ELSEVIER

Journal of Experimental Marine Biology and Ecology
292 (2003) 187–197

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan

Zainudin Bachok*, Prosper L. Mfilinge, Makoto Tsuchiya

Laboratory of Ecology and Systematics, Faculty of Science, University of the Ryukyus, Senbaru-1, Nishihara, Okinawa 903-0213, Japan

Received 21 November 2002; received in revised form 10 March 2003; accepted 31 March 2003

Abstract

Fatty acid compositions in the tissues of the clam *Geloina coaxans* collected from Oura mangal, Okinawa, Japan, during the cold and warm seasons (January and July 2001, respectively) were compared with those in suspended materials (SM) in order to assess the clams' diet. In both seasons, the suspended mangrove detritus at the sediment–water interface was high as indicated by the mean percentage of even-numbered long-chain fatty acids in SM (12.8–18.4%). The contribution of this marker in the clam tissues, especially during the cold season (3.9%), indicates the consumption of mangrove detritus in considerable amounts by the clams. The occurrence of the fatty acids 16:1ω7, 18:1ω9, 18:2ω6 and 18:3ω3 in SM was most likely due to the mangrove detritus sources, whereas in the SM they together constituted 12.9% and 23.9% of total fatty acid contents during the cold and warm seasons, respectively. As a result, their contribution in the clam tissues was high in the cold (15.4%) and warm seasons (19.0%). These results indicate that mangrove detritus play a significant role in the clams' diet. The mean percentages of bacterial markers (odd-numbered branched fatty acids and vaccenic acid, 18:1ω7) in the SM and tissues during both seasons ranged from 8.1% to 9.5%. This indicates that the clam diet is also dependent on the attached bacteria on the partially decomposed leaf detritus suspended at the sediment–water interface. The relative contribution by microalgae markers (18:4ω3, 20:5ω3 and 22:6ω3) in clam tissues ranged from 4.3% to 7.6%, suggesting considerable microalgae sources in the diets.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Bivalve; Diet; Fatty acid markers; *Geloina coaxans*; Mangrove

* Corresponding author. Tel.: +81-98-895-8540; fax: +81-98-895-8576.
E-mail address: zainudinb@hotmail.com (Z. Bachok).

0022-0981/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0022-0981(03)00160-6

1. Introduction

Subtropical mangals are among the most productive ecosystems in the lower-latitude regions. Even though mangrove production (through leaf litter) could be utilized directly by macrozoobenthos in the mangrove forest (Slim et al., 1997), in some cases, the products of primary production become more useful in the form of organic detritus after fragmentation and heterotropic biodegradation processes (Wafar et al., 1997). The mangrove litter, for example, contributed to the formation of dissolved (Alongi, 1989) and particulate organic materials (Mfilinge et al., 2002), which may then either be directly absorbed by animals, or may serve in the particulate fraction as a substrate for metabolism of heterotrophic microorganisms (Alongi, 1994). Such bacteria and associated microorganisms may then form a principal food resource for certain mangrove invertebrates (Bano et al., 1997).

Bivalve molluscs comprise a considerable proportion of animal taxa in mangrove ecosystems of Okinawa, Japan (Yoshimi, 1993; Shokita, 2000). The mud clam *Geloina coaxans*, from the family Corbiculidae, is a typical mangrove bivalve, which lives semi-infaunally on the soft sediment that accumulates around the roots of the mangrove trees (Kubo and Kurozumi, 1995). In the mangrove forest of Oura, Okinawa, *G. coaxans* is the only bivalve species that coexists successfully, together with other invertebrates such as crabs and gastropods (personal observation). Shokita (1983) studied the food chains and energy flows in Okinawan mangrove ecosystems. However, to date, no study has been done on the diet of mangrove bivalves such as *G. coaxans*.

Fatty acids are the major constituents of lipids, which are a compact and concentrated form of energy for plant and animals (Sargent, 1976). Previous studies have demonstrated that the specific fatty acid markers available for different groups of primary producers and consumers (Perry et al., 1979; Volkman et al., 1989) could represent a useful tool, especially since information on dietary composition is integrated over a longer time scale than in conventional approaches, i.e. gut-content analyses. Thus the concentration of particular fatty acid markers could provide insight into the food sources utilized by animals (e.g. see Kharlamenko et al., 2001).

The nature of the habitat and the available organic material influence bivalve diet. For instance, Odum and Heald (1972) found that the identified organic materials in the stomachs of the mangrove bivalves, *Brachidontes exustus* and *Congeria leucophaeata*, consisted mainly of vascular plant detritus (69–73%), followed by epiphytic diatoms (~ 16%) and phytoplankton (6–9%). Furthermore, stable isotope studies indicated that the oyster, *Crassostrea virginica*, and the marsh mussel, *Geukensia demissa*, utilize *Spartina* (marsh grass) detritus (Langdon and Newell, 1990), and suggest that the consumption and assimilation of particles is related to particle abundance and that detrital carbon is assimilated into bivalve tissue. In another study, Stuart et al. (1982) found that kelp detritus was absorbed with an efficiency of about 50% while kelp bacteria were absorbed with an efficiency of about 70% by the ribbed mussel *Aulacomya ater*.

This study was done in order to investigate the diet of *G. coaxans* and bridge the existing gap in the food webs of this subtropical mangrove forest. We hypothesized that detritus particles form the main component of the diet that are efficiently assimilated. In many cases, near shore suspension-feeding bivalves mostly assimilate phytoplankton.

Detritus is abundant in the mangrove ecosystem and organic detritus may be a major food source for the bivalves.

2. Materials and methods

2.1. Study area

The study area was the subtropical mangrove forest of Oura, located in the northern part of Okinawa Island (26°N, 128°E; Fig. 1) in southern Japan. The mangrove forest consists of mixed stands of *Bruguiera gymnorrhiza*, *Kandelia candel* and *Rhizophora stylosa*, covering an area of approximately 10 ha. The forest is unique because it is situated in an enclosed bay and the effects of wave action are relatively small. Allochthonous inputs that are channelled through the Oura River come from surrounding mountain forests, sugar cane plantations and human settlements (Fig. 1). The sediment surface of the mangrove forest is comprised mainly of the silt-clay fraction.

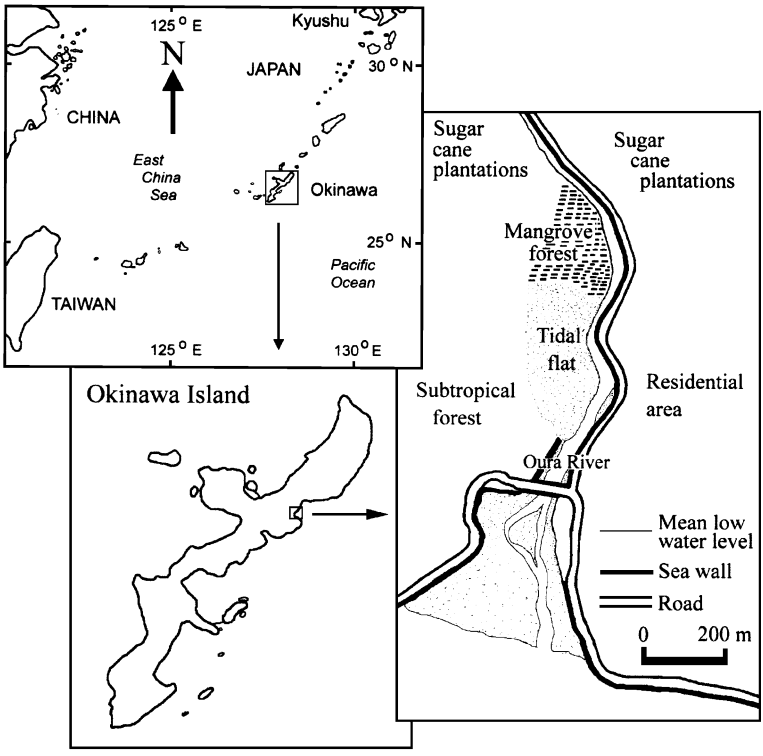


Fig. 1. Location of Okinawa Island and Oura mangrove forest.

2.2. Sample collection and preparation

Clam and sediment samples were collected on two separate occasions. The first sampling was during the cold season (end of January 2001, mean air temperature was 14 °C), and the second sampling was during the warm season (end of July 2001, mean air temperature 28 °C).

All collected clams were kept in filtered seawater for gut-content clearance and then the clam shells were opened to get tissue samples. The tissues were cleaned thoroughly in filtered seawater, chopped finely and between 5 and 6 g of tissue (wet weight) was used for lipid extraction. The sediment samples were collected by scraping the top 2 mm of surface sediments around the clams. In the laboratory, the sediment samples were resuspended in containers filled with distilled water. The water with suspended materials was then centrifuged and the supernatant discarded. The suspended materials (SM) were collected and 6 g wet weight of samples was used for lipid extraction. Tissues from six different clams and six samples of SM were prepared for lipid extractions.

2.3. Lipid extraction

Lipid was extracted by following a slightly modified version of the method of Bligh and Dyer (1959). Lipids were extracted ultrasonically for 20 min with a mixture of distilled water/methanol/chloroform (1:2:1, 20 cm^3, v/v/v). Lipids were then transferred into the lower chloroform phase and improved by centrifugation [3000 rpm (650 × *g*), 5 min]. After evaporation of the solvent under nitrogen, the lipid extracts were saponified, transmethylated and separated in order to get fatty acid methyl esters (FAMEs) (detailed in Meziane et al., 2002). FAMEs were then analysed by a GC 14-B Shimadzu gas chromatograph equipped with flame ionisation. FAMEs were separated with an FFAP-polar capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film thickness). Hydrogen was used as a carrier gas. After injection at 60 °C, the oven temperature was raised to 150 °C at a rate of 40 °C min^{-1}, then to 230 °C at 3 °C min^{-1}, and finally held constant for 30 min. The flame ionisation was held at 240 °C. FAMEs were identified by comparing their retention times with those of a standard.

The percentage of fatty acids was compared using ANOVA. Sample type (animals and SM) and seasons were entered as fixed factors (independent) and data were arcsine P-transformed if necessary to produce normal distribution before analysis. Fisher's PLSD post hoc test showed significant differences in the means.

3. Results

The concentration of FAMEs (mg g^{-1} wet wt.) and the fatty acid compositions (%) in the tissues of *G. coaxans* and suspended materials (SM) from Oura mangal are listed in Table 1. The mean concentration of FAMEs was significantly higher in the clam tissues (7.64–9.40

Notes to Table 1:
Values for individual fatty acids are the mean % of total fatty acids from six different samples. Values in parentheses are standard errors; –: not detected; *: location of double bonds unknown.

Table 1
Fatty acids in the tissues of *G. coaxans* and suspended materials collected from Oura mangrove

	Tissues		Suspended materials	
	Cold	Warm	Cold	Warm
14:0	1.3 (1.2)	5.2 (3.1)	2.1 (0.4)	1.5 (1.2)
14:1	–	–	–	0.4 (0.3)
15:0 iso	0.1 (0.2)	0.6 (0.3)	2.7 (0.2)	1.1 (0.6)
15:0 anteiso	–	–	1.6 (0.2)	1.0 (0.5)
15:0	0.2 (0.3)	0.9 (0.2)	1.3 (0.2)	1.4 (0.9)
16:0 iso	0.4 (0.4)	0.3 (0.3)	1.5 (0.2)	0.8 (0.2)
16:0 anteiso	0.1 (0.2)	0.5 (0.8)	1.0 (0.1)	0.3 (0.4)
16:0	11.1 (5.6)	20.1 (5.4)	23.2 (4.1)	18.9 (5.1)
16:1ω9	1.5 (0.8)	1.5 (0.7)	1.5 (0.3)	7.5 (5.8)
16:1ω7	–	3.6 (1.1)	2.6 (1.0)	5.2 (0.9)
17:0 iso	2.7 (0.9)	2.1 (0.5)	1.9 (0.2)	1.5 (0.6)
17:0 anteiso	0.8 (0.4)	1.0 (0.2)	1.0 (0.1)	0.7 (0.1)
16:2ω4	0.7 (0.4)	0.6 (0.3)	0.5 (0.3)	0.3 (0.4)
17:0	2.9 (0.6)	2.2 (0.6)	1.3 (0.2)	1.0 (0.5)
17:1	0.2 (0.3)	0.9 (0.4)	–	1.0 (0.6)
18:0 iso	0.7 (0.4)	0.3 (0.2)	–	–
18:0 anteiso	0.2 (0.5)	0.4 (0.3)	–	–
18:0	8.6 (0.9)	5.7 (1.2)	4.3 (2.3)	6.2 (1.5)
18:1ω9	11.9 (0.9)	9.6 (1.0)	9.7 (1.4)	14.0 (6.1)
18:1ω7	5.5 (1.0)	4.8 (1.1)	1.9 (1.5)	5.1 (1.8)
18:2ω6	2.5 (1.3)	3.3 (0.7)	0.6 (0.8)	4.0 (2.7)
18:3ω6	0.6 (0.3)	0.5 (0.1)	–	0.6 (0.6)
18:3ω4	0.5 (0.2)	0.7 (0.2)	–	–
18:3ω3	1.0 (0.6)	2.5 (0.7)	–	0.7 (0.5)
18:4ω3	0.3 (0.3)	1.0 (0.3)	–	0.8 (0.7)
20:0	1.0 (0.5)	0.1 (0.3)	3.5 (0.3)	2.0 (0.2)
20:1ω9	11.0 (3.8)	6.1 (1.7)	–	0.4 (0.6)
20:1ω7	4.0 (2.9)	3.9 (2.2)	–	0.1 (0.2)
20:1*	0.8 (0.2)	1.8 (1.9)	–	–
20:2*	2.4 (0.9)	1.7 (0.8)	0.8 (1.2)	–
21:0	0.6 (0.3)	–	–	0.8 (1.1)
20:4ω6	6.0 (1.8)	4.8 (2.6)	–	0.6 (0.3)
20:5ω3	0.6 (0.5)	2.4 (0.7)	–	2.2 (2.3)
22:0	0.5 (0.3)	0.8 (0.9)	4.9 (0.6)	2.7 (1.6)
22:1*	1.0 (0.7)	0.1 (0.2)	–	–
22:4ω3	–	–	1.1 (0.5)	0.2 (0.3)
22:5ω6	3.8 (1.7)	1.6 (1.0)	0.3 (0.6)	0.1 (0.3)
22:5ω3	2.7 (1.3)	1.2 (0.7)	–	0.2 (0.4)
24:0	1.6 (0.7)	1.2 (0.3)	9.9 (1.2)	6.0 (1.0)
22:6ω3	3.4 (1.3)	4.2 (1.0)	–	–
25:0	0.8 (1.7)	–	2.2 (0.7)	1.0 (1.2)
26:0	1.2 (1.7)	0.2 (0.2)	8.8 (1.2)	6.6 (3.1)
27:0	–	–	–	0.7 (0.9)
28:0	0.3 (0.4)	–	3.2 (3.5)	1.9 (2.0)
29:0	–	–	–	0.7 (0.9)
30:0	0.1 (0.2)	–	5.9 (4.3)	–
32:0	0.6 (1.3)	–	–	–
Unknown	3.9 (0.5)	1.3 (0.9)	0.6 (0.6)	0.2 (0.2)
Total FAMEs (mg g^{-1} wet wt.)	9.4 (1.7)	7.6 (1.3)	1.0 (0.3)	0.9 (0.2)

mg g^{-1}) than in the SM (0.89–0.99 mg g^{-1}). The FAME content was higher during the cold season compared to the warm season in both tissues and SM (Table 1). The fatty acid profiles did not differ between the tissues and SM, or between the cold and warm seasons ($p>0.05$).

The fatty acids in the tissues of *G. coaxans* in the cold and warm seasons were dominated by palmetic acid (16:0) and the ω9 series of monounsaturated fatty acids (MUFAs). During the cold season, the percentages of fatty acids in descending order were 18:1ω9, 16:0 and 20:1ω9 (11.9%, 11.1% and 11.0%, respectively), whereas in the warm season, the order was 16:0, 18:1ω9, and 20:1ω9 (20.1%, 9.6% and 6.1%, respectively). In the SM, the fatty acid 16:0 was dominant in both seasons (18.9–23.2%). However, unlike in the tissues of *G. coaxans*, the fatty acid profile in SM was characterized by the higher contribution of even-numbered long-chain fatty acids (even-LCFAs 26:0 to 32:0), whereas in the cold season, even-LCFAs constituted 18.4% of total fatty acids content, while in the warm season, the amount was lower (12.8%). The percentage of 18:1ω9 in the SM was relatively high during the cold and warm seasons (9.7% and 14.0%, respectively). Furthermore, the fatty acid profile in the SM was also characterized by a lower contribution of polyunsaturated fatty acids (PUFAs) during both seasons (3.6–8.8%), compared to the tissues of *G. coaxans* in which the percentage of PUFAs was much higher during both seasons (21.0–23.8%). The ω6 PUFAs were more abundant compared to the ω3 PUFAs (Table 1) in the clam tissues, especially during the cold season; the sum of ω6 PUFAs was two times greater than ω3 PUFAs.

The percentage of odd-numbered branched fatty acids (odd-BrFAs 15:0 and 17:0, iso and anteiso) in the tissues of *G. coaxans* was almost similar during both seasons (4.0–4.1%; Table 1). In the SM, however, the percentage was much higher during the cold season (7.0%) compared to the warm season (2.9%). The percentage of vaccenic acid 18:1ω7 in the clam tissues was found to be higher during the cold than the warm season but it was reversed in the SM (Table 1). The fatty acid 20:4ω6 was relatively higher in clam tissues (4.8–6.0%) compared to the SM (<0.6%). The contribution of other PUFAs 20:5ω3, 22:6ω3, etc. was found to be lower than 5% of the total fatty acid content.

Table 2 shows the summary statistics from ANOVAs comparing the content of selected fatty acids and the sum of fatty acids, which are used as markers. The mean contribution of

Table 2
Summary of two-way ANOVA for content of fatty acid in tissues of *G. coaxans*, suspended materials and seasons (cold and warm)

	Sample types		Seasons	
	$F_{1,20}$	P	$F_{1,20}$	P
Σeven-LCFAs[a]	72.6290	<0.0001***	15.8170	0.0007***
(16:1ω7 + 18:1ω9 + 18:2ω6 + 18:3ω3)[b]	0.1910	0.6665[ns]	12.0330	0.0024**
(Σodd-BrFAs + 18:1ω7)[c,d]	0.3300	0.532[ns]	0.0420	0.8389[ns]
(18:4ω3 + 20:5ω3 + 22:6ω3)[e,f,g]	34.9730	<0.0001***	17.7640	0.0004***

References relating to fatty acids and sum of fatty acids, which are used as markers: [a]Scribe et al. (1991); [b]Sargent et al. (1990), [c]Gillan and Johns (1986); [d]Perry et al. (1979); [e]Volkman et al. (1989); [f]Pond et al. (1998); [g]Napolitano et al. (1997).

ns: not significant.

** $p<0.01$.

*** $p<0.001$.

even-LCFAs and the sum of 18:4ω3, 20:5ω3 and 22:6ω3 was significantly different between the clam tissues and SM, and between the seasons ($p < 0.001$). The percentages of the sum of 16:1ω7, 18:1ω9, 18:2ω6 and 18:3ω3 was similar between the clam tissues and SM ($p > 0.05$) but differed significantly between the cold and warm seasons ($p < 0.01$). The sum of odd-BrFAs and 18:1ω7 did not differ significantly between the tissues and SM, or between the cold and warm seasons ($p > 0.05$).

4. Discussion

4.1. Contribution of mangrove sources

The fatty acid profiles of *G. coaxans* showed a considerable contribution of even-LCFAs (which is normally scarce in suspension-feeding bivalves) (Canuel et al., 1995), in their tissues during the cold and warm seasons (3.9% and 0.2%, respectively). The even-LCFAs are generally associated with the waxy leaf coating of vascular plant species (Scribe et al., 1991; Colombo et al., 1996) and have been used as tracers of vascular plants in marine food webs (Meziane et al., 1997). In the SM, the significantly higher percentage of even-LCFAs during the cold season (January; 18.4%) than the warm season (July; 12.8%) suggests a higher input of mangrove organic matter to the detrital fraction that is resuspended at the sediment–water interface during the cold season compared to the warm season. Meziane and Tsuchiya (2000) found that organic matter from this mangrove forest was exported to the adjacent intertidal flat during the rainy season (May) and warm season (July). Therefore, the higher contribution of mangrove organic matter in SM during the cold season indicated that it was less exported to the adjacent area. Most remains within the mangrove. As a result, the even-LCFAs were detected more in the clam tissues during the cold than the warm season (Table 1). This indicates the usefulness of mangrove detritus as a potential food source for clams.

The fatty acid profile of *G. coaxans* was characterized by high levels of PUFAs (21.0–23.8%) and low levels of even-LCFAs (<4%). However, the fatty acid profile of SM was characterized by high levels of even-LCFAs (12.8–18.4%) and low levels of PUFAs (3.6–8.8%). PUFAs play a significant role in animals. They are generally derived either from the diet directly or following conversion of dietary components. As noted by Sargent et al. (1990), photosynthetic organisms biosynthesise both ω3 and ω6 PUFAs readily, initially by converting newly biosynthesised 16:0 and 18:0 to 16:1ω7 and 18:1ω9, respectively. Unlike animals, plants can continue the further desaturation of the 18:1ω9 to 18:2ω6 and 18:3ω3 fatty acids. Therefore in this study, the occurrence of the fatty acids 16:1ω7, 18:1ω9, 18:2ω6 and 18:3ω3 indicates mangrove detritus sources. These fatty acids constituted 23.9% of total fatty acids in the SM during the warm season and 12.9% during the cold season. This may be due to the high amount of mangrove organic matter in SM as indicated by the percentages of even-LCFA (12.8–18.4%). In this study, the mean percentages of the sum of 16:1ω7, 18:1ω9, 18:2ω6 and 18:3ω3 did not differ significantly between the clam tissues and SM ($p > 0.05$) and their contribution in the clam tissues was also significantly higher during the warm season (19.0%) than the cold season (15.4%), suggesting that these fatty acids were obtained

from mangrove detritus suspended at the sediment–water interface. The above facts, therefore, support the conclusion that mangrove bivalves assimilate mangrove detritus, thus indicating the importance of mangrove organic materials in the diet of clams in Oura mangrove forest.

4.2. Bacteria sources

The odd-BrFAs and vaccenic acid (18:1ω7) are predominant in bacteria (Perry et al., 1979; Gillan and Johns, 1986), and have previously been used as bacterial markers in marine food webs (Kharlamenko et al., 1995). In SM, the higher concentration of odd-BrFA and 18:1ω7 (8.8–9.4%), indicates bacterial sources. In addition, the higher concentration of MUFAs (11.7–35%) compared to PUFAs (3.6–8.8%) in the SM during both seasons also indicates food sources related to bacteria (Pranal et al., 1996). These may be due to the bacterial colonization of the detritus during the degradation of mangrove litter on the sediment surface (Benner and Hodson, 1985). The mean percentage of bacterial markers in the clam tissues (8.1–9.5%) was not significantly different, with the means in SM (8.8–9.4%; $p>0.05$). The contribution of MUFAs was also high (31.0–31.9%). Therefore, the high concentration of fatty acid markers for bacteria and the high level of MUFAs in the tissues suggest that the clams assimilated bacteria attached to decomposing mangrove litter.

4.3. Microalgal sources

The fatty acid percent concentration of *G. coaxans* was less than in other suspension-feeding bivalves, e.g. the low level of eicosapentaenoic acid (20:5ω3; $<2.4\%$) and docosahesaenoic acid (22:6ω3; $<4.2\%$), which are typical and essential for the growth and survival of suspension-feeding bivalves (e.g. see Trider and Castell, 1980; Landgon and Waldock, 1981). Compared to five coexisting suspension-feeding bivalve species on Tomigusuku tidal flat, Okinawa, Japan (in preparation), the mean percentage of 20:5ω3 and 22:6ω3 was over 8.4% of total fatty acids. The fatty acid 20:5ω3 was predominant in diatoms (Pond et al., 1998) and has been used as a benthic diatom marker in food webs (Kharlamenko et al., 1995). In this study, the detection of the fatty acid 20:5ω3 in the SM only during the warm season though in low concentration (2.2%) probably indicates that low light intensity inside the mangrove forest limits the growth of benthic diatoms (Admiral and Peletier, 1977). Because of this, the concentration of the fatty acid 20:5ω3 in the clam tissues was also low during both seasons (0.6–2.4%), suggesting minor benthic diatom sources in the clams diet. On the other hand, the fatty acid 22:6ω3, which is a dinoflagellate marker (Joseph, 1975), was detected in the clam tissues during both seasons (3.4–4.2%), indicating that dinoflagellates were also assimilated by the clams. The fatty acid 18:4ω3, which is also typical of dinoflagellates (Mansour et al., 1999), was present in the clam tissues in low concentration ($<1.0\%$). Furthermore, the low concentration of fatty acid markers for dinoflagellates in SM ($<0.8\%$) indicates the absence of dinoflagellates in the sediments. However, their occurrence in the clam tissues suggests an input of dinoflagellates during high tide. The relative contribution of the microalgal markers (18:4ω3, 20:5ω3 and 22:6ω3) in the clam tissues, which ranged from 4.3% to

7.6%, suggests that microalgae contribute less to the diets of *G. coaxans* than other food sources.

4.4. Other sources

Falk-Petersen et al. (2002) have shown that the lipids of copepods contained 20:1ω9 and 20:1ω11, which together constituted 60% of total fatty acids. When ingested by predators, the long-chain monoenes partially accumulate in the predators' tissue lipids (e.g. Hopkins et al., 1993; Raclot et al., 1998). In this study the relatively high concentration of the fatty acid 20:1ω9 in the tissues of bivalves (11.0% and 6.1% of total fatty acids) in the cold and warm seasons, respectively (Table 1), suggests that *G. coaxans* consumes considerable quantities of zooplankton. The use of zooplankton as food has also been reported in the oyster *Ostrea edulis* (Knox, 1986), though the fatty acid markers for zooplankton in suspension-feeding bivalves was rarely reported.

To conclude, our results support the hypothesis that mangrove detritus particles form the main component of the bivalve diet. However, its importance seems to vary among seasons depending on the quantity of detritus accumulated in the mangal. The fatty acids of clams also revealed bacteria attached to mangrove detritus to be the second major food source, while microalgae such as diatoms and dinoflagellates are minor sources in the diet. The bivalve *G. coaxans* plays an important role in the transfer of energy to higher tropic levels at the Oura mangrove ecosystem.

Acknowledgements

Z.B. is grateful to the Ministry of Education, Culture, Sports, Science and Technology, Japan (Monbukagakusho), for scholarship grant during his study. Thanks are given to Dr. T. Meziane for assistance on fatty acid analyses, Mr. M.I. Salim for critical reading of an earlier version of the manuscript, and all students in the ecology laboratory for encouragement and help. **[SS]**

References

Admiral, W., Peletier, H., 1977. Influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. Br. J. Phycol. 14, 197.

Alongi, D.M., 1989. Effect of exported mangrove litter on bacterial productivity and dissolved organic carbon fluxes in adjacent tropical nearshore sediments. Mar. Ecol. Prog. Ser. 56, 133–144.

Alongi, D.M., 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. In: Sasekumar, A., Marshall, N., Macintosh, D.J. (Eds.), Ecology and Conservation of Southeast Asian Marin and Freshwater Environments Including Wetlands. Kluwer Academic Publishing, Belgium, pp. 19–32.

Bano, N., Nisa, M., Khan, N., Saleem, M., Harrison, P.J., Ahmed, S.I., Azam, F., 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. Mar. Ecol. Prog. Ser. 157, 1–12.

Benner, R., Hodson, R.E., 1985. Microbial degradation of the leachable and lignocellulosic components of leaves and wood from *Rhizophora mangle* in a tropical mangrove swamp. Mar. Ecol. Prog. Ser. 51, 221–230.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Canuel, E.A., Cloern, J.E., Ringelberg, D.G., Guckert, J.B., Rau, G.H., 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco bay. Limnol. Oceanogr. 40, 67–81.

Colombo, J.C., Silverberg, N., Gearing, J.N., 1996. Lipid biochemistry in the Laurentian Trough: 1. Fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. Org. Geochem. 25, 211–225.

Falk-Petersen, S., Dahl, T.M., Scott, C.L., Sargent, J.R., Gulliksen, B., Kwasniewski, S., Hop, H., Millar, R., 2002. Lipid biomarkers and troph linkages between ctenophores and copepods in Svalbard waters. Mar. Ecol. Prog. Ser. 227, 187–194.

Gillan, F.T., Johns, R.B., 1986. Chemical markers for marine bacteria: fatty acids and pigments. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Environment. Elsevier, Amsterdam, pp. 291–309.

Hopkins, C.C.E., Sargent, J.R., Nilssen, E.M., 1993. Total lipid content, and lipid and fatty acid composition of the deep-water prawn *Pandalus borealis* from Balsfjord, northern Norway: growth and feeding relationships. Mar. Ecol. Prog. Ser. 96, 217–228.

Joseph, J., 1975. Identification of 3,6,9,12,15-octapentaenoic acid in laboratory-cultured photosynthetic dinoflagellates. Lipids 10, 395–403.

Kharlamenko, V.I., Zhukova, N.V., Khotimchenko, S.V., Svetashev, V.I., Kamenev, G.M., 1995. Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Mar. Ecol. Prog. Ser. 120, 231–241.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., Vyshkvartzev, D.I., 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Mar. Ecol. Prog. Ser. 220, 103–117.

Knox, G.A., 1986. Estuarine Ecosystems: A Systems Approach, vol. I. CRC Press, p. 198.

Kubo, H., Kurozumi, T., 1995. Molluscs of Okinawa. Okinawa Shuppan, p. 197. In Japanese.

Langdon, C.J., Newell, R.I.E., 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. Mar. Ecol. Prog. Ser. 58, 299–310.

Landgon, C.J., Waldock, M.J., 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. J. Mar. Biol. Assoc. U.K. 62, 431–448.

Mansour, M.P., Volkman, J.K., Jackson, A.E., Blackburn, S.I., 1999. The fatty acid and sterol composition of five marine dinoflagellates. J. Phycol. 35, 710–720.

Meziane, T., Tsuchiya, M., 2000. Fatty acids as tracers of organic matter in the sediment and food web of mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar. Ecol. Prog. Ser. 200, 49–57.

Meziane, T., Bodineau, L., Retiere, C., Thoumelin, G., 1997. The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt marsh-flat ecosystem of Mont-Saint-Michel Bay, France. J. Sea Res. 38, 47–58.

Meziane, T., Sanabe, M.C., Tsuchiya, M., 2002. Role of fiddler crabs of a subtropical intertidal flat on the fate of sedimentary fatty acids. J. Exp. Mar. Biol. Ecol. 270, 191–201.

Mfilinge, P.L., Atta, N., Tsuchiya, M., 2002. Nutrient dynamics and leaf litter decomposition in a subtropical mangrove forest at Oura Bay, Okinawa, Japan. Trees 16, 172–180.

Napolitano, G.N., Pollero, R.J., Gayoso, A.M., Macdonald, B.A., Thompson, R.J., 1997. Fatty acids as tropic markers of phytoplankton blooms in the Bahia Blanca Estuary (Buenos Aires, Argentina) and Trinity Bay (Newfoundland, Canada). Biochem. Syst. Ecol. 25 (8), 739–755.

Odum, W.E., Heald, E.J., 1972. Trophic analyses of an estuarine mangrove community. Bull. Mar. Sci. 22 (3), 671–738.

Perry, G.J., Volkman, J.K., Johns Jr., R.B., 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Acta 43, 1715–1725.

Pond, D.W., Bell, M.V., Harris, R.P., Sargent, J.R., 1998. Microplanktonic polyunsaturated fatty acid markers: a mesocosm trial. Estuar. Coast. Shelf Sci. 46 (Suppl. A), 61–67.

Pranal, V., Fiala-Medioni, A., Guezennec, J., 1996. Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific. Mar. Ecol. Prog. Ser. 142, 175–184.

Raclot, T., Groscolas, R., Cherel, Y., 1998. Fatty acid evidence for the important of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. Mar. Biol. 132, 523–533.

Sargent, J.R., 1976. The structure, metabolism and function of lipids in marine organisms. Biochem. Biophys. Perspect. Mar. Biol. 3, 149–212.

Sargent, J.R., Bell, M.V., Hendersen, R.J., Tocher, D.R., 1990. Polyunsaturated fatty acids in marine and terrestrial food webs. In: Mellinger, J. (Ed.), Animal Nutrition and Transport Processes: 1. Nutrition in Wild and Domestic Animals. Comp. Physiol. Karger, Basel, pp. 11–23.

Scribe, P., Fillaux, J., Laureillard, J., Denant, V., Saliot, A., 1991. Fatty acids as biomarkers of planktonic inputs in the stratified estuary of the Kraka River, Adriatic Sea: relationship with pigments. Mar. Chem. 32, 299–312.

Shokita, S., 1983. Macrofauna community structure and food chains at the mangals. Training courses on life history of selected species of flora and fauna in mangrove ecosystems. Natl. Res. Counc. Thail., Bangkok 2, 1–40.

Shokita, S., 2000. The role of aquatic animal in a mangrove ecosystem. Proceedings of an International Workshop, Okinawa, Japan, 26–30 March 2000 by Asia-Pacific Cooperation on Research for Conservation of Mangroves. The United Nations University, pp. 1–30.

Slim, F.J., Hemminga, M.A., Ochieng, C., Jannink, N.T., de la Moriniere, C., van der Velde, G., 1997. Leaf litter removal by snail *Terebralia palustris* (Linnaeus) and sesarmid crabs in an East African mangove forest (Gazi Bay, Kenya). J. Exp. Mar. Biol. Ecol. 215, 35–48.

Stuart, V., Field, J.G., Newell, R.C., 1982. Evidence for absorption of kelp detritus by mussel *Aulacomya ater* using a new ^{51}Cr-labeled microsphere technique. Mar. Ecol. Prog. Ser. 9, 263–271.

Trider, D.J., Castell, J.D., 1980. Effect of dietary lipids on growth, tissues composition and metabolism of the oyster (*Crassostrea virginica*). J. Nutr. 110, 1303–1309.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D., 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. 128, 219–240.

Wafar, S., Utawale, A.G., Wafar, M., 1997. Litter fall and energy flux in a mangrove ecosystem. Estuar. Coast. Shelf Sci. 44, 111–124.

Yoshimi, M., 1993. Mangrove Creatures of Iriomote Island, Okinawa, Japan. Nirai. In Japanese.