Journal of Sustainability Science and Management 2006 Volume 1(1): 92-111

FOOD SOURCES OF COEXISTING SUSPENSION-FEEDING BIVALVES AS INDICATED BY FATTY ACID BIOMARKERS, SUBJECTED TO THE BIVALVES ABUNDANCE ON A TIDAL FLAT

Z. BACHOK, P. L. MFILINGE, M. TSUCHIYA

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

T. MEZIANE

School of Environmental and Applied Sciences, Griffith University,
Gold Coast PMB 50 GCMC, Qld 9726, Australia

Abstract: Resources partitioning among co-existing suspension-feeding bivalves - *Cyclina sinensis, Gafrarium tumidum, Katelysia japonica, Psammotaea elongata* and *Semele carnicolor* on Tomigusuku intertidal flat, Okinawa was investigated using fatty acid (FA) biomarkers during the cold (January 2001) and warm seasons (July 2001). *P. elongata* is the most dominant infaunal species. Other species are semi-infaunal and minority on the tidal flat. The total FA methyl esters (FAMEs) content during both seasons was significantly higher in the tissues of *P. elongata* and *S. carnicolor* than in *C. sinensis, G. tumidum* and *K. japonica*. *P. elongata* showed most unique fatty acid characteristics compared to other species during the cold–season: low percentage of ω3 and ω6 polyunsaturated FAs (PUFA; 11.5% of total FAMEs) compared to others (23.6 to 37.3%), highest percentage of odd-numbered branched FAs (odd-BrFAs; 5.7 %), the revelation of even-numbered long-chain FAs (0.7%), and the lowest value of PUFA/saturated FA (SAFA), PUFA/monounsaturated FA (MUFA), 16:1ω7/16:0 and ω3/ω6 PUFA ratios. Analysis of specific FA markers (irrespective to their mean percentage) showed a significant contribution of diatom (16:1ω7 and 20:5ω3), dinoflagellates (18:4ω3 and 22:6ω3), bacterial (odd-BrFAs and 18:1ω7) and green macroalgal (18:2ω6 and 18:3ω3) markers in all bivalves during the cold-warm seasons. These indicate that the coexisting bivalves on Tomigusuku tidal flat utilize the same food sources, originating from phytoplankton, benthic microalgae, macroalgae detritus and bacteria. However, with references to the concentration of total FAMEs in all species, the level of most FAs (SAFA, MUFA, PUFA) and FA markers of food sources was significantly higher in *P. elongata* and *S. carnicolor*, suggesting that these bivalve species accumulate food more than other species. Because *P. elongata* is a deep burrower, this behaviour might have increased its survival rate and therefore its greater abundance on Tomigusuku tidal flat compared to other suspension-feeding bivalves.

KEYWORDS: Abundances, burrowing-depth, fatty acid, intertidal flat, suspension-feeding bivalves

Correspondence: Zainudin Bachok, Kolej Universiti Sains dan Teknologi Malaysia, 21030 Kuala Terengganu, Terengganu D. I., Malaysia

Introduction

Suspension-feeding bivalves represent a substantial proportion of intertidal communities. They play a significant role on primary production and in the production of biodeposit (Heip *et al.*, 1995; Chiantore *et al.*, 1998; Peterson and Heck, 1999). Beside larval supply and transport (Underwood and Keough, 2001), population dynamics of suspension-feeding bivalves is controlled by predation, which stimulates development of effective defenses against predators in prey species by living deep below sediment surface (Peterson, 1982). Also competition may result in elimination of suspension-feeding bivalves living in tidal flats (e.g. Carlson *et al.*, 1984; Peterson and Black, 1991). However, continuous competition among similar species shows niche partition. Therefore coexistence of species with similar food sources is possible either by resources partitioning, or by feeding on different food sources (Levinton, 2001).

The infaunal or semi-infaunal bivalve species on Okinawa Island inhabit shallow coastal water where large numbers of detrital particles, benthic algae and bacteria are often resuspended at sediment-water interface, and filtered relatively easily. Food sources of suspension-feeding bivalves on the intertidal flats are not yet fully investigated. In addition it is not known whether the bivalves compete or partition the resources available. In Mugu Lagoon, California, Peterson and Andre (1980) suggested that food is not the limiting factor for the coexisting suspension-feeding bivalves because all species feed by means of a siphon connected with the surface and even vary in density. We therefore hypothesized those suspension-feeding bivalves on Okinawa intertidal flat utilize the same food sources, which is not only contributed by phytoplankton but also by benthic microalgae, macroalgae, vascular plant detritus and bacteria.

In order to determine the bivalve food sources, it is necessary to take into account the pool sources that are utilized simultaneously by animals. But the impracticability for identifying a number of primary producers and microorganisms has constrained investigators to use gut analysis methodology, and hence variety of other methods, including fatty acid analyses have been established to determine animal food sources (e.g. Kharlamenko *et al.*, 2001). The biomarkers give information on dietary composition that have been integrated over a longer time scale, and may provide insights into the food sources that have been assimilated simultaneously by animals (Auel *et al.*, 2002).

The variability in bivalve morphology reflects the behavior (Morse and Zardus, 1997), which is one of the factors that influence species survival in a particular ecosystem. Blundon and Kennedy (1982) reported that burrowing-depth is animal specific characteristic and adaptation against predation and natural disturbance. Therefore, the level of exploitation of food source and the burrowing-depth could be among other factors that influence the variability in abundances of bivalve species on a tidal flat. The main objectives of this paper are 1) to find if there are any distinct fatty acid characteristics among bivalves, and 2) to find whether the coexisting bivalves utilize the different sources.

Material and methods

Study area

Samples were collected at Tomigusuku tidal flat, which is located in the southern part of Okinawa Island (Fig. 1). The area consists of a small portion of tidal flat that remains after the land reclamation (on the northern part), which began at the end of 1998. The tidal flat is in between the two major towns of the southern region of Okinawa. On the eastern side, the site is neighbored by Tomigusuku City, a small stream and a large roadside drainage that discharges into the tidal flats. On the western side it is neighbored by Itoman City, the Mukue River that also empties into the tidal site. The other anthropogenic features that surround the study area includes housing, a small industry, cultivated fields, etc. Physical feature of the flat is characterized by sand sediment

associated with the coral rubbles. In addition to that, there are considerable patches of muddy sediments and in places rock flats interspersed with sandy pockets.

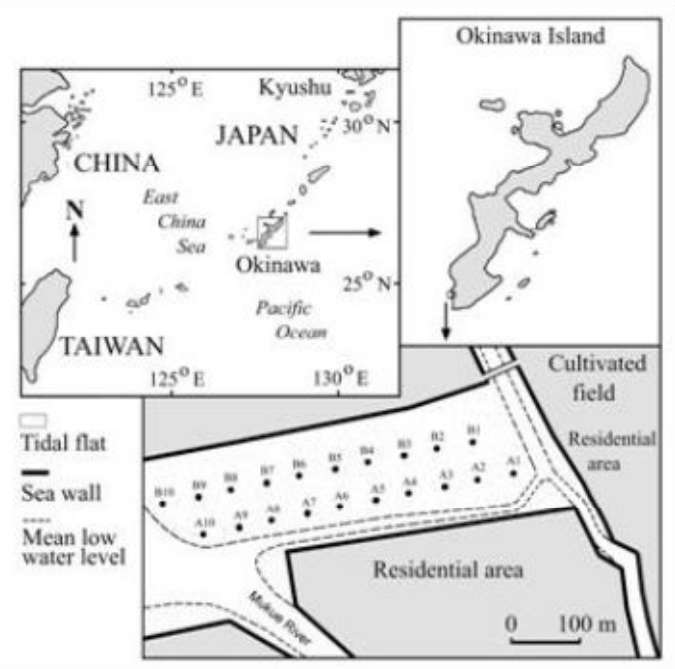


Figure 1. Location of Okinawa Island and sampling site at Tomigusuku intertidal flat

The tidal flats have a thick cover of green macroalgae, mainly of the species *Enteromorpha intestinalis* and *Ulva pertusa*, which grow during the cold season (December to March). These dense macroalgae tend to decompose with the onset of the rainy season (April to June). Within the upper and middle parts of the intertidal flats, the macrozoobenthos from 5 species of fiddler crab – *Uca crassipes, U. dussumieri, U. lactea, U. vocans* and *U. tetragonon,* and the gastropod of species *Batillaria zonalis* dominate. From the middle toward lower intertidal zone, these organisms are absent, but the deposit-feeding bivalve *Quidnipagus palatum* occur abundantly. On the small rock flat scattered on tidal flat, the sessile animals such as mussel dominate. Other macrozoobenthos include polychaete worms, benthic fishes, mudskippers, benthic shrimps and hermit crabs. This tidal flat is also a feeding and nursery ground for migratory species, in particularly birds. Based on the annual report of an environmental survey by the Okinawa Prefecture at the study site, the major benthic microalgae are *Coconeis* sp., *Nitzschia* spp., *Navicula* sp. and *Pleurosigma* sp. The concentration of chlorophyll *a* was higher in the warm season (27-29 July 1999; 5.5 to 6.4 μg g^{-1} dry wt) than in the cold season (23-25 January 2000; 2.4 to 2.5 μg g^{-1} dry wt). The mean biomass of aerobic and anaerobic bacteria in warm season were 1.6×10^6 CFU g^{-1} and 1.6×10^5 CFU g^{-1}, respectively; while in cold season they dropped to 6.2×10^5 CFU g^{-1} and 6.0×10^4 CFU g^{-1}, respectively.

Study species

Preliminary survey was done at the end of the year 1998, five species of benthic suspension-feeding bivalves were observed: *Cyclina sinensis* (Veneridae), *Gafrarium tumidum* (Veneridae), *Katelysia*

japonica (Veneridae), *Psammotaea elongata* (Psammobiidae) and *Semele carnicolor* (Semelidae) co-existing on Tomigusuku tidal flat. During the observation it was relatively easier to collect the bivalves on the tidal flat, especially *C. sinensis, G. tumidum, K. japonica* and *S. carnicolor* because upper parts of their shells protruded above sediment surface, and made them more visible. However, *P. elongata*, could only be collected by scraping the tidal flat sediment to a considerable depth. The bivalves were recognized as suspension-feeders by examining their internal and external morphology (Brusca and Brusca, 1990).

To study the bivalve feeding modes in detail, animals with the shell length within 3 to 5 cm were collected from intertidal flat, just after the tidal flat was exposed during low tide. The bivalves were brought immediately to the laboratory, and were kept in running filtered seawater. For every species fifteen individuals were placed on the sediment surface of experiment tank having dimensions measuring 36 cm (W) x 69 cm (L) x 31 cm (H) and contained 3300 cm^3 tidal flat sediments (grain size <4 mm). The animals that did not burrow or non-active within several hours were replaced with the new one. The experiment tanks were kept in the large 900-liter fiberglass container (Fig. 2). Fresh, aerated and filtered seawater was supplied into large 900-liter fiberglass container and the water channeled into experiment tanks from the bottom part. The water level in the tank was controlled by a Water Level Controller. Seawater from the field was supplied into experiment tanks and controlled by Seawater Input Controller. Aeration processes provided artificial physical mechanism for mixing and maintenance of particle suspension in uniform state inside experiment tanks. Outflow Pump attached with a timer controlled the tidal rhythm, at a particular set time, the water inside the big container was siphoned out and new filtered seawater entered the container. A series of observation was done: between 22 February 1999 and 22 April 1999, 1 July 1999 and 29 August 1999, and between 1 September 1999 and 24 September 1999. The animals feeding behavior and the type of materials thrown out of the siphon and its deposition were observed and recorded. At the end of each experiment the bivalves that appeared at the sediment surface (including semi-infaunal individual) were removed and counted as surface individuals. The sediments in the experiment tanks were then scrapped carefully from the upper layer down to a depth of 2 cm. The uppermost end of bivalve shells that occurred from the sediment surface to the beginning and end of two cm sections were recorded.

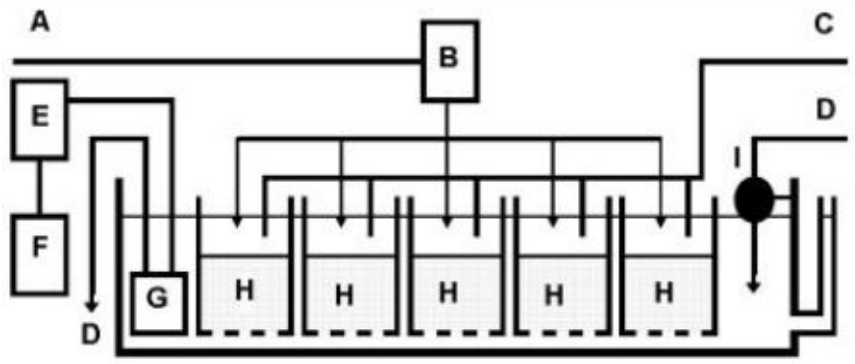


Figure 2. Schematic diagram of a simulated tidal flat system. A, seawater input; B, seawater input controller; C, aeration; D, filtered sea water; E, outflow controller; F, timer; G, outflow pump; H, sediment; I, water level controller.

Quantitative sampling of the bivalve species was carried out on 2 different periods, from May to October 1999 (rainy season – warm season – typhoon seasons), and from November 2000 to April

2001 (Okinawa autumn – cold season – beginning of rainy season). All sampling was done in the daytime during the spring tide. Two transect lines A and B was set with 20 sampling stations (Fig. 1). Stations A1 to A10 were located along transect A while stations B1 to B10 were located along transect B. The stations are located within the high- and low-water marks, with interval of 50 meters. At each station, 4 replicates of a quadrate (25 cm x 25 cm) were set randomly for the study. Benthos were obtained by scraping the upper layer using a small scoop. The benthos samples were divided into 3 layers, depth of 0-5, 5-10 and > 10 cm. All benthos samples were sieved immediately, initial using a 4-mm mesh sieve and then followed by a 1-mm mesh sieve. The bivalves remaining on the sieve were collected and were brought to the laboratory located next to the tidal flat. The shell length (mm), defined as the longest distance of shell (anterior to posterior) was determined.

To examine the sediment characteristics, the sediment samples were randomly collected from all studied stations to a depth of 5 to 10 cm with a small scoop in July 2000. The sediment samples were first oven-dried at 60°C for 48 hours to a constant weight, weighed and wet-sieved using a 0.063 mm mesh sieve to separate the silt-clay fraction. The samples were then re-dried in the oven and re-weighed. After removal of the coral rubbles (>4 mm), the sediments were dry-sieved (Buchanan, 1984) through a series of sieves, consisting of 2, 1, 0.5, 0.25, 0.125 and 0.063 mm mesh openings by a mechanical shaker. Fraction retained on each sieve were weighed and recorded. The fraction removed during the first (wet) sieving was mixed to the <0.063 mm fraction obtained in the dry-sieving process.

Sample collection and preparation for fatty acid study

Animal specimens (within 20 to 50 mm in shell length) were collected at low tide (Fig. 1). Sampling was done on two different occasions. The first sampling was held on 27-30 January 2001, the coldest month in Okinawa (mean air temperature was 18°C) and the second sampling on 20-24 July 2001, which is the middle of the warm season in Okinawa (mean air temperature was 28°C). The animals were carried immediately to the laboratory where they were kept in filtered seawater (~15 hours) for gut content clearance, and then stored immediately by freezing at – 20°C. Prior to lipid extraction, the clam shells were opened to collect their tissues. The tissues were cleaned thoroughly in filtered seawater, chopped finely and between 2.3 to 7.2 g of tissue (wet weight) was used for lipid extraction. Tissues from three different individuals for each species were prepared for lipid extractions.

Lipid extraction

Lipids were 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). They 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 extracts were saponified under reflux (2 h, 100°C) with a 2 mol dm^{-3} NaOH solution in methanol and distilled water (2:1, v:v). After acidification with ultra pure HCL solution (37.5%), 2 x 2 cm^3 of chloroform were added successively to recover lipids. However, due to the saponification process, a small portion of phospholipids remains in the water layer and was not recovered. The solvent was evaporated under a nitrogen stream and the fatty acids were converted to methyl esters under reflux with 1 ml of 14% BF_3-methanol for 10 min. Total lipid extracts were re-extracted with cloroform and washed with distilled water. After evaporation under a nitrogen stream, the extracts were weighed and redissolved in chloroform/methanol (2:1, v:v).

Lipids were purified by the thin-layer chromatography technique (TLC) using Merck plates coated with kieselgel 60 silica (Darmstadt, Germany). The solvent used for developing was a mixture of hexane/diethyl ether/acetic acid (70:30:1). Band containing fatty acid methyl esters (FAMEs) were scraped and collected in a mixture of chloroform/methanol (2:1, v;v) at 40°C for 60

min. FAMEs were then isolated in the same solution until analysis by gas chromatography. For all samples, a second plate was prepared in order to estimate the proportion of FAMEs in the obtained lipids (Yamashiro *et al.*, 1999). The chromatogram was immersed in phosphoric acid/33% acetic acid/sulphuric acid/0.5% copper sulfate (5:5:0.5:90, v:v:v:v). After drying, the plate was heated at high temperature to make the lipid spots visible and then was scanned (Epson GT-9000), and the image was stored with Adobe Photo-Shop software (Adobe system). An image analysis program (NIH 6 image) was used to estimate the relative contribution of the fatty acids, in the lipids, by integrating the chromatogram.

The FAMEs were analyzed by a GC 14-B Shimadzu gas chromatograph equipped with flame ionization. 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 ionization was held at 240°C. FAMEs were identified by comparing their retention times with those of a standard. Fatty acids are designated as *A*:*B*ω*C*, where *A* is the no. of carbon atoms, *B* is the no. of double bonds and *C* is the position of the ultimate double bond from the terminal methyl group.

Data analysis

The feeding behaviors were qualitatively compared among the species. These included the texture of materials thrown out of the siphon and its deposition. The frequency of occurrence of bivalve species at sediments surface and every 2 centimeters depth was measured and compared. To evaluate quantitative aspect of the bivalve community, the percentage of total number of individuals of each species to the total number of benthic bivalve community in 2 sampling periods (May-October 1999 and November 2000-April 2001) was measured. The total number of each bivalve species in each sampling month was measured from the pool data of all quadrates (5 m^2). The total number of each bivalve species was divided into three layers, 0-5, 5-10 and >10 cm and the mean density in all sampling months was measured. The total number of bivalves for the pooled data of the 4 quadrates (0.25 m^2) was measured at every station and the mean density in all sampling months was measured. For sediment data, the relative importance of sand, coral rubbles and silt + clay fractions were expressed in percentages of dry weight. The relationships of each sediment fractions with the mean density of bivalves were determined using simple regression analysis.

The total FAMEs concentration, the percentages of fatty acids and the fatty acid ratios in the tissues of all species and seasons were compared using ANOVA. Species (*C. sinensis, G. tumidum, K. japonica, P. elongata* and *S. carnicolor*) and seasons (cold and warm) were entered as fixed factors. The data were arcsine p-transformed before analysis (Zar, 1999). The analyses were performed by using Stat View 5 software at 95% confidence intervals. Fatty acid markers in the tissue of bivalve were analyzed to determine the food sources. The distinct fatty acid characteristics of the bivalves subjected to their population density on tidal flat were investigated.

Results

Feeding modes and burrowing depth of bivalve species

The bivalves *C. sinensis* and *P. elongata* eject very fine particles from exhalant siphon [Fig. 3-A-(a), D-(e)]. These particles were deposited and scattered around the siphon in a wide area on sediment surface [Fig. 3-A-(b), D-(f)]. The ejected materials of *G. tumidum, K. japonica* and *S. carnicolor,* easily identified, were deposited near the entrance of the siphons. In the case of *G. tumidum*, about one to two millimeters long pellets were accumulated just in front of the siphon [Fig. 3-B-(c)] while in *K. japonica* the ejected materials were characterized by accumulation of numerous long pellets

[Fig. 3-C-(d)]. However, in *S. carnicolor* two types of ejected materials were observed, cylindrical and several millimeters long pellet [Fig. 3-E-(g), (h)].

The mean frequency of occurrence (%) of bivalve species at sediment surface and every 2 cm depth at the end of laboratory experiment shows that *P. elongata* was deep-burrowing species (Fig. 3-D). Their mean frequency of occurrence was the highest at the depth of >10 cm (~28%). The occurrence of *P. elongata* at the sediment surface was low (<3%). *C. sinensis* and *S. carnicolor* burrowed down to 6 and 8 cm depth, respectively (Fig. 3-A, E). However, the mean frequency of occurrence of both *C. sinensis* and *S. carnicolor* were highest at the depth of 2-4 cm (41 and 33%, respectively). *G. tumidum* and *K. japonica* burrowed to the depth of 2 cm but their frequency of occurrence was generally high at the sediment surface (>55%; Fig. 3-B, C).

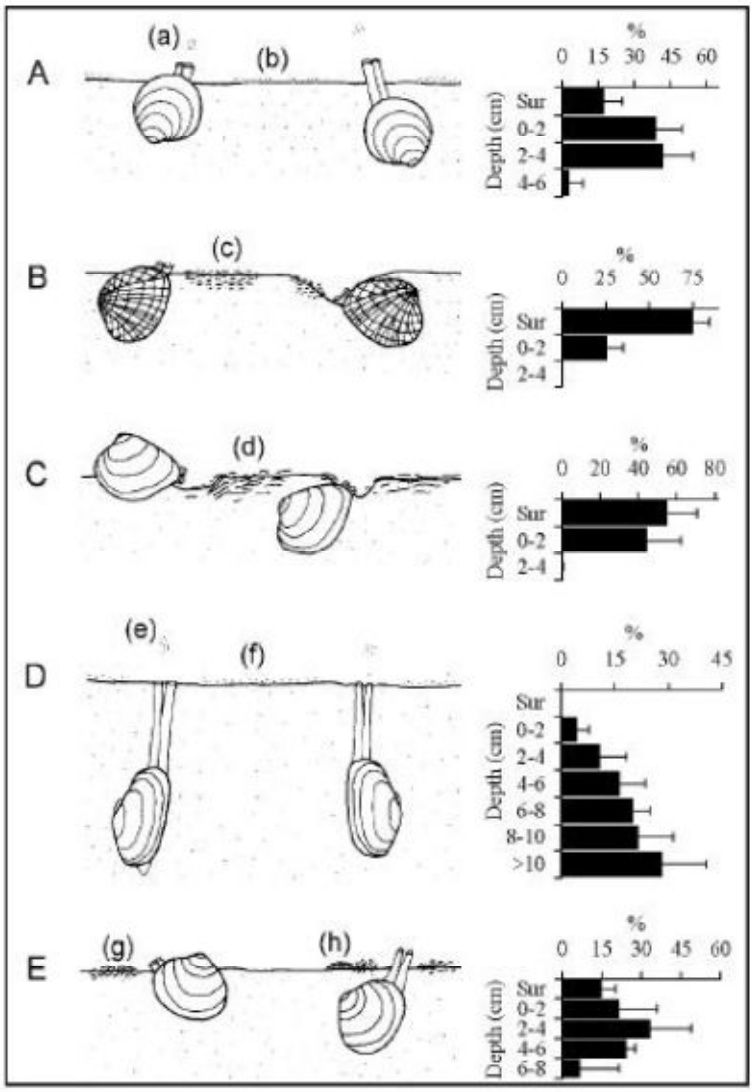


Figure 3. Schematic diagram of feeding modes of bivalve species (left) and their frequency of occurrence at particular depth (right). Values are mean percentage ± SD of 3 experimental observations. A, Cyclina sinensis (shell length 3.2 to 4.7 cm, n = 15); B, Gafrarium tumidum (shell length 3.5 to 5.0 cm, n = 15); C, Katelysia japonica (shell length 3.8 to 4.9 cm, n = 15); D, Psammotaea elongata (shell length 3.0 to 5.0 cm, n = 15); and E, Semele carnicolor (shell length 3.2 to 4.5 cm, n = 15). (a) to (h) are materials thrown from the siphons by the animals

Abundances of bivalve species

The result of abundances study of this bivalve community throughout two sampling periods, May to October 1999 and November 2000 to April 2001, indicated that *P. elongata* comprises 45 to 88% of the total abundances of suspension-feeding bivalves on tidal flat. Other species represent <25% of the total abundances. Comparison between the 2 sampling periods (May-October 1999 and November 2000-April 2001) show an increase in the mean density of *P. elongata* in November 2000-April 2001 and a decrease in other species (Table 1).

The bivalves are more abundant from the mid toward the lower tidal area, and showed a particularly highest density at the mean low water levels (Fig. 4). The percentages of sand, coral rubbles and silt + clay from the upper tidal level toward the lower tidal area are shown in Fig. 5. Sand fraction was the major component in most areas on tidal flat (73 to 96 %), except at the station A1, in which the composition of silt + clay was highest (64 %). In other stations, the composition of silt + clay in sediment ranged from 2.6 to 22.7 %. For both transects, the composition of coral rubbles increased from the mid tidal flat toward the lower tidal area. Among the sediment fractions, only coral rubbles showed strong correlation (r^2 = 0.941) with bivalves mean density.

Comparison of bivalve mean density at different depths 0-5, 5-10 and >10 cm on tidal flat shows the increase with depth in *P. elongata* (Fig. 6). The densities were always higher at the layer deeper than 10 cm than 5-10 cm depth. The mean density of *S. carnicolor* was highest at the depth of 5-10 cm while for *C. sinensis* at the depth of 0-5 cm. *G. tumidum* and *K. japonica* were only observed at the depth of 0-5 cm (Fig. 6).

Table 1 Density (individuals/5 m^2)* and size range (mm) of bivalve species collected from Tomigusuku intertidal flat.

	Cyclina sinensis		*Gafrarium tumidum*		*Katelysia japonica*		*Psammotaea elongata*		*Semele carnicolor*	
	Density	Size	Density	Size	Density	Size	Density	Size	Density	Size
11-18 May 99	16 (19)	10 - 45	4 (5)	36 - 44	8 (10)	8 - 26	37 (45)	8 - 62	18 (22)	18 - 39
18-25 June 99	10 (12)	4 - 37	1 (1)	42 - 42	9 (11)	21 - 47	46 (56)	10 - 75	16 (20)	24 - 41
12 - 14 July 99	1 (2)	17	5 (10)	43 - 43	6 (12)	20 - 43	31 (61)	14 - 67	8 (16)	23 - 40
26-28 Aug 99	4 (13)	9 - 20	1 (3)	45 - 45	—	—	19 (63)	7 - 63	6 (20)	24 - 36
26-30 Sept 99	—	—	6 (8)	39 - 48	5 (6)	10 - 43	54 (70)	11 - 64	12 (16)	26 - 41
25-26 Oct 99	2 (4)	15 - 25	3 (5)	39 - 44	2 (4)	11 - 30	40 (71)	11 - 70	9 (16)	25 - 38
24-25 Nov 00	—	—	5 (10)	33 - 48	4 (8)	14 - 25	34 (71)	15 - 65	5 (10)	22 - 36
13 & 19 Dec 00	—	—	—	—	1 (2)	18	42 (88)	5 - 71	5 (10)	19 - 36
26-27 Jan 01	—	—	1 (2)	36	2 (4)	21 - 34	35 (70)	16 - 71	12 (24)	24 - 41
25-27 Feb 01	1 (1)	28	2 (2)	44 - 48	4 (5)	19 - 30	65 (78)	11 - 68	11 (13)	21 - 39
24 & 27 Mar 01	1 (2)	23	1 (2)	43	5 (8)	14 - 30	50 (76)	7 - 63	9 (14)	13 - 39
24-27 Apr 01	1 (2)	20	—	—	7 (13)	14 - 20	43 (81)	10 - 68	2 (4)	29 - 39

Values in parathenses are % of total bivalve density in each month.
**: Sampling was done at 20 stations on tidal flat. At each station, 4 quadrates (25 cm x 25 cm) were set randomly. The total number of each bivalve species from the pool data of all 80 quadrates (5 m^2) was measured in each sampling month. —: not found during sampling.*

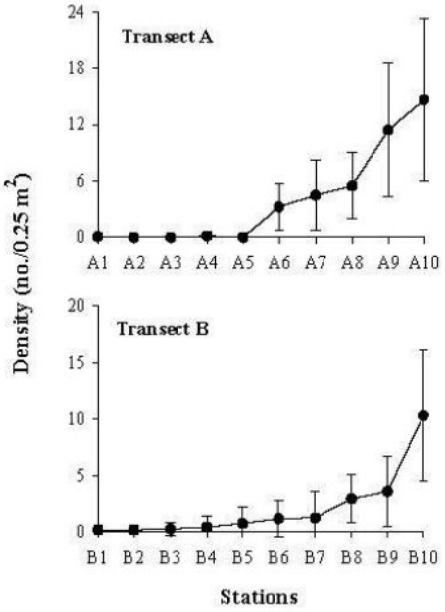


Figure 4. Density of benthic bivalves (no./0.25 m^2) at each stations on two transect lines A and B. Values are mean ± SD of all sampling months (May to October 1999 and November 2000 to April 2001).

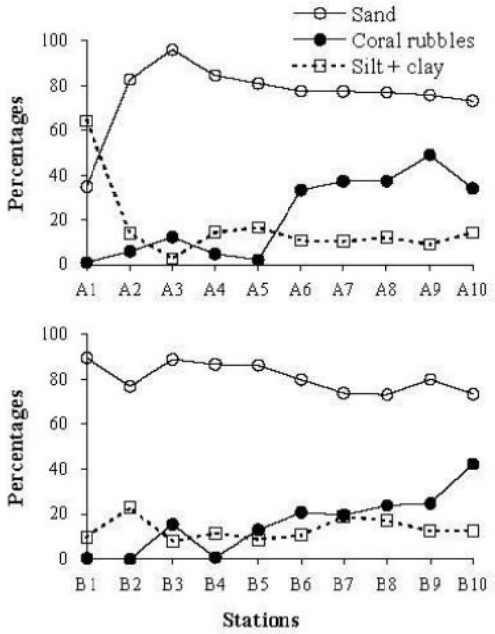


Figure 5. Percentages of sand (63 µm to 2 mm), coral rubbles (> 4 mm) and silt + clay (< 63 µm) at each stations on two transect lines A and B

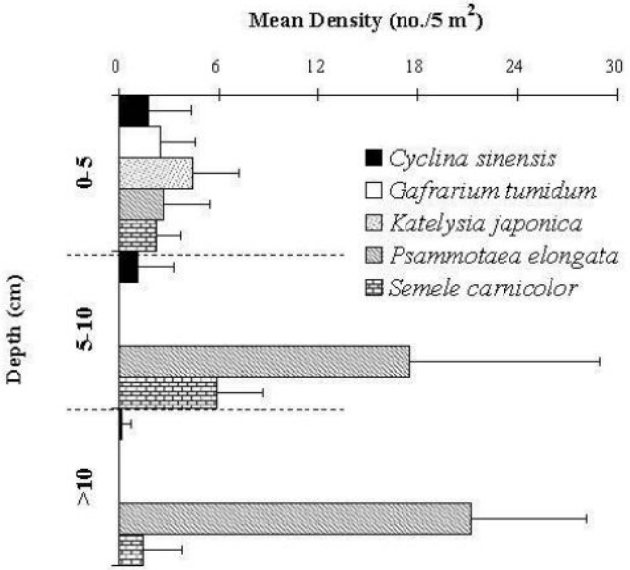


Figure 6. Density (no./5 m^2) of benthic bivalves at the depth of 0-5, 5-10 and >10 cm at Tomigusuku intertidal flat. Values are mean ± SD of all sampling months (May to October 1999 and November 2000 to April 2001).

Fatty acid of bivalve species

A total of 38 identifiable FAs, that contributed = 0.5 % of total FAMEs, were recorded in the tissues of bivalves. The percentages of some FAs are shown in Table 2. Table 3 shows the statistical summary from ANOVAs, comparing the mean percentage of FAs and FA ratios among bivalve species, and between the cold and warm seasons. Palmitic acid (16:0) was the main FA in the tissues of all species except for the cold-season samples of *K. japonica* (Table 2). The mean percentages of palmitic acids were significantly higher in warm season (20.7 to 29.5%) than cold season (13.1 to 19.2%) in *C. sinensis, G. tumidum* and *K. japonica* while in *P. elongata* and *S. carnicolor* the mean percentages were almost similar in both seasons (22.4 to 26.3%). Stearic acid (18:0) was also high, especially in the tissue samples of *G. tumidum* (10.0%) and *P. elongata* (15.5%) during the cold season, as well as in both seasons in *K. japonica* (11.3 to 15.5%). In other samples the mean percentages of stearic acids were found to be below 8.4% of total FAMEs.

Table 2. Fatty acid (FA) in the tissues of *Cyclina sinensis, Gafrarium tumidum, Katelysia japonica, Psammotaea elongata* and *Semele carnicolor* collected from Tomigusuku tidal flat.

	Cyclina sinensis		*Gafrarium tumidum*		*Katelysia japonica*		*Psammotaea elongata*		*Semele carnicolor*	
Fatty acids	Cold	Warm	Cold	Warm	Cold	Warm	Cold	Warm	Cold	Warm
Σ FAMEs (mg g^{-1})*	1.2 (0.2)	2.1 (0.6)	1.6 (0.2)	1.4 (0.1)	0.9 (0.2)	0.7 (0.1)	6.3 (1.3)	6.7 (2.0)	4.8 (1.3)	4.3 (0.5)
16:0	19.2 (0.5)	24.3 (6.4)	16.8 (1.4)	20.7 (5.5)	13.1 (0.8)	29.5 (4.7)	26.3 (3.3)	24.6 (0.9)	22.4 (3.6)	23.2 (4.0)
18:0	6.6 (1.2)	5.7 (0.6)	10.0 (1.4)	5.4 (1.4)	11.3 (0.2)	11.9 (6.4)	15.5 (2.6)	8.4 (1.4)	6.8 (0.7)	6.9 (2.9)
15:0 iso	-	n.d	n.d	n.d	-	n.d	1.1 (0.5)	n.d	0.6 (0.4)	-
17:0 iso	2.7 (0.4)	2.8 (0.5)	3.3 (0.3)	2.0 (0.5)	1.7 (0.1)	3.6 (0.6)	4.5 (3.5)	2.8 (1.0)	2.6 (0.8)	1.7 (0.3)
17:0 anteiso	0.8 (0.1)	1.0 (0.3)	1.0 (0.1)	n.d	0.8 (0.1)	0.7 (0.3)	n.d	n.d	0.6 (0.4)	0.7 (0.2)
16:1?7	4.1 (0.0)	5.7 (1.8)	4.4 (0.3)	7.0 (2.0)	3.3 (0.2)	7.3 (1.5)	3.9 (0.6)	9.0 (0.6)	8.1 (1.7)	8.0 (2.3)
18:1?7	5.9 (0.2)	6.5 (1.9)	5.3 (0.6)	4.6 (0.5)	3.5 (0.9)	4.3 (0.4)	3.8 (2.7)	5.3 (0.3)	7.7 (1.3)	6.1 (1.1)
18:1?9	6.1 (0.4)	7.7 (1.8)	8.2 (0.7)	2.3 (0.8)	10.4 (1.5)	3.5 (0.8)	11.3 (3.5)	6.8 (1.2)	7.5 (1.5)	7.8 (2.4)
18:2?6	1.9 (0.0)	2.7 (0.8)	1.9 (0.2)	0.8 (0.2)	1.1 (0.1)	1.0 (0.3)	1.6 (0.4)	1.8 (0.4)	3.2 (0.0)	2.4 (0.4)
18:3?3	3.4 (0.4)	3.9 (0.9)	2.6 (0.3)	1.7 (0.4)	2.2 (1.2)	1.5 (0.7)	0.6 (0.4)	2.4 (0.5)	3.5 (0.5)	3.1 (0.3)
18:4?3	2.5 (0.4)	3.0 (0.4)	2.1 (0.6)	2.2 (0.5)	1.3 (0.5)	1.7 (0.7)	1.3 (0.9)	1.5 (1.1)	2.8 (0.6)	2.1 (0.3)
20:5?3	6.4 (0.8)	8.6 (1.2)	5.5 (1.5)	15.9 (3.2)	3.6 (0.5)	8.4 (2.9)	0.9 (0.6)	10.6 (2.7)	8.2 (2.7)	10.1 (1.7)
22:6?3	8.7 (1.4)	6.3 (1.7)	6.6 (1.2)	6.9 (2.3)	14.6 (0.6)	4.9 (2.1)	1.5 (0.7)	3.8 (0.7)	2.7 (0.3)	3.5 (0.6)
Σ SAFAs¶	31.6 (1.7)	36.8 (8.4)	33.6 (2.4)	32.6 (5.2)	28.4 (1.1)	49.7 (10.9)	49.9 (5.5)	42.8 (5.0)	36.5 (4.1)	37.4 (3.7)
Σ odd-BrFAs	3.5 (0.6)	3.8 (0.8)	4.2 (0.3)	2.0 (0.5)	2.5 (0.1)	4.3 (0.8)	5.7 (4.0)	2.8 (1.0)	3.8 (1.6)	2.4 (0.3)
Σ MUFAs¶	26.6 (0.7)	27.1 (2.3)	28.2 (1.5)	19.8 (2.3)	31.0 (1.6)	19.8 (3.2)	27.3 (2.9)	26.7 (1.2)	29.0 (1.3)	30.9 (1.6)
Σ PUFAs¶	35.7 (0.5)	30.4 (5.7)	31.1 (3.5)	40.7 (8.2)	33.6 (3.1)	23.6 (7.8)	15.6 (3.1)	25.8 (4.5)	26.6 (2.4)	27.8 (3.2)
Σ even-LCFAs¶	n.d	n.d	n.d	n.d	n.d	n.d	0.7 (0.5)	n.d	n.d	n.d
Σ Other FAs **	1.9 (1.2)	0.7 (0.6)	1.2 (0.3)	2.4 (0.5)	3.8 (3.0)	1.8 (0.4)	n.d	1.0 (0.0)	3.5 (3.8)	1.0 (0.0)
Σ Unidentified FAs	0.6 (0.1)	1.3 (0.2)	1.6 (0.4)	2.3 (0.6)	0.5 (0.4)	1.1 (0.2)	0.9 (0.0)	0.8 (0.1)	1.0 (0.0)	0.6 (0.1)

Values for individual and sum of fatty acids are the mean % of total fatty acid methyl esters (FAMEs). Values in parentheses are standard deviations (n=3). n.d: not detected; - : <0.5 %; ¶: includs fatty acids not shown in this table; SAFAs: saturated FAs; odd-BrFAs: odd-numbered branched FAs; MUFAs: monounsaturated FAs; PUFAs: polyunsaturated FAs; even-LCFAs: even-numbered long-chain FAs.

** Concentration data are expressed per gram wet tissues. The average water content in the tissues of all bivalve species is 69.7 ± 5.3 %. The average percentage of total FAMEs to the total lipids in all sample is 46.6 ± 9.8 %.*

*** Other FAs: sum of 16:0 iso, 16:0 anteiso, 18:0 iso, 18:0 anteiso, 25:0, 27:0 and 29:0.*

Despite the dominance of saturated FA (SAFA), the mean percentage of polyunsaturated FA (PUFA) was higher than monounsaturated FA (MUFA) in all species except in *P. elongata* and *S. carnicolor* (Table 2). With the exception of the cold–season samples of *P. elongata*, PUFAs were characterized by high percentages of ω3 and ω6 FA series (23.6 to 37.3% of total FAMEs). The FA of *P. elongata* during the cold–season was characterized by the low percentage of ω3 and ω6 PUFAs (11.5%), relatively higher percentage of odd-numbered branched FAs (odd-BrFAs; 5.7 %) compared to other samples (=4.2%), even long-chain FAs though in very small percentages (=0.7%), and the lowest value of PUFA/SAFA, PUFA/MUFA, 16:1 ω7/16:0 and ω3/ω6 PUFA ratios (Fig. 7).

Table 3 Statistical summary from ANOVAs comparing the percentages of fatty acids and fatty acid ratio among species (*Cyclina sinensis, Gafrarium tumidum, Katelysia japonica, Psammotaea elongata* and *Semele carnicolor*) and between seasons (cold and warm).

	Species		Seasons			Species		Seasons	
Fatty acids	$F_{4, 20}$	P	$F_{1, 20}$	P	Fatty acids	$F_{4,20}$	P	$F_{1, 20}$	P
16:0	1.604	0.2121 ns	8.562	0.0084 **	(odd-BrFA + 18:1 ω7) [g]	1.340	0.2899 ns	0.827	0.3740 ns
18:0	4.524	0.0091 **	5.031	0.0364 *	(16:1ω7 + 20:5 ω3) [h]	6.247	0.0020 **	76.341	<0.0001 ***
16:1ω7 [a]	3.517	0.0249 *	18.605	0.0003 ***	(18:2ω6 + 18:3 ω3) [c, i]	11.852	<0.0001 ***	0.061	0.8072 ns
18:1ω7 [b]	3.937	0.0162 *	0.052	0.8221 ns	(18:4ω3 + 22:6 ω3)	17.186	<0.0001 ***	6.080	0.0228 *
18:4ω3 [c]	3.619	0.0224 *	0.048	0.8295 ns	Σω3 PUFAs	6.200	0.0021 **	4.826	0.0400 ns
20:5ω3 [d]	4.300	0.0113 *	40.836	<0.0001 ***	Σω6 PUFAs	2.601	0.0671 ns	13.136	0.0017 **
22:6ω3 [e]	20.694	<0.0001 ***	8.499	0.0086 **	PUFA/SAFA	3.799	0.0112 *	0.018	0.8944 ns
ΣSAFAs	3.367	0.0292 *	2.278	0.1469 ns	PUFA/MUFA	6.218	0.0008 *	4.466	0.0452 *
Σodd-BrFAs [f]	0.386	0.8162 ns	2.025	0.1702 ns	(16:1ω7/16:0) [j]	7.714	0.0002 ***	62.551	<0.0001 ***
ΣMUFAs	4.751	0.0074 **	15.843	0.0007 ***	ω3/ω6 PUFA	9.228	<0.0001 ***	40.494	<0.0001 ***
ΣPUFAs	5.817	0.0028 **	0.299	0.5904 ns					

References relating to fatty acids, sum of fatty acids and fatty acid ratios that have previously been related to food sources; [a] *Dunstan et al., (1994),* [b] *Gillan and Johns (1986),* [c] *Meziane and Tsuchiya (2000),* [d] *Volkman et al. (1989),* [e] *Pond et al. (1998),* [f] *Perry et al. (1979),* [g] *Johns et al. (1979),* [h] *Kharlamenko et al. (2001),* [i] *Napolitano et al. (1997),* [j] *Kharlamenko et al. (1995).*

ns *not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.*

The FAs 20:5 ω3 and 22:6ω3 were the more dominant PUFAs in the examined bivalves compared to 18:2 ω6, 18:3 ω3 and 18:4 ω3 (Table 2). The FA 20:5 ω3 dominated the PUFAs in the warm season rather than the cold season for all species. In contrast to 20:5 ω3, 22:6ω3 dominated the PUFAs in the cold season of all species except in *P. elongata* and *S. carnicolor* (Table 2). The high amounts of MUFAs in bivalves were indicated by the high contributions of FAs 16:1 ω7 and 18:1 ω9. The mean percentage of 16:1 ω7 was significantly higher in the warm season for all species, except for *S. carnicolor* (Table 2). The mean percentages of 18:1 ω7 did not differ significantly between the cold and warm season, but was significantly different among bivalves (Table 3).

Discussion

Abundances, burrowing-depth and feeding modes of bivalve species

The increase of individuals collected for *P. elongata* and consequently the decrease for other species during November 2000 to April 2001 may suggest a slight change of abundances of suspension-feeding bivalves on Tomigusuku intertidal flat over two sampling periods (Table 1). The higher level of abundance in *P. elongata* on the Tomigusuku tidal flat compared to other species indicates that it is a major component of suspension-feeding bivalves and may have a significant role on the intertidal flat ecology. The bivalve vertical distribution in laboratory and field (Figs 3 and 6) characterized *P. elongata* as deep burrowing species (>10 cm), *C. sinensis* and *S. carnicolor* were medium burrowing (~4 cm), and *G. tumidum* and *K. japonica* live just below the sediment surface. However, this burrowing-depth could be overestimated because the bivalves in the study might migrate deeper as a response to the low tide condition prior to sampling, and/or the bivalves might increase their burrows due to disturbance when the surface sediments in the experiment tanks is scrap at the end of experiments (Roberts *et al.*, 1989). Zaklan and Ydenberg (1997) found that shallowly buried bivalves had a lower survival rate than those buried more deeply. At Tomigusuku tidal flat, typhoon winds occur from July to September. Rees *et al.* (1979) found that the coastal zone processes that cause mass striding especially during their extreme season, play a part in the formation of benthic associations as well as their destruction. Therefore, the deeper the burrow, the lower the risk of being washed out (Zwarts and Wanink, 1984). Thus this could suggest that *P. elongata* might have higher survival rate than other species on tidal flat due to their behavior that burrow deep below sediment surface.

The mean densities of bivalves were increased from the middle stations towards the stations nearer the low tide level (Fig. 4). Benthic bivalves have pelagic larval phases and are dependent on offshore currents to carry mature larvae into suitable intertidal flat area for settlement (Young, 2001). Jensen (1992) discussed briefly the importance of submersion time and intraspecific density for recruitment and growth of cockles. He found that annual growth rates of individual cockles and the abundance of spat along the tidal gradient were positively correlated with submersion time. Furthermore, a decrease in submersion time also determines the foraging time available for intertidal bivalves, and an increase in submersion time may have adverse physiological effects, because they may be exposed to harmful salinity and temperatures (Peterson and Andre, 1980). Thus, this may explain the higher density of bivalve community at stations nearer to the mean low water level of the tidal flat.

The mean density of bivalve increased with the percentage of coral rubbles in the tidal flat sediments (simple regression $P < 0.01$). A previous study in an intertidal sand flat on the west coast (Yellow sea) of Korea, indicates that after seawall construction sediment size became coarser and total abundance and biomass of macrobenthic community increased (Ahn and Choi, 1998). In the bivalve *Mactra veneriformis*, the abundance increased ten times, seemingly to benefit from the coarser grain size. In addition, Thorin *et al.* (2001) found that the area which is more unstable (strong currents, strong effect of swell and to river channels which have very unstable courses) sustained higher densities of bivalves. The coarse sediments are more resistant to movement during strong water current than fine sediments, thus the bivalves that live in this habitat might have higher protection from natural disturbance as well as their dispersal (Commito *et al.*, 1995).

Bivalves pump and filter considerably volumes of water in order to consume sufficient food to survive (Meyhofer, 1985). Newell (1979) discussed the ciliary's mechanism of food collection in Eulamellibranchia whereas the water is drawn into the inhalant siphon, the current was created by means of a ciliated gill, which strained, sorted particles and transport of captured particles to the mouth. Production of feces and pseudofeces or biodeposition by suspension feeders is influenced by many factors such as internal morphology of bivalve species, and the quality and quantity of suspension materials (Foster-Smith, 1975; Shumway *et al.*, 1985; Wong and Cheung, 1999). The present study could not confirm the differences of internal structure of the gill filaments among the bivalve species and how they function during feeding. However, the variable characteristics of

materials thrown away from the siphons of suspension-feeding bivalves (Fig. 3) could suggest the differences in sensitivity of each species to suspended particulate materials in the water.

Fatty acid of suspension-feeding bivalves

Differences in total fatty acid content were revealed in *P. elongata* and *S. carnicolor* compared to other species. These were particularly due to high total FA content. In both seasons the concentration of total FAs in the tissues of *P. elongata* and *S. carnicolor* was significantly higher (6.3 to 6.7 and 4.3 to 4.8 mg g^{-1} wet wt, respectively) compared to *C. sinensis, G. tumidum* and *K. japonica* (0.71 to 2.10 mg g^{-1} wet wt).

In this study, the average percentage of total FAMEs to the total lipids in all sample was 46.6 ± 9.8 %. The amount of lipids in animals could be attributed to physiological condition of animals and food supply. For instance, the study on krill populations in western Norway found that the high levels of lipids were related to ovary maturation (Bamstedt, 1976). However, according to Buchholz and Prado-Fiedler (1987) lipid accumulation was related to food supply and the lipid reserves were used for metabolic needs rather than for gonad maturation and spawning. Moreover, Cook *et al.* (2000) suggested that in the gonad of sea urchin *Psammechinus miliaris* the significant differences in lipid contents particularly between the sea urchin fed *Laminaria saccharina* (7.1 % wet sample) and those collected from the salmon cages (3.5 % wet sample) was due to the differences in uptake rates and/or storage differences of fatty acids within their diet. On the other hand, different species of suspension-feeding bivalves show differential absorption of food sources (Langdon and Newell, 1990). Thus the variation of total FAMEs concentration in bivalves (Table 2) may suggest differences in dietary intake, anabolism and catabolism of the studied bivalves.

Many studies revealed that fatty acids are species-specific. For example those in photosynthetic organisms are rich in ω3 and ω6 polyunsaturated fatty acids (PUFAs), due to the organisms capability to biosynthesize these PUFAs through desaturation and chain elongation of newly biosynthesized fatty acids (Sargent *et al.*, 1990; Vazhappilly and Chen, 1998). Since animals are incapable of synthesizing adequate amounts of ω3 and ω6 PUFAs, they obtain additional amounts of these fatty acids through feeding on photosynthetic organisms (Kanazawa *et al.*, 1979). Parkes and Taylor (1983) reported that odd-numbered branched fatty acids 15:0 and 17:0, iso and anteiso (odd-BrFAs), and some monounsaturated fatty acids are predominantly synthesized by bacterial communities and Kharlamenko *et al.* (1995) observed that the fatty acids contribute significant proportion in marine invertebrates, and hence indicate the presence of bacteria in the diet. Therefore, the contributions of FA compounds with 18, 20 and 22, carbon atoms and odd-BrFAs in the bivalve tissues (Table 2) indicate potential algal and bacterial sources in the bivalves' diets.

The high levels of long-chain PUFAs (carbon atoms 20 and 22) dominated by ω3 FA series is typical of marine microalgae (Viso and Marty, 1993). This indicates a significant contribution of microalgae to the bivalve diets. The high contribution of PUFAs with carbon atoms 18 and ω6 FA series is also shown in macroalgae species (Kayama *et al.*, 1989); thus the occurrence of these FAs in bivalve tissues may be due to the contribution of macroalga diet.

However, the differences shown in *P. elongata* and *S. carnicolor* were likely due to their PUFA/MUFA ratio, which was always lower than 1.0 in both seasons, compared to other species (Fig. 7). Moreover, during the cold season, the ratio values of PUFA/SAFA, PUFA/MUFA, 16:1 ω7/16:0 and ω3/ω6 in *P. elongata* were much lower than other samples, which indicated that *P. elongata* has relatively divergent FA characteristics.

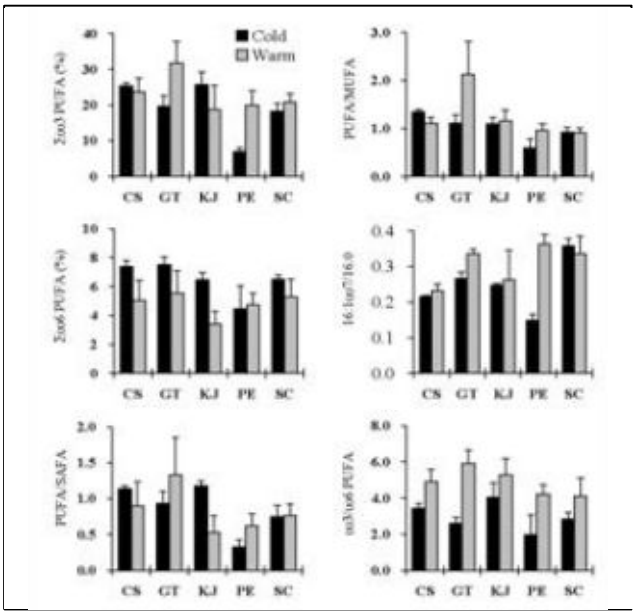


Figure 7. The percentages of ?3 PUFA, ?6 PUFA, the ratios of PUFA/SAFA, PUFA/MUFA, 16:1?7/16:0 and ? 3/?6 PUFA in the tissues of *Cyclina sinensis* (CS), *Gafrarium tumidum* (GT), *Katelysia japonica* (KJ), *Psammotaea elongata* (PE) and *Semele carnicolor* (SC) at Tomigusuku intertidal flat during the cold and warm seasons. Values are mean ± SD (n = 3).

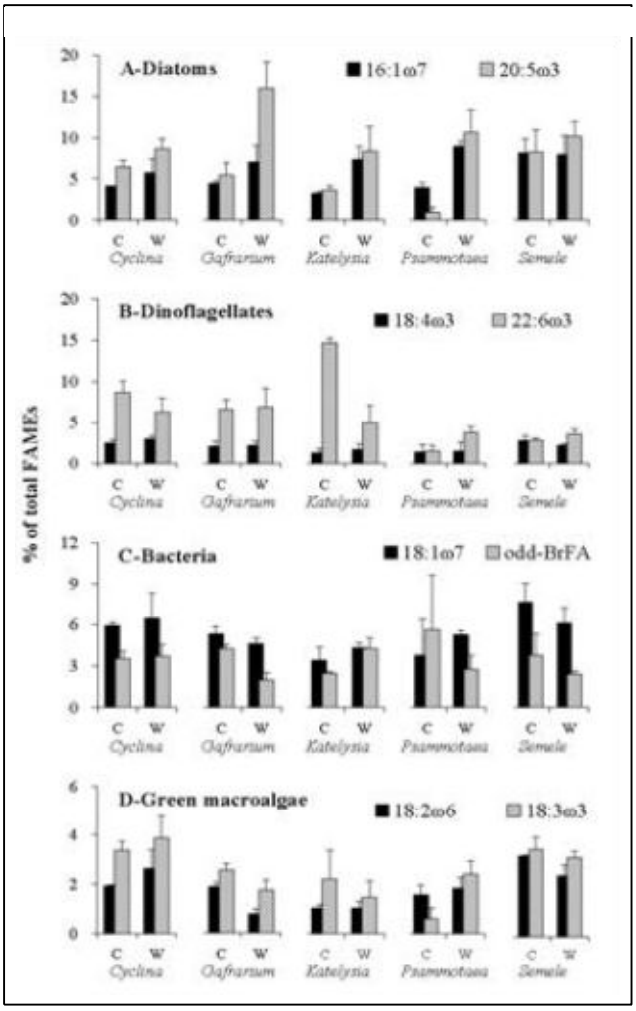


Figure 8. Relative contributions (% of total FAMEs) of fatty acid markers (A) diatoms, (B) dinoflagellates, (C) bacteria, and (D) green macroalgae in the tissues of *Cyclina sinensis, Gafrarium tumidum, Katelysia japonica, Psammotaea elongata* and *Semele carnicolor* at Tomigusuku intertidal flat during the cold (C) and warm seasons (W). Values are mean ± SD (n = 3).

Food sources of bivalves as indicates by fatty acid biomarkers

The FAs 20:5ω3 and 22:6ω3 are also found in many suspension-feeding bivalve species (DeMoreno *et al.*, 1980; Canuel *et al.*, 1995) and they have been found to be essential for bivalve survival and growth (Trider and Castell, 1980). The PUFA 20:5ω3 and the MUFA 16:1 ω7 predominate in diatoms (Volkman *et al.*, 1989; Dunstan *et al.*, 1994) for which they are often used as a marker (Kharlamenko *et al.*, 1995). In this study, the significantly high percentage of these FAs in the bivalve tissues during the warm season (Fig. 8A), suggested that diatoms contribute significantly to bivalve diet. In *S. carnicolor,* both FAs 20:5ω3 and 16:1ω7 also predominated the PUFAs and MUFAs during the cold seasons, suggesting that diatoms were major food source during the cold season. Comparison of the relative contribution of diatoms markers (20:5 ω3 + 16:1 ω7) between species in the warm season indicated that the mean percentage was highest in the tissues of *G. tumidum* (22.9%), followed by *P. elongata* (19.6%), *S. carnicolor* (18.1%), *K. japonica* (15.7 %) and *C. sinensis* (14.4 %).

Flagellated algae contain the PUFA 22:6ω3, with lower amounts of FAs 16:1ω7 and 20:5 ω3 (Joseph, 1975; Pond *et al.*, 1998). Mansour *et al.* (1999) found that the PUFA 18:4 ω3 is also typical of dinoflagellates. Although there were some differences in the conc. of this FA among bivalves, the mean percentages of 18:4 ω3 did not differ significantly in both seasons and was relatively lower than 22:6ω3 (Fig. 8B). The FA 22:6 ω3 was the predominant PUFA during the cold season in all species except in *P. elongata* and *S. carnicolor* (Table 2), which indicate that dinoflagellates are important food sources during the cold season for *C. sinensis, G. tumidum* and *K. japonica.*

Bacteria and macroalgal food sources

At Tomigusuku tidal flat, the degradation of large amounts of organic material from the green macroalga *U. pertusa* (personal observation) may have lead to immediate bacterial colonization of organic detritus (Alongi, 1994). Stable isotope studies indicate that kelp bacteria are absorbed with an efficiency of about 70% while kelp detritus was absorbed with an efficiency of about 50% by the ribbed mussel *Aulacomya ater* (Stuart *et al.*, 1982). The vaccenic acid (18:1ω7) and odd-BrFAs are characteristic of bacteria FAs (Perry *et al.*, 1979; Gillan and Johns, 1986) and was previously used as a bacterial marker in the food web (Kharlamenko *et al.*, 1995). On the other hand, the presence of 18:1 ω7 FA in the bivalves could be due to the occurrence of endobacteria that are attached to gill cells. This study observed relatively low percentage (3.5 to 7.7%) of the FA 18:1ω7 compared to bivalves with the bacterial-symbiont, e.g. *Solemya velum* (24.8%; Conway and Capuzzo, 1991). Although the relative contribution of FA markers for bacteria (odd-BrFAs + 18:1ω7) was higher in the tissues of *S. carnicolor* during the cold season (11.5%) compared to others (6.0 to 10.3%), generally, the results of this study indicate similarity in the mean percentage of this markers among bivalve species, and between the cold and the warm seasons ($P > 0.05$, Fig. 8C).

Higher level of MUFAs than PUFAs indicates that the nutrition of animals was related to bacterial supply (Conway and Capuzzo, 1991). In addition, the ratio of 16:1ω7 to 16:0 has been used to compare the level of bacteria in the food web (Volkman *et al.*, 1980). In marine invertebrates, the addition of bacteria to food, produces a significant decrease in the 16:1ω7/16:0 ratio but if the animals are fed on other sources, such as diatoms, the ratio increases (Parkes and Taylor, 1983). Therefore, the significantly lower values of PUFA/MUFA and 16:1ω7/16:0 ratios, 0.57 and 0.15, respectively, that were observed during the cold-season in *P. elongata*, when compared to the others species (Table 2) suggest a higher level of bacteria in the diet.

At Tomigusuku tidal flat, the PUFA 18:2ω6 has been used as a marker of green macroalgae due to their high contribution in *E. intestinalis* and *U. pertusa* (Meziane and Tsuchiya, 2000). Other studies also claim that the PUFA 18:2ω6, together with 18:3ω3 are the major FAs in green

macroalgae (Johns *et al.*, 1979; Vascovsky *et al.*, 1996); hence the occurrence of these FAs in all species (Fig. 8D) suggests that green macroalgae also contributed to the bivalves diet. Despite the relative contribution of these markers (18:2ω6 + 18:3ω3), they were not abundant throughout the samples (2.5 to 6.7%), however they did vary in the tissues of bivalves, with a marked increase in *C. sinensis* and *S. carnicolor* (5.3 to 6.7%).

The even-numbered long-chain FAs (even-LCFAs) with greater than 24 carbon atoms indicate organic source from vascular plants (Scribe *et al.*, 1991), but were not detected in the animal tissues except in the cold-season samples of *P. elongata* (Table 2). Although the contribution of even-LCFAs in the tissues of *P. elongata* during the cold season was small (Table 2), it may indicate considerable contribution of vascular plant detritus in the bivalves' diets.

In conclusion, the results indicate that the coexisting bivalves on Tomigusuku tidal flat utilized the same food sources; phytoplankton, benthic microalgae, macroalgae detritus and bacteria. With reference to the concentration of total FAMEs in all species, the level of most food sources was significantly higher in *P. elongata.* This suggests that *P. elongata* has a higher accumulation efficiency of fatty acids compared to other species (*C. sinensis, G. tumidum, K. japonica* and *S. carnicolor*). In addition, *P. elongata* showed unique fatty acid characteristics compared to other species during the cold–season, likely due to its low percentage of ω3 and ω6 PUFAs, relatively higher percentage of odd-BrFAs, the even-LCFAs, and the lowest value of PUFA/SAFA, PUFA/MUFA, 16:1ω7/16:0 and ω3/ω6 PUFA ratios. Furthermore, the deep burrowing behavior in *P. elongata* might have increased the survival rate and abundance on Tomigusuku tidal flat compared to other suspension-feeding bivalves.

Acknowledgement

Z. B. is grateful to the Ministry of Education, Science, Sport and Culture, Japan (Monbukagakusho) for scholarship grant during his study. We thank the members of Laboratory of Ecology and Systematics for technical assistance.

References

Ahn, I-Y, & Choi, J-W., 1998. Macrobenthic communities impacted by anthropogenic activities in an intertidal sand flat on the west coast (Yellow Sea) of Korea. Marine Pollution Bulletin. 36: 808-817.

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 Marine and Freshwater Environments including Wetlands. Kluweer Academic Publishing, Belgium, pp. 19-r32.

Auel, H., Harjes, M., Rocha, R., Stubing, D., & Hagen, W. 2002. Lipid biomarkers indicate different ecological niches and trophic relationships of the Artic hyperiid amphipods *Themisto abyssorum* and *T. libellula.* Polar Biol. 25: 374-383.

Bamstedt, U., 1976. Studies on the deep-water pelagic community of Korsfjorden, western Norway. Changes in the size and biochemical composition of *Megenyctiphanes norvegica* (Euphausiacea) in relation to its life cycle. Sarsia. 61: 15-30.

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

Blundon, J.A., & Kennedy, V.S., 1982. Refuges for infaunal bivalves from blue crab, *Callinectes sapidus* (Rathbun), predation in Chesapeake Bay. J. Exp. Mar. Biol. Ecol. 60: 17-34.

Brusca, R.C., & Brusca, G.J., 1990. Invertebrates. Siauer Associates, Inc

Buchanan, J.B., 1984. Sediment analysis. *In* N. A. Holme and A. D. McIntyre (Eds.), Methods for the Study of Marine Benthos (pp. 41-65). Oxford London. Blackwell Scientific Publications.

Buchholz, F., & Prado-Fiedler, R. 1987. Studies on the seasonal biochemistry of the northern krill *Meganyctiphanes norvegica* in the Kattegat. Helgol Meeresunters. 41: 433-452.

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

Carlson, D. J., Townsend, D. W., Hilyard, A. L., & Eaton, J. F. 1984. Effect of an intertidal mudflat on plankton of the overlaying water column. Can. J. Fish. Aquat. Sci. 41: 1523-1528.

Chiantore, M., Cattaneo-Vietti, R., Albertelli, G., Misic, C., & Fabiano, M., 1998. Role of filtering and biodeposition by *Adamussium colbecki* in circulation of organic matter in Terra Nova Bay (Ross Sea, Antarctica). J. Mar. Syst. 17: 411-424.

Commito, J.A., Thrush, S.F., Pridmore, R.D., Hewitt, J.E., & Cummings, V.J., 1995. Dispersal dynamics in a wind-driven benthic system. Limnol. Oceanogr. 40: 1513-1518.

Conway, N., & Capuzzo, J.M., 1991. Incorporation and utilization of bacterial lipids in the *Solemya velum* symbiosis. Mar. Biol. 108: 277-291.

Cook, E.J., Bell, M.V., Black, K.D., & Kelly, M.S., 2000. Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: trophic and nutritional implications. J. Exp. Mar. Biol. Ecol. 255: 261-274.

DeMoreno, J.E.A., Moreno, V.J., & Brenner, R.R., 1980. Lipids and fatty acids of mussels (*Mytilus platensis* d'Orbigny) from South Atlantic waters. J. Exp. Mar. Biol. Ecol. 48: 263-276.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.-M., & Jeffrey, S.W., 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochem. 35: 155-161.

Foster-Smith, R.L., 1975. The effect of concentration of suspension on the filtration rates and pseudofecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J. Exp. Mar. Biol. Ecol. 17: 1-22.

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 Sci Publisher B.V., Amsterdam, pp. 291-309.

Heip, C.H.R., Goosen, N.K., Herman, P.M.J., Kromkamp, J., Middelburg, J.J., & Soetaert, K., 1995. Production and consumption of biological particles in temperate tidal estuaries. In: Ansell, A.D., Gibson, R.N., Barnes, M. (Eds.), Oceanography and Marine biology: An Annual Review. UCL Press, pp. 1-149.

Jensen, K.T., 1992. Dynamics and growth of the cockle, *Cerastoderma edule*, on an intertidal mud-flat in the Danish Wadden sea: Effects of submersion time and density. J. Sea Res. 28: 335-345.

Johns, R.B., Nichols, P.D., & Perry, G.J., 1979. Fatty acid composition of ten marine algae from Australian waters. Phytochem. 18: 799-802.

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

Kanazawa, A., Teshima, S., & Ono, K., 1979. Relationship between the essential fatty acid requirements of aquatic animals and their capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comp. Biochem. Physiol. 63: 295-298.

Kayama, M., Arakaki, S., & Sato, S., 1989. Lipids of marine plants. In: Ackman, R.F. (Ed.), Marine Biogenic Lipids, Fats and Oils. Vol. 2. CRC Press, Boca Raton, Florida, pp. 3-48.

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.

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.

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.

Levinton, J.S., 2001. Marine Biology: Function, Biodiversity and Ecology. Oxford University Press, New York.

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.

Meyhofer, E., 1985. Comparative pumping rates in suspension-feeding bivalves. Mar. Biol. 85: 137-142.

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.

Morse, M.P., & Zardus, J.D. 1997. Bivalvia. In: Harrison, F.W., Kohn, A.J. (Eds.), Microscopic Anatomy of Invertebrates, Volume 6A: Mollusca II. Wiley-Liss, Inc, pp. 7-118.

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, Arge3ntina) and Trinity Bay (Newfoundland, Canada). Biochem. Syst. Ecol. 25(8), 739-755.

Newell, R.C., 1979. Biology of Intertidal Animals. Marine Ecological Survey Ltd, Faversham, Kent.

Parkes, R.J., & Taylor, J., 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuar. Coastal Shelf Sci. 16: 173-189.

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

Peterson, B.J., & Heck, K.L., 1999. The potential for suspension feeding bivalves to increase seagrass productivity. J. Exp. Mar. Biol. Ecol. 240: 37-52.

Peterson, C.H., & Andre, S.H., 1980. An experimental analysis of interspecific competition among marine filter feeders in a soft-sediment environment. Ecol. 61: 129-139.

Peterson, C.H., & Black, R., 1991. Preliminary evidence for progressive sestonic food depletion in incoming tide over a broad tidal sand flat. E. Coast. Shelf Sci. 32: 405-413.

Peterson, C.H., 1982. The important of predation and intra- and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella.* Ecol. Monogr. 52: 437-475.

Pond, D.W., Bell, M.V., Harris, R.P., & Sargent, J.R., 1998. Microplanktonic polyunsaturated fatty acid markers: a mesocosm trial. E. Coast. Shelf Sci. 46 (Supplement 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.

Rees, E.I.S., Nicholaidou A., & Laskaridou, P., 1979. The effects of storms on the dynamics of shallow water benthic associations. In: Keegan, B.F., Ceidigh, P.O. (Eds.), Biology of Benthic Organisms. Pergamon Press Ltd., pp. 465-474.

Roberts, D., Rittschof, D., Gerhart, D.J., Schmidt, A.R., & Hill, L.G., 1989. Vertical migration of the clam *Mercenaria mercenaria* (L.) (Mollusca: Bivalvia): environmental correlates and ecological significance. J. Exp. Mar. Biol. Ecol. 126: 271-280.

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. Basel, Karger, 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.

Shumway, S.E., Cucci, T.L., Newell, R.C., & Yentsch, C.M., 1985. Particle selection, ingestion, and absorption in filter-feeding bivalves. J. Exp. Mar. Biol. Ecol. 91: 77-92.

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.

Thorin, S., Radureau, A., Feunteun, E., & Lefeuvre, J-C., 2001. Preliminary results on a high east–west gradient in the macrozoobenthic community structure of the macrotidal Mont Saint-Michel bay. Continental Shelf Research. 21: 2167-2183.

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

Underwood, A.J., & Keough, M.J., 2001. Supply-side ecology: The nature and consequences of variations in recruitment of intertidal organisms. In: Bertness, M.D., Gaines, S.D., Hay, M.E. (Eds.), Marine Community Ecology. Sinauer Associates, Inc., Sunderland, pp. 183-200.

Vascovsky, V.E., Khotimchenko, S.V., Xia, B., & Hefang, L., 1996. Polar lipids and fatty acids of some marine macrophytes from the Yellow sea. Phytochem. 42: 1347-1356.

Vazhappilly, R., & Chen, F. 1998. Heterotrophic production potential of omega-3 polyunsaturated fatty acids by microalgae and algae-like microorganisms. Bot. Mar. 41:553-558.

Viso, A.C., & Marty, J.C., 1993. Fatty acids from 28 marine microalgae. Phytochem. 34 (6): 1521-1533.

Volkman, J.K., Bavor, H.J., Gillan, F.T., Johns, R.B., & Perry, G.J., 1980. Microbial lipids of an intertidal sediment. 1. Fatty acids and hydrocarbons. Geochim. Cosmochim. Acta. 44: 1133-1143.

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.

Wong, W.H., & Cheung, S.G., 1999. Feeding behaviour of the green mussel, *Perna viridis* (L.): Responses to variation in seston quantity and quality. J. Exp. Mar. Biol. Ecol. 236: 191-207.

Yamashiro, H., Oku, H., Higa, H., Chinen, I., & Sakai, K., 1999. Composition of lipids, fatty acids and sterols in Okinawan corals. Come. Biochem. Physiol. 122B: 397-407.

Young, C.M., 2001. Atlas of Marine Invertebrate Larvae. Academic Press

Zaklan, S.D., & Ydenberg, R., 1997. The body size-burial depth relationship in the infaunal clam *Mya arenaria*. J. Exp. Mar. Biol. Ecol. 215: 1-17.

Zar, J.H., 1999. Biostatistical analysis, 4th edn. Prentice-Hall, New York.

Zwarts, L., & Wanink, J., 1984. How oystercatchers and curlews successively deplete clams. In: Evans, P.R., Goss-Custard, J.D., Hale, W.G. (Eds.), Coastal Waders and Wildfowl in Winter. Cambridge University press, Cambridge, pp. 69-83.