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Carbon sources and trophic structure in a macrophyte-dominated polyculture pond assessed by stable-isotope analysis

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SUMMARY

1. Freshwater polyculture systems should efficiently utilise natural food resources and pond habitats with the consequent effects of reducing costs and increasing productivity. However, understanding of the primary carbon sources and food web within polyculture ponds, and especially macrophyte-dominated ponds, remains limited.
2. Stable-isotope analysis was used to identify the main carbon sources and describe the trophic patterns of freshwater fauna in a polyculture pond around Lake Gucheng, China. An isotope mixing model (SIAR) was also used to estimate the relative contribution of four basal sources (suspended particulate organic matter [SPOM], sedimentary organic matter [SOM], macrophytes and epiphytes) to invertebrates and different fish trophic groups.
3. Basal resources showed distinct δ^{13}C values, ranging from −24.3 to −20.5‰, which allowed for a powerful discrimination of carbon sources. Consumers were also well separated in δ^{13}C (−24.0 to −20.0‰ for invertebrates and −24.4 to −18.9‰ for fish), suggesting considerable differences in ultimate sources of carbon. Results from the SIAR model indicated that epiphytes were the most important carbon sources for crustaceans, herbivorous fish, omnivorous fish and piscivorous fish, while SPOM made the highest contribution to zooplankton, chironomids, molluscs, annelids, planktivorous fish and benthivorous fish.
4. Mean δ^{15}N values, combined with calculated food source contributions, were used to elucidate the food-web structure of this polyculture pond, which suggested that carbon fixed by primary producers could be transported up the food chain to piscivorous fish through four main trophic pathways: one based mainly on SPOM, one on both SPOM and SOM, one on SPOM, SOM, macrophytes and epiphytes, and one based on both macrophytes and epiphytes.
5. Our results emphasised the trophic importance of epiphytes on submerged macrophytes, a key component in the polyculture ponds, for the main culture species: Chinese mitten crab (*Eriocheir* sinensis: Varunidae), freshwater shrimp (*Macrobrachium nipponense*: Palaemonidae), and high-value piscivorous fish. This study also revealed the high level of complexity of polyculture pond food webs, and suggests that a diversity of major trophic pathways may be a characteristic of macrophyte-dominated aquatic ecosystems.

Keywords: epiphytes, fish community, food webs, SIAR, trophic pathways

Introduction

Polyculture systems have been used for centuries in China and are probably the best examples of successful sustainable aquaculture (Xie *et al*., 2013). The idea of polyculture is based on the principle that each species stocked has its own feeding niche that does not completely overlap with the feeding niches of other species. This type of system might efficiently utilise natural food resources (e.g. phyto- and zooplankton, benthos,

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detritus, macrophytes and epiphytes) and water habitats (i.e. surface, pelagic and benthic) of the aquaculture ecosystem with the consequent effects of reducing costs and increasing productivity (Bardach, Ryther & McLarney, 1972; Tacon & De Silva, 1997).

The Chinese mitten crab *Eriocheir sinensis* (Varunidae) is native to the coastal rivers and estuaries of the Yellow Sea. Due to its high nutritional value and unique flavour, this crab has become a species of commercial importance for Chinese fisheries. Its annual production has increased during the past decades in China from 8000 tonnes in 1991 to 640 000 tonnes in 2011 (Zeng *et al*., 2013). Gaochun (a district of Nanjing city), located around the reclamation area of Lake Gucheng, is well known for Chinese mitten crab and commercial fish culture, with a production of 41 900 tonnes in 2013. Polyculture of crab with the freshwater shrimp *Macrobrachium nipponense* (Palaemonidae) and high-value fishes, such as topmouth culter *Culter alburnus* (Cyprinidae) and mandarin fish *Siniperca chuatsi* (Serranidae), has become a successful and popular culture model in this region, with significant economic and other benefits (Xie *et al*., 2013). These culture ponds are characterized by a submersed macrophyte community, which is species-rich and complex, both in terms of food sources and biotic interactions (Wang *et al*., 2006). Although carbon sources that support macrophyte food webs are still debated, the recent studies in macrophyte-dominated ecosystems imply strong trophic linkages between epiphytic and benthic algae and consumers, whereas fresh macrophyte leaves are assumed to be of minor importance (France, 1996; Jones & Waldron, 2003; Jaschinski, Brepohl & Sommer, 2011). However, polyculture systems vary in the mix of culture species stocked, and studies of trophic relationships and food-web structure are an essential step in clarifying the functioning of these ecosystems and providing a baseline for sustainable aquaculture management. Here, we describe the food web by characterising carbon sources supporting the community and trophic pathways among the components in the polyculture ponds.

It is not possible to elucidate the structure in food webs in these complex macrophyte-dominated pond systems by using conventional gut content analyses alone (Jones & Waldron, 2003). However, stable-isotope tracers ($\delta^{13}C$ and $\delta^{15}N$) provide a powerful tool for identifying the trophic relationships and the fluxes of carbon sources in food webs (Peterson & Fry, 1987; Post, 2002). The application of this technique is based on the assumption that the ratios of naturally occurring stable isotopes of carbon and nitrogen in consumer tissues reflect those of diets actually assimilated by consumers (Minagawa & Wada, 1984). Compared to the traditional dietary observation methods, stable-isotope analysis has the advantage of providing time-integrated food assimilation instead of the instant food ingestion (Hobson, Ambrose & Renaud, 1995; Peterson, 1999). Several isotopic studies have been carried out in different types of polyculture systems to assess the relative trophic role of each primary producer and the trophic relationships among the diverse cultured species. Guo *et al.* (2014) described the food-web structure in the sea ponds of an optimised culture model (jellyfish–shellfish–fish–prawn) located in Donggang, Liaotung Peninsula. Feng *et al.* (2014) also identified the trophic relationships among primary producers, benthic macro-invertebrate and fish from a commercial marine polyculture pond in Jinghai Bay. However, these studies have aimed at clarifying the food sources for the economic animals and trophic structure in marine polyculture ecosystems, and to our knowledge, little information exists on the carbon sources and food web within freshwater polyculture ponds, especially macrophyte-dominated culture ponds. Therefore, the present study is an important supplement to the food-web research of freshwater ecosystems.

The objectives of this study were therefore to: (i) identify the main carbon sources sustaining the polyculture pond, a less frequently investigated aquatic ecosystem, (ii) to estimate the trophic levels and feeding habits of all organisms from the pond, and (iii) to describe the possible pathways of carbon flow and trophic relationships between components of the pond food web.

Methods

Experimental pond

Lake Gucheng (118°52′–118°57′E, 31°14′–31°18′N) is located at the downstream of Yangtze River, on the border of Nanjing City, Jiangsu Province, China. The total surface area of Lake Gucheng was about 65 km^2 in the early 1960s, but the frequent land reclamation around the lake between the 1960s and 1980s caused the continuous reduction in the surface area of the lake to 30.9 km^2 in 1979 (Yu, Fang & Ru, 2010). The experimental ponds for crustaceans and fish polyculture were located in the reclamation area of Lake Gucheng. At present, this region is well known for Chinese mitten crab and commercial fish culture with a production of 41 900 tonnes in 2013. About two hundred culture ponds, which are similar in size, are distributed in our study area. The area of each pond was *c.* 6000 m^2 with a mean water depth of 1.5 m. Three sampling ponds were selected randomly for sample collection, while stocking, feeding and harvesting were managed homogeneously.

The sampling ponds were fully covered by a submersed macrophyte community comprised mainly of *Elodea nuttallii* and *Hydrilla verticillata* (Hydrocharitaceae). The main cultured species included Chinese mitten crab, the freshwater shrimp *M. nipponensis*, topmouth culter *C. alburnus*, dark sleeper *Odontobutis potamophila* (Eleotridae), and mandarin fish *S. chuatsi*. Planktivorous fishes such as silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis* (Cyprinidae) were also stocked in the pond for controlling the planktonic algae. Fish such as *Rhodeus ocellatus* (Cyprinidae), *Acheilognathus chankaensis* (Cyprinidae), *Hemiculter leucisculus* (Cyprinidae), *Pseudorasbora parva* (Cyprinidae), *Sarcocheilichthys nigripinnis* (Cyprinidae), *Rhinogobius giurinus* (Cobiidae) and *Channa argus* (Channidae) were wild species that occurred naturally within the pond or entered ponds during water exchange.

The stocking density and biomass of the main cultured species Chinese mitten crab, freshwater shrimp and commercial fish were 1.20, 22.49 and 0.22 ind m^{-2} and 17.40, 7.42 and 6.78 g m^{-2}, respectively, while their average yield were 94.45, 27.59 and 44.68 g m^{-2} respectively (unpubl. data, Mao *et al.*). Wild fish species contributed only a small fraction to total cultured species biomass. Some forage fish (small oceanic fish which were frozen) were provided to crabs as an important animal protein feed, but no artificial feed or fertiliser was supplied for polyculture fishes during culture.

Sample collection and preparation

Samples for stable-isotope analysis were collected in October 2013 from three polyculture ponds, which were located around Lake Gucheng. Organic matter was collected from both the water column and sediment. Samples of surface water (20 cm depth) were collected with a 12-L Niskin bottle in the study area. Water samples were pre-filtered through a 200-μm mesh to remove zooplankton and large detritus. Suspended particulate organic matter (SPOM) samples were obtained by filtering 1 L of water on precombusted (450 °C, 4 h) Whatman GF/F filters under moderate vacuum (10 mbar). Sedimentary organic matter (SOM) samples were collected using a Peterson grab sampler (1/16 m^2), whereby the top 1 cm layer was sliced off and stored in plastic bags. Samples of the dominant submerged macrophytes, *E. nuttallii* and *H. verticillata*, were collected by hand and washed with distilled water to remove fine detritus and attached animals. Epiphytes were carefully scraped from the *E. nuttallii* leaves, transferred to small amounts of distilled water using a soft toothbrush and filtered on precombusted (450 °C, 4 h) Whatman GF/F filters.

Zooplankton (composed mostly of copepods and cladocerans) was sampled by towing a net (64-μm mesh size) behind a boat for *c.* 10 min (Zeng *et al.*, 2013), while macrozoobenthos (composed mostly of molluscs, oligochaetes and chironomids) were collected with a Peterson grab. Both zooplankton and macrozoobenthos were kept alive for 24 h in the laboratory to allow evacuation of gut contents. Fish were caught with a dip net and crustaceans were collected with a small fish cage. All the sampled macrozoobenthos, crustaceans and fish were identified, enumerated, measured (total length) and weighed. A total of 21 fish species were collected and these fish were assigned to different feeding ecology (e.g. planktivorous fish *A. nobilis*, herbivorous fish *Xenocypris microlepis* and piscivorous fish *S. chuatsi* and *O. potamophila*) based on information in the literature (Li, 1999; Li *et al.*, 2009; Zhou *et al.*, 2009a,b).

We took muscle tissue samples only from the adult crustaceans and fish, since it is less variable in $\delta^{13}C$ and $\delta^{15}N$ than other tissue types (Pinnegar & Polunin, 1999), whereas the whole body mass of zooplankton and macrozoobenthos were processed. Moreover, muscle samples were unacidified in this study, because no debris of exoskeletons or any other inorganic carbonates were included in the samples (Stenroth *et al.*, 2006).

Samples of SPOM, SOM, primary producers, invertebrates and fish (n = 2–6) were collected from the ponds for stable-isotope analysis. All samples were then dried to a constant weight for at least 48 h at 60 °C and crushed to a fine powder using a mortar and pestle.

Stable-isotope analysis and trophic levels

Carbon and nitrogen isotope ratios were determined at Nanjing Institute of Geography and Limnology using a Flash EA CN elemental analyser coupled to a Thermo Finnigan Delta Plus mass spectrometer. Stable isotope ratios were expressed in conventional δ notation as parts per mille (‰) according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standare}} - 1] \times 1000 \tag{1}$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. R standard values were based on Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen. Precision of the isotopic analysis was

0.1‰ for both carbon and nitrogen. Elemental results were expressed as a percentage of the considered element (C or N) relative to the total dry weight.

As δ^{15}N values provide an indication of the trophic position of a consumer (Minagawa & Wada, 1984), the following formula was used for the calculation of trophic levels (TL):

$$\mathrm{TL} = \left(\delta^{15}\mathrm{N}_{\mathrm{consumer}} - \delta^{15}\mathrm{N}_{\mathrm{primary\ consumer}}\right)/2.2 + 2 \qquad (2)$$

where TL is the trophic level of the consumer, $\delta^{15}\mathrm{N}_{\mathrm{consumer}}$ is the δ^{15} value of the consumer, $\delta^{15}\mathrm{N}_{\mathrm{primary\ consumer}}$ (8.2 ± 0.4‰, n = 4) is calculated as the mean δ^{15}N of zooplankton collected at each sampling pond. The assumed enrichment in δ^{15}N between successive TL is 2.2, which has been identified as an average trophic nitrogen enrichment for aquatic consumers (McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003).

Isotope mixing model

The SIAR (Stable Isotope Analysis in R) package (Parnell *et al.*, 2010) in R 3.0.3 was used to assess relative contributions from different food items to fish diet. Trophic enrichment factors with measures of variability derived for invertebrates and fish from the literature were used in the models. δ^{13}C enrichment values for consumers analysed in whole (i.e. zooplankton, chironomids and annelids) was 0.3 ± 0.2‰ and for consumers analysed as muscle tissue was 1.3 ± 0.3‰, while δ^{15}N for consumers was 2.2 ± 1.6‰ (McCutchan *et al.*, 2003; Vanderklift & Ponsard, 2003). We are aware that the trophic enrichment levels can deviate from the values we used, but it was beyond the scope of this study to experimentally determine the precise trophic enrichment factors for every taxon surveyed.

Statistical analyses

Statistical analyses were conducted with the programs SPSS 19.0 (SPSS Inc., Chicago). Kruskal–Wallis tests were used to test for differences in δ^{13}C and δ^{15}N composition among the basal food sources, invertebrates, fish and different fish feeding types because of unequal sample sizes. In the cases that Kruskal–Wallis tests indicated significant differences among variables, the Mann–Whitney U test using Bonferroni correction to adjust the probability was applied for multiple comparisons. Since fish species seemed to form different groups based on their trophic categories, a cluster analysis (Euclidian distance, average grouping methods) was performed on fish δ^{13}C and δ^{15}N values.

Results

Stable-isotope composition of basal resources and consumers

Organic matter, submerged macrophytes and epiphytes were considered as primary organic carbon sources. Their mean δ^{13}C and δ^{15}N values are listed in Table 1. Basal sources were distinguishable based on their δ^{13}C values, ranging from −24.3 to −20.5‰ (Fig. 1). The δ^{15}N of basal sources were more comparable, and varied from 5.7‰ (SPOM) to 7.0‰ (epiphytes) (Fig. 1). Significant differences were observed among potential carbon resources for δ^{13}C (Kruskal–Wallis, H = 16.1, d.f. = 4, P = 0.003) and δ^{15}N (H = 11.8, d.f. = 4, P = 0.019), which allowed for designating their role as trophic base for the associated fauna. Epiphytes were the most ^{13}C-enriched sources in the study area, while SPOM showed the most ^{13}C-depleted compositions (Mann–Whitney, P < 0.05 after Bonferroni correction) (Table 1). δ^{13}C values of submerged macrophytes *E. nuttallii* and *H. verticillata* and SOM were intermediate between those of epiphytes and SPOM.

δ^{13}C and δ^{15}N values of consumers (10 invertebrates and 21 fish species) are presented in Table 1 and plotted together in Fig. 1. Animals were well separated in δ^{13}C, with invertebrates ranging from −24.0 to −20.0‰, and fish ranging from −24.4 to −18.9‰. The herbivorous fish *Rhodeus sinensis* was the most ^{13}C-enriched consumer in the area, while the planktivorous fish *A. nobilis* showed the most ^{13}C-depleted compositions. δ^{15}N values of consumers also displayed a wide range, with invertebrates ranging from 7.6 to 9.5‰, and fish ranging from 8.8 to 12.1‰. The predatory fish *S. chuatsi* was the most ^{15}N-enriched consumer, while the benthic annelid *Limnodrilus hoffmeisteri* (Tubificidae) showed the most ^{15}N-depleted composition. On the whole, fish had higher δ^{15}N than invertebrates and species of lower trophic levels had lower δ^{15}N than species of higher trophic levels, generally in accordance with their presumed diet (based on literature data) (Fig. 1). Significant differences were observed among 10 invertebrate consumers for δ^{13}C (Kruskal–Wallis, H = 40.2, d.f. = 9, P < 0.001) and δ^{15}N (H = 30.9, d.f. = 9, P < 0.001), and also among 21 fish consumers for δ^{13}C (H = 83.2, d.f. = 20, P < 0.001) and δ^{15}N (H = 81.9, d.f. = 20, P < 0.001) (Table 1).

Table 1 Size ranges and stable-isotope compositions ($\delta^{13}C$ and $\delta^{15}N$, mean ± SD) for basal sources and consumers in polyculture ponds around Lake Gucheng (*n*: number of samples analysed; C/N: the atomic ratios (w : w); TL: trophic level; SPOM: suspended particulate organic matter; SOM: sediment organic matter). Significant differences (Bonferroni-adjusted *P* values were used, $P < 0.05$) in $\delta^{13}C$ and $\delta^{15}N$ are indicated by different letters among five basal sources (normal), 10 invertebrate consumers (italic) and 21 fish consumers (bold).

Species	Size (cm)	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N	TL
Basal sources						
SPOM		4	$-24.3 \pm 0.6^{\mathrm{a}}$	5.7 ± 0.4	5.47	0.84
Elodea nuttallii		4	$-22.5 \pm 0.6^{\mathrm{ab}}$	6.8 ± 0.3	9.37	1.34
Hydrilla verticillata		4	$-21.4 \pm 0.8^{\mathrm{ab}}$	6.2 ± 0.4	9.54	1.07
Epiphytes		4	$-20.5 \pm 0.4^{\mathrm{b}}$	7.0 ± 0.7	9.10	1.44
SOM		4	$-22.6 \pm 0.6^{\mathrm{ab}}$	6.0 ± 0.3	5.88	0.99
Invertebrates						
Zooplankton		4	-24.0 ± 0.5^{a}	8.2 ± 0.4	6.72	2.00
Chironomids		4	-23.4 ± 0.7^{ab}	8.2 ± 0.5	4.90	1.98
Molluscs						
Bellamya aeruginosa	2–3	4	-23.5 ± 0.9^{a}	8.5 ± 0.2	4.09	2.13
Radix swinhoei	1–2	4	-23.2 ± 0.4^{ab}	8.5 ± 0.6	3.69	2.12
Alocinma longicornis	2–3	4	-21.6 ± 0.8^{abc}	7.9 ± 0.5	5.29	1.84
Annelids						
Limnodrilus hoffmeisteri	3–6	4	-22.6 ± 0.5^{ab}	7.6 ± 0.5	3.11	1.70
Branchiura sowerbyi	8–12	4	-23.7 ± 0.6^{a}	7.9 ± 0.5	2.59	1.83
Crustaceans						
Macrobrachium nipponense	7–9	6	-20.0 ± 0.4^{c}	9.5 ± 0.5	3.48	2.56
Procambarus clarkii	10–14	6	-20.0 ± 0.4^{c}	7.9 ± 0.5	3.38	1.83
Eriocheir sinensis	6–7	6	-20.9 ± 0.3^{bc}	9.2 ± 0.4	3.68	2.43
Fish						
Planktivore						
Hypophthalmichthys molitrix	28–38	5	−24.0 ± 0.8	9.0 ± 0.3	3.48	2.34
Aristichthys nobilis	32–42	4	−24.4 ± 0.2	9.6 ± 0.5	3.43	2.61
Herbivores						
Xenocypris microlepis	14–19	4	−19.0 ± 0.7	8.8 ± 0.5	3.39	2.25
Rhodeus sinensis	4–6	6	−18.9 ± 0.8	9.4 ± 0.2	3.43	2.51
Rhodeus ocellatus	3–5	3	−19.8 ± 0.4	8.8 ± 0.5	3.49	2.24
Acheilognathus chankaensis	6–9	4	−19.9 ± 0.4	9.0 ± 0.5	3.35	2.33
Omnivores						
Hemiculter leucisculus	12–15	5	−21.1 ± 0.9	9.3 ± 0.2	3.62	2.50
Carassius auratus	18–22	6	−20.7 ± 0.4	9.2 ± 0.5	3.55	2.45
Parabramis pekinensis	12–18	3	−20.9 ± 0.2	9.9 ± 0.5	3.17	2.74
Pseudorasbora parva	9–11	6	−21.9 ± 0.6	9.8 ± 0.2	3.23	2.87
Benthivore						
Sarcocheilichthys nigripinnis	7–10	4	−21.7 ± 0.6	10.2 ± 0.4	3.27	2.91
Misgurnus anguillicaudatus	10–12	2	−22.2 ± 0.5	9.7 ± 0.3	3.65	2.65
Micropercops swinhonis	4–6	6	−23.2 ± 0.3	10.0 ± 0.6	3.25	2.79
Abbottina rivularis	9–11	3	−22.6 ± 0.6	10.5 ± 0.2	3.34	3.03
Piscivores						
Siniperca chuatsi	29–32	6	−19.8 ± 0.4	12.1 ± 0.6	3.72	3.76
Culter alburnus	22–27	6	−21.3 ± 0.5	11.8 ± 0.7	3.53	3.63
Cultrichthys erythropterus	10–16	6	−21.1 ± 0.8	11.2 ± 0.5	3.45	3.35
Odontobutis potamophila	12–16	4	−20.0 ± 0.9	11.7 ± 0.3	3.56	3.56
Pelteobagrus fulvidraco	12–15	3	−22.0 ± 0.2	11.0 ± 0.5	3.15	3.27
Rhinogobius giurinus	6–8	6	−20.3 ± 0.2	11.1 ± 0.6	3.15	3.34
Channa argus	20–25	3	−19.7 ± 0.4	11.2 ± 0.3	3.62	3.36

Trophic levels

The TL of invertebrates and fish were characterised by $\delta^{15}N$ values. Using the assumption of a $\delta^{15}N$ trophic enrichment of 2.2‰ per TL, we suggest that this aquatic food web had *c.* 4 TL with invertebrates and herbivorous fish as the intermediate level (Table 1 and Fig. 1). From these calculations, all primary sources were in the first trophic level (TL values from 0.84 to 1.44). Nine invertebrates and six fish species were in the second trophic level of primary consumers (TL values from 1.70 to 2.50). The third trophic level (TL 2.76–3.48)

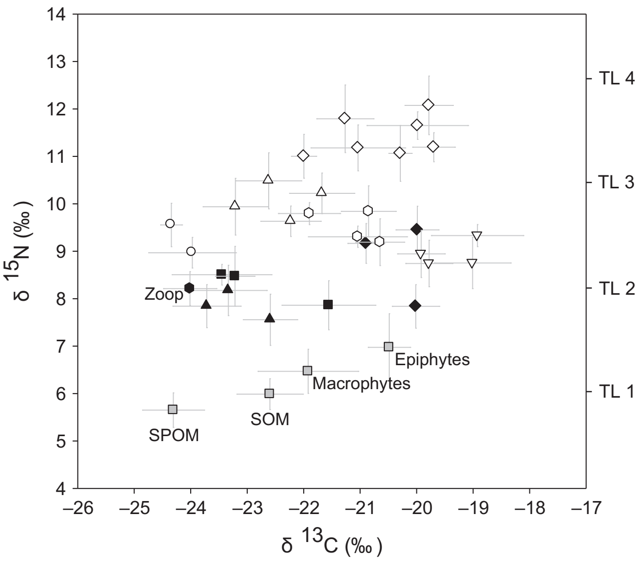

Fig. 1 Stable-isotope compositions (δ^{13}C and δ^{15}N, mean ± SD) for basal sources (grey symbols), invertebrates (black symbols) and fish (white symbols) in polyculture ponds around Lake Gucheng. (Invertebrates: ⬢ – zooplankton; ■ – molluscs; ◆ – crustaceans; ▲ – annelids and chironomids. Fish: ○ – planktivores; ▽ – herbivores; ⬡ – omnivores; △ – benthivores; ◇ – piscivores. Zoop: zooplankton; SPOM: suspended particulate organic matter; SOM: sediment organic matter).

contained one invertebrates and 12 fish species. The species that approached a fourth trophic level were piscivorous fish that consumed variable amounts of crustaceans and small fish: *S. chuatsi*, *C. alburnus* and *O. potamophila* (TL 3.56–3.76). However, a trend of higher δ^{15}N enrichment with a higher trophic position can nevertheless be seen.

A cluster analysis based on δ^{13}C and δ^{15}N values of the 21 fish species suggested the identification of three major groups (Fig. 2). Group I was the most heterogeneous since it included planktivores and benthivores as well as one omnivorous fish. Group II was mainly composed of herbivores and omnivores and Group III included mostly piscivorous fish. The clustering results were in good agreement with postulated trophic groups. All fish feeding types showed wide ranges of δ^{15}N values covering about 1.3‰ in planktivores and up to 2.5‰ in piscivores (Fig. 3). Planktivorous and herbivorous fish had relatively lower δ^{15}N values (mean = 9.2 ± 0.5‰ and mean = 9.0 ± 0.5‰ respectively), while piscivorous fish displayed higher levels (mean = 11.5 ± 0.7‰). Significant differences were observed among all fish feeding types for δ^{13}C (Kruskal–Wallis, $H = 66.1$, d.f. = 4, $P < 0.001$) and δ^{15}N ($H = 74.1$, d.f. = 4, $P < 0.001$) (Table 1). And significantly higher δ^{15}N values of benthivorous and piscivorous fish (Mann–Whitney, $P < 0.05$ after Bonferroni correction) were recorded as compared to other fish feeding types.

Contributions of the basal resources

Results from the SIAR model indicated that epiphytes were identified as the most important carbon sources for crustaceans (a range of contributions 52–65%), herbivorous fish (40–78%), omnivorous fish (31–43%) and piscivorous fish (43–69%), while SPOM made up the highest contribution to zooplankton (25–44%), chironomids (28–47%), molluscs (33–47%), annelids (38–54%), planktivorous fish (20–43%) and benthivorous fish (40–56%) (Table 2). It is worth noting that macrophytes and SOM also made a substantial contribution for consumers in the study region, with macrophytes ranging 17.6–30.8% and SOM ranging 10.9–27.1% according to different consumer groups (Table 2).

Food web

Based on the evidence of trophic relationships suggested by stable isotope results, a general model for the macrophyte-dominated polyculture pond food web was constructed, in which the main trophic chains transporting energy from primary producers were represented (Fig. 4). Relative contributions of the potential prey items determined by a mixing model were also reported as the arrows of different thicknesses in Fig. 4. This trophic web likely contained four main trophic chains transporting carbon source through three or four trophic steps. In this food web, the most ^{13}C depleted trophic pathway seemed to be based on SPOM (mostly of phytoplankton), and zooplankton consumed SPOM at the TL 2, while at the upper trophic levels were planktivorous fish species (close to TL 3). The second trophic pathway obtained carbon sources from SPOM and SOM. The benthic organic matter transferred at least four trophic steps: SPOM and SOM at the base, macrozoobenthos (e.g. molluscs and annelids) which consumed detritus and/or suspended particles at the TL 2, while at the upper trophic levels some benthivorous fish were found which mainly fed on benthic prey. In turn, some of these benthivorous fish might be captured by top piscivores *S. chuatsi* and *O. potamophila* (close to TL 4). The third trophic chain had all four primary resources at its base. The carbon of SPOM and SOM was transported to benthic invertebrates (TL 2), which were in turn consumed by omnivorous fish such as *Carassius auratus* (Cyprinidae) and *Parabramis pekinensis* (Cyprinidae), while another pathway transported

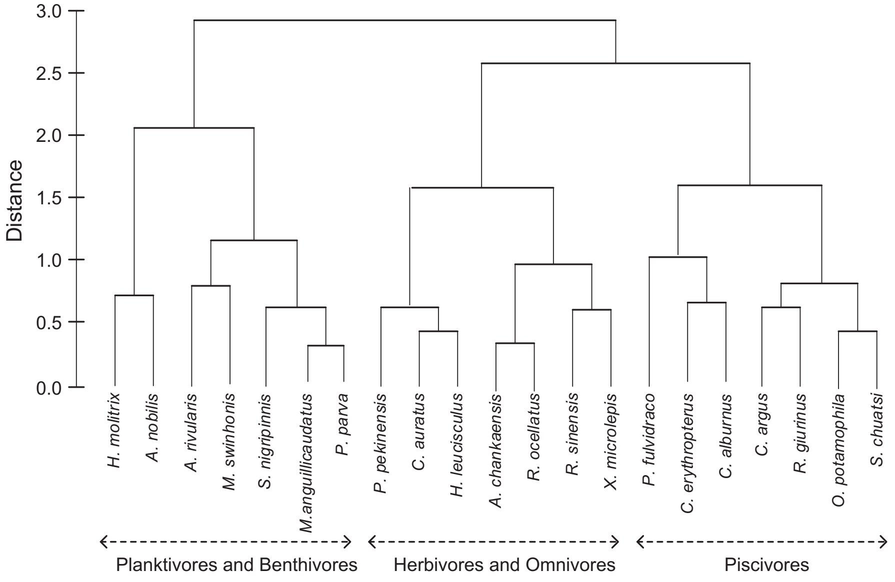

Fig. 2 Dendrogram from cluster analysis (Euclidian distance) for δ^{13}C and δ^{15}N values for 21 fish species from polyculture ponds.

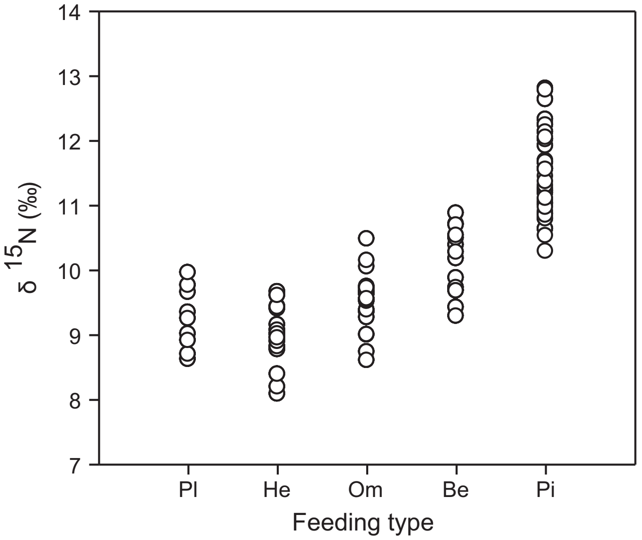

Fig. 3 Distribution of δ^{15}N values among different feeding types of fish species from polyculture ponds. Pl: planktivores, mean = 9.2‰ ± 0.5; He: herbivores, mean = 9.0‰ ± 0.5; Om: omnivores, mean = 9.5‰ ± 0.5; Be: benthivores, mean = 10.1‰ ± 0.5; Pi: piscivores, mean = 11.5‰ ± 0.7.

carbon from epiphytes and macrophytes directly to omnivorous fish. In turn, these omnivores contributed to the diet of large piscivorous fish (close to TL 4). The last trophic pathway was mainly based on a combination of submerged macrophytes and epiphytes, which was incorporated directly by herbivorous shrimps and fishes (e.g. *X. microlepis* and the shrimp *M. nipponense*, TL 2), that were in turn consumed by predatory fishes (close to TL 4).

Discussion

Discrimination and composition of primary producers

Vegetated aquatic systems are complex, heterogeneous environments with a variety of sources of primary production available for exploitation (Jeppesen, Sondergaard & Christoffersen, 1998). Within the polyculture ponds around Lake Gucheng, there are four potential carbon sources for the primary consumers: SPOM, SOM, submerged macrophytes and epiphytes. The isotopic compositions of these basal resources showed a wide variation in the δ^{13}C content. SPOM δ^{13}C was the most depleted and could be mainly composed of phytoplankton cells, plants detritus and other organic material (Gu, 2009). Indeed, microscopic observation of suspended bulk showed that the SPOM was composed mainly of Cryptomonas and diatoms (standing crop, 388–471 cell mL^{-1}) during the course of sampling (unpubl. data, Mao *et al.*). In addition, the low C:N atom ratios of SPOM (5.5) may highlight the contribution of phytoplankton to the suspended particles rather than plants detritus (Vizzini *et al.*, 2002) (Table 1). The SOM δ^{13}C value was intermediate between those of the macrophytes and SPOM, suggesting that these primary sources contributed significantly in SOM composition. However, several stable isotopic and fatty acid studies have confirmed that benthic algae also largely contribute to SOM composition. SOM was thus mainly constituted by benthic algae, with a significant contribution of macrophytes and SPOM (Doi *et al.*, 2006; Lebreton *et al.*, 2011). Macrophytes and the associated epiphytes had the most enriched δ^{13}C and δ^{15}N values. Macrophytes could make an important contribution to the organic carbon pool in freshwater systems, but may not be a direct food sources for animals (Keough, Sierszen & Hagley, 1996). Past research suggests that epiphytes on submerged plants (mostly filamentous chlorophytes and diatoms) may be important carbon sources in these communities (Jones & Waldron, 2003; Rodusky & Anderson, 2013).

Table 2 SIAR model estimates of mean percent of basal sources' relative contributions to invertebrates and different feeding types of fish species collected in polyculture ponds (*n*: number of samples of each consumer group; values in parentheses are minimum and maximum values; basal sources with highest contribution to consumers are indicated in bold). SPOM, suspended particulate organic matter; SOM, sedimentary organic matter.

Sources	Zooplankton (*n* = 4)	Chironomids (*n* = 4)	Molluscs (*n* = 12)	Annelids (*n* = 8)	Crustaceans (*n* = 18)	Planktivores (*n* = 9)	Herbivores (*n* = 17)	Omnivores (*n* = 20)	Benthivores (*n* = 15)	Piscivores (*n* = 34)
SPOM	**37.3 (25–44)**	**39.4 (28–47)**	**41.2 (33–47)**	**46.9 (38–54)**	4.4 (0–6)	**38.2 (20–43)**	4.5 (0–6)	14.3 (8–19)	**48.4 (40–56)**	6.7 (0–10)
SOM	25.0 (17–36)	25.8 (17–36)	26.6 (20–36)	27.1 (19–39)	11.9 (0–18)	21.4 (7–29)	12.1 (0–18)	18.0 (2–19)	21.3 (0–33)	10.9 (0–16)
Macrophytes	21.6 (12–31)	20.9 (6–28)	20.9 (12–29)	17.6 (3–19)	26.0 (17–37)	22.0 (2–33)	23.3 (7–38)	30.8 (23–41)	20.2 (2–24)	26.2 (4–42)
Epiphytes	16.0 (0–24)	13.9 (0–21)	11.3 (0–17)	8.4 (0–12)	**57.7 (52–65)**	18.4 (0–22)	**60.0 (40–78)**	**36.9 (31–43)**	10.0 (0–15)	**56.2 (43–69)**

Basal resources supporting consumers: importance of epiphytes

Our stable-isotope analysis strongly supported the assumption of a food web based mainly on epiphytes and SPOM and to a lesser degree on macrophytes and SOM in the polyculture ponds. Stable isotope studies are increasingly being used for identifying the relative contributions of different sources of primary production to higher trophic levels in a multitude of aquatic ecosystems. Nevertheless, it was possible to separate the different carbon sources of primary production in our study ponds using stable-isotope analysis, with separation being most pronounced in δ^{13}C.

SPOM (mostly of phytoplankton) was the most important contributor to the carbon source of the most invertebrate species in our study. Zooplankton was depleted in δ^{13}C compared to other invertebrates and showed great similarity to the SPOM compositions, suggesting the exploitation of pelagic resources in the water column by copepods and cladocerans (mean 37.3% SPOM-derived carbon). Chironomids, molluscs and annelids had a similar mean δ^{13}C situated between the SPOM and SOM carbon compositions and results from the mixing model also emphasised the importance of SPOM for these macrozoobenthos species. By contrast, the high-value polyculture crustaceans (e.g. Chinese mitten crab and *M. nipponensis*) were the most enriched invertebrate species collected, with δ^{13}C values relatively close to epiphytes, suggesting a substantial reliance on epiphytes as a carbon source (mean 57.7% epiphytes-derived carbon). The main food sources of these decapod crustaceans in the polyculture ponds have also been discussed in detail by Mao *et al.* (2016). Their results showed that forage fish and macrozoobenthos were also an important for the crustaceans.

In the fish community, the feeding strategies of different species are important factors in the complex relationships that emerge from stable-isotope analysis (Davenport & Bax, 2002). SPOM was the most important contributors to the nutrition of the planktivorous fish (*A. nobilis* and *H. molitrix*) in our study, and this result

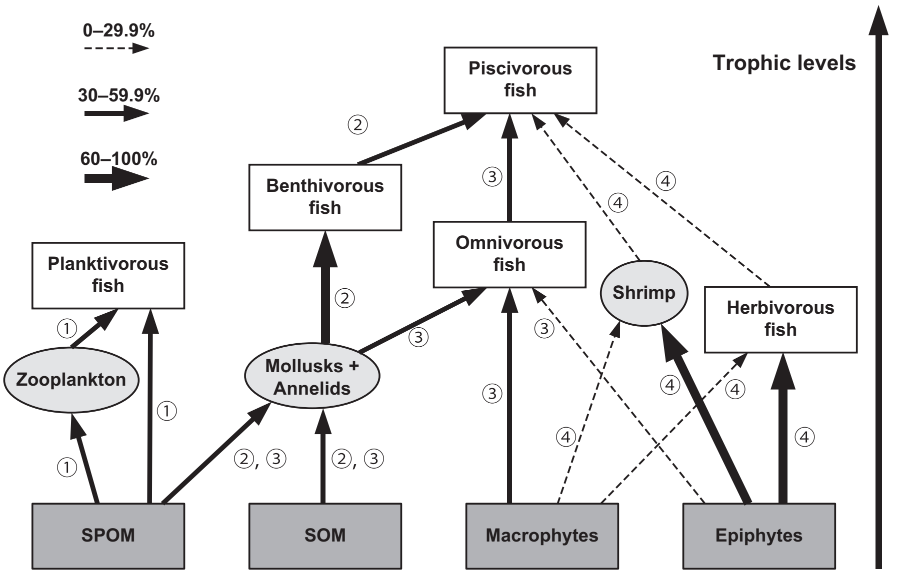

Fig. 4 Food-web structure in polyculture ponds as revealed by $\delta^{13}C$ and $\delta^{15}N$ values and SIAR model estimates. Arrows point from prey to consumer. Darker arrows suggest stronger trophic links, dashed arrows suggest weaker links and light arrows indicate links of intermediate importance. Associated percentages above arrows indicate a range of contributions of prey to consumers. The four main food chains are indicated: ① SPOM based; ② SPOM and SOM based; ③ SPOM, SOM, macrophytes and epiphytes based and ④ macrophytes and epiphytes based.

is in good accordance with a previously reported feeding ecology of these two fish species by Zhou *et al.* (2009a,b). Stable isotope values also indicated that the benthivorous fish mainly depend on carbon of phytoplankton, SOM, submerged macrophytes and epiphytes in about equal proportions. However, the contribution of phytoplankton carbon to higher trophic levels was assumed to be of minor importance in these plant-dominated ponds, while the planktivorous and benthivorous fish in this system contributed only a small fraction to total consumer biomass. In contrast, herbivorous and omnivorous fish had $\delta^{13}C$ values closest to those of epiphytes and macrophytes. Traditionally, omnivorous fish are considered generalist feeders, although preferring aquatic invertebrates, but may take macrophytes and the associated epiphytes when more nutritious food is scarce (Jaschinski *et al.*, 2011). Piscivorous fish such as topmouth culter and mandarin fish were the top predators in this macrophyte-dominated polyculture system and had the highest $\delta^{15}N$ values. These piscivores consume a mixture of prey items, including benthic crustaceans, copepods and small fish, which may explain their high trophic positions (Mao *et al.*, 2014). Taking into account the high biomass of fish prey, shrimps and small fish are likely to be crucial links between primary production and higher trophic levels in plant-dominated systems. They are consumed by both the adjacent and higher levels and appear as important processors of organic matter. This would suggest a ‘wasp-waist’ control of the pond food web: that is, control by the species that predominate quantitatively (Cury *et al.*, 2000).

Ultimately, our results indicated that epiphytes were the most important basal resources for the main cultured species (i.e. Chinese mitten crab, freshwater shrimp and high-value piscivorous fish) in the aquaculture ponds. In fact, the importance of seagrass, mangroves or freshwater macrophytes versus epiphytic algae is still under debate (Abrantes & Sheaves, 2009; Jaschinski *et al.*, 2011). However, previous studies have found that epiphytes, rather than macrophytes, are a more important food invertebrates and fishes (Moncreiff & Sullivan, 2001; Jaschinski *et al.*, 2011). Epiphytes usually contain more nitrogen and phosphorus in relation to carbon compared to macrophytes, and lack the toxins of feeding deterrents present in many macrophytes (Harrison, 1982; Jaschinski, Brepohl & Sommer, 2008). Furthermore, previous studies show that epiphytic algae had higher primary production rates than macrophytes, and they could make equal contributions to annual system carbon production (Thom, 1990; Jaschinski & Sommer, 2008). Therefore, submerged vascular plants were supposed to be primarily important as substratum for epiphytes and a refuge from predation, while food is supplied indirectly by providing space for attached epiphytes.

Food-web structure of the macrophyte-dominated polyculture pond

Although only 10 invertebrate and 21 fish species were analysed and a relatively small number of individuals per species were used, these species contributed most to the total biomass in the area and they were distributed throughout each trophic group. Using the assumption of a δ^{15}N trophic enrichment of 2.2‰ per TL, we suggest that the food web of this polyculture system is composed of 3.76 TL, which is slightly lower comparing to the reported estimation by Feng *et al.* (2014) for a commercial marine polyculture pond in Jinghai Bay (4.14) but similar to the results of Guo *et al.* (2014) for the sea ponds in Donggang, Liaotung Peninsula (3.54). Overall, there was a good agreement between the postulated TL of most consumers identified based on the literature and those derived from δ^{15}N measurements (Li, 1999; Li *et al.*, 2009; Zhou *et al.*, 2009a,b).

The detailed analyses of this food web illustrated the high level of complexity of polyculture pond food webs, and a diversity of major trophic pathways seems to be a characteristic of macrophyte-dominated aquatic ecosystems (Fig. 4). Over a range of nutrient concentrations, the communities of aquatic systems can be dominated either by aquatic plants and have clear water, or by phytoplankton and have turbid water (Canfield *et al.*, 1984). In contrast to algae-dominated ponds, ponds dominated by macrophytes are species-rich with complex structure and food webs. This pattern could be explained by more variety in their carbon sources and biotic interactions (Jeppesen *et al.*, 1998). As a consequence, these factors make the analysis of stable-isotope composition of polyculture pond organisms complex and difficult to interpret.

However, in the present study, details of carbon sources and trophic pathways could be obtained from the analysis of polyculture pond food web. Firstly, this was in great part possible due to the presence of basal sources with distinct δ^{13}C values such as SPOM and epiphytes in close proximity. Moreover, a cluster analysis was performed on fish δ^{13}C and δ^{15}N values and the cluster results were consistent with the ecological information of different fish feeding types observed. The mixing model of Parnell *et al.* (2010) was also used to determine the extent to which primary producers supported the diets of different biotic trophic groups. Finally, it is important to note that background knowledge on diet and behaviour of the different components of the food web is necessary to avoid erroneous conclusions. For example, large planktivorous fish were not considered as a potential food for piscivorous fishes.

To our knowledge, there are few isotopic data exist on the food-web structure within freshwater polyculture ponds, especially macrophyte-dominated systems. Therefore, this study is an important supplement to the food-web research of freshwater ecosystems. The isotopic analyses of carbon and nitrogen presented here suggest the trophic importance of epiphytes that grow on submerged macrophytes, a key component in these polyculture ponds, for the main cultured species. In addition, the feeding habits of the consumers deduced from the isotopic analysis are in agreement with those concerning the diets of the well-studied species. Our study also revealed that carbon fixed by primary producers could be transported up to piscivorous fish through four main trophic pathways. Therefore, this type of freshwater culture ecosystem efficiently utilises the potential range of natural food resources within polyculture ponds.

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