

Seasonal variation in carbon sources of mesograzers and small predators in an eelgrass community: stable isotope and fatty acid analyses

Sybill Jaschinski^{1,*}, Daniela C. Brepohl^{1,2}, Ulrich Sommer¹

¹Experimental Ecology, Department of Marine Ecology, IFM-GEOMAR Leibniz Institute of Marine Sciences, Düsternbrooker Weg 20, 24105 Kiel, Germany

²ICBM-TERRAMARE, Schleusenstr. 1, 26382 Wilhelmshaven, Germany

ABSTRACT: We assessed the seasonal change in trophic interactions at the mesograzer and small predator level in a temperate eelgrass system. This was done through stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and fatty acid analyses of eelgrass *Zostera marina*, attached epiphytes, sand microflora, the red alga *Delesseria sanguinea*, and the 4 most common mesograzer and predator species. Sampling took place monthly in the western part of the Baltic Sea during a whole year (April 2002 to February 2003). The trophic importance of microalgae was corroborated in all studied species. Red algae were consumed to a lesser extent and eelgrass was of minor importance. The degree of dependence on the main carbon sources was species-specific and varied with time. The small gastropod, *Rissoa membranacea*, mostly grazed epiphytes (73% on average), whereas the fractions of epiphytes and sand microflora showed a more balanced pattern in the other mesograzer species. Stable carbon data and fatty acid composition strongly suggested that epiphytic algae were the primary source of organic matter for mesograzers in late spring and autumn. In summer, sand microflora were of greater importance as carbon sources, except for the amphipod *Gammarus oceanicus* for which red algae was the most important. Stable nitrogen values indicated that the degree of carnivory was size-dependent in both omnivorous crustacean species studied; larger individuals generally occupied a higher trophic position than did smaller ones. Furthermore, the isopod *Idotea baltica* was more herbivorous in summer than in the other seasons. Our results confirm the importance of species-specific and temporal variability for the effect of mesograzers in eelgrass systems. The significance of mesograzers as determinants of food-web structure via the reduction of epiphytes is further corroborated by our study, and the observed high plasticity of mesograzers concerning food sources may promote the stability of eelgrass food webs despite strong seasonal variations in the biomass of primary producers.

KEY WORDS: Seagrass · Grazing · *Idotea* · *Gammarus* · *Littorina* · *Rissoa* · Trophic interactions · Nutrition

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Recent studies have strongly demonstrated how trophic interactions can influence coastal seagrass ecosystems (Hauxwell et al. 1998, Hughes et al. 2004, Heck & Valentine 2007). By feeding preferentially on fast-growing epiphytic algae, and thus facilitating growth and survival of the seagrass, small invertebrate grazers play an important role in these communities

(Duffy et al. 2001, Jaschinski & Sommer 2008a). Additionally, large parts of the energy flux to higher trophic levels including commercially important fish species are channelled through these consumers (Klumpp et al. 1992, Edgar & Shaw 1995). Although the relevance of mesograzers is widely acknowledged, it is a challenge to draw general conclusions on their ecological role in macrophyte systems because of the great spatial and temporal variability in the abundance of meso-

*Email: sjaschinski@ifm-geomar.de

grazers and their food (Edgar & Shaw 1995, Nelson & Waaland 1997, Douglass et al. 2010). In temperate systems, abiotic and biotic conditions change considerably in the course of the year with possible consequences for food web interactions (Polis et al. 1996). The strong seasonal variation of primary producer biomass and composition has a relevant influence on the abundance and composition of the mesograzers assemblage in seagrass ecosystems (Nelson & Waaland 1997, Gohse-Reimann 2007).

Most common mesograzers in temperate regions are considered generalists and can feed on a wide range of food sources from micro- to macroalgae, and some species are even omnivorous (Norton et al. 1990, Duffy & Harvilicz 2001, Orav-Kotta & Kotta 2003). This capacity may enable these species to switch between food sources and feed on the most abundant prey (Chesson 2000). Such density-dependant prey-switching is thought to stabilize food web dynamics.

The results of laboratory experiments on feeding preference are limited in their conclusions concerning natural ecosystems because it is difficult to replicate the exact conditions that determine preferences in the field. Large scale ecological field studies, however, are expensive and time consuming and therefore scarce in macrophyte systems (Connolly et al. 2005). Traditionally, the analysis of gut contents is used to study the nutrition of consumer species. This method provides detailed taxonomic information on the ingested food, but is very time consuming. It also requires expert taxonomic knowledge and reveals only the ingested but not the assimilated food. Furthermore, only the food items consumed in a short period of time can be assessed with this method. Most importantly, gut content analysis also provides a biased image of the diet with an over-representation of food items that contain indigestible, structural material.

Stable isotope and fatty acid analysis, in contrast, have the benefit of supplying time-integrated estimates of assimilated food (Hobson & Welch 1992, Dahl et al. 2003). The ratios of naturally occurring stable carbon ($^{13}\text{C}/^{12}\text{C}$, $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) isotopes can be used in food web analyses in different ways considering that all biochemical reactions tend to accumulate the heavier isotope (fractionation). The fractionation of $\delta^{13}\text{C}$ is thought to be low—maximum of 1‰ per trophic level—and $\delta^{13}\text{C}$ is therefore useful to identify different carbon sources (France & Peters 1997). The $\delta^{15}\text{N}$ content is enriched by 3 to 4‰ per trophic level on average and the results can be used to construct the structure of a food web, assuming $\delta^{15}\text{N}$ of the basal food sources is known (Vanderklift & Ponsard 2003). Fatty acid analysis can be a reliable method to trace food sources in aquatic food webs, since laboratory experiments have proven that transfer of specific

fatty acids is conservative (Lee et al. 1971, Brett et al. 2006). A number of 'indicator' fatty acids specific for algal groups, like diatoms or dinoflagellates, can be used as biomarkers (Viso & Marty 1993, Desvillettes et al. 1997), and the quantitative pattern of all fatty acids, the fatty acid signature, can provide additional information (Dahl et al. 2003).

The use of stable isotope analysis is well established in the research of seagrass ecosystems (Kharlamenko et al. 2001, Connolly et al. 2005, Douglass et al. 2010), but most studies focus on 1 or 2 temporal sampling points. As a high temporal variability in the abundance of consumers and their food is common in benthic ecosystems (Edgar & Shaw 1995, Nelson & Waaland 1997, Douglass et al. 2010), a broader approach is necessary to assess the effect of mesograzers in seagrass meadows. We used stable isotope and fatty acid analyses to study the seasonal variation in carbon sources of common mesograzers and small predator species in an eelgrass system in the Baltic Sea.

MATERIALS AND METHODS

Study area. The research site was an eelgrass meadow adjacent to Falkenstein Beach in the inner Kiel Fjord, Germany (Fig. 1). The Kiel Fjord is located in the Kiel Bight, a part of the western Baltic Sea. The eelgrass meadow extends over an area of 23 ha and is interrupted by small, unvegetated patches (Bobsien 2006). Owing to the special hydrological situation in the Baltic Sea, salinity ranges between 10 and 20 depending on discharge rates, prevailing winds and season. The astronomical tide range is negligible, but storm events can cause changes in the water level. The studied eelgrass meadow extends from approximately 1.5 to 6 m depth. The sediment is sandy (grain sizes: 0.5 to 1 mm, 42%; >1 mm, 51%) and the content of organic matter is low (<1%). Grain size was analysed with a set of standardised sieves and sediment organic content was determined by drying (60°C, 48 h) and combusting (450°C, 24 h) the samples. The difference in weight was the ash-free dry weight of the organic content.

Sample collection. Samples for analyses were collected monthly from April 2002 to February 2003 at 3 m water depth. In November the sampling had to be cancelled because of a breakdown of the research vessel. All samples of macrophytes and consumers were collected by slowly dredging 3 times parallel to the coast at approximately the same position for about 10 min. The sampled plants and animals were placed in plastic containers with water from the collection site and transported to the laboratory for sorting and further processing. Samples of *Zostera marina*, attached epiphytes, the main red alga *Delesseria sanguinea* and

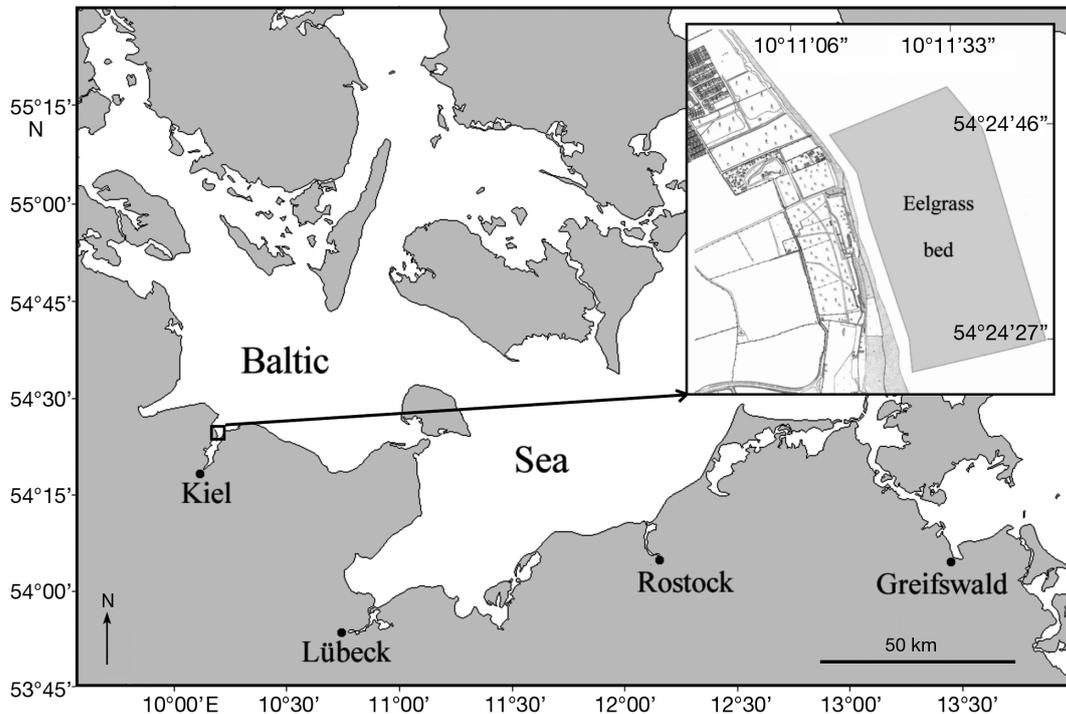


Fig. 1. Study area in the outer Kiel Fjord, Baltic Sea, Germany (Bobsien 2006). Inset shows detailed location of eel grass bed

the most common mesograzers—the isopod *Idotea baltica*, the amphipod *Gammarus oceanicus*, and the 2 gastropods *Rissoa membranacea* and *Littorina littorea* (Gohse-Reimann 2007)—were analysed in this study. Additionally, we sampled the most common small predators: the green crab *Carcinus maenas*, the sea stickleback *Spinachia spinachia* and 2 pipefish species, *Nerophis ophidion* and *Syngnathus typhle* (Bobsien 2006, Gohse-Reimann 2007). The sand microflora was directly measured as detritus-free substrate. For that purpose SCUBA divers collected 15 sediment cores (1 cm diameter) within the eelgrass bed.

Sample processing. In the laboratory, all plant materials were cautiously cleaned in 0.2 μm filtered seawater to remove detrital fragments and attached organisms. Epiphytes were carefully scraped from the eelgrass blades into small amounts of filtered seawater with a special plastic scraper and a scalpel. This suspension was filtered on precombusted (450°C, 24 h) GF/F filters (Whatman). All consumer species were kept alive overnight in filtered seawater to clear their intestinal tracts and then rinsed with distilled water. Samples for the fatty acids analysis were deep-frozen at -80°C until further processing. Samples for stable isotope analyses were dried to constant weight (60°C, 48 h), and plant and animal samples were ground with an agate mortar and pestle as fine as possible and then stored in airtight plastic vials. The shells of the gastropods were discarded as far as was feasible before

this procedure. Mesograzers were processed as whole organisms and only muscle tissue of predators was analysed. Filters were stored in a dessicator. The sediment cores were deep-frozen, the top 0.5 cm was cut off, and cores were pooled so that 1 sample contained 5 cores. Visible detritus was manually removed and the sediment samples were carefully rinsed with 0.2 μm filtered seawater. Observations made under a dissecting microscope before and after the cleaning procedure revealed the successful removal of unwanted material. The samples were then dried to constant weight (60°C, 48 h) for stable isotope analysis or deep-frozen for fatty acid analysis.

Stable isotope analysis. We measured 10 eelgrass shoots, epiphyte samples, *Delesseria sanguinea* and 3 sediment samples separately each month. Ten individuals of each mesograzer species were measured, with the exception of the small gastropod *Rissoa membranacea* for which 10 individuals were pooled into 3 samples to obtain enough material for the analyses. The isopod *Idotea baltica* and the amphipod *Gammarus oceanicus* were separated in 2 size classes. Five individuals of each predator species were analysed. Eelgrass and algae subsamples were transferred into tin cups. The mesograzer subsamples were transferred into silver cups, treated with 0.2 μl 10% HCl to remove carbonates and then dried again.

All samples were combusted in a CN-analyser (Fisons 1500N) connected to a mass spectrometer

(Finnigan Delta plus). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios were calculated as:

$$\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where $X = {}^{15}\text{N}$ or ${}^{13}\text{C}$ and $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$. Pure N_2 and CO_2 gas were used as the primary standard and calibrated against International Atomic Energy Agency (IAEA) reference standards (N1, N2, N3, NBS22 and USGS24). Acetanilide was used as an internal standard after every sixth sample. The overall analytical precision was $\pm 0.1\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

To determine the carbon sources the model of Phillips & Gregg (2003), which provides a range of feasible source mixtures, was used as follows:

$$\delta_M = f_A \delta_A + f_B \delta_B + f_C \delta_C \quad \text{and} \\ 1 = f_A + f_B + f_C$$

where f_A , f_B and f_C are the proportion of source isotopic signatures (δ_A , δ_B and δ_C) that coincide with the observed signature for the mixture (δ_M). All possible combinations of primary producer contributions were analysed with an increment of 1%. These predicted mixture signatures were compared with the measured values. If values were within a tolerance of 0.1%, they were considered feasible solutions. We used only $\delta^{13}\text{C}$ values in the modelling because of the sensitivity of the model to fractionation corrections (Connolly et al. 2005). The fractionation is much larger for ${}^{15}\text{N}$ than for ${}^{13}\text{C}$ and can vary considerably between different species. We chose 0.5‰ as average fractionation increase of $\delta^{13}\text{C}$ per trophic level for estuarine ecosystems (France & Peters 1997). Calculations were carried out with IsoSource, a Visual Basic program, provided by D. L. Phillips (Phillips & Gregg 2003).

We used epiphytes, sand microflora and the red alga *Delesseria sanguinea* as ultimate carbon sources for all consumers because the fatty acid analysis showed that the biomarker fatty acid for eelgrass, 18:4(n-3), was only present in low amounts in all consumers (0 to 2.8%) throughout the year. Therefore, we consider the contribution of eelgrass to the studied food web as negligible. As primary producer carbon reaches the omnivorous and carnivorous consumers through other consumers, we considered the trophic position of these consumers in the calculation of the fractionation for each consumer and month. Our unpublished data from laboratory experiments showed that the mesograzers we studied had a relatively quick adaptation to changes in stable isotope signature (significant changes in stable isotope values occurred within 10 d). The carbon source of main mesograzers species in the studied eelgrass system could only be calculated between April and October. In winter, because most consumer $\delta^{13}\text{C}$ values were higher than those found in the possible food sources, the IsoSource model could not be used.

Trophic levels were calculated according to the model of Hobson & Welch (1992) as follows:

$$\text{TL} = 1 + (\text{N}_m - \text{N}_b)/\text{TE}$$

where TL is the trophic level of the consumer, N_m is the $\delta^{15}\text{N}$ value of the consumer, N_b is the average basis $\delta^{15}\text{N}$ value and TE the trophic enrichment factor in this system. A TL value close to 2 is consistent with herbivorous nutrition, whereas a $\text{TL} \geq 3$ suggests a carnivorous diet. We used the average $\delta^{15}\text{N}$ value of epiphytes, sand microflora and *Delesseria sanguinea* as N_b and 1.5 as TE (Jaschinski et al. 2008).

Fatty acid analyses. We measured 3 replicates of all samples. Macrophytes were processed as individuals; mesograzers were pooled into 3 replicate samples each containing 3 individuals. *Rissoa membranacea* replicates contained 10 individuals to obtain sufficient material for the analyses. The macrophyte and mesograzers samples were freeze-dried for 48 h, ground with an agate mortar and pestle and then weighed. Fatty acids were extracted, esterified and analysed on a gas chromatograph (GC, Hewlett Packard 5890 Series II) according to Wiltshire et al. (2000), with the GC temperature settings of von Elert (2002). To quantify the fatty acid content, an internal standard of heptadecanoic (17:0) and tricosanoic (23:0) fatty acid methyl esters was used.

Statistical analysis. Fatty acid data of mesograzers were log transformed and subjected to nonmetric multidimensional scaling with the program package PRIMER 5.0. Calculations for fatty acid signatures were only performed for fatty acids represented by at least 1 value above 1%. A 1-way analysis of similarities (ANOSIM) with 999 permutations was used to test for differences in the fatty acid composition of the studied mesograzers species.

The seasonal differences in stable isotope values for primary producers and consumers were analysed with 1-way ANOVA followed by a Tukey's test ($p < 0.05$). The differences between primary producers, mesograzers species and size, and the influence of seasonality and the interaction between these factors, were tested with 2-way factorial ANOVA.

The variation in the trophic position of the omnivorous species, *Idotea baltica* and *Gammarus oceanicus*, was tested with seasonality and size as factors in a 2-way factorial ANOVA.

RESULTS

Biomarker fatty acids in main mesograzers species

Throughout the year (Fig. 2), the biomarker fatty acid for eelgrass, 18:4(n-3), was present in only insignificant amounts in *Idotea baltica* ($\leq 2.8\%$). The biomarker fatty

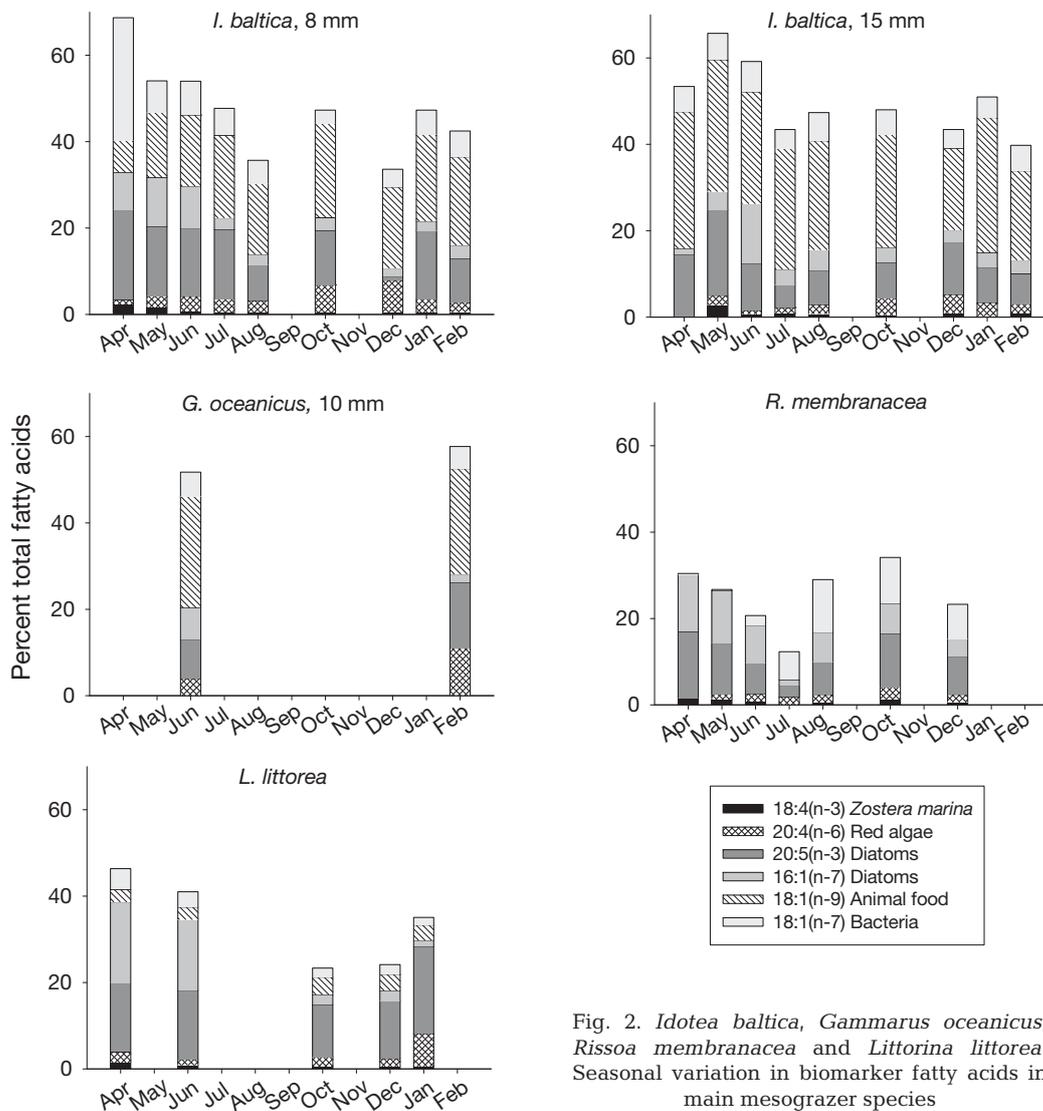


Fig. 2. *Idotea baltica*, *Gammarus oceanicus*, *Rissoa membranacea* and *Littorina littorea*. Seasonal variation in biomarker fatty acids in main mesograzers species

acid for red algae, 20:4(n-3), occurred in small amounts ($\leq 7.4\%$) displaying the highest values in October and December. Fatty acids characteristic for diatoms, 16:1(n-7) and 20:5(n-3), were more pronounced in spring and in early summer than in the rest of the year. The fatty acids 18:1(n-9) and 18:1(n-7), typical for animals and aerobic bacteria, respectively, were found in relatively constant amounts in both size classes with the exception of April. In this month 18:1(n-9) showed the highest values in large *I. baltica* (31.8%) and the lowest in small *I. baltica* (7.4%). In contrast, the bacteria biomarker fatty acid 18:1(n-7) was observed at high levels in small *I. baltica* (28.4%). Small *Gammarus oceanicus* contained relatively similar amounts of biomarker fatty acids as in small *I. baltica*, but no 18:4(n-3) characteristic for eelgrass was present. In winter the amount of 20:4(n-3), the indicator fatty acid for red algae, nearly doubled.

In *Rissoa membranacea* the levels of biomarker fatty acids for diatoms were highest in spring, declined during the summer and increased again in autumn. The fatty acid 18:1(n-7) (bacteria) increased from May to August (0.2 to 12.1%) and remained at this level until December. Fatty acids typical for eelgrass and red algae were found in small amounts throughout the year. Until December, *Littorina littorea* showed the same pattern concerning these 2 biomarker fatty acids, but in January an increase in 20:4(n-3) occurred (red algae). The amounts of fatty acids characteristic for diatoms and bacteria were lower in spring and early summer than in autumn and winter.

The fatty acid signatures of all mesograzers were subjected to nonmetric multidimensional scaling (MDS) and an ANOSIM to evaluate similarities. The MDS-plot showed differences in the fatty acid composition of the studied species, and the fatty acid composi-

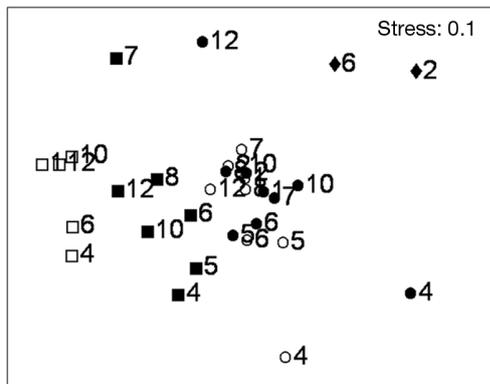


Fig. 3. *Idotea baltica*, *Gammarus oceanicus*, *Rissoa membranacea* and *Littorina littorea*. Multidimensional scaling (MDS) plot of the total fatty acid composition of mesograzers. Stress = 0.1 gives a good representation in an MDA analysis. ○: *I. baltica*, 8 mm; ●: *I. baltica*, 15 mm; ◆: *G. oceanicus*, 10 mm; ■: *R. membranacea*; □: *L. littorea*. Numbers represent the month of the year

tion of single species varied with the time of the year (Fig. 3). Most values for *Idotea baltica* clustered into 2 groups: one containing those from the months of May and June and another containing other values. April values and, for large specimens, the December values were not similar to those from other months. Fatty acid composition of *Gammarus oceanicus* was clearly distinct from the other mesograzers. The fatty acid composition of *Rissoa membranacea* was relatively similar in spring and clearly different in July. The values for *Littorina littorea* were grouped in spring/early summer and autumn/winter values.

The ANOSIM R statistic gives an absolute measure of how similar groups are on a scale of 0 to 1. An R value < 0.25 indicates that the groups are barely separated at all; R > 0.5 shows that the groups are clearly different but overlapping, and R > 0.75 indicates well-separated groups.

Table 1. *Idotea baltica*, *Gammarus oceanicus*, *Rissoa membranacea*, *Littorina littorea*. Results of the pairwise comparison (ANOSIM) of the fatty acid composition of the mesograzers studied. Is: *I. baltica*, 8 mm; Ib: *I. baltica*, 15 mm; Gs: *G. oceanicus*, 10 mm; Rm: *R. membranacea*; L: *L. littorea*

Groups	R	p	Permutations
Ib, Is	0.120	0.017	999
Ib, Rm	0.537	0.001	999
Ib, L	0.867	0.001	999
Ib, Gs	0.802	0.18	55
Is, Rm	0.593	0.001	999
Is, L	0.899	0.001	999
Is, Gs	0.874	0.018	55
Rm, L	0.773	0.001	792
Rm, Gs	1	0.028	36
L, Gs	1	0.048	21

The ANOSIM verified that there are significant differences between the fatty acid composition of the mesograzers studied (R = 0.611, p = 0.001). The results of the pairwise tests indicating that all mesograzers species can be separated by their fatty acid composition with the exception of small and large *Idotea baltica* are shown in Table 1.

Stable isotope ratios of primary producers

We found significant effects of seasonality (MS = 40.9, F = 45.3, p < 0.001) and primary producer type (MS = 6304.9, F = 6981.1, p < 0.001) on stable carbon isotope ratios. The interaction of the 2 factors was also significant (MS = 9.8, F = 10.9, p < 0.001).

Three primary producers were important as carbon sources for the studied consumers: epiphytes attached to *Zostera marina*, sand microflora and the red alga *Delesseria sanguinea*. Epiphytes and sand microflora mainly consisted of small pennate diatoms. The stable carbon isotope ratios of epiphytes, sand microflora and *D. sanguinea* were significantly different from each other (p < 0.001) and therefore it was possible to calculate their relative importance as food sources at higher trophic levels. Epiphytes were the most enriched carbon source, the red algae the most depleted carbon source and sand microflora showed intermediate values (Fig. 4A). In December, the $\delta^{13}\text{C}$ value for epiphytes was essentially identical with the value for sand microflora. For epiphytes, the $\delta^{13}\text{C}$ values remained relatively constant from May to September, and for sand microflora and the red alga from April to October (Fig. 4A). In winter all 3 primary producers were depleted in ^{13}C compared with the spring and summer situation.

The stable nitrogen isotope ratios had a significant seasonal variation (MS = 3.8, F = 8.5, p < 0.001) and depended on the primary producer type (MS = 30.4, F = 67.9, p < 0.001). The interaction of the 2 factors was also significant (MS = 14.1, F = 31.4, p < 0.001).

The $\delta^{15}\text{N}$ values of primary producers ranged from 5.77 to 11.67‰ in epiphytes, from 2.38 to 6.47‰ in sand microflora and from 7.69 to 11.65‰ in the red alga *Delesseria sanguinea* (Fig. 4B). The $\delta^{15}\text{N}$ values remained relatively stable from April to September, when significant changes occurred. Sand microflora became increasingly depleted in ^{15}N , whereas epiphytes and the red alga showed enrichment in ^{15}N as a function of time.

Stable isotope ratios of main mesograzers species

We found significant effects of seasonality (MS = 35.2, F = 57.8, p < 0.001) and grazer species (MS = 80.5,

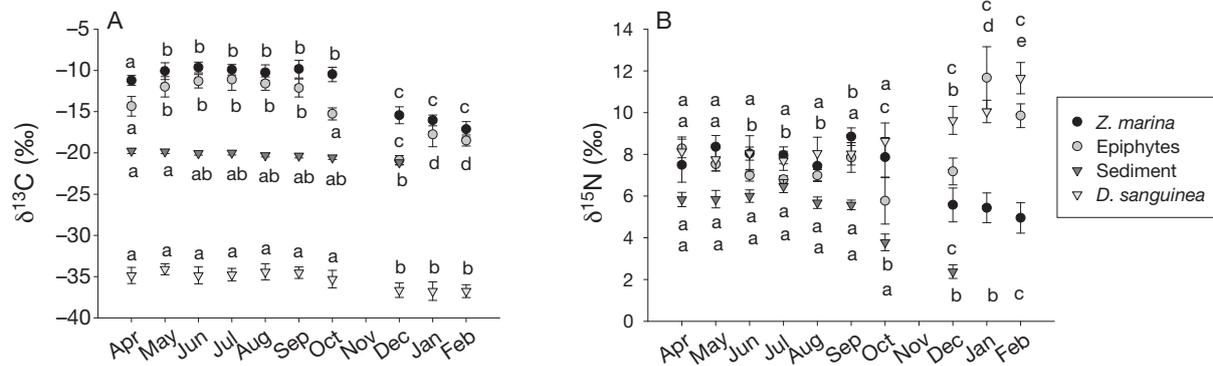


Fig. 4. Seasonal variation (mean \pm SD) of the stable isotopes (A) $\delta^{13}\text{C}$ and (B) $\delta^{15}\text{N}$ in *Zostera marina*, epiphytes, sand microflora and *Delesseria sanguinea*. Different letters accompanying symbols indicate significant differences between sampling dates ($p \leq 0.05$)

$F = 132.3$, $p < 0.001$) on stable carbon isotope ratios. The interaction of the 2 factors was also significant ($MS = 22.1$, $F = 36.3$, $p < 0.001$).

The development of stable carbon isotope ratios of small and large *Idotea baltica* showed an even pattern for both size classes with low values in April and July and for small specimens also in winter (Fig. 5). The $\delta^{13}\text{C}$ signals of small *I. baltica* were significantly lower than those found in the larger specimens ($p < 0.001$), which suggested a larger amount of red algae in their diet. The $\delta^{13}\text{C}$ values of *Gammarus oceanicus* had a larger range than those of *I. baltica*. There were significant differences between size classes ($p = 0.006$). The $\delta^{13}\text{C}$ values indicated that in summer large *G. oceanicus* depended more on carbon derived from red algae than carbon from smaller algae, whereas the situation was reversed in winter.

The $\delta^{13}\text{C}$ values of *Rissoa membranacea* remained at the same level from April to July, decreased to a minimum in August and increased again in autumn (Fig. 5). The range of $\delta^{13}\text{C}$ values in *Littorina littorea* was generally lower than in *R. membranacea* with the exception of the month of May, suggesting that this gastropod feeds mainly on epiphytes during this month.

Stable nitrogen isotope ratios had a significant seasonal variation ($MS = 3.5$, $F = 32.7$, $p < 0.001$), but a weaker one than that observed for the $\delta^{13}\text{C}$ values, and depended on the mesograzers species ($MS = 14.4$, $F = 135.7$, $p < 0.001$). The interaction of the 2 factors was also significant ($MS = 0.9$, $F = 8.1$, $p < 0.001$).

The stable nitrogen isotope ratios of the omnivorous isopod *Idotea baltica* ranged from 8.3 to 10.65‰ (Fig. 5). Larger specimens generally had significantly higher $\delta^{15}\text{N}$ values than did smaller ones indicating a more carnivorous diet ($p < 0.001$). Both size classes had significantly lower $\delta^{15}\text{N}$ values in summer pointing to a more herbivorous diet in this season (Fig. 5). The seasonal variation in $\delta^{15}\text{N}$ values of the likewise omnivorous amphipod *Gammarus oceanicus* showed a similar,

but less strongly pronounced pattern than that in *I. baltica*. The $\delta^{15}\text{N}$ values were higher in the larger specimens and the difference was significant ($p < 0.001$). Significantly lower $\delta^{15}\text{N}$ values in both amphipod size classes were found in summer (Fig. 5).

The gastropod *Rissoa membranacea* had distinctly lower $\delta^{15}\text{N}$ values than did the crustacean species in correspondence with its herbivorous feeding mode. The $\delta^{15}\text{N}$ signal of *Littorina littorea* had the highest variation among individuals. The enriched $\delta^{15}\text{N}$ values compared with *R. membranacea* were not an indication of a higher trophic position, but resulted probably from a different fractionation of stable nitrogen isotopes.

Carbon sources of mesograzers according to the feasible source mixture model

In all seasons epiphytes and sand microflora were most likely to substantially contribute to mesograzers nutrition (means over all seasons and species, 49 and 37%, respectively), while the red alga *Delesseria sanguinea* had a lower contribution (mean, 14%) (Fig. 6). Epiphytes were the most important contributors to the carbon in *Idotea baltica* (45% in small specimens, 47% in larger ones). The dependence on epiphyte-derived carbon for small and large specimens had a peak in May, was lowest in July (33 and 30%, respectively) and increased again in autumn (Fig. 6). The diet of *Gammarus oceanicus* consisted of about 30% epiphytes in spring and summer. The contribution of this carbon source increased in autumn up to 64% for small and 68% for larger specimens. Throughout the growing season, epiphytes were the most important contributors to *Rissoa membranacea* nutrition (Fig. 6). In summer, the relative contribution of this primary producer decreased to 45%. *Littorina littorea* obtained about 40% of its carbon from epiphytes with

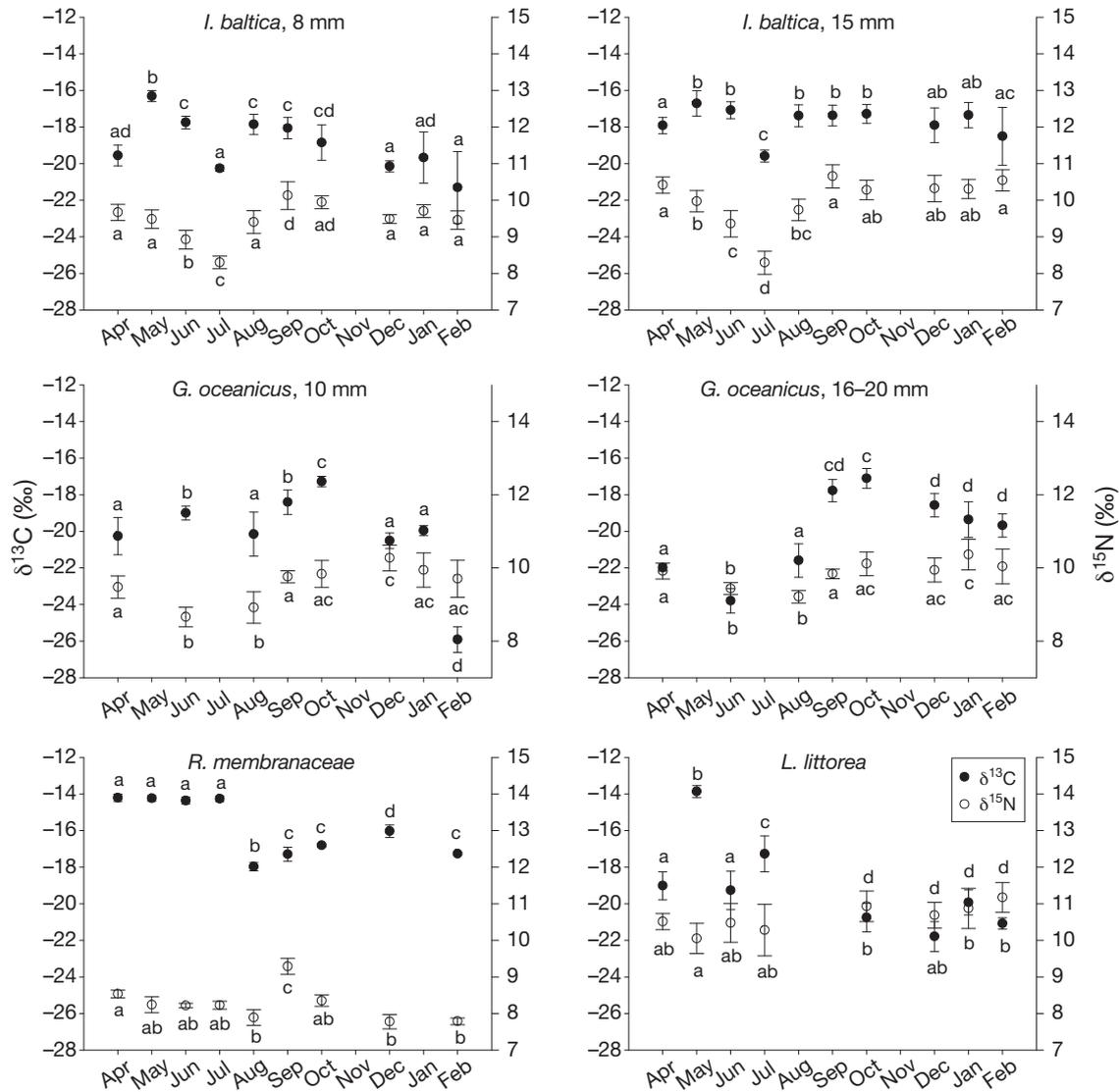


Fig. 5. *Idotea baltica*, *Gammarus oceanicus*, *Rissoa membranacea* and *Littorina littorea*. Seasonal variation (mean \pm SD) of the stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the most abundant mesograzers. Different letters accompanying symbols indicate significant differences ($p \leq 0.05$) between sampling dates; letters above represent $\delta^{13}\text{C}$, those below represent $\delta^{15}\text{N}$

the exception of the month of May, where the contribution nearly doubled to 77% epiphyte-derived carbon.

Sand microflora increased as the carbon source when epiphytes became less important (Fig. 6). Red algae seem to be of minor importance for most of the studied mesograzers (Fig. 6); only large *Gammarus oceanicus* had a high contribution of this carbon source to their nutrition in summer (45%).

Trophic position

The trophic position of main mesograzers species in the eelgrass system studied could only be calculated be-

tween April and October, when $\delta^{15}\text{N}$ values of primary producers remained relatively similar. In winter, strong changes in primary producer $\delta^{15}\text{N}$ values in combination with reduced growth rates and lower metabolic activity of animals prevented the computing of realistic results at this time of the year. The isopod *Idotea baltica* showed a significant seasonal variation in trophic position ($MS = 3.6$, $F = 104.3$, $p < 0.0001$), changing from nearly exclusively carnivorous in spring to herbivorous in summer and back to a more carnivorous feeding mode again in winter (Fig. 7). Smaller individuals generally held a significant lower trophic position than did larger ones ($MS = 2.7$, $F = 78.6$, $p < 0.0001$), suggesting that they became more carnivorous as they grew.

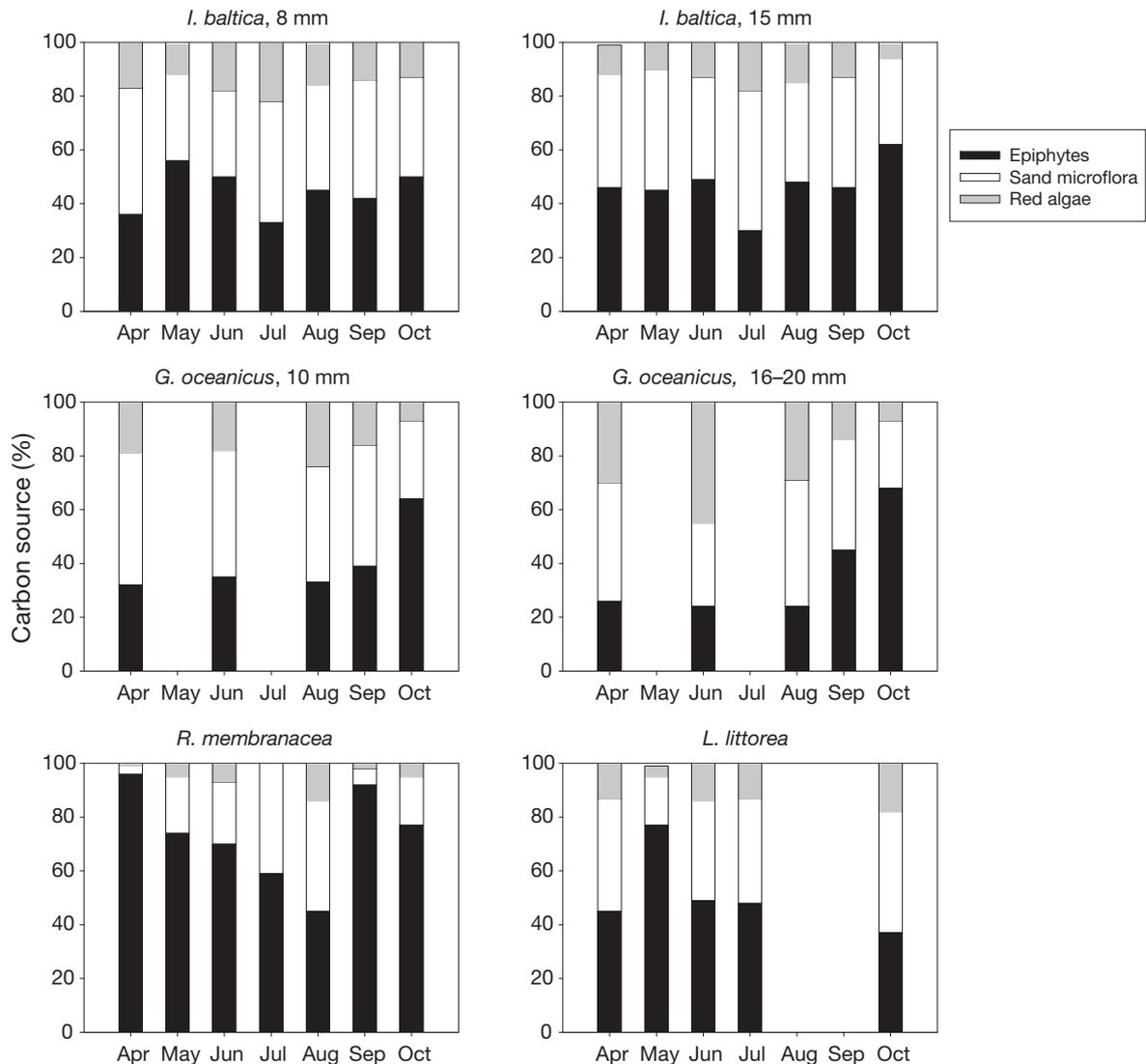


Fig. 6. *Idotea baltica*, *Gammarus oceanicus*, *Rissoa membranacea* and *Littorina littorea*. Seasonal variation in ultimate carbon sources (epiphytes, sand microflora, red algae *Delesseria sanguinea*) for main mesograzers species. Shown are the mean contributions calculated with IsoSource according to the model of Phillips & Gregg (2003), which could only be calculated from April to October

Stable carbon isotope ratios and carbon sources of small predators

All the small predator species studied—the green crab *Carcinus maenas*, the shrimp *Crangon crangon*, the sea stickleback *Spinachia spinachia*, the straight-nose pipefish *Nerophis ophidion* and the broad-nosed pipefish *Syngnathus typhle*—had a significant seasonal variation in their $\delta^{13}\text{C}$ values (Fig. 8).

All predators depended mainly on epiphyte carbon, which ranged from an annual mean of 39% for the broad-nosed pipefish to 53% for the green crab (Fig. 9). Sand microflora was the second most important carbon

source and annual mean contribution ranged from 35 to 45%. Red algae were a less crucial carbon source (annual mean contribution, 12 to 16%). The seasonal variation in the dependence on epiphyte carbon that we found for mesograzers, which showed a summer minimum, was also found for the green crab and the straightnose pipefish. The broad-nosed pipefish had a more balanced contribution of this carbon source to its diet and the sea stickleback showed an increase in dependence on epiphyte carbon in autumn; we did not find the stickleback in our study area in spring. In general, the seasonal variation of carbon sources was less pronounced in predators than in mesograzers.

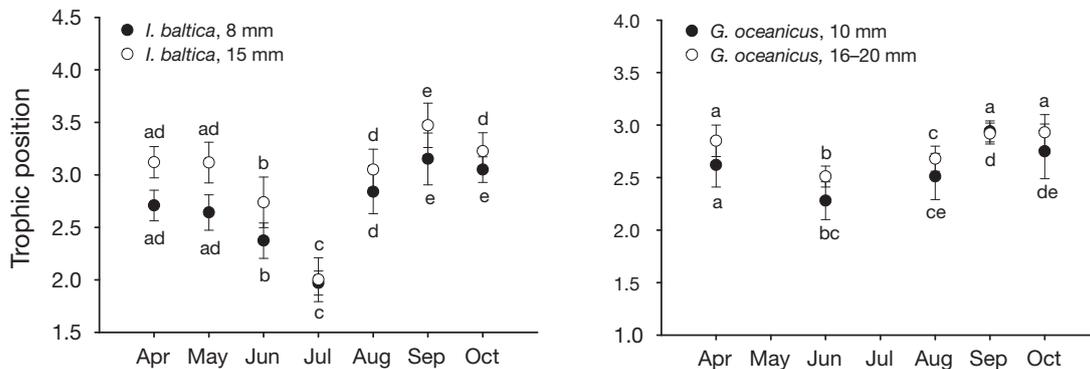


Fig. 7. *Idotea baltica* and *Gammarus oceanicus*. Seasonal variation (mean ± SD) in trophic position of omnivorous mesograzer species. Different letters accompanying symbols indicate significant differences between sampling dates ($p \leq 0.05$)

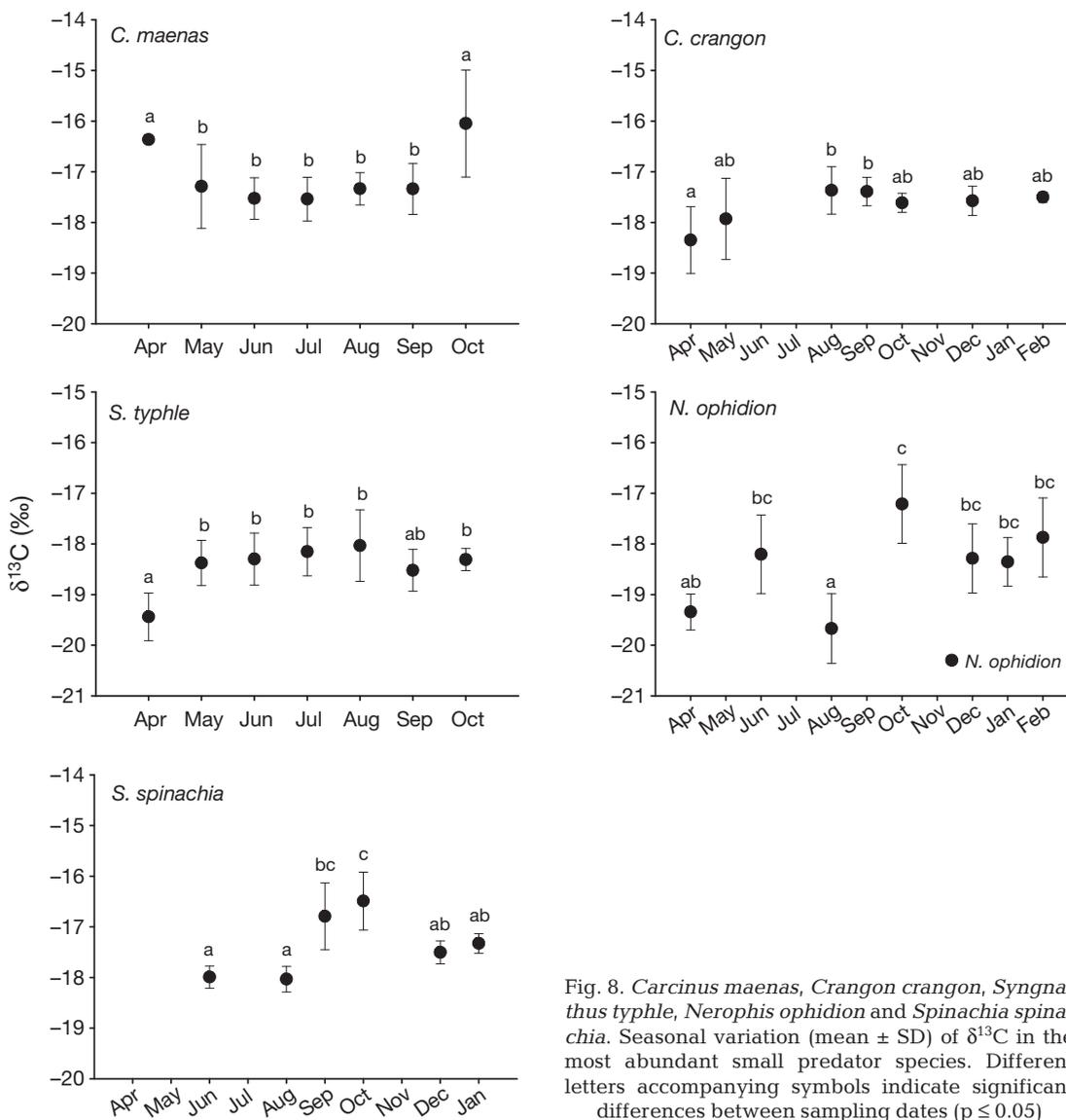


Fig. 8. *Carcinus maenas*, *Crangon crangon*, *Syngnathus typhle*, *Nerophis ophidion* and *Spinachia spinachia*. Seasonal variation (mean ± SD) of $\delta^{13}\text{C}$ in the most abundant small predator species. Different letters accompanying symbols indicate significant differences between sampling dates ($p \leq 0.05$)

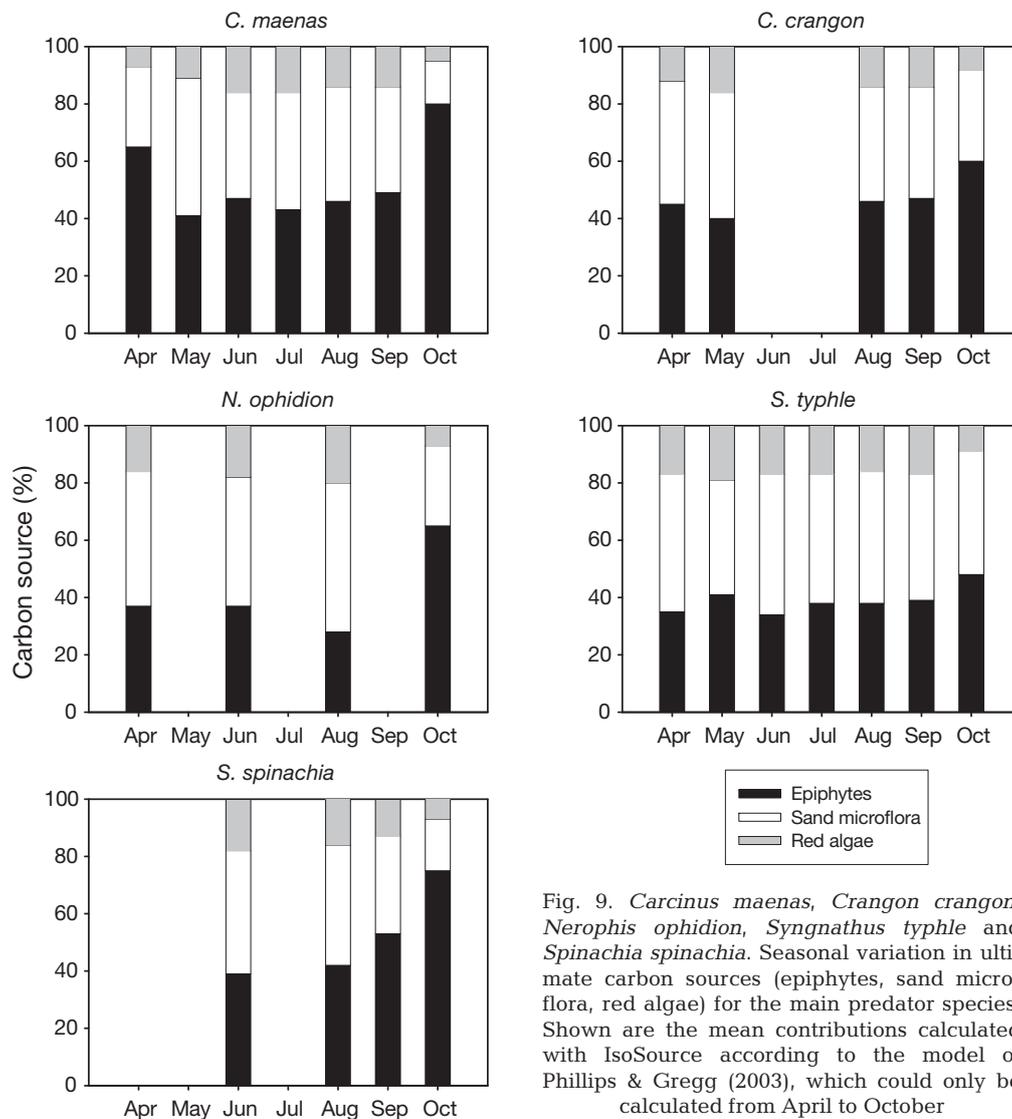


Fig. 9. *Carcinus maenas*, *Crangon crangon*, *Nerophis ophidion*, *Syngnathus typhle* and *Spinachia spinachia*. Seasonal variation in ultimate carbon sources (epiphytes, sand microflora, red algae) for the main predator species. Shown are the mean contributions calculated with IsoSource according to the model of Phillips & Gregg (2003), which could only be calculated from April to October

DISCUSSION

The present study suggests that epiphytes were the most important carbon source for common mesograzers and small predators in a subtidal eelgrass community throughout the year. However, the dependence on this food source showed clear seasonal and species-specific variability. The trophic importance of microalgae in benthic macrophyte systems was confirmed in recent stable isotope studies (Créach et al. 1997, Connolly et al. 2005, Douglass 2008, Jaschinski et al. 2008). Our results support the assumption that fresh macrophyte leaves are of minor relevance for the nutrition of mesograzers in temperate seagrass systems (Lepoint et al. 2000, Moncreiff & Sullivan 2001, Douglass 2008, Jephson et al. 2008). Throughout the year, the fatty acid 18:4(n-3), which is characteristic of living eelgrass

(Khotimchenko 1993), was only present in negligible amounts in *Idotea baltica*, *Littorina littorea* and *Rissoa membranacea*.

All studied mesograzers had the smallest contribution of epiphytes to their diet in early summer, which corresponded to a minimum in epiphyte biomass (Jaschinski & Sommer 2008b). This indicates the capacity of mesograzers to control epiphyte biomass on eelgrass (Hughes et al. 2004, Jaschinski & Sommer 2008a). The seasonal pattern was most pronounced in the small gastropod *Rissoa membranacea*, in which a reduction from total dependence on epiphytes in spring and autumn to about 45% in summer was observed and agreed with the model of Phillips & Gregg (2003). The decrease in epiphyte abundance caused this herbivorous species to switch to sand microflora as an alternative food source, a behaviour

that we also observed in laboratory experiments (Jaschinski & Sommer 2008a). The fatty acid composition of *R. membranacea* confirmed the conclusions determined from the stable carbon signatures. The biomarker fatty acids for diatoms, 20:5(n-3) and 16:1(n-7), decreased in summer. These fatty acids are found in epiphytes and sand microflora communities, both of which consist mainly of diatoms, but the absolute content is much lower in sand microflora than in epiphytes (S. Jaschinski, D. C. Brepohl, U. Sommer unpubl. data). The increase of 18:1(n-7), a fatty acid characteristic of bacteria, starting in June, could indicate detrital material as an additional food source. The biomarker fatty acids for eelgrass and red algae, 18:4(n-3) and 20:4(n-6), respectively, were only found in low amounts throughout the year.

The periwinkle *Littorina littorea* had a strong dependence on epiphyte carbon in May. The rest of the year, this species mostly fed on sand microflora according to stable isotope data. Red algae seem to be of minor importance in the diet; however, in December when *Delesseria sanguinea*, which grows mainly below the eelgrass meadows, is found more often in the eelgrass meadow (S. Jaschinski pers. obs.), the biomarker fatty acid for red algae in *L. littorea* increased.

Stable isotope data and biomarker fatty acids indicated a seasonally varying importance of epiphyte carbon for both size classes of the omnivorous isopod *Idotea baltica*, with high values occurring in spring and autumn and a summer depression in accordance with epiphyte development. Red algae also seemed to be more important from October to December. Small *I. baltica* had very high values of 18:1(n-7) in April, indicating that detritus was an important food source in early spring when the biomass of primary producers is low. Large specimens seemed to have a different strategy during this period when fresh herbal food was limited. The fatty acid 18:1(n-9), the presence of which suggests a carnivorous diet, was especially high in April (32% of fatty acids). Seasonally, the trophic position of *I. baltica* changed with a more herbivorous diet in summer. Smaller individuals were always less carnivorous than the larger ones, according to stable isotope and fatty acid data. Gut content analyses support the omnivorous diet of *I. baltica* (Douglass 2008).

The amphipod *Gammarus oceanicus* had the strongest contribution of epiphytes to its diet in autumn. Nutrient input caused by storm events promoted the growth of diatom chains during this season (S. Jaschinski, pers. obs.), which are selectively eaten by this amphipod, probably because the uptake of smaller, more strongly adhering diatom species is not possible for *G. oceanicus* (Jaschinski et al. 2010a). The stable isotope data indicated that smaller individuals compensated for the decrease in epiphytes in summer

by consuming more sand microflora and larger individuals compensated by consuming more red algae. Unfortunately, most of the fatty acid data on *G. oceanicus* were lost during analyses, therefore only an increase in red algae as a food source in winter can be deduced from the biomarker fatty acids. The trophic position of this potentially omnivorous species increased in small specimens in winter; the larger individuals were more carnivorous than were the smaller ones in summer. Gut content analyses of other *Gammarus* species support the assumption of the omnivorous diet of this genus (Douglass 2008, Moksnes et al. 2008).

The total fatty acid composition of the 4 mesograzers studied supported the species-specific and seasonal variability in nutrition in the eelgrass system. These mesograzers are common consumers in temperate shallow marine benthic ecosystems. These species have a broad range of possible food sources, but distinct preferences if several food items are offered (Norton et al. 1990, Warén 1996, Franke & Janke 1998, Orav-Kotta & Kotta 2004, Gohse-Reimann 2007). Our study indicated that the mesograzers species and size classes used different strategies to compensate for the limitation of the main food source of epiphytes in early spring, summer and winter. A shift or switching of prey, when preferred food sources are limited, has also been found for other invertebrate species in eelgrass systems. For example, the omnivorous crustacean *Palaemon* spp. switched to the qualitatively inferior macroalgae if the preferred amphipods became scarce (Jephson et al. 2008, Persson et al. 2008). The cause of the limitation of preferred food sources can be natural prey-consumer cycles as seen in our study, seasonal nutrient limitation, overfishing, which can promote competitors via a trophic cascade, or even hypoxia as a consequence of eutrophication, which reduces the number of small invertebrates available as potential prey for omnivores (Jephson et al. 2008, Fox et al. 2009).

In the eelgrass meadow we studied, the summer minimum of epiphyte biomass was probably caused by a combination of nitrogen limitation and intense grazing pressure. In autumn, there was also an even smaller increase in mesograzers abundance, but without a strong negative effect on epiphyte biomass. Increasing values of dissolved nutrients and especially a strong decrease in epiphyte C:N values suggested an increase in nitrogen supply and thus a break-up/avoidance of nitrogen limitation (Jaschinski & Sommer 2008a). Experiments support strong interactions of bottom-up and top-down effects on epiphytes (Douglass et al. 2007, Jaschinski & Sommer 2008b). The biomass of sand microflora followed the same pattern as that for epiphyte biomass, but the summer minimum was less pronounced (S. Jaschinski unpubl. data). The cause

could be bioturbation during the grazing process, which can promote primary production via nutrient release from the sediment (Lohrer et al. 2004). We also found a positive effect of mesograzers on epiphyte productivity in this system (Jaschinski & Sommer 2010), emphasising the complex interactions of abiotic and biotic factors, which determine biomass and probably species composition even if only the primary producer and herbivore level was considered.

The seasonal fluctuations in carbon sources at the mesograzer level were also present at the next trophic level, but less pronounced. The main second-order consumers in the eelgrass system were the green crab, a shrimp, the sea stickleback and 2 pipefish species. The contributions of the different primary producers as carbon source of these small predators had a summer minimum, with the exception of the broad-nosed pipefish. Epiphytes (annual mean, 47% for all predators) again provided the most likely carbon source, followed by sand microflora (39%) and red algae (14%). Gut content analyses showed that amphipods and isopods are important parts of the nutrition of these predators (Bobsien 2006, Moksnes et al. 2008). Nordström et al. (2009) found that seasonal fluctuations of carbon contribution at the herbivore level were strongly diminished at the predator level, but they sampled only from June to September, a period of time when our data suggested similarly stable carbon sources. As the importance of seasonal variation in basic $\delta^{13}\text{C}$ values depends on the growth rates of the species, the observed low variability in carbon sources of small predators is in accordance with their lower turnover rate compared with the smaller fast-growing mesograzers. Nevertheless, there are significant changes in carbon sources detectable at the predator level in the course of the year. This is supported by Nordström et al. (2009), who found that $\delta^{15}\text{N}$ values and thus trophic position changed significantly from month to month.

We showed that trophic interactions at the mesograzer level can be dynamic at a temporal scale during the growth season in a temperate eelgrass community. Earlier studies with lower temporal resolution than ours indicated that these processes are relevant in macrophyte systems in which epiphytes and/or annual macroalgae are important food sources (Orav-Kotta & Kotta 2003, Vizzini & Mazzola 2003, Connolly et al. 2005, Nordström et al. 2009). Trophic interactions in benthic food webs that depend more on macrophyte detritus seem to be characterised by relatively stable dynamics (Akin & Winemiller 2006, Douglass 2008). The strong seasonality of epiphytic and annual algae biomass and the high selectivity of many mesograzer species for food items that are of high quality with respect to nutrient content or digestibility result in a seasonally varying limitation of optimal food sources.

This seems to promote a community of generalist mesograzers, which are able to react with great flexibility to the changing food conditions and thus contribute to the stability of the whole system.

Acknowledgements. We thank S. Flöder for helpful comments and T. Hansen for analysing the stable isotope samples. We acknowledge the financial support from the German Research Foundation (So 145/20) and the Brazilian National Counsel of Technological and Scientific Development (CNPq).

LITERATURE CITED

- Akin S, Winemiller KO (2006) Seasonal variation in food web composition and structure in a temperate tidal estuary. *Estuaries Coasts* 29:552–567
- Bobsien I (2006) The role of small fish species in eelgrass food webs of the Baltic Sea. Christian-Albrechts-University, Kiel
- Brett MT, Müller-Navarra DC, Ballantyne AP, Ravet JL, Goldman CR (2006) *Daphnia* fatty acid composition reflects that of their diet. *Limnol Oceanogr* 51:2428–2437
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* 31:343–366
- Connolly RM, Hindell JS, Gorman D (2005) Seagrass and epiphytic algae support nutrition of a fisheries species, *Sillago schomburgkii*, in adjacent intertidal habitats. *Mar Ecol Prog Ser* 286:69–79
- Créach V, Schricke MT, Bertru G, Mariotti A (1997) Stable isotopes and gut analyses to determine feeding relationships in saltmarsh macroconsumers. *Estuar Coast Shelf Sci* 44:599–611
- Dahl TM, Falk-Petersen S, Gabrielsen GW, Sargent JR, Hop H, Millar RM (2003) Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: a trophic study from an Arctic fjord. *Mar Ecol Prog Ser* 256: 257–269
- Desvillettes C, Bourdier G, Amblard C, Barth B (1997) Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoa and microalgae. *Freshw Biol* 38: 629–637
- Douglass JG (2008) Community dynamics in submersed aquatic vegetation: intermediate consumers as mediators of environmental change. PhD dissertation, The College of William and Mary, Williamsburg, VA
- Douglass JG, Duffy JE, Spivak AC, Richardson JP (2007) Nutrient versus consumer control of community structure in a Chesapeake Bay eelgrass habitat. *Mar Ecol Prog Ser* 348:71–83
- Douglass JG, France KE, Richardson JP, Duffy JE (2010) Seasonal and interannual changes in a Chesapeake Bay eelgrass community: insights into biotic and abiotic control of community structure. *Limnol Oceanogr* 55:1499–1520
- Duffy JE, Harvilicz AM (2001) Species-specific impacts of grazing amphipods in an eelgrass-bed community. *Mar Ecol Prog Ser* 223:201–211
- Duffy JE, MacDonald KS, Rhode JM, Parker JD (2001) Grazer diversity, functional redundancy, and productivity in seagrass beds: an experimental test. *Ecology* 82:2417–2434
- Edgar GJ, Shaw C (1995) The production and trophic ecology of shallow-water fish assemblages in southern Australia. III. General relationships between sediments, seagrasses, invertebrates and fishes. *J Exp Mar Biol Ecol* 194:107–131

- Fox SE, Teichberg M, Olsen YS, Heffner L, Valiela I (2009) Restructuring of benthic communities in eutrophic estuaries: lower abundance of prey leads to trophic shifts from omnivory to grazing. *Mar Ecol Prog Ser* 380:43–57
- France RL, Peters RH (1997) Ecosystem differences in the trophic enrichment of ^{13}C in aquatic food webs. *Can J Fish Aquat Sci* 54:1255–1258
- Franke HD, Janke M (1998) Mechanisms and consequences of intra- and interspecific interference competition in *Idotea baltica* (Pallas) and *Idotea emarginata* (Fabricius) (Crustacea: Isopoda): a laboratory study of possible proximate causes of habitat segregation. *J Exp Mar Biol Ecol* 227:1–21
- Gohse-Reimann S (2007) Untersuchungen zur Ernährungsökologie benthischer Invertebraten im Makrophytensystem: ein marin-limnischer Ansatz. Christian-Albrechts-University, Kiel
- Hauxwell J, McClelland J, Behr PJ, Valiela I (1998) Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. *Estuaries* 21:347–360
- Heck KL Jr, Valentine JF (2007) The primacy of top-down effects in shallow benthic ecosystems. *Estuaries Coasts* 30:371–381
- Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar Ecol Prog Ser* 84:9–18
- Hughes AR, Bando KJ, Rodriguez LF, Williams SL (2004) Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. *Mar Ecol Prog Ser* 282:87–99
- Jaschinski S, Sommer U (2008a) Functional diversity of mesograzers in an eelgrass–epiphyte system. *Mar Biol* 154:475–482
- Jaschinski S, Sommer U (2008b) Top-down and bottom-up control in an eelgrass–epiphyte system. *Oikos* 117:754–762
- Jaschinski S, Sommer U (2010) Positive effects of mesograzers on epiphytes in an eelgrass system. *Mar Ecol Prog Ser* 401:77–85
- Jaschinski S, Brepohl DC, Sommer U (2008) Carbon sources and trophic structure in an eelgrass *Zostera marina* bed, based on stable isotope and fatty acid analyses. *Mar Ecol Prog Ser* 358:103–114
- Jaschinski S, Flöder S, Sommer U (2010a) Consumer identity, abundance, and nutrient concentration affect epiphyte diversity in an experimental eelgrass system. *Oikos* 119:1745–1754
- Jephson T, Nyström P, Moksnes PO, Baden SP (2008) Trophic interactions in *Zostera marina* beds along the Swedish coast. *Mar Ecol Prog Ser* 369:63–76
- Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulphur stable isotope ratio and fatty acid analyses. *Mar Ecol Prog Ser* 220:103–117
- Klumpp DW, Salita-Espinosa JS, Fortes MD (1992) The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. *Aquat Bot* 43:327–349
- Khotimchenko SV (1993) Fatty acids and polar lipids of seagrasses from the Sea of Japan. *Phytochemistry* 33:369–372
- Lee RF, Nevenzel JC, Paffenhofer GA (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Mar Biol* 9:99–108
- Lepoint G, Nyssen F, Gobert S, Dauby P, Bouquegneau JM (2000) Relative impact of a seagrass bed and its adjacent epilithic algal community in consumer diets. *Mar Biol* 136:513–518
- Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. *Nature* 431:1092–1095
- Moksnes PO, Gullström M, Tryman K, Baden S (2008) Trophic cascade in a temperate seagrass community. *Oikos* 117:763–777
- Moncreiff CA, Sullivan MJ (2001) Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Mar Ecol Prog Ser* 215:93–106
- Nelson TA, Waaland JR (1997) Seasonality of eelgrass, epiphyte, and grazer biomass and productivity in subtidal eelgrass meadows subjected to moderate tidal amplitude. *Aquat Bot* 56:51–74
- Nordström M, Aarnio K, Bonsdorff E (2009) Temporal variability of a benthic food web: patterns and processes in a low-diversity system. *Mar Ecol Prog Ser* 378:13–26
- Norton TA, Hawkins SJ, Manley NL, Williams GA, Watson DC (1990) Scraping a living: a review of littorinid grazing. *Hydrobiologia* 193:117–138
- Orav-Kotta H, Kotta J (2003) Seasonal variations in the grazing of *Gammarus oceanicus*, *Idotea baltica*, and *Palaemon adspersus* on benthic macroalgae. *Proc Estonian Acad Sci Biol Ecol* 52:141–148
- Orav-Kotta H, Kotta J (2004) Food and habitat choice of the isopod *Idotea baltica* in the northeastern Baltic Sea. *Hydrobiologia* 514:79–85
- Persson M, Andersson S, Baden S, Moksnes PO (2008) Trophic role of the omnivorous grass shrimp *Palaemon elegans* in a Swedish eelgrass system. *Mar Ecol Prog Ser* 371:203–212
- Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–269
- Polis GA, Holt RD, Menge BA, Winemiller KO (1996) Time, space, and life history: influences on food webs. In: Polis GA, Winemiller KO (eds) *Food webs. Integration of patterns and dynamics*. Chapman & Hall, London, p 435–460
- Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* 136:169–182
- Viso AC, Marty JC (1993) Fatty acids from 28 marine microalgae. *Phytochemistry* 34:1521–1533
- Vizzini S, Mazzola A (2003) Seasonal variations in the stable carbon and nitrogen isotope ratios ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) of primary producers and consumers in a western Mediterranean coastal lagoon. *Mar Biol* 142:1009–1018
- Von Elert E (2002) Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnol Oceanogr* 47:1764–1773
- Warén A (1996) Ecology and systematics of the north European species of *Rissoa* and *Pusillina* (Prosobranchia: Rissoidae). *J Mar Biol Assoc UK* 76:1013–1059
- Wiltshire KH, Boersma M, Möller A, Buhtz H (2000) Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). *Aquat Ecol* 34:119–126