Environ Biol Fish (2015) 98:1965–1978
DOI 10.1007/s10641-015-0414-4

Diet of *Leptobotia elongata* revealed by stomach content analysis and inferred from stable isotope signatures

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Received: 4 July 2014 / Accepted: 16 April 2015 / Published online: 8 May 2015

Abstract The diet of *Leptobotia elongata* in the Yibin reach of the Yangtze River, China was investigated by stomach content analysis and by stable isotope analysis from muscle. The results of the two methods were agreement. Both stomach contents and isotope analysis indicated that *L. elongata* fed in spring mainly on plankton, shrimp and fish, and secondarily on benthic invertebrates and aquatic insect larvae. For the stomach content analysis, the diet composition showed significant differences among the size classes in relative weight of prey items, with *L. elongata* changing feeding habits at *c.*110 mm standard length. The smaller individuals fed on benthic invertebrates and aquatic insect larvae, whereas individuals >109 mm fed mainly on shrimp and fish. A similar shift to piscivory at *c.*110 mm standard length was found using the stable isotope mixing model to reveal dietary ontogeny by IsoSource software, and the trend in variation of the $\delta^{13}C$ and $\delta^{15}N$ was similar with increased body length, and the plankton is important prey item in all size classes. The $\delta^{13}C$ and $\delta^{15}N$ values in similar sized individuals showed significant seasonal differences ($\delta^{13}C$, ANOVA, $F=76.33$, $p<0.001$ and $\delta^{15}N$, ANOVA, $F=144.56$, $p<0.001$), indicating a temporal dietary and trophic level shift. *L. elongata* is an important commercial species, and the results of the study form part of a detailed investigation of feeding ecology of *L. elongata* that provides basic data for studying the food web of the upper Yangtze River.

Keywords *Leptobotia elongata* · Dietary shift · Body length · Season · Stomach contents · Stable isotope

Introduction

Leptobotia elongata is a benthic freshwater fish endemic in the upper reaches of the Yangtze River, China (Anonymous 1976; Chen 1980; Liang 2000). It is the largest species of Cobitidae in the world, reaching to 3 kg (Ding 1994), mainly inhabiting the river's middle stretch (Ku 1999). In recent years, *L. elongata* populations have declined greatly due over-fishing and to environmental deterioration, including the destruction of spawning grounds and prey (Le and Chen 1998; Sun et al. 2010). *L. elongata* was classified as a

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vulnerable species by the Endangered Species Scientific Commission in China in 1998 (Le and Chen 1998). The annual catch was 10 000 kg prior to the 2000s, but has decreased to no more than 2000–3000 kg per year in recent years (Duan et al. 2008). *L. elongata* is an important commercial species in the Yibin reach of the upper Yangtze River in spring, but difficult to catch in other seasons (Li et al. 2013). It has considerable ornamental value. Recovery of *L. elongate* populations requires augmentation of stock through release of fish into the upper Yangtze River. A stocking program has been in place since 2010, supported by the China Three Gorges Corporation, the largest water conservation and hydropower facility in China.

Dietary shifts are a common phenomenon of the early life of many fish species (Eloranta et al. 2010) and are usually the result of morphologic constraints, migration, or change in habitat (Grey 2001). Thus far, most studies of *L. elongata* have concentrated on its taxonomy and reproductive biology. Information regarding the feeding strategies of the species, particularly in the Yibin reach of the Yangtze, is scarce. Studies of *L. elongata* conducted in the Yichang reach in 1999 used percent frequency of occurrence in stomach content to evaluate ontogenetic shifts in diet (Ku 1999). The distance from Yibin reach to Yichang reach is approximately 1040 km. Research on the dietary shifts of *L. elongata* is important to improve understanding of its trophic ecology to contribute to its management and conservation.

The goals of the present study were (1) to describe the diet of the *L. elongata* on the Yangtze River, (2) to characterize ontogenetic dietary changes by stomach content analysis and stable isotope analysis, and (3) to investigate seasonal variations in the diet of the *L. elongata* by the stable isotope technique. The study forms part of a detailed investigation of feeding ecology of *L. elongata*, and will provide basic data for studying the food web of the upper Yangtze River. The results may also provide information for management plans of rare and endemic fish species in the National Nature Reserve of the upper reaches of the Yangtze River.

Materials and methods

Study area

The area of the Upper Yangtze above Yichang is an exceptional ecosystem with unique geology, topography, and climate, with up to 286 fish species. Construction of the Three Gorges Dam created a reservoir in a stretch of approximately 600 km from Yichang to Chongqing. The National Nature Reserve for rare and endemic fish species in the upper reaches of the Yangtze River was established in 2005 and includes the main stream of the Yangtze between Xiangjiaba Dam and Masngxi Bridge at Chongqing (approximately 387 km) (Wei 2012). Annual mean air temperature is 18 °C, and a flood period extends from June through October (Wei 2012). The present study was conducted from Nanguang to Nanxi reach in Yibin reach (28°45’50” to 28°48’17”N, 104°39’35” to 104°57’11”E), located below the junction of the Jinshajiang and Minjiang Rivers in the central area of the reserve (Fig. 1), which includes spawning grounds of *L. elongata* (Zhao 1995). And *L. elongata* is an important commercial fish in Yibin reach, representing 3.16 % of fish harvested annually and 9.53 % of the annual harvest by weight (Li et al. 2013).

Sample collection

A total of 427*L. elongata* specimens were collected by fishermen with drift gill nets from Nanguang to Nanxi reach in the Yibin reach of the upper Yangtze River (Fig. 1) in spring (April) 2012, n=413; summer (July) 2012, n=5; autumn (October) 2012, n=5; and winter (January) 2013, n=4. Fish were weighed to the nearest 0.1 g, and body length (standard length) was measured to the nearest 1.0 mm. The 413 specimens captured in spring were used to analyze dietary shift in ontogeny by stomach content, and 67 of those were used for analysis of dietary shift by stable isotope in spring according to different body length of the *L. elongata*, and the grouping of the body length for the stable isotope analysis was consistent with the stomach content. Nineteen specimens collected throughout the year with drift gill nets (spring, n= 5; summer, n=5; autumn, n=5; winter, n=4) were used to analyze seasonal variation in diet.

Stomach content analysis

Immediately after their collection, individuals were euthanized, and stomachs were dissected and placed in

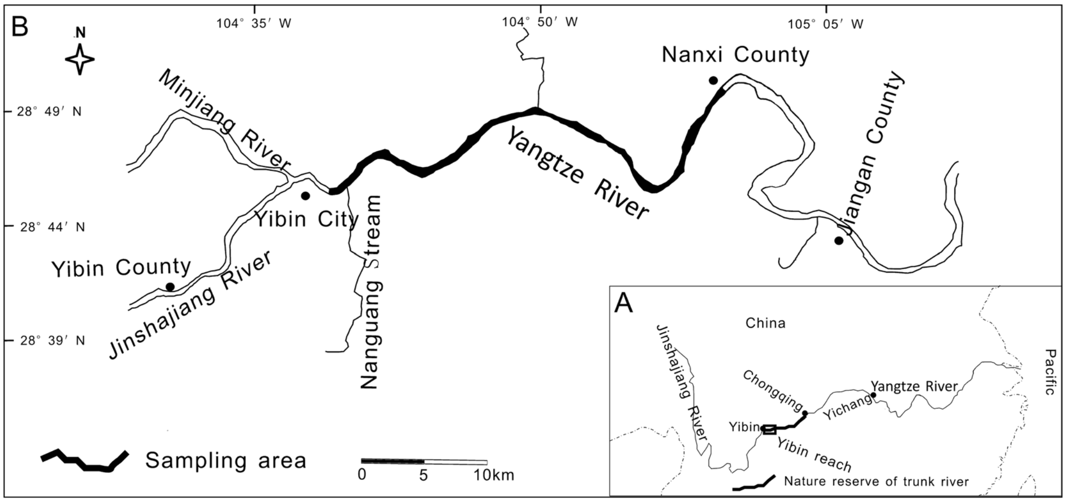


Fig. 1 **a** location of Yibin reach in the Yangtze River, China, **b** sampling area in Yibin reach

10 % buffered formalin until processing. In the laboratory, stomach contents were separated using a dissecting microscope, and the food items were identified to the lowest possible taxonomic level using standard taxonomic keys (Ding 1994; Liang et al. 1996; Zhao 2005; Zhou and Chen 2011). Items of each identified taxon were counted, and wet mass of each prey item was weighted to the nearest 0.1 mg by electronic analytical balance for per stomach. Food items were grouped into eight categories to reduce error caused by comparisons among taxonomic levels (Cortés 1997): fish, shrimp, gammarid, mollusk, benthic invertebrates, aquatic insect larvae, plankton, and phytodetritus. prey compositions were expressed as a percentage weight (*W*%) and percentage numerical composition (*N*%), and percentage frequency of occurrence (*F*%) of a prey item in stomachs with prey was calculated (Hyslop 1980). The index of relative importance (*IRI*, Pinkas et al. 1971) was also calculated as follows: $IRI = (W\% + N\%)F\%$. Fish that had regurgitated, had food in the mouth or pharynx, and those with slack, thin-walled empty stomachs were excluded from the analysis. Because of the difficulty in capturing larger individuals of *L. elongata* (Wei 2012; Li et al. 2013), the number of individuals of body length >229 mm obtained in this study was not sufficient for stomach content analysis. *L. elongata* were grouped into eight body length classes, (70–89 mm, 90–109 mm, 110–129 mm, 130–149 mm, 150–169 mm, 170–189 mm, 190–209 mm, and 210–229 mm), where selection was based on available stomach contents data. Unidentifiable food items were excluded from further analysis (Schafer et al. 2002).

Stable isotope analysis

Muscle tissue was removed from the dorsal-anterior section of the body immediately after capture and stored at −20 °C until use. Each sample was oven dried at 60 °C for 48 h and ground to a fine homogeneous powder using a mortar. Sixty-seven specimens captured in April were separated into 11 size classes: 70–89 mm, $n=5$; 90–109 mm, $n=10$; 110–129 mm, $n=9$; 130–149 mm, $n=7$; 150–169 mm, $n=6$; 170–189 mm, $n=8$; 190–209 mm, $n=6$; 210–229 mm, $n=5$; 230–249 mm, $n=3$; 250–269 mm, $n=3$; and >270 mm, n=5. The grouping of size classes for stable isotope analysis was partially consistent with the grouping of size classes for stomach content analysis. *Leptobotia elongata* is difficult to capture except in spring, so no analysis of dietary shift was conducted in other seasons.

Potential *L. elongata* food items were collected in April. Plankton was collected using a 30 cm diameter net (64 μm mesh) at the surface for 10 min. Six samples were filtered through pre-combusted fiberglass filters (Whatman GF/C) (450 °C, 4 h) for isotopic analysis, and 6 additional samples were used for species identification by microscopy. The major phytoplankton species were Bacillariophyta, Euglenophyta, Chlorophyta, Pyrrophyta, Cyanophyta, and Cryptophyta, and zooplankton Copepoda, Cladocera, Protozoa, and rotifers. A single dominant shrimp species, *Macrobrachium nipponense*, and small fish were also collected. The shrimp were dissected and the soft tissue was used for isotopic analysis. Aquatic insect larvae, gammarid, and organic detritus were collected with dip nets. Benthic invertebrates were obtained with a Pedersen dredge, and all species were transferred into distilled water to allow gut evacuation. We also collected a filter-feeding mussel *Limnoperna lacustris*. Specimens of the same species were combined for isotope analysis when the individuals were not large enough to provide a sufficient sample (Karl et al. 2002). In the laboratory, each taxon was identified and separated, and all samples were dried at 60 °C for at least 48 h and ground to a homogeneous powder using a mortar and pestle.

The dominant food items in the stomachs of *L. elongata* were classified into eight groups according to the similarity of stable isotope values of food items, fish, shrimp, gammarid, mollusks, aquatic insect larvae, benthic invertebrates, plankton, and organic detritus, and the proportion of each was estimated. The contributions of the dominant food items in the 67 tested specimens were determined by IsoSource software. We used the fractionation factors 1.0‰ for δ^{13}C and 3.4‰ for δ^{15}N (Vander et al. 1999) to account for trophic fractionation.

Stable carbon and nitrogen isotope ratios were analyzed with a continuous flow isotope ratio mass spectrometer (Delta V Advantage) directly coupled to an elemental analyzer (flash EA1112 HT). The equations to calculate δ^{13}C and δ^{15}N were:

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

$$\text{and } \delta^{15}\mathrm{N}(‰) = \left[\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{sample}}/\left({}^{15}\mathrm{N}/{}^{14}\mathrm{N}\right)_{\mathrm{standard}}-1\right] \times 10^{3}, \text{ respectively.}$$

^{13}C/^{12}C and ^{15}N/^{14}N are the ratios of the heavy isotope to the light isotope of the sample or standard. δ denoted stable isotope values of the sample as parts per thousand (‰) difference from international standards. The international standards were the Pee Dee Belemnite for ^{13}C and atmospheric N for δ^{15}N. The samples were expressed as δ^{13}C and δ^{15}N, and duplicates were run every five samples with a mean standard error for δ^{13}C measurements below ±0.1‰ and for δ^{15}N measurements below ±0.2‰.

Statistical analyzes

The Kruskal-Wallis nonparametric rank test and χ^2 test of independence were used to assess the ontogenetic differences in mean stomach fullness index (*IF*) and percentage of empty stomachs (Zar 1984), respectively. The PRIMER v. 5 package was used for cluster analysis, and the Bray-Curtis similarity index was employed to describe ontogenetic variations in diet composition (Bray and Cyrtis 1957; Clarke and Warwick 2001). Williams et al. (2001) reported that when diet data was not normally distributed, non-parametric tests may be employed to test the data.

The size-related effect on the C and N isotope signatures of *L. elongata* collected from the spring was investigated using Pearson's correlations, and Pearson's correlations were also used to determine the correlation between $^{\delta13}$C and $^{\delta15}$N for all individuals in spring. ANOVA was used to determine the isotope signatures of the *L. elongata* individuals among seasons. All statistical tests were conducted with SPSS 16.0. A level of $p<0.05$ was considered significant when the null hypothesis was rejected (SPSS Inc., Chicago, USA).

Results

Diet composition

Of the 413 specimens collected in April (spring) 2012, 289 (69.98 %) had food in the stomach. Food items were classified into eight groups, fish, shrimp, Gammarid, mollusks, benthic invertebrates, aquatic insect larvae, plankton and phytodetritus (Table 1). Stomach contents of *L. elongata* were composed mainly of plankton, fish and shrimp in terms of *IRI*. The *F*% was generally high, but the *W*% was low for benthic invertebrates, aquatic insect larvae and plankton, whereas both the *F*% and *W*% was high for fish and shrimp (Table 1). The most important prey items of *L. elongata* was plankton in terms of both *F*% (100 %) and *N*% (99.99 %), and the *IRI* value was the highest among all the prey items, whereas the *W*% value (0.071 %) was the lowest. But the fish was the most important prey in terms of *W*%, and accounted for 44.54 % of the total weight of stomach content, and followed by shrimp (*W*%, 35.36 %).

Table 1 Diet composition of *Leptobotia elongata* indicated by percentage frequency of occurrence (*F*%), percentage weight (*W*%), percentage numerical composition (*N*%) and index of relative importance (*IRI*) in spring of 2012

Prey items	*F*%	*W*%	*N*%	*IRI*
Fish	36.68	44.54	<0.01	1633.76
Gobioninae	9.00	5.85	<0.01	52.65
Botiinae	17.99	30.90	<0.01	555.91
Homalopterinae	1.04	0.35	<0.01	0.36
Siluridae	1.04	1.08	<0.01	1.12
Gobiidae	2.08	1.93	<0.01	4.01
Unidentified fish	17.99	4.42	<0.01	79.52
Gammarid	23.88	2.95	<0.01	70.47
Shrimp	32.87	35.36	<0.01	1162.28
Mollusk	5.88	0.60	<0.01	3.53
Limnoperna lacustris	5.88	0.60	<0.01	3.53
Benthic invertebrates	30.80	8.52	<0.01	262.42
Hirudinea	27.68	7.76	<0.01	214.80
Oligochaeta	3.11	0.75	<0.01	2.33
Polychaeta	1.04	0.02	<0.01	0.02
Aquatic insect larvae	67.47	7.98	0.01	539.09
Odonata	7.96	3.70	<0.01	29.45
Ephemeroptera	58.48	1.85	<0.01	108.54
Trichoptera	6.92	2.13	<0.01	14.74
Chironomidae	10.04	0.02	<0.01	0.21
Corydalidae	2.08	0.0003	<0.01	<0.01
Unidentified aquatic insects	5.88	0.28	<0.01	1.65
Plankton	100.00	0.071	99.99	10006.00
Zooplankton	66.78	0.041	0.14	11.89
Phytoplankton	100.00	0.03	99.85	9988.20
Phytodetritus	6.92	0.034	<0.01	0.24

For isotopic analysis, 81 specimens of *L. elongata* with body length from 84 mm to 405 mm were used (Table 2). Sixty-seven specimens were used to analyze the diet composition of the *L. elongata* by δ^{13}C and δ^{15}N in spring, and a strong positive relationship was observed between the δ^{13}C and δ^{15}N values (n=67, r=0.734, P<0.01, Fig. 2). During the spring, the dominant food items in *L. elongata* were classified into eight groups: fish (Cobitidae, *Saurogobio dabryi*, *Squalidus argentatus*, *Ctenogobius girrinus*, and *Lepturichthys fimbriata*, body length 47–125 mm), shrimp (*Macrobrachium nipponense*, body length 31–46 mm), gammarid, mollusks (*Limnoperna lacustris*), aquatic insect larvae (Ephemeroptera, Odonata, Chironomidae, and Trichoptera), benthic invertebrates (Tubificidae, caddis, clamworms, leeches), plankton (phytoplankton and zooplankton), and organic detritus (Fig. 3). After correction for isotope values of *L. elongata*, all individuals fell within the irregular polygon delineated by the site of each source (Fig. 3), confirming that we sampled all major food items (Jonathan et al. 2006). The δ^{13}C and δ^{15}N values of organic detritus were the lowest (δ^{13}C, −27.28±0.64‰; δ^{15}N, 0.75±0.58‰) among all potential food items, and the isotope values of shrimp was similar to that the values of fish. For all the food items, the δ^{13}C values for *L. elongata* approached the values of fish, shrimp and plankton (Fig. 3).

Diet variation and body length

The samples were divided into eight categories of body length (Fig. 4). Fish exceeding 229 mm body length were excluded, since the number of individuals collected was insufficient for analysis. Both the mean±S.E. stomach fullness index (Kruskal-Wallis test, Hc=87.25, p<0.001) and the percent of empty stomachs (χ^2 test, χ^2=34.90, p<0.001) showed significant differences among the categories (Fig. 4). The percent of *L. elongata* with empty stomachs increased with body length. The rates were the lowest at body lengths <110 mm and highest when the body length ranged from 210 to 229 mm (Fig. 4). The mean stomach fullness was lower when the body length <110 mm and increased steeply with body lengths ranging from 110 to 129 mm, declining at body lengths >209 mm (Fig. 4).

Food items were separated into fish, shrimp, gammarid, benthic invertebrates, aquatic insect larvae, and others (mollusks, plankton, phytodetritus) for easy analysis of percentage of total prey weight. The diet composition showed significant differences among the size classes in relative weight of prey items (Fig. 5). Benthic invertebrates and aquatic insect larvae were the primary prey for the smaller individuals (70–109 mm), accounting for 60.75 % in fish of body length 70–89 mm and 27.28 % for body length 90–109 mm. The proportions of aquatic insect larvae were 31.81 % for 70–89 mm fish and 46.20 % for 90–109 mm fish. Fish and shrimp were more important prey when body length >109 mm, the lowest values were 38 and 34 % in different size classes of the *L. elongata*, respectively. Fish content was higher than that of shrimp when body length ranged from 110 to 209 mm, with presence of

Table 2 The δ^{13}C and δ^{15}N of *Leptobotia elongata* from 2012 to 2013 in Yibin reach

Size classes (mm)	Number	Season (mean±SD)							
		Spring(n=67)		Summer(n=5)		Autumn(n=5)		Winter(n=4)	
		δ^{13}C(‰)	δ^{15}N(‰)	δ^{13}C(‰)	δ^{15}N(‰)	δ^{13}C(‰)	δ^{15}N(‰)	δ^{13}C(‰)	δ^{15}N(‰)
70–89	5	−21.54±0.19	8.48±0.49	—	—	—	—	—	—
90–109	10	−21.60±0.50	8.95±0.66	—	—	—	—	—	—
110–129	9	−21.01±0.46	9.91±0.48	—	—	—	—	—	—
130–149	7	−20.96±0.49	9.78±0.53	—	—	—	—	—	—
150–169	6	−20.72±0.37	9.90±0.22	—	—	—	—	—	—
170–189	8	−20.80±0.52	9.87±0.41	—	—	—	—	—	—
190–209	6	−20.84±0.57	10.10±0.49	—	—	—	—	—	—
210–229	5	−20.55±0.20	10.59±0.33	—	—	—	—	—	—
230–249	3	−20.52±0.07	10.60±0.21	—	—	—	—	—	—
250–269	3	−20.50±0.15	10.61±0.04	—	—	—	—	—	—
>270	5	−20.45±0.14	10.65±0.08	−23.49±0.22	11.44±0.20	−23.36±0.69	12.15±0.21	−21.39±0.18	9.83±0.22

"—": No data

fish significantly lower than that of shrimp when the body length ranged from 210 to 229 mm (Fig. 5).

Cluster analysis based on percentage weight for the eight size classes discriminated two main groups, A: fish 70–109 mm and B: fish >109 mm (Fig. 6). The dissimilarity between the two groups was 50.44 %. The average similarity among fish in group A and in group B was 81.28 and 89.62 %, respectively. The *L. elongata* in group A mainly fed on aquatic insect larvae and benthic invertebrates, and those in group B mainly fed on fish and shrimp with shrimp the dominant prey of *L. elongate* >209 mm.

For isotopic analysis, 67 specimens were used in different size classes of *L. elongata* in spring (Fig. 2). The samples were separated into eleven size classes. The δ^{13}C values for *L. elongata* ranged from −22.47 to −20.25‰, and showed a significant positive relationship with increasing fish body length (n=67, r=0.594,

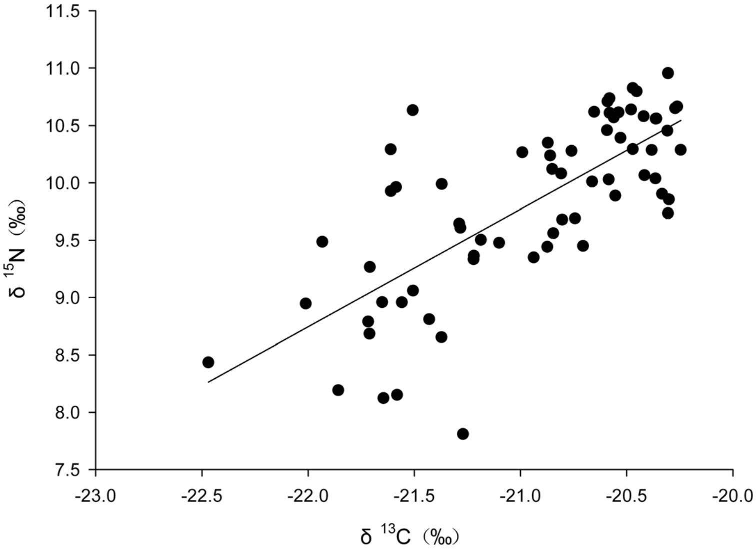
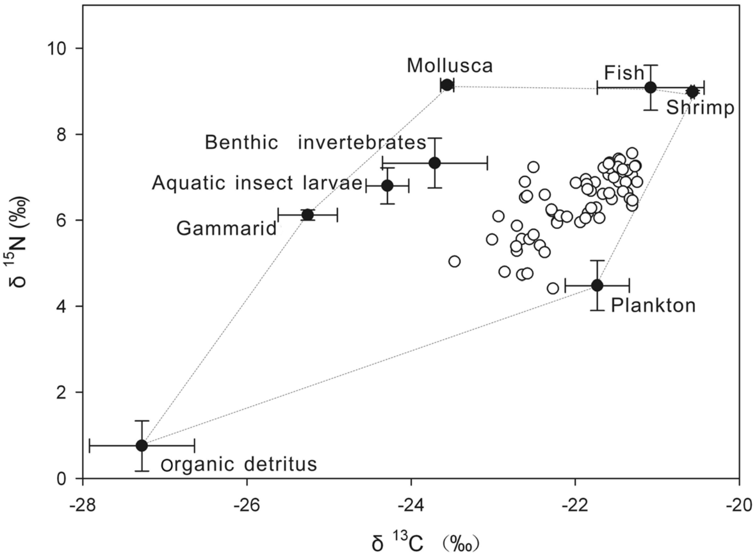


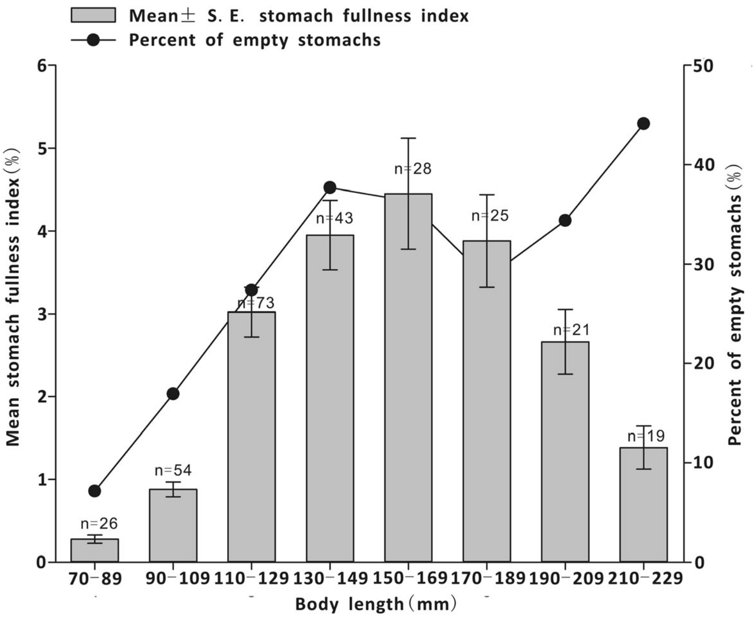
Fig. 2 Variations in δ^{13}C and δ^{15}N signatures in spring (The *line* represents the relationships among the δ^{13}C and δ^{15}N values (‰))

Fig. 3 Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of the dominant potential food items (*solid circles*, mean±SD) and *Leptobotia elongata* (*open circles*, $\delta^{13}C$ and $\delta^{15}N$ values of individuals) from the Yibin reach in spring 2012



$p<0.01$, Fig. 7). There were two points of increase of the $\delta^{13}C$ mean values in all size classes: The $\delta^{13}C$ mean values increased from −21.60‰ in the 90–109 mm group to −21.01‰ in the 110–129 mm group and also from −21.84‰ in the 190–209 mm group to −20.55‰ in the 210–229 mm group. The $\delta^{15}N$ values varied from 7.81 to 10.95‰, and showed a significant positive relationship as the fish grew ($n=67$, $r=0.705$, $p<0.01$, Fig. 7). The $\delta^{15}N$ mean values also showed two points of increase related to fish size. First, the $\delta^{15}N$ mean values increased from 8.95‰ in body length <109 mm to 9.91‰ in body length 110–129 mm, as well as from 10.10‰ in body length 190–209 mm to 10.59‰ in body length 210–229 mm (Table 2). We also investigated the proportional contribution of the food items in different size classes in spring using IsoSource software, and chose three feasible contribution values to express the results, minimal values, MINV; maximum values,

Fig. 4 Mean±S.E. of stomach fullness and percent of empty stomachs for each size class



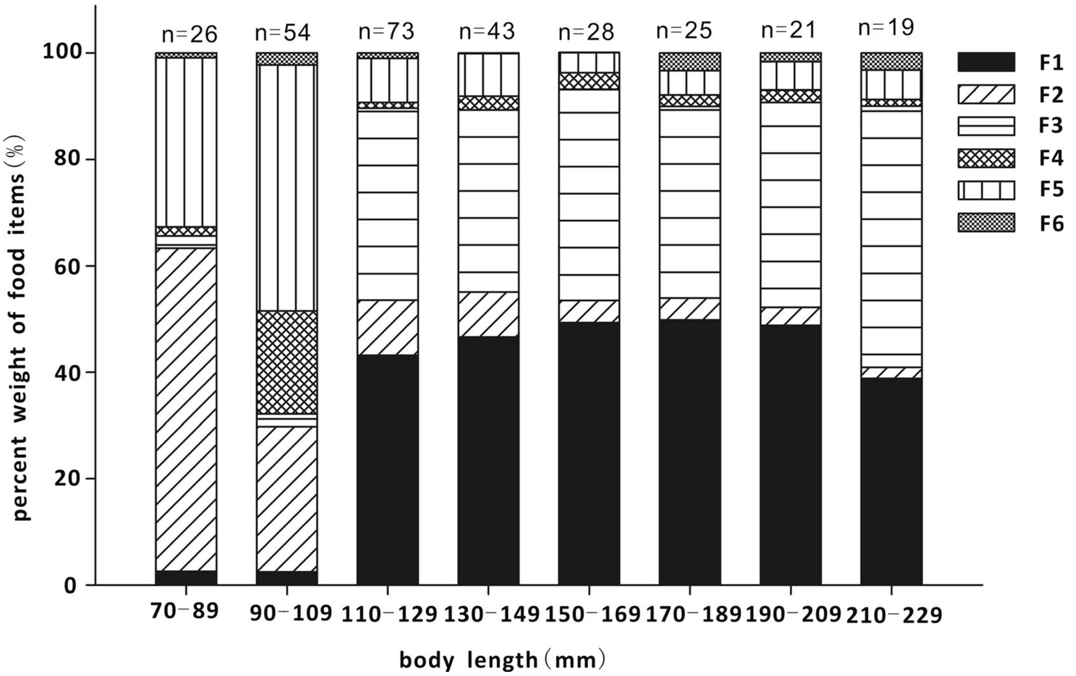


Fig. 5 Variation in the diet composition indicated by percent weight of prey. F1: fish; F2: benthic invertebrates; F3: shrimp; F4: gammarid; F5: aquatic insect larvae; F6: other)

MAXV; mean values, MEANV). The feasible MINV contribution of plankton to *L. elongata* averaged 28 % to 56 % in the different size classes, and the MAXV of feasible contribution averaged 40 % to 80 % (Table 3). The MEANV of feasible contribution of plankton ranged from 35.9 to 70.3 % (Fig. 8). The MINV of feasible contribution of plankton was declined with body length of *L. elongata* (Table 3). The MINV of feasible contribution of fish and shrimp to *L. elongata* was 0 in all size classes, while the MAXV ranged from 26 to 66 % and from 24 to 60 %, respectively. The MAXV and MEANV of the feasible contribution of shrimp and fish increased with body length increase (Table 3, Fig. 8). The MINV of feasible contribution of benthic invertebrates, gammarid, aquatic insect larvae, mollusks, and organic detritus was also 0, and the MAXV was lower than that of other food items fish, shrimp, and plankton (Table 3). The MEANV of feasible contribution of benthic invertebrates, gammarid, aquatic insect larvae, and organic detritus declined with body

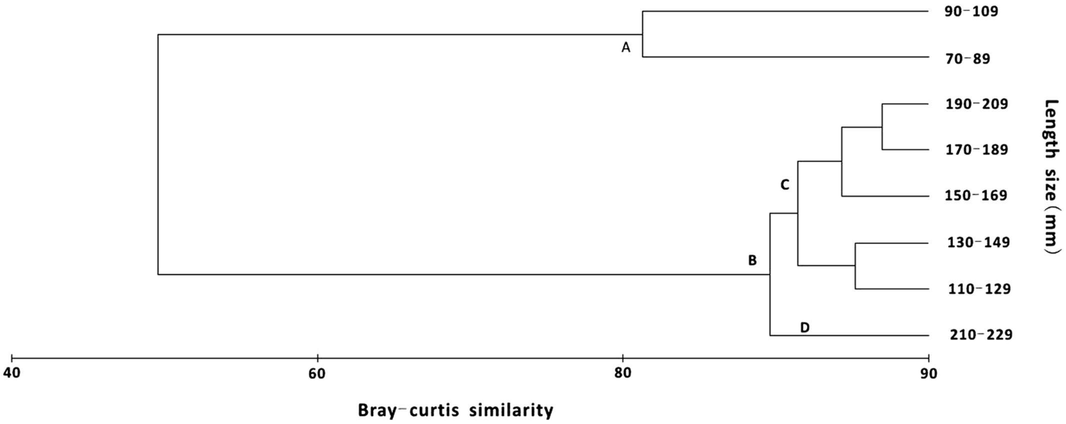
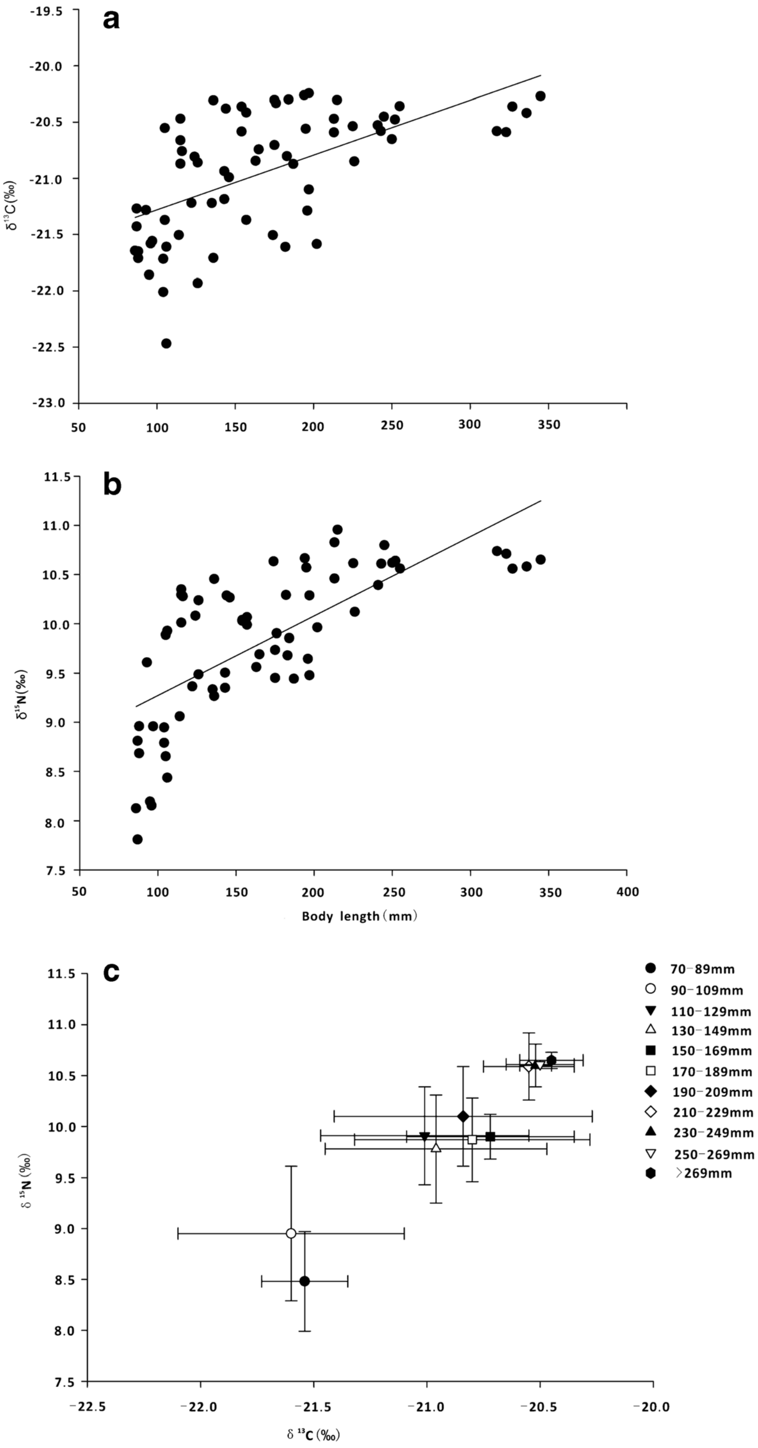


Fig. 6 Dendrogram of the cluster analysis based on the percent mass values

Fig. 7 δ^{13}C and δ^{15}N signatures in *Leptobotia elongata* according to body length (mm) in spring **a** δ^{13}C of different body lengths; **b** δ^{15}N of different body lengths; **c** δ^{13}C and δ^{15}N composition (mean±S.D.) of different size classes. The *lines* represent the relationships among the isotopes values (δ^{13}C, δ^{15}N) and body length



length increase from 90 to 109 mm to 110–129 mm: benthic invertebrates, from 6.1 to 4.1 %; gammarid, from 4.8 to 2.6 %; aquatic insect larvae, from 5.7 to 3.4 %; and organic detritus, from 5.3 to 2.2 %.

Diet variation and season

We chose similar size *L. elongata* to investigate whether a seasonal dietary shift occurred by stable isotopes

Table 3 The percent contribution of food items by IsoSource software for *Leptobotia elongata* in the Yibin reach (spring 2012)

Size classes (mm)	The ratio of contribution of the food items															
	Shrimp		Plankton		Fish		Benthic invertebrates		Mollusk		Aquatic insect larvae		Gammarid		Organic detritus	
	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %	Min. %	Max. %
70–89	0	26	56	80	0	24	0	24	0	16	0	24	0	20	0	16
90–109	0	40	38	72	0	36	0	36	0	26	0	30	0	24	0	20
110–129	0	56	30	56	0	50	0	24	0	20	0	20	0	16	0	14
130–149	0	52	36	58	0	46	0	20	0	18	0	18	0	14	0	12
150–169	0	50	42	56	0	46	0	12	0	10	0	10	0	8	0	6
170–189	0	50	40	56	0	44	0	14	0	14	0	14	0	10	0	8
190–209	0	58	30	52	0	52	0	18	0	16	0	16	0	12	0	10
210–229	0	64	28	42	0	60	0	12	0	12	0	10	0	8	0	6
230–249	0	66	28	40	0	60	0	12	0	10	0	10	0	8	0	6
250–269	0	66	28	40	0	60	0	12	0	10	0	10	0	8	0	6
>270	8	66	28	40	0	52	0	10	0	8	0	8	0	6	0	6

(*min.* minimum, *max.* maximum)

analysis. Nineteen specimens were analyzed in all seasons: spring, $n=5$; summer, $n=5$; autumn, $n=5$; and winter, $n=4$. Results showed that the isotopic composition of *L. elongata* was significantly different among different seasons (Fig. 9). The δ^{13}C values of *L. elongata* in spring (−20.44±0.14‰) (Fig. 9), summer (−23.49±0.22‰), autumn (−23.36±0.69‰), and winter (−21.39±0.18‰) showed significant differences by ANOVA ($F=76.33$, $p<0.001$). The δ^{13}C values in spring and winter were significantly higher

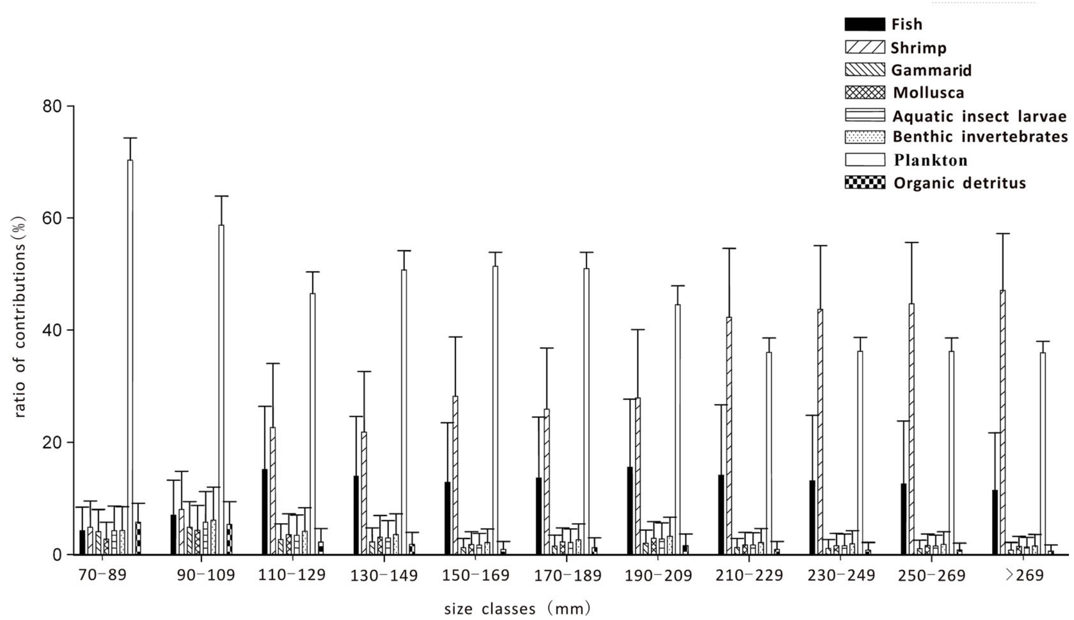
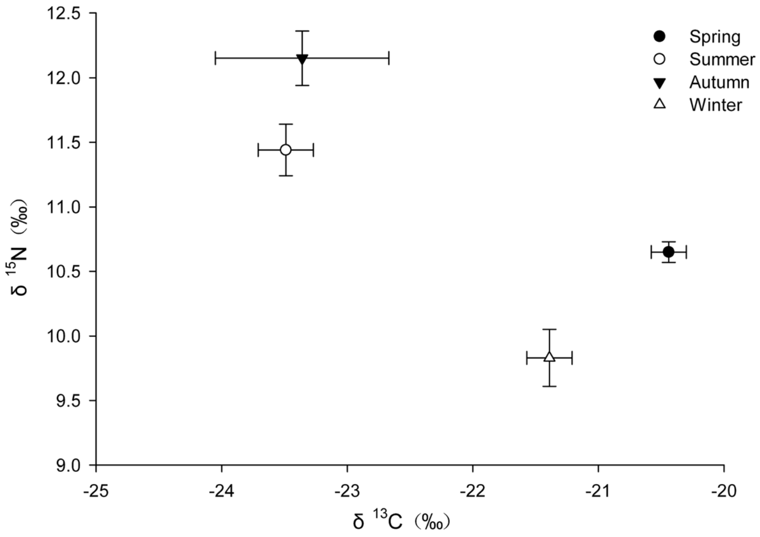


Fig. 8 Contribution of prey items in fish size classes in spring by IsoSource software (mean±SD)

Fig. 9 δ^{13}C and δ^{15}N composition (mean±S.D.) in all seasons (*dot*: spring; *circle*: summer; filled *triangle*: autumn; *hollow triangle*: winter)

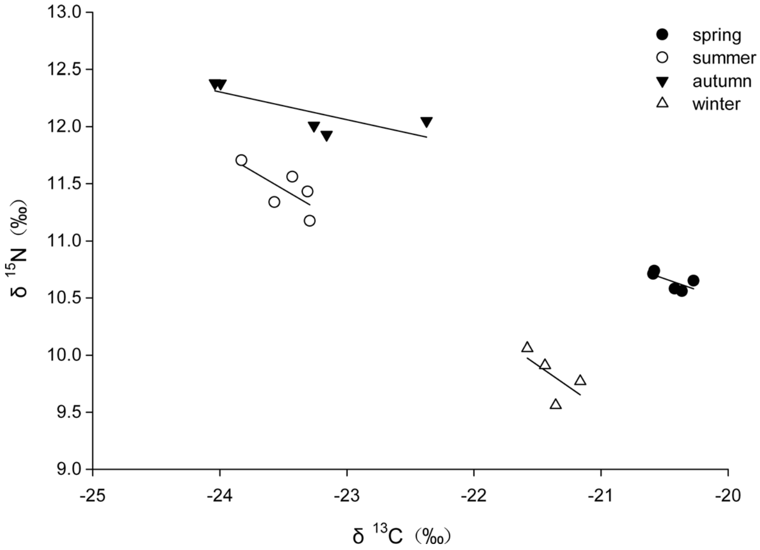


than those in summer and autumn, indicating that *L. elongata* consumed more δ^{13}C-rich food in winter and spring. The δ^{15}N values of *L. elongata* in spring (10.65±0.08‰, $n=5$) (Fig. 9), summer (11.44±0.20‰, $n=5$), autumn (12.15±0.21‰, $n=5$), and winter (9.83±0.22‰, $n=4$) showed significant differences by ANOVA ($F=144.56$, $p<0.001$), indicating a significant seasonal shift in diet. No significant relationship was observed between the values of δ^{13}C and δ^{15}N in spring ($r=-0.690$, $p>0.1$, Fig. 10), summer ($r=-0.722$, $p>0.1$, Fig. 10), autumn ($r=-0.778$, $p>0.1$, Fig. 10), or winter ($r=-0.621$, $p>0.1$, Fig. 10).

Discussion

In studies of other reaches of the Yangtze, *L. elongata* was shown to prey primarily on shrimp and fish (Ku 1999), so the stomach content analysis in the present study are partial consistent with those results. Because of those study only used Percentage frequency of occurrence (%F) to analyze the diet composition, so those results might be biased. In the present study, the stomach content analysis was consistent with the isotopic analysis, which also indicated that *L. elongata* primarily feeds on plankton, shrimp and fish.

Fig. 10 Variations in δ^{13}C and δ^{15}N signatures among seasons. The *lines* represent the relationships between the δ^{13}C and δ^{15}N values of *Leptobotia elongata* in the seasons. (*dot* : spring; *circle*: summer; *filled triangle*: autumn; *hollow triangle*: winter)



The effect of size on the diet of *L. elongata* in the Yibin reach has not previously been investigated. Therefore, we could only compare the data of the stomach content analysis to that of stable isotopic analysis conducted in the present study. We investigated diet shifts with a combination of stomach content and stable isotopic analysis, the results demonstrated that the diet shifts occurred when the body length of *L. elongata* was increased, which is consistent with findings for other fish species (Bacha and Amara 2009; Eloranta et al. 2010; Santiago et al. 2010). Metabolic rate directly impacts the food demand of fish (Moyle 2004) with metabolic rate of larger individuals slower than that of smaller individuals (Sims 1996). Hence, the results show that small individuals of *L. elongata* show a lower percent of empty stomachs.

L. elongata is an omnivorous species that the small individuals primarily consume small-sized prey such as plankton, benthic invertebrates, and aquatic insects, with larger individuals feeding mainly on shrimp and fish. Such a phenomenon presumably reflects the morphological adaptations of *L. elongata*. Santiago et al. (2010) found that size-related shift in diet is probably related to morphological limitations. And many reports show that ontogenetic changes in the diet of fishes have been related to changes in the size of the mouth (Schael et al. 1991; Lucifora et al. 2000). Swimming speed is also correlated with feeding of fish, and larger pectoral fins are able to facilitate accurate and stable feeding (Higham 2007). Larger individuals are more active predators (Scharf et al. 2000) and have larger pectoral fins relative to small individuals in the same population. Thus, the fact that *L. elongata* of different body lengths ingested prey of different sizes may be related to morphological characteristics and swimming speed.

The increased proportion of larger prey with larger fish size may be related to the enhancing effect of optimal foraging behavior on growth (Wanink and Joordens 2007) and reproduction. The caloric value of prey partly depends on the size of the prey consumed (Kikolsky 1963). The caloric content of plankton, benthic invertebrates, and aquatic insect larvae is lower than that of shrimp and fish. A major characteristic of *L. elongata* is high growth rate (Chen 1980; Liang et al. 2000), with sexual maturity reached when body length is approximately 230 mm (Ku and Wen 1997). Hence diet shift at body length >209 mm may be related to reproduction. Protein-rich teleosts are consumed by larger individuals to satisfy their demands for energy (Santiago et al. 2010). Thus, the size-related dietary shifts of *L. elongata* are likely related to the demand for more energy required for the rapid growth and reproduction.

The size shifts in diet may also reflect the occupation of different habitats for avoidance of intraspecific overlap in resource use (Schoener 1974). Some studies have reported that small-sized brown trout mainly live in littoral habitats, while larger individuals occupy the pelagic zone (Hegge et al. 1993; Hesthagen et al. 1997), and larger individuals are more capable of migration across habitat boundaries for foraging (Grey 2001). Small anchovies occupy littoral habitats for more favorable feeding and to reduce the risk of being preyed upon (Bacha and Amara 2009). Ku (1999) found that larger *L. elongata* individuals were mainly caught in the middle zone of the river in spring, which is indirect prove the results of the above-mentioned studies of *L. elongata*. Smaller individuals of *L. elongata* probably occupy primarily littoral habitats for more favorable food sources and to reduce the risk of being preyed upon, and larger individuals move into habitats further from the littoral zone and often forage across the habitat boundaries.

Because the number of larger individuals was not sufficient, we chose individuals of similar size for the diet analysis of different seasons. Significant seasonal variations in δ^{13}C and δ^{15}N values of *L. elongata* were observed in this study, and this phenomenon has also been found in other fish (Eloranta et al. 2010; Zhou et al. 2011; Zhang et al. 2013). The δ^{13}C values were the highest in spring and the lowest in summer, and the δ^{15}N values in autumn were higher than any other season and were lowest in winter, probably related to the diet shift of *L. elonga*ta. Flood seasons occur in summer and autumn, and spring and winter constitute the dry season. Thus, the δ^{13}C and δ^{15}N values of *L. elonga*ta showed clear differences between the flood season and the dry season. The nutrient input and stable isotope signatures of plankton have strong relationships with hydrologic changes (Hein et al. 2003). The increased anthropogenic input from the upper reaches of the river during flooding changes the primary productivity and correspondingly increases heavy isotopes in the environment (Xu et al. 2005; Benson et al. 2008). Mook and Tan (1991) found that because of the respiration of phytoplankton to reuse biogenic CO_2, plankton show more δ^{13}C depletion and more δ^{15}N abundance during the high-water seasons (Mook and Tan 1991). Plankton and shrimp were found to be the main energy sources for *L. elonga*ta in spring. The seasonal changes in δ^{13}C and δ^{15}N values of *L. elonga*ta probably had a strong relationship with hydrologic changes.

Acknowledgments We thank H.S. Xu, L. Ren, B.B. Li, Z.H. Hu, X. Xie, H.R. Hu, Y.T. Zhou, and X.G. Yang for their assistance during fieldwork and W. Guo for help in the laboratory. We also thank Q. Zhou assistance in preparing the manuscript and the Xiangjiaba Dam hydrological stations for provision of hydrological data in the Yibin reaches of the Yangtze River. The research was partially supported by the scientific projects of China Three Gorges Corporation (0701979)

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