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Seasonal variations in stable isotope ratios of two biomanipulation fishes and seston in a large pen culture in hypereutrophic Meiliang Bay, Lake Taihu

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ARTICLE INFO

Article history:

Received 27 February 2007

Received in revised form

24 October 2007

Accepted 24 November 2007

Keywords:

Stable isotopes

Silver carp

Bighead carp

Seston

Seasonal variation

ABSTRACT

This paper reports on seasonal changes in stable carbon and nitrogen isotope ratios of seston and muscle tissue of silver carp and bighead carp during 2004 and 2005, focusing primarily on the carbon sources and trophic relationships among phytoplankton, zooplankton and silver carp and bighead carp in a large fish pen of Meiliang Bay (Lake Taihu, China). $\delta^{13}\text{C}$ showed a minimal value in March 2005 and a maximal value in August 2005 in seston both inside and outside the pen, whereas $\delta^{15}\text{N}$ of seston showed the minimum in winter and the maximum during algal blooms. A positive correlation between $\delta^{13}\text{C}$ of silver carp and that of seston suggested that temporal variation of $\delta^{13}\text{C}$ in seston was preserved in fish via the food chain. The differences of $\delta^{13}\text{C}$ among seston, zooplankton and muscle tissue of silver carp and bighead carp ranged only 0.2–1.7%, indicating that plankton production was the primary food source of filter-feeding fishes. According to a mass balance model, we estimated that the contributions of zooplankton to the diets of silver carp and bighead carp were 45.7% and 54.3%, respectively, based on the $\delta^{15}\text{N}$ values of zooplankton and planktivorous fishes.

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1. Introduction

Farmed silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) are among the so-called “four major fish species” in Chinese freshwater aquaculture (Nie and Yao, 2000). They are the main focus of the non-traditional biomanipulation technique (Xie, 2003), and have been applied in many Chinese lakes (e.g., Lake Dianchi in Yunnan Province, Lake Chaohu in Anhui Province) for managing dense cyanobacterial blooms. Feeding habits of these two fishes have been the focus of many ichthyologists and ecologists. Nie and Chiang (1954) have suggested that silver

carp and bighead carp are planktivorous fishes, and that silver carp feeds mainly on phytoplankton and bighead carp on zooplankton. Cremer and Smitherman (1980) show agreement with the conclusions of Nie and Chiang (1954).

In recent decades, more studies have been reported about the utilization of silver carp and/or bighead carp in managing algal community structure (e.g., Xie, 1996; Turker et al., 2003). However, the digestibility of algae by stomachless filter-feeding fishes has been debated (Xie, 1999). Silver carp is reported to be effective in manipulating nuisance phytoplankton blooms in eutrophic lakes (Starling, 1993; Xie, 1996; Fukushima et al., 1999) and aquaculture systems (Smith, 1985; Turker et al., 2003). It has been recognized, however, that silver

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doi:10.1016/j.ecoleng.2007.11.009

carp cannot digest many algal species (Nie and Chiang, 1954; Herodek et al., 1989). Furthermore, experimental stocking of silver carp for managing phytoplankton biomass in lakes and ponds has often failed to reduce algae (Laws and Weisburd, 1990). Other researchers indicate that zooplankton is the primary composition of food ingested by silver carp (Kajak et al., 1977). There is less uncertainty about food sources of bighead carp (Gu et al., 1996a,b), which largely feeds on zooplankton (Cremer and Smitherman, 1980).

Stable isotopes of carbon and nitrogen provide a powerful tool to estimate carbon flow and trophic positions for consumers in food webs (Post, 2002). Field investigations and laboratory experiments suggest that $\delta^{15}\text{N}$ generates a progressive enrichment of approximately 3–4% from prey to predator (Minagawa and Wada, 1984; Fry, 1988), and hence the stable nitrogen isotope ($\delta^{15}\text{N}$) has been used to define trophic levels in food webs (Fry, 1991). Stable isotope ratios of carbon also produce a trophic enrichment (0–1%) for clarifying carbon sources in aquatic ecosystems and tracing carbon flow from primary producers to top consumers (Gu et al., 1996a,b; Vizzini et al., 2002; Persic et al., 2004). Nevertheless, stable isotope signatures of phytoplankton are naturally variable over time, and short-term studies may lead to inaccurate assessment about food web structure based on phytoplankton (Kendall et al., 2001). Therefore, temporal dynamics of stable carbon and nitrogen isotope ratios need to be investigated for understanding the food web structure and the effect of bio-control on algae blooms through use of filter-feeding carps.

Early studies on silver carp and bighead carp rely mainly on the feeding habits through conventional analysis of gut content (e.g., Nie and Chiang, 1954; Cremer and Smitherman, 1980). Few examinations engaging stable isotopes have been executed on the feeding relationships of planktivorous silver carp and bighead carp. Gu et al. (1996a,b) have indicated that silver carp and bighead carp obtained a mean 60% overlap of their food from the same trophic level. Xu and Xie (2004) reported the results of the food web structure of Lake Donghu with stable isotope ratios of carbon and nitrogen, showing that the contributions of zooplankton to the food of silver carp and bighead carp are 54% and 74%, respectively.

In the present study, together with other measures of lake restoration such as nutrient removal by floating aquatic macrophytes (Sooknah and Wilkie, 2004) and the restoration of submerged macrophytes (Qiu et al., 2001), silver carp and bighead carp stocked in the pen are utilized to control algal blooms for improving drinking water quality in Meiliang Bay of Lake Taihu. Stable isotope studies on carbon sources and trophic relationships among phytoplankton, zooplankton and carp are necessary for estimating the algae-controlled efficiency of silver carp and bighead carp. The main objectives of this study are: (1) to investigate the temporal dynamics of seston, zooplankton and planktivorous fishes, silver carp and bighead carp in a large pen of Meiliang Bay (Lake Taihu, China); (2) to discuss the possible mechanisms underlying the patterns; (3) to determine the carbon sources and trophic relationships among phytoplankton, zooplankton and silver carp and bighead carp and (4) to assess the contributions of zooplankton to the growth of silver carp and bighead carp by mass balance model of stable isotopes.

2. Materials and methods

2.1. Study sites description

Lake Taihu, with an area of 2428 km² and a mean water depth of 1.9 m, is one of the famous five great freshwater lakes in China. A total of 35 million people live in the Lake Taihu drainage area. Due to the development of industry and agriculture in the lake region, as well as a rapid increase in the population, the water quality is decreasing in Lake Taihu and its surrounding river-lake systems (Pu et al., 1998). Meiliang Bay (31°31'–325'N, 120°09'–340'E), located in the northern part of Lake Taihu, acts as principal water resource and recreational spot for Wuxi City, Jiangsu Province. In recent decades, due to annual outbreaks of *Microcystis* blooms, this region has been one of the most hypertrophic parts in Lake Taihu (Ke et al., 2007).

2.2. Experimental designing and sample collection

The fish pen (surface area 1.035 km²) was located near the bank of Meiliang Bay where heavy cyanobacterial blooms occurred in the warm seasons. The bay has a mean water depth of 2.1 m. Water temperature averaged 21.3 °C throughout the whole year. Fingerlings of similar sizes and weight (with average of 18.4 ± 2.8 cm, 133.3 ± 55.2 g for silver carp and 21.4 ± 1.3 cm, 242.1 ± 44.4 g for bighead carp, respectively) were stocked in the pen in March 2004, in order to control heavy cyanobacterial surface blooms. Silver and bighead carp samples were collected monthly from April 2004 to December 2005. Generally, three to five individuals of each carp were taken monthly from the pen on each sampling date. Water samples used for POM and nutrient analyses were taken from the surface and bottom layers using a 5-l modified Patalas's bottle sampler. Water samples were well mixed and then filtrated through glass fiber filter (Whatman GF/C) using vacuum. Particulate organic matter kept on the filter was analyzed as seston sample and filtrated water was used for further nutrient analyses. POM was collected monthly from both inside (three sites) and outside (one site) the pen during September 2004 and October 2005. Zooplankton sample collection included three processes: (i) filtering lake water inside the pen through a plankton net with 64 μm mesh size, (ii) light-illuminating to detach zooplankton from large-sized algal community and (iii) collecting bulked zooplankton sample through a plankton net with 112 μm mesh size.

2.3. Nutrient analysis and stable isotope analysis

Ammonium was measured by the Nessler method, nitrite by the a-naphthylamine method and nitrate by the UV spectrophotometry method (Eaton et al., 1995). Total inorganic nitrogen (TIN) was the sum of ammonium, nitrate and nitrite concentrations. Total nitrogen (TN) was analyzed by alkaline potassium persulfate digestion-UV spectrophotometric method (Nydahl, 1978). Total phosphorus (TP) was digested with potassium persulfate and measured by the ammonium molybdate method (Prepas and Rigler, 1982).

Dorsal muscle tissues were anatomized from carps. Both muscle tissues and zooplankton samples were roasted at 60 °C to constant weight and ground to a fine powder using a mortar and pestle for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Prior to measuring, all seston samples were acidified with 1N HCl and roasted to a constant weight at 60 °C then ground to a fine powder using a mortar and pestle. Samples were analyzed with a Carlo Erba EA-1110 elemental analyzer accompanied by a Delta Plus Finnigan Mat isotope ratio mass spectrometer via continuous flow II interface. Isotopic ratios were expressed relative to international standards (Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). Delta values were defined as: $\delta R = [(X_{\text{sample}} - X_{\text{standard}})/X_{\text{standard}}] \times 10^3 (\%)$, where $R = ^{13}\text{C}$ or ^{15}N and X is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Analytical deviations for replicates were less than 0.4% and 0.5% for carbon and nitrogen, respectively.

2.4. Statistical analysis

The correlations between isotopic compositions of seston and chemical nutrients (TN, TIN and TP) were executed with SPSS 11.5 version. Simultaneously, the correlations between isotopic compositions of seston and two carps inside the pen were investigated to indicate how silver carp and bighead carp responded to temporal variations of seston. Pearson t-test was made to elucidate if there were significant differences between isotopic compositions of seston inside and outside the pen. The correlation was expressed by p -value and r -value, and the difference test by p -value only.

2.5. Mass balance model

The proportion (f) of zooplankton consumed by filter-feeding fishes can be calculated using the following mass balance model provided by Gu et al. (1996a,b): $f (\%) = (x - y) \times 100/e$, where x is $\delta^{15}\text{N}$ of filter-feeding fishes and y is $\delta^{15}\text{N}$ of zooplankton; e is the enrichment factor of $\delta^{15}\text{N}$ from one trophic level to the upper one. Here we defined the average e value as 3.5%, as reported from other studies (e.g., Minagawa and Wada, 1984; Fry, 1988).

3. Results

3.1. Seasonal variation in stable isotopic compositions of seston

Seston consisted of a mixture of phytoplankton, aquatic invertebrates and other organic detritus. During the study period, microscopic observations showed that predominant species of phytoplankton were *Microcystis* spp. in summer and autumn and *Ulothrix* spp. in Winter and Spring. Phytoplankton production was high during algal blooms (from May to November), indicating the importance of phytoplankton in particulate organic matter (seston).

During the study period, $\delta^{13}\text{C}$ of seston samples showed remarkable seasonal variations both inside and outside the pen (Fig. 1). $\delta^{13}\text{C}$ of the seston samples showed a gradual decline to the minimal value of -32.4% in March, followed by a clear increase up to the maximal -25.7% in August inside

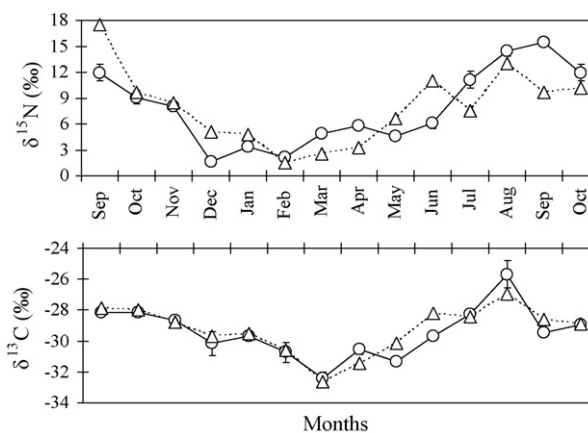


Fig. 1 – Seasonal variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of seston inside (○) and outside (△) the fish pen from September 2004 to October 2005 (mean \pm 1S.D.). Three samples were taken monthly for seston inside the pen and one sample for seston outside the fish pen. No significant difference was found between the stable isotope compositions of seston inside and outside the fish pen.

the pen, while $\delta^{13}\text{C}$ of seston samples outside the pen showed a minimal value of -32.6% in March and the maximal -26.9% in August. Similar variations occurred in $\delta^{15}\text{N}$ of seston samples inside and outside the pen (Fig. 1). $\delta^{15}\text{N}$ of seston samples inside the pen reached the minimal (1.7%) in December 2004, followed by an intermittent increase up to the maximal 15.4% in September 2005. $\delta^{15}\text{N}$ of seston samples outside the pen had the maximal value of 17.5% in September 2004, and then underwent a remarkable decline to a minimal value of 1.5% in February 2005, followed by an intermittent increase afterwards.

There was no significant difference between isotopic compositions of seston inside and outside the pen ($p=0.400$ and $p=0.996$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). The correlations between stable isotopes of seston samples and nutrients (including TN, TIN and TP) inside and outside the pen were evaluated. $\delta^{15}\text{N}$ of seston inside the pen was negatively correlated with both TN ($r=-0.699$, $p<0.01$) and TIN ($r=-0.756$, $p<0.01$) inside the pen, while there was no correlation with all the nutrients outside the pen. $\delta^{15}\text{N}$ of seston outside the pen was roughly correlated with TN and TIN inside the pen ($r=-0.518$, $p=0.069$ and $r=-0.546$, $p=0.054$, respectively). Furthermore, $\delta^{13}\text{C}$ of seston had no significant correlation with all the nutrients both inside and outside the pen. TP and TDP concentrations were not correlated with stable isotope ratios of seston both inside and outside the pen.

3.2. Seasonal variation in stable isotopic compositions of filter-feeding fishes

Stable isotope signatures of silver carp showed temporal variations similar to those of bighead carp (Figs. 2 and 3). There were lower values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for silver carp and bighead carp in the initial stages. For silver carp, $\delta^{13}\text{C}$ ranged between -31.5% in April 2004 and -25.9% in July 2005, while $\delta^{15}\text{N}$ varied from 7.5% in May 2004 to 18.8% in December

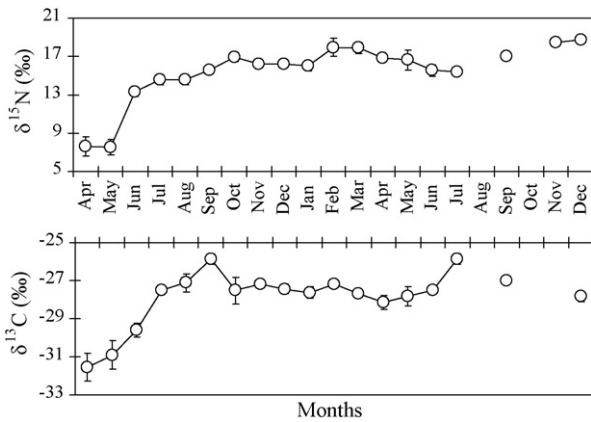


Fig. 2 – Seasonal variations in muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of silver carp in the fish pen (mean \pm 1S.D.). Three samples were taken monthly for muscle tissue of silver carp except that no sample was collected in August, October or November of 2005.

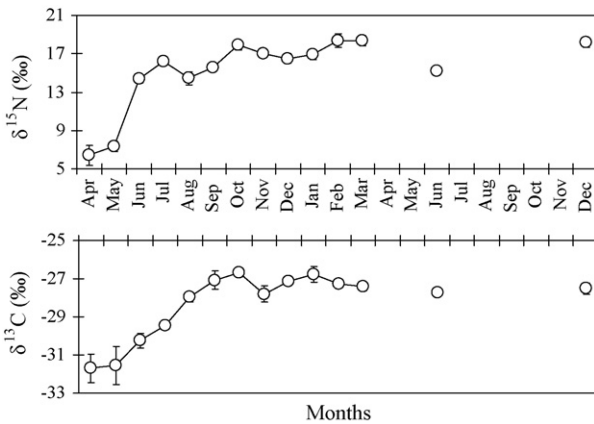


Fig. 3 – Seasonal variations in muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bighead carp in the fish pen (mean \pm 1S.D.). Three to five samples were taken monthly for muscle tissue of bighead carp except for one or two samples in April, May and July of 2004 and in June and December of 2005.

2005. The $\delta^{13}\text{C}$ value of bighead carp varied from -31.7% in April 2004 to -26.7% in October 2004. $\delta^{15}\text{N}$ of bighead carp showed significant variation, ranging from 6.4% in April 2004 to 18.4% in March 2005. However, both silver carp and big-

Table 1 – Relationships between stable isotope ratios of seston and muscle tissue of silver carp (SC) and bighead carp (BH)

		SC		BH	
		$\delta^{13}\text{C}$ (%)	$\delta^{15}\text{N}$ (%)	$\delta^{13}\text{C}$ (%)	$\delta^{15}\text{N}$ (%)
SI	$\delta^{13}\text{C}$	$r=0.631^*$	$r=-0.649^*$	$r=0.243$	$r=-0.474$
	$\delta^{15}\text{N}$	$r=0.606^*$	$r=-0.266$	$r=0.012$	$r=-0.324$

SI represents seston inside the pen.
* Represents $p < 0.05$.

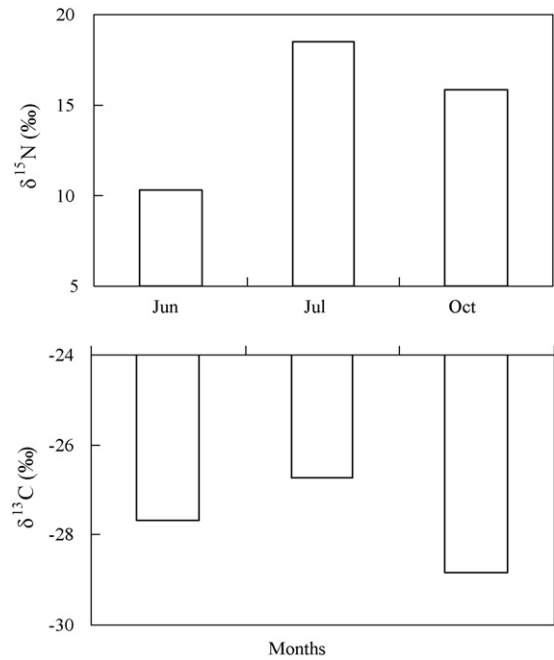


Fig. 4 – Temporal variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of zooplankton collected during the outbreak of water bloom. Hundreds of individual zooplankton were incorporated into each sample to obtain enough analytical mass.

head carp had much higher $\delta^{15}\text{N}$ values than those of seston (Fig. 6).

Statistical analyses between isotopic compositions of the two carps and seston inside the pen are shown in Table 1. $\delta^{13}\text{C}$ of silver carp showed positive correlations with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of seston, while $\delta^{15}\text{N}$ of silver carp was negatively correlated with $\delta^{13}\text{C}$ of seston only. There was no significant correlation between isotopic compositions of bighead carp and seston.

3.3. $\delta^{13}\text{C}$ signatures of seston, zooplankton and filter-feeding fishes

$\delta^{13}\text{C}$ of seston showed non-significant difference between inside and outside the pen, with average (\pm S.D.) of $-29.4 \pm 1.6\%$ ($n=42$) and $-29.3 \pm 1.5\%$ ($n=14$), respectively. Zooplankton had more enriched $\delta^{13}\text{C}$ than that of seston ($-27.7 \pm 1.1\%$ for zooplankton ($n=3$) and $-29.4 \pm 1.6\%$ for seston ($n=56$), respectively) (Fig. 4), while there was very little difference (1.7%) between $\delta^{13}\text{C}$ of zooplankton and seston. $\delta^{13}\text{C}$ of silver carp and bighead carp were $-27.9 \pm 1.5\%$ ($n=51$) and $-28.1 \pm 1.6\%$ ($n=35$), respectively, suggesting that they had similar values of $\delta^{13}\text{C}$. As a result, the $\delta^{13}\text{C}$ of seston, zooplankton and muscle tissue of silver carp and bighead carp ranged from 0.2% to 1.7% (Fig. 5).

3.4. $\delta^{15}\text{N}$ signatures of seston, zooplankton and filter-feeding fishes

$\delta^{15}\text{N}$ of seston showed non-significant difference between inside and outside the pen, with average (\pm S.D.) of $7.9 \pm 4.5\%$, $n=42$ and $7.9 \pm 4.4\%$, $n=12$, respectively. $\delta^{15}\text{N}$ of zooplankton

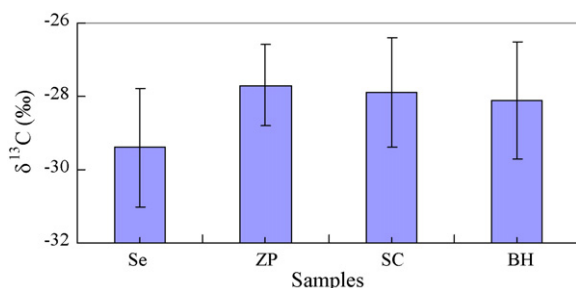


Fig. 5 – Comparison of stable carbon isotope values (mean ± 1S.D.) among seston (Se), zooplankton (ZP), muscle tissues of silver carp (SC) and bighead carp (BH). The corresponding δ¹³C values were averaged monthly in the study period, respectively.

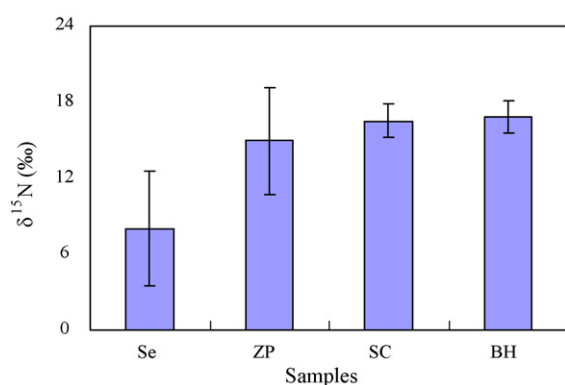


Fig. 6 – Comparison of stable nitrogen isotope values (mean ± 1S.D.) among seston (Se), zooplankton (ZP), muscle tissue of silver carp (SC) and bighead (BH). The corresponding δ¹⁵N values were averaged monthly in the study period, respectively.

was the average of δ¹⁵N values in June, July and October, which was 7.0‰ higher than that of seston. Prior to stocking, silver carp and bighead carp were cultured in ponds feeding on commercial forage with distinct isotopic signatures. Therefore, δ¹⁵N values from April to June 2004 were excluded from the data pool, considering that the time-lagged isotopic signatures of commercial forage for fingerlings in the initial stages could affect the interpretation of isotopic signatures in the field. Muscle δ¹⁵N of silver carp and bighead carp were averaged from July 2004 to December 2005. Great variations of δ¹⁵N among seston, zooplankton and fish muscle were found (Fig. 6), with ranges from 6.9‰ (between seston and zooplankton) to 8.8‰ (between seston and bighead carp). Nevertheless, δ¹⁵N of silver carp and bighead carp were 1.6‰ and 1.9‰ higher than that of zooplankton.

4. Discussion

POM can be a significant nutrient source for local ecosystems and also provides a historical record of natural and anthropogenic activities in sewage drainage systems (Hedges et al., 1986). Seasonal variations of isotopic compositions have been found in organisms like plankton with rapid growth

and isotope turnover rates (O'Reilly and Hecky, 2002), whereas isotopic compositions of consumers can experience enrichments of approximately 1‰ for δ¹³C (Michener and Schell, 1994) and 3.5‰ for δ¹⁵N between predator and prey (Minagawa and Wada, 1984). Consequently, stable isotope ratios of silver carp and bighead carp can range temporally owing to seasonal variations of isotopic compositions of plankton as diets of filter-feeding fishes.

Vizzini and Mazzola (2003) observed wide seasonal variations of isotopic compositions in organic matter sources and fishes and progressively showed the general trend that isotopic compositions were enriched in summer and depleted in winter. In the present study, positive correlation was found between δ¹³C of silver carp and that of seston in the pen, suggesting that temporal variation of δ¹³C in seston was preserved in filter-feeding fishes via the food chain.

δ¹³C of seston and fishes underwent consistent variations, with the minimal value in cold seasons and the maximal value during plankton blooms. However, there was no significant correlation between isotopic ratios of bighead carp and those of seston in the pen. δ¹³C and δ¹⁵N of bighead carp were 1.3‰ and 8.8‰ higher than seston, respectively, indicating that bighead carp assimilated carbon sources mainly from the pelagic food web and occupied a trophic position about two trophic levels higher than phytoplankton.

Perrson and Hansson, 1999 studied two fishes (*Perca fluviatilis* and *Abramis brama*) and suggested that stable isotope signatures of consumers have not been found in the tissue until 3 months after their trophic shifts. Silver carp fry had already finished their diet shift during the larval stages, and revealed feeding habits similar to adult fish (Feng and Zhou, 1995). This indicated that there was no trophic shift from fingerlings stocked in the pen to adult silver carp. Fingerlings of silver carp and bighead carp were stocked in cultivated ponds in another city before they were transferred into the pen. The transition from the ponds to the lake inevitably made them utilize food sources with different isotopic compositions in the initial stages of this study. This should be responsible for clearly low values of stable carbon and nitrogen isotopes for silver carp and bighead carp in the initial phases.

The differences of δ¹³C among seston, zooplankton and muscle tissue of silver carp and bighead carp showed little variations from each other, indicating an identical carbon source. Similar δ¹³C of zooplankton and muscle tissue of silver carp and bighead carp made it difficult to evaluate the contribution of zooplankton to the growth of filter-feeding fishes using δ¹³C value. In our study, average muscle δ¹⁵N of silver carp and bighead carp was, respectively, 1.6‰ and 1.9‰ higher than that of zooplankton, and 8.5 and 8.8‰ higher than that of seston (mainly phytoplankton). According to the relatively consistent enrichment of δ¹⁵N (3.4‰) from prey to predator (Minagawa and Wada, 1984; Vizzini and Mazzola, 2003), two carps were more than two trophic levels higher than seston and lower than one trophic level compared with zooplankton. Therefore, δ¹³C and δ¹⁵N indicated that zooplankton, silver carp and bighead carp derived their carbon sources from phytoplankton and silver carp and bighead carp fed on both phytoplankton and zooplankton. On the basis of a mass balance model, we estimated that the contributions of zooplankton to the diets of silver carp and bighead carp were

45.7% and 54.3%, respectively, suggesting that silver carp and bighead carp belonged to the same trophic position. Ke et al. (2007) investigated temporal dynamics in food utilization of silver carp and bighead carp and showed seasonal variations in prey species compositions in our study area. Their results were in agreement with our study of food utilization by silver carp and bighead carp, showing that both carps fed on phytoplankton and zooplankton, but bighead carp assimilated more zooplankton. Based on stable carbon and nitrogen isotope ratios, there was a dietary superposition between silver carp and bighead carp to some extent, which was in agreement with a previous report by Gu et al. (1996a,b) on polyculture ponds in Zhanjiang, China.

5. Conclusions

Seasonal variations of isotopic compositions of seston and filter-feeding fishes were studied in a large fish pen of Meiliang Bay, Lake Taihu, and positive correlation was found between $\delta^{13}\text{C}$ of silver carp and that of seston, indicating that temporal variation of $\delta^{13}\text{C}$ in seston was preserved in filter-feeding fishes via the food chain. Similar $\delta^{13}\text{C}$ values among seston, zooplankton and muscle tissue of silver carp and bighead carp showed that growth of filter-feeding fishes was supported by plankton production. According to a mass balance model, the contributions of zooplankton to the diets of silver carp and bighead carp were 45.7% and 54.3%, respectively.

Acknowledgements

The authors are grateful to D. Zhang, T. Qiu and Y. Liu for their assistance with sample collection. This research was funded by an 863 project titled "Study on Eco-techniques for Improving Water Quality of Drinking Water Source in Meiliang Bay, Lake Taihu (Grant No. 2002AA601011).

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