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*Primary Research Paper*

# Variation in stable isotope signatures of seston and a zooplanktivorous fish in a eutrophic Chinese lake

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## Abstract

Temporal and spatial changes in $\delta^{13}$C and $\delta^{15}$N of seston (mainly phytoplankton) and isotopic relationship between seston and the lake anchovy (*Coilia ectenes*) were studied in the large eutrophic freshwater Lake Chaohu in China. Much of the spatial and temporal variation in $\delta^{13}$C of lake anchovies was explained by variation in seston, indicating a strong link between pelagic primary production and higher order consumers. Because the lake is shallow, there were no significant differences in $\delta^{13}$C and $\delta^{15}$N of seston between surface and overlying waters. Spatially, the relatively high $\delta^{13}$C and $\delta^{15}$N of seston in the western part of the lake might be due to high levels of anthropogenically derived N and C introduced from the surrounding cities through sewage drainage systems. The trophic position of the lake anchovy in the food web of Lake Chaohu was estimated to be 2.9–4.1 (3.5 ± 0.4), which agrees well with the previous stomach content analysis suggesting that the lake anchovy fed both on zooplankton and small planktivorous fishes.

## Introduction

Since the $\delta^{15}$N and $\delta^{13}$C isotopic signatures of an animal are a reflection of its trophic position and the carbon sources on which it is feeding, respectively (Vander Zanden et al., 1997; Kelly, 2000), stable isotope analysis is increasingly used to provide a time-integrated measure of food web relationships based on energy flow (e.g. Kling et al., 1992; Hesslein et al., 1993; Gu et al., 1994; France, 1995; Vander Zanden et al., 1997; Pinnegar & Polunin, 2000; Post, 2002). Temporal and spatial isotopic variations in phytoplankton and aquatic consumers make it difficult to construct food webs in natural lakes (Yoshioka & Wada, 1994; Beaudoin et al., 2001). Since stable isotope signatures of phytoplankton are variable in nature, the information obtained from short-term studies may result in inaccurate assessment on food web structure based on phytoplankton (Kendall et al., 2001). Therefore, examination on temporal and spatial variations of stable carbon and nitrogen isotopes in phytoplankton and dominant consumers will be important for the understanding of food web structure constructed by stable isotope.

The present study was conducted in the highly eutrophic Lake Chaohu during April and October 2003. The aims were to describe the temporal, spatial and vertical variations of stable carbon and nitrogen isotopes in seston, and to describe temporal variations of stable carbon and nitrogen isotopes of the lake anchovy (*Coilia ectenes*), a predominant planktivorous fish in the lake, and to discuss the possible mechanisms underlying these variations.

## Materials and methods

### *Study site*

Lake Chaohu, located in the delta of the Yangtze River, is one of the five largest freshwater lakes in China and has a mean surface area of 770 $km^2$, a mean depth of 2.7 m and a storage capability of 2.1 billion $m^3$ (Fig. 1). Because of heavy discharge of domestic sewage, the lake is eutrophic with dense cyanobacterial blooms (mainly composed of *Microcystis* and *Anabaena*) in the warm seasons of each year. The lake anchovy is the most important commercial fish in this lake.

### *Sampling*

Sampling of seston was conducted monthly from April to October in 2003 at six sites across the lake (Fig. 1). Surface (0 m) and overlying (0.5 m above the surface of sediment) water of each sampling site were filtered onto percombusted glass fibre filters (GF/C whatman). The filters were then wrapped in aluminium foil, put into plastic baggies, preserved in an ice box and brought back to laboratory. In the laboratory, the filters were acidified with superfluous 1N HCl solution to dissolve possible calcium carbonate ($CaCO_3$), followed by a rinse in distilled water. They were then dried to a constant weight at 50 °C, ground and homogenized to a fine powder with mortar and pestle, and then stored in a desiccator with silica gel for subsequent stable isotope analysis. The lake anchovy *C. ectenes* was collected monthly by casting nets and/or from fishermen from April to October in 2003. White dorsal muscle of the fish was taken for analysis, since it is less variable in terms of $\delta^{13}C$ and $\delta^{15}N$ than other tissues (Pinnegar & Polunin, 1999). The fish samples were dried at 60 °C to a constant weight, ground and homogenized to a fine powder and sealed in a desiccator with a silica gel desiccant for stable isotope analysis. Sample sizes of seston and lake anchovy were shown in captions of Figures 2–4.

### *Analysis*

Total dissolved inorganic carbon (DIC) was determined with 1010 Total Organic Carbon Analyzer. Ammonium was determined by Nessler's reagent colorimetric method, nitrate by ultraviolet spectrophotometric screening method, and nitrite by the $\alpha$-naphthylamine method. Total dissolved inorganic nitrogen (DIN) was the sum of ammonium, nitrate and nitrite concentrations. Total nitrogen (TN) was measured by alkaline potassium persulfate digestion–UV spectrophotometric method. Total phosphorus (TP) was digested with potassium persulfate and measured by molybdenum blue colorimetric method.

Stable carbon and nitrogen isotope ratios were analyzed with Delta plus (Finnigan) continuous flow isotope ratio mass spectrometer (CF-IRMS) directly coupled to an EA1110 elemental analyzer (Carlo Erba) for combustion. 20% of the tissues and seston samples were analyzed two or more times as replicates. Two samples of an internal reference material were analyzed after every 5–10 measurements in order to calibrate the system and compensate for drift with time. Isotope ratios were expressed as parts-per-thousand (‰) differences from a standard reference material using the equation: $\delta X(‰) = (R_{sample}/R_{standard} - 1) \times 1000$, where $X$ is $^{15}N$ or $^{13}C$, $R$ is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$ and $\delta$ is the measure of heavy to light isotope in the sample, whereby higher $\delta$ values denote a greater proportion of the heavy isotope. The standard reference materials were Pee Dee Belemnite (PDB) and atmospheric nitrogen for carbon and nitrogen, respectively (Craig, 1957; Mariotti, 1983). International reference materials were IAEA-NBS18, IAEA-USGS24, IAEA-USGS25 and IAEA-USGS26. The standard deviations of $\delta$ $^{13}C$ and $\delta^{15}N$ replicate analyses were less than 0.2‰ and 0.4‰, respectively.

## Results

Seston was a mixture of phytoplankton, other minute organisms and organic detritus. During the study period, phytoplankton production was high with dense water blooms of *Microcystis* spp. and *Anabaena* spp., indicating the importance of phytoplankton in seston.

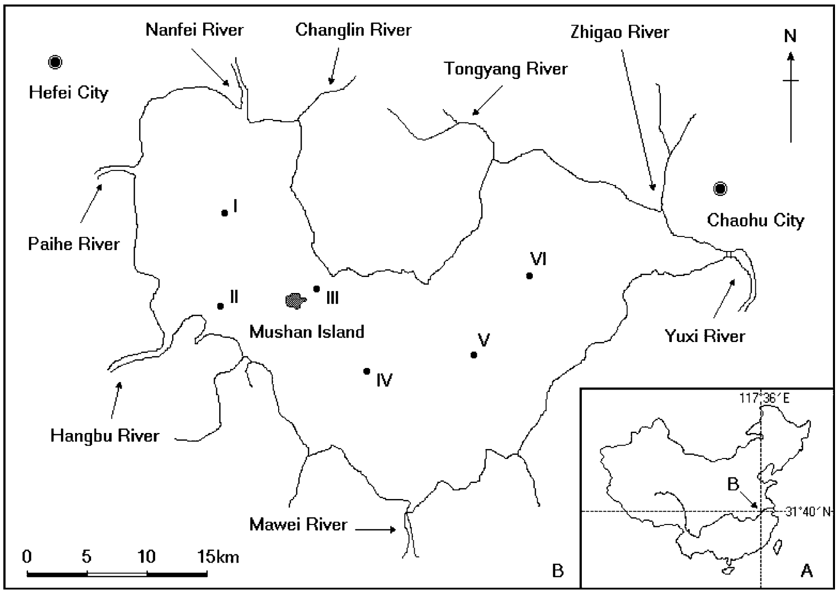


*Figure 1.* Lake Chaohu in the Yangtze Basin (A) and locations of sampling sites (B).

The $\delta^{13}C$ of seston samples showed a minimal value of −28.2‰ in April, followed by a remarkable increase up to the maximal −24.5‰ in July and a gradual decline afterwards (Fig. 2). $\delta^{13}C$ of seston was negatively correlated with DIC concentrations ($r = -0.92$, $p < 0.05$). $\delta^{13}C$ of the lake anchovy showed a positive correlation with that of seston ($r = 0.87$, $p < 0.05$). The difference in $\delta^{13}C$ between the fish muscle and seston was less than 1‰ except in June and October (1.2‰). $\delta^{15}N$ of seston showed significant variation, ranging from 5.2‰ to 8.7‰, while $\delta^{15}N$ of the fish muscle remained quite stable throughout the study period. There was no significant correlation between $\delta^{15}N$ of seston and DIN ($r = -0.28$, $p > 0.1$) or $\delta^{15}N$ of fish muscle ($r = -0.60$, $p > 0.1$).

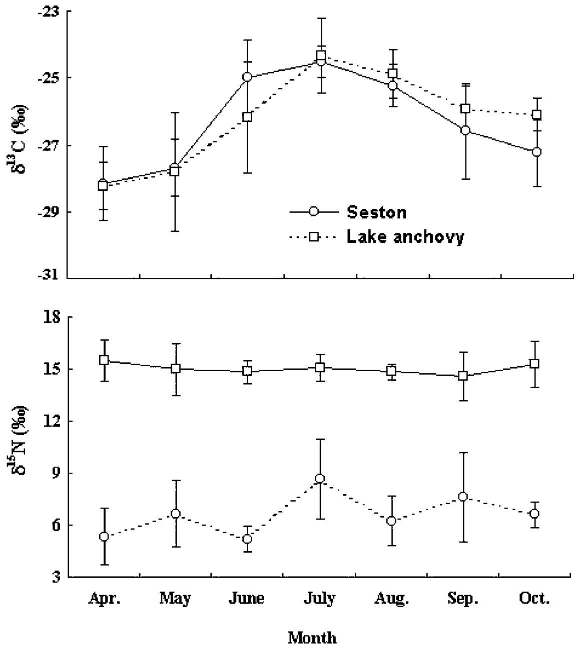


*Figure 2.* Temporal variation in $\delta^{13}C$ and $\delta^{15}N$ of seston and lake anchovy (means ± 1 SD). The number of samples analyzed was 12 per month for seston except in October ($n = 2$) and 17–30 per month for fish except in August ($n = 4$).

There were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ of seston and DIC and DIN concentrations between surface and overlying waters (Fig. 3). Both $\delta^{13}C$ and $\delta^{15}N$ values of seston decreased from western to eastern sites (Fig. 4). $\delta^{13}C$ showed a significantly positive correlation with DIC concentrations ($r = 0.95$, $p < 0.01$), while $\delta^{15}N$ showed positive correlations with DIN ($r = 0.93$, $p < 0.01$), TP ($r = 0.92$, $p < 0.01$) and TN ($r = 0.83$, $p < 0.05$). No correlation in space was found between stable isotope signatures of fish and those of seston.

## Discussion

In the present study, the average $\delta^{13}C$ values of seston samples are within the range (−42‰ ~ −24‰) of freshwater plankton reported in the

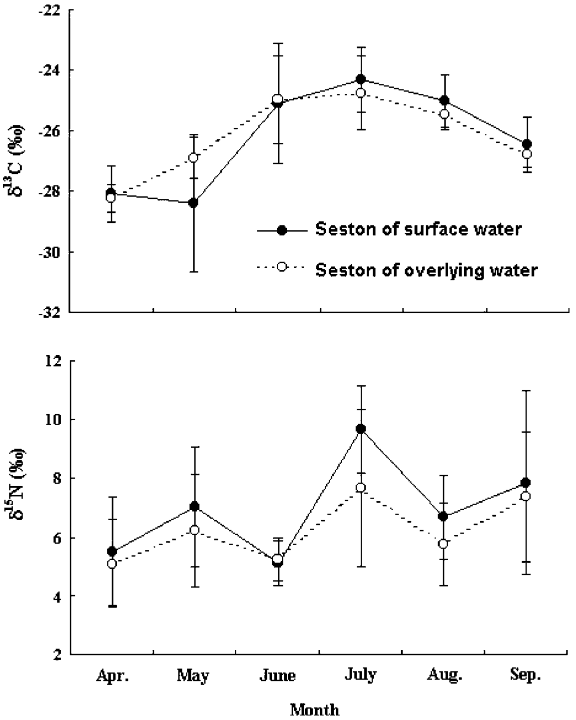


*Figure 3*. Temporal and vertical differences in $\delta^{13}C$ and $\delta^{15}N$ of seston (means ± 1 SD). The number of samples analyzed was 6 per month for seston from both surface water and overlying water.

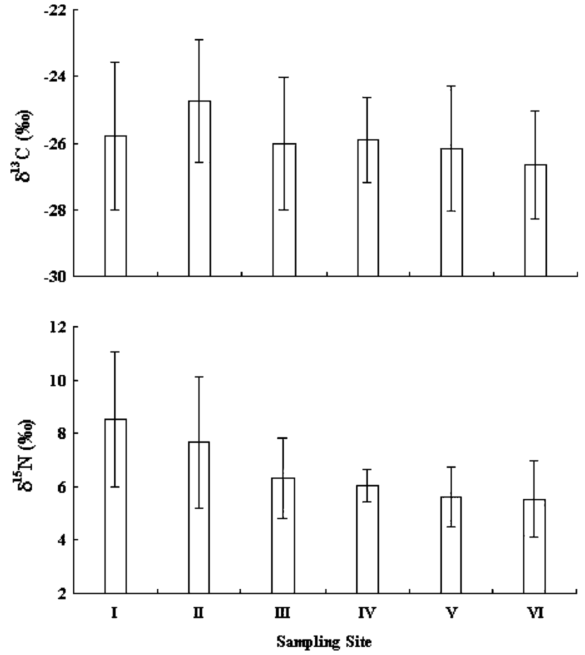


*Figure 4*. Spatial variation in $\delta^{13}C$ and $\delta^{15}N$ values of seston across the six sample sites (see Fig. 1; means ± 1 SD). $\delta^{13}C$ and $\delta^{15}N$ values are means of all sampling dates from each site ($n = 12$).

literature (Kendall et al., 2001). The $\delta^{13}C$ value of phytoplankton is affected by DIC availability, its isotopic content, and phytoplankton physiology (Paterson & Whitfield, 1997). The observed negative correlation between DIC and $\delta^{13}C$ has been reported in some eutrophic lakes (Stiller & Nissenbaum, 1980; Yoshioka et al., 1989; Yoshioka & Wada, 1994). Takahashi et al. (1990) suggested that DIC concentration available for photosynthesis was the primary factor that governs carbon isotope fractionation during photosynthesis of phytoplankton, and also suggested that low DIC concentration during summer bloom could result in relatively high $\delta^{13}C$ of phytoplankton. Generally, the difference between the $\delta^{15}N$ of DIN and phytoplankton is greatest when DIN is sufficient and growth rate of phytoplankton is low, but smallest when DIN is scare and/or growth rate of phytoplankton is high (Kendall et al., 2001). However, in present study, the negative correlation between DIN and $\delta^{15}N$ of seston was not significant. In June, $\delta^{15}N$ of seston was minimum when biomass of the $N_2$-fixing *Anabaena* reached its maximum, accounting for more than 50% of total algal biomass (D. G. Deng, personal communication). It seems that the variation in $\delta^{15}N$ of seston in Lake Chaohu might be caused, to some extent, by different biological fractionation between non-fixation species and $N_2$-fixation species which assimilate $N_2$ from atmosphere and have $\delta^{15}N$ value near to 0‰ (Yoshioka & Wada, 1994; Kendall et al., 2001).

Because Lake Chaohu is shallow and vertical mixing of the lake water occurs all the year, the vertical variations of $\delta^{13}C$ and $\delta^{15}N$ of seston were not observed in present study. Both $\delta^{13}C$ and $\delta^{15}N$ values of seston decreased form western sites to eastern ones. The relatively high $\delta^{13}C$ and $\delta^{15}N$ of seston in the western part of the lake might be due to high levels of anthropogenically derived N and C introduced from the surrounding cities through sewage drainage systems, since it is known that anthropogenical sewage and aquatic animal waste usually have high $\delta^{15}N$ (Mariotti, 1986; Kendall et al., 2001; Kendall & Aravena, 2000), and that nitrogen and carbon metabolism concomitant with eutrophication in lake sediment including biochemical and physicochemical processes also cause the enrichment of $^{13}C$ and $^{15}N$ (Miyake & Wada, 1971; Yoshioka et al., 1988; Gu et al., 1996; Ogawa et al., 2001; Havens et al., 2003).

The lake anchovy predominated in the fish community of Lake Chaohu throughout the year. No correlation in space was found between stable isotope signatures of fish and those of seston, which may suggest that fish are moving (and feeding) extensively through the lake. The positive correlation between $\delta^{13}C$ of the lake anchovy and that of seston indicated that temporal variation of $\delta^{13}C$ in primary producers was conserved in consumers higher up the food chains since it has been reported that modification of $\delta^{13}C$ during feeding process is within 0–1‰ (France, 1995). Wada et al. (1987) established a relationship between $\delta^{15}N$ value and trophic level, TL (trophic level) = $(\delta^{15}N_{animal} - \delta^{15}N_{algae})/3.3 + 1$, in an Antarctic ecosystem. Using the $^{15}N$ enrichment factor of 3.3‰ per trophic level, the trophic position of *C. ectenes* in the food web of Lake Chaohu was estimated to be 2.9–4.1 (3.5 ± 0.4), indicating that the fish deriving their biomass from not only zooplankton but also other fishes. The isotope results are consistent with previous stomach content analysis indicating that anchovies fed on both zooplankton and small planktivorous fishes, such as small lake anchovy, ice fish (*Hemisalranx prognathus*) and halfbeak (*Hyporhamphus intermedius*) (Diao & Luo, 1982; Diao & Wu, 1982).

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