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Lipid Contents and Fatty Acid Compositions of *Idotea baltica* and *Sphaeroma serratum* (Crustacea: Isopoda) as Indicators of Food Sources

Ermelinda Prato[1,*], Antonio Danieli[2], Michele Maffia[2], and Francesca Biandolino[1]

[1]*CNR – Institute for Coastal Marine Environment, Section of Taranto, Via Roma 3, Taranto 74100, Italy*

[2]*Laboratory of General Physiology, Department of Biological and Environmental Science and Technology. Univ. of Lecce, Monteroni, Lecce I-73100, Italy*

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Ermelinda Prato, Antonio Danieli, Michele Maffia, and Francesca Biandolino (2012) Lipid contents and fatty acid compositions of *Idotea baltica* and *Sphaeroma serratum* (Crustacea: Isopoda) as indicators of food sources. *Zoological Studies* **51**(1): 38-50. The lipid and fatty acid (FA) compositions of *Idotea baltica* and *Sphaeroma serratum*, from Mar Piccolo basin at Taranto (Ionian Sea), Italy, were analyzed during winter and summer to assess their feeding habits. The 2 isopods showed strong similarities in total lipid contents. Phospholipids (PLs) were the major lipid class in both species, followed by triacylglycerols (TAGs). A low proportion of energy-storage lipids suggested a regular food supply. Twenty-seven fatty acids were identified in the species studied. Unsaturated FAs (UFAs) represented the predominant proportion in both species in the seasons studied. Among them, monounsaturated FAs (MUFAs) showed higher levels. Regarding FAs corresponding to the potential food of the 2 isopods studied, *I. baltica* and *S. serratum* displayed different FA profiles. Large amounts of 18:2n-6 and18:3n-3 were found, especially in *S. serratum* suggesting a specific selection of phytodetritus from green algae or terrestrial material of neighboring vegetation. The FA marker for diatoms of *I. baltica* differed from that of *S. serratum*, although both species showed major consumption of diatoms during summer. *Idotea baltica* showed higher levels of 22:6n-3 and 20:4n-6 in winter suggesting a preference for dinoflagellates and macroalgae in this period. High levels of the carnivorous marker (the 18:1n-9/18:1n-7 ratio) reflected consumption of animal materials, especially in winter. Examination of trophic markers indicated that *I. baltica* and *S. serratum* consumed a mixed diet, showing that they have the ability to choose among available food sources. http://zoolstud.sinica.edu.tw/Journals/51.1/38.pdf

Key words: *Idotea baltica*, *Sphaeroma serratum*, Lipids, Fatty acids, Trophic markers.

Predicting food web patterns is fundamental to understanding the processes involved in estuarine ecosystems. Food webs in estuaries are often complex, largely due to the high diversity of both producers and consumers inhabiting these ecosystems, as well as relatively extreme and variable environmental conditions.

Among major approaches, the use of fatty acid (FA) biomarkers to establish trophic interactions within estuarine ecosystems is widely used as a reliable method for determining important dietary information over an extended time span (Graeve et al. 1994, Lee 1995, Shi et al. 2001, Dalsgaard et al. 2003, Richoux and Froneman 2008), unlike stomach-content analyses that are extremely time consuming and can only provide an indication of the most recent ingestion of food (Sano et al. 2003). Previous studies used FAs as biomarkers for bacteria (Rajendran et al. 1993), diatoms (Parrish et al. 2000), dinoflagellates (Parrish et al. 2000), zooplankton (Falk-Petersen et al. 2002), macroalgae (Johns et al. 1979, Khotimchenko and

*To whom correspondence and reprint requests should be addressed. E-mail:linda.prato@iamc.cnr.it

Vaskovsky 1990), and vascular plants (Wannigama et al. 1981). FAs form structural and functional components of membranes, and because of biochemical restrictions on the synthesis of FAs in many marine organisms, it is possible to recognize FAs derived from their diet (Arts et al. 2001). These lipid components are not selectively processed during food intake and incorporation, and hence in many circumstance, are integrally and markedly transferred through aquatic food webs (Dalsgaard et al. 2003). In this way, a predator's FA composition can reveal dietary sources of the lipids. Thus, FAs can be used to follow the transfer of energy through a marine food web and qualitatively assess the relative trophic position of an organism (Sargent and Whittle 1981, Dalsgaard et al. 2003, Falk-Petersen et al. 2004). Because recent works reported that rotifers and daphinids synthesize long-chain FAs such as 20:5n-3 even if these are not provided by their diet (Weithoff and Wacker 2007, Wacker and Weithoff 2009, Martin-Creuzburg et al. 2010), this can be a restriction to the use of trophic biomarkers.

Isopods are dominant components of estuarine macrobenthos worldwide (Bruce 1992, Dias and Sprung 2003, Gonçalves et al. 2005), and in most regions of the world, they were recorded in close association with submerged macrophyte beds (Henninger et al. 2008, Wang et al. 2010). However, they are recognized omnivores, capable of utilizing a wide range of diets ranging from carnivory (including cannibalism), to herbivory and detritivory (Briones-Fourzán and Lozano-Alvarez 1991, Newman et al. 2007). In particular, they consume benthic microalgae, filamentous algae, macroalgae, detritus, bacteria present on sediment surfaces, small invertebrates, and even conspecifics (Nicotri 1980, Franke and Janke 1998). Among isopods, *Idotea baltica* (Pallas) and *Sphaeroma serratum* are the most common isopods in the littoral zone of the Mar Piccolo basin (Ionian Sea, southern Italy), where they are numerically dominant and are important food for many predators in this ecosystem (crustaceans, fish, and birds) (De Nicola et al. 1989).

Previous studies were undertaken to characterize the ecology and geographical distribution of these isopods (Naylor 1955, Sywula 1964), feeding habits, and habitat selection (Orav-Kotta and Kotta 2004), as well as their potential use as a tool in ecotoxicological studies (De Nicola et al. 1989, Prato et al. 2006, Annicchiarico et al. 2007). In Mar Piccolo, they are often associated with macroalgae and particularly with drifting mats of the green algae *Enteromorpha intestinalis*, *Chaetomorpha linum*, and *Ulva laetevirens* and red algae such as *Gracilaria gracilis*, *G. dura*, and *Gracilariopsis longissima*. However, it is unclear whether the association is favorable for the isopods because they use algae as food, protection from predators, or both. In addition *S. serratum* was found under stones (pers. observ.), where it may find food deposited on bottom sediments. To date, the degree of omnivory in the diets of these isopod species and their trophic positions in the Mar Piccolo basin have not been assessed, and although they are numerically the most abundant macrobenthic component in this basin, little is known about their diets.

The FA approach can be useful for exploring habitat preferences, feeding strategies, and food sources of these isopod species from Mar Piccolo basin. The roles that FAs play as trophic markers of lipid flow through food webs were examined in marine ecosystems in a number of studies (Sargent and Whittle 1981, Graeve et al. 1994 2001, Phleger et al. 1998, Dalsgaard et al. 2003). Since marked changes in environmental conditions affect metabolic rates and can alter the production, storage, and conversion of FAs, and mask trophic links, FA compositions should mainly be considered qualitative indicators of trophic links (Dalsgaard et al. 2003).

The aim of this study was to analyze, for the 1st time, total lipid contents and FA compositions of *I. baltica* and *S. serratum* from the Mar Piccolo estuary, in order to elucidate the spectrum and variety of their food sources and trophic relationships. Since specific FAs are used as trophic biomarkers, they can highlight the ability of *I. baltica* and *S. serratum* to use food sources available in their environment, which can be a key factor in ecosystem functioning. In addition, the FA compositions of these isopods were compared between winter and summer collections to determine how seasonal environmental conditions influence their FA compositions.

MATERIALS AND METHODS

Study area

Mar Piccolo is located in the northern part of the town of Taranto (Fig. 1) with a total surface area of 20.72 km^2 structured in 2 parts, a 1st inlet and a 2nd inlet, which have maximum depths of 13 and 10 m, respectively. There is restricted

circulation: water exchanges occur with the Gulf of Taranto (on the Ionian Sea), through 2 channels, and this water flow is subject to tidal effects with the average variation between high and low tides not exceeding 30-40 cm. In terms of hydrographic characteristics, Mar Piccolo can be compared to an estuarine ecosystem. Salinity is influenced by the input of fresh water derived from small tributary rivers and by freshwater springs called *citri*. The low hydrodynamism and reduced water exchange with the nearby Mar Grande create high water stratification mainly in summer. In addition, urban expansion and intensive agriculture have caused increased nutrient and organic-matter levels (Cardellicchio et al. 1991) particularly at the 2nd inlet where the mild hydrodynamism allows organic matter to be deposited on the bottom.

Sampling strategy

Idotea baltica and *S. serratum* were sampled during winter and summer months (of 2008) in the 2nd inlet of the Mar Piccolo estuary (Ionian Sea, Italy; 40°29'17"E; 17°14'23"N). Sampling was carried out once a month, and materials from 3 mo were pooled as replicates. The euryhaline *I. baltica* is a littoral and sublittoral crustacean of tidal shores, and was recorded in close association with macrophyte beds of *Chaetomorpha linum*, *Enteromorpha intestinalis*, *Ulva laetevirens*, *Gracilaria gracilis*, *G. dura*, and *Gracilariopsis longissima*. *Sphaeroma serratum* usually lives in coastal marine or brackish waters, which are often subject to variations in salinity. It lives under stones close to the shore or among seaweed, in 5-80-cm-deep water, and it usually moves within a range of only a few meters.

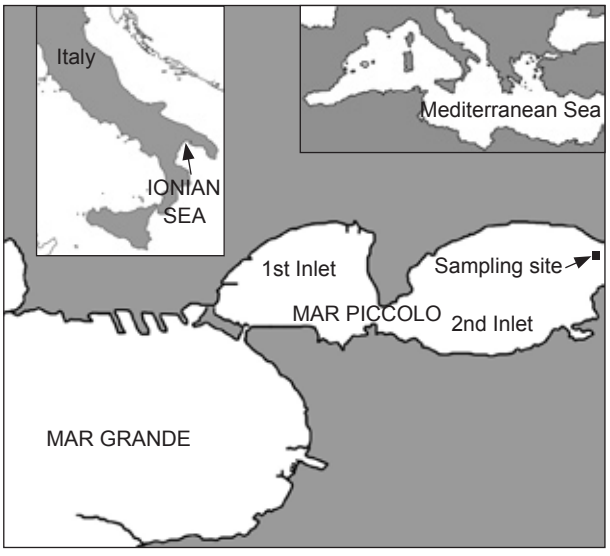

Fig. 1. Location of isopod sampling sites in Mar Piccolo basin (southern Italy).

Isopods were collected under stones and by hand from macroalgae, and small quantities of sediment were sieved through a 1-mm stainless-steel sieve. Individuals were placed in a clean plastic container with water and sediment collected *in situ*. This container was immediately carried to the laboratory where the animals were placed in aquaria. In order to remove gut contents, the animals, prior to processing, were starved for 1 d, and after about 24 h, samples of each species were prepared for biochemical analysis. During the sampling period, water temperatures were 12.2 ± 3.4 and 26.7 ± 1.5°C; salinities were 35.5 ± 0.67 and 34.9 ± 0.6 psu; dissolved O_2 values were 8.1 ± 1.8 and 9.5 ± 0.4 mg/L, and pH values were 8.3 ± 0.2 and 8.4 ± 0.2 in winter and summer, respectively.

Sample preparation for lipid determination

All isopods analyzed were adults with total lengths of 10.7 ± 0.8 mm for *S. serratum* and 14.2 ± 1 mm for *I. baltica*. Samples of each species were dried to a constant weight at 50°C and ground to powder in a mortar. About 500 mg of dry weight pooled from 35-50 samples was used for each extraction, including 3 replicates for each species.

Lipids were extracted using a solvent mixture of chloroform: methanol (2: 1, v/v) following Folch et al.'s (1957) method. The chloroform layer, containing dissolved lipids, was collected, washed with 0.88% potassium chloride, and completely removed using a rotary evaporator. The total lipid content was determined gravimetrically. Lipid analyses were carried out in triplicate, and the results were expressed as mg/g of dry weight (DW). Triacylglycerols (TAGs) and total cholesterol (CHL) were measured by the colorimetric enzymatic Trinder method (1969), using a commercial kit (SGM, Rome, Italy). Phospholipids (PLs) were quantified by a colorimetric enzymatic method (Takayama et al. 1977) with a commercial kit (SGM). TAG, PL, and CHL levels were expressed as a percentage of total lipids.

FA analysis

FAs of total lipids were transesterified to methyl esters (FAMEs) in a boron trifluoride-

catalyzed methanol: benzene solution (1: 2, v/v). The mixture was shaken, and then heated in boiling water for 45 min (Allinger 1986). Samples were allowed to cool, then 1 ml of distilled water was added followed by vigorous shaking. FAMEs were recovered in the upper benzene phase. Benzene phases were concentrated under nitrogen and kept at -20°C until further analysis.

Analysis of FAMEs was performed by gas chromatography (GC) using an HP 6890 series GC (Hewlett Packard, Wilmington, DE, USA) equipped with flame ionization detector. FAMEs were separated with a Omegawax 250 capillary column (Supelco, Bellafonte, PA, USA) (30 m long, 0.25-mm internal diameter, and 0.25-mm film thickness). Helium was used as the carrier gas at a flow rate of 1 ml/min. The column temperature program was as follows: 150 to 250°C at 4°C/min and then held at 250°C. FAMEs were identified by comparing retention times with a standard (Supelco 37 Component FAME Mix). FAs were quantified by integrating areas under peaks in the GC traces, with calibration derived from an external standard containing different methyl esters. FA biomarkers (specific FAs and ratios of FAs) of the major potential food sources found at Mar Piccolo were identified by comparisons with published literature (Table 1).

Table 1. Trophic and dietary fatty acid markers used in this paper

Source	Trophic markers
Diatoms[a]	20:5n-3 16:1/16:0 20:5n-3/22:6n-3 >1
Dinoflagellates[b]	22:6n-3 20:5n-3/22:6n-3 <1
Bacteria[c]	Σ 15 + 17
Terrestrial detritus or green algae[d]	18:2n-6 + 18:3n-3
Carnivory[e]	18:1n-9/18:1n-7 20:1 + 22:1
Macroalgae[f]	20:4n-6
Detritus[g]	PUFA/SAFA

[a]Dunstan et al. (1994) and Parrish et al. (2000). [b]Graeve et al. (1994), Parrish et al. (2000), and Nelson et al. (2001). [c]Kaneda (1991) and Rajendran et al. (1993). [d]Budge and Parrish (1998) and Dalsgaard et al. (2003). [e]Phleger et al. (1998) and Falk-Petersen et al. (2000). [f]Khotimchenko and Vaskovsky (1990) and Graeve et al. (2001). [g]Fahl and Kattner (1993). PUFA, polyunsaturated fatty acid; SAFA, saturated fatty acid.

Statistical analysis

For each species, the data obtained (total lipid content, lipid classes, and trophic markers, expressed as percent of total FAMEs) were arcsine-transformed and analyzed using a two-way analysis of variance (ANOVA). Means were separated at or below the 5% probability level, using Tukey's honest significant difference (HSD) post-hoc test. Data were tested for normality prior to being analyzed using the Kolmogorov-Smirnov test. Bartlett's test was used to test for homogeneity of variances (Zar 1996). Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA), and 95% confidence intervals (CI) are given.

To aid in visualizing the results obtained, a principle component analysis (PCA) and hierarchical cluster analysis were performed to investigate variations in FA signatures between species and seasons and identify the FAs most responsible for those variations. These analyses were carried out using STATISTICA 8 (StatSoft, Tulsa, OK, USA).

RESULTS

Total lipid contents

Total lipid contents of *I. baltica*, expressed on a DW basis, were 30.13 ± 2.77 mg/g DW in winter and 33.45 ± 1.50 mg/g DW in summer. *Sphaeroma serratum* showed similar values of 33.71 ± 1.56 mg/g DW in winter and 32.89 ± 2.46 mg/g DW in summer. Results of the statistical analysis indicated that lipid contents of each species did not significantly vary between winter and summer, or between species in the same season (ANOVA, $p > 0.05$) (Table 2).

Lipid classes

PLs were the major lipid class especially in *I. baltica* (74.70% in winter and 65.84% in summer) followed by TAGs that in *S. serratum* accounted for 31.20% of total lipids in winter and 39.75%, in summer (Fig. 2). Statistical analysis (two-way ANOVA) revealed significant differences in PL

and TAG contents between species and seasons ($p < 0.05$); however the interaction between species and seasons was not significant ($p > 0.05$) (Table 2). *Sphaeroma serratum* showed the significantly highest CHL level in winter (ANOVA; Tukey's test $p < 0.05$). Post-hoc (Tukey's) test results are reported in figure 2.

FAs

Mean values ± standard deviation (SD) of the FA composition are given in table 3. At least 27 FAs with numbers of carbon atoms of 14-24 were identified in the species studied. On average, unsaturated FAs (UFAs) represented the predominant portion in *I. baltica* and *S. serratum*

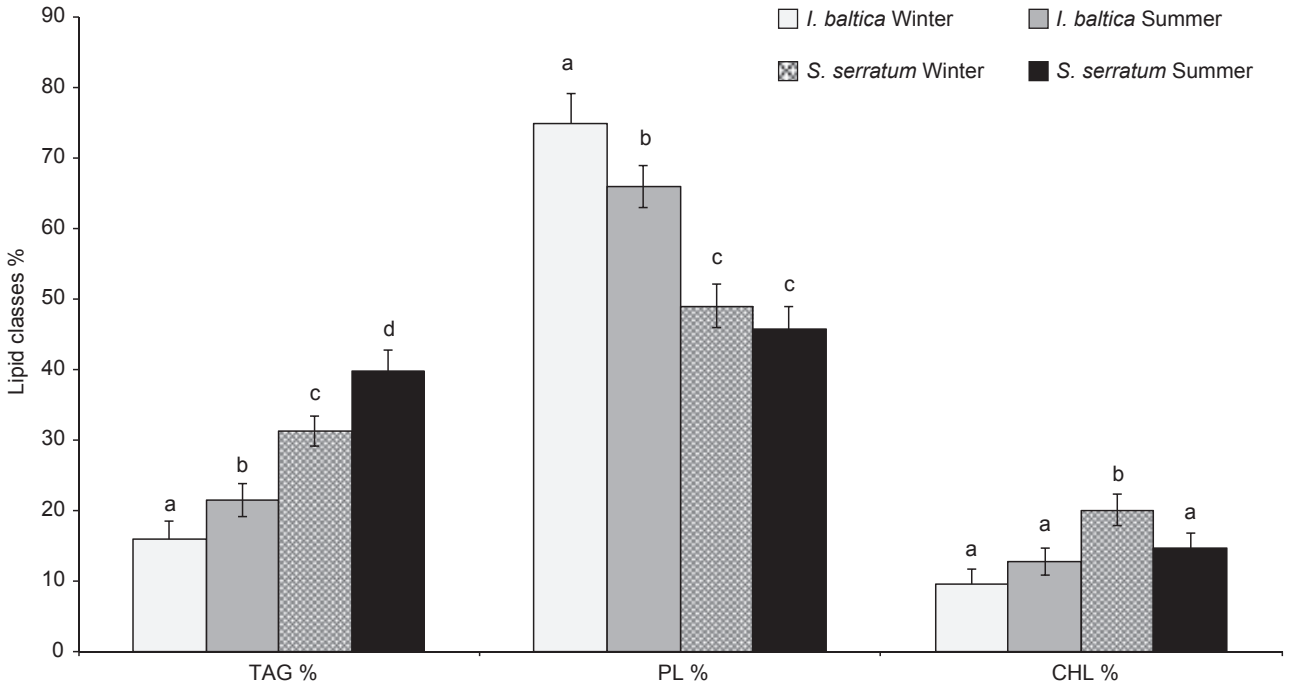

Fig. 2. Lipid class composition of *Idotea baltica* and *Sphaeroma serratum* collected in winter and summer. Different letters (a, b, and c) indicate a statistically significant difference between intra- and interspecific groups (Tukey's test; $p < 0.05$).

Table 2. Results of a two-way ANOVA performed on lipids, triacylglycerols (TAGs), phospholipids (PLs), and cholesterol (CHL) contents

Source	*d.f.*	Mean square	*F* Ratio	*p*	Source	*d.f.*	Mean square	*F* Ratio	*p*
Total Lipid					TAG				
Species	1	0.00	1.15	ns	Species	1	1.27	111.48	***
Seasons	1	0.00	1.50	ns	Seasons	1	0.22	19.76	**
Interaction	1	0.01	3.04	ns	Interaction	1	0.03	0.27	ns
Error	8				Error	8			
PL					CHL				
Species	1	0.47	131.23	***	Species	1	0.60	20.77	**
Seasons	1	0.03	7.99	*	Seasons	1	0.00	0.00	ns
Interaction	1	0.02	0.67	ns	Interaction	1	0.28	9.80	*
Error	8				Error	8			

Level of significance: ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 3. Fatty acid (FA) composition (% of total FAs) of 2 isopod species (*Idotea baltica* and *Sphaeroma serratum*) from Mar Piccolo of Taranto, Italy

	I. baltica Winter (*n* = 35)	± S.D.	*I. baltica* Summer (*n* = 50)	± S.D.
	30.13	2.77	33.45	1.50
Lipid content (mg/g dry weight)				
14:0	4.83	0.54	5.15	0.08
15:0	1.91	0.13	0.60	0.02
16:0	20.02	0.98	32.01	1.39
17:0	1.72	0.02	1.24	0.07
18:0	9.93	0.65	8.39	0.21
20:0	2.75	0.62	0.23	0.00
22:0	2.12	1.11	0.37	0.06
24:0	2.46	0.21	0.49	0.03
SAFA	**45.74**	4.3	**48.49**	2.60
14:1	2.47	0.28	2.32	0.39
15:1	0.5	0.01	0.78	0.05
16:1	1.72	0.02	9.51	0.20
17:1	0.69	0.03	0.68	0.10
18:1n-9	11.42	1.13	10.39	1.36
18:1n-7	9.93	0.65	11.26	1.82
20:1n-9	0.21	0.01	0.65	0.18
22:1n-9	1.32	0.3	0.17	0.00
24:1n-9	1.88	0.42	2.71	0.04
MUFA	**30.14**	2.85	**38.49**	1.68
18:2n-6	6.41	0.84	3.43	0.00
18:3n-6	0.45	0.01	1.99	0.01
18:3n-3	2.89	0.39	1.89	0.01
20:2	1.17	0.13	0.22	0.00
20:3n-6	0.81	0.02	0.29	0.01
20:3n-3	1.63	0.06	0.84	0.01
20:4n-6	4.76	0.13	1.52	0.01
20:5n-3	1.34	0.23	1.40	0.01
22:2	0.72	0.01	0.21	0.00
22:6n-3	3.94	0.28	1.22	0.01
PUFA	**24.12**	2.2	**13.02**	0.95
	S. serratum Winter (*n* = 50)	± S.D.	*S. serratum* Summer (*n* = 50)	± S.D.
	33.71	1.56	32.89	2.46
Lipid content (mg/g dry weight)				
14:0	3.35	0.78	3.75	0.26
15:0	1.08	0.08	0.83	0.04
16:0	19.87	1.15	24.45	1.14
17:0	1.95	0.34	1.32	0.12
18:0	8.73	0.75	7.21	0.37
20:0	1.74	0.08	0.12	0.02
22:0	2.01	0.07	0.26	0.03
24:0	1.87	0.02	0.51	0.1
SAFA	**40.60**	3.3	**38.45**	1.83
14:1	0.47	0.02	0.19	0.00
15:1	0.51	0.03	0.64	0.01
16:1	9.18	0.32	11.96	0.23
17:1	2.04	0.11	0.95	0.08
18:1n-9	10.97	0.95	9.04	0.87
18:1n-7	6.41	0.38	10.89	1.05
20:1n-9	1.35	0.1	1.28	0.16
22:1n-9	1.37	0.25	1.96	0.41
24:1n-9	3.43	0.31	3.29	0.92
MUFA	**35.73**	2.47	**40.20**	1.87
18:2n-6	5.11	0.83	4.47	0.51
18:3n-6	1.00	0.06	1.29	0.09
18:3n-3	5.18	0.62	4.16	0.15
20:2	1.40	0.06	0.21	0.04
20:3n-6	0.98	0.05	0.23	0.00
20:3n-3	0.87	0.07	0.32	0.05
20:4n-6	3.85	0.59	4.18	0.84
20:5n-3	1.22	0.41	2.57	0.71
22:2	0.84	0.04	0.34	0.02
22:6n-3	3.22	0.18	3.58	0.23
PUFA	**23.67**	2.9	**21.35**	1.64

SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

in both seasons studied, and among UFAs, these species showed a higher level of mono-UFAs (MUFAs) than poly-UFAs (PUFAs).

The mean percentage of saturated FAs (SFAs) significantly differed between the 2 species collected in summer (two-way ANOVA; $p < 0.05$), with higher values exhibited by *I. baltica*. Regarding MUFAs, *I. baltica* and *S. serratum* demonstrated significantly higher levels in summer than winter (two-way ANOVA; $p < 0.05$), and in winter *S. serratum* had significantly higher levels of MUFAs than *I. baltica* (two-way ANOVA; $p < 0.05$). The PUFA content in *I. baltica* was significantly higher in winter than summer (two-way ANOVA; $p < 0.05$), while there was no consistent difference between summer and winter for *S. serratum*. Regarding comparisons between species, *S. serratum* displayed a significantly higher PUFA level than that found in *I. baltica* during the summer period only (two-way ANOVA; $p < 0.05$) (Table 3).

Palmitic (16:0) and stearic (18:0) acids were the most abundant SFAs recorded in the 2 isopods in both seasons, although lower palmitic acid levels were found in winter in both species than in summer. High amounts of MUFAs were indicated by considerable levels of oleic (18:1n-9) and vaccenic acids (18:1n-7) in both isopods; in addition, considerable levels of palmitoleic acid (16:1n-7) were detected in both species, except for *I. baltica* collected in winter which showed a lower level (1.72% of total FAs). Among the PUFAs observed in this study, linoleic (18:2n-6), arachidonic (AA, 20:4n-6), and docosaesaenoic acids (DHA, 22:6n-3) were the most abundant in *I. baltica*, especially in winter samples, while in *S. serratum*, linoleic acid (18:2n-6), α linolenic acid (18:3n-3), AA, and DHA were the main PUFAs in both seasons (Table 3).

FA trophic markers

In general, the pattern of change in trophic markers varied between seasons and between species (two-way ANOVA), which indicates that season and species had effects on the concentrations of FAs in the organisms' tissues (Table 4).

In both isopods, the diatom biomarkers 20:5n-3, 16:1n-7+18:1n-7, 20:5n-3/22:6n-3, and 16:1n-7/16:0 were well represented, especially in *S. serratum* collected in summer (Fig. 3). The 20:5n-3/22:6n-3 ratio was > 1 in *I. baltica* collected in summer, while it was < 1 in *S. serratum* in both seasons.

Idotea baltica showed significant differences between seasons for almost all trophic markers ($p < 0.05$), while *S. serratum* showed a lower seasonal variation and only for some trophic markers, such as 20:5n-3, 16:1n-7+18:1n-7, 20:5n-3/22:6n-3, branched and odd-numbered FAs, and 18:1n-9/18:1n-7 (Fig. 3). Differences in biomarker levels between *I. baltica* and *S. serratum* were significantly higher in summer than in winter (Tukey's test; $p < 0.05$) (Fig. 3).

The 18:1n-9/18:1n-7 ratio, proposed as an indicator of carnivorous behavior in different aquatic organisms, presented values of 1.15 in winter and 0.92 in summer in *I. baltica*, and values of 1.71 in winter and 0.83 in summer in *S. serratum*, thus characterizing these species as more carnivorous in the winter period. A two-way ANOVA showed significant seasonal differences ($p < 0.05$) (Table 4). The other 2 FA biomarkers for a carnivorous diet, 20:1n-9 and 22:1, were significantly higher in *S. serratum* than *I. baltica*, in both seasons examined ($p < 0.05$). The FA signature of dinoflagellates (22:6-n3) showed moderate amounts in *S. serratum* in both seasons (3.22% and 3.58% of total FAs), similar to that observed in *I. baltica* collected in winter, while in summer, its 22.6n-3 content was significantly lower ($p < 0.05$). AA (20:4n-6), used as a general marker of macroalgae, showed similar amounts in *S. serratum* collected in winter and summer and in *I. baltica* in winter ($p > 0.05$), with percentages of 3.85% and 4.18% of total FAs (4.76% of total FAs) respectively (Fig. 3).

Specific bacterial marker FAs (the sum of odd-numbered and branched FAs) were significantly higher in winter than summer in both isopods ($p < 0.05$), but no differences were detected between species in winter samples ($p > 0.05$) (Fig. 3).

In addition, 18:2n6+18:3n3, used as a marker of terrestrial input, showed high values in both isopods studied. The statistical analysis revealed significant differences between seasons in *I. baltica* ($p < 0.05$), while on the other hand, *I. baltica* showed similar values of this trophic marker to that *S. serratum* in winter ($p > 0.05$) (Fig. 3).

Figures 4 and 5 present the distributions of the most significant trophic markers along the 2 first principal components and groupings and/or differences among samples. Three principal components were extracted which accounted for 87% of the variance, and the scatterplot of scores on the 1st 2 principal components (PC1 and PC2) showed a separation between species

(Fig. 4). Loading of variables on the 1st 2 principal components showed that PUFA/SFA, 22:6n-3, 20:4n-6, 18:2n-6+18:3n-3, and branched and odd-numbered FAs, were the dominant variables for PC1, and the 2 species showed similar values in winter. FA trophic markers (FATMs) that contributed most to the separation of groups along PC2 were 20:1n-9+22:1, 20:5n-3, 16:1n-7/16:0, and 16:1n-7+18:1n corresponding to the highest values in *S. serratum* collected in summer (Fig. 4).

DISCUSSION

In isopod tissues examined, low levels of total lipids were detected with strong similarities among them, in both seasons. Low levels of storage lipids (TAG) were also observed especially in winter, and comparisons of the 2 species showed that *S. serratum* had higher TAG levels than *I. baltica*. Since TAGs are short-term energy reserves, differences in TAG contents are likely indicative of

Table 4. Results of a two-way ANOVA performed on fatty acid trophic markers

Source	*d.f.*	Mean square	*F* Ratio	*p*	Source	*d.f.*	Mean square	*F* Ratio	*p*
20:5n-3					16:1+18:1n-7				
Species	1	0.14	3.55	n.s.	Species	1	0.11	41.69	***
Seasons	1	0.34	8.89	*	Seasons	1	0.69	256.89	***
Interaction	1	0.28	7.19	*	Interaction	1	0.03	10.80	*
Error	8				Error	8			
22:6n-3					20:4n-6				
Species	1	0.44	103.94	***	Species	1	0.37	20.71	**
Seasons	1	0.68	160.82	***	Seasons	1	0.73	40.62	***
Interaction	1	0,99	236.87	***	Interaction	1	0.95	53.37	***
Error	8				Error	8			
18:2n-6+18:3n-3					18:1n-9				
Species	1	0.25	41.04	***	Species	1	0.02	7.53	*
Seasons	1	0.40	64.28	***	Seasons	1	0.06	19.02	**
Interaction	1	0.11	17.34	**	Interaction	1	0.01	2.18	n.s.
Error	8				Error	8			
20:1n-9+22:1					15:0+17:0				
Species	1	1.40	292.34	***	Species	1	0.00	0.54	n.s.
Seasons	1	0.07	9.62	*	Seasons	1	0.68	410.49	***
Interaction	1	0.30	42.28	***	Interaction	1	0.07	44.45	***
Error	8				Error	8			
PUFA/SAFA					20:5n-3/22:6n-3				
Species	1	0.07	18.58	**	Species	1	0.05	2.14	n.s.
Seasons	1	0.05	14.79	**	Seasons	1	0.68	27.52	**
Interaction	1	0.04	9.95	*	Interaction	1	0.10	4.02	*
Error	8				Error	8			
16:1n-7/16:0					18:1n-9/18:1n-7				
Species	1	0.22	124.92	***	Species	1	0.04	1.51	n.s.
Seasons	1	0.04	20.57	***	Seasons	1	0.37	12.30	**
Interaction	1	0.03	14.40	**	Interaction	1	0.12	4.03	n.s.
Error	8				Error	8			

n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

different feeding strategies (Lee and Patton 1989).

Lipids of both species were dominated by PLs, which are important membrane components, and indicates a low dependence on lipid reserves, in agreement with data on benthic amphipods from cold waters (Graeve et al. 1997, Kawashima et al. 1999).

Like most animals, marine invertebrates have certain lipid requirements that must be fulfilled through their diet. EFAs cannot be synthesized by organisms at rates sufficient to meet their basic biochemical requirements and thus must largely be obtained through the diet (Arts et al. 2001). FATMs are useful tools to study trophic

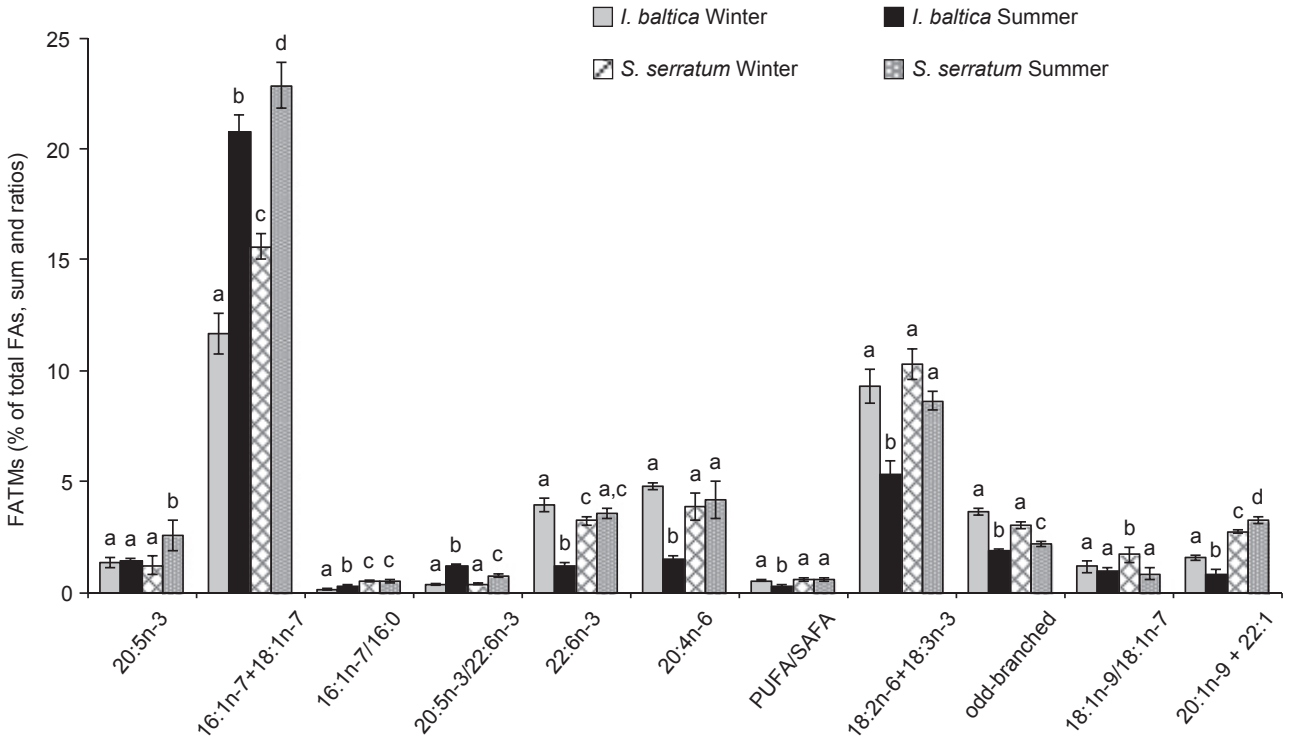

Fig. 3. Fatty acid trophic markers (FATMs) of the isopods *Idotea baltica* and *Sphaeroma serratum* collected in winter and summer. Different letters (a, b, and c) indicate a statistically significant difference between intra- and interspecific groups (Tukey's test; $p < 0.05$).

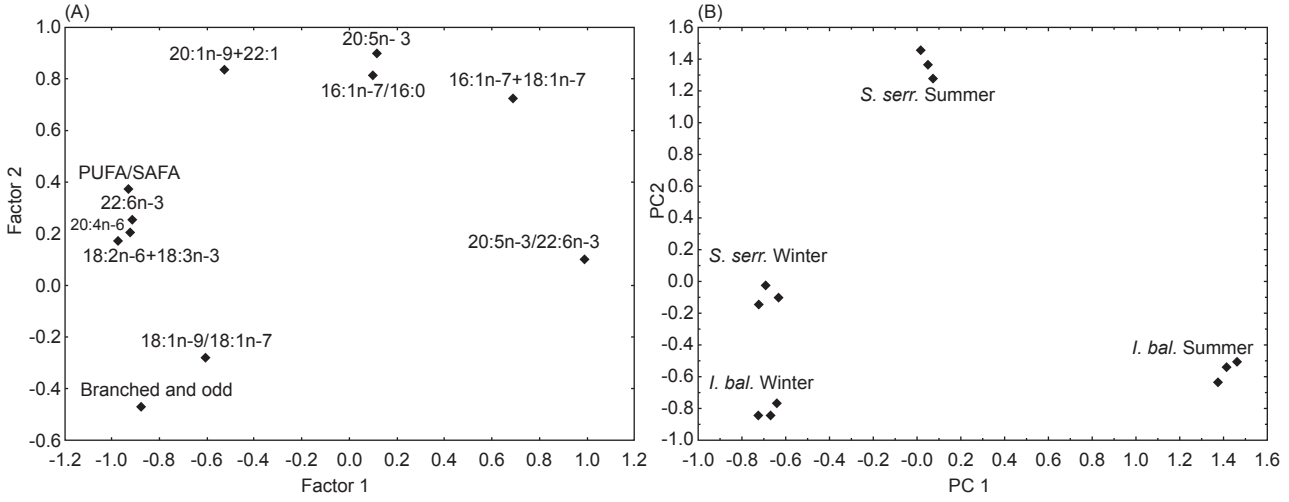

Fig. 4. Plots of loadings and scores for the principal component analysis of fatty acid trophic markers of the 2 isopod species in winter and summer.

ecology and determine food web connections. Contrary to more-traditional gut content analyses, which provide information only on recent feeding, FATMs provide information on dietary intake and food constituents leading to the sequestration of lipid reserves over a longer period of time (St John and Lund 1996, Kirsch et al. 1998, Auel et al. 2002). A previous study conducted on *Gammarus aequicauda* from the same area as this study showed the effectiveness of this approach (Biandolino and Prato 2006). However, because consumers selectively metabolize FAs and can convert some forms to others, FAs can only be used as semiquantitative food web tracers (Dalsgaard et al. 2003). In this study, the FATM approach showed differences in diet between *I. baltica* and *S. serratum* collected in Mar Piccolo of Taranto.

Sphaeroma serratum showed higher variability of food source use in both seasons, and it did not seem to be limited by food availability (i.e., by reduced algal biomass in winter). Indeed, in contrast to *I. baltica*, *S. serratum* was able to build up energy storages (in the form of TAGs), indicating a steady food supply. The relative contributions of carnivory, detritivory, and herbivory to the diet of these isopod species were shown to vary with season. The PCA grouped *I. baltica* and *S. serratum*, collected in winter, in a cluster separate from the remaining isopods collected in summer, yet also showed greater intraspecific differences between seasons. The FAs mainly responsible for similarities in the diets of the 2 isopods in winter were greater amounts of 22:6n-3, 20:4n-6, 18:2n-6+18:3n-3, and FAs which indicated greater dinoflagellate, macroalgal, detrital, and bacterial inputs to the diets of both species. This similarity between *I. baltica* and *S. serratum* collected in winter, may have been due to a decrease in the phytoplankton community recorded in the Mar Piccolo basin in this season (Marino 1988, Carrada et al. 1992, Caroppo and Cardellicchio 1995). In summer, *S. serratum* substantially shifted this feeding strategy when diatoms and material derived from animals formed greater components of its diet. Although the sum of 16:1n-7+18:1n-7 significantly differed between the 2 species and between the 2 seasons, higher levels found in summer in both species indicated that diatoms formed a greater component of the diet in this season. This is in agreement with the typical seasonal development of phytoplankton populations in Mar Piccolo and other coastal areas of the Mediterranean Sea, that are characterized by a predominance of diatoms during summer months, followed by a decrease in winter (Marino 1988, Carrada et al. 1992, Caroppo and Cardellicchio 1995). Thus the low level of this trophic marker in winter, potentially reflects the reduced availability of diatoms in this season.

Again, the ratio 16:1n-7/16:0 was significantly higher in *S. serratum* than in *I. baltica* in both seasons. This could have been due to the presence of diatoms in detritus samples or benthic diatoms which are often found in greater quantities on sediment surfaces (Alfaro et al. 2006). These results could reflect differences in feeding strategies, that are mostly determined by physiological requirements, which in turn are influenced by the feeding pattern and the ability to make use of food potentially available to them. This may explain the high level of diatom markers observed in *S. serratum* in winter, when diatoms are quite scarce (Caroppo and Cardellicchio 1995).

Since diatoms contain a high level of 20:5n-3 (and also high levels of 16:1n-7 and 18:1n-7),

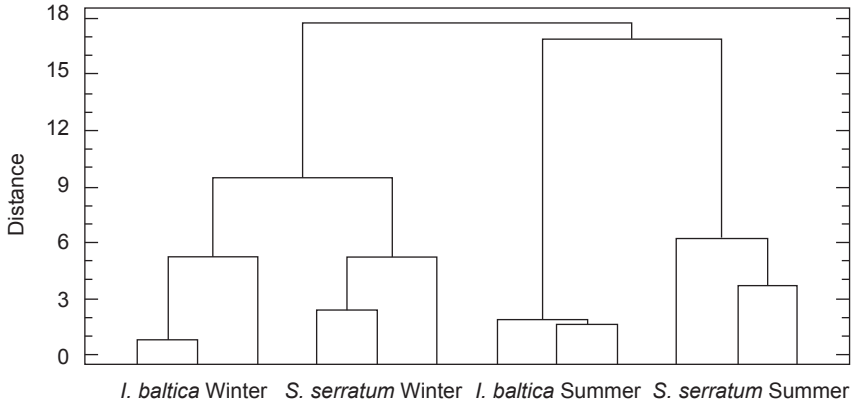

Fig. 5. Cluster analysis of fatty acid trophic markers of *I. baltica* and *S. serratum* in winter and summer.

whereas dinoflagellates are usually rich in 22:6n-3, the 20:5n-3/22:6n-3 ratio allows differentiation between a diatom- and a flagellate-based diet (Graeve et al. 1994, Nelson et al. 2001). Although in this study, tissues of *I. baltica* and *S. serratum* showed low amounts of these FAs, *I. baltica* showed a ratio of > 1 for 20:5n-3/22:6n in summer, suggesting a low consumption of dinoflagellates in this season.

Low values of the PUFA/SAFA ratio determined in the 2 isopods were linked to high levels of palmitic acid (16:0), suggesting a contribution of vegetal detritus in the diet (Reemtsma et al. 1990). It is important to highlight that 16:0 is widespread in all organisms, and most of them (such as crustaceans) can synthesize this FA on their own; hence it cannot be considered a useful FA biomarker, and certain caution is necessary in interpreting its amount. Although the environmental and biological context in which the isopods live allowed us to imagine the use of detritus-based food, another explanation could be that when the isopods have access to more resources, they synthesize their own lipids, one of which is 16:0.

In contrast, a recent study reported that polymethylene-interrupted (PMI)-FAs possess an unusual methylene substitution pattern and, as such, occur much less frequently in nature. In marine environments, it is thought that PMI-FAs are primarily de novo synthesized in bivalves and carnivorous gastropods and further accumulate and are transferred, to varying extents (depending on diet), through the food web (Albu et al. 2011). Therefore they were identified as a useful biomarker. Indeed, in a previous study, Prato et al. (2010) reported that PMI-FA 22:2 was a dominant FA among PUFAs for *M. galloprovincialis* from Mar Grande of Taranto. In the present study, we only found traces of this PMI-FA (22:2), suggesting that the isopods are unable to synthesize these acids, and the low amounts detected suggest the ingestion of animal detritus (such as bivalves and gastropods).

Sphaeroma serratum showed considerably higher values of trophic markers typically derived from animals (20:1n-9+22:1 and 18:1n-9/18:1n-7), which supports evidence of a more-carnivorous diet.

TPUFAs in green algae predominantly consist of 18:2n-6 and 18:3n-3, and these FA compositions are similar to those of terrestrial (vascular) plants since they have common ancestors (Harwood and Russel 1984, Raven et al. 1992). In this study, the sum of 18:2n-6 and 18:3n-3, as green algae or terrestrial markers, was high, indicating that these isopods receive considerable inputs of green algae present in the basin and of terrestrial material from neighboring vegetation. In particular in winter, *S. serratum* and *I. baltica* reflected a greater consumption of algal material in their diets as evidenced by the elevated presence of algal FAs. Between the 2 species, *S. serratum* showed major utilization of this food, probably also derived from phytodetritus which is found in the shoreline zone. Terrestrial organic matter can also be associated with bacteria or fungi, and constitutes an attractive and energetically utilizable food source for peracarida and other invertebrates (Hieber and Gessner 2002, Barlocher and Corkum 2003). In this study, the odd-branched FAs, as an indicator of a bacterial contribution, suggested a food supply for *I. baltica* and *S. serratum* from decaying organic matter especially in winter. AA, which is related to a dietary origin (macrophytes and leaves) or to synthesis from the respective precursor, linoloeic acid, showed high proportions in both species, except in *I. baltica* collected in summer, which showed a preference for a more-reliable diet based on phytoplankton or detritus. Although *I. baltica* was found exclusively on submerged macroalgae in Mar Piccolo basin in both summer and winter, the results suggested that in summer, the macroalgae serve primarily as shelter.

This study represents a 1st attempt to shed some light on food sources commonly utilized by *I. baltica* and *S. serratum* in the Mar Piccolo basin (Ionian Sea, southern Italy). The FA biomarkers proved useful in revealing differences between isopod species in the 2 seasons investigated and in feeding strategies adopted, such as detritivory, herbivory, and carnivory. In conclusion, we found that *I. baltica* was highly selective in its food choice, mainly depending on algal biomass, whereas *S. serratum* was a generalist which also utilizes detritus, benthic biofilms, bacteria, and decomposing organic material to large extent, with diet shifts when food availability changes, suggesting that this species could occupy a wider niche. In addition, significant differences recorded between the 2 species, especially in summer, could be explained by high competition for food resources, as the 2 species coexist in the same intertidal area; this leads to different distributions of resources at different times. Moreover, the results confirm that these species play essential roles in energy recycling in this ecosystem.

In the future, additional feeding experiments could help clarify the ecofunctional and trophodynamic roles of *I. baltica* and *S. serratum* in this ecosystem.

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