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Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses

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ABSTRACT: Carbon and sulfur stable isotope ratios, as well as fatty acid composition of tissues, of dominant consumer species were determined and compared to those of potential food sources in an isolated community of *Zostera marina* in a shallow, semi-enclosed inlet of the Sea of Japan. Of the 6 dominant species of invertebrates, 4 species were enriched in ^{13}C, compared to all sampled carbon sources alternative to *Z. marina*. Among them, the grazing gastropods *Littorina squalida* and *Homalopoma sangarense* exhibited the most enriched $\delta^{13}C$ values. On the dual $\delta^{13}C$ versus $\delta^{34}S$ plot, these mollusks occupy an intermediate position between *Z. marina* and epiphytes, suggesting nearly equal proportions of organic carbon from both nutritional sources. In lipids of *H. sangarense* there was a high content of the 18:1(n-7) acid characteristic of aerobic bacteria; however, another grazer (*L. squalida*) showed the lowest content of bacterial fatty acids among all consumers. Other highly ^{13}C-enriched consumers were the surface-deposit-feeding mollusks, the gastropod *Batillaria cumingii* and the bivalve *Macoma incongrua*; however, their $\delta^{34}S$ values were markedly lower than those of any of the primary producers sampled, including *Z. marina*. Although the high $\delta^{13}C$ values of grazers and surface-deposit feeders are suggestive of a great contribution of *Z. marina* organic carbon, no substantial concentrations of seagrass marker fatty acids were detected. Significant interspecific variations of both the sulfur isotope ratios and the fatty acid composition of these consumers suggest that there are a variety of pathways by which seagrass organic matter reaches invertebrates at lower trophic levels of the community food web. Dominant filter feeders, the bivalves *Ruditapes philippinarum* and *Pillucina pisidium*, had carbon drastically different in isotopic composition from *Z. marina* organic matter. Body tissues of *P. pisidium* and especially its gills, which bear symbiotic bacteria, were dramatically depleted both in ^{13}C and ^{34}S compared to all sources of photosynthetically fixed carbon in the bay. Very low $\delta^{13}C$ and $\delta^{34}S$ values of *P. pisidium* body together with a high content of 18:1(n-7) acid suggest a leading role of sulfur-oxidizing symbiotic bacteria in the nutrition of this species. *R. philippinarum* was only slightly ^{13}C-enriched, compared to POM, and was the only consumer which had the high concentration of fatty acids characteristic of plankton, particularly 22:6(n-3). At the same time, it was much more ^{34}S-depleted than would be expected, assuming negligible contribution of *Z. marina* detritus to its food. This mollusk showed a high content of branched fatty acids, especially the iso17:0 and anteiso17:0 acids characteristic of bacteria from sediment; this suggests that *R. philippinarum* assimilated notable amounts of bacteria from resuspended sediment. Furthermore, the contribution of ^{34}S-depleted bacteria, which inhabit reduced sediment, to *R. philippinarum* nutrition was high enough to result in the observed depletion of ^{34}S in mollusks. Further progress in food web studies of seagrass ecosystems using a complex of multiple stable isotope and fatty acid analyses would appear possible on the basis of analysis of separate components of the seagrass epiphytic community and micro- and meiobenthic organisms, inhabiting surface sediments.

KEY WORDS: Eelgrass community · Mollusks · Stable carbon isotope · Stable sulfur isotope · Fatty acids · Food web

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INTRODUCTION

Seagrass communities are widely distributed in marine shallow water all over the world and are among the most productive marine ecosystems (McRoy &

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McMillan 1977, Zieman & Wetzel 1980, Duarte 1989). Live tissues of marine vascular plants are of limited use as food for animals; therefore, other primary producers or the detrital food chain supports secondary production in these communities (Mann 1972, Fenchel 1977, Kikuchi 1980). A number of constraints (small size of some consumers, impossibility of identifying a number of primary producers and intermediate links in the food web) have compelled investigators to abandon gut analysis methodology and have led to a use of a variety of other methods, including stable isotope ratio analysis (SIRA), to determine seagrass community food webs (Klump et al. 1989).

The contribution of seagrass organic matter to food webs can be estimated by carbon ($^{13}C/^{12}C$) SIRA of the consumers, since seagrasses and other primary producers in communities usually differ in the isotopic composition of organic carbon (Thayer et al. 1978, McConnaughey & McRoy 1979, Fry et al. 1987, McClelland & Valiela 1998). Because little change occurs in the $^{13}C/^{12}C$ ratio on each trophic level (DeNiro & Epstein 1978), it is possible to identify in animal tissues the proportion of carbon originally synthesized by seagrass; however, it is impossible to follow the pathway by which it enters the consumers. Moreover, if organic matter is derived from more than 2 sources, additional information is needed for unambiguous estimation of the contribution of each source to the food web by carbon SIRA (Fry & Sherr 1984). Sulfur ($^{34}S/^{32}S$) SIRA could significantly improve the method (Fry et al. 1987), but appears not to have been attempted in seagrass systems (Hemminga & Mateo 1996).

The sulfur SIRA has been widely used to examine organic matter flows in saltmarsh food webs (Peterson et al. 1985, 1986, Sullivan & Moncreiff 1990, Currin et al. 1995). Marine vascular plants deeply rooted in reduced sediments are considerably ^{34}S-depleted compared to algae, due to incorporation of ^{34}S-depleted sulfides (Fry et al. 1982, Trust & Fry 1992). This allows the use of $\delta^{34}S$ as a marker in the analysis of the fate of organic matter synthesized by vascular plants in the near-shore zone (Newell et al. 1995, Deegan & Garritt 1997, Stribling & Cornwell 1997). The fact that eelgrass *Zostera marina* is also ^{34}S-depleted (Mekhtieva et al. 1976), compared to marine algae, encourages us to believe that the stable sulfur isotope ratio will be useful as a second isotope marker in eelgrass community food webs.

Using SIRA alone, it is difficult to assess the contribution of the main component of the detritus food chain — heterotrophic microorganisms; hence, in addition to isotopes, lipid markers were used. Bacteria, microalgae, macroalgae and seagrasses contain different specific fatty acids. The presence of these specific fatty acids in the animal tissues may indicate which organisms are food items for a given animal (Sargent & Whittle 1981). However, a combination of carbon SIRA and lipid markers has rarely been used for analyzing trophic relationships in seagrass meadows (Nichols et al. 1985, 1986) or other marine environments (Canuel et al. 1995).

In this work, we have made an attempt to identify the major food sources of the dominant invertebrate species in a relatively isolated *Zostera marina* meadow in the shallow, semi-enclosed inlet of the Sea of Japan, using a combination of carbon and sulfur SIRA and lipid markers. With this aim in view, carbon and sulfur stable isotope ratios, as well as the fatty acid composition, of tissues of the dominant invertebrate species were determined and compared with the same variables in the potential food sources.

MATERIALS AND METHODS

Study area and sample collection. The study was carried out in microtidal Novgorodskaya Bight (Possyet Bay, Sea of Japan). This bight has no marked runoff of freshwater. The broad, semi-enclosed inward part of the bight communicates with the rest of the bay through a shallow channel. The water depth in the inward part does not exceed 3.6 m, and at this location most of the bottom area (about 15 km^2) is covered by a subtidal *Zostera marina* meadow (Fig. 1). The results of intensive hydrobiological studies of this community (Vyshkvartzev & Peshekhodko 1982, Lebedev & Vyshkvartzev 1988, 1990) indicated that *Z. marina* comprises 83% of the total community biomass in this part of the bight, followed by the seagrass *Z. japonica* and the green alga *Codium fragile* (4% and 2%, respectively). Animals represent 11% of the total community biomass, and only 6 species of invertebrates comprise >90% of the macrozoobenthic biomass. All of the most abundant species are bivalves and gastropods, with different modes of feeding (Table 1). They were selected for the study of the isotope and fatty acid composition of the community's main consumers.

Samples for analysis were collected in July 1996 by SCUBA diving. All samples of macrophytes and consumers were collected in the same area (150 to 200 m^2) at a distance of 160 m from the shore and at a depth of 2 m, in order to minimize the spatial differences in the food supply of animals.

All animal and plant samples for SIRA and lipid analysis consisted of at least 10 individuals of the same species that had been collected concurrently at the same site. For the food web studies by SIRA and lipid analysis, when high cost of analyses allows only low replication, such pooling of samples has the advantage

of reducing intraspecific variability in the signals of isotopic and lipid markers.

During the sampling period, there were only 3 major sources of plant material in the investigated community: eelgrass, epiphytic algae and phytoplankton. The Novgorodskaya Bight has no direct river or creek drainage; therefore, terrestrial plant material is not a major source. Microscopic examination of the top layer of bottom sediment using epifluorescent microscopy at a magnification of 200× revealed no notable amount of benthic microalgae or terrestrial plant detritus. The macroalga *Codium fragile* was found mainly outside the eelgrass community, at a distance 4 km from the sampling plot.

Composite samples of *Zostera marina* comprised green leaves, old brown leaves and rhizomes, which were pooled separately. Fallen decomposing leaves were collected from the bottom. The leaf blades were carefully cleared of epiphytes using a razor blade.

Total samples of epiphytic algae were collected from mature green leaves using a razor blade. Samples were examined using microscope for detection of seagrass debris, which was then removed.

Suspended particulate organic matter (POM) was collected from the water column with a net (mesh size 150 µm) directly above the *Zostera marina* bed. The net tow samples from the *Z. marina* meadow consisted mainly of zooplankton, primarily copepods.

Samples of benthic POM from the sediment-water interface were collected using a vacuum sampling device (Levin 1987). A subsample of small particles of *Zostera marina* detritus was selected for lipid analysis from these samples using a stereo microscope. Samples of the top 1 cm of sediment were collected with a glass tube 5 cm in diameter.

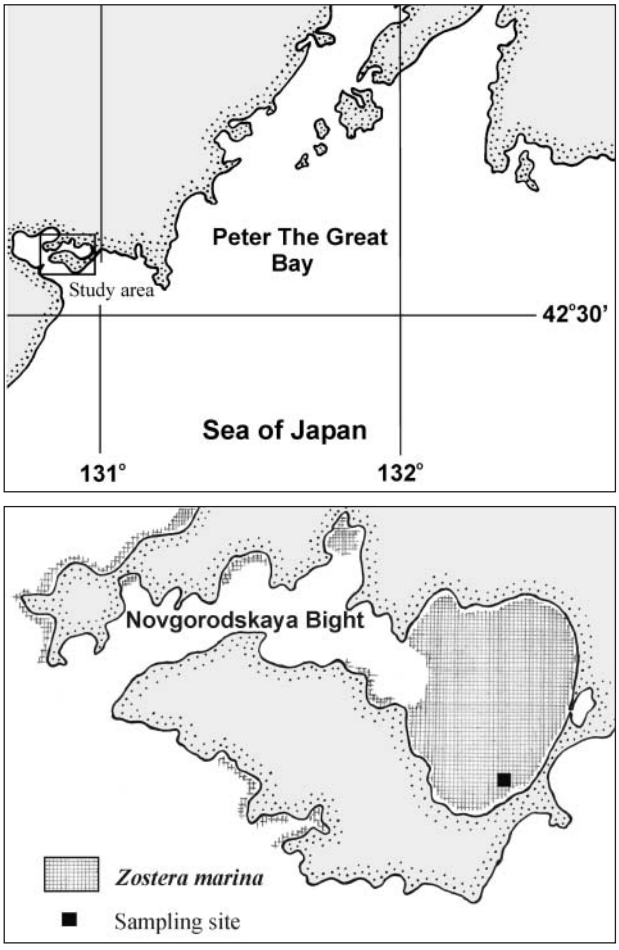

Fig. 1. Location of *Zostera marina* community and position of sampling site in the Novgorodskaya Bight (Possyet Bay, Sea of Japan)

Table 1. Characteristics of dominant invertebrate species in *Zostera marina* community (based on data from: Vyshkvartzev & Peshekhodko 1982, Lebedev & Vyshkvartzev 1988, 1990)

Invertebrate species	Habitat	Feeding mode	Maximal biomass (g m^{-2})	% of total zoobenthos biomass	% of total community biomass
Pillucina pisidium (Dunker)	Infauna (in sediment)	Filter feeding and symbiotrophy	75	1.3	0.1
Littorina squalida (Broderip et Sowerby)	Epifauna of eelgrass blades	Grazing	52	2.8	0.3
Batillaria cumingii (Grosse)	Epifauna (on sediment)	Surface-deposit feeding	234	3.6	0.4
Homalopoma sangarense (Schrenk)	Epifauna of eelgrass blades	Grazing	97	4.8	0.5
Ruditapes philippinarum (Adams et Reeve)	Infauna (in sediment)	Filter feeding	445	4.9	0.5
Macoma incongrua (Martens)	Infauna (in sediment)	Surface-deposit feeding and filter feeding	354	72.9	7.9

For isotope and lipid analyses, parts consisting mainly of muscle tissues were excised from the body of mollusks; the digestive organs and the gonads, which are rich in lipids, were discarded. For the bivalve *Pillucina pisidium* (in addition to the body tissues), the gills containing symbiotic bacteria (Rodionov & Yushin 1991) were analysed separately.

Isotopic analysis. Since the sulfur SIRA requires a considerable amount of material, due to the relatively low content of organic sulfur (especially in plants), tissues of 10 to 100 individuals were pooled into 1 sample, depending on their size. All samples were washed with distilled water, dried at 60°C and ground to fine powder using an agate mortar and pestle.

For the carbon SIRA, subsamples of 1 mg were combusted with CuO in vacuum-sealed Pyrex tubes in a muffle furnace at 590°C (Sofer 1980). Prior to combustion, subsamples of epiphytes, POM and sediments were treated with 1 N HCl to remove carbonates, washed with distilled water and then dried again. After combustion, the resulting CO_2 was purified by cryogenic distillation for analysis. The intercomparison material IAEA-C-6 (sucrose) was used as the running standard to control the entire procedure of sample preparation and carbon isotope analysis.

For sulfur isotope analysis, the remainder of each dried and ground sample was resuspended in deionized water (1:4, v/v) and then centrifuged. The procedure was repeated 3 times in order to fully remove seawater sulfate from the samples (Peterson et al. 1986). The precipitate was dried at 60°C and ground again to fine powder. For converting organic sulfur to sulfate subsamples, 120 to 150 mg of the animal tissues or 300 mg of the plant tissues were mixed with KNO_3 and combusted in vacuum-sealed Pyrex tubes (Dornblaser et al. 1994), to which CaO had been added to absorb excessive CO_2 (B. Fry pers. comm.). After combustion, the contents of the tubes were dissolved by 1 N HCl, solutions were diluted with deionized water and filtrated, and then sulfur was precipitated with barium chloride to $BaSO_3$. Pure SO_2 was obtained after $BaSO_3$ decomposition and cryogenic distillation. Samples of 1.5 mg of IAEA-S-4 (elemental sulfur) served as the running standard to check the entire procedure of sample preparation and sulfur isotope analysis.

Isotope ratios were measured in CO_2 and SO_2 on a modified (for precise SIRA of gases) MI-1201V mass spectrometer in δ notation:

$$\delta X\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3 \quad (1)$$

where X is ^{13}C or ^{34}S and R is $^{13}C/^{12}C$ or $^{34}S/^{32}S$. The δ values are expressed in parts per thousand relative to PDB (carbon) and CDT (sulfur) standards. Reference materials (NBS-19 and NBS-127) were used for calibration. Replicates using intercomparison materials (IAEA-C-6 and IAEA-S-4) indicate overall analytical errors of ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{34}S$.

The contributions of 1 of 2 alternative sources to consumers were calculated by inserting $\delta^{13}C$ or $\delta^{34}S$ values into the simple mixing equation (McConnaughey & McRoy 1979):

$$C_{(S1)} = \frac{\delta_C - \delta_{S2} - F}{\delta_{S1} - \delta_{S2}} \times 100\% \quad (2)$$

where $C_{(S1)}$ is the contribution of Primary Source 1 to the consumer; δ_C, δ_{S1} and δ_{S2} are $\delta^{13}C$ or $\delta^{34}S$ values of consumer, Primary Source 1, and Primary Source 2, correspondingly; F is the trophic shift in consumer stable isotope ratios (+1‰ for carbon and 0‰ for sulfur).

Lipid extraction and analysis of fatty acids. The samples were transferred to a glass homogenizer and homogenized in a mixture of chloroform-methanol (1:1, v/v). Lipids were extracted according to Bligh & Dyer (1959). Fatty acid methyl esters (FAME) were prepared by the consecutive treatment of the total lipids with 1% sodium methylate in methanol and 5% HCl in methanol according to Carreau & Dubacq (1978) and purified by thin layer chromatography in benzene. A GC-9A gas chromatograph (Shimadzu, Kyoto, Japan) was used for analysis. Separation of FAME was performed on a fused quartz capillary column (30 m × 0.25 mm i.d.) coated with Supelcowax 10M (Supelco Co., Bellefonte, PA). The column and detector temperature was 210°C, the injector temperature was 240°C. Helium was used as a carrier gas, and the split ratio was 1:30. Individual peaks of FAME were identified by equivalent chain length measurements (Christie 1988) and by comparing retention times with those of the authentic standards of fatty acids. Chromatographic data were calculated with a Shimadzu Chromatopac C-R3A integrator.

Fatty acids were designed as the ratio of the number of carbon atoms to the number of double bonds. Double bond position (n) is numbered from the methyl end of the fatty acid.

Significant differences ($p < 0.05$) in fatty acid concentrations and SIRA data were tested between species or food sources using a 1-way ANOVA. All tests were performed using Statistica 4.0 statistical software.

RESULTS

Carbon and sulfur stable isotopes in potential food sources

The main sources of organic matter in the community exhibited a wide range (14.2‰) of carbon isotope ratios (Table 2). The $\delta^{13}C$ values varied from –6.7‰ for

green eelgrass leaves to –20.9‰ for suspended POM. A much narrower range (within 5.5‰) was shown for sulfur isotope ratios of food sources. The maximum difference in $\delta^{34}S$ values was also found between eelgrass and suspended POM.

Live green leaves of *Zostera marina*, senescent brown leaves and fallen decomposing leaves showed very similar $\delta^{13}C$ values (an average of –7.0 ± 0.5‰, N = 6), close to the maximum reported for this species (Hemminga & Mateo 1996). Rhizomes of *Z. marina* were ^{13}C-depleted in comparison to leaves; the average $\delta^{13}C$ value of rhizomes was 1.9‰ less than that of leaves. The eelgrass leaves and rhizomes were substantially depleted in ^{34}S relative to seawater sulfate, and showed $\delta^{34}S$ values from +8.9 to +11.3‰. There was no significant difference in the sulfur isotopic composition between leaves and rhizomes (Table 2). Such depletion is common in higher marine plants rooted in reduced sediment (Trust & Fry 1992); however, *Z. marina* from Novgorodskaya Bight had relatively high $\delta^{34}S$ values in comparison with the –0.4 to +10‰ range known for this species from other marine basins (Mekhtieva et al. 1976, Peterson et al. 1986).

The structure of the epiphytic community of eelgrass blades in Novgorodskaya Bight is characteristic of seagrass meadows of the northwestern Sea of Japan, where they usually consist of the diatom *Cocconeis scutellum*, the small crustose coralline alga *Pneophyllum lejolisii*, fungi and bacteria (Kharlamenko & Lysenko 1991b). Epiphytic algae were ^{13}C-depleted compared to the eelgrass blades on which they grew; $\delta^{13}C$ values were on average 6.5‰ lower than those for *Z. marina* blades and showed intermediate values (mean = –13.5‰) between those of *Z. marina* and POM (Table 2). We were not able to measure sulfur isotopic composition of epiphytes because of the small amount of material in the samples. The $\delta^{34}S$ value for epiphytic algae in subtidal environments, such as the studied seagrass meadow, is expected to be close to that of seawater sulfate — about +20.3‰ (Nriagu et al. 1991), as is the case of *Thalassia testudinum* epiphytes (Fry et al. 1987) and many other marine algae (Trust & Fry 1992); however, for epiphytes of saltmarsh grasses a very low (–0.4‰) value has been reported (Currin et al. 1995).

The $\delta^{13}C$ values (mean = –20.9‰) of suspended POM (mainly zooplankton) were similar to that of phytoplankton (mainly diatoms *Chaetoceros* spp.) collected 6 km apart from the seagrass meadow (–20.7‰). These $\delta^{13}C$ values were in the typical range of marine temperate plankton (Gearing et al. 1984). The average $\delta^{34}S$ value (+14.5‰) of suspended POM was lower than the +18.9 to +21.0‰ range reported for plankton of near-shore waters of the Sea of Japan (Kiyashko et al. 1998). Visible absence of eelgrass detritus in these POM samples and their extremely low $\delta^{13}C$ values compared to those of *Zostera marina* suggest that POM $\delta^{34}S$ values represent mainly the isotopic composition of copepods, which markedly dominated the structure of the seston in the *Z. marina* community during the sampling period. We suspect that the lower $\delta^{34}S$ values of suspended POM in the shallow waters of Novgorodskaya Bight are related to contamination of net tow samples by particles of resuspended sedimentary sulfide, as in the case of low $\delta^{34}S$ values of plankton samples from other muddy estuaries (Chanton & Lewis 1999), and do not correctly reflect the isotopic composition of plankton organic sulfur, which should be close to that of seawater sulfate (Peterson et al. 1986, Kiyashko et al. 1998).

Benthic POM was substantially ^{13}C-enriched in comparison to suspended POM, and showed a $\delta^{13}C$ value (–15.5‰) very close to that of sedimentary organic matter (SOM) (Table 2). Eelgrass meadow SOM had a higher $\delta^{13}C$ value (–15.2‰) than the values reported earlier (–18.1 to –21.3‰) for sediments from the open, vegetation-free part of Novgorodskaya Bight (Zhakin & Kiyashko 1991). This indicates that ^{13}C-enriched detritus of *Zostera marina* or epiphytes make a substantial contribution to SOM in this ecosystem.

Table 2. Carbon and sulfur stable isotope ratios for potential food sources in the *Zostera marina* community of Novgorodskaya Bight, the Sea of Japan. Mean values marked by the same letter are not significantly different ($p > 0.05$); unmarked mean values are significantly different (1-way ANOVA Tukey HSD-test, $p < 0.05$). POM: particulate organic matter

Source	$\delta^{13}C$‰ Mean ± SD	N	$\delta^{34}S$‰ Mean ± SD	N
Eelgrass *Zostera marina*				
Green leaves	–6.7 ± 0.1	4	10.0[a] ± 1.0	4
Senescent brown leaves	–7.9			
Fallen decomposing leaves	–6.9			
Rhizomes	–8.9 ± 0.1	2	9.1[a] ± 0.2	2
Epiphytic algae (diatoms + *Pneophyllum lejolisii*)	–13.5 ± 0.3	2		
Phytoplankton[1] (mainly *Chaetoceros* spp.)	–20.7			
Suspended POM (zooplankton)	–20.9 ± 0.3	3	14.5 ± 0.2	2
Benthic POM	–15.5			
Sedimentary organic matter	–15.2			

[1]Net tow sample collected outside seagrass meadow

Fatty acids in potential food sources

The major fatty acids of *Zostera marina* were 18:2(n-6), 18:3(n-3) and 16:0 (Table 3). These acids comprised 82.4% of the total fatty acids in live blades and 72.6% in rhizomes. Maximum concentrations of 18:3(n-3) occurred in the blades (mean = 41.3%), and 18:2(n-6) was the major fatty acid in the rhizomes (mean = 39.0%). The 18:2(n-6) and 18:3(n-3) acids are usually observed at high levels in seagrass blades (Nichols et al. 1982, Khotimchenko 1993), and the content of these acids in degraded seagrasses is usually markedly decreased (Tenore et al. 1984).

Decomposing leaves of *Zostera marina* lost a large portion of 18:2(n-6) and 18:3(n-3). The total content of 18:2(n-6) and 18:3(n-3) in degraded blades was diminished by a factor of 10.4. The fatty acids 18:2(n-6) and 18:3(n-3) made up 3.9% and 2.1%, respectively, of the total fatty acids in lipids of *Z. marina* detritus (Table 3).

The major fatty acids in the total epiphyte samples were 14:0, 16:0, 16:1(n-7), 18:1(n-9) and 20:5(n-3). Their total content was 65.8%. The predominance of diatom fatty acids has been reported for the epiphytic community of the seagrass *Posidonia australis* (Nichols et al. 1985).

In lipids of the suspended POM, the dominant fatty acids were 16:0,18:0, 20:5(n-3) and 22:6(n-3). The sum of these acids comprised 67.2% of the total fatty acids of suspended POM. The fatty acid composition of suspended POM was typical for marine POM in the period of abundant development of zooplankton, with a characteristic high content of 22:6(n-3) (Sargent & Whittle 1981).

Stable isotope ratios in dominant animals

The composite samples of mollusks exhibited very low intraspecific variations of stable isotope ratios which commonly did not exceed 1‰ (Table 4); this was evidently a result of pooling more than 10 individuals in each sample. A wide range of interspecific differences (maximal difference = 16.4‰) was found in $\delta^{13}C$ for mollusks. Nevertheless, the average $\delta^{13}C$ values of 4 out of 6 species of animals were narrowly clustered in the −9.1 to −12.3‰ range. Among these, the grazing

Table 3. Fatty acid composition of potential food sources in the *Zostera marina* community of Novgorodskaya Bight, the Sea of Japan (% of total fatty acids, mean ± SD, N = 3). Table contains only fatty acids with concentrations >1% in 1 of the food sources. Mean values marked by the same letter are not significantly different ($p > 0.05$); unmarked mean values (except data for *Z. marina* detritus) are significantly different (1-way ANOVA Tukey HSD test, $p \leq 0.05$). POM: particulate organic matter; SOM: sedimentary organic matter

Fatty acid	*Z. marina* green leaves	*Z. marina* detritus	*Z. marina* rhizomes	Epiphytes of *Z. marina*	Suspended POM	Benthic POM	SOM
14:0	$0.4^{a} \pm 0.0$	3.4	$0.6^{a} \pm 0.0$	$5.6^{b} \pm 0.5$	$3.4^{b} \pm 0.4$	$3.9^{b} \pm 0.5$	$3.4^{b} \pm 0.3$
14:1	$0.1^{a} \pm 0.0$	0.4	$0.3^{a} \pm 0.0$	$1.1^{b} \pm 0.3$	$0.2^{a} \pm 0.2$	$1.2^{b} \pm 0.3$	$0.7^{b} \pm 0.1$
i15:0	0	1.4	$0.1^{a} \pm 0.1$	$0.3^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	1.0 ± 0.1	1.6 ± 0.3
ai15:0	0	0.7	$0.2^{a} \pm 0.0$	$0.4^{ab} \pm 0.2$	$0.2^{ab} \pm 0.1$	$1.1^{b} \pm 0.4$	2.8 ± 0.8
15:0	$0.2^{a} \pm 0.0$	1.3	$0.6^{ab} \pm 0.1$	$1.0^{bc} \pm 0.3$	$0.8^{b} \pm 0.1$	$1.3^{cd} \pm 0.2$	$1.7^{d} \pm 0.1$
16:0	$19.9^{ab} \pm 0.4$	20.6	$19.5^{ab} \pm 1$	$23.8^{b} \pm 2.9$	$22.4^{ab} \pm 1.1$	$16.6^{a} \pm 2.0$	$16.7^{a} \pm 1.7$
16:1(n-9)	0.5 ± 0.0	0.0	$2.0^{a} \pm 0.1$	0	$1.7^{a} \pm 1.1$	0	0
16:1(n-7)	$0.8^{a} \pm 0.1$	5.1	0	$18.2^{b} \pm 1.8$	$2.1^{a} \pm 0.4$	12 ± 1.4	$15.8^{b} \pm 1.7$
ai17:0	0	0.4	$0.1^{a} \pm 0.0$	$0.4^{b} \pm 0.0$	$0.2^{a} \pm 0.1$	$0.5^{b} \pm 0.1$	1.1 ± 0.2
17:0	$0.2^{a} \pm 0.0$	0.7	$0.7^{ab} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.8^{b} \pm 0.4$	$0.6^{ab} \pm 0.2$	$1.0^{b} \pm 0.0$
17:1(n-9)	$0.1^{a} \pm 0.0$	0.7	$0.2^{a} \pm 0.0$	$1.5^{b} \pm 0.2$	$0.3^{a} \pm 0.2$	$1.9^{b} \pm 0.5$	$1.9^{b} \pm 0.2$
16:3(n-3)	3.5 ± 0.5	1.0	0	$0.2^{a} \pm 0.1$	$0.2^{a} \pm 0.1$	$0.5^{ab} \pm 0.2$	$0.9^{b} \pm 0.1$
18:0	$1.5^{a} \pm 0.2$	6.9	$2.2^{ab} \pm 0.4$	$3.8^{bc} \pm 1.0$	$5.2^{c} \pm 0.4$	$3.8^{bc} \pm 0.1$	$3.2^{abc} \pm 0.6$
18:1(n-9)	$1.5^{a} \pm 1.0$	12.9	$3.2^{ab} \pm 0.4$	$10.5^{c} \pm 1.1$	$2.5^{ab} \pm 0.8$	$7.2^{bc} \pm 2.9$	$5.9^{b} \pm 1.4$
18:1(n-7)	$0.5^{a} \pm 0.2$	4.1	$1.5^{ab} \pm 0.4$	$2.4^{b} \pm 0.5$	$1.9^{ab} \pm 0.1$	$3.9^{b} \pm 1.2$	6.4 ± 0.8
18:2(n-6)	21.2 ± 0.2	3.9	39.0 ± 1.3	$3.2^{a} \pm 0.1$	$1.5^{a} \pm 0.2$	$3.7^{a} \pm 1.7$	$3.5^{a} \pm 0.8$
18:3(n-3)	41.3 ± 3.7	2.1	14.1 ± 1.1	$0.9^{a} \pm 0.3$	$3.6^{a} \pm 0.7$	$1.3^{a} \pm 0.3$	$2.0^{a} \pm 0.6$
18:4(n-4)	0	0.1	0	$0.6^{a} \pm 0.2$	4.4 ± 0.9	$0.9^{a} \pm 0.4$	$0.7^{a} \pm 0.1$
20:0	$0.7^{ab} \pm 0.0$	0.8	$1.2^{a} \pm 0.2$	$1.0^{a} \pm 0.1$	$0.1^{b} \pm 0.1$	$0.9^{a} \pm 0.3$	$1.2^{a} \pm 0.7$
20:4(n-6)	0	0.7	0	$1.3^{a} \pm 0.2$	$0.5^{a} \pm 0.1$	$1.3^{a} \pm 0.5$	$3.1^{a} \pm 0.8$
20:4(n-3)	0	0.3	0	$0.3^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.4^{a} \pm 0.7$	$0.1^{a} \pm 0.1$
20:5(n-3)	0.3 ± 0.0	1.3	0	$7.7^{a} \pm 2.0$	18.6 ± 2.4	$5.8^{a} \pm 2.0$	$4.9^{a} \pm 1.7$
22:0	$1.9^{a} \pm 0.1$	1.4	$2.9^{a} \pm 0.2$	$0.5^{a} \pm 0.1$	$0.2^{a} \pm 0.2$	$0.9^{a} \pm 0.8$	$1.6^{a} \pm 0.8$
22:2(n-6)	$0.4^{a} \pm 0.7$	0.7	0	0	0	$2.3^{a} \pm 3.7$	0
24:0	$0.7^{a} \pm 0.1$	1.0	3.3 ± 0.4	$0.4^{a} \pm 0.4$	0	$0.3^{a} \pm 0.3$	$1.1^{a} \pm 0.6$
22:6(n-3)	0	3.3	$0.7^{a} \pm 0.9$	$0.8^{a} \pm 0.2$	21.0 ± 1.5	$5.2^{a} \pm 6.8$	$1.1^{a} \pm 0.2$

Table 4. Carbon and sulfur stable isotope ratios of consumer organisms in the *Zostera marina* community of Novgorodskaya Bight, the Sea of Japan. Mean values marked by the same letter are not significantly different ($p > 0.05$); unmarked mean values are significantly different (1-way ANOVA Tukey HSD test, $p \leq 0.01$)

Species	$\delta^{13}C$ (‰)			$\delta^{34}S$ (‰)		
	Mean ± SD	Range	N	Mean ± SD	Range	N
Pillucina pisidium						
Body	−26.2 ± 0.3	−26.4 to −25.9	3	−16.0 ± 1.0	−16.8 to −15.0	3
Gills	−27.8 ± 0.1	−27.9 to −27.7	3	−0.2[1]		1
Littorina squalida	−9.8[a] ± 0.9	−10.9 to −9.1	4	14.1 ± 0.1	14.0 to 14.2	3
Batillaria cumingii	−12.0[b] ± 0.3	−12.3 to −11.7	3	7.6 ± 0.5	7.1 to 8.1	3
Homalopoma sangarense	−10.0[a] ± 0.4	−10.3 to −10.5	3	12.0[c] ± 0.4	11.5 to 12.3	3
Ruditapes philippinarum	−19.4 ± 0.2	−19.6 to −19.3	3	12.2[c] ± 0.3	12.0 to 12.5	3
Macoma incongrua	−11.0[ab] ± 0.3	−11.3 to −10.7	3	−3.8 ± 0.2	−3.9 to −3.5	3

[1]This value represents mainly composition of inorganic elemental sulfur deposits

gastropods *Littorina squalida* (mean $\delta^{13}C$ = −9.8‰) and *Homalopoma sangarense* (mean $\delta^{13}C$ = −10.0‰) had the highest and nearly identical values of $\delta^{13}C$. Also, no significant difference was found between $\delta^{13}C$ values of the deposit-feeding bivalve *Macoma incongrua* and grazers (Table 4). Another surface-deposit feeder, the gastropod *Batillaria cumingii*, showed slightly lower $\delta^{13}C$ values (mean = −12‰) than those of grazers.

The filter feeder *Ruditapes philippinarum* was much more ^{13}C-depleted (mean $\delta^{13}C$ = −19.4‰) than grazers and deposit feeders. However, another filter-feeding bivalve, *Pillucina pisidium*, was an extremely ^{13}C-depleted component of the studied community. The $\delta^{13}C$ values of *P. pisidium* muscle tissues (−26.4 to −25.9‰) were at least 5‰ lower than those of suspended POM, which was the most ^{13}C-depleted food source in the community (Tables 2 & 4). Gills of *P. pisidium*, which contained dense populations of chemoautotrophic bacteria, were significantly more depleted in ^{13}C (on average 1.6‰ less) than muscle tissues (Table 4).

The $\delta^{34}S$ range for consumers (about 30‰) was much greater than that expected for producers. Among consumers, the interspecific differences in $\delta^{34}S$ values were considerably greater than the differences inferred from the carbon isotope ratios. Most species, with the exception of *Ruditapes philippinarum* and *Homalopoma sangarense*, significantly differ in their $\delta^{34}S$ values (Table 4). The grazing periwinkle *Littorina squalida* was most ^{34}S-enriched (mean $\delta^{34}S$ = +14.1‰) among consumers. Slightly lower $\delta^{34}S$ values (about +12‰) were shown for another grazer (*H. sangarense*) and the filter feeder *R. philippinarum*. The deposit feeder *Macoma incongrua* (mean $\delta^{34}S$ = −3.8‰) and particularly the symbiont-bearing bivalve *Pillucina pisidium* (mean $\delta^{34}S$ = −16.0‰) were extremely depleted in ^{34}S. Intermediate $\delta^{34}S$ values (+7.6‰) were found for another surface-deposit feeder (*Batillaria cumingii*).

Fatty acid composition of dominant animal species

In decreasing order of importance, 16:0 and 18:0 were the dominant saturated fatty acids of all macroconsumers studied (Table 5). A high concentration of 14:0 was found only in *Pillucina pisidium*. The major monoenic acids (content >5%) were 16:1(n-7) in *P. pisidium*, 18:1(n-9) in *Macoma incongrua* and *Homalopoma sangarense*, 18:1(n-7) in *H. sangarense* and *P. pisidium*, and 20:1(n-11) in *M. incongrua* and *Littorina squalida*. Polyenoic acids with a content of >5% were 18:2(n-6) in *Batillaria cumingii*, 20:4(n-6) in all gastropods and in the bivalve *M. incongrua*, 20:5(n-3) in all mollusks, except *P. pisidium*, and 22:6(n-3) in *M. incongrua* and particularly *Ruditapes philippinarum*. We note that non-methylene-interrupted (NMI) acids were present in all species studied (Table 5).

Isotopic and lipid markers in the community food web

The stable isotopic composition of consumers and the main primary producers were superimposed on the $\delta^{34}S$ versus $\delta^{13}C$ diagram (Fig. 2). Only 2 species of animals fell within the area, determined by $\delta^{13}C$ and $\delta^{34}S$ values of the main primary producers; the eelgrass blade epifaunal gastropods *Littorina squalida* and *Homalopoma sangarence* occupied an intermediate position between *Zostera marina* and epiphytes.

All bottom dwelling species differed in ^{34}S and/or ^{13}C from the primary producers sampled. The $\delta^{13}C$ values of the filter feeder *Ruditapes philippinarum* and the deposit feeders *Batillaria cumingii* and *Macoma*

Table 5. Fatty acid composition of consumers in the *Zostera marina* community of Novgorodskaya Bight, the Sea of Japan (mean ± SD, N = 3, % of total fatty acids). Table contains only fatty acids with concentrations >1% in 1 of the consumers. Mean values marked by the same letter are not significantly different ($p > 0.05$); not marked mean values are significantly different (1-way ANOVA Tukey HSD test, $p \leq 0.05$). Pa: phytanic acid

Fatty acid	*Pillucina pisidium*	*Pillucina pisidium* (gills)	*Littorina squalida*	*Batillaria cumingii*	*Homalopoma sangarense*	*Ruditapes philippinarum*	*Macoma incongrua*
14:0	8.1 ± 1.3	1.8^{ab} ± 0.3	2.9^{a} ± 0.2	1.6^{ab} ± 0.2	2.5^{ab} ± 1.2	1.2^{b} ± 0.3	1.3^{ab} ± 0.4
15:0	0.5^{a} ± 0.0	0.7^{a} ± 0.2	0.4^{a} ± 0.1	0.9^{a} ± 0.1	0.9^{a} ± 0.3	0.6^{a} ± 0.0	1.6 ± 0.2
16:0	15.0^{ab} ± 2.4	10.5^{bc} ± 0.8	8.5^{c} ± 0.4	7.3^{c} ± 0.8	16.7^{a} ± 3.4	14.8^{ab} ± 0.7	9.4^{c} ± 1.6
16:1(n-9)	0	0	0.5^{a} ± 0.8	0.6^{a} ± 1.1	1.2^{a} ± 2.2	0.3^{a} ± 0.5	0.4^{a} ± 0.7
16:1(n-7)	10.1 ± 1.0	19.6 ± 0.7	2.1^{a} ± 0.7	2.5^{a} ± 0.7	4.1^{a} ± 0.9	2.8^{a} ± 1.1	3.3^{a} ± 1.4
i17:0	0.4^{a} ± 0.0	0.3^{a} ± 0.1	0.4^{a} ± 0.2	1.0^{b} ± 0.1	0.6^{a} ± 0.1	1.3^{b} ± 0.1	1.3^{b} ± 0.2
ai17:0	0.3^{ab} ± 0.0	0.3^{a} ± 0.0	0.2^{a} ± 0.0	0.9^{b} ± 0.1	0.3^{a} ± 0.1	1.7 ± 0.1	0.5^{ab} ± 0.0
16:2+Pa	0.3^{a} ± 0.0	0.4^{a} ± 0.0	0.4^{a} ± 0.1	1.1^{b} ± 0.2	0.2^{a} ± 0.1	1.4^{b} ± 0.4	0.4^{a} ± 0.0
17:0	0.7^{a} ± 0.0	1.0^{b} ± 0.1	0.3^{c} ± 0.0	1.4^{d} ± 0.1	1.4^{d} ± 0.0	1.3^{d} ± 0.0	0.6^{a} ± 0.0
18:0	7.9^{a} ± 0.8	5.6^{b} ± 0.3	8.1^{a} ± 0.9	6.9^{ab} ± 0.6	4.9^{ab} ± 1.0	7.1^{ab} ± 0.4	6.4^{ab} ± 1.2
18:1(n-9)	1.7^{a} ± 0.1	3.6^{ab} ± 0.6	4.0^{ab} ± 0.8	3.5^{ab} ± 0.4	5.5^{b} ± 1.2	3.2^{ab} ± 0.2	9.2 ± 0.9
18:1(n-7)	11.1^{a} ± 0.8	15.9 ± 0.6	1.7^{b} ± 0.3	1.7^{b} ± 0.1	9.3^{a} ± 0.7	2.3^{b} ± 0.5	1.1^{b} ± 0.2
18:2(n-6)	1.5^{a} ± 0.1	2.8^{ab} ± 0.5	3.5^{abc} ± 1.1	6.4^{c} ± 0.8	4.1^{abc} ± 1.4	0.8^{a} ± 0.3	3.6^{ab} ± 2.6
19:1	0.4^{a} ± 0.3	0.3^{a} ± 0.4	1.2^{a} ± 2.1	0.6^{a} ± 0.9	0.4^{a} ± 0.7	0.2^{a} ± 0.3	0.6^{a} ± 0.8
18:3(n-3)	1.4^{a} ± 0.2	1.3^{a} ± 0.1	1.5^{a} ± 0.3	1.2^{a} ± 0.3	0.7^{a} ± 0.1	0.8^{a} ± 0.2	0.9^{a} ± 0.4
18:4(n-3)	0.2^{a} ± 0.0	0.1^{a} ± 0.1	0.3^{a} ± 0.0	0.3^{a} ± 0.1	0	0.7^{a} ± 0.2	0.5^{a} ± 0.2
20:0	0.3^{a} ± 0.0	0.2^{a} ± 0.0	0.1^{a} ± 0.0	0.2^{a} ± 0.0	0.1^{a} ± 0.1	0.3^{a} ± 0.2	0.1^{a} ± 0.0
20:1(n-11)	2.6^{a} ± 0.1	1.4^{a} ± 0.6	9.3^{b} ± 1.3	4.7^{a} ± 0.5	3.3^{a} ± 0.5	4.0^{a} ± 0.3	11.1^{b} ± 2.7
20:1(n-9)	1.7^{ab} ± 0.1	1.1^{a} ± 0.1	0.6^{c} ± 0.5	0.4^{c} ± 0.1	0	1.9^{b} ± 0.2	0.6^{c} ± 0.5
20:1(n-7)	5.6 ± 0.4	4.1^{c} ± 0.2	2.1^{a} ± 0.2	1.2^{b} ± 0.1	0.3^{b} ± 0.0	1.9^{a} ± 0.1	3.8^{c} ± 0.7
20:2nmi	1.7^{a} ± 0.2	0.8^{a} ± 0.1	0.1^{a} ± 0.1	0.3^{a} ± 0.0	0	0.1^{a} ± 0.1	0.2^{a} ± 0.1
20:2(n-6)	1.0^{a} ± 0.2	0.7^{a} ± 0.1	6.7^{b} ± 1.7	4.4^{ab} ± 0.1	1.6^{ab} ± 0.1	2.5^{ab} ± 0.2	1.5^{ab} ± 0.7
20:4(n-6)	3.9^{a} ± 0.4	4.2^{a} ± 0.1	12.3^{b} ± 2.3	17.1 ± 1.1	13.2^{b} ± 1.9	3.3^{a} ± 0.1	6.4^{a} ± 1.4
20:4(n-3)	2.1^{a} ± 0.3	1.5^{a} ± 0.1	0.2^{b} ± 0.0	0.2^{b} ± 0.1	0	0.5^{b} ± 0.1	0.2^{b} ± 0.2
20:5(n-3)	3.1^{a} ± 0.3	2.3^{a} ± 0.2	12.8 ± 2.4	6.6^{a} ± 0.9	5.7^{a} ± 0.9	7.1^{a} ± 1.2	7.4^{a} ± 3.5
22:2Δ7,13	1.1^{a} ± 0.1	1.3^{a} ± 0.2	5.5^{b} ± 0.9	4.0^{b} ± 0.4	0.9^{a} ± 0.2	2.2 ± 0.2	0.1^{a} ± 0.0
22:2Δ7,15	4.2^{a} ± 0.1	5.1^{a} ± 0.1	3.1 ± 0.5	5.1^{a} ± 0.5	6.0 ± 1.0	4.9^{a} ± 0.2	0
22:4(n-6)	0.4^{a} ± 0.4	0.6^{a} ± 0.0	0.7^{a} ± 0.1	2.3^{b} ± 0.3	1.9^{b} ± 0.4	1.7^{b} ± 0.3	1.4^{b} ± 0.2
22:5(n-3)	0.3^{a} ± 0.0	0.2^{a} ± 0.2	2.7^{b} ± 0.4	3.9^{b} ± 0.6	4.7 ± 0.8	2.7^{b} ± 0.2	2.7^{b} ± 1.0
22:6(n-3)	0.8^{a} ± 0.3	0.7^{a} ± 0.3	0.7^{a} ± 0.6	1.8^{a} ± 0.5	1.4^{a} ± 1.0	16.4 ± 0.7	5.6^{a} ± 0.5
24:1	0	1.4 ± 1.2	0	0	0	0	0

incongrua corresponded to those of a mixture of phytoplankton and eelgrass in different proportions, but all these mollusks were much more ^{34}S-depleted than would be expected from their feeding on such a mixture (Fig. 2). Consumption of other non-sampled food sources, such as ^{34}S-depleted benthic diatoms or bacteria, could be inferred for these animals from consideration of isotopic data only. The infaunal bivalve *Pillucina pisidium*, which bears chemoautotrophic bacterial symbionts, greatly differed in isotopic composition (both carbon and sulfur) from all photosynthetic producers in the community (Fig. 2).

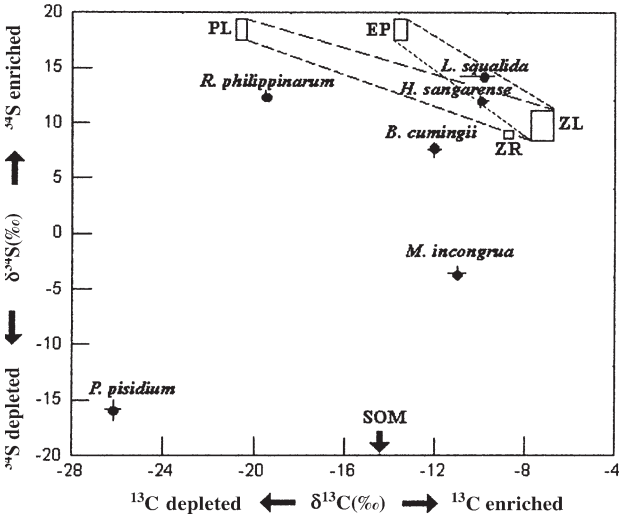

Fig. 2. Plot of $\delta^{13}C$ versus $\delta^{34}S$ values for consumers and food sources from the *Zostera marina* community of Novgorodskaya Bight, expressed as parts per thousand (‰). Mean value and range are shown for consumers. Food sources include: PL, phytoplankton; EP, epiphytes of eelgrass blades; ZL, *Z. marina* leaves; ZR, *Z. marina* rhizomes. The $\delta^{34}S$ values for PL and EP are based on literature values for marine algae. Broken lines outline the area of mixing of alternative food sources

The fatty acids and fatty acid ratios used to define food sources in the *Zostera marina* community are shown in Table 6. Only insignificant amounts of the acids 18:2(n-3) and 18:3(n-3), which are characteristic of *Z. marina* blades, were found in all studied animals (Fig. 3A). Even in *Littorina squalida* and *Homalopoma sangarence*, which are the closest to *Z. marina* by isotopic markers, the levels of these fatty acids were low. No significant differences were observed in the amounts of these fatty acids between studied mollusks.

The diatoms should be characterized by a 16:1/16:0 ratio ≥ 1 along with a high content of the acid 20:5(n-3). In all consumers, including the epifaunal gastropods of *Zostera marina* blades, the 16:1/16:0 ratio was much less than 1. An exception was found in the bivalve *Pillucina pisidium*; however, it had a low content of 20:5(n-3) (Fig. 3B).

A high concentration of C_{18} and C_{20} polyunsaturated fatty acids (PUFA) is typical of many macroalgae (Kayama et al. 1989). Among the animals from the *Zostera marina* community, high levels of C_{18} and C_{20} PUFA occurred only in gastropods, both grazers and deposit feeders. It should be pointed out that very high concentrations of 20:4(n-6) acid were detected in gastropods (Fig. 3C).

Among the studied consumers of the community, a high concentration of 22:6(n-3), specific to zooplankton, was observed only in the filter feeder *Ruditapes philippinarum* (Fig. 3D), which was also very similar to POM based on its isotopic composition (Fig. 2).

The tissues of the investigated consumers of the *Zostera marina* community contained an appreciable quantity of the acid 18:1(n-7), typical for bacteria. Very high concentrations of 18:1(n-7) occurred in both the muscle tissue and the bacteria-bearing gills of the bivalve *Pillucina pisidium* and in the muscle tissue of the grazing gastropod *Homalopoma sangarence*. *P. pisidium* significantly differed ($p < 0.01$) from other mollusks by the highest 18:1(n-7)/18:1(n-9) ratio. Elevated levels of branched fatty acids, characteristic of bacteria inhabiting marine sediments, were found in the infaunal bivalves *Ruditapes philippinarum* and *Macoma incongrua*, as well as in the surface-deposit-feeding gastropod *Batillaria cumingii* (Fig. 3E).

DISCUSSION

The structure of the eelgrass community in Novgorodskaya Bight is typical of many seagrass ecosystems. In such ecosystems, the organic matter of seagrasses dominated the primary producer biomass, while consumer biomass is dominated by bivalves and gastropods. Of the consumers, we consider those species of invertebrates which comprise the main part of community biomass. Unlike the traditional comparative analysis of representatives from different trophic groups, this approach has allowed a more adequate representation of the major fluxes to the upper trophic levels of the ecosystem.

Table 6. Fatty acids and fatty acid ratios that were used as markers for food sources in the *Zostera marina* ecosystem. Main sources are abundant groups with high levels of marker acids. Other important sources are less abundant groups or groups having species with high levels of marker fatty acids

Fatty acid	Main sources	Other important sources	Ratio	Marker for:
16:1(n-7)	Diatoms (1)	Bacteria (2)	16:1(n-7)/16:0	Diatoms (1), bacteria (4)
iso17:0; anteiso17:0	Bacteria (3)			
18:1(n-7)	Bacteria (4)		18:1(n-7)/18:1(n-9)	Bacteria (10)
18:2(n-6)	*Z. marina* (5)	Algae (6), fungi (7), protozoa (9)	18:2(n-6) +18:3(n-3)/ C_{20} +C_{22} PUFAs	*Z. marina* (11)
18:3(n-3)	*Z. marina* (5)	Fungi (7), algae (6)		
18:4(n-3)		Dinoflagellates (8), cryptomonads (8)		
20:4(n-3)		Fungi (7), protozoa (9), algae (6)		
20:5(n-3)	Diatoms (1), *Pneophylum lejolisii* (5)	Algae (6)		
22:6(n-3)	Zooplankton (5)	Protozoa (9), dinoflagellates (8), cryptomonads (8)		

References: (1) Ackman et al. (1968), (2) Gillian & Hogg (1984), (3) Findlay et al. (1990), (4) Volkman et al. (1980), (5) present study, (6) Kayama et al. (1989), (7) Erwin (1973), (8) Sargent et al. (1987), (9) Zhukova & Kharlamenko (1999), (10) Pond et al. (1997), (11) Nichols et al. (1986)

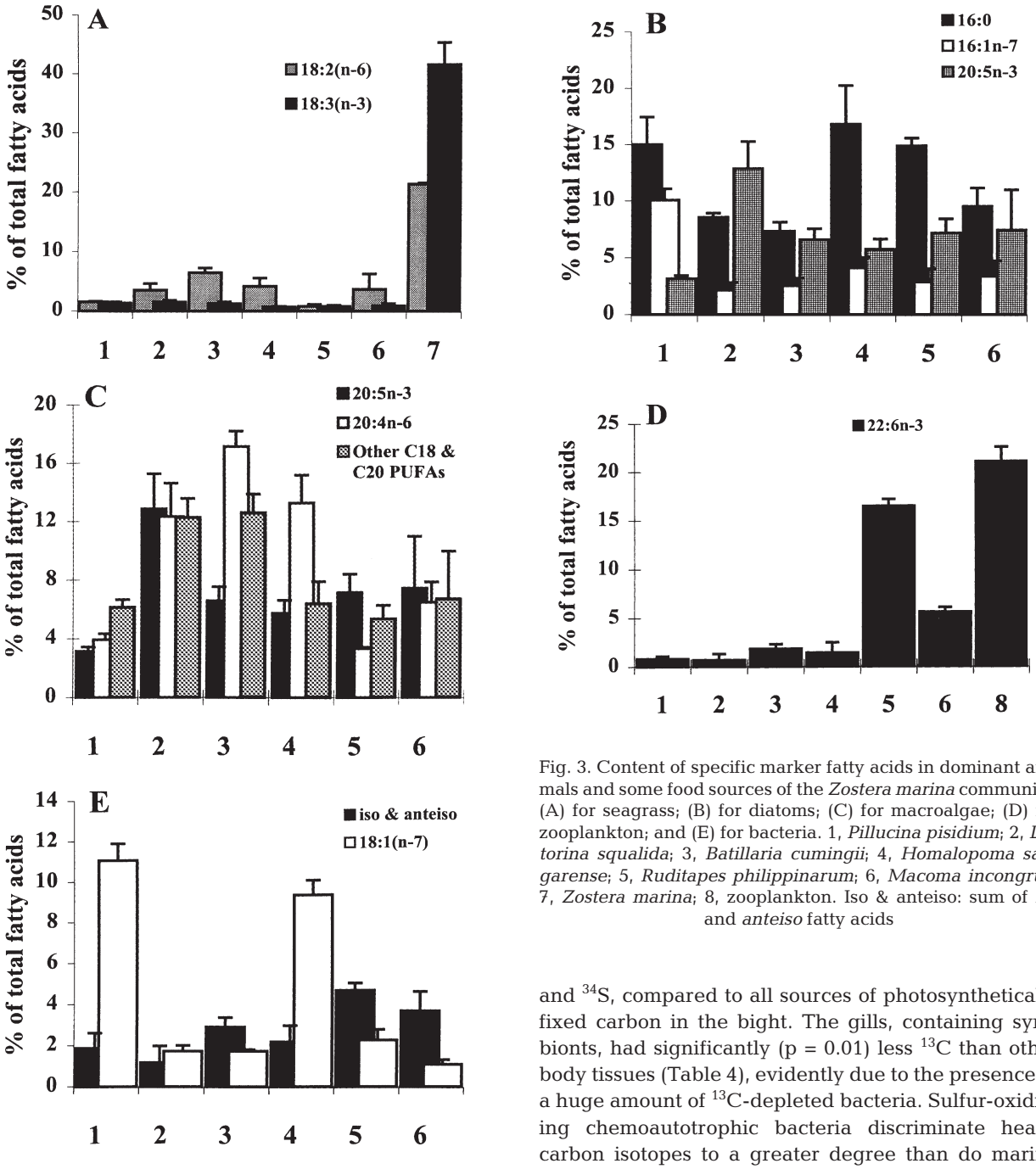

Fig. 3. Content of specific marker fatty acids in dominant animals and some food sources of the *Zostera marina* community: (A) for seagrass; (B) for diatoms; (C) for macroalgae; (D) for zooplankton; and (E) for bacteria. 1, *Pillucina pisidium*; 2, *Littorina squalida*; 3, *Batillaria cumingii*; 4, *Homalopoma sangarense*; 5, *Ruditapes philippinarum*; 6, *Macoma incongrua*; 7, *Zostera marina*; 8, zooplankton. Iso & anteiso: sum of *iso* and *anteiso* fatty acids

Of the dominant consumers in the *Zostera marina* community of Novgorodskaya Bight, the tiny bivalve *Pillucina pisidium* comprised 1.3% of total zoobenthos biomass. Similarly to other members of the family Lucinidae, this species can feed as a filter feeder and also has numerous chemoautotrophic bacterial endosymbionts inhabiting cells of its enlarged gills (Rodionov & Yushin 1991).

Symbiont-free body tissues of *Pillucina pisidium* were dramatically depleted in heavy isotopes, both ^{13}C and ^{34}S, compared to all sources of photosynthetically fixed carbon in the bight. The gills, containing symbionts, had significantly ($p = 0.01$) less ^{13}C than other body tissues (Table 4), evidently due to the presence of a huge amount of ^{13}C-depleted bacteria. Sulfur-oxidizing chemoautotrophic bacteria discriminate heavy carbon isotopes to a greater degree than do marine photoautotrophs (Ruby et al. 1987), and very low $\delta^{13}C$ values (–34.0 to –31.7‰) were shown for sulfur-oxidizing bacteria isolated from symbiont-bearing bivalves (Cary et al. 1989, Conway et al. 1989).

Total sulfur content in the gill sample of *Pillucina pisidium* exceeded 15% of its dry weight due to the presence of elemental sulfur reserves in the cells of sulfur-oxidizing symbionts; therefore, $\delta^{34}S$ value of the gill sample (+0.2‰) reflects the isotope composition of elemental sulfur, rather than symbiont and host organic sulfur. Organic sulfur of symbiont-free tissues

was greatly depleted in ^{34}S, as a result of a major contribution of reduced sulfur from interstitial water of the anaerobic sediment throughout symbiotrophic feeding upon sulfur-oxidizing bacteria.

Contribution of chemoautotrophs to *Pillucina pisidium* feeding was calculated, using $\delta^{13}C$ values for symbiotic bacteria (mean = –33‰) (Cary et al. 1989, Conway et al. 1989) and suspended POM (–20.9‰) as endmembers in Eq. (2). This conservative estimate shows that at least 52% of host organic carbon was derived from symbionts.

The fatty acid composition of this mollusk is characterized by a high content of 18:1(n-7). The increased concentration of this acid has been reported for other symbiont-bearing mollusks (Conway & McDowell Capuzzo 1991), as well as *Pillucina pisidium* from Vostok Bay in the Sea of Japan (Zhukova et al. 1992). Another distinctive feature of mollusks with bacterial symbionts, namely a very low level of (n-3) PUFAs, was detected in this animal. The sum of essential PUFAs [20:4(n-6), 20:5(n-3) and 22:6(n-3)] in the tissue of this mollusk was significantly lower ($p = 0.05$) than in any studied invertebrate from this ecosystem.

The gastropod *Littorina squalida* constitutes only 2.3% of total consumer biomass in the community studied. Epiphytic algae and *Zostera marina* are accessible primary producers for this grazing consumer. It has been demonstrated that some species of grazing gastropods actually feed on seagrasses, and sometimes they are the only consumers of seagrass among all animals studied in the ecosystem (Stephenson et al. 1986). *L. squalida* exhibited the highest $\delta^{13}C$ and $\delta^{34}S$ values among consumers. On the dual isotope plot (Fig. 2), this mollusk occupies an intermediate position between *Z. marina* and epiphytic algae, suggesting a nearly equal consumption of organic matter from both sources; the contribution of eelgrass ranges from 41% (estimated from $\delta^{13}C$ data) to 52% (estimated from $\delta^{34}S$ values). However, only small amounts of 18:3(n-3) and 18:2(n-3), which are markers of consumption of live eelgrass, were detected in the lipids of this mollusk. The ratio of 18:3(n-3) and 18:2(n-3) to non-NMI acids (C_{20} and C_{22} PUFAs) was also low — 0.17. This ratio was much higher (up to 1.22) in lipids of animals feeding on seagrass blades (Nichols et al. 1986). This may be because *L. squalida* assimilates *Z. marina* organic matter via intermediate links in the microheterotrophic community. A significant part of the organic carbon produced by *Z. marina* leaves was found in epiphytic microheterotrophs (Kirchman et al. 1984). By feeding on these microheterotrophs, epifauna may obtain ^{13}C-enriched organic matter void of seagrass marker fatty acids. In such a case, the $\delta^{34}S$ values of mollusks would be similar to the $\delta^{34}S$ values of seawater or epiphytes. *L. squalida* was the most ^{34}S-enriched among the consumer species.

The fatty acid composition of *Littorina squalida* reflected the ingestion of several components of the epiphytic community. A high level of 20:5(n-3) in *L. squalida* was due to feeding on *Pneophyllum lejolisii* or diatoms. Lipids of *L. squalida* contain large amounts of 20:4(n-6), which is probably derived by the assimilation of some component of the eelgrass blade community besides diatoms and *P. lejolisii*. This component may be a species of fungi rich in 20:4(n-6); many such fungi are encountered on seagrasses (Novak 1984) and may contain high concentrations of 20:4(n-6) (Kim et al. 1998).

Unlike *Littorina squalida*, the less ^{13}C-enriched gastropod *Batillaria cumingii* (3.6% of total zoobenthos biomass) was an obligatory bottom dweller. Benthic POM is therefore likely to be the main food source of this consumer. However, *B. cumingii* was >3‰ more enriched in ^{13}C in comparison to benthic POM or SOM. Thus, it can be presumed that the proportion of ^{13}C-enriched *Zostera marina* organic carbon was higher in *B. cumingii* nutrition than in SOM or benthic POM. *B. cumingii* was significantly (on average 2‰) depleted in ^{34}S, compared to *Z. marina* leaves and rhizomes ($p = 0.018$). Moreover, the main feature of *B. cumingii* lipid composition was an unusually high content of 20:4(n-6) (average 17.1%). Fungi and protozoa are the most probable components of the microbial community, which may contain high concentrations of 20:4(n-6); furthermore, fungi, bacteria and diatoms can assimilate inorganic sulfur, including some part of the ^{34}S-depleted sulfide from reduced sediment. At the water-sediment interface we found no significant numbers of benthic diatoms. There were high levels of bacterial fatty acids in the lipids of *B. cumingii*, including the branched fatty acids typical for sulfate-reducing bacteria; however, the majority of bacteria do not have PUFAs. These indications led us to believe that *B. cumingii* can selectively ingest both fungi and bacteria, which may be important intermediate links between dead *Z. marina* and this deposit feeder. Total contribution of eelgrass carbon to *B. cumingii* nutrition may be very high — up to 57% (evaluated versus plankton-derived carbon using Eq. 2), but overestimation is probable because neither the proportion of epiphytic algae lost nor ^{13}C-fractionation by fungi was considered.

The gastropod *Homalopoma sangarense* was the most abundant grazer in the studied community, constituting 4.8% of total consumer biomass. This species was found on seagrass blades only. On the dual $\delta^{13}C/\delta^{34}S$ isotope plot *H. sangarense* falls into both overlapping areas determined by eelgrass and epiphytes or eelgrass and plankton (Fig. 2). Estimates of

the *Zostera marina* contribution to *H. sangarense* carbon varied from 38% (evaluated versus epiphytes) to 71% (evaluated versus suspended POM).

The most striking features of the fatty acid composition of *Homalopoma sangarense* were a high content of 18:1(n-7), comparable with the concentration of this acid in *Pillucina pisidium*, which bears symbiotic bacteria, and a high 18:1(n-7)/18:1(n-9) ratio (Table 5). The *H. sangarense*, which belongs to the more primitive *Archaeogastropoda* with brush-like radula, can probably obtain the bulk of eelgrass organic matter via selective feeding on epiphytic bacteria. Bacteria were important among the microheterotrophs using the organic matter of *Zostera marina* (Kirchman et al. 1984). Bacterial production was high enough to meet most of the trophic requirements of *H. sangarense* (Kharlamenko & Lysenko 1991a). High content of 20:4(n-6) in *H. sangarense* may indicate an important contribution of fungi to grazer nutrition, as in the case of *L. squalida*.

The filter feeder *Ruditapes philippinarum*, found mostly on the periphery of the seagrass meadow, was only slightly ^{13}C-enriched compared to plankton (Fig. 2). At the same time, it was much more ^{34}S-depleted than could be expected assuming similarity of the δ^{34}S value of plankton organic sulfur with that of seawater sulfate. Low contribution of *Zostera marina* (<4%) to *R. philippinarum* food, estimated on the basis of δ^{13}C values, cannot explain observed ^{34}S-depletion of the tissues of this mollusk.

Among macroconsumers of the eelgrass meadow, only *Ruditapes philippinarum* had the high concentrations of 22:6(n-3) characteristic of zooplankton (Table 6). Mollusks of this species can consume a variety of food items, including zooplankton (Sorokin & Giovanardi 1995), and the correspondence of the *R. philippinarum* fatty acid composition to the fatty acid composition of its food has been shown (Albentosa et al. 1996). This mollusk showed a high content of branched fatty acids, especially iso- and anteiso17:0, which are characteristic of sediment bacteria (Fig. 3E). It suggests that *R. philippinarum* assimilated a notable amount of bacteria from resuspended sediment. Contribution of ^{34}S-depleted bacteria, inhabiting reduced sediment, to *R. philippinarum* nutrition could be high enough to result in the observed depletion of ^{34}S in mollusks.

The bivalve mollusk *Macoma incongrua* constituted the predominant part (72.9%) of total consumer biomass in the eelgrass meadow of Novgorodskaya Bight. The highest population density and the use of 2 feeding modes (filter feeding and surface-deposit feeding) differentiate *M. incongrua* from other bivalves of the eelgrass community.

Macoma incongrua contained much less 22:6 (n-3), typical for zooplankton, than filter-feeding *Ruditapes philippinarum* ($p = 0.003$). We therefore conclude that this mollusk obtained food mainly from the water-sediment interface or from bottom sediment. However, the δ^{13}C values of *M. incongrua* were on average 4‰ greater than those of benthic POM. This mollusk was at least 13‰ more depleted in ^{34}S than eelgrass. Moreover, *M. incongrua* is one of the most ^{34}S-depleted species in comparison to other marine or estuarine animals (Peterson et al. 1986, Currin et al. 1995). With the exception of mollusks with symbiotic sulfur bacteria (Conway et al. 1989), only *Polymesoda erosa*, the mangrove intertidal bivalve, had a δ^{34}S value (–4.3‰) (Newell et al. 1995) similar to *M. incongrua*, but only 5‰ lower than mangrove leaves. It could be supposed that other unsampled primary producers or microheterotrophs, which assimilated ^{34}S-depleted reduced sulfur from interstitial waters, were important in the *M. incongrua* diet.

We can exclude benthic diatoms as an important ^{34}S-depleted primary food source for *Macoma incongrua*: δ^{34}S values lower than those known to occur in the most ^{34}S-depleted benthic microalgae (+3.9 to +5.4‰, Currin et al. 1995, Stribling & Cornwell 1997), relatively small amounts of 20:5(n-3) and low 16:1(n-7)/16:0 ratio lipids (Fig. 3B) do not confirm the importance of live diatoms in the diet of this consumer. Moreover, microscopic examination did not reveal notable amounts of benthic microalgae in surface sediment or in samples of benthic POM from the eelgrass community studied.

The microbial food chain, based on bacteria and fungi that are capable of assimilation of ^{34}S-depleted sulfide from reduced sediments, can be assumed to be the most probable source of ^{34}S-depleted organic matter for *Macoma incongrua*. However, no apparent indication of preferential assimilation of some particular component of the microbial community, bacteria, fungi or protozoa, can be inferred from the fatty acid composition of consumer tissues.

Thus, we can suppose that eelgrass, epiphytes and plankton remain the main primary sources for the detritus food chain, supporting the deposit feeder *Macoma incongrua*. Maximal contribution of *Zostera marina* organic carbon to *M. incongrua* was 64%, evaluated using δ^{13}C values of eelgrass leaves versus suspended POM as endmembers in Eq. (2). The most conservative case, using δ^{13}C values of eelgrass leaves versus epiphytes, gives minimal *Z. marina* contribution to *M. incongrua* of 38%.

The dominant mollusk, *Macoma incongrua*, seems to be the species which can directly assimilate large portions of eelgrass organic matter as detritus. The benthic microbial food chain, based on mixed detritus of plankton, epiphytes and eelgrass, also plays an important part in *M. incongrua* nutrition as a protein-rich

source. Lipid markers of specific food sources are less pronounced in this consumer, reflecting a more diverse spectrum of food items than in other consumer species of the *Zostera marina* community. The use of several food sources by animals at the upper level of the detritus food chain is quite justified. Bacteria, which provide the first live link in the detritus food chain, are fairly inactive during the greater part of the year, because of the low water temperature in this area of the sea (Kharlamenko & Orlova 1990, Kharlamenko & Lysenko 1991a). Seagrass detritus and bacteria lack a number of essential fatty acids and amino acids. The use of several food sources compensates for these drawbacks in the detritus food web.

In conclusion, based on $\delta^{13}C$ data, eelgrass carbon plays an important role as a food resource for the dominant consumers in the *Zostera marina* community of Novgorodskaya Bight. At least 4 of the 6 animal species dominating the biomass, including the gastropods *Littorina squalida*, *Homalopoma sangarense*, *Batillaria cumingii* and the most abundant surface-deposit-feeding bivalve *Macoma incongrua*, derived on average ca 50% of their carbon from eelgrass.

The eelgrass carbon was only of minor importance for filter-feeding bivalves: *Ruditapes philippinarum* fed mainly on suspended POM, and *Pillucina pisidium* derived its carbon mainly from chemoautotrophic endosymbionts. Both these findings were supported by characteristic fatty acid markers.

The fatty acid analysis showed that none of the consumer species studied directly assimilated fresh organic matter of eelgrass. Interspecific variations of both the fatty acid compositions and the sulfur isotope ratios of consumers suggest that there is a variety of pathways, frequently complex, by which eelgrass organic matter reaches invertebrates at lower trophic levels of the community food web. In the case of epifaunal grazers, fatty acid markers showed that epiphytic bacteria (for *Homalopoma sangarense*) or eukaryotic microorganisms (for *Littorina squalida*) were the most important links between eelgrass and grazers. The fatty acid markers were much less pronounced in deposit feeders, reflecting an absence of prevailing kinds of food items.

The $\delta^{34}S$ data turned out to be of limited use for calculation of the contributions of different primary sources to the *Zostera marina* community food web, despite negligible trophic fractionation of sulfur isotopes (Peterson et al. 1986). Comparison of $\delta^{34}S$ data and fatty acid markers in consumers allows us to suppose that heterotrophic microorganisms at the base of the detritus food web could change, to various degrees, the isotopic composition of organic sulfur flowing through the food web, due to assimilation of inorganic sulfur with different ^{34}S contents.

As a result, the $\delta^{34}S$ data allow recognition of consumers with a high contribution of sulfur-oxidizing chemoautotrophic bacteria (symbiont-bearing *Pillucina pisidium*) in their diets and those with a major proportion of heterotrophic microorganisms from reduced bottom sediment. In the community studied, deposit-feeding *Macoma incongrua* showed the most pronounced $\delta^{34}S$ signal, which indicated utilization of ^{34}S-depleted links from the detritus food web; however, fatty acid composition of this consumer did not show a high content of the fatty acid markers specific for bacteria. Notable contribution of benthic bacteria to the filter feeder *Ruditapes philippinarum* was confirmed by both the $\delta^{34}S$ data and the bacterial fatty acid markers.

Further progress in food web studies of seagrass ecosystems using the complex of multiple SIRA and fatty acid analysis is possible based on the analysis of separate components of the seagrass epiphytic community and micro- and meiobenthic organisms inhabiting surface sediments.

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