Food sources of dominant macrozoobenthos between native and non-native mangrove forests: a comparative study

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Abstract

The macrozoobenthos is an important link of the food web in coastal wetlands. Diet-habitat relationships may significantly depend on qualitative differences and seasonal availability of food sources. Increasing interest has been shown in food web structure altered by non-native plants. In particular, however, a non-native mangrove species from Bangladesh, *Sonneratia apetala*, has been widely planted in China, but little is known about its possible impact on food sources of macrozoobenthos living in these non-native mangrove forests. Therefore, in this study, we used fatty acid analysis to compare the food sources of one littorinid snail and two grapsid crab species between two native mangrove forests and one non-native *S. apetala* plantation in the Zhanjiang Mangrove National Nature Reserve of China. We found that the sediment of all three forests had high diatom and bacteria signals, but low mangrove leaf signals, while the opposite patterns were detected in the three macrozoobenthos. Specifically, the gastropod *Littoraria melanostoma* relied mainly on mangrove leaves and brown algae as food sources, with significant differences among the three mangrove forests, and showed significant seasonal variation in its diet. The grapsidae species (*Perisesarma bidens* and *Parasesarma plicatum*) mainly grazed on mangrove litter, brown and green algae, and occasionally consumed diatoms and bacteria, also showing significant seasonal variation in their diet. Overall, Principle Components Analysis (PCA) of

29 the fatty acid profiles showed a significant overlapping in food sources among the
30 macrozoobenthos living in the non-native and native mangrove forests, but significant
31 seasonal variations in their food sources. This suggests that the planting of non-native *S.*
32 *apetala* near original mangrove forests has had little effect on the feeding behavior of
33 macrozoobenthos some 10 years after planting.

34 **Key words:** Coastal wetland; *Sonneratia apetala*; Macrofauna; Food web; Fatty acids;
35 Invasion

1. Introduction

Mangrove wetlands are regarded as refuge and habitat for benthic fauna, and they sustain high detrital production, which is a major food resource for macrozoobenthos (Odum and Heald, 1972; Nordhaus et al., 2011). Brachyurn crabs and gastropods are important groups of macrozoobenthos in mangrove ecosystems (Lee and Williams, 2002). These macrozoobenthic organisms feed on organic matter in mangrove sediments, mangrove fragments, algae, and bacteria. They are scavengers, predators, cannibals and also serve as food resources for fish, birds and mammals in mangrove ecosystems (Nordhaus et al., 2011; Herbon and Nordhaus, 2013). Crabs of the families Sesamidae and Grapsidae, together with gastropods of the family of Littorinidae, often dominate mangrove fauna in number and biomass, and they play a significant role in nutrient recycling in mangrove ecosystems (Alfaro, 2008).

As detritivores, Grapsidae crabs feed on mangrove leaf litter and detritus in the water column and on the sediment as an important trophic pathway (Herbon and Nordhaus, 2013). *Littoraria melanostoma* is the most common fauna species in Chinese mangrove forests. It occurs predominantly on either shoots or new leaves of mangrove trees with a vertical distribution less than two meters from the ground (Lee and Williams, 2002). In addition to leaf litter, fungi, microalgae and macroalgae, *Littoraria* species also feed on live or dead animal tissue to supplement nitrogen as a result of the low nutritive value of mangrove leaves (Nordhaus et al., 2011).

However, both of these macrozoobenthic taxa rely on leaf litter or detritus for food and may be sensitive to changes in food quantity and quality associated with habitat change. Non-native plant species have been shown to affect the structure and dynamics of benthic macrofauna by changing both environment and food sources (Demopoulos and Smith, 2010). In 1985, the non-native Bangladesh mangrove species *Sonneratia apetala* was introduced to China by the Guangdong Forestry Bureau (Liao et al., 2003). Because of its fast growth rate, it has since been used extensively in mangrove reforestation programs (Chen et al., 2009a). In a previous study, we found that *S. apetala* had lower leaf nitrogen content than native mangrove species (Chen et al., 2008), which may result in lower nutritional value of the leaf litter and, consequently, lower nutrient transfer to the macrozoobenthos. However, little is

known about the contribution of non-native *S. apetala* to the constantly shifting, but dominant, benthic macrofauna in invaded habitats. Large scale seasonal variations of food sources, such as macroalgae and leaf litter production, have previously been found in mangrove forests (Chen et al., 2009b; Zhang et al., 2014). Nonetheless, possible seasonal variations in the diet of these dominant species associated with planting of the non-native *S. apetala* remain to be elucidated.

Fatty acids, as qualitative biomarkers, have frequently been applied as one of the most promising approaches to study food web relationships in marine and coastal ecosystems (Iverson, 2009; Magnone et al., 2015). Primary producers develop certain fatty acid patterns that may be transferred conservatively through the food chain to and, therefore, recognized in primary consumers (Dalsgaard et al., 2003). Signature fatty acids include individual fatty acids and unique ratios of commonly occurring fatty acids, as reflected in the fatty acid profile of consumers (Iverson, 2009). Previous studies found that different primary producers synthesized specific fatty acids, which could be identified as dietary fatty acids in their consumers (Findlay et al., 1990; Graeve et al., 1997; Budge and Parrish, 1998; Meziane and Tsuchiya, 2000). In this study, we used fatty acid analysis to compare the food sources of one littorinid snail and two grapsid crab species between two native mangrove communities and one non-native *S. apetala* plantation with the aim of testing the hypothesis that the non-native *S. apetala* plantation would change the diets of dominant macrozoobenthos in the native mangrove ecosystem by its bottom-up effect.

2. Materials and methods

2.1 Study sites

The present study was carried out in the Gaoqiao mangrove area of Zhanjiang Mangrove National Nature Reserve (21°34'N, 109°45'E), Zhanjiang City, Guangdong Province, China (Figure 1). This area is located northeast of the Beibu Gulf, which is connected to the South China Sea. The Gaoqiao mangrove area is a core zone of the Zhanjiang Mangrove National Nature Reserve, the largest mangrove nature reserve in China with a total mangrove area of approximately 1200 ha (Figure 1). This region consists of seven native mangroves species, including *Rhizophora stylosa*, *Bruguiera gymnorrhiza*, *Kandelia obovata*, *Aegiceras*

corniculatum, *Acrostichum aureum* and *Acanthus ilicifolius*, and one non-native mangrove species, *S. apetala*. Three mangrove forests within the Gaoqiao region were selected for this study. One of the native mangrove forests at the study site was dominated by *A. corniculatum* and another by *B. gymnorrhiza*, the two largest communities at this site having approx. 500 ha and 300 ha of the total area of each species, respectively (Lu et al., 2014). *S. apetala* was introduced to the study site in 1999 for afforestation after the destruction of a hundred hectares of mangroves during the construction of fishponds. Five hectares of *S. apetala* were initially planted. Most of these plantations were monoculture stands of *S. apetala*, but some patches were mixed with the 50-year- old *A. corniculatum*. Most of the non-native plantations formed dense forests after establishment. We selected the largest *A. corniculatum* and *B. gymnorrhiza* forests for our native sampling sites, while the monoculture *S. apetala* forest was chosen as the non-native sampling site, as shown in Figure 1.

Forest structure and soil properties of the sampling sites are shown in Table 1. Tree height in the *S. apetala* plantation is greater, but tree density is lower, than the two native mangrove forests. Soil salinity and soil pH values were similar for all three mangrove forests. Soil organic carbon content was lower in the *S. apetala* plantation.

Air temperature in the Gaoqiao region ranged from 8.7 to 37.3°C, with an annual mean of 23.2°C. The annual precipitation in the region is 1200 mm, and summer is considered the rainy season. This region experiences regular diurnal tides with the spring tides about 190 cm above the sediment surface.

2.2 Field sampling

Five 10 m × 10 m sample plots at least 10 m apart and parallel to the river bank were selected as replicates of each mangrove forest. The five sample plots within each forest were regarded as the replicates for our analyses. All samples for fatty acid analysis were collected in the winter (January) and the summer (July) of 2010. Five mature green sun leaves were randomly collected from the edge of the mangrove canopy in each sample plot. Five brown, senescent leaves (hereinafter denoted as leaf litter) were collected randomly from the sediment surface in each sample plot. All vegetation samples were rinsed with distilled water and placed in a cooler.

In each of the five sample plots, surface sediment in each sample plot was collected by scraping off the top 1 cm of soil from five subplots (20 cm × 20 cm) using a plastic spatula. The five subsamples were homogenized in a plastic bag and then placed in a cooler for transport. In the laboratory, samples were stored at -20°C for one day before freeze-drying. Vegetation and sediment samples were ground into a fine powder, sieved through a 0.25 mm mesh, and then stored at -80°C until further treatment and analysis.

One species of gastropod (*Littoraria melanostoma*) and two crab species (*Perisesarma bidens* and *Parasesarma plicatum*) were selected for sample collection because they were the dominant species of benthic macrofauna across all sample plots. From the soil surface of each sample plot, we collected three to five individuals of each crab species with carapace widths >1 cm. Ten snails with diameter >1 cm were collected from the branches or stems of trees in each sample plot. All macrozoobenthos samples were rinsed with distilled water and placed in a cooler for immediate transport. In the laboratory, the muscle tissues were dissected from the gastropods and crabs, rinsed with distilled water, and stored at -20°C for one day before freeze-drying. Each individual muscle tissue sample was ground into a fine powder and then stored at -80°C until further treatment and analysis. Fatty acid analysis requires 0.10-0.15 g of sample; therefore, we combined ground muscle tissue from all individuals of each species within the same sample plot to create one sample.

2.3 Lipid extraction and determination

We used 0.15 g of powdered sample tissue to extract fatty acids from all sample types. The fatty acids were then methylated to create fatty acid methyl esters (FAMES) using the Ester-linked extraction method (Schutter and Dick, 2000). First, samples were extracted ultrasonically with 5 ml 0.2 M KOH in methanol by incubating in a 60°C water bath for 1 h, during which ester-linked fatty acids were released and methylated. One mL of 1.0 M acetic acid was then added to neutralize the pH. An aqueous-organic two-layer system was formed by adding 3 mL of hexane. The bilayer mixture was centrifuged (3500 rpm, 5 min), and the hexane layer (upper layer) was transferred to a clean glass tube to which an internal standard, nonadecanoic acid methyl ester (C19:0), was also added to the extract. The extract was kept at -80°C until further analysis.

FAMES were analyzed by a Varian CP3800 gas chromatography-mass spectrometer (Varian, Palo Alto, CA, USA). Separation was performed with a VARIAN CPWAX 52 CB capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness; Varian, Palo Alto, CA, USA), using nonadecanoic acid methyl ester (C19:0) as the internal standard (Sigma-Aldrich). Nitrogen was used as the carrier gas. After injection at 50°C, the oven temperature was raised to 170°C at a rate of 10°C/min, then to 205°C at a rate of 3°C/min, and, finally, held constant for 5 min. Individual fatty acids were then identified by their characteristic retention times and were confirmed where necessary by mass spectrometer.

2.4 Statistical analysis

Diagnosis of fatty acids was used to classify different microbial groups (Table 2). Concentrations of individual fatty acids were calculated, and the percentages of different fatty acid grouped as saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids were calculated. Two-way ANOVA (analysis of variance) was used to investigate variations in fatty acid biomarkers of sediment and macrozoobenthos samples by season and by forest. If the difference was significant at $P < 0.05$, a post-hoc test was employed to determine biomarkers responsible for the difference. One-way ANOVA following least significance difference (LSD) test was used to investigate differences in fatty acid biomarkers in macrozoobenthos tissues among different mangrove forests. Principal Components Analysis (PCA) of the fatty acid profiles of muscle tissue and corresponding potential food sources was employed to investigate the variations in fatty acid biomarkers of macrozoobenthos in the three mangrove forests. PCA included all of the fatty acids presented rather than those that had been identified *a priori* as biomarkers. This resulted in reducing the number of dimensions produced by the large number of variables. Also, PCA used linear correlations to identify those fatty acids contributing most to the separation among observed groups (Best and Laposata, 2003). All statistical analyses were performed using SPSS16.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1 Fatty acid profiles of potential food sources

The green leaves of the three mangrove species were composed of 48-54% 18:2n6 (linoleic acid (LA)) and 18:3n3 (alpha-linoleic acid (ALA)) with significant differences among the mangrove forests ($F=3.770$, $p<0.05$) (Table A.1, Table 3). For leaf litter, the proportion of LA and ALA in the *B. gymnorrhiza* forest was significantly lower than that in the *A. corniculatum* forest, thus confirming significant differences among the three forests (Table A.1, Table 3).

As shown in Table 2, the fatty acid biomarkers in the sediment samples of the three mangrove forests, when respectively ranked from high to low, were diatom, followed by bacteria, and then mangrove leaves in both seasons (Figure 2). However, in winter, sediment samples of *S. apetala* and *B. gymnorrhiza* forests had a fatty acid profile similar to each other, but significantly different from that of the *A. corniculatum* forest.

3.2 Fatty acid profiles of dominant gastropod species

In total, 21 fatty acids were identified in the muscle tissue of the dominant species of macrozoobenthos in these mangrove forests (Table A. 2). Using these fatty acid biomarkers to identify probable food sources of macrozoobenthos in mangrove ecosystems (Table 2), we analyzed the fatty acid signals in *L. melanostoma* (Figure 3). The fatty acid profiles in *L. melanostoma* were found to be seasonally variable with significant differences among forests. In winter, zooplankton and mangrove leaves respectively contributed more than 10% of the food sources, while in summer, mangrove leaves and brown algae contributed about 10-15% of the gastropod diet.

A two-way ANOVA for fatty acid biomarkers in *L. melanostoma* (Table 3) showed significant differences for forests and seasons. Fatty acid signals of three *L. melanostoma* biomarkers, including green algae, diatom, and mangrove leaf, were much lower in winter compared to summer. However, the fatty acid signal of its dinoflagellate biomarker was significantly higher in winter compared to summer.

3.3 Fatty acid profiles of dominant crab species

Brown algae, dinoflagellates, green algae and mangrove leaves constituted the main food sources of two crab species among samples collected in winter. In the summer collection, the most abundant fatty acid content was 20:3n3, which constituted approximately 20% of the

total fatty acid contents in *P. bidens* and *P. plicatum* (Figure 3). As determined from muscle tissue, fatty acid food sources, when ranked from high to low, were from brown algae, followed by mangrove and then diatom.

3.4 Results of Principal Components Analysis

According to PCA scores, the same macrozoobenthos species from the native mangrove forests and non-native mangrove plantation overlapped well with each other in both seasons. C14:0, the diatom biomarker, largely contributed to the separation of *L. melanostoma* diets in two seasons. That is, mangrove leaves and zooplankton were dominant food sources in the winter, and mangrove leaves were dominant in the summer. C22:6n3, the dinoflagellate biomarker, largely contributed to the classification of two groups of gastropod crabs. Overlapping diets were observed for *P. bidens* and *P. plicatum* in the same season. These crabs fed primarily on green algae, dinoflagellates and diatoms in the winter and then diatoms and brown algae in the summer. For the three species, seasonality accounted for the major differences of diets within species. Diets for the same macrozoobenthic species from all three forests overlapped in winter, but were slightly separated in summer.

4. Discussion

4.1 Potential food sources for mangrove macrozoobenthos

It is assumed that invasive plants can potentially contribute to niche diversification and different food sources for consumers in the invaded habitats (Demopoulos et al., 2007; Deudero et al., 2011). In mangrove ecosystems, sediment receives organic matter from a variety of sources, and the main organic inputs come from algal production, marine debris, diatoms, benthic microalgae, and mangrove litter (Odum and Heald, 1972). Although these are the main potential food sources for benthic fauna, previous studies indicated that the role of mangrove litter in sustaining epibenthic faunal communities was very limited as a consequence of the high concentrations of tannins in mangrove leaves (Bouillon et al., 2004; Hall et al., 2006). In this study, we found that the most common compound in mangrove signals (LA and ALA) accounted for 50% of the total fatty acid content in both green leaves and leaf litter, a finding consistent with the results of previous studies (Budge and Parrish,

1998; Napolitano, 1999; Meziane et al., 2007). Fatty acids from diatom and bacteria were the most important constituents in the sediment of the three mangrove forests. At the same time, low diatom and bacteria signals, but high mangrove leaf signals, were detected in all three macrozoobenthic taxa, which could be attributed to the large proportion of leaves consumed by littorinid snails and grapsid crabs (Lee and Williams, 2002; Nardhaus et al., 2011). In addition to seasonal variation, differences in fatty acid biomarkers of diatom in the sediment of the studied forests could most likely be attributed to the patterns of consumer diets (Figure 2 and Table 3). However, neither green leaves nor leaf litter in non-native *S. apetala* significantly contributed to variation of fatty acids in the sediment of this new habitat; instead, such variation more likely resulted from non-significant differences in fatty acid profiles among these mangrove species.

4.2 Seasonal variation and forest differences of consumer diets

Some *Littorinidae* species are arboreal, and their vertical distribution is determined mainly by behavioral responses (Reid, 1985). They consume a large amount of mangrove leaves at flooding tide when food is generally limited, but feed on various food sources at ebb tide (Alfaro, 2008). Similarly, we found that mangroves contributed the biggest percentage of fatty acid biomarkers of *L. melanostoma* (18:2n6 and 18:3n3), suggesting that *L. melanostoma* can ingest mangrove fragments directly (litter or green leaves) as key food sources. At the same time, significant seasonal variation in the diet of *L. melanostoma* was found when they fed more on mangrove leaves and green algae, but less on dinoflagellates, in summer compared to winter. These seasonal shifts in feeding behavior were consistent with seasonal variation in the availability of food sources. This significant shift in feeding behavior might be explained by a pursuit for foods with high PUFA contents (e.g., 22:6n3), which are essential for their survival in winter (Trider and Castell, 1980). Furthermore, *L. melanostoma* in this study relied more heavily on brown algae, mangroves, and dinoflagellates, as food sources, in the non-native *S. apetala* plantations compared to the two native forests. In *L. melanostoma*, differences in biomarkers of these sources among the three mangrove forests were greater than those between native and non-native forests, suggesting that plantations of the non-native *S. apetala* may not have any significant effect on the feeding behavior of this

species. This result implied that *L. melanostoma* could be a bioindicator species for forest change, especially in summer (Figure 4).

As detritivorous species, grapsid crabs play a significant role in nutrient recycling in mangrove ecosystems. They prefer leaves with low C:N ratio, but high nutrient levels (Linton and Greenway, 2007). In the present study, both grapsid crabs consumed a higher proportion of plant fragments in *S. apetala* plantations than they did in two native forests in summer, when foods were adequate (Figure 3), most likely because lower leaf nitrogen content was found in *S. apetala* leaves compared to native mangrove leaves (unpublished data). Also, the lower concentration of tannin and cellulose in *S. apetata* fragments would make them more palatable, again when compared to the two native mangrove species (Mun et al., 2011; Wang et al., 2014).

Zhanjiang mangroves support high abundances of brown and green algae, such as *Catenella impudica*, *Caloglossa leprieurii*, and *Enteromorpha clathrat*, especially in spring and summer (Zhang et al., 2014). Consistently, we found that brown algae comprised the greatest food source for both crab species at 16-23%. Compared to mangrove leaves, high N contents, low tannin content and smooth leaf structure make macroalgae palatable and easier to digest for sesarmid crabs (Linton and Greenway, 2007; Kristensen, 2008). Kristensen (2008) also found that the muscles of Grapsidae had high compositions of macroalgae fatty acid biomarkers, such as *Ulva sp.* signals, a kind of green algae.

Some diatom signals contributed to the diet of all macrozoobenthos as well, which was consistent with the fatty acid compositions of sediment. Diatom signals in macrozoobenthos in *S. apetala* were lower than those in the native mangroves, suggesting lower contribution of diatom as a food source for both crabs in the non-native plantation. Furthermore, the diatom signals of all macrozoobenthos were higher in summer, which was consistent with the higher level of diatom signals in summer compared to winter. During the summer, diatoms predominated in the organic matter of sediment. It follows that diatom signals found in sediment would correspondingly increase, explaining, in turn, the higher diatom signals of all macrozoobenthos in summer. Furthermore, dinoflagellates comprised 12-20% of the diet of both Grapsidae species in winter, a much higher percentage than that observed in summer.

Similar to *L. melanostoma*, this could be caused by the increased consumption of foods, such as dinoflagellates, which are rich in PUFAs (e.g., 22:6n3); moreover, PUFAs can protect against the relatively low winter temperatures (Meziane et al., 1997; Parrish et al., 2000).

4.3 Influence of non-native mangroves on macrozoobenthos diet

Macrozoobenthic taxa are the consumers of primary producers and the food sources of higher trophic levels. They are diverse in resource utilization (e.g., Bouillon et al., 2002; Thimda et al., 2001). In our study site, the non-native mangrove species *S. apetala* was planted about 10 years ago; therefore, we expected significant changes in the diets of the dominant macrozoobenthos post-planting. Our results showed significant differences in the fatty acid profiles of the potential food sources among the three mangrove communities, except for bacteria. Furthermore, the differences in the fatty acid signals of the consumers among communities might also be caused by the quantity of available food resources. From the results of PCA, we found that three macrozoobenthos were widely separated by season (Figure 6), implying a significant seasonal shift of food sources as a result of resource availability. At the same time, only slight species differences in food preferences were found in the two crab species, which was consistent with the results of fatty acid biomarker contents. However, different species have different digestive and metabolic processes which, in this case, may have contributed to the difference in biomarker signals in the crab species studied, as found in other sesamid crabs (Kristensen et al., 2010), suggesting the need to incorporate metabolism as an aspect of further studies of macrozoobenthos food sources. We had hypothesized that planting non-native *S. apetala* would change the diets of the dominant macrozoobenthos in these ecosystems. However, this cannot be supported because differences of fatty acid biomarkers among macrozoobenthos could only be found among the three mangrove forests, as suggested by PCA results, making it difficult to identify the non-native mangrove forest in this study as a significant contributor to dietary shift. However, significant seasonal variations were found between food sources of these macrozoobenthos, as expected.

Previous studies found that non-native plants significantly altered the food web structure in both mangroves (Demopoulos et al., 2007) and salt marsh ecosystems (Shang et al., 2008;

Brusati and Grosholz, 2009). Demopoulos et al. (2007) reported that the alteration of food web structure in the invaded mangrove forests was a consequence of forest age in Hawaii, where exotic mangrove species had been introduced to non-mangrove-dominant coasts nearly 100 years earlier. We originally proposed that the planting of the non-native *S. apetala* mangrove species would change nutrient relationships and, hence, further alter the food web structure in these mangroves. However, our results indicated that the three macrozoobenthic species in this study were not sensitive to this change. Unlike these previous cases, the introduction of *S. apetala* in the present study does not involve a food source with greatly different stable isotope signatures (cf. C3/C4 plants shifting as a result of *Spartina* invasion in mangroves or the introduction of mangroves to non-mangrove-dominant coasts), as shown in those previous studies (e.g., Demopoulos et al., 2007; Feng et al., 2014). Therefore, natural abundance stable isotope analysis cannot detect shifts in consumption of different mangrove species; inversely, fatty acid signals can, to some extent, detect this difference among them, as shown in this study. Compared to mangrove fragments, which accounted for 10-15% of macrozoobenthic diets in this study, food sources, such as microalgae, contributed much more to consumer diets. The methods to quantify the shift to another food source driven by a slight difference in nutrient content of the native/non-native mangroves must be better developed, such as *in situ* feeding experiments. Thus, a correction would need to be made for a given consumer potentially retaining compounds from a given source other than a specified biomarker. In addition, multiple-location and long-term studies of mangrove benthic fauna are still needed to determine the influence of non-native *S. apetala* plantations on mangrove food web structure.

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Table 1 Forest structure and soil properties of the native and non-native mangrove forests in Zhanjiang City, China.

Forest	Forest structure			Soil properties				
	Density	Height	LAI	salinity	SOC	C/N	pH	Soil texture
	(Ind/100 m ²)	(m)		(mg/g)	(%)	ratio		
<i>Aegiceras corniculatum</i>	917.0	2.40	2.20	16.9	4.17	16.6	5.6	Silty clay
<i>Bruguiera gymnorrhiza</i>	44.3	3.08	1.84	17.1	3.15	14.6	5.9	Silty clay
<i>Sonneratia apetala</i>	15.6	11.16	0.88	16.0	2.70	13.6	5.6	Silty clay

* LAI: leaf area index; SOC: soil organic matter; Soil C/N ratio: soil carbon and nitrogen ratio.

478 **Table 2 Fatty acid biomarkers used to identify food sources of macrozoobenthos in**
 479 **mangrove ecosystems**

Source	Biomarker	References
Brown algae	C20:3n3, C20:3n6	Perry et al., 1979; Khotimchenko, 2002
Green algae	C18:3n6	Meziane and Tsuchiya, 2000
Diatoms	C14:0, C16:1n7, C20:5n3	Graeve et al., 1997; Napolitano et al., 1997
Bacteria	Sum of odd C no. and branched chain fatty acids, C18:1n7	Findlay et al., 1990; Kharlamenko et al., 2001
Mangroves	C18:2n6, C18:3n3 C22:0, C24:0	Meziane et al., 2007; This study
Zooplankton	C20:1, C22:1	Hagen and Auel., 2001
Dinoflagellates	C22:6n3	Sargent et al., 1987

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Table 3 F-values (Two-way ANOVA and post hoc tests) for fatty acid groups (shown as the sources of fatty acid biomarkers) from sediment and the three dominant macrozoobenthic species within the three mangrove study forests over two seasons.

					Mangrove	Brown	Green	
Items		df	Diatoms	Bacteria	leaves	algae	algae	Dinoflagellates
Green leaves	Season (S)	1			0.412			
	Forest (F)	2			3.770*			
	Season ×Forest	2			0.544			
Leaf litter	Season (S)	1			0.701			
	Forest (F)	2			15.23***			
	Season ×Forest	1			0.397			
Sediment	Season (S)	1	9.837**	18.958***	0.152			
	Forest (F)	2	5.473*	1.484	2.928			
	Season ×Forest	2	15.969***	2.278	4.224*			
<i>Littoraria</i>	Season (S)	1	60.0**	0.1	587.4**	2.3	25.4**	3969***
<i>melanostoma</i>	Forest (F)	2	23.5**	8.2**	23.8**	20.4**	2.1	171.3**
	Season ×Forest	2	5.2*	1.4	11.2**	12.6**	45.4**	156.7**
<i>Perisesarma</i>	Season (S)	1	1640***	26.7**	107.2**	47.1**	3.1	2591***
<i>bidens</i>	Forest (F)	2	40.6**	1.7	460.8**	2.6	201.2**	171.3**
	Season ×Forest	2	73.4**	2.7	384.0**	25.7**	10.6*	2.6
<i>Parasesarma</i>	Season (S)	1	11340***	2.5	53.9**	36.4**	177.4**	2260***
<i>plicatum</i>	Forest (F)	2	43.7**	1.0	84.0**	9.8*	38.9**	59.8**
	Season ×Forest	2	46.1**	1.9	6.5*	1.8	62.7**	77.6**

*: p<0.05, **: p<0.01, ***: p<0.001

485 **Figure captions**

486 Figure 1 Distribution of sampling sites in the Gaoqiao region of Zhanjiang Mangrove Nature
487 Reserve in Guangdong Province, China.

488 Figure 2 Mean content of fatty acid groups (mass % of total) in the sediment among the three
489 mangrove forests in two seasons. Three groups of biomarkers were shown, which were the sum of
490 diatom biomarkers (C14:0+C16:1n7+C20:5n3), the sum of bacterial biomarkers (C15:0+C17:0),
491 and the sum of mangrove leaf biomarkers (C18:2n6+C18:3n3). *Ac: Aegiceras corniculatum*, *Bg:*
492 *Bruguiera gymnorrhiza*, *Sa: Sonneratia apetala*.

493 Figure 3 Mean content of fatty acid groups (mass % of total) found in three macrozoobenthos
494 species among the three mangrove forests in two seasons. Seven groups of biomarkers were
495 shown, which were the sum of diatom biomarkers (C14:0+C16:1n7+C20:5n3), the sum of
496 bacterial biomarkers (C15:0+C17:0), the sum of mangrove leaf biomarkers (C18:2n6+C18:3n3),
497 brown algae biomarkers (C20:3n3), green algae biomarkers (C18:3n6), dinoflagellate biomarker
498 (C22:6n3), and the sum of zooplankton biomarkers (C20:1+C22:1). *Ac: Aegiceras corniculatum*,
499 *Bg: Bruguiera gymnorrhiza*, *Sa: Sonneratia apetala*.

500 Figure 4 Scores and associated loadings plot of PCA (Principal Component Analysis) of fatty acid
501 profiles from consumer tissues indicating variance in consumer diet. The first (PC1) and second
502 (PC2) Principal Components explained 49.0% and 16.1% of the variability, respectively. *Arrows*
503 are labeled according to the results from the loadings plot, indicating that the fatty acids
504 contributed most to the distribution of species along each component. The results from the scores
505 plot of the PCA of the consumer fatty acid profiles are labeled as splattering, indicating the diet

506 variance of consumers.

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508 Figure 1

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