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Feeding ecology of *Limnoperna fortunei* in southern China: insights from stable isotopes and fatty-acid biomarkers

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ABSTRACT

Limnoperna fortunei (*L. fortunei*) is one of the most abundant freshwater bivalves in southeast Asia, with wide-ranging direct and indirect impacts on ecosystems. To estimate material flows in the habitats of *L. fortunei*, a combination of stable-isotope and fatty-acid analyses were applied to assess the feeding spectrum of *L. fortunei* in southern China. Using the isotope-mixing model, the contribution proportions to the diet of *L. fortunei* were estimated as 19.8%–28.2% for plankton, 57.6%–65.2% for particulate organic matter (POM) and 10.2%–21.1% for sediment organic matter. We conclude that POM is the principal food source of *L. fortunei*. The $\delta^{13}C$ enrichment of fixed carbon from POM to *L. fortunei* was 0.67%–2.41%. Based on the fatty acid data, it was estimated that *L. fortunei* consumed or selectively accumulated Chlorophyceae, Cryptophyceae, Dinophyceae, bacteria and terrestrial organic matter. The feeding spectrum of *L. fortunei* is similar to that of *Dreissena polymorpha*. We suggest that *L. fortunei* is able to differentiate suitable food items using chemical cues and the surface properties of particles.

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Limnoperna fortunei; feeding ecology; stable isotope; fatty acid

Introduction

The bivalve mussel, *Limnoperna fortune (L.fortunei)*, is a benthic suspension feeder with a widespread distribution in fresh waters of Southeast Asia and South America (Paolucci et al. 2010; Zhang et al. 2014; Zhang et al. 2015). Adults of *L.fortunei* are able to firmly attach to hard substrata using the byssus (Nishino 2012). The density of *L.fortunei* is extremely high, reaching 10,000 individuals m^{-2} near the bank of the Xizhijiang River, southern China (Xu et al. 2009). With individual filtration rates up to 350 mL h^{-1}, *L.fortunei* has a marked effect on suspended and sediment organic matter (SSOM), which modified the nutrient supply (Boltovskoy et al. 2009; Di Fiori et al. 2012). Therefore, *L.fortunei* provides an important link between SSOM and consumers in fresh water. Consequently, to understand the energy and material flows in the habitats of *L.fortunei*, it is necessary to clarify the composition of diet of this species.

Physical methods to determine the diet of bivalves include analysis of stomach contents and faecal pellets (Lehane and Davenport 2004). Stomach-contents analysis of *L.fortunei* living in the Middle Paraná River indicated that the main food ingested comprised plankton, particularly Euglenophyta, Rotifera, Chydoridae and Bosminidae (Molina et al. 2010). However, this method

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did not identify which dietary items were assimilated and can provide only rough estimates of recent feeding activity (seconds to hours) (Kang et al. 1999). More recently, biochemical methods, such as the analysis of stable isotopes or fatty acids, have been used with considerable success in determining the diet of bivalves (Kürten et al. 2013; Najdek et al. 2013; Braeckman et al. 2015). Stable-isotope analysis is a useful approach for identifying the food sources assimilated by bivalve mussels (Vaughn and Hakenkamp 2001). Consumers typically contain more heavy isotopes than is present in their diets, with stepwise heavy-isotope enrichment occurring at each trophic level (Peterson and Fry 1987). The ratio of $^{13}C/^{12}C$ ($\delta^{13}C$) is preferentially used as a dietary tracer because there is little fractionation (ca. 1.0%) between the consumer and its food sources (Asante et al. 2010; Sun et al. 2012). In contrast, the method of using fatty acids as dietary tracers in aquatic ecosystems relies on the observation that different primary producers (e.g. diatoms, dinoflagellates, bacteria) generally have specific fatty-acid profiles in their biomass. Furthermore, these fatty acid profiles tend to be transferred conservatively and thus are present in consumers' tissues in an unchanged form (Shin et al. 2008). Therefore, it is possible to distinguish dietary and non-dietary compounds in the body of a consumer via fatty-acid analysis. These new approaches are therefore able to improve the accuracy of information on the diet of *L.fortunei*. Such information is essential for understanding energy and material flows in the habitats in which it occurs.

Bivalves are considered to be herbivores and it is assumed that plankton, particulate organic matter (POM) and sediment organic matter (SOM) are the main components of their diet (Molina et al. 2010; Zhao et al. 2013). In this work, we used a combination of stable-isotope and fatty-acid analyses to evaluate the contribution of plankton, POM and SOM to the diet of *L.fortunei*. Using these data, we assessed the feeding spectrum of *L.fortunei* in the Xijiang River, southern China.

Materials and methods

Sample collection and preparation

Triplicate samples of the plankton, POM and SOM were collected monthly between March and November 2013. The sampling site (23°08′12″ N, 112°48′7″ E) (Figure 1) is locate at the Xijiang River

Figure 1. Sampling site (23°08′12″ N, 112°48′7″ E) locating at the Xijiang River is about 140 kilometres from seaport. The Xijiang River cover a full distance of over 2214 kilometres.

(south China), and its riverbed is rock based. The water quality of sampling site belong to Class I of the Surface water Environmental Quality Standard (2002). Samples of plankton were collected with a net of 60 μm mesh by repeated multiple horizontal short duration tows (5–10 min) carried out between 2–8 m depth. About 30 L of fresh water were collected and stored in acid-cleaned polyethylene bottles to obtain samples of POM. SOM samples were collected at depths of 8–10 m via the sediment corer (300 mm in diameter, PC-300, Mooring Systems Inc., Cataumet, MA). Adult *L.fortunei* ($\geq$22 mm) were collected from the riverbed at same site using SCUBA at depths of 2–10 m. All samples were immediately stored at 4 °C before being transported to the laboratory.

In the laboratory, samples of plankton were freeze-dried, ground into powder using a pestle and mortar, and stored in acid-cleaned polyethylene bags at −80 °C. For samples of POM, water samples were pre-sieved through a 200 μm mesh net to remove large particles and then filtered using Whatman GF/F glass fibre (pre-combusted at 550 °C for 5 h). The filter papers were rinsed with ultrapure water, freeze-dried and stored in acid-cleaned polyethylene bags at −80 °C. Samples of SOM were freeze-dried, sieved through a 300 μm stainless steel screen, homogenised and stored in acid-cleaned polyethylene bags at −80 °C. After depuration in filtered water for 24 h, samples of *L.fortunei* tissues were dissected with a plastic knife and rinsed with ultrapure water.

For each analysis, 10 individuals of *L.fortunei* were pooled, freeze-dried, homogenised, and stored in acid-cleaned polyethylene bags at −80 °C.

Stable-isotope analyses

For stable-isotope measurements, plankton, POM and SOM samples were acidified with 10% HCl, rinsed with distilled water and oven-dried at 40 °C for 24 h, to remove carbonates (Deniro and Epstein 1978). When production of CO_2 bubbles ceased, the samples were dried and stored in acid-cleaned polyethylene bags. For analysis, about 1 mg of the powdered sample was packed into a 4 × 6-mm tin capsule.

Samples were combusted in an elemental analyser (Vario MICRO cube, Elementar Analysensysteme GmbH, Lagensebold, Germany) attached to an isotope-ratio mass spectrometer (MAT 253, Thermo Fisher Scientific, Waltham, MA) to determine $^{13}C/^{12}C$. The value of $\delta^{13}C$ was expressed as the deviation from a standard in parts per thousand (‰) according to the following equation: $\delta^{13}C = [(R_{sample}/R_{standard})-1] \times 1000$ where R is the corresponding ratio of $^{13}C/^{12}C$. Carbon values were referenced to standard Pee Dee Belemnite (PDB). Measurements were made with a precision of approximately 0.2‰.

Fatty acid analyses

Lipids were extracted from plankton, POM, SOM and *L.fortunei* samples following the method of Folch et al. (1957) and Zhao et al. (2013). Lipids were extracted ultrasonically for 10 min using a solvent mixture (two parts chloroform to one part methanol). The lower chloroform phase containing lipids was collected and separated further by centrifugation. The lipid extracts were saponified, transmethylated, separated and purified to transform fatty acids to fatty-acid methyl esters (FAMEs). FAMEs were analysed using a gas chromatograph (GC-9A; Shimadzu, Tokyo, Japan) on a DB-FFAP capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film). Hydrogen was used as the carrier gas. The injector temperature was 250 °C. FAMEs were identified by comparing their retention times with those of standards.

Data analyses

Statistical analyses were performed using SPSS software (Version 19.0; SPSS, Chicago, IL). Significant differences ($P < 0.05$) in $\delta^{13}C$ were tested using Student's t-test and one-way analysis of

Table 1. Fatty acids as biochemical markers of certain taxonomic groups that occur in the Xijiang River, southern China. SFAs, MUFAs, PUFAs and BrFAs refer to saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids and branched fatty acids, respectively. DHA refers tor C22:6(n-3) and EPA refers to C20:5(n-3).

Group	Fatty acid markers	References
Chlorophyceae	C18:2(n-6); C18:3(n-3); C16 PUFAs n-3 and n-6	Viso and Marty 1993 Napolitano 1999 Petkov and Garcia 2007
Cryptophyceae	Simultaneous occurrence of C18:3(n-3) and C18:4(n-3); C20:5(n-3); C22:6(n-3)	Desvilettes et al. 1997 Dijkman and Kromkamp 2006 Brett et al. 2009 Guevara et al. 2011
Dinophyceae	C18:4(n-3); C22:6(n-3); C16:1(n-7)/C16:0 < 1; DHA/EPA > 1	Napolitano 1999 Bergé and Barnathan 2005 Zhao et al. 2013
Bacillariophyceae	C16 PUFAs n-4; C16:1(n-7)/C16:0 > 1; DHA/EPA < 1	Shin et al. 2000 Dijkman and Kromkamp 2006 Prato et al. 2012 Zhao et al. 2013
Cyanobacteria	C18:3(n-3) or 18:3(n-6)	Gugger et al. 2002
Heterotrophic bacteria	BrFAs 15-17 and C18:1(n-7)	Napolitano 1999 Green and Scow 2000
Decomposed material	C18:0	Hama 1999
Plant detritus particle	C20 and C22 SFAs	Shorland 1963 Napolitano 1999
Copepoda	C20 and C22 MUFAs	Hagen 1993 Kattner et al. 2007
Terrestrial organic matter	$\sum$C18:2(n-6) + C18:3(n-3) > 2.5	Zhao et al. 2013

variance (ANOVA). For fatty acid data, means, standard errors (SE) and Fisher's least significant difference (LSD) post-hoc tests were calculated conventionally.

To evaluate the relative contributions of plankton, POM and SOM to the diet of *L.fortunei*, the isotope-mixing model (Philips 2001) was used with slight modifications. The fractionation of δ^{13}C values for bivalves was set at 0.8 in the model (Fukumori et al. 2008), defined as follows: $\delta^{13}C_{Lf} = f_{pl}(\delta^{13}C_{pl}+0.8)+f_p(\delta^{13}C_p+0.8)+f_s(\delta^{13}C_s+0.8)$ where the subscripts Lf, pl, P and S refer to *L.fortunei*, plankton, POM and SOM, respectively, and $f_{pl,}$ f_p and f_s are the fractional contributions of plankton, POM and SOM, respectively.

Fatty acids that are commonly used as biochemical markers for particular taxonomic groups that occurred in our study are shown in Table 1.

Results

Characteristics of stable carbon isotopes in plankton, POM, SOM and L.fortunei

Mean δ^{13}C values for plankton, POM, SOM and *L.fortunei* tissue sample are shown in Table 2. Significant differences in δ^{13}C values were observed among plankton, POM, SOM and *L.fortunei* samples (one-way ANOVA, $P < 0.05$). The δ^{13}C values varied between −23.38‰ and −20.68‰ for plankton, between −23.52‰ and −20.99‰ for POM, and between −26.72‰ and −19.30‰ for SOM. The δ^{13}C values for *L.fortunei* ranged from −21.62‰ to −19.91‰.

Contribution of plankton, POM and SOM to the dietary regime of L.fortunei

The relative contributions of plankton, POM and SOM in the diet of *L.fortunei*, calculated using the isotope-mixing model, are presented in Figure 2. The contribution of POM to the carbon content of *L.fortunei* ranged between 57.6% and 65.2%, which was significantly higher than that of plankton (19.8%–28.2%) and SOM (10.2%–21.1%) (*t*-tests, $P < 0.05$).

Table 2. δ^{13}C values (‰) for the plankton, POM, SOM and *L.fortunei* tissues in the Xijiang River between March and November in 2014. POM, particulate organic matter; SOM, sediment organic matter. δ^{13}C values (‰) are means ± SD (n = 3).

	Plankton	POM	SOM	*L.fortunei*
Mar	−21.74(0.40)	−20.99(0.26)	−22.87(0.25)	−19.81(0.06)
Apr	−23.38(0.43)	−22.14(0.51)	−22.65(0.30)	−20.87(0.03)
May	−21.81(0.70)	−21.05(0.25)	−26.17(0.21)	−20.38(0.11)
Jun	−22.86(0.34)	−23.52(0.56)	−26.72(0.20)	−21.62(0.18)
Jul	−20.68(0.71)	−23.97(0.47)	−22.88(0.22)	−21.56(0.23)
Aug	−20.87(0.45)	−23.55(0.60)	−21.92(0.20)	−21.59(0.16)
Sep	−21.46(0.54)	−21.92(0.30)	−19.30(0.23)	−19.83(0.03)
Oct	−22.21(0.44)	−21.75(0.21)	−20.36(0.18)	−19.91(0.24)
Nov	−20.77(0.79)	−21.64(0.28)	−19.63(0.21)	−19.39(0.15)

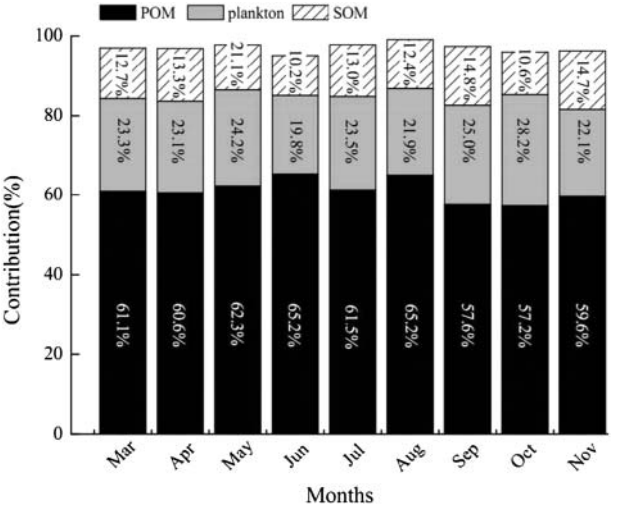

Figure 2. Contributions (%) of plankton, particulate organic matter (POM) and sediment organic matter (SOM) to the diet of *L.fortunei* in the Xijiang River between March and November 2014.

Fatty acid profiles of plankton, POM, SOM and L.fortunei

The proportions of 45 prominent FAs in the samples of plankton, POM, SOM and *L.fortunei* are shown in Table 3. The level of saturated fatty acids (SFAs) was highest in SOM (49.73%), followed by POM, plankton and *L.fortunei*. Among SFAs, C14:0, C16:0 and C18:0 dominated in all samples, although their ratios significantly differed among the samples (one-way ANOVA, $P < 0.05$). Monoenoic acids (MUFA) ranged between 21.85% and 29.99% in the samples and were primarily represented by C16:1(n-7 + n-9), C18:1(n-7), C18:1(n-9), C20:1(n-9) and C22:1(n-9). However, MUFA profiles differed among *L.fortunei* and their food items. *L.fortunei* had highest level of C18:1(n-7) and C20:1(n-9), while plankton, POM and SOM showed significantly higher levels of C16:1(n-7 + n-9) and C22:1(n-9). The levels of polyunsaturated fatty acids (PUFA) varied from 11.23% to 33.93% of the samples. The lowest value for PUFA was found in SOM, while the highest value was in *L.fortunei* tissue. Dominant PUFAs, shown in bold in Table 2, were particularly represented in some taxonomic groups. Branched fatty acids (BrFAs) comprised mostly 15-iso, 16-iso, 16-anteiso and 17-iso and their levels varied from 2.45% to 13.64% of the samples. The levels of 15-iso and 17-iso were significantly higher in SOM than in the other samples (Table 3).

Table 1 lists the fatty acid biomarkers that were used to identify the food sources of *L.fortunei*. Compared to its food items, the *L.fortunei* samples contained higher levels of biomarkers for Cryptophyceae (C18:4(n-3), C20:5(n-3) and C22:6(n-3); Figure 3(b)) and Dinophyceae (C18:4(n-3), C22:6(n-3), C16:1

Table 3. Fatty acid profiles (%) of plankton, POM, SOM and *L.fortunei* in the Xijiang River (mean ± SD, $n = 3$). Values indicate concentration (%), while 'n.d' and 'tr.' mean non-detected and trace, respectively. Fisher's LSD test was used to compare the fatty acids indicated in bold among samples; values labelled with the same letter are not significantly different ($P < 0.05$). POM, particulate organic matter; SOM, sediment organic matter. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; BrFAs, branched fatty acids.

Fatty acid	Plankton	POM	SOM	*L.fortunei*
C14:0	**13.64 (1.08) b**	**5.10 (0.23) c**	**6.53 (0.52) cd**	**2.10 (0.07) hi**
C15:0	0.85 (0.12)	1.15 (0.06)	0.91 (0.34)	0.57 (0.03)
C16:0	**12.38 (0.85) c**	**24.52 (1.46) a**	**22.65 (2.80) a**	**28.92 (1.14) a**
C17:0	0.62 (0.09)	1.91 (0.35)	7.69 (1.03)	1.41 (0.20)
C18:0	**7.96 (1.32) d**	**4.83 (0.71) cd**	**7.86 (1.41) c**	**2.36 (0.35) gh**
C20:0	**2.07 (0.21) hij**	**3.35 (0.93) ef**	**2.72 (0.40) fghi**	**0.32 (0.06) j**
C22:0	0.36 (0.09)	0.80 (0.23)	1.37 (0.51)	n.d.
∑SFAs	**37.88 (2.68)**	**41.66 (2.27)**	**49.73 (3.84)**	**35.68 (1.36)**
C14:1(n-5)	0.45 (0.02)	n.d.	n.d.	n.d.
C16:1(n-5)	0.33 (0.04)	n.d.	n.d.	0.18 (0.02)
C16:1(n-7 + n-9)	**16.88 (0.57) a**	**9.75 (0.36) b**	**10.83 (0.69) b**	**5.73 (0.20) d**
C17:1(n-9)	n.d.	n.d.	n.d.	0.31 (0.06)
C18:1(n-5)	0.15 (0.02)	n.d.	n.d.	0.23 (0.05)
C18:1(n-7)	**1.91 (0.07) ij**	**4.08 (0.25) cde**	**2.75 (0.42) fgh**	**5.69 (1.35) d**
C18:1(n-9)	**1.52 (0.13) jk**	**10.55 (0.62)b**	**2.31 (0.58) ghij**	**5.30 (1.21) de**
C20:1(n-7)	0.18 (0.05)	n.d.	n.d.	0.57 (0.14)
C20:1(n-9)	**0.62 (0.15) k**	**1.74 (0.35)gh**	**0.85 (0.25) jk**	**2.65 (0.37) gh**
C22:1(n-9)	**4.15 (0.62) f**	**3.87 (1.28)de**	**5.11 (0.95) de**	**0.87 (0.26) ij**
C22:1(n-11)	n.d.	n.d.	n.d.	0.39 (0.12)
∑MUFAs	**26.19 (1.75)**	**29.99 (1.86)**	**21.85 (2.16)**	**21.92 (1.25)**
C16:2(n-4)	**1.95 (0.12) ij**	**1.50 (0.37) gh**	**0.60 (0.30) jk**	**0.45 (0.02) j**
C16:3(n-3)	1.36 (0.06)	tr.	tr.	0.81 (0.19)
C16:3(n-4)	**2.20 (0.42) hij**	**1.60 (0.21) gh**	**1.80 (0.42) ghij**	**tr.**
C16:4(n-3)	1.25 (0.12)	0.80 (0.15)	1.05 (0.11)	n.d.
C18:2(n-4)	0.24 (0.05)	n.d.	n.d.	0.19 (0.01)
C18:2(n-6)	**3.03 (0.29) gh**	**1.95 (0.40) gh**	**1.02 (0.58) hijk**	**4.16 (1.43) ef**
C18:3(n-3)	**3.73 (0.09) fg**	**1.68 (0.57) gh**	**0.97 (0.30) ijk**	**1.04 (0.35) ij**
C18:3(n-6)	0.33 (0.05)	n.d.	n.d.	0.25(0.01)
C18:4(n-3)	**1.78 (0.23) ij**	**2.60 (0.25) fg**	**n.d.k**	**3.49 (0.36) fg**
C20:2(n-6)	0.43 (0.02)	0.53 (0.10)	tr.	0.35 (0.03)
C20:2(n-9)	0.27 (0.08)	n.d.	n.d.	0.35 (0.04)
C20:3(n-3)	n.d.	n.d.	n.d.	0.18 (0.03)
C20:3(n-6)	0.15 (0.02)	n.d.	n.d.	0.23 (0.02)
C20:4(n-3)	0.51 (0.03)	n.d.	n.d.	0.77 (0.08)
C20:4(n-6)	**2.01 (0.62) ij**	**0.84 (0.39) h**	**1.58 (0.65) hijk**	**4.65 (1.17) def**
C20:5(n-3)	**5.23 (0.39) e**	**4.72 (0.51) cd**	**3.37 (1.40) fg**	**7.35 (0.76) c**
C22:3(n-9)	n.d.	n.d.	n.d.	0.58 (0.05)
C22:4(n-6)	n.d.	n.d.	n.d.	0.15 (0.02)
C22:5(n-3)	0.28 (0.07)	0.67 (0.31)	n.d.	0.55 (0.12)
C22:5(n-6)	n.d.	n.d.	n.d.	0.21 (0.07)
C22:6(n-3)	**2.68 (0.11) hi**	**1.75 (0.68) gh**	**0.84 (0.15) jk**	**9.43 (0.29) b**
∑PUFAs	**27.43 (1.72)**	**18.64 (2.23)**	**1.23 (0.93)**	**33.93 (2.40)**
14-iso	0.32 (0.06)	n.d.	1.60 (0.45)	0.19 (0.04)
14-antiiso	0.20 (0.08)	n.d.	0.95 (0.40)	0.37 (0.12)
15-iso	0.15 (0.01)	0.55 (0.12)	3.37 (0.55)	0.28 (0.05)
16-iso	0.42 (0.15)	0.36 (0.10)	2.11 (0.30)	1.39 (0.22)
16-antiiso	0.15 (0.01)	0.47 (0.31)	1.38 (0.50)	0.31 (0.06)
17-iso	**1.21 (0.30) jk**	**3.52 (0.20) ef**	**4.23 (0.80) ef**	**0.47 (0.05) j**
∑BrFAs	**2.45 (0.37)**	**4.90 (1.65)**	**13.64 (2.43)**	**3.01 (1.02)**
Total	**93.95 (2.91)**	**95.19 (3.23)**	**96.45 (3.85)**	**94.54 (2.45)**

(n-7)/C16:0 = 0.19, DHA/EPA = 1.28; Figure 3(c)). In contrast, higher level of biomarkers for Chlorophyceae (C18:3(n-3) and C16:4(n-3); Figure 3(a)), Bacillariophyceae (C16:2(n-4), C16:3(n-4), C16:1 (n-7)/C16:0 = 0.19, DHA/EPA = 1.28; Figure 3(d)), cyanobacteria (C18:3(n-3) in Figure 3(e)), decomposed material (C18:0; Table 3) and plant detritus (C20:0 and C22:0; Figure 3(g)) were found in the food items of *L.fortunei*.

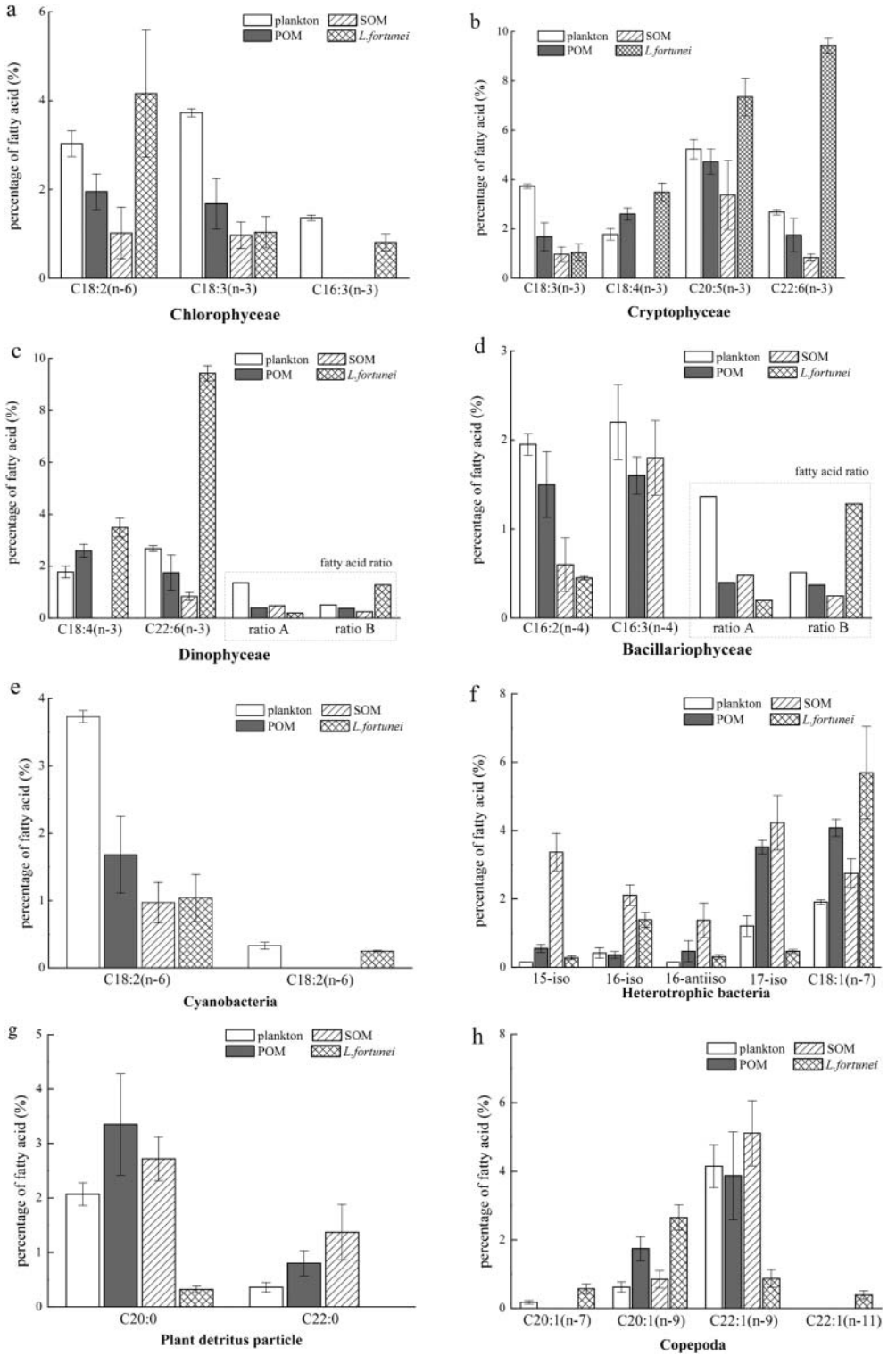

Figure 3. Fatty acid biomarkers in plankton, POM, SOM and in *L.fortunei* tissues in the Xijiang River. a, Chlorophyceae; b, Cryptophyceae; c, Dinophyceae; d, Bacillariophyceae; e, Cyanobacteria; f, heterotrophic bacteria; g, plant detritus particle; h, Copepoda. Column shading: white, plankton; grey, POM; diagonal, SOM; cross-hatch, *L.fortunei,* tissues.

Discussion

In the present study, the $\delta^{13}C$ signatures of plankton, POM and SOM were significantly different. These distinct signatures made it possible to calculate the relative contributions of plankton, POM and SOM to the diet of *L.fortunei* using a mixing model. These calculations indicated that *L.fortunei* primarily feeds on POM, followed by plankton and SOM (Figure 2). Previous studies on the feeding ecology of bivalves indicated that *Pinctada fucata martensii* obtained 78% (Kanaya et al. 2005) and *Ruditapes philippinarum* 61.0% (Fukumori et al. 2008) of their carbon from POM in their natural habitats. Our results are consistent with their conclusion that POM is the principal food source of bivalves. The stable-isotope approach assumes a fixed isotopic enrichment between the bivalve and its food items. *Ruditapes philippinarum*, *Mactra veneriformis* and *Nihonotrypaea japonica* were reported to be enriched by 0.6%–2.0% for $\delta^{13}C$ relative to POM (Yokoyama et al. 2005). Therefore, our observation of the $\delta^{13}C$ enrichment of *L.fortunei* relative to POM (0.67%–2.41%) (Table 1) is consistent with those findings.

However, plankton, POM and SOM are heterogeneous mixtures of phytoplankton, bacteria, benthic microalgae and other OM (Dalsgaard et al. 2003). The isotopic signatures of these sources often overlap in natural conditions, making it difficult to separate specific components (Phillips and Gregg 2003). Primary producers, such as diatoms, dinoflagellates and bacteria, are characterised by distinct fatty-acid profiles (Kharlamenko et al. 2001). Therefore, these profiles can be used to identify the relative contribution of each component to the plankton, POM and SOM mixtures.

According to the fatty acid profiles of *L.fortunei* food items (Figure 3), plankton, POM and SOM differ in the percentages of several fatty acid markers, indicating high levels Chlorophyceae, Cryptophyceae, Bacillariophyceae and cyanobacteria in plankton, high levels of plant detritus in POM, and high levels of heterotrophic bacteria and Copepoda in SOM. Although the dominant taxa in the phytoplankton were Chlorophyceae, Cryptophyceae, Dinophyceae and Bacillariophyceae, fatty acid markers of these algae were not abundant in plankton, and were particularly scarce in POM. The relatively low proportion of algal biomass in the water column is a possible explanation of this result. The higher percentages of SFAs (C18:0, C20:0 and C22:0) and BrFAs in POM and SOM suggest that they contain high proportions of detrital particles derived from plant debris, pseudofaeces, faeces and other molluscan excreta (Makhutova et al. 2011).

The fatty acid compositions of various bivalve taxa are highly variable. As in other freshwater bivalve, e.g. *D. polymorpha* and *D. bugensis* (Makhutova et al. 2011), *L.fortunei* possesses high levels of C20:5(n-3) and C22:6(n-3), which are considered physiologically crucial and probably are conservatively retained in tissues relative to other compounds (Gladyshev et al. 2011; Kelly and Scheibling 2012). In addition, many researchers emphasised the importance of the ratio C22:6(n-3)/C20:4(n-6) for the growth and reproduction of the zoobenthos (Ahlfren et al. 2009). Data collected from the literature (Makhutova et al. 2011) indicated values for the C22:6(n-3)/C20:4(n-6) ratio in *D. polymorpha* and *D. bugensis* of 1.44 and 1.49, respectively, and in *Potamocorbula amurensis* the ratio was ca. 2 (Canuel et al. 1995). In the present study, the ratio in *L. fortunei* was somewhat higher (2.02; Table 3).

Algal fatty acids are used as energetic resources and are catabolised in animal tissues (Brett and Goldman 2006; Gladyshev et al. 2011). The percentage of C18:2(n-6) (Figure 3a), an essential fatty acid synthesised by algae (Maria-José et al. 2010), was higher in *L.fortunei* than in its food items. Likewise, percentages of the essential fatty acid, C18:4(n-3), which was abundant in Cryptophyceae and Dinophyceae (Gugger et al. 2002; Maria-José et al. 2010), was also higher in *L.fortunei* (Table 3). Apparently, *L.fortunei* consumed or selectively accumulated C18:2(n-6) and C18:4(n-3) from certain algae species, i.e. Chlorophyceae, Cryptophyceae and Dinophyceae. In contrast, Bacillariophyceae are abundant in plankton, POM and SOM, but are deficient in *L.fortunei* (Figure 3(d)). Nevertheless, we could not estimate the consumption of Bacillariophyceae by *L.fortunei* by using fatty-acid markers because bivalves are reported to preferentially store fatty acids from Bacillariophyceae as triacylglycerols and to use them for catabolism (Gladyshev et al. 2011). Heterotrophic bacterial fatty acids are reported to be a significant source of organic carbon and nitrogen for some

bivalve molluscs (Nichols and Garling 2000). Bacterial taxa differ markedly in fatty markers (Napolitano 1999), e.g. bacterial i17:0 and 17:0 for sulfate-reducing bacteria, and C18:1(n-7) for cyanobacteria, sulphur-oxidising and aerobic bacteria. Thus, *L.fortunei* in the present paper apparently consumed the latter bacteria, which usually dwell in the water column. Decomposed material, particles of plant detritus and copepods can also be potential food sources for *L.fortunei* but the high percentage of their fatty acid markers in plankton, POM and SOM, and low levels in *L.fortunei* indicated that *L.fortunei* did not prefer these food items. The high level of the biomarkers of terrestrial OM in *L.fortunei* (Table 3) suggest the indirect assimilation of terrestrial OM via heterotrophic bacteria, but could also result from the direct assimilation of terrestrial OM using cellulase and hemicellulase in this species (Mcleod and Wing 2009).

According to the fatty-acid marker analyses, *L.fortunei* preferred planktonic algae and bacteria. This feeding spectrum is similar to that of *D. polymorpha* (Cole and Solomon 2012; Makhutova et al. 2013) and could be explained in terms of active selection, more efficient assimilation of the selected diet from POM and/or preferential ingestion. Although some bivalve species appear to capture their diet indiscriminately (Ward et al. 1997), *L.fortunei* is able to differentiate between suitable and unsuitable particles by gill sorting mechanisms. This selection mechanism appears not to be based on particle size but on chemical cues and their surface properties (Wong and Cheung 1999).

Conclusions

The feeding spectrum of *L.fortunei* in the Xijiang River in summer was described. Using the isotope-mixing model, the relative contributions to the diet of *L.fortunei* were estimated as 19.8%–28.2% for plankton, 57.6%–65.2% for POM and 10.2%–21.1% for SOM. Fatty acid biomarkers specific to Chlorophyceae, Cryptophyceae, Dinophyceae, heterotrophic bacteria and terrestrial OM were identified in the tissues of *L.fortunei*, indicating that there were substantial algal, bacterial and terrestrial inputs into the diet of *L.fortunei*. However, the present study did not include the winter months. The possibility of seasonal variation in the levels of plankton, POM and SOM in the water column should be addressed in future research.

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Disclosure statement

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