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PRIMARY RESEARCH PAPER

Submerged macrophytes facilitate dominance of omnivorous fish in a subtropical shallow lake: implications for lake restoration

Jinlei Yu · Zhengwen Liu · Hu He · Wei Zhen · Baohua Guan · Feizhou Chen · Kuanyi Li · Ping Zhong · Franco Teixeira-de Mello · Erik Jeppesen

Abstract Biomanipulation based on removal of coarse fish, piscivorous fish stocking and sometimes also planting of submerged macrophytes has been used to restore temperate eutrophic shallow lakes. However, in warmer lakes, omnivorous fish are more abundant and apparently less well controlled by the piscivores. We investigated the food web structure and energy pathways of fish in the restored part of subtropical Lake Wuli, China, using gut contents analysis (GCA) and the IsoSource model based on stable isotope analysis (SIA) data. We found that omnivores dominated the fish community in terms of numbers. GCA showed that cyclopoid copepods constituted the main food item for the planktivores, while all adult omnivorous fish fed mainly on macrophytes. The IsoSource SIA model supported these results. Furthermore, piscivores consumed shrimps rather than juvenile omnivores, and the SIA analysis revealed no trophic links between piscivores and adult omnivores or zooplanktivores. We conclude that macrophytes constituted an important food item for omnivores, potentially promoting population growth of omnivores as control by piscivores was weak. This may yield a high predation pressure on both zooplankton and on macrophytes, possibly preventing the establishment of a stable macrophyte state following restoration of eutrophic lakes unless the fish density is regularly controlled.

Keywords Submerged macrophytes · Fish diets · Alternative prey · Stable isotope · Food web · Lake restoration

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Introduction

Submerged macrophytes affect the physical and chemical environment and biota in shallow lakes (Carpenter & Lodge, 1986; Jeppesen et al., 1998), and play a key role in maintaining a clear water state in such environments (Moss, 1990; Scheffer & Jeppesen, 1998) through a number of different mechanisms such as suppression of algal growth through competition with phytoplankton for nutrients (van Donk et al., 1993) and by providing refuge for zooplankton against fish predation (Lauridsen et al., 1996), which leads to a higher grazing pressure on phytoplankton (Jeppesen et al., 2002). Although macrophytes play an important structuring role in shallow lakes, phytoplankton and phytobenthos are usually considered as the basic primary carbon sources for freshwater fishes (Araujo-Lima et al., 1986; Forsberg et al., 1993; Keough et al., 1998). However, herbivorous (Nurminen et al., 2003) and omnivorous (Hansson et al., 1987) fish may consume macrophytes, and Mendonça et al. (2013) showed that macrophytes can play an important role as a carbon source for fish in shallow lakes.

In temperate lakes, a goal in the restoration of eutrophicated lakes is to enhance grazer control by herbivorous zooplankton on phytoplankton. This may in part be achieved by establishing submerged macrophytes as these plants favour visually foraging piscivores, which in turn, reduce the abundance of plankti-benthivorous fish through predation and competition. This, in turn, leads to higher proportions of large-bodied zooplankton and a higher zooplankton:phytoplankton biomass ratio and thus higher grazing pressure on phytoplankton, further facilitating the growth of submerged macrophytes (Moss, 1990; Scheffer et al., 1993; Lauridsen et al., 1994). By contrast, re-establishment of submerged macrophytes may have a much weaker effect on the food webs in warm lakes that are characterised by high fish diversity among the plants (Meerhoff et al., 2007), high fish abundance (Mazzeo et al., 2003; Zhao et al., 2006), widespread omnivory (Winemiller, 1990; Teixeira-de Mello et al., 2009; Moss, 2010; González-Bergonzoni et al., 2012) and low abundances of strictly piscivorous fish (Quirós, 1998). The relative contribution of omnivores to fish species richness is typically higher in (sub)tropical than in temperate regions (Teixeira-de Mello et al., 2009; Moss, 2010; González-Bergonzoni et al., 2012). The low-latitude fish species are not only smaller but also grow faster, mature earlier, have shorter life spans and allocate less energy to

reproduction than populations at higher latitudes in Europe (Blanck & Lammouroux, 2007).

Many of the omnivorous species in warm lakes are herbivorous (González-Bergonzoni et al., 2012). A study of Nile tilapia (*Oreochromis niloticus* Linnaeus*)* in restored Huizhou West Lake, China (Rao et al., 2015), showed an ontogenetic shift in the diet from feeding mainly on zooplankton in the juvenile stage to preying mainly on macrophytes as adults. By feeding on macrophytes, tilapia may not only exert a significant grazing pressure on the macrophytes but also indirectly affect their growth through high fish recruitment, resulting in low abundance of cladocerans and weak grazing on phytoplankton, as observed in the restored sites of Huizhou West Lake (Liu et al., 2014). Similar studies from subtropical shallow lakes in Asia, including also other omnivores, are, however scarce, making it difficult to draw generalisations. Moreover, although the results are ambiguous (Starling et al., 2002; Jeppesen et al., 2007), most studies from warm lakes indicate poor control of prey by piscivorous fish in South America (Meerhoff et al., 2012) and also in China (Chen et al., 2009; Gao et al., 2014), and weak piscivory may further promote the grazer control of macrophytes and predation on zooplankton. It is an open question, however, why piscivores stocked during restoration, as in Huizhou West Lake, do not exert a strong top-down control on prey fish as otherwise seen in temperate lakes.

In the present study, we used a combination of gut contents analysis (GCA) and stable isotope analysis (SIA) to investigate the food web structure and food sources of fish in a restored subtropical shallow lake dominated by submerged macrophytes. The isotopic model (Phillips & Gregg, 2003) was used to estimate the contribution of potential carbon sources for fishes. We hypothesise that submerged macrophytes act as a significant carbon source to omnivorous fish and facilitate dominance of omnivores in shallow lakes with high macrophyte coverage. We also seek to elucidate why stocked piscivores apparently are poor predators on prey fish in such an environment.

Materials and methods

Description of the study lake

Lake Wuli is a shallow hypereutrophic bay in Lake Taihu, situated in Wuxi City in the Jiangsu Province,

China. It has a mean depth of 2.1 m, a maximum depth of 3.4 m and a surface area of 860 ha. In the 1950s, 98% of the lake area was covered by submerged macrophytes, and the water was clear (Zhu, 1959; Wu, 1962), but from the 1960s, because of fish farming and increased external and internal nutrient loading, the submerged macrophytes disappeared and the lake became turbid and eutrophic (Chen et al., 2013).

Aiming at improving the water quality, a sub-basin of Lake Wuli, completely isolated from the main lake by dams built under two bridges, was selected to test the effect of ecological restoration. This sub-basin had a mean depth of about 2.0 m and a surface area of 5 ha. Removal of coarse fish (mainly from the Cyprinidae family, 431 kg ha^{-1}), reestablishment of submerged macrophytes by planting adult plants (eel grass *Vallisneria spinulosa* Yan, hydrilla *Hydrilla verticillata* Royle, Eurasian watermilfoil *Myriophyllum spicatum* L., pondweeds *Potamogeton maackianus* Benn and *Potamogeton malaianus* Miq. and coontail *Ceratophyllum demersum* L.), and piscivorous fish stocking (51 kg ha^{-1} at one time) were carried out in 2010. After restoration, the water cleared up, and during our sampling period average phytoplankton chlorophyll *a* (13.1 µg l^{-1}) and key nutrient concentrations (total nitrogen, 0.74 mg l^{-1}, total phosphorus, 0.041 mg l^{-1}) were low and transparency high (Secchi depth 127 cm, average depth in this basin was 203 cm). For the unrestored part of the lake, however, average phytoplankton chlorophyll *a* (21.9 µg l^{-1}) and key nutrient concentrations (total nitrogen, 1.43 mg l^{-1}, total phosphorus, 0.065 mg l^{-1}) were relatively high and Secchi depth was low (36 cm, average depth in this part was 224 cm). Submerged macrophytes became the dominant primary producer, with *M. spicatum, P. malaianus* and *P. maackianus* as key species in 2011.

Sampling of aquatic biota and potential carbon sources

We monitored the fish community structure (80 × 1.5 m gillnets with four mesh sizes—10, 15, 25 and 40 mm, three gillnets were randomly set in the morning and retrieved after 2 h) every two months after the clear water state was established in August 2010. We found clear dominance of omnivorous fish in the lake and plenty of macrophytes in the guts of sharpbelly, such as a species considered to be zooplanktivorous in a previous study (Li et al., 2008), which encouraged us to study the diet patterns of the fish in more detail. Thus, we conducted a more comprehensive sampling programme, including gut content and stable isotope (^{13}C and ^{15}N) analysis, for the remaining part of the summer (July–October, 2011). Dominant species, representing all trophic levels and potential carbon sources, were sampled from three different sites located along the midcourt line of the lake (the same sites sampled by Zhang et al., 2012) in the middle of each sampling month, encompassing stable isotope samples of fish, invertebrates (zooplankton and snails) and primary producers (phytoplankton in the form of suspended particulate organic matter (SPOM), periphyton, and submerged macrophytes).

The water samples, pooled from three sub-samples collected at different depths (0.5, 1.0 and 1.5 m), were collected with a 5 l water sampler at each sampling site; additionally, a 5 l subsample was taken from each site, and all samples were brought back to the laboratory. SPOM samples ($n = 3$) were prepared by filtering 2 l of lake water through pre-weighed and pre-combusted GF/C filters, which were then dried at 60°C. Surface (~ 1 cm) sediment samples ($n = 3$) were collected with a Peterson grab sampler (1/40 m^2) and dried at 60°C for analysis of the isotopic composition of sedimentary organic matter (SOM).

Zooplankton samples were collected by towing a 64-µm mesh-sized net in the water column from the bottom to the water surface for approximately 15 min from the three sites in each sampling month, after which the animals were kept in deionized water for 24 h in the laboratory to allow emptying their guts. Afterwards, the dominant zooplankton species (at least one sample per site) and bulk zooplankton (at least one sample per site) were placed in a capsule under a dissecting microscope and dried at 60°C for subsequent stable isotope analyses. Periphyton (at least two samples per site per month) were sampled from the surface of the submerged macrophytes by vigorously shaking a sample of the plants in algal-free water in order to remove most of the attached algae. The resultant suspension was passed through GF/C glass fibre filters in the same way as SPOM. Green leaves of macrophytes (at least one sample of each species per site), used for periphyton sampling, were then washed repeatedly with distilled water in the laboratory to ensure that all the attached organic

matter was removed, after which they were put into the oven and dried at 60°C for 36–48 h. Thereafter, the macrophytes were ground into fine powder using a mortar and a pestle for further stable carbon and nitrogen analyses.

For each fish captured, total length (TL) and wet weight were measured. The dorsal white muscle of the fish (at least 6 individuals for each species) used for stable isotope analysis was removed and dried at 60°C. Then the fish guts were removed and preserved in 4% formalin for stomach content analyses. Frequency of occurrence (FO%) and per cent diet composition (P%) of each prey species per fish species were calculated as the proportion of zooplanktivorous and piscivorous fish stomachs containing a particular prey taxon. Based on analysis of the stomachs of some species, we classified each fish species into trophic groups following the classification of Teixeira-de Mello et al. (2009): zooplanktivorous fish feed only on zooplankton, omnivorous fish feed on both plant and animal food sources, and piscivores feed on fish but include also shrimps in their diet. In total, 84 omnivorous (size ranges between 3.5 and 19.8 cm in total length), 11 piscivorous (size ranges between 15.0 and 28.0 cm in total length) and 13 zooplanktivorous (size ranges between 10.0 and 15.5 cm in total length) fish guts were analysed to determine the diet compositions.

Snails were gathered in the littoral area only as no snails were found in the pelagic. Grass shrimp specimens were caught by a trap net, and muscle tissue of 6 individuals was dried at 60°C for stable isotope analysis.

Stable isotope analysis

All samples were analysed to determine $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios using a SerCon 20–20 isotope ratio mass spectrometer at the Department of Ecology and Institute of Hydrobiology, Jinan University, Guangzhou, China. Isotope abundance was expressed using the conventional delta notation against the conventional international standards (Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$):

$$\delta X(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where $X = {}^{13}C$ or ^{15}N and R is the ratio of $^{13}C:^{12}C$ or $^{15}N:^{14}N$. The precision of repeated measurements was ca. ±0.3%.

As $\delta^{15}N$ signatures an indicator of the trophic position of the consumer (Minagawa & Wada, 1984), the following formula was used for the calculation of trophic levels (TLs):

$$\text{TLs} = \left(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}} \right)/3.4 + 2$$

where 3.4 is the assumed enrichment of $\delta^{15}N$ between successive TLs, which has been identified as an average trophic nitrogen fractionation for aquatic consumers (Post, 2002). $\delta^{15}N$ of snails was used as the food web baseline and level 2 was consequently assigned to the snail group.

Data analyses

In order to determine the relative contribution of different primary producers in the diets of consumers, an isotopic mixing model, IsoSource designed by Phillips & Gregg (2003), was employed. This model determines the relative contribution of all the potential prey species with distinct isotopic signatures to the fish diet. The initial tolerance was set to 0.5‰; if mixture isotope values were out of bounds, the tolerance value was increased by 0.5‰, up to a maximum of 5‰. The output of the model was expressed as the mean and plotted by Systat Sigmaplot version 10.0 to compare the potential sources of the fish diets.

In our study, the means of the four sampling times were regarded as replicates (as no significant differences ($P > 0.05$) in the $\delta^{13}C$ of adult similar-sized omnivorous fish were found between months using one-way ANOVA). At each sampling event, isotope values of SPOM, periphyton (collected from the leaves of submerged macrophytes) and zooplankton were obtained from three samples, while isotope values for all fish, shrimps and snails were calculated from the mean value of at least six samples. The relationships between fish body size and stable carbon and nitrogen signatures of omnivorous fish were tested by linear regressions. The variations in $\delta^{15}N$ and $\delta^{13}C$ between different primary producers (SPOM and submerged macrophytes), and between different consumers (zooplankton, shrimps and fish), were analysed by one-way ANOVA ($P < 0.05$). Post hoc multiple comparisons of treatment means were performed using Tukey's least significant difference procedure. All these comparisons were performed with the statistical package SPSS version 16.0 (IBM Corporation, Somers, NY, USA).

Results

Fish community structure

Although the majority of the fish were removed before restoration, the fish community had somehow recovered within approximately a year after the clear water state was reached. Seven fish species were caught in the restored part of Lake Wuli: the zooplanktivore *Toxabramis swinhonis* Günther; the omnivores sharpbelly *Hemicculter leuciclus* Basilewsky, topmouth gudgeon *Pseudorasbora parva* Temminck & Schlegels, crucian carp *Carassius carassius* Linnaeus and bitterling *Acheilognathus macropterus* Bleeker; and the two piscivores mandarin fish *Siniperca chuatsi* Basilewsky and snakehead fish *Channa argus* Cantor.

During the whole sampling period, the percentage abundances of omnivorous fish were much higher ($F_{2,11} = 22.07$, $P < 0.001$) than those of both zooplanktivorous and piscivorous fish (Fig. 1). While the percentage biomasses of piscivorous fish were higher ($F_{2,11} = 5.53$, $P < 0.05$) than that of zooplanktivorous fish, no significant differences ($F_{1,7} = 3.688$, $P > 0.05$) were found between omnivores and piscivores.

Isotopic analysis

The isotopic composition of primary producers was distinguishable based on their $\delta^{13}C$ values (ANOVA, $F_{2,11} = 127.18$, $P < 0.001$), with a broad mean value ranging from -28.89 to -16.83‰; however, a
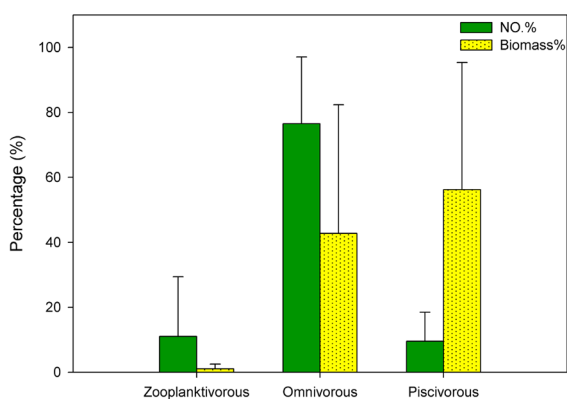

Fig. 1 Percentage of fish trophic groups in the restored part of Lake Wuli. *Error bars* represent the standard deviation of different sites and months

relatively narrow range (3.22–6.46‰) was found for $\delta^{15}N$ (Fig. 2). The submerged macrophyte *M. spicatum* was the most ^{13}C-enriched, while SPOM was the most ^{13}C-depleted (Fig. 2). Periphyton from the macrophyte leaf surfaces was the most ^{15}N-depleted primary producer, and the submerged macrophytes species *P. malaianus* and *P. maackianus* were the most ^{15}N-enriched.

Significant differences were observed among consumers for $\delta^{13}C$ (ANOVA, $F_{2,11} = 15.36$, $P < 0.05$) and $\delta^{15}N$ (ANOVA, $F_{2,11} = 37.67$, $P < 0.001$,). Cyclopoid copepods and bulk zooplankton were the most ^{13}C-depleted consumer taxa, with $\delta^{13}C$ values matching those of SPOM (Fig. 2). Juvenile omnivorous fish and zooplanktivorous fish had intermediate values, and the adult omnivorous fish were the most ^{13}C-enriched group, matching the macrophyte group (Fig. 2). The average $\delta^{13}C$ of zooplankton and shrimps differed by ~ 5‰, indicating dietary differences. The $\delta^{15}N$ values of zooplankton and shrimps were 3–4‰ heavier than their diets.

The monthly average $\delta^{13}C$ signatures of omnivorous fish ranged from -26.32 to -18.82‰, with juvenile sharpbelly fish being most depleted and adult topmouth gudgeon most enriched (Fig. 2). Hence, adult omnivorous fish were significantly more ^{13}C-enriched than juvenile omnivorous fish (ANOVA, $F_{1,7} = 47.12$, $P < 0.001$), with $\delta^{13}C$ values matching those of the macrophyte group (Fig. 2). The juvenile omnivorous fish were thought to be planktivores but exhibited a $\delta^{13}C$ isotopic composition between zooplankton and the macrophyte group, indicating diet diversity, however, except for topmouth gudgeon, their $\delta^{13}C$ values were closer to those of zooplankton.

Omnivorous fish had a broad range of $\delta^{13}C$ and $\delta^{15}N$ signatures. The $\delta^{13}C$ and $\delta^{15}N$ of fish differed between species and particularly between the size classes of the same fish species. All the omnivorous fish species became significantly ($P < 0.05$) more ^{13}C-enriched with size (Fig. 3A), except sharpbelly fish (Fig. 3A, L1, $P > 0.05$), and $\delta^{15}N$ tended to increase with size (Fig. 3B).

The piscivorous species, mandarin fish and snakehead fish, had similar $\delta^{13}C$ and $\delta^{15}N$ values, suggesting similar diets and similar trophic levels (Fig. 2). However, the $\delta^{15}N$ data demonstrated that these two piscivorous fish had no trophic or isotopic relationship with adult omnivorous and zooplanktivorous fish in Lake Wuli (Fig. 2).
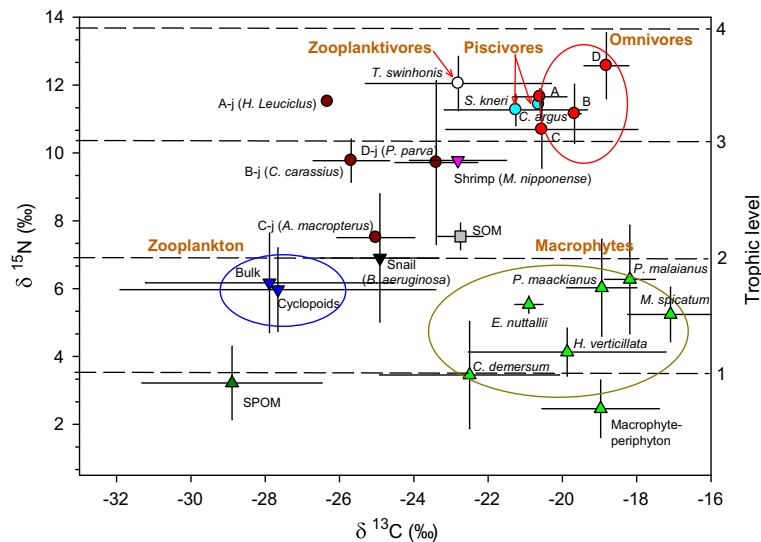
Fig. 2 Dual isotope plot of biota from the restored part of Lake Wuli. *Error bars* represent the standard deviation of different individuals/sites and months. *Triangle*, primary producer; *green triangle* (submerged macrophytes) and *dark green triangle* (phytoplankton, SPOM). *Inverted triangle*, invertebrate primary consumer; blue inverted triangle (cyclopoids and bulk zooplankton), *black inverted triangle* (snails) and *purple inverted triangle* (shrimps). *Circle*, fish; *red circles* (adult omnivores) and *dark red circles* (juvenile omnivores): *A* and *A-j*, adult and

juvenile sharpbelly, respectively; *B* and *B-j*, adult and juvenile crucian carp, respectively; *C* and *C-j*, adult and juvenile bitterling, respectively; *D* and *D-j*, adult and juvenile topmouth gudgeon, respectively, *blank circle* (zooplanktivore) and *cyan circles* (piscivores). *Square*, sediment organic matter (SOM). Trophic levels were calculated using the assumption of a 3.4‰ enrichment in $\delta^{15}N$ from one trophic level to another (Post, 2002)

Fish guts

Only zooplankton was found in the guts of the zooplanktivorous *T. swinhonis*. The small-bodied cladoceran *Bosmina* sp. was seldom found compared other food items, such as *Moina* sp. and *Diaphanosoma* sp., exhibiting a percentage occurrence of less than 20%, while cyclopoid copepods occurred in all *T. swinhonis* gut samples. On average 90% of the food items consisted of cyclopoid copepods, which were the main and most important food item for *T. swinhonis* (Fig. 4).

In the omnivorous fish guts, both macrophytes and zooplankton were found. However, the diet of adult fish mainly consisted of macrophytes, approximately 99.8% for sharpbelly, 99.4% for crucian carp, 99.6% for topmouth gudgeon and 98.3% for bitterling in volume, while no macrophytes occurred in the juvenile fish guts. The macrophyte in the guts were highly digested and thus difficult to determine to species level. Juvenile fish and shrimps were found in the piscivorous mandarin and snakehead fish stomachs. The prey fish species of piscivores were topmouth

gudgeon and bitterling, and the maximum body size of the prey fish was 3.5 cm in total length. The per cent occurrences of topmouth gudgeon, bitterling and shrimps in the guts of mandarin fish were 50, 16.7 and 66.7%, respectively, indicating that topmouth gudgeon and shrimps were the main food source. No food items were found in the guts of snakehead (only two individuals were caught in August).

Proportion of food items in the diet

The relative contributions of different food items to the diets of fish were determined using the isotopic mixing model (Phillips & Gregg, 2003). The model data showed a clear diet difference relative to the size of omnivorous fish (Fig. 5). Juvenile omnivores depended more on zooplankton than did adult omnivores, juvenile sharpbelly and crucian carp showing a higher preference for zooplankton than bitterling and topmouth gudgeon (Fig. 5), and the relative contribution of macrophytes to the diets of juvenile bitterling and topmouth gudgeon was higher than that of zooplankton (Fig. 5). Adult omnivores depended
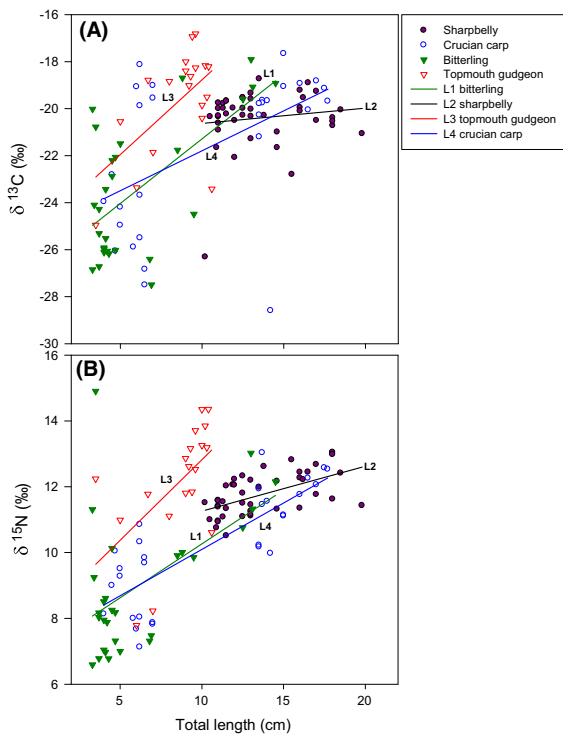
Fig. 3 Relationships between total lengths (TL) of omnivorous fish with: **a** $\delta^{13}C$ composition and **b** $\delta^{15}N$ composition. **a** Sharpbelly showed no statistical significance in its TL-$\delta^{13}C$ relationship $r^2_{adj} = 0.00$, $P = 0.3733$, but the other three species showed significant relationships, $r^2_{adj} = 0.39$, $P = 0.0002$ for bitterling, $r^2_{adj} = 0.28$, $P = 0.011$ for topmouth gudgeon and $r^2_{adj} = 0.24$, $P = 0.0043$ for crucian carp. **b** Significant relationships were found for all species separately ($r^2_{adj} = 0.26$, $P = 0.0034$ for bitterling, $r^2_{adj} = 0.31$, $P < 0.0001$ for sharpbelly, $r^2_{adj} = 0.25$, $P = 0.0163$ for topmouth gudgeon, $r^2_{adj} = 0.64$, $P < 0.0001$ for crucian carp)

more on macrophytes than did juvenile fish (Fig. 5). The relative contribution of macrophytes to the diets of adult omnivorous fish was much higher than that of zooplankton, ranging from 68 to 79% for macrophytes and 20–31% for zooplankton. Thus, both juvenile and adult bitterling and topmouth gudgeon were observed to feed mainly on macrophytes; zooplankton constituted the primary food source for juvenile sharpbelly and crucian carp, and macrophytes were the main food source for the adults. Only zooplankton was found in the guts of zooplanktivorous fish. As the isotopic composition of zooplankton was only analysed for dominant species or bulk zooplankton, the relative contributions of the different zooplankton species to the diet of zooplanktivorous fish were not estimated.
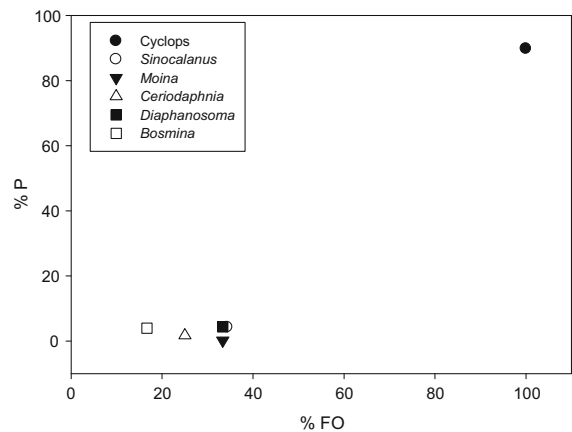

Fig. 4 Frequency occurrence (FO%) and per cent diet composition (P%) of food items in the gut contents of *T. swinhonis* as plotted according to Costello (1990)

Piscivorous fish were stocked to control zooplanktivores and benthivores, but only juvenile topmouth gudgeon and bitterling and shrimps were found in their guts. The mixing model estimation showed that the food of mandarin fish and snakehead fish mainly consisted of shrimps, constituting 90.8% and 93.1% of the diet of mandarin and snakehead, respectively. While juvenile fish represented a small proportion of the piscivore diet, bitterling contributed 2.2% and 1.6% and topmouth gudgeon 7% and 5.3% to the diet of mandarin and snakehead, respectively (Fig. 6).

Discussion

In the restored basin of Lake Wuli, characterised by high macrophyte biomass and coverage, the GCA and SIA data both suggest that submerged macrophytes constituted a significant percentage of the food sources for adult omnivores, while the juveniles obtained more carbon from zooplankton. The piscivores mandarin and snakehead were abundant in terms of biomass but mainly foraged on shrimps rather than on fish (juvenile topmouth gudgeon and bitterling), indicating weak piscivory. The latter is supported by the SIA results reveal no trophic or isotopic relationships between potential piscivores and adult planktivores and omnivores. While the number of the potential piscivorous fish analysed from Lake Wuli was scarce, rendering the conclusion of low piscivory somewhat uncertain, the results concur with findings in other restoration
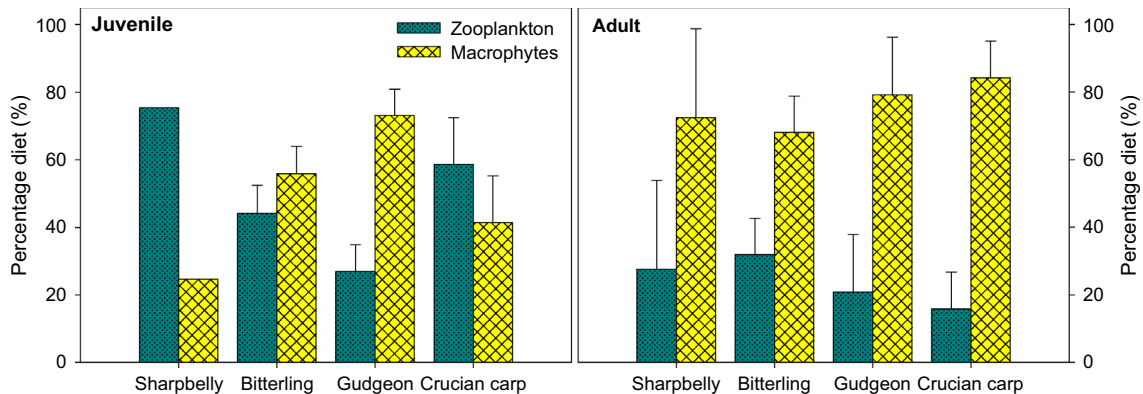
Fig. 5 Percentage contribution of zooplankton and macrophytes to the diet of juvenile and adult omnivorous fish as estimated in the mixing isotope model IsoSource (Phillips & Gregg, 2003). *Note* Total length of juvenile crucian carp was ≤6 cm, ≤7 cm of topmouth gudgeon and ≤5 cm of bitterling. *Error bars* represent the standard deviation of different individuals and months
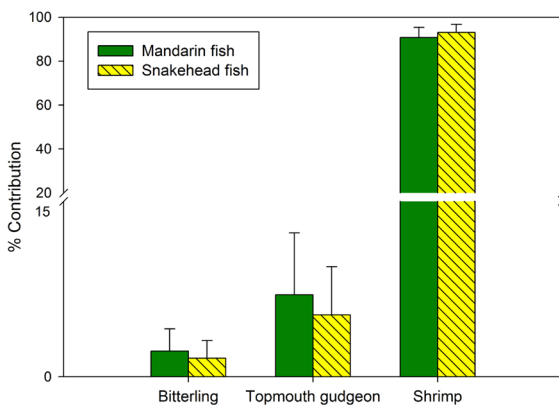

Fig. 6 The relative contributions of juvenile fish (bitterling and topmouth gudgeon) and shrimps to the diets of mandarin (mandarin fish) and snakehead (snakehead fish). *Error bars* represent the standard deviation of different individuals and months

case studies conducted in subtropical (Chen et al., 2009) and tropical lakes (Gao et al., 2014) in China where stocking of mandarin and/or snakehead only led to a limited control effect on the population of omnivorous fish. Mandarin fish is a typical non-active stalking (sit and wait) predator relying on its vision when feeding (Liang et al., 1998; Wu & Hardy, 1988), and similar feeding modes have been found for snakehead (Liu et al., 1998). Most small fish in Lake Wuli, especially topmouth gudgeon and bitterling, are considered to be closely associated with macrophytes (Ye et al., 2006), and the high coverage and biomass of

submerged macrophytes in this lake may, therefore, not only have functioned as a refuge for prey fish but also reduced the foraging success of the visually hunting piscivores. Moreover, several studies have shown that piscivorous fish turn to an alternative prey in subtropical lakes, such as shrimps (facultative piscivores, sensu Lazzaro et al., 2003) which can be abundant in subtropical lakes (Collins, 1999), not least among plants (Meerhoff et al., 2007). Other studies have shown stronger piscivory in the absence of alternative prey [*Hoplias malabaricus* feeding on the omni-planktivorous *Jenynsia multidentata* (Mazzeo et al., 2010)]. Moreover, in a set of tropical Brazilian reservoirs enhancement of the biomass ratio of facultative piscivores to omnivores by stocking induced a top-down effect on the omnivorous fish, with a subsequent cascading effect down to phytoplankton (Lazzaro et al., 2003). More studies are clearly needed to elucidate the role of piscivores in warm lakes with contrasting prey fish and alternative prey conditions to draw general conclusions.

Dietary shifts during growth are common in size-structured fish populations (see review in Werner & Gilliam, 1984), including many cyprinids (Mark et al., 1987), and it divides the populations into life history stages with different trophic effects on food webs. In the present study, the isotope composition of the four omnivorous species changed with size (Fig. 3), indicating ontogenetic dietary shifts, this being confirmed by the IsoSource model estimations and GCA: juveniles had a high proportion of zooplankton in their

diet, while adults mainly consumed macrophytes (Fig. 5). However, other studies based on gut content analysis have shown minor importance of macrophytes in the diet of the omnivores found in the present study. For instance, topmouth gudgeon was observed to prefer animal diets in both turbid shallow Lake Neusiedler See (benthic invertebrates and zooplankton) (Wolfram-Wais et al., 1999) and in clear shallow Lake Liangzi in which macrophytes were abundant (Yang et al., 2004); crucian carp were found to prey more on benthic invertebrates in the littoral habitats of Lake Balaton, which is covered by the reed *Phragmites australis* (Specziár et al., 1997); sharpbelly were recorded to feed mainly on zooplankton in turbid shallow Lake Taihu (Li et al., 2008), while bitterling preferred algae in rivers (Solomon et al., 1985; Koutrakis et al., 2003). Diet shifts of omnivorous fish species may be driven by temperature, as seen in a subtropical stream, where the omnivore *Bryconamericus iheringii* was found to eat more vegetal food in summer than in winter (González-Bergonzoni et al., 2016).

In the present study, submerged macrophytes clearly acted as an important food source for the omnivorous fish. A predator–prey relationship may be uncoupled (Spencer & Collie, 1995; Lazzaro, 1997; Yafe et al., 2002) when alternative food sources are available, allowing an even higher predation pressure by predators on their ideal prey. For instance, *Daphnia magna* as an alternative prey has been observed to enhance the predation rate on mosquito (*Culex pipiens*) larvae by the cyprinodontid *Aphanius mento* (Blaustein & Byard, 1993), but in other studies the presence of alternative prey has been found to decrease the predation on ideal prey (Svenning et al., 2005; Aditya et al., 2012). The effects of alternative prey therefore depend on the prey species and the selective feeding strategy of the predators. In Lake Wuli, the abundant submerged macrophytes, acting as the main carbon source for omnivores, may promote the population development of omnivorous fish, which, in turn, may enhance the predation on zooplankton in the juvenile state and, eventually, when the fish become adult, on the submerged macrophytes.

Our results have implications for subtropical shallow lake restoration in the region. In the present study, macrophytes were found to constitute an important food source for omnivores, thereby promoting

omnivore population growth due to the low control by piscivores. This, in turn, results in a potentially high predation pressure on both zooplankton (when juvenile) and on macrophytes (adult fish) (Fig. 5) as seen in the restored Huizhou West Lake, China (Liu et al., 2014). This may impair the top-down control by zooplankton of phytoplankton, potentially reducing the chances of maintaining submerged macrophytes in the long term. A weak top-down control of piscivores as seen in Lake Wuli and other restored Chinese lakes also indicates a higher risk of returning to the turbid state. Better growth and a prolonged growing season for macrophytes (all year around) may, however, somehow counterbalance this weakness. More studies are needed to reach firm conclusions about when and how to conduct biomanipulation in warm lakes following a nutrient loading reduction in order to restore and maintain a clear water state.

References

Aditya, G., S. Pal, N. Saha & G. K. Saha, 2012. Efficacy of indigenous larvivorous fishes against *Culex quinquefasciatus* in the presence of alternative prey: implications for biological control. Journal of Vector Borne Diseases 49: 217–225.

Araujo-Lima, C., B. R. Forsberg, R. Victoria & L. Martinelli, 1986. Energy sources for detritivorous fishes in the amazon. Science 234: 1256–1258.

Blanck, A. & N. Lammouroux, 2007. Large-scale intraspecific variation in life-history traits of 44 European freshwater fish. Journal of Biogeography 34: 862–875.

Blaustein, L. & R. Byard, 1993. Predation by a cyprinodontid fish, *Aphanius mento*, on *Culex pipiens*: effects of

alternative prey and vegetation. Journal of the American Mosquito Control Association 9: 356–358.

Carpenter, S. R. & D. M. Lodge, 1986. Effects of submerged macrophytes on ecosystem processes. Aquatic Botany 26: 341–370.

Chen, K., C. Bao & W. Zhou, 2009. Ecological restoration in eutrophic Lake Wuli: a large enclosure experiment. Ecological Engineering 35: 1646–1655.

Chen, F., T. Shu, E. Jeppesen, Z. Liu & Y. Chen, 2013. Restoration of a subtropical eutrophic shallow lake in China: effects on nutrient concentrations and biological communities. Hydrobiologia 718: 59–71.

Collins, P. A., 1999. Feeding of *Palaemonetes argentinus* (Decapoda: Palaemonidae) from an oxbow lake of the Paraná River, Argentina. Journal of Crustacean Biology 19: 485–492.

Costello, M. J., 1990. Predator feeding strategy and prey importance: a new graphical analysis. Journal of Fish Biology 36: 261–263.

Forsberg, B. R., C. A. R. M. Araujo-Lima, L. A. Martinelli, R. L. Victoria & J. A. Bonassi, 1993. Autotrophic carbon sources for fish of the central Amazon. Ecology 74: 643–652.

Gao, J., Z. Liu & E. Jeppesen, 2014. Fish community assemblages changed but biomass remained similar after lake restoration by biomanipulation in a Chinese tropical eutrophic lake. Hydrobiologia 724: 127–140.

González-Bergonzoni, I., E. Jeppesen, N. Vidal, F. Teixeira-de Mello, G. Goyenola, A. López-Rodríguez & M. Meerhoff, 2016. Potential drivers of seasonal shifts in fish omnivory in a subtropical stream. Hydrobiologia 768: 183–196.

González-Bergonzoni, I., M. Meerhoff, T. A. Davidson, F. Teixeira-de Mello, A. Baattrup-Pedersen & E. Jeppesen, 2012. Meta-analysis shows a consistent and strong latitude pattern in fish omnivory across ecosystems. Ecosystems 15: 492–503.

Hansson, L.-A., L. Johansson & L. Persson, 1987. Effect of fish grazing on nutrient release and succession of primary producers. Limnology and Oceanography 32: 723–729.

Jeppesen, E., T. L. Lauridsen, T. Kairesalo & M. R. Perrow, 1998. Impact of submerged macrophytes on fish-zooplankton interactions in lakes. In Jeppesen, E., Ma Søndergaard, Mo Søndergaard & K. Christoffersen (eds.), The Structuring Role of Submerged Macrophytes in Lakes. Ecological Studies Series. Springer Verlag, New York: 91–114.

Jeppesen, E., Ma Søndergaard, Mo Søndergaard, K. Christoffersen, J. Theil-Nielsen & K. Jürgens, 2002. Cascading trophic interactions in the littoral zone: an enclosure experiment in shallow Lake Stigsholm, Denmark. Archiv für Hydrobiologie 153: 533–555.

Jeppesen, E., M. Meerhoff, B. A. Jacobsen, R. S. Hansen, M. Søndergaard, J. P. Jensen, T. L. Lauridsen, N. Mazzeo & C. W. C. Branco, 2007. Restoration of shallow lakes by nutrient control and biomanipulation – the successful strategy varies with lake size and climate. Hydrobiologia 581: 269–285.

Keough, J. R., C. A. Hagley, E. Ruzycki & M. Sierszen, 1998. δ^{13}C composition of primary producers and role of detritus in a freshwater coastal ecosystem. Limnology and Oceanography 43: 734–740.

Koutrakis, E. T., A. K. Kokkinakis, A. C. Tsikliras & E. A. Eleftheriadis, 2003. Characteristics of the European bitterling *Rhodeus amarus* (Cyprinidae) in the Rihios River, Greece. Journal of Freshwater Ecology 18: 615–624.

Lauridsen, T. L., E. Jeppesen & M. Søndergaard, 1994. Colonization and succession of submerged macrophytes in shallow Lake Væng during the first five years following fish manipulation. Hydrobiologia 275(276): 233–242.

Lauridsen, T. L., L. J. Pedersen, E. Jeppesen & M. Søndergaard, 1996. The importance of macrophyte bed size for cladoceran composition and horizontal migration in a shallow lake. Journal of Plankton Research 18: 2283–2294.

Lazzaro, X., 1997. Do the trophic cascade hypothesis and classical biomanipulation approaches apply to tropical lakes and reservoirs? Verhandlungen der Internationale Vereinigung für Limnologie 26: 719–730.

Lazzaro, X., M. Bouvy, R. A. Ribeiro-Filho, V. S. Oliviera, L. T. Sales, A. R. M. Vasconcelos & M. R. Mata, 2003. Do fish regulate phytoplankton in shallow eutrophic Northeast Brazilian reservoirs? Freshwater Biology 48: 649–668.

Li, Y., J. Ye, F. Chen, Z. Liu, W. Wang & X. Liu, 2008. Feeding ecology of *Hemiculter leucisculus* in the Meiliang Bay of Taihu Lake. Journal of Hohai University (Natural Sciences) 36: 82–85. (In Chinese with English abstract).

Liang, X. F., J. K. Liu & B. Y. Huang, 1998. The role of sense organs in the feeding behavior of Chinese perch. Journal of Fish Biology 52: 1058–1067.

Liu, J., Y. Cui & J. Liu, 1998. Food consumption and growth of two piscivorous fishes, the mandarin fish and the Chinese snakehead. Journal of Fish Biology 53: 1071–1083.

Liu, Z. W., P. Zhong, X. Zhang, J. Ning, S. E. Larsen & E. Jeppesen, 2014. Successful restoration of a tropical shallow eutrophic lake: strong bottom-up but weak top-down effects recorded. In: G. Kattel (eds), Australia–China wetland network research partnership. Proceedings of the Australia–China Wetland Network Research Partnership Symposium, Nanjing, China: 78–86.

Mark, W., R. Hofer & W. Wieser, 1987. Diet spectra and resource partitioning in the larvae and juveniles of three species and six cohorts of cyprinids from a subalpine lake. Oecologia 71: 388–396.

Mazzeo, N., L. Rodríguez-Gallego, C. Kruk, M. Meerhoff, J. Gorga, G. Lacerot, F. Quintans, M. Loureiro, D. Larrea & F. García-Rodríguez, 2003. Effects of *Egeria densa* Planch beds in a shallow lake without piscivorous fish. Hydrobiologia 506–509: 591–602.

Mazzeo, N., C. Iglesias, F. Teixeira-de Mello, A. Borthagaray, C. Fosalba, R. Ballabio, D. Larrea, J. Vilches & E. Jeppesen, 2010. Trophic cascade effects of *Hoplias malabaricus* (Characiformes, Eritrinidae) in subtropical food webs: a mesocosm approach. Hydrobiologia 644: 325–335.

Meerhoff, M., J. M. Clemente, F. Teixeira-de Mello, C. Iglesias, A. R. Pedersen & E. Jeppesen, 2007. Can warm climate-related structure of littoral predator assemblies weaken the clear water state in shallow lakes? Global Change Biology 13: 1888–1897.

Meerhoff, M., F. Teixeira-de Mello, C. Kruk, C. Alonso, I. González-Bergonzoni, J. P. Pacheco, G. Lacerot, M. Arim, M. Beklioğlu, S. Brucet, G. Goyenola, C. Iglesias, N. Mazzeo, S. Kosten & E. Jeppesen, 2012. Environmental warming in shallow lakes: a review of potential changes in

community structrue as evidenced from space-for-time substitution approaches. Advances in Ecological Research 46: 259–349.

Mendonça, R., S. Kosten, G. Lacerot, N. Mazzeo, F. Roland, J. P. Ometto, E. A. Paz, C. P. Bove, N. C. Bueno, J. Henrique, C. Gomes & M. Scheffer, 2013. Bimodality in stable isotope composition facilitates the tracing of carbon transfer from macrophytes to higher trophic levels. Hydrobiologia 710: 205–218.

Minagawa, M. & E. Wada, 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. Geochimica Cosmochimica Acta 48: 1135–1140.

Moss, B., 1990. Engineering and biological approaches to the restoration from eutrophication of shallow lakes in which aquatic plant communities are important components. Hydrobiologia 200(201): 367–377.

Moss, B., 2010. Climate change, nutrient pollution and the bargain of Dr Faustus. Freshwater Biology 55: 175–187.

Nurminen, L., J. Horppila, J. Lappalainen & T. Malinen, 2003. Implications of rudd (Scardinius erythrophthalmus) herbivory on submerged macrophytes in a shallow eutrophic lake. Hydrobiologia 506–509: 511–518.

Phillips, D. & J. W. Gregg, 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136: 261–269.

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

Quirós, R., 1998. Fish effects on trophic relationships in the pelagic zone of lakes. Hydrobiologia 361: 101–111.

Rao, W., J. Ning, P. Zhong, E. Jeppesen & Z. Liu, 2015. Size-dependent feeding of omnivorous Nile tilapia in a macrophyte-dominated lake: implications for lake management. Hydrobiologia 749: 125–134.

Scheffer, M. & E. Jeppesen, 1998. Alternative stable states. In Jeppesen, E., M. Søndergaard & K. Christoffersen (eds.), The Structuring role of submerged macrophytes in lakes. Springer-Verlag, New York: 397–406.

Scheffer, M., S. Hosper, M. Meijer, B. Moss & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology and Evolution 8: 275–279.

Solomon, G., M. Shimizu & Y. Nose, 1985. The feeding habits of rose bitterling in the Shin Tone River. Bulletin of the Japanese Society of Scientific Fisheries 51: 711–716.

Specziár, A., L. Tölg & P. Bíró, 1997. Feeding strategy and growth of cyprinids in the littoral zone of Lake Balaton. Journal of Fish Biology 51: 1109–1124.

Spencer, P. D. & J. S. Collie, 1995. A simple predator-prey model of exploited marine fish populations incorporating alternative prey. ICES Journal of Marine Science 53: 615–628.

Starling, F., X. Lazzaro, C. Cavalcanti & R. Moreira, 2002. Contribution of omnivorous tilapia to eutrophication of a shallow tropical reservoir: evidence from a fish kill. Freshwater Biology 47: 2443–2452.

Svenning, M.-A., R. Borgstrøm, T. O. Dehli, G. Moen, R. T. Barrett, T. Pedersen & W. Vader, 2005. The impact of marine fish predation on Atlantic salmon smolts (Salmo salar) in the Tana estuary, North Norway, in the presence of an alternative prey, lesser sandeel (Ammodytes marinus). Fisheries Research 76: 466–474.

Teixeira-de Mello, F., M. Meerhoff, Z. Pekcan-Hekim & E. Jeppesen, 2009. Substantial differences in littoral fish community structure and dynamics in subtropical and temperate shallow lakes. Freshwater Biology 54: 1202–1215.

Van Donk, E., R. D. Gulati, A. Iedema & J. T. Meulemans, 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia 251: 19–26.

Werner, E. E. & J. F. Gilliam, 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15: 393–426.

Winemiller, K. O., 1990. Spatial and temporal variation in tropical fish trophic network. Ecological Monographs 60: 331–367.

Wolfram-Wais, A., G. Wolfram, B. Auer, E. Mikschi & A. Hain, 1999. Feeding habits of two introduced fish species (Lepomis gibbosus, Pseudorasbora parva) in Neusiedler See (Austria), with special reference to chironomid larvae (Diptera: Chironomidae). Hydrobiologia 408(409): 123–129.

Wu, X. W., 1962. Investigation of limnology in Lake Wuli in 1951. Acta Hydrobiologica Sinica 1: 63–113. (In Chinese).

Wu, Z. L. & S. Hardy, 1988. A preliminary ethological analysis on the feeding behaviour of mandarin fish. Freshwater Fisheries 5: 18–21.

Yafe, A., M. Loureiro, F. Scasso & F. Quintans, 2002. Feeding of two cichlidae species (Perciformes) in a hypertrophic urban lake. Iheringia, Série Zoologica 92: 73–79.

Yang, R., S. Bian, J. Zhou & C. Xie, 2004. Study on food habits of Pseudorasbora parva in Liangzi Lake. Journal of Huazhong Agricultural University 23: 331–334. (In Chinese with English abstract).

Ye, S., Z. Li, S. Lek-Ang, G. Feng, S. Lek & W. Cao, 2006. Community structure of small fishes in a shallow macrophytic lake (Niushan Lake) along the middle reach of the Yangtze River, China. Aquatic Living Resources 19: 349–359.

Zhang, M., J. Yu, H. He, K. Li, F. Chen, B. Guan, Y. Hu, Y. Su, Y. Du & Z. Liu, 2012. Effects of ecological restoration on water quality of Wuli Bay, Lake Taihu. Ecological Science 31: 240–244. (In Chinese with English abstract).

Zhao, S., J. Fang, C. Peng, Z. Tang & S. Piao, 2006. Patterns of fish species richness in China's lakes. Global Ecology and Biogeography 15: 386–394.

Zhu, S. P., 1959. Variety of water quality in whole year in the northern of Taihu Lake. Oceanologia et Limnologia Sinica 2: 146–162. (In Chinese).