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# Size-related shifts in reliance on benthic and pelagic food webs by lake anchovy<sup>1</sup>

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> Abstract: This paper reports large variations in stable carbon and nitrogen isotope ratios of lake anchovy (Coilia ectenes taihuensis) from Lake Chaohu, China. The lake anchovy exhibited a significant <sup>13</sup>C- and <sup>15</sup>N- enrichment in relation to increasing fish length, and the isotopic compositions of small lake anchovy ( $\leq$  130 mm) were significantly more enriched than those of large lake anchovy (> 130 mm). The significant differences in the isotopic compositions of small and large lake anchovy suggested that their assimilated diets differed over a period of time and reflected the size-related diet shift of this fish. Bellamya aeruginosa and Corbicula fluminea were used to establish the baseline carbon signal of benthic and pelagic food webs, and these data were used to parameterize a 2-source mixing model to estimate in consumers the contribution of carbon derived from benthic versus pelagic food webs. Mixing models showed that small lake anchovy derived only 37% of their carbon from benthic food web, indicating increased reliance on pelagic prey, whereas benthic prey contributed 71% of large lake anchovy diet, suggesting greater use of benthic sources. These data indicate that there was a change in lake anchovy feeding strategy related to their size, suggesting a role in dynamic coupling between pelagic and benthic food chains. The trophic position of small lake anchovy averaged 3.0, indicating a zooplankton-based diet, compared with 3.6 in large lake anchovy, indicative of an increase in piscivorous diet. Overlap in the isotopic compositions of small and large lake anchovy probably indicated that these fish occasionally shared common diets, as suggested by stomach content studies, and/or resulted from the differences in the rate of isotopic turnover depending on differences in growth rate and metabolic turnover between small and large anchovy during diet shift from pelagic to benthic food webs. This study presents the contributions of benthic and pelagic food webs supporting lake anchovy and indicates that the intraspecific isotopic dynamic should be considered when applying stable isotope analyses to infer trophic interactions in aquatic ecosystems.

Keywords: benthic and pelagic food webs, lake anchovy, size-related diet shift, stable isotopes.

Résumé : Cette étude rapporte de grandes variations dans les rapports des isotopes stables du carbone et de l'azote chez l'anchois (Coilia ectenes taihuensis) du lac Chaohu, Chine. Les anchois démontraient un enrichissement significatif en <sup>13</sup>C et <sup>15</sup>N en lien avec une augmentation de la longueur du poisson et la composition isotopique des petits anchois ( $\leq$  130 mm) était significativement plus enrichie que celle des anchois de grande taille (> 130mm). La différence significative entre les compositions isotopiques des anchois de petite et de grande taille suggère que leurs diètes différaient depuis un certain temps et reflétaient un ajustement en fonction de la taille chez ce poisson. Bellamya aeruginosa et Corbicula fluminea ont été utilisés afin d'établir le signal de base du carbone des chaînes alimentaires benthique et pélagique. Ces données ont ensuite été utilisées pour déterminer les paramètres d'un modèle combinant 2 sources pour estimer chez les consommateurs la contribution du carbone provenant des chaînes alimentaires benthique et pélagique. Les modèles ont démontré que les anchois de petite taille obtenaient seulement 37 % de leur carbone de la chaîne alimentaire benthique indiquant une augmentation de la dépendance sur les proies pélagiques alors que les anchois de grande taille consommaient des proies benthiques dans une proportion de 71 % suggérant une plus grande utilisation des sources benthiques. Ces données indiquent que les anchois ont des stratégies d'alimentation qui diffèrent selon leur taille et que ceci joue un rôle dans le couplage dynamique entre les chaînes alimentaires pélagique et benthique. La position trophique des anchois de petite taille était de 3,0 en moyenne indiquant une diète basée sur le zooplancton, en comparaison, la position trophique de 3,6 des anchois de grande taille indiquait plutôt une augmentation des poissons dans leur diète. Le chevauchement des compositions isotopiques des anchois de petite et de grande taille indique que ces poissons partagent occasionnellement une diète commune tel que suggéré par les études de contenus stomacaux et/ou résulte de différences dans les taux de renouvellement isotopique (qui sont dépendants du taux de croissance et du renouvellement métabolique) entre les anchois de petite et de grande taille durant le changement de diète de la chaîne alimentaire pélagiques à benthique. Cette étude décrit les contributions respectives des chaînes alimentaires benthique et pélagique au support des anchois de lac et indique que la dynamique isotopique intraspécifique devrait être considérée lorsque des analyses d'isotopes stables sont utilisées pour inférer les interactions trophiques dans les écosystèmes aquatiques.

*Mots-clés* : anchois de lac, ajustement de la diète en fonction de la taille, chaînes alimentaires pélagique et benthique, isotopes stables.

Nomenclature: Zhu, 1995.

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### Introduction

Stable isotopes, particularly those of carbon and nitrogen, are increasingly used to study the structure and dynamics of food webs and trophic relationships in a wide variety of ecosystems (Cabana & Rasmussen, 1996; Vander Zanden, Casselman & Rasmussen, 1999; Post, 2002; Xu et al., 2004). Within the food web of an ecosystem, stable carbon and nitrogen isotopic signatures of a consumer are indicators of its assimilated diet and its trophic position over a period of time, respectively (France, 1995; Vander Zanden, Cabana & Rasmussen, 1997; Pinnegar & Polunin, 2000; Post, 2002). The popularity of stable isotope analysis for studying individual species feeding ecology is due, in part, to the fact that food sources often have specific isotope signatures that are acquired by consumers (Fry, 1988). If 2 individuals consume 2 separate food sources with differing isotopic signatures, they will have different isotopic compositions. The primary advantage of the stable isotope approach is that stable isotopic signatures represent the composition of the assimilated intake from diets over a long time period, from weeks to years (Bootsma et al., 1996). In contrast, examination of stomach contents provides information about feeding habits immediately prior to capture, which may vary considerably over temporal scales. In addition to providing snapshots of diet, stomach content information is vulnerable to biases related to which ingested items are recognizable after being masticated or digested in the stomach, since different prey types are digested and evacuated at different rates, and the importance of rapidly digested prey (e.g., softer bodied components) may be significantly underestimated (e.g., MacDonald, Waiwood & Green, 1982).

Recent studies have shown that stable isotope signatures could be used to identify intraspecific shifts in feeding strategies of many aquatic organisms (Jardine et al., 2003). For example, stable isotope analysis has been used to investigate changes in diet and food web structure after species invasions in lakes (Mitchell et al., 1996; Kidd et al., 1999; Vander Zanden, Casselman & Rasmussen, 1999) and diet shifts of island rodents and beetles from terrestrial sources in wet years to marine sources in dry years (Stapp, Polis & Sanchez Pinero, 1999). Diet shifts in. e.g., brown trout (Grey, 2001), dusky grouper (Renones, Polunin & Goni, 2002), brook charr (Power et al., 2002), opossum shrimp (Branstrator et al., 2000), and deposit-feeding polychaetes (Hentschel, 1998) have also been demonstrated using stable isotope analysis. Shifts in feeding strategies are suggested to be associated with changes in morphology, behaviour, habitat, and distribution and with changes in diets of species (Stoner & Zimmerman, 1988; Polis & Strong, 1996) and to be a consequence of changes in selection pressure throughout the life history (Holbrook & Schmitt, 1988; Grey, 2001; Adams et al., 2003). However, in recent years there have been repeated recommendations that caution must be used when drawing conclusions about any species' trophic position in aquatic ecosystems because of the complexity of the environment and of the interactions between organisms in aquatic ecosystems (Gu, Schelske & Hoyer, 1997; Vander Zanden et al., 1998; Adams et al., 2003).

Most limnologists study either the benthic or, more often, the pelagic habitat of lake ecosystems (Vadeboncoeur et al., 2002). However, interactions between functional groups and habitat linkage of benthic and pelagic food webs can profoundly affect aquatic ecosystems (France, 1995; Vadeboncoeur et al., 2002; Vander Zanden & Vadeboncoeur, 2002; Vadeboncoeur et al., 2003; Vander Zanden et al., 2003). For example, primary producers (e.g., phytoplankton and benthic algae) compete for light and nutrients at the bottom of food webs through which the pelagic and benthic food chains are linked (Vadeboncoeur et al., 2003). Benthic and pelagic food webs can be linked in a variety of ways, including flexible foraging between habitats of fishes. Many fishes undergo diet shifts that deviate from their presumed food chain, and piscivores often feed on both pelagic and benthic food webs (Vander Zanden & Vadeboncoeur, 2002), thereby energetically linking pelagic and benthic food chains.

In light of these facts, stable carbon and nitrogen isotopes were used to investigate intraspecific changes in feeding habits of a non-migratory lake anchovy, Coilia ectenes taihuensis, in the large, shallow, eutrophic Lake Chaohu, China. This fish has a widespread distribution and inhabits lakes in the middle and lower reaches of the Yangtze River; it is the most exploited fish species in Lake Chaohu and accounts for more than 50% of the total fishery production in recent years (Guo, 2005). Generally, lake anchovy reaches maturity at > 50 mm total fish length, spawning begins in April, with the peak in June and July, and larvae first appear in May, with the most abundant population in July and August (Diao & Luo, 1982). Maximum age attained is 4+ y, while about 97% of the population is age-0 (Diao & Luo, 1982; Guo, 2005). Under good conditions larval fish can attain 20-50 mm after 40 d and 70-100 mm after 5 months of life (Diao & Luo, 1982). The primary aims of the study were to examine any variability in isotopic ratios exhibited by lake anchovy relative to size and to determine the potential cause of the observed changes. We also sought to evaluate size-related variations of lake anchovy in trophic level and percentage concentration of benthic-derived carbon sources using simple 2-source mixing models, in order to determine the role of lake anchovy in linking the benthic and pelagic food webs of the lake ecosystem.

#### Methods

Lake Chaohu, located in the delta of the Yangtze River in southeastern China (Figure 1a), is one of the 5 largest freshwater lakes in China. Table I gives an overview of the morphometrical and limnological characteristics of the lake during 2002 and 2003 (Deng, 2004; Geng *et al.*, 2005; Guo, 2005; Xu *et al.*, 2005; Zhang *et al.*, 2006). The lake receives a large anthropogenic import from the watershed, and eutrophication of the lake has taken place as indicated by the dense summer cyanobacterial blooms that have occurred since the 1950s (Deng, 2004; Geng *et al.*, 2005). Floristically, the lake can be classified as a phytoplanktondominated system. Vascular aquatic plants are scarce, and their contribution to total primary production of the system is negligible (Song & Lu, 1982; Guo, 2005). Historical



FIGURE 1. a) Location of Lake Chaohu in Yangtze Basin, China. b) Lake Chaohu and the Yuxi River, through which Lake Chaohu links with the Yangtze River. Black area is Mushan Island and shaded area denotes approximate location from which samples were collected in the present study.

records of fish fauna surveys indicate that Lake Chaohu previously harboured 94 fish species belonging to 20 families (Wu, Diao & Yiao, 1982). Linking through the Yuxi River, Lake Chaohu is a downstream lake of the Yangtze River (Figure 1b). Artificial dams built in the early 1970s have separated Lake Chaohu from the Yangtze River for several decades, resulting in serious declines or even extinctions of many migratory and semi-migratory species, and only 54 species of fishes belonging to 16 families were recorded during the survey from 2002 to 2004, among which 86% were non-migratory inhabitants of Lake Chaohu (Guo, 2005).

The major fishing season for lake anchovy in Lake Chaohu is July-October. Sixty-one samples were collected from a fisher's catch in the region around Mushan Island on 15 September 2003 (Figure 1). The fishing gear used was a pair-trawl with a cod end mesh of  $8 \times 8$  mm. Lake water from the fishery area was filtered onto precombusted glass fibre filters (Whatman GF/C-pore size 1.2 µm) to collect seston samples. The filters were then wrapped in aluminium foil and put into plastic bags. Samples of epilithic algae were scraped from the surface of rocks in the sampling area and repeatedly rinsed with distilled water to remove large coarse detritus particulates. Macroinvertebrates, the surface-grazing freshwater snail Bellamva aeruginosa and the filter-feeding mussel Corbicula fluminea (Asiatic clam), were also sampled in the fishing area. All samples were kept in an ice box and brought back to the laboratory. In the lab-

TABLE I. Average values and ranges for selected morphometrical and limnological characteristics in Lake Chaohu from August 2002 to July 2003 (Deng, 2004; Geng *et al.*, 2005; Xu *et al.*, 2005; Zhang *et al.*, 2006). TP: total phosphorus, TN: total nitrogen, TC: total carbon, and DIC is dissolved inorganic carbon.

Parameter	Range	Average
Surface area (km <sup>2</sup> )	nd	770
Storage capability (m <sup>3</sup> )	nd	$2.1 \times 10^{9}$
Depth (m)	2.5-5.9	2.7
Oxygen saturation (mg· $L^{-1}$ )	7.2-16.0	11.7
Secchi depth (cm)	20.6-95	48.9
pH	6.92-9.44	8.04
Conductivity ( $\mu s \cdot cm^{-1}$ )	185.8-400.4	256.7
Temperature (OC)	3.5-29.3	17.9
Total algal biomass (mg·L <sup><math>-1</math></sup> )	5.2-22.7	11.4
Chlorophyll <i>a</i> ( $\mu g \cdot L^{-1}$ )	9.4-47.9	17.1
TP (mg·L <sup><math>-1</math></sup> )	0.06-0.23	0.11
Ortho-P (mg·L <sup><math>-1</math></sup> )	0.002-0.046	0.022
$TN (mg \cdot L^{-1})$	0.9-6.1	2.9
$NO_3 - N (mg \cdot L^{-1})$	0.499-2.653	1.562
$NH_4 - N (mg \cdot L^{-1})$	0.117-0.667	0.385
TC (mg·L <sup><math>-1</math></sup> )	4.6-16.6	12.7
DIC (mg·L <sup>-1</sup> )	3.8 <b>–9.8</b>	6.1

Note: nd means no data.

oratory, total fish length was measured to the nearest 1 mm, and fish weight was recorded to the nearest 0.1 g. A fillet of dorsal muscle (white muscle) was removed from each fish since these tissues have been shown to be representative of the overall stable isotope signature in fish (Hesslein, Hallard & Ramlal, 1993). Macroinvertebrates with a hard shell (B. aeruginosa and C. fluminea) were dissected and a sample of muscle tissue was taken for stable isotope analysis because shell material is enriched in <sup>13</sup>C and does not reflect what is actually assimilated by consumers (Mitchell et al., 1996). The filters of seston samples were acidified with superfluous 1M HCl solution to dissolve possible carbonate contamination (Xu et al., 2005) and then rinsed in distilled water. The muscle tissues of fish, B. aeruginosa, and C. fluminea, and the seston and epilithic algae, were dried at 60 °C to a constant weight, ground to a fine homogeneous powder with a mortar and pestle, and sealed in glass vials with silica gel for later analysis.

Stable carbon and nitrogen isotope ratios were analyzed with a Finnigan Delta Plus (Thermo Scientific, Waltham, Massachusetts, USA) continuous flow isotope ratio mass spectrometer (CF-IRMS) directly coupled to an EA1110 elemental analyzer (Carlo Erba). Two samples of an internal reference material were analyzed after every 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 following equation:

$$\delta X(\%_0) = \left(\frac{A_{\text{sample}}}{A_{\text{standard}}} - 1\right) \times 1000$$
[1]

where X is <sup>15</sup>N or <sup>13</sup>C, A is the corresponding ratio <sup>15</sup>N/<sup>14</sup>N or <sup>13</sup>C/<sup>12</sup>C, and  $\delta$  is the measure of heavy to light isotope in the sample, with higher  $\delta$  values denoting a greater proportion of the heavy isotope. The standard reference materials

were Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen for carbon and nitrogen, respectively. International reference materials were carbonatite (IAEA-NBS18) and graphite (IAEA-USGS24) for  $\delta^{13}$ C and ammonium sulfate (IAEA-USGS25 and IAEA-USGS26) for  $\delta^{15}$ N. On a daily basis, an internal working standard, urea ( $\delta^{13}$ C = -49.44‰ and  $\delta^{15}$ N = -1.53‰), was used for both  $\delta^{13}$ C and  $\delta^{15}$ N. More than 20% of the samples were analyzed 2 or more times, and the standard deviations of  $\delta^{13}$ C and  $\delta^{15}$ N replicate analyses were less than 0.2‰ and 0.3‰, respectively.

We initially expected to use the isotopic compositions of seston and epilithic algae to estimate the trophic position of lake anchovy and the contribution of pelagic and benthic food webs to their diet. However, the stable isotope compositions of both seston and epilithic algae showed temporal variations and overlapped with each other during this study (Xu et al., 2005) and thus were not suitable to precisely assess the trophic position and contribution of the derived carbon source of lake anchovy. It has been documented that the freshwater snail *B. aeruginosa* grazes mainly on epiphytic algae (Xu, Wang & Zhang, 2003), whereas the Asiatic clam C. fluminea filters phytoplankton from the water column (Havens et al., 2003). These long-lived macroinvertebrates are considered to be less sensitive to seasonal fluctuations of the isotopic compositions of primary producers and can reflect the base of the benthic and pelagic food webs, respectively, providing an isotopic baseline for estimating the trophic position of higher-trophic-level consumers in lake ecosystems (Vander Zanden, Casselman & Rasmussen, 1999; Post, 2002). Therefore, in this study, the long-lived primary consumers the surface-grazing freshwater snail *B. aeruginosa* and the filter-feeding mussel C. *fluminea* were used to quantify the isotopic baseline for the benthic and pelagic food webs, respectively, following the methodology outlined in previous studies (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999; Post, 2002; Vander Zanden et al., 2003).

To compare variations in reliance on benthic *versus* pelagic prey in lake anchovy, a mass balance mixing model was used to estimate the contribution of benthic secondary production to fishes using the formula:

Percent benthic = 
$$\frac{\delta^{13}C_{\text{fish}} - \delta^{13}C_{\text{pelagic}}}{\delta^{13}C_{\text{benthic}} - \delta^{13}C_{\text{pelagic}}} \times 100$$
[2]

where  $\delta^{13}C_{\text{fish}}$ ,  $\delta^{13}C_{\text{pelagic}}$ , and  $\delta^{13}C_{\text{benthic}}$  are the mean  $\delta^{13}C$ of lake anchovy, pelagic baseline (mean  $\delta^{13}C$  of *C. fluminea*), and benthic baseline (mean  $\delta^{13}C$  of *B. aeruginosa*), respectively. To capture potential spatial heterogeneity in  $\delta^{15}N_{\text{baseline}}$  for fishes that feed on both pelagic and benthic food webs, trophic position was calculated as follows:

$$\frac{\text{Trophic}}{\text{position}} = \frac{\delta^{15} N_{\text{fish}} - [\delta^{15} N_{\text{pelagic}} \times (1 - \alpha) + \delta^{15} N_{\text{benthic}} \times \alpha]}{3.4 + \lambda} [3]$$

where 3.4 is the generally assumed per trophic level enrichment of  $\delta^{15}N$  (Post, 2002);  $\alpha$  is the proportion of nitrogen in the consumer ultimately derived from the base of the benthic food web and is estimated using carbon isotopes in the formula mentioned above (Post, 2002);  $\lambda = 1$  for primary producers,  $\lambda = 2$  for primary consumers, and so on;

 $\delta^{15}N_{fish}$ ,  $\delta^{15}N_{pelagic}$ , and  $\delta^{15}N_{benthic}$  represent the mean  $\delta^{15}N$  of lake anchovy, pelagic baseline (mean  $\delta^{15}N$  of *C. fluminea*), and benthic baseline (mean  $\delta^{15}N$  of *B. aeruginosa*), respectively. Note that these models use primary consumers (rather than primary producers) as isotopic endpoints and thus estimate the contributions of pelagic and benthic secondary production, assuming that there is no trophic enrichment in  $\delta^{13}C$  (trophic fractionation = 0‰) and that mixing is linear (Vander Zanden, Casselman & Rasmussen, 1999; Post, Pace & Nelson, 2000; Post, 2002; Vander Zanden & Vadeboncoeur, 2002).

#### **Results**

The weight and length of the lake anchovy ranged from 0.5 to 66.0 g and from 54 to 290 mm, respectively, and exhibited a typical length–weight relationship (ANCOVA,  $y = 0.0035x^{3.0}$ , n = 61, r = 0.99, P < 0.01, Figure 2a). The lake anchovy also exhibited great variability in  $\delta^{13}$ C values,



FIGURE 2. Changes in lake anchovy weight (a) and muscle  $\delta^{13}$ C (b) and  $\delta^{15}$ N (c) signatures with increasing fish length. Open squares represent small lake anchovy ( $\leq 130$  mm, n = 36) and filled squares represent large lake anchovy (> 130 mm, n = 25).

with a range from -28.7% to -22.9% (average -26.1%) and showed a significant <sup>13</sup>C-enrichment in relation to increasing fish length when all lake anchovy were pooled (ANCOVA, n = 61, r = 0.73, P < 0.01, Figure 2b). The  $\delta^{15}$ N ratios ranged from 10.8‰ to 16.6‰, with an average of 13.5%. The lake anchovy became more enriched in <sup>15</sup>N as the fish length increased (ANCOVA, n = 61, r = 0.77, P < 0.01, Figure 2c). In view of the distinct variations in stable isotopes in anchovy, we selected a fish length of 130 mm as the arbitrary boundary between large and small anchovy. Plotting  $\delta^{15}$ N with  $\delta^{13}$ C revealed a distinct change in both isotope ratios between small ( $\leq 130$  mm, n = 36) and large lake anchovy (> 130 mm, n = 25, Figure 3b).  $\delta^{15}N$  (15.3 ± 0.9‰) and  $\delta^{13}C$  (-24.6 ± 0.8‰) of large lake anchovy were significantly higher than those  $(\delta^{15}N = 12.9 \pm 1.3\%, \ \delta^{13}C = -27.1 \pm 1.1\%)$  of small lake anchovy (*t*-test, t = -8.272, df = 59, P < 0.001 for  $\delta^{15}$ N; t = -9.813, df = 59, P < 0.001 for  $\delta^{13}$ C).

 $\delta^{15}$ N of the samples increased from primary producers (seston and epilithic algae) to consumers (lake anchovy, freshwater snail B. aeruginosa, and Asian clam C. fluminea) at different trophic positions (Figure 3a), indicating that the heavy nitrogen isotope (<sup>15</sup>N) was retained preferentially during nutrient assimilation and incorporation into animal tissues.  $\delta^{13}$ C values of seston were depleted relative to epilithic algae (Figure 3a), reflecting differential expression of fractionation during the uptake of dissolved inorganic carbon. Corbicula fluminea had depleted  $\delta^{13}$ C and  $\delta^{15}$ N values relative to B. aeruginosa, indicative of their different food sources. The trophic position of individual lake anchovy ranged from 2.3 to 4.2, and the trophic positions of small lake anchovy  $(2.9 \pm 0.4)$  were significantly lower than those of large lake anchovy  $(3.6 \pm 0.3)$  (*t*-test, t = -8.272, df = 59, P < 0.001). Estimation of pelagic versus benthic



#### Discussion

Stable isotope analyses revealed significant changes in the isotopic signatures of lake anchovy with increasing body size. Small lake anchovy (≤ 130 mm) were depleted in  $\delta^{13}$ C and  $\delta^{15}$ N compared with large anchovy (> 130 mm), indicating that they must have been feeding on different food sources for some considerable time (Gu, Schelske & Hoyer, 1997). The wide range of stable carbon isotope ratios cannot be attributed to different isotopic fractionation during food assimilation, since modification of  $\delta^{13}$ C value by feeding process is usually within 1‰, and animals that feed on the same food source presumably exhibit similar  $\delta^{13}$ C value (DeNiro & Epstein, 1978). Lipids are typically depleted in <sup>13</sup>C relative to muscle tissue and can interfere with interpretation of stable carbon isotopic data. Although the muscle tissue of lake anchovy used in the current study was not treated to remove lipids, the large variation of  $\delta^{13}C$ (6‰) is not likely attributable to different amounts of lipids in muscle tissue since differences between the treated and untreated muscle tissues of some fishes have been reported to be within 4‰ (Gu, Schelske & Hoyer, 1996; Grey, 2001). Variation in  $\delta^{15}N$  (6‰) was also much greater than the expected 3 to 4‰ enrichment per trophic transfer (Minagawa & Wada, 1984; Vander Zanden, Cabana & Rasmussen, 1997; Post, 2002). Therefore, the significantly distinct  $\delta^{13}$ C and  $\delta^{15}$ N signatures between small and large lake anchovy imply differential reliance on benthic and pelagic food webs.



FIGURE 3. (a) Mean ( $\pm$  SD) isotopic composition of small and large anchovy, *B. aeruginosa*, *C. fluminea*, and primary producers. (b) Expanded section of Figure 3(a); markers represent isotopic signatures of all individuals of lake anchovy (n = 61).

The carbon isotope differences between the benthic and pelagic habitats were found to be conserved up the food chain in the present study and could thus be used to estimate the contributions of benthic and pelagic resources to higher trophic levels (Vander Zanden, Casselman & Rasmussen, 1999; Post, 2002). Stable isotopic signatures of seston and epilithic algae overlapped with each other to some extent during the period of this study, and also showed temporal variations, complicating their direct use as indicators of baseline values of stable isotope ratios for consumers higher up the food web (Cabana & Rasmussen, 1996). Post (2002) found that long-lived primary consumers fall within the range of time-series data of primary producers and that there is no significant difference between the primary consumers and the median of the time-series data of primary producers, despite considerable temporal variation in primary producers. However, in this study, the isotopic composition of snails and mussels substantially differed from benthic algae and seston. This may be due to a combination of isotopic carryover from previous years, coarse temporal sampling, the presence of recalcitrant material in time-series samples that was not assimilated, and small physiological differences in isotope fractionation (Post, 2002). Another reason for the difference in stable isotopes values between clam and seston might be acidification of the filters of the seston samples. Acidification of samples can result in either increased or decreased variations in carbon and nitrogen stable isotope values due to the loss of C and N molecules (Bunn, Loneragan & Kempster, 1995; Pinnegar & Polunin, 1999; McCutchan et al., 2003; Carabel et al., 2006). For instance, acidification of suspended particulate organic matter led to a variation of over 1‰ in stable carbon isotope (Carabel et al., 2006). Therefore, B. aeruginosa and C. corbicula were used to establish the baseline signals of the benthic and pelagic food webs, respectively, in this study, and these data were used to parameterize the 2-source mixing model to estimate in consummers the contribution of carbon derived from benthic versus pelagic secondary production.

 $\delta^{13}$ C signatures of lake anchovy muscle tissues provide specific evidence of food source differences between the 2 size classes.  $\delta^{13}$ C signatures of small lake anchovy averaged -27.1%, indicating reliance on planktonic prey, while  $\delta^{13}C$ of large lake anchovy was -24.6‰, suggesting greater use of benthic prey. Two-source mixing models using pelagic (C. fluminea) and benthic (B. aeruginosa)  $\delta^{13}$ C endpoints indicate that small lake anchovy fed on 63% (average) planktonic prey, compared with only 29% for the large lake anchovy. These data indicate that there was a change in feeding strategy of lake anchovy related to their size, suggesting a role in dynamic coupling between pelagic and benthic food chains. This is in agreement with the results of previous studies indicating that omnivorous fishes are one of many vectors to link benthic and pelagic food webs of lake ecosystems and that benthic production provides important support to a variety of lacustrine fishes (Vadeboncoeur et al., 2002; Vander Zanden & Vadeboncoeur, 2002; Vadeboncoeur et al., 2003; Vander Zanden et al., 2003). Our findings contribute to a growing body of evidence that pelagic-benthic habitat coupling is both energetically significant and important in food-web dynamics (Polis & Strong, 1996). Recent studies demonstrated that the division of lakes into benthic and pelagic habitats to be studied in isolation could distort the perception of lake food webs (Vadeboncoeur *et al.*, 2002), since most of the world's lakes are relatively small and have high ratios of benthic surface area to pelagic volume (Wetzel, 1990).

The trophic position of the 2 size classes of lake anchovv was estimated by comparing muscle  $\delta^{15}$ N values to the  $\delta^{15}$ N of C. fluminea and B. aeruginosa (Vander Zanden, Casselman & Rasmussen, 1999; Post, 2002). The trophic position of small lake anchovy averaged 3.0, indicating a zooplankton-based diet, compared with 3.6 in large lake anchovy, indicative of an increase of piscivorous diet. This finding was confirmed by previous gut content analysis, which showed that the natural diet of small lake anchovy (fish length 70-130 mm) consisted mainly of zooplankton (89%, n = 173), with only minor contribution of fishes (0.7%) (Diao & Wu, 1982). In contrast, large lake anchovy (fish length 190–220 mm) increased their fish prey to 19%, while zooplankton declined to 46% (Diao & Wu, 1982). The high percentage of completely empty stomachs of large lake anchovy observed by Guo (2005) in this lake could also indicate piscivory, as found in other fish species (Chapman, Mackay & Wilkinson, 1989).

Overlap in the stable isotopic compositions of small and large lake anchovy probably indicates that they occasionally shared common diets as reported by the previous stomach content analysis (Diao & Wu, 1982; Guo, 2005). Stomach content studies indicated that the young-of-theyear lake anchovy fed primarily on zooplankton, including copepods and cladocerans, and then increased benthic prey, such as chironomid larvae and shrimps, and fish forage as fish size/age increased (Diao & Wu, 1982; Guo, 2005). This is consistent with the significantly positive relationships between the isotopic compositions of lake anchovy and increasing fish length observed in the present study. Another possible explanation for the observed overlap of isotopic compositions is that the rate of isotopic turnover depends on the growth rate and metabolic turnover of lake anchovy during their diet shift from pelagic to benthic food webs. It has been suggested that young, rapidly growing organisms could reach equilibrium relatively quickly compared with older and slow-growing ones (Hesslein, Hallard & Ramlal, 1993; Herzka, 2005), and metabolic turnover, defined as the breakdown and replacement of existing tissues through de novo synthesis, could also increase the rate at which equilibrium is reached (Herzka, 2005).

Consequentially, stable-isotope–based estimates of lake anchovy provided firm evidence of divergence of trophic position and ultimate carbon sources between small and large lake anchovy. This study also indicates that intraspecific isotopic dynamics should be considered when applying stable isotope analyses to infer trophic interactions in aquatic ecosystems (Gu, Schelske & Hoyer, 1997; Adams *et al.*, 2003).

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