N$ · Freshwater ecosystems · Anthropogenic contamination

Stable isotope ratios of nitrogen ($\delta^{15}N$) have been used successfully in some studies to identify sources of nitrogen-contaminated surface and ground waters (e.g., Lindau et al., 1997). Nitrate derived from commercial fertilizers has typical $\delta^{15}N$ values that range from about –2 to +4‰; $\delta^{15}N$ values of +3 to +9‰ have been reported for soil organic nitrogen-nitrate; human and animal waste nitrate $\delta^{15}N$ values vary from approximately +10 to +22‰ (Heaton, 1986). Many factors may be involved in establishing the $\delta^{15}N$ value at a site, but studies have related increases in $\delta^{15}N$ values in aquatic systems to the extent of anthropogenic activity in watersheds and the input of wastewater (Cabana and Rasmussen, 1996). Constant nitrogen isotope fractionation has been found during uptake and assimilation of nitrogen nutrients by phytoplankton (Pennock et al., 1996) and during trophic transfer along food chains (Post, 2002). For example, the constant enrichment of $\delta^{15}N$ 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, λ=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 $\delta^{15}N$ per trophic level (Post, 2002; Xu et al., 2005b). Arguably, fish may be preferred indicators of anthropogenic contamination because they integrate waste inputs over long time periods and fish-muscle ^{15}N reflects movement of waste through the food chain. $\delta^{15}N$ increases might be expected along the trophic gradient because wastewater generally has a higher $\delta^{15}N$ value than other inputs to aquatic systems (Schlacher et al., 2005).

Icefishes or noodlefishes (*Salangidae*) are small transparent fishes with a life cycle of about one year. They are widely distributed in both inland and coastal waters of China. Some species, e.g., *Protosalanx hyalocranius*, *Neosalanx taihuensis* and *N. pseudotaihuensis*, have played important roles in freshwater fisheries (Wang et al., 2005). During the past 20 years, icefishes have been successfully introduced into a number of lakes and reservoirs r to enhance fish production (Wang et al., 2005). Unlike other fish species, such as lake anchovy who exhibited size-related changes in diets (Xu et al., 2007a), icefishes shod relatively

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stable diet contents and trophic positions. Gut contents analysis revealed that the major components of the icefish diet were zooplankton (Liu, 2001). Thus, as a secondary consumer with a large distribution in freshwater systems, icefishes were expected to reflect anthropogenic pollution across multiple systems through ^{15}N analysis.

In light of these facts, we investigated differences in $\delta^{15}N$ of seston and icefishes from seven freshwater ecosystems with different trophic states in China (Fig. 1). Since no direct measures of wastewater input were available for the sites in the present study, we used the measured total nitrogen and phosphorus concentrations to indicate anthropogenic pollution and compared these values with the $\delta^{15}N$ values of seston and icefishes. Our principal objectives were to examine the relationships between $\delta^{15}N$ of seston and icefishes and total nitrogen and phosphorus concentrations among these sites, and to assess how well the icefishes perform as bio-indicators of anthropogenic pollution in these freshwater systems.

Materials and Methods

Lake water for seston was sampled from one to eight sites in each freshwater system, and then filtered onto pre-combusted glass-fibre filters (GF/C Whatman).

Icefishes (n = 1–23) were collected using gill nets near the sites where seston were sampled. The filters and icefishes (whole body) for isotope analyses were dried to a constant weight at 60°C in a drying oven, 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. Stable nitrogen isotope ratios were analyzed in parts-per-thousand (‰) differences from a standard reference material using the equation: $\delta^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where R is $^{15}N/^{14}N$. The standard reference 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‰. To examine the correlations between $\delta^{15}N$ values of seston, icefishes, and total nitrogen and phosphorus concentrations, STATISTICA for Windows statistical software (version 6.0) was used for the relative analyses.

Results and Discussion

In our test systems, total nitrogen and phosphorus concentrations ranged from 0.19 to 5.71 mg/L, and from 0.02 to 0.44 mg/L, respectively (Fig. 2). $\delta^{15}N$ of seston varied from 3.1‰ to 11.9‰, with an average of 6.0‰, while $\delta^{15}N$ of icefishes varied from 10.1‰ to 19.3‰, with an average of 14.7‰. $\delta^{15}N$ of both icefishes and seston varied among the freshwater systems (Figs. 2 and 3). $\delta^{15}N$ of icefishes were always higher (ranging from 5.8‰ to 14.3‰) than those of seston. The increase of seston $\delta^{15}N$ values was accompanied by an increase of total nitrogen (TN) and phosphorus (TP) concentrations (r = 0.59, $p < 0.05$ for TN; r = 0.68, $p < 0.01$ for TP; Fig. 2). Icefishes from the eutrophic systems had substantially higher $\delta^{15}N$ values (up to 9.2‰) compared to those from oligotrophic ones. Significantly positive correlations were observed between $\delta^{15}N$ of icefishes and $\delta^{15}N$ of seston (r = 0.66, $p < 0.01$), total nitrogen (r = 0.78, $p < 0.001$) and phosphorus (r = 0.45, $p < 0.05$) concentrations (Fig. 3, Table 1).

Assessment of anthropogenic pollution in aquatic ecosystems with $\delta^{15}N$ isotopic method has mostly concentrated on plants, sediment, and invertebrates (McKinney et al., 2002; Cole et al., 2004; Xu et al., 2005a, Xu et al., 2007b), with comparatively fewer applications of fish as

Fig. 1 Map of China showing the locations of our test freshwater ecosystems. 1, Lake Erhai; 2, Lake Fuxian; 3, Lake Xingyun; 4, Yangtze River; 5, Three Gorge Reservoir; 6, Lake Chaohu; 7, Lake Taihu

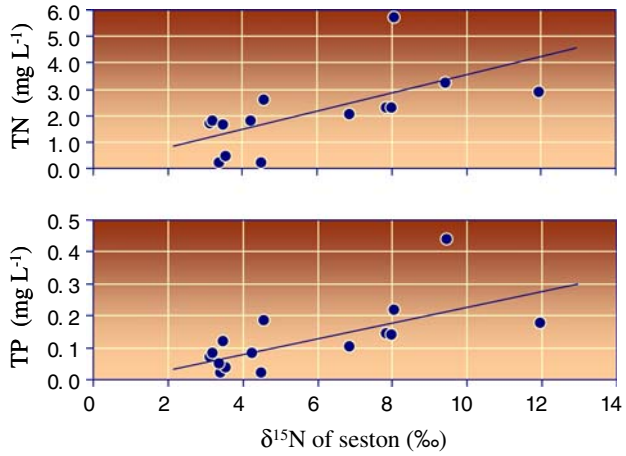

Fig. 2 Relationship between δ^{15}N values of seston and total nitrogen and phosphorus concentrations of lake water. Significant positive correlations were found between δ^{15}N values of seston and total nitrogen ($r = 0.59$, $p < 0.05$) and phosphorus ($r = 0.68$, $p < 0.01$) concentrations of lake water. Average values per site were used in the regression analysis and showed in the figures

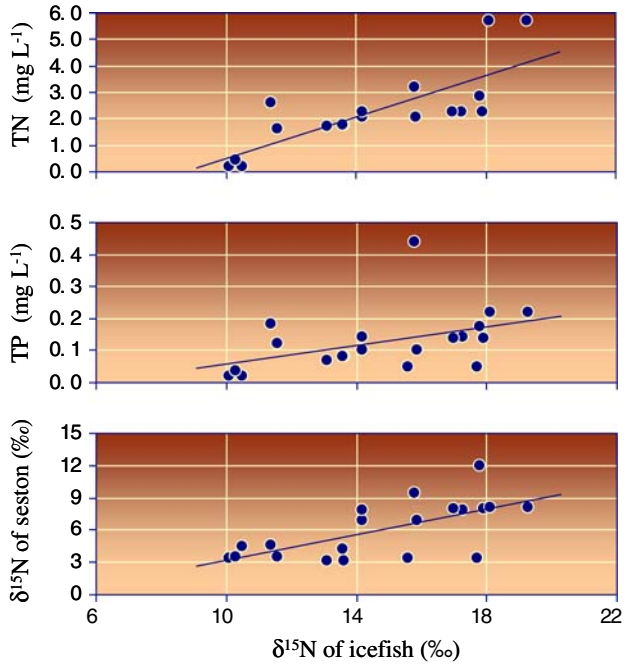

Fig. 3 Relationship between δ^{15}N values of icefishes and δ^{15}N values of seston, total nitrogen, and phosphorus concentrations of lake water. Significant positive correlations were found between δ^{15}N values of icefishes and δ^{15}N of seston ($r = 0.66$, $p < 0.01$), total nitrogen ($r = 0.78$, $p < 0.001$) and phosphorus ($r = 0.45$, $p < 0.05$) concentrations of lake water. Average values per site were used in the regression analysis and are shown in the figures

biological indicators (Lake et al., 2001; Schlacher et al., 2005). In this study, relationships between the δ^{15}N of seston and total nitrogen and phosphorus concentrations indicated that δ^{15}N of seston was a valid descriptor of the

Table 1 Average isotope values, standard deviations (SD), and sample size of icefish and seston in our test freshwater ecosystems

Lake	Site	Icefish*			Seston		
		δ^{15}N	SD	n	δ^{15}N	SD	N
Lake Erhai	1	10.3	0.7	7	3.6	0.2	3
Lake Fuxian	1	10.1	0.4	4	3.4	0.6	2
	2	10.5	0.4	23	4.5	0.8	25
Lake Xingyun	1	15.8	0.6	5	9.5	3.1	4
Yangtze River	1	13.1	0.6	5	3.1		1
Three Gorge Reservoir	1	13.6	0.7	5	3.2	0.4	3
	2	13.6	1.0	5	4.2	0.3	3
	3	11.6	0.8	5	3.5		1
Lake Chaohu	1	14.2	0.6	5	6.9	0.7	6
	2	15.9	0.1	3	6.7	0.6	6
	3	14.2	0.6	5	7.7	2.1	6
	4	17.3	1.1	4	7.9	3.1	6
Lake Taihu	1	17.9	0.6	16	8.0	4.5	54
	2	17.0	0.5	5	8.0	4.5	54
	3	17.8	0.3	2	11.9	0.3	4
	4	19.3	0.7	3	8.1	0.4	4
	5	18.1	0.4	3	8.1	0.4	4
	6	17.7	0.0	1	3.4	0.7	4
	7	15.6	1.1	5	3.4	0.7	4
	8	11.4	1.3	4	4.6	0.6	4

* No relationship was found between δ^{15}N values and the length of icefishes in this study

trophic state in these systems. Generally, the tissue turnover rate of fishes is slower than that of algae and invertebrates, which suggests that the δ^{15}N value of fishes fluctuates less over time than small, primary producers and consumers and thus provides better precision (Cabana and Rasmussen, 1996). Recently, the suitability of fish as bioindicators of wastewater nitrogen loads was investigated in subtropical estuaries, and the results indicated that fish δ^{15}N was a suitable indicator of wastewater not only in systems that receive large loads, but also for the detection of more-subtle nitrogen inputs (Schlacher et al., 2005). Schlacher et al. (2005) also suggested δ^{15}N in fish for the detection of the nitrogen isotope signal in waste input in the fish habitat, and that it represents an integration of nutrient movements along food chains rather than by the direct uptake of inorganic nitrogen as is the case for plants (Cole et al., 2004). This is especially important when assessing the influence of wastewater pollution on entire food webs. Significant positive correlations between δ^{15}N of icefishes and δ^{15}N of seston, total nitrogen, and phosphorus concentrations observed in this investigation also demonstrated that icefishes could be preferred indicators of anthropogenic contamination in test systems because they

integrated waste inputs over long time periods and reflected the movement of waste through the pelagic food chain.

In conclusion, the consistent shifts in $\delta^{15}N$ of icefishes between our tested freshwater systems that differ in trophic states, together with pervious studies (Schlacher et al., 2005; and references therein), have demonstrated that stable nitrogen isotopes are an effective tool to assess anthropologic pollution. It is possible that a similar approach may be applied to enable us to follow and estimate the nutrients input from the surrounding environment.

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References

Cabana G, Rasmussen JB (1996) Comparison of aquatic food chains using nitrogen isotopes. Proc Natl Acad Sci 93: 10844–10847

Cole ML, Valiela I, Kroeger KD, Tomasky GL, Cebrian J, Wigand C, McKinney KA, Grady SP, Silva MHC (2004) Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. J Environ Qual 33: 124–132

Heaton THE (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem Geol 59: 87–102

Lake JL, McKinney RA, Osterman FA, Pruell RJ, Kiddon J, Ryba SA, Libby AD (2001) Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Can J Fish Aquat Sci 58: 870–878

Lindau CW, Delaune RD, Alford DP (1997) Monitoring nitrogen pollution from sugarcane runoff using ^{15}N analysis. Water Air Soil Pollut 89: 389–399

Liu Z (2001) Changes in abundance of the icefish *Neosalanx pseudotaihuensis* Zhang (Salangidae) and the impacts on the zooplankton community of Xujiahe Reservoir (central China). Hydrobiologia 445: 193–198

McKinney RA, Lake JL, Charpentier MA, Ryba S (2002) Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. Environ Monit Assess 74: 167–192

Pennock JR, Velinski DJ, Ludham DJ, Sharp JH, Fogel MJ (1996) Isotope fractionation of ammonium and nitrate during the uptake by *Skeletonema costatum*: implications for the $\delta^{15}N$ dynamics under bloom conditions. Limnol Oceanogr 41: 451–459

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83: 703–718

Schlacher TA, Liddell B, Gaston TF, Schlacher–Hoenlinger M (2005) Fish track wastewater pollution to estuaries. Oecologia 144: 570–584

Wang Z, Lu C, Hu H, Zhou Y, Xu C, Lei G (2005) Freshwater icefishes (Salangidae) in the Yangtze River basin of China: Spatial distribution patterns and environmental determinants. Environ Biol Fish 73: 253–262

Xu J, Li S, Xie P (2005a) Differences in $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter from the deep oligotrophic Lake Fuxian connected with the shallow eutrophic Lake Xingyun, China. Bull Environ Contam Tox 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 (2007a) Size-related shifts in reliance on benthic and pelagic food webs by lake anchovy. Ecoscience 14 (2): 170–177

Xu J, Zhang M, Xie P (2007b) Stable carbon isotope variations in surface bloom scum and subsurface seston among shallow eutrophic lakes. Harmful Algae doi: 10.1016/j.hal.2007.02.002