

# Temporal variation in stable isotope composition ( $\delta^{13}\text{C}$ , $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ ) of a temperate *Zostera marina* food web

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**ABSTRACT:** Simultaneous triple stable isotope analysis of carbon, nitrogen and sulphur was employed to study the temporal variation in the food web of a subtidal eelgrass *Zostera marina* bed in the western Baltic Sea. Samples of 3 potential food sources (eelgrass, epiphytes and seston) and consumer species were collected biweekly from March through September 2011. Temporal variation of stable isotope compositions was observed in primary producers and consumer species. However, variation between replicates, particularly omnivores, often exceeded variation over time. The high degree of omnivory among the generalist feeders in this eelgrass community allows for generalist feeders to flexibly switch food sources, thus enhancing food-web stability. As coastal systems are subject to seasonal changes, as well as alterations related to human disturbance and climate, these food webs may retain a certain resilience due to their plentiful omnivores.

**KEY WORDS:** Trophic level · Eelgrass · Consumer · Primary producer · Baltic Sea

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## INTRODUCTION

Seagrass meadows constitute highly complex ecosystems with regards to biodiversity and are among the most productive autotrophic communities on earth (Hemminga & Duarte 2000). Despite its high productivity, the direct contribution of eelgrass *Zostera marina* to the carbon flow up the food web is negligible (Jaschinski et al. 2008a). Nelson (1997) and Valentine & Duffy (2006) argued that *Z. marina* contributes little to nothing to macroinvertebrate diets; instead, mesograzers feed primarily on epiphytic algae attached to eelgrass. In contrast, Connolly et al. (2005), Kharlamenko et al. (2001) and Lonergan et al. (1997) demonstrated the importance of seagrass production to the diets of a variety of smaller invertebrates living in seagrass meadows. These contradictions between studies may be a result of insufficient methods, such as direct observation,

gut content analysis and feeding experiments, which provide only a snapshot in time and do not necessarily capture the long-term behaviour of consumers.

Stable isotopes provide time-integrated information on what has been digested and assimilated into an organism's tissue.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analyses are standard in food web studies and are well documented (reviewed by Grey 2006).  $\delta^{13}\text{C}$  values have been mainly used to identify the primary carbon sources of food webs, while  $\delta^{15}\text{N}$  values are used to determine the trophic level. However, the frequently observed similarity between  $\delta^{13}\text{C}$  values of seagrass and epiphytes has prevented a distinction between these 2 carbon sources based on stable isotope analysis (Connolly et al. 2005, Jaschinski et al. 2008a, Olsen et al. 2013). Sulphur inclusion in the stable isotope analysis might allow for separation of seagrass and its epiphytes, because epiphytes obtain sulphur mostly from seawater sulphate, whereas seagrass

leaves at least partially obtain their sulphur from the isotopically different interstitial waters of the sediment (Kharlamenko et al. 2001, Moncreiff & Sullivan 2001). It is generally assumed that producers that mainly utilize seawater sulphates (e.g. phytoplankton, epiphytes) are enriched in  $^{34}\text{S}$  ( $\delta^{34}\text{S}$  of seawater:  $\sim 22.9\%$ ) and producers that gain the necessary sulphur from sediments (e.g. seagrass) are depleted ( $\delta^{34}\text{S}$  of sediment:  $\sim 1\%$ ; Kharlamenko et al. 2001, Connolly et al. 2004, Michener & Kaufman 2007). Thus, using the triple isotope approach, a distinction between the primary carbon sources at the base of the food web is possible.

Temporal and seasonal changes in biotic and abiotic factors, e.g. light, temperature and nutrient concentrations, can considerably alter rates of primary production and thus reshape aquatic food web interactions. It has been observed that the isotopic composition of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of phytoplankton in the Baltic Sea can change several times during a year (Rolff 2000). Herbivores respond to these changes with successional patterns in abundance, community structure and trophic interactions (Möller et al. 1985, Blomqvist & Bonsdorff 1986). Generalist consumers switch between food sources (Fox et al. 2009, Nordström et al. 2009, Olsen et al. 2013) as their availability changes, and thus may stabilize trophic dynamics by remaining on the same trophic level. Fluctuations in  $\delta^{13}\text{C}$  in herbivores are weakly transferred to the predator level (Nordström et al. 2009). Stable isotope values of animals are influenced by individual size, condition, tissue turnover time and temperature (Rolff 2000, Rossi et al. 2004, Vizzini & Mazzola 2004). Small species with high tissue turnover rates are more susceptible to changes in stable isotope values than larger species which respond more slowly to isotopic changes in food sources. Temporal variability has been observed in zooplankton, benthic invertebrates, mesograzers and fish (Nordström et al. 2009, Jaschinski et al. 2011a).

Technical limitations in the analysis of sulphur isotopes have previously impeded sample analyses, producing studies with low replications/sample sizes. Here we employed

simultaneous triple stable isotope analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  to evaluate the temporal variability in a seagrass food web in the Baltic Sea throughout the main growing season of *Z. marina* from March to September. The benthic and pelagic organisms analysed belong to different trophic levels, ranging from primary producers to herbivores and filter feeders to omnivorous and carnivorous invertebrates as well as fish. To our knowledge, this is the first high temporal resolution study of a seagrass food web including all trophic levels present that applies simultaneous triple stable isotope analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$ .

## MATERIALS AND METHODS

### Study area

The study site (Fig. 1) was a *Zostera marina* meadow in the inner Kiel Fjord, Germany ( $54^{\circ}21' \text{N}$ ,  $10^{\circ}9' \text{E}$ ). The Kiel Fjord is part of the Kiel Bight in the western Baltic Sea. The *Z. marina* meadow site is approximately 23 ha in size and is interrupted by

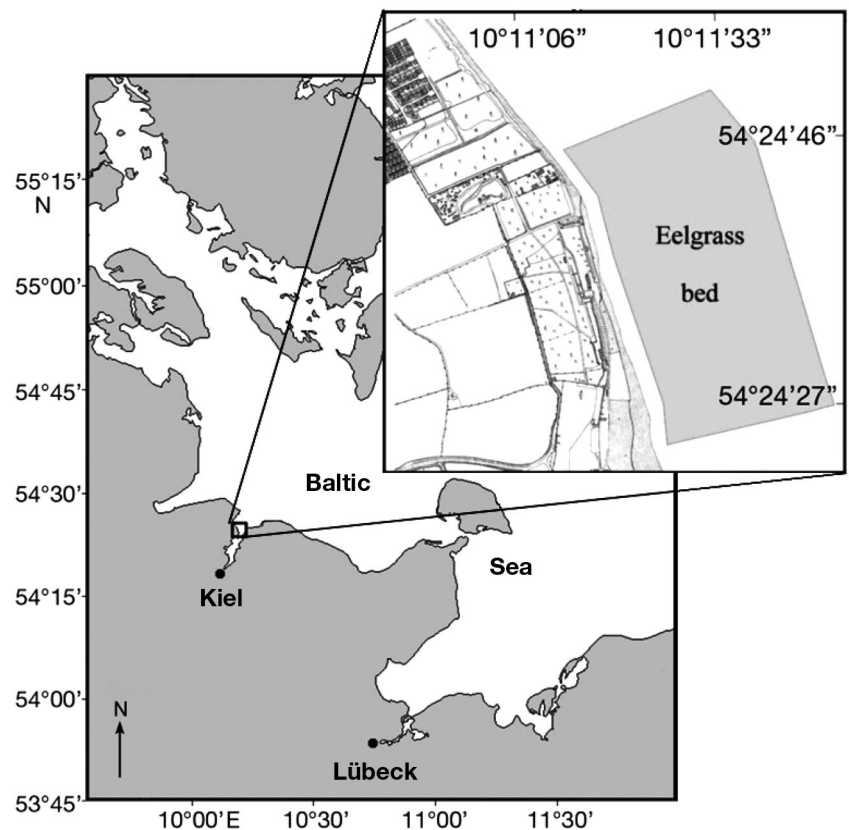


Fig. 1. Study area in the outer Kiel Fjord, Baltic Sea, Germany (adapted from Bobsien 2006). Inset shows the location of the eelgrass bed

small, unvegetated patches (Bobsien 2006). During the sampling period (March through September 2011), salinity at the collection site ranged between 13 and 18 PSU, depending on season, discharge rates and prevailing winds. The sediment in this area is sandy (grain size: 0.5 to 1 mm, 42%; >1 mm, 51%) with low organic matter content (<1%; Jaschinski et al. 2011a). Astronomical tides are negligible in this area, but storm events can cause temporary changes in sea level.

### Sample collection

Samples of seston, zooplankton, *Z. marina*, attached epiphytes, macrozoobenthic organisms and pelagic species were collected for this study. Since phytoplankton cannot be separated reliably from similar-sized heterotrophic or detrital particulate organic carbon (POC) for stable isotope analysis, seston samples were treated as a proxy for phytoplankton, even though they are not identical and also contain bacteria and protozoa. Samples were collected biweekly over a period from March through September 2011. Phytoplankton samples were collected at 3 m water depth. Zooplankton was sampled by vertical net hauls (mesh size 150  $\mu\text{m}$ ) from 3 m depth to the surface. *Z. marina*, attached epiphytes and macrozoobenthic organisms were collected with a Van Veen grab (opening: 1000  $\text{cm}^2$ ) from depths of 0.5 to 10.8 m. Pelagic macro-organisms were sampled with an 8 m beach seine with a mesh size of 0.5 cm. Animals and plants were collected in plastic containers with water from the sampling site and transported to the laboratory for identification and further processing. In total, 59 consumer species were identified over the entire sampling season and were included in the analysis.

### Sample processing

In the laboratory, epiphytes were carefully removed from *Z. marina* blades with plastic scrapers, put into distilled water and desiccated in small watch glasses. Whole leaves of *Z. marina* were processed in order to avoid bias of isotopic variability within a leaf. Seston samples were sieved through a 20  $\mu\text{m}$  mesh to separate zooplankton prior to being filtered on 0.8  $\mu\text{m}$  cellulose acetate filters (Sartorius) and carefully scraped off into distilled water with plastic cell scrapers before being desiccated in small watch glasses. Zooplankton samples were identified and

transferred directly into tin capsules (3.2  $\times$  4 mm, Hekatech) filled with distilled water. Species composition of epiphytes consisted of non-calcifying diatoms, and therefore acidification was not necessary. All consumer species were rinsed in deionized water, fixed in liquid nitrogen and processed as whole organisms, with hard parts (shells for molluscs, skin for Asteroidae) removed as practicable. All samples were dried to constant weight (60°C, 48 h). Samples were not acidified because the effects of acid washing on  $\delta^{15}\text{N}$  are still controversial. Bunn et al. (1995) showed that acid washing changed stable isotope values, particularly in  $\delta^{15}\text{N}$ , while Bosley & Wainright (1999) found no significant acid washing effects on  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  in a decapod species. Carabel et al. (2006) reported a decrease in  $\delta^{15}\text{N}$  in organisms with carbonated structures after acid washing, but for seaweeds and fish muscle tissue, acid washing is not necessary. According to Jaschinski et al. (2008b), samples should only be acidified if absolutely necessary because nitrogen in exoskeletons originates from the diet and may be relevant in the determination of food sources. Furthermore, acidifying subsamples for a separate  $\delta^{13}\text{C}$  analysis was not considered, as the simultaneous analysis of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  ratios was central to this study. After drying, animal and plant samples were ground with an agate mortar and pestle as fine as possible and stored in airtight glass vials. Subsamples of ground material (0.05 mg for animal and 0.25 mg for plant material) were weighed and placed into tin capsules. To facilitate complete combustion, ~0.25 mg vanadium pentoxide ( $\text{V}_2\text{O}_5$ ) was added to every sample. Individuals with insufficient dry weight to reach the minimum biomass levels were pooled.

### Stable isotope analysis

For this study, 1526 samples were analysed by simultaneously measuring  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values. Samples were combusted in an elemental analyser system (NA 110, Thermo) connected to a temperature controlled gas chromatography (GC) oven (SRI 9300, SRI Instruments), connected to an isotope ratio mass spectrometer (Delta<sup>plus</sup> Advantage, Thermo Fisher Scientific) as described by Hansen et al. (2009).  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values were calculated as:

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

where  $X = \delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  or  $\delta^{34}\text{S}$  and the ratio  $R = {}^{15}\text{N}/{}^{14}\text{N}$ ,  ${}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{34}\text{S}/{}^{32}\text{S}$ .  $\text{N}_2$ ,  $\text{CO}_2$  and  $\text{SO}_2$  gases were used as reference gases for each respective

analysis and were calibrated against International Atomic Agency (IAEA) reference standards (NBS123, NBS127, NBS600, S1, S2). Acetanilide, hay, cadmium sulphide (CdS) and 2,5-bis(5-tert-butyl-benzoxazol-2-yl)thiophene (BBOT) were used as internal standards after every sixth sample. The overall stability (SD) was  $\pm 0.47\%$  for  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  and  $\pm 0.12\%$  for  $\delta^{13}\text{C}$ .

Seston, epiphytes and *Z. marina* were used as ultimate carbon sources for all consumers. *Z. marina* roots and rhizomes were treated as 1 group of *Z. marina* below-ground biomass and later excluded for the purpose of this study, as they are not a relevant food source in the studied system (Mittermayr et al. 2014). As the carbon of these primary producers travels to the top of the food web, it passes through herbivorous, omnivorous and carnivorous consumers.

To determine carbon sources and the relative contributions of food sources to consumer diets, the mixing model MixSIR (Semmens & Moore 2008) was used. MixSIR is a graphical user interface built on MATLAB that employs an algorithm based on a Bayesian framework to determine the probability distributions for proportional contributions of each food source to the mixture (Moore & Semmens 2008). This model allows for allocation of different fractionation values and standard deviation around those averaged fractionation values for each element and source, respectively. A fractionation increase of  $0.5 \pm 0.5\%$  was chosen for  $\delta^{13}\text{C}$  (France & Peters 1997, Jaszinski et al. 2011b) and  $2.4 \pm 1.1\%$  and  $3.4 \pm 1.1\%$  fractionation increase in  $\delta^{15}\text{N}$  for the first and following trophic level transfers, respectively (DeNiro & Epstein 1981, Peterson & Fry 1987, Vander Zanden

& Rasmussen 1999). Fractionation for  $\delta^{34}\text{S}$  was assumed to be  $0 \pm 0.2\%$  (Peterson & Fry 1987, Currin et al. 1995, Michener & Kaufman 2007). Sufficient iterations were carried out to ensure at least 1000 posterior draws for each mixing model. Only consumers with  $n > 1$  were considered in the mixing model.

The trophic position of these consumers was calculated according to:

$$\text{TL} = 1 + \Sigma(\text{TL}_S \times C_S) \quad (2)$$

where TL is the trophic level of the consumer,  $\text{TL}_S$  is the trophic level of the source and  $C_S$  is the contribution of the source to the food mix. TL of primary producers is always assumed to be 1, thus true herbivores will always be at TL 2.

### Statistical analysis

Differences in stable isotope compositions were tested with non-metric multi-dimensional scaling (nMDS) and 1-way analysis of similarity (ANOSIM) using the PRIMER software package (Clarke & Warwick 2001). nMDS was used to identify similarities between primary producers and resulted in a stress value of 0.09, representing a good 2-dimensional ordination with no real prospect of misleading interpretation (Clarke & Warwick 2001). ANOSIM was used to test for similarities among primary producers and trophic levels of functional groups. ANOSIM results in an R-value and a p-value. The R-value is scaled from  $-1$  to  $+1$ . R-values close to 0 indicate groups that are barely different,  $R > 0.3$  shows that groups are clearly different but overlapping, and  $R > 0.5$  indicates well separated groups. The p-value is similar to that of an ANOVA, with  $p < 0.05$  indicating significance of the R-value (Clarke & Warwick 2001). Additionally, Pearson's correlation was used to describe temporal variability.

## RESULTS

### Isotopic composition and nutrient content of primary producers and consumers

Stable isotope ratios of producers significantly differed from one another. nMDS analysis of C, N and S stable isotopes showed distinct separation of the 3 primary producers, *Zostera marina*, epiphytes and seston (Fig. 2); an nMDS stress value of 0.09 represents a good 2-D ordination for clear interpretation (Clarke & Warwick 2001). Furthermore, ANOSIM

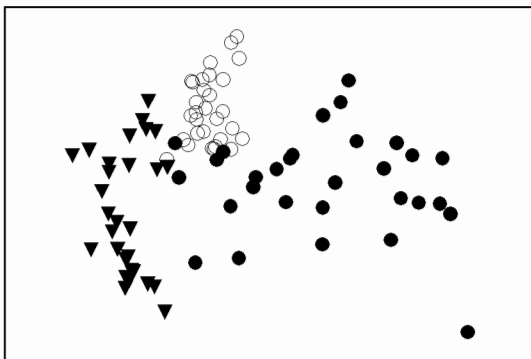


Fig. 2. Non-metric multi-dimensional scaling (nMDS) plot based on a similarity matrix calculated on a data matrix of  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values of *Zostera marina* leaves (●), epiphytes (○) and seston (▼). Axes are dimensionless and proximity (nearness in space between any 2 points) indicates that samples are similar in isotopic makeup (stress = 0.09)

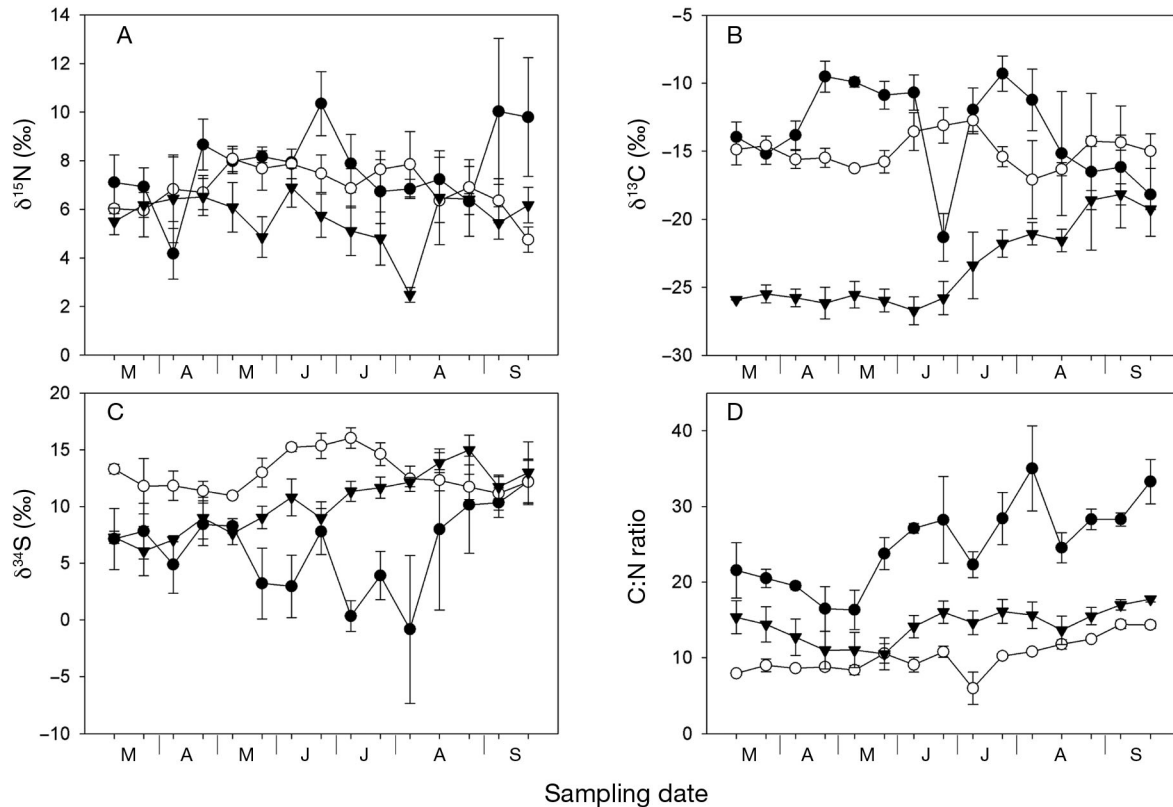


Fig. 3. Mean  $\pm$  SE of the stable isotopes. (A)  $\delta^{13}\text{C}$ , (B)  $\delta^{15}\text{N}$ , (C)  $\delta^{34}\text{S}$  and (D) carbon to nitrogen ratios in *Zostera marina* leaves (●), seston (▲) and epiphytes (○)

results clearly separated *Z. marina* from seston ( $p = 0.001$ ,  $R = 0.72$ ) and epiphytes ( $p = 0.002$ ,  $R = 0.554$ ), and seston from epiphytes ( $p = 0.002$ ,  $R = 0.69$ ). Analyses of temporal trends in stable isotopes of producers showed variable results (Fig. 3).  $\delta^{13}\text{C}$  and  $\delta^{34}\text{S}$  values of seston increased with the progression of the growing season, as reflected by a positive correlation with time ( $r = 0.86$ ,  $n = 30$ ,  $p < 0.001$ ;  $r = 0.85$ ,  $n = 30$ ,  $p < 0.001$ , respectively; Fig 3B,C). Stable isotope ratios of *Z. marina* and its epiphytes did not exhibit significant correlations with time, suggesting that, if present, temporal differences were not linear. Further analysis using ANOSIM showed significant temporal differences in stable isotopes for *Z. marina* leaves and seston, but not for epiphytes (Fig. 3, and see Table S1 in the Supplement; [www.int-res.com/articles/suppl/m505p095\\_supp.xlsx](http://www.int-res.com/articles/suppl/m505p095_supp.xlsx)). There were also no significant effects of depth on *Z. marina* isotopes within our study site ( $r = 0.03$ ,  $N = 30$ ,  $p > 0.1$ ; data not shown). Carbon to nitrogen ratios of all primary producers (Fig. 3D) showed significant correlations with time (seston:  $r = 0.57$ ,  $n = 30$ ,  $p < 0.001$ ; epiphytes:  $r = 0.74$ ,  $n = 30$ ,  $p < 0.001$ ; *Z. marina*:  $r = 0.71$ ,

$n = 30$ ,  $p < 0.001$ ), with an increase in C:N over the growing season.

Consumers were arranged into the following functional groups according to the literature (Encyclopedia of Life, [www.eol.org](http://www.eol.org); World Register of Marine Species, [www.marinespecies.org](http://www.marinespecies.org)): zooplankton, suspension feeders, omnivores and predators (Table 1). However, several species exhibited stable isotope values atypical for the feeding type suggested by the literature. Fig. 4 shows the relative frequency of occurrence of  $\delta^{15}\text{N}$  values of individuals of known omnivores (*Gammarus locusta*, *Palaemon adspersus*, *Idotea balthica*, *Nephtys hombergii*, *Nereis diversicolor* and *Nereis pelagica*) and those of *Littorina littorea* (grazer), *Edwardsia longicornis* (predator), *Cryptocelides loveni* (predator) and *Asterias rubens* (predator). Omnivore diets create a mix of  $\delta^{15}\text{N}$  values representative of herbivores ( $< 7\text{‰}$ ) and predators ( $> 10\text{‰}$ ), resulting in values between 7 and 10‰. The majority of isotopic values for individuals of known omnivore species are between 7 and 10‰. Also, for all 6 representative omnivore species, some individuals were strictly herbivorous and others were



Table 1.  $\delta^{13}\text{C}$ ,  $\delta^{14}\text{N}$ ,  $\delta^{34}\text{S}$  values and C:N (mean  $\pm$  SE) of primary producers and consumers according to functional groups in an eelgrass *Zostera marina* bed in the Kiel Bight, Germany, March to September 2011. Data for each sampling date can be found in Table S1 in the Supplement; [www.int-res.com/articles/suppl/m505p095\\_supp.xlsx](http://www.int-res.com/articles/suppl/m505p095_supp.xlsx)

Species	n total	% present	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{34}\text{S}$	C:N ratio
<b>Primary producers</b>						
<i>Zostera marina</i>	30	100	7.5 $\pm$ 1.2	-13.4 $\pm$ 3.3	5.8 $\pm$ 3.6	24.9 $\pm$ 5.9
Epiphytes	30	100	6.9 $\pm$ 1.1	-15 $\pm$ 1.5	12.8 $\pm$ 1.9	10.3 $\pm$ 2.4
Seston	30	100	5.7 $\pm$ 1.3	-23.4 $\pm$ 3.1	10.3 $\pm$ 2.8	14.3 $\pm$ 2.3
<b>Zooplankton</b>						
<b>Zooplankton</b>	<b>1490</b>	<b>100</b>	<b>7.1 <math>\pm</math> 1.3</b>	<b>-24.1 <math>\pm</math> 1.3</b>	<b>19 <math>\pm</math> 1.7</b>	<b>5.5 <math>\pm</math> 0.7</b>
<i>Acartia</i> sp.	562	100	7 $\pm$ 1.8	-24.2 $\pm$ 2.4	18.4 $\pm$ 3	6.2 $\pm$ 1.9
<i>Calanus</i> sp.	20	13	7.9 $\pm$ 0.7	-21.9 $\pm$ 0.6	18.6 $\pm$ 2.2	4.8 $\pm$ 0.2
Chaetognatha larvae	10	7	7.6 $\pm$ 0.2	-21.5 $\pm$ 0.6	19.7 $\pm$ 0.0	5.2 $\pm$ 0.2
Cirripedia larvae	145	33	6.3 $\pm$ 1.5	-24.7 $\pm$ 1.6	20.3 $\pm$ 1.1	5.2 $\pm$ 0.5
Cladocera larvae	61	33	6.6 $\pm$ 1.7	-22.5 $\pm$ 1.5	17.5 $\pm$ 3.3	6.6 $\pm$ 0.7
<i>Oithona</i> sp.	396	53	6.9 $\pm$ 2.1	-25.3 $\pm$ 0.8	20.1 $\pm$ 1.9	5.4 $\pm$ 1.3
<i>Pseudocalanus</i> sp.	126	33	8.1 $\pm$ 1.7	-24.8 $\pm$ 1.4	18.8 $\pm$ 3.9	4.9 $\pm$ 0.6
<i>Temora</i> sp.	158	60	7.4 $\pm$ 1.4	-25 $\pm$ 1.3	19.5 $\pm$ 2.8	5.4 $\pm$ 0.8
Veliger	12	7	7.7 $\pm$ 0.1	-25.3 $\pm$ 0.5	18.6 $\pm$ 0.5	5.4 $\pm$ 0.3
<b>Suspension feeders</b>						
<b>Suspension feeders</b>	<b>235</b>	<b>100</b>	<b>8.6 <math>\pm</math> 1.8</b>	<b>-20.5 <math>\pm</math> 2.3</b>	<b>15 <math>\pm</math> 3</b>	<b>5.6 <math>\pm</math> 0.6</b>
<i>Abra prismatica</i>	9	27	8.6 $\pm$ 1.7	-19.4 $\pm$ 2.5	14.9 $\pm$ 3.3	5 $\pm$ 0.7
<i>Ascidia aspersa</i>	4	13	9.4 $\pm$ 2.5	-17.8 $\pm$ 2.1	19.2 $\pm$ 1.3	6.5 $\pm$ 0.2
<i>Balanus improvisus</i>	4	13	9.6 $\pm$ 1.6	-19.8 $\pm$ 1.4	16.5 $\pm$ 2	6 $\pm$ 1.0
<i>Cerastoderma edule</i>	60	100	8.7 $\pm$ 1.8	-20.3 $\pm$ 2.2	14.8 $\pm$ 3.5	5.5 $\pm$ 0.9
<i>Chalinula limbata</i>	2	7	7.8 $\pm$ 0.5	-22.1 $\pm$ 0.5	16.4 $\pm$ 0.5	5.8 $\pm$ 0.6
<i>Ciona intestinalis</i>	14	27	8.6 $\pm$ 1.7	-19.3 $\pm$ 3.1	15.7 $\pm$ 3.4	6 $\pm$ 0.6
<i>Halichondria panicea</i>	13	27	8.2 $\pm$ 2.1	-21.4 $\pm$ 1.9	16.3 $\pm$ 1.2	6.5 $\pm$ 0.7
<i>Lacuna vineta</i>	2	7	9.3 $\pm$ 3.8	-18.5 $\pm$ 0.2	10.8 $\pm$ 3.2	4.9 $\pm$ 0.4
<i>Macoma balthica</i>	31	53	8.6 $\pm$ 1.5	-20.1 $\pm$ 2.4	10.6 $\pm$ 3.8	5.5 $\pm$ 1.2
<i>Mya arenaria</i>	10	20	8.1 $\pm$ 1.3	-19.6 $\pm$ 3.1	11.4 $\pm$ 6	4.9 $\pm$ 0.4
<i>Mytilus edulis</i>	81	100	8.5 $\pm$ 2.1	-20.9 $\pm$ 2.5	14.4 $\pm$ 3.5	5.2 $\pm$ 0.8
<i>Suberites ficus</i>	5	13	9 $\pm$ 3.1	-22.6 $\pm$ 2.5	15.5 $\pm$ 1	5.8 $\pm$ 0.1
<b>Omnivores</b>						
<b>Omnivores</b>	<b>585</b>	<b>100</b>	<b>9.3 <math>\pm</math> 2.1</b>	<b>-19.4 <math>\pm</math> 2.1</b>	<b>13.3 <math>\pm</math> 3.1</b>	<b>5.6 <math>\pm</math> 0.6</b>
<i>Arenicola marina</i>	3	13	11.2 $\pm$ 0.4	-18.1 $\pm$ 0.4	10.1 $\pm$ 1	5.2 $\pm$ 0.1
<i>Asterias rubens</i>	28	67	8.3 $\pm$ 1.7	-21.6 $\pm$ 2.5	14.3 $\pm$ 2.9	6.6 $\pm$ 1.4
<i>Buccinum undatum</i>	5	20	11.9 $\pm$ 0.7	-19.9 $\pm$ 1.7	15.9 $\pm$ 1.1	5.4 $\pm$ 0.4
<i>Capitella punctata</i>	5	7	11 $\pm$ 2.5	-20.4 $\pm$ 1.4	11.4 $\pm$ 5	4.9 $\pm$ 0.1
<i>Carcinus maenas</i>	4	27	10.5 $\pm$ 1.4	-18.6 $\pm$ 3.1	13.5 $\pm$ 2.9	6 $\pm$ 0.4
<i>Corophium voluntator</i>	9	33	7.9 $\pm$ 1.4	-21.3 $\pm$ 1.1	13.8 $\pm$ 3.6	5.1 $\pm$ 0.4
<i>Cryptocelides loveni</i>	12	40	8.3 $\pm$ 1.9	-18 $\pm$ 4	16.6 $\pm$ 2.1	6.2 $\pm$ 1.1
<i>Edwardsia longicornis</i>	10	33	8.5 $\pm$ 1.4	-20.6 $\pm$ 2.3	11.3 $\pm$ 2.5	4.5 $\pm$ 0.3
<i>Etone longa</i>	4	20	10.1 $\pm$ 2.2	-20.5 $\pm$ 1.4	8.9 $\pm$ 2.6	4.5 $\pm$ 0.2
<i>Gammarus locusta</i>	80	93	8.4 $\pm$ 1.7	-19.7 $\pm$ 2.3	13.2 $\pm$ 3.3	5.7 $\pm$ 1.6
<i>Idotea balthica</i>	112	100	8.3 $\pm$ 1.9	-18.9 $\pm$ 2.7	14.9 $\pm$ 3.5	6.1 $\pm$ 1.1
<i>Lepidontus squamatus</i>	12	53	9.4 $\pm$ 2.7	-20.5 $\pm$ 3.2	13.4 $\pm$ 2.3	5.2 $\pm$ 1.6
<i>Lineus ruber</i>	5	20	10.5 $\pm$ 2.3	-19.6 $\pm$ 1.2	12.4 $\pm$ 3.5	4.9 $\pm$ 1.2
<i>Littorina littorea</i>	33	87	9.5 $\pm$ 1.9	-18.9 $\pm$ 2.7	15.3 $\pm$ 2.7	5.1 $\pm$ 1
<i>Mysis mixta</i>	10	20	8.2 $\pm$ 1.3	-20.2 $\pm$ 2.5	13.2 $\pm$ 2.3	5 $\pm$ 0.7
<i>Neoamphitrite figulus</i>	6	13	9 $\pm$ 2.2	-20.6 $\pm$ 2.1	14.9 $\pm$ 3.5	4.9 $\pm$ 0.9
<i>Nephtys hombergii</i>	25	47	9.2 $\pm$ 2.2	-18.8 $\pm$ 2.5	11.4 $\pm$ 3.8	3.3 $\pm$ 2.5
<i>Nereis diversicolor</i>	64	93	10.2 $\pm$ 2.3	-19 $\pm$ 2.5	11.9 $\pm$ 4.4	4.8 $\pm$ 1.5
<i>Nereis pelagica</i>	90	100	10.2 $\pm$ 1.8	-19.1 $\pm$ 1.7	11.6 $\pm$ 3.3	4.8 $\pm$ 0.8
<i>Nereis virens</i>	8	7	10.7 $\pm$ 1.9	-18.7 $\pm$ 1.1	13.9 $\pm$ 3.3	4.9 $\pm$ 0.5
<i>Palaemon adspersus</i>	13	20	10.4 $\pm$ 1.1	-17.3 $\pm$ 2.7	15.8 $\pm$ 1.7	4.8 $\pm$ 0.7
<i>Phascolion strombi</i>	8	13	8.8 $\pm$ 2.3	-19.1 $\pm$ 1.5	12.6 $\pm$ 3.9	4.8 $\pm$ 0.4
<i>Polydora ciliata</i>	3	7	10.2 $\pm$ 3.1	-20.2 $\pm$ 0.6	17.7 $\pm$ 0.3	5.5 $\pm$ 0.3
<i>Praunus flexuosus</i>	36	73	9.7 $\pm$ 1.7	-20.1 $\pm$ 1.9	13.5 $\pm$ 2.4	5.4 $\pm$ 2.2
<b>Predators</b>						
<b>Predators</b>	<b>166</b>	<b>80</b>	<b>10.4 <math>\pm</math> 1.6</b>	<b>-19.7 <math>\pm</math> 2.1</b>	<b>14.6 <math>\pm</math> 2.4</b>	<b>5.2 <math>\pm</math> 1.3</b>
<i>Ammodytes tobianus</i>	7	13	11.4 $\pm$ 1.9	-20.4 $\pm$ 4.7	16.1 $\pm$ 1.1	4.2 $\pm$ 0.2
<i>Belone belone</i>	2	7	12.9 $\pm$ 0.9	-19.5 $\pm$ 0.7	18.2 $\pm$ 0.0	4.9 $\pm$ 0.8
<i>Crangon crangon</i>	21	40	10.7 $\pm$ 1.8	-18 $\pm$ 1.9	11.1 $\pm$ 3.3	4.5 $\pm$ 0.5
<i>Cyclopterus lumpus</i>	11	13	9.2 $\pm$ 1.8	-19 $\pm$ 1.9	14.2 $\pm$ 2.8	5.2 $\pm$ 0.7
<i>Gasterosteus aculeatus</i>	19	53	11.3 $\pm$ 1.9	-21.4 $\pm$ 2.1	16.4 $\pm$ 1.6	5.6 $\pm$ 1.2
<i>Gattyana cirrosa</i>	13	53	10.2 $\pm$ 2	-20.2 $\pm$ 1.3	14.5 $\pm$ 2.4	3.3 $\pm$ 2.2
<i>Gobiusculus flavescens</i>	15	33	10.2 $\pm$ 2	-19.7 $\pm$ 2.4	15.9 $\pm$ 3	4.7 $\pm$ 1.2
<i>Nerophis ophidion</i>	18	33	9.6 $\pm$ 1.7	-19.5 $\pm$ 3.1	14.2 $\pm$ 3.1	5.7 $\pm$ 1.4
<i>Pomatoschistus minutus</i>	14	33	10.1 $\pm$ 2.4	-19.3 $\pm$ 2.4	13.6 $\pm$ 4	5.3 $\pm$ 2.8
<i>Spinachia spinachia</i>	10	20	10.5 $\pm$ 1.8	-17 $\pm$ 2.9	15.1 $\pm$ 2.2	4.2 $\pm$ 0.5
<i>Syngnathus rostellatus</i>	13	33	9.8 $\pm$ 1.5	-20.3 $\pm$ 1.9	14.9 $\pm$ 2.3	5.2 $\pm$ 1.0
<i>Syngnathus typhle</i>	23	40	10.4 $\pm$ 1.6	-21.2 $\pm$ 2.5	16 $\pm$ 1.1	5.6 $\pm$ 1.4

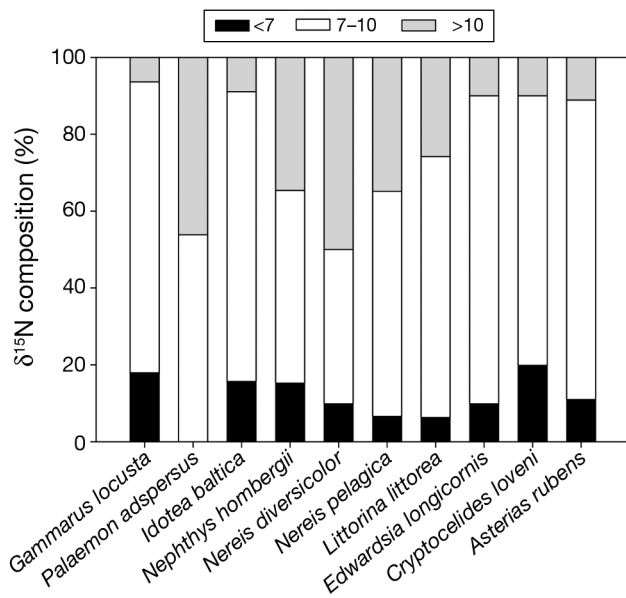


Fig. 4. Frequency of  $\delta^{15}\text{N}$  values measured; these were used to assign species to the appropriate functional groups.  $\delta^{15}\text{N}$  values:  $<7\text{‰}$  = herbivore;  $7\text{--}10\text{‰}$  = omnivore;  $>10\text{‰}$  = predator

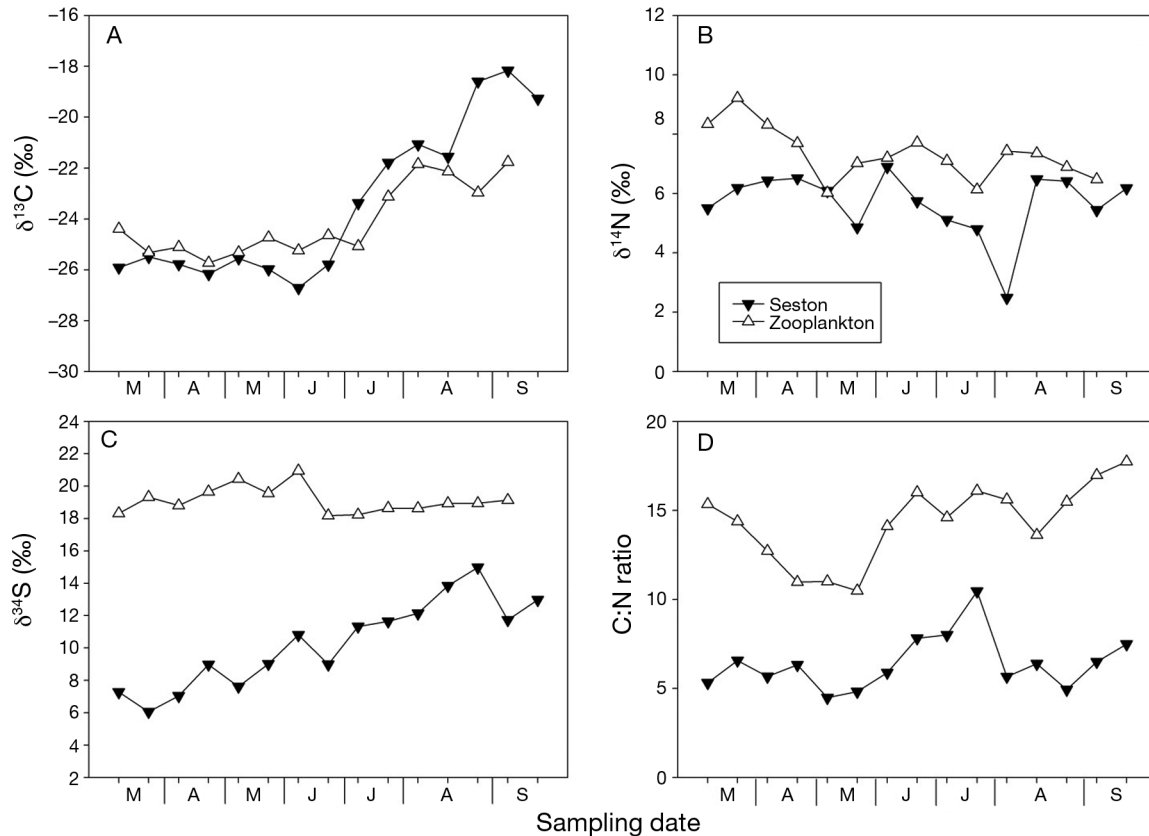


Fig. 5. Mean stable isotopes and C:N ratios of primary sources (seston) and their respective primary consumers (zooplankton) over the sampling period. Additional data can be found in Table S1 in the Supplement; [www.int-res.com/articles/suppl/m505p095\\_supp.xlsx](http://www.int-res.com/articles/suppl/m505p095_supp.xlsx)

strict carnivores. Based on the frequency of occurrences of  $\delta^{15}\text{N}$  values within the omnivore range, *L. littorea* (grazer), *E. longicornis* (predator), *C. loveni* (predator) and *A. rubens* (predator) were moved into the omnivore functional group. *L. littorea* has previously been classified as a scavenger by Petraitis (2002), and Jaschinski et al. (2008a) have discussed herbivory by *A. rubens*.

Isotopic values of grazers tended to follow producers (Fig. 5), particularly so in  $\delta^{13}\text{C}$  and for seston and zooplankton, where the tightest coupling was observed (Fig. 5A). Pearson correlation could not significantly explain more than 33% of stable isotope ratios over time for any functional group (all groups not shown).

#### Diet composition and trophic level estimated by the mixing model

Temporal variation in diets and the resulting trophic levels of consumer functional groups differed depending on position in the food web (Fig. 6,

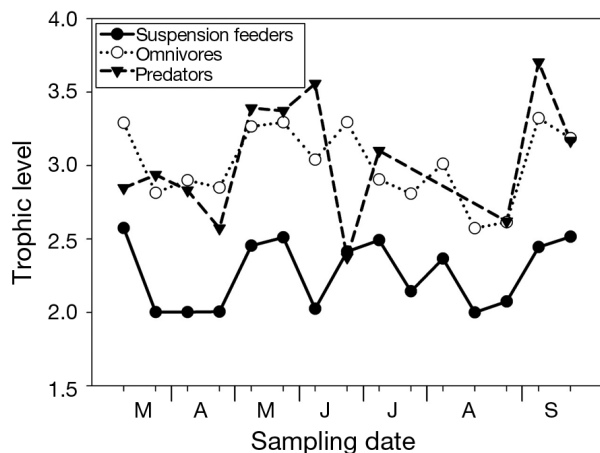


Fig. 6. Trophic levels of consumer groups over the sampling period from March to September 2011

Table 2. Trophic levels (TL) of functional groups and analysis of similarity (ANOSIM) comparison of stable isotope values over time. Samples are similar when ANOSIM  $R < 0.3$ . Significance is indicated by \* $p < 0.05$ , \*\*\* $p < 0.001$

	TL (mean $\pm$ SE)	TL min.	TL max.	R	p-value
Zooplankton	2.0	2.0	2.0	0.3	***
Suspension feeders	2.4 $\pm$ 0.3	2.0	2.7	0.1	*
Omnivores	3.1 $\pm$ 0.3	2.6	3.7	0.3	***
Predators	3.2 $\pm$ 0.5	2.4	3.9	0.1	***

Tables 2 & S1). Primary consumer diets showed little temporal variation. Zooplankton diet consisted of seston (>92%; Fig. 7A) and remained at TL = 2 throughout the sampling period (Tables 2 & S1). As the  $\delta^{34}\text{S}$  and  $\delta^{13}\text{C}$  values of benthic and pelagic suspension feeders were significantly similar ( $p = 0.001$ ,  $R = 0.06$  and  $p = 0.002$ ,  $R = 0.101$ , respectively), indicating a mix of benthic and pelagic food sources for both groups, all suspension feeders were treated as 1 group (Table 1). The same was observed for benthic and pelagic omnivores ( $p = 0.001$ ,  $R = 0.009$ ) and for benthic and pelagic predators ( $p = 0.003$ ,  $R = 0.123$ ), which were treated as omnivores and predators, respectively. Suspension feeders relied on zooplankton, seston, epiphytes and *Z. marina* as food sources with TL = 2 to 2.6 (Tables 2 & S1, Fig. 7B). They fed primarily from the water column with different relative ratios of seston and zooplankton over the sampling period. They ingested an average of ~37% seston and ~35% zooplankton from March to September. Recurring peaks in zooplankton consumption in early March, May, early July, early August

and September resulted in an increase in trophic level from TL = 2 to a maximum of 2.7 (Tables 2 & S1). Omnivore diet was made up primarily of suspension feeders (~65%) resulting in TL = 3 (Fig. 7C). A peak in zooplankton consumption in August (53%) caused TL to drop to 2.6. The contribution of epiphytes and *Z. marina* was low (3.6 and 4.4%, respectively). Predators fed on suspension feeders (>40%) and a mix of omnivores, zooplankton, *Z. marina*, epiphytes and seston (<17%), with trophic levels reaching a maximum of 3.7 in early September (Fig. 7, Tables 1 & S1).

## DISCUSSION

We found substantial temporal variation in stable isotopes of producers, particularly in seston and *Zostera marina*. Temporal variation was also observed in stable isotopes of consumer functional groups; however, variation within a species, particularly for omnivores, often exceeded variation in consumer group isotopes over time.  $\delta^{15}\text{N}$  values outside the range of 7 to 10‰, representing strict herbivory (0–20%) or carnivory (6–50%) were observed in a significant portion of individuals from many omnivore species (Fig. 4). Omnivory defined the diets of 40 to 80% of individuals from the representative omnivore species. For individuals of the species *Idotea balthica*, for example, 15% were herbivorous, 75% were omnivorous, and 10% were carnivorous (Fig. 4). This supports the hypothesis of high flexibility of omnivores concerning food sources. Omnivore flexibility and opportunistic switching of food sources are of extraordinary importance to food web stability (McCann et al. 2005).

Seston and *Z. marina* exhibited significant temporal differences in stable isotope ratios. This variation over time suggests changes in sources, physiological demands and/or allocations over the *Z. marina* growing season from March to September (Hemminga & Mateo 1996, Adams & Sterner 2000). Warmer water temperatures in summer and early fall and the resulting alteration of biological processes cause inorganic carbon and nitrogen chemistry in the water column and sediments to adapt, thereby shifting the availability for uptake by producers. Changes in availability of essential elements lead to differences in isotope discrimination and isotopic ratios. Sampling was undertaken within the same area of the seagrass bed throughout the study to avoid spatial variation (Hyndes et al. 2013). Variability in stable isotope ratios of primary producers was generally higher than those of consumers, which can be expected, as



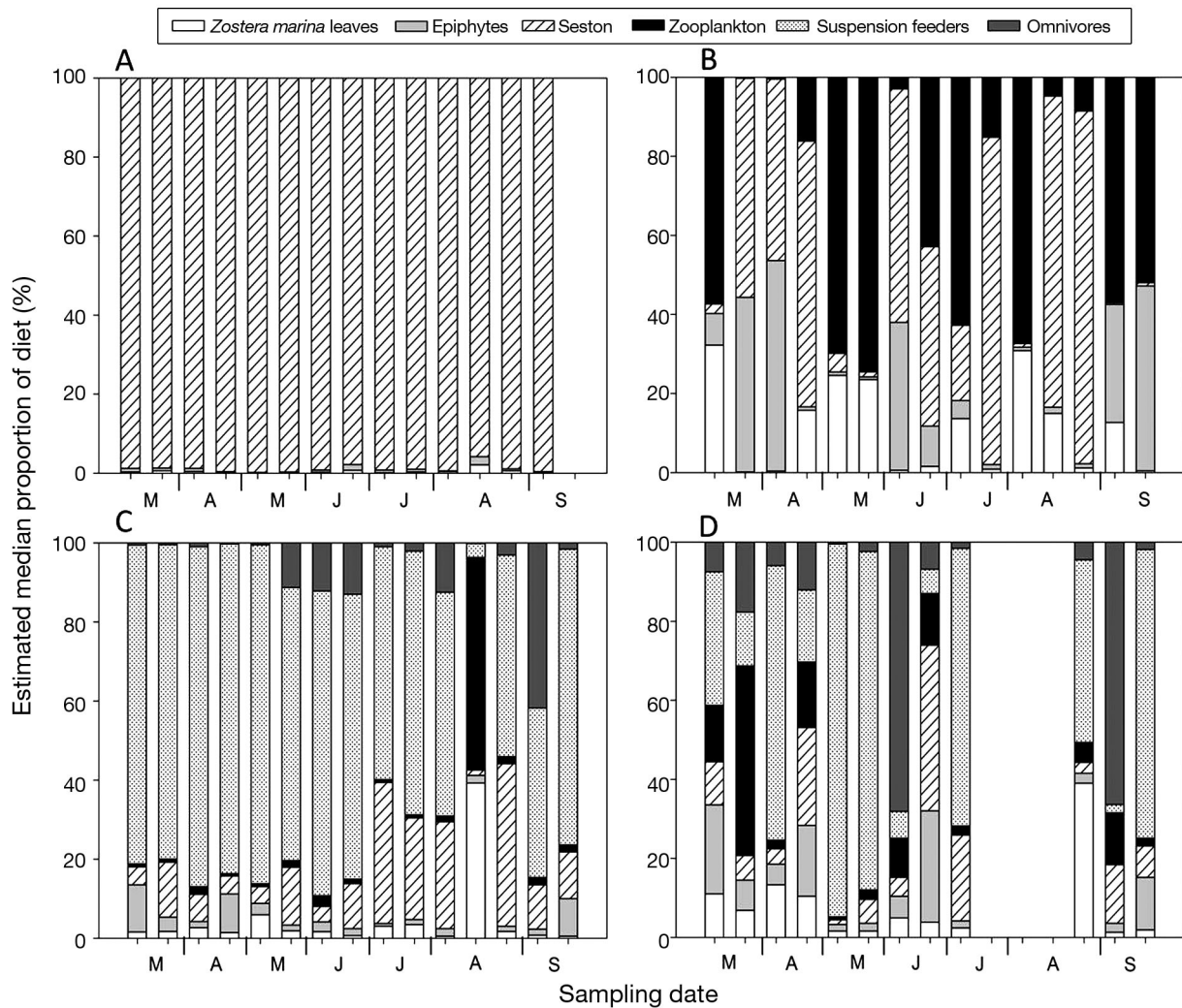


Fig. 7. Summary of mixing model (MixSIR) outputs of (A) zooplankton, (B) suspension feeders, (C) omnivores and (D) predators showing the respective food sources and their contributions to the carbon mix for every sampling date

stable isotope ratios of consumers reflect a longer time integrated assimilation of food sources because of slower tissue turnover rates.

Seston was the most important original carbon source in this *Z. marina* system, followed by epiphytes. *Z. marina* appears to be of little importance as a food source but gained in relevance for some species, particularly *I. balthica*, towards the end of the sampling season when epiphytes declined (A. Mittermayr pers. obs.). Although there may be increased direct consumption of *Z. marina* by *I. balthica*, much of the increase in contribution to consumer diet might be explained by increases in  $\delta^{34}\text{S}$  of *Z. marina* in August and September (Fig. 3). The increase in  $\delta^{34}\text{S}$  made epiphytes and *Z. marina* leaves indistinguishable as food sources in September ( $p = 0.032$ ,  $R = 0.16$ ). Thus, the mixing model would be

unable to correctly determine consumer diets that consist of epiphytes and/or *Z. marina* leaves. When modelled individually, *I. balthica* showed significant *Z. marina* leaf consumption (~42%) after the  $\delta^{34}\text{S}$  shift in *Z. marina* leaves (data not shown). Trophic levels for *I. balthica* remained stable over the sampling period at  $\text{TL} = 2.4$ , suggesting that a large proportion of its omnivorous diet came from primary producers. We identified 15% of *I. balthica* as strict herbivores which were present throughout sampling and thus cannot be responsible for the diet shift.

Temporal patterns in suspension feeder diets (Fig. 7B) suggest that changes in food availability may be driving their feeding habits. Multiple storm events observed throughout the sampling period (Maritime Meteorology, GEOMAR) caused mixing of the water column and detachment of epiphytes from

*Z. marina* leaves, making this food source available to filter feeders. Suspension feeders showed a substantial epiphyte contribution to their diet at the beginning and the end of the sampling season (~53% and ~46%, respectively). The trophic levels observed were highest when zooplankton intake was highest and exhibited variability over time (Figs. 6 & 7B).

Food sources for omnivores were dominated by suspension feeders (65%; Fig. 7C) which caused a similar pattern of variability in trophic levels (Fig. 5), propagating up the food web. As expected, trophic levels decreased when primary producer uptake increased. In July and August, the lower carbon values suggest a large contribution by pelagic sources. The diet mix of epiphytes and seston is found among many omnivore species with  $\delta^{13}\text{C}$  values near  $-20\text{‰}$ . Other potential sources to this food web procuring such a signal might include suspended POC and benthic microalgae (Moncreiff & Sullivan 2001, Maier et al. 2011), which were not sampled in this study.

Predators were predominantly fish, with the exception of 1 polychaete species (Table 1). The main carbon source for predators (Fig. 7D) was suspension feeders (43%). Omnivore contribution peaked twice, in June (68%) and September (66%), which may result from the high abundance of *Gasterosteus aculeatus* during those sampling dates.

In this study, we did not sample detritus or epibenthic microalgae, which are both known to play significant roles in seagrass food webs (Nordström et al. 2009, Ouisse et al. 2012). Additionally, sand microflora also exhibit  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values similar to seston (Jaschinski et al. 2011a), and demand a detailed triple stable isotope analysis. Furthermore, the fact that copepods feed on protozoa (Sommer et al. 2002) could not be taken into account due to technical sampling methodology limitations. Future seagrass food web studies should use the triple stable isotope approach to distinguish epiphytes from *Z. marina* and should include more carbon sources at the base of the food web, e.g. benthic diatoms and benthic seston.

As previously described by Jaschinski et al. (2011a), *Z. marina* in the Kiel Bight mainly provides critical habitat, shelter and substrate for epiphyte growth, but food is primarily supplied by seston and epiphytes. Thus, this system depends on eelgrass as an ecosystem engineer or keystone species, but substantially less as a direct food resource. Epiphytes, dominated by benthic diatoms (Jaschinski et al. 2008a), are considered to be a highly nutritious food source (Creach et al. 1997), and together with seston support the invertebrate food web in this *Z. marina*

bed. Even though the studied eelgrass system is characterized by benthic-pelagic coupling, the majority of food sources are of pelagic origin.

Temporal patterns in  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{34}\text{S}$  in this *Z. marina* bed differ between functional groups. This *Z. marina* bed is characterized by a high degree of omnivory and many generalist feeders that are able to respond to changes in food source availability which is supported by the highly variable stable isotope compositions within species. Although the base of the food web may change over time in this dynamic seagrass bed, there is evidence that in response, consumers flexibly change diets, thus maintaining a stable food web structure throughout the growth season.

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