1   Running head: Seasonal nutrition of mesograzers

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3   **Seasonal variation in carbon sources of mesograzers and small predators in an eelgrass**

4   **community: stable isotope and fatty acid analyses**

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26   ABSTRACT: We assessed the seasonal change in trophic interactions at the mesograzer and

27   small predator level in a temperate eelgrass system. This was done through stable isotope

28   (δ^{13}C and δ^{15}N) and fatty acid analyses of eelgrass (*Zostera marina*), attached epiphytes, sand

29   microflora, the red algae *Delesseria sanguinea*, and the four most common mesograzer and

30   predator species. Sampling took place in the western part of the Baltic Sea monthly during a

31   whole year (April 2002 to February 2003). The trophic importance of microalgae was

32   corroborated in all studied species. Red algae were consumed to a lesser extent and eelgrass

33   was of minor importance. The degree of dependence on the main carbon sources was species-

34   specific and varied with time. The small gastropod *Rissoa membranacea* mostly grazed

35   epiphytes (73% on the average), whereas the fractions of epiphytes and sand microflora

36   showed a more balanced pattern in the other mesograzer species. Stable carbon data and fatty

37   acid composition strongly suggested that epiphytic algae were the primary source of organic

38   matter for mesograzers in late spring and autumn. In summer sand microflora and for the

39   amphipod *Gammarus oceanicus* red algae were of greater importance as carbon sources.

40   Stable nitrogen values indicated that the degree of carnivory was size-dependant in both

41   studied omnivorous crustacean species; larger individuals generally occupied a higher trophic

42   position than smaller ones. Furthermore, *Idotea baltica* was more herbivorous in summer than

43   in the other seasons. Our results confirm the importance of species-specific and temporal

44   variability for the impact of mesograzers in eelgrass systems. The significance of mesograzers

45   as structuring force via the reduction of epiphytes is further corroborated by our study and the

46   found high plasticity of mesograzers concerning food sources may promote the stability of

47   eelgrass food web despite strong seasonal variations in the biomass of primary producers.

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50   KEY WORDS: Seagrass, Grazing, *Idotea*, *Gammarus*, *Littorina*, *Rissoa*, Trophic interactions,

51   Nutrition

INTRODUCTION

52 **INTRODUCTION**

53 Recent studies have strongly demonstrated the structuring force of trophic interactions in

54 coastal seagrass ecosystems (Hauxwell et al. 1998, Hughes et al. 2004, Heck & Valentine

55 2007). By feeding preferentially on fast-growing epiphytic algae, and thus, facilitating growth

56 and survival of the seagrass, small invertebrate grazers are supposed to play an important role

57 in these communities (Duffy et al. 2001, Jaschinski & Sommer 2008a). Additionally, large

58 parts of the energy flux to higher trophic levels including commercially important fish species

59 are channelled through these consumers (Klumpp et al. 1992, Edgar & Shaw 1995). Although

60 the relevance of mesograzers is widely acknowledged, general conclusions on their ecological

61 role in macrophyte systems are challenging because of the great spatial and temporal

62 variability in the abundance of mesograzers and their food (Edgar & Shaw 1995, Nelson &

63 Waaland 1997, Douglass 2010). In temperate systems abiotic and biotic conditions change

64 considerably in the course of the year with possible consequences for food web interactions

65 (Polis et al. 1996). The strong seasonal variation of primary producer biomass and

66 composition has a relevant influence on the abundance and composition of the mesograzer

67 assemblage in seagrass ecosystems (Nelson & Waaland 1997, Gohse-Reimann 2007).

68 Most common mesograzers in temperate regions are considered generalists and can feed on a

69 wide range of food sources from micro- to macroalgae and some species are even omnivorous

70 (Norton et al. 1990, Duffy & Harvilicz 2001, Orav-Kotta & Kotta 2003). This capacity may

71 enable these species to switch between food sources and feed on the most abundant prey

72 (Chesson 2000). Such density-dependant prey-switching is thought to stabilize food web

73 dynamics.

74 The results of laboratory experiments on feeding preference are limited in their conclusions

75 concerning natural ecosystems, because it is difficult to replicate the exact conditions that

76 determine preferences in the field. Large scale ecological field studies, however, are

77 expensive and time-consuming and therefore scarce in macrophyte systems (Connolly et al.

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78   2005). Traditionally, the analysis of gut contents is used to study the nutrition of consumer

79   species. This method provides detailed taxonomic information on the ingested food, but is

80   very time-consuming. It also requires expert taxonomic knowledge and reveals only the

81   ingested but not the assimilated food. Furthermore, only the food items consumed in a short

82   period of time can be assessed with this method. Most importantly, gut content analysis also

83   provides a biased image of the diet with an over-representation of food items which contain

84   indigestible, structural material.

85   Stable isotope and fatty acid analysis, in contrast, have the benefit of supplying time-

86   integrated estimates of assimilated food (Hobson & Welch 1992, Dahl et al. 2003). The ratios

87   of naturally occurring stable carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) isotopes can

88   be used in food web analyses in different ways considering the fact that all biochemical

89   reactions tend to accumulate the heavier isotope (fractionation). The fractionation of $\delta^{13}C$ is

90   thought to be low – maximal 1‰ per trophic level – and $\delta^{13}C$ is therefore useful to identify

91   different carbon sources (France & Peters 1997). The $\delta^{15}N$ content is enriched by 3 to 4‰ per

92   trophic level on average and the results can be used to construct the structure of a food web,

93   assuming $\delta^{15}N$ of the basal food sources is known (Vanderklift & Ponsard 2003). Fatty acid

94   analysis has been shown to be a reliable method to trace food sources in aquatic food webs,

95   since laboratory experiments proved that transfer of specific fatty acids is conservative (Lee et

96   al. 1971, Brett et al. 2006). A number of "indicator" fatty acids specific for algal groups like

97   diatoms or dinoflagellates can be used as biomarkers (Viso & Marty 1993, Desvilettes et al.

98   1997) and the quantitative pattern of all fatty acids, the fatty acid signature, can provide

99   additional information (Dahl et al. 2003).

100   The use of stable isotope analysis is well established in the research of seagrass ecosystems

101   (Kharlamenko et al. 2001, Conolly et al. 2005, Douglass 2010), but most studies focus on one

102   or two temporal sampling points. As a high temporal variability in the abundance of

103   consumers and their food is common in benthic ecosystems (Edgar & Shaw 1995, Nelson &

104   Waaland 1997, Douglass 2010), a broader approach is necessary to assess the effect of

105   mesograzers in seagrass meadows. We used stable isotope and fatty acid analyses to study the

106   seasonal variation in carbon sources of common mesograzer and small predator species in an

107   eelgrass system in the Baltic Sea.

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109   **MATERIAL AND METHODS**

110   **Study area.** The research site was an eelgrass meadow adjacent to Falkenstein Beach in the

111   inner Kiel Fjord, Germany (Fig. 1). The Kiel Fjord is located in the Kiel Bight, a part of the

112   Western Baltic Sea. The eelgrass meadow extends over an area of 23 ha and is interrupted by

113   small, unvegetated patches (Bobsien 2006). Due to the special hydrological situation in the

114   Baltic Sea, salinity ranges between 10 and 20 PSU depending on discharge rates, prevailing

115   winds and season. The astronomical tide range is negligible, but storm events can cause

116   changes in the water level. The studied eelgrass meadow extends from approximately 1.5 to 6

117   m depth. The sediment is sandy (grain size: 0.5-1 mm = 42%, >1 mm = 51%). The content of

118   organic matter is low (< 1%). Grain size was analysed using a set of standardized sieves and

119   sediment organic content was determined by drying (60^0C, 48 h) and combusting (450^oC,

120   24h) the samples. The difference in weight gives the ash-free dry weight of the organic

121   content.

122   **Sample collection.** Samples for analyses were collected monthly from April 2002 to February

123   2003 at 3 m water depth. In November the sampling had to be cancelled because of a

124   breakdown of the research vessel. All samples of macrophytes and consumers were collected

125   by slowly dredging three times parallel to the coast at approximately the same position for

126   about 10 minutes. The sampled plants and animals were placed in plastic containers with

127   water from the collection site, and transported to the laboratory for sorting and further

128   processing. Samples of *Zostera marina*, attached epiphytes, the main red alga *Delesseria*

129   *sanguinea* and the most common mesograzers the isopod *Idotea baltica*, the amphipod

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130 *Gammarus oceanicus*, and the two gastropods *Rissoa membranacea* and *Littorina littorea*

131 (Gohse-Reimann 2007) were analysed in this study. Additionally we sampled the most

132 common small predators: the green crab *Carcinus maenas*, the sea stickleback *Spinachia*

133 *spinachia* and two pipefish species *Nerophis ophidion* and *Syngnathus typhle* (Bobsien 2006,

134 Gohse-Reimann 2007). The sand microflora was directly measured as detritus-free substrate.

135 For that purpose 15 sediment cores (1cm Ø) were taken within the eelgrass bed by SCUBA-

136 divers.

137 **Sample processing**. In the laboratory, all plant materials were cautiously cleaned in 0.2μm

138 filtered sea water to remove detrital fragments and attached animals. Epiphytes were carefully

139 scraped from the eelgrass blades into small amounts of filtered sea water using a special

140 plastic scraper and a scalpel. This suspension was filtered on precombusted (450°C, 24h)

141 Whatmann GF/F filters. All consumer species were kept alive overnight in filtered sea water

142 to clear their guts and then rinsed with distilled water. Samples for the fatty acids analysis

143 were deep-frozen at -80^{0}C until further processing. Samples for stable isotope analyses were

144 dried to constant weight (60^{0}C, 48 h), plant and animal samples were ground with an agate

145 mortar and pestle as fine as possible and then stored in airtight plastic vials. The shells of the

146 gastropods were discarded as far as feasible before this procedure. Mesograzer were

147 processed as whole organisms and only muscle tissue was analysed for predators. Filters were

148 stored in a dissicator. The sediment cores were deep-frozen, the top 0.5 cm was cut off, and 5

149 at a time were pooled to one sample. Visible detritus was manually removed and the sediment

150 samples were carefully rinsed with 0.2 μm filtered sea water. Observations with a dissecting

151 microscope before and after the cleaning procedure showed the successful removal of

152 unwanted material. The samples were then dried to constant weight ($60 \, ^{o}$C, 48 h) for stable

153 isotope analysis or deep-frozen for fatty acid analysis.

154 **Stable isotope analysis.** We measured ten eelgrass shoots, epiphyte samples, *D. sanguinea*

155 and three sediment samples separately each month. Ten individuals of mesograzer species and

156 size were measured, with the exception of the small gastropod *R. membranacea*; here ten

157 individuals respectively were pooled into three samples to obtain enough material for the

158 analyses. Five individuals of each predator species were analysed.

159 Eelgrass and algae subsamples were transferred into tin cups. The mesograzer subsamples

160 were transferred into silver cups, treated with 0.2µl 10% HCl to remove carbonates and then

161 dried again.

162 All samples were combusted in a CN-analyser (Fisons, 1500N) connected to a Finnigan Delta

163 plus mass spectrometer. $\delta^{15}N$ and $\delta^{13}C$ ratios were calculated as

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165 $$\delta X (\text{‰}) = [(R_{sample}/R_{standard})-1] \times 1000$$

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167 were $X = {}^{15}N$ or ${}^{13}C$ and $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. Pure N_2 and CO_2 gas were used as primary

168 standard and calibrated against IAEA reference standards (N1, N2, N3, NBS22 and USGS24).

169 Acetanilide was used as internal standard after every sixth sample. The overall analytical

170 precision was ±0.1‰ for $\delta^{15}N$ and $\delta^{13}C$.

171 To determine the carbon sources the model of Phillips and Gregg (2003) was used that

172 provides a range of feasible source mixtures.

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174 $$\delta_M = f_A\delta_A + f_B\delta_B + f_C\delta_C$$

175 $$1 = f_A + f_B + f_C$$

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177 f_A, f_B and f_C are the proportion of source isotopic signatures (δ_A, δ_B and δ_C) which coincide

178 with the observed signature for the mixture (δ_M). All possible combinations of primary

179 producer contributions were analysed with an increment of 1%. These predicted mixture

180 signatures were compared with the measured values. If they lay within a tolerance of 0.1%,

181 they were considered feasible solutions. We used only $\delta^{13}C$ values in the modelling because

182   of the sensitivity of the model to fractionation corrections (Connolly et al. 2005). The

183   fractionation is much larger for ^{15}N than for ^{13}C and can vary considerably between different

184   species. We chose 0.5‰ as average fractionation increase of δ^{13}C per trophic level for

185   estuarine ecosystems (France & Peters 1997). Calculations were carried out with IsoSource, a

186   Visual Basic program, provided by Phillips (Phillips & Gregg 2003).

187   We used epiphytes, sand microflora and the red alga *D. sanguinea* as ultimate carbon sources

188   for all consumers, because the fatty acid analysis showed that the biomarker fatty acid for

189   eelgrass 18:4(n-3) was only present in low amounts in all consumers (0-2.8%) throughout the

190   year. Therefore, we consider the contribution of eelgrass to the studied food web as

191   negligible. As primary producer carbon reaches the omnivorous and carnivorous consumers

192   through other consumers, we considered the trophic position of these consumers in the

193   calculation of the fractionation for each consumer and month. Own unpublished data from

194   laboratory experiments showed that the studied mesograzers had a relatively quick adaptation

195   to changes in stable isotope signature (significant changes in stable isotope values occurred

196   within 10 days). The carbon source of main mesograzer species in the studied eelgrass system

197   could only be calculated between April and October. In winter the fact that most consumer

198   δ^{13}C values were higher than the possible food sources prevented the use of the IsoSource

199   model.

200   Trophic levels were calculated according to the model of Hobson and Welch (1992):

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202                        $TL = 1 + (N_m - N_b) / TE$

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204   where TL is the trophic level of the consumer, N_m is the δ^{15}N value of the consumer, N_b is the

205   average basis δ^{15}N value and TE the trophic enrichment factor in this system. A TL close to 2

206   is consistent with herbivorous nutrition, whereas a TL $\geq$ 3 suggests a carnivorous diet.

207 We used the average $\delta^{15}N$ value of epiphytes, sand microflora and *D. sanguinea* as N_b and 1.5

208 as TE (Jaschinski et al. 2008).

209 **Fatty acid analyses.** We measured three replicates of all samples. Macrophytes were

210 processed as individuals; mesograzers were pooled into three replicate samples containing

211 three individuals. *R. membranacea* replicates contained 10 individuals to obtain sufficient

212 material for the analyses. The macrophyte and mesograzer samples were freeze-dried for 48 h,

213 ground with an agate mortar and pestle and weighted. Fatty acids were extracted, esterified

214 and analysed on a gas chromatograph (Hewlett Packard 5890 Series II.) according to

215 Wiltshire *et al.* (2000), using the GC temperature settings of von Elert (2002). To quantify the

216 fatty acid content an internal standard of heptadecanoic (17:0) and tricosanoic fatty (23:0)

217 acid methyl esters was used.

218 **Statistics.** Fatty acid data of mesograzers were log-transformed and subjected to non-metric

219 multi-dimensional scaling using the program package PRIMER 5.0. Calculations for fatty

220 acid signatures were only performed for fatty acids represented with at least one value above

221 1%. A 1-way ANOSIM with 999 permutations was used to test for differences in the fatty

222 acid composition of the studied mesograzer species.

223 The seasonal differences in stable isotope values for primary producers and consumers were

224 analysed with 1-way ANOVAs followed by a Tukey-test ($P < 0.05$). The differences between

225 primary producers, respectively mesograzer species and size, and the influence of seasonality

226 and the interaction between these factors were tested with 2-way factorial ANOVAs.

227 The variation in the trophic position of the omnivorous species *I. baltica* and *G. oceanicus*

228 was tested with seasonality and size as factors in a 2-way factorial ANOVA.

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RESULTS

234   **Biomarker fatty acids in main mesograzer species**

235   Throughout the year (Fig. 2), the biomarker fatty acid for eelgrass 18:4(n-3) was present in

236   only insignificant amounts in *I. baltica* ($\leq 2.8\%$). 20:4(n-3) the biomarker fatty acid for red

237   algae occurred in small amounts ($\leq 7.4\%$) displaying the highest values in October and

238   December. Fatty acids characteristic for diatoms (16:1(n-7), 20:5(n-3)) were more

239   pronounced in spring and in early summer than in the rest of the year. The fatty acids 18:1(n-

240   9) and 18:1(n-7), typical of animals and aerobic bacteria respectively were found in relative

241   constant amounts in both size classes with exception of April. In this month 18:1(n-9) showed

242   the highest values in large *I. baltica* (31.8%) and the lowest in small *I. baltica* (7.4%). In

243   contrast, the bacteria biomarker fatty acid 18:1(n-7) was observed at high levels in small *I.*

244   *baltica* (28.4%). Small *G. oceanicus* contained relatively similar amounts of biomarker fatty

245   acids as small *I. baltica*, but no 18:4(n-3) characteristic for eelgrass was present. In winter the

246   amount of 20:4(n-3) nearly reduplicated (red algae).

247   In *R. membranacea* the levels of biomarker fatty acids for diatoms were highest in spring,

248   declined during the summer and increased again in autumn. The fatty acid 18:1(n-7) (bacteria)

249   increased from May to August (0.2 to 12.1%) and remained on this level until December.

250   Fatty acids typical for eelgrass and red algae were found in small amount throughout the year.

251   Until December, *L. littorea* showed the same pattern concerning these two biomarker fatty

252   acids, but in January an increase in 20:4(n-3) occurred (red algae). The amounts of fatty acids

253   characteristic for diatoms and bacteria were lower in spring and early summer than in autumn

254   and winter.

255   The fatty acid signatures of all mesograzers were subjected to non-metric multi-dimensional

256   scaling (MDS) and an ANOSIM to evaluate similarities. The MDS-plot showed differences in

257   the fatty acid composition of the studied species and the fatty acid composition of single

258   species varied with the time of the year (Fig. 3). Most *I. baltica* values were in two groups

259   containing the May and June, respectively the other values. April values and for large

260   specimen the December values were not similar to other months. *G. oceanicus* was clearly

261   distinct from the other mesograzers. The fatty acid composition of *R. membranacea* was

262   relatively similar in spring and clearly different in July. The *L. littorea* values were grouped in

263   spring/early summer and autumn/winter values.

264   The ANOSIM R statistic gives an absolute measure how similar groups are on a scale of 0 to

265   1. R < 0.25 indicates that the groups are barely separated at all; R > 0.5 shows that the groups

266   are clearly different, but overlapping and R > 0.75 stands for well separated groups.

267   The ANOSIM verified that there are significant differences between the fatty acid

268   composition of the studied mesograzers (R = 0.611, P = 0.001). Table 1 shows the results of

269   the pairwise tests indicating that all mesograzer species can be separated by their fatty acid

270   composition with the exception of small and big *I. baltica*.

271

272   **Stable isotope ratios of primary producers**

273   We found significant effects of seasonality (MS = 40.9, F = 45.3, P < 0.001) and primary

274   producer type (MS = 6304.9, F = 6981.1, P < 0.001) on stable carbon isotope ratios. The

275   interaction of the two factors was also significant (MS = 9.8, F = 10.9, P < 0.001).

276   Three primary producers were important as carbon sources for the studied consumers:

277   epiphytes attached to *Zostera marina*, sand microflora and the red alga *Delesseria sanguinea*.

278   Epiphytes and sand microflora mainly consisted of small pennate diatoms. The stable carbon

279   isotope ratios of epiphytes, sand microflora and *D. sanguinea* were significantly different

280   from each other (P <0.001) and therefore it was possible to calculate their relative importance

281   as food sources at higher trophic levels. Epiphytes were the most enriched carbon source, the

282   red algae the most depleted carbon source and sand microflora showed intermediate values

283   (Fig. 4A). In December the $\delta^{13}C$ value for epiphytes was essentially identical with the value

284   for sand microflora. For epiphytes the $\delta^{13}C$ values remained relatively constant from May to

285 September, for sand microflora and the red alga from April to October (Fig. 4A). In winter all

286 three primary producers were depleted in ^{13}C compared to the spring and summer situation.

287 The stable nitrogen isotope ratios had a significant seasonal variation (MS = 3.8, F = 8.5, P <

288 0.001) and depended on the primary producer type (MS = 30.4, F = 67.9, P < 0.001). The

289 interaction of the two factors was also significant (MS = 14.1, F = 31.4, P < 0.001).

290 The stable nitrogen values of primary producers ranged from 5.77 to 11.67‰ in epiphytes,

291 from 2.38 to 6.47‰ in sand microflora and from 7.69 to 11.65‰ in the red alga *D.sanguinea*

292 (Fig. 4B). The δ^{15}N values remained relatively stable from April to September, when

293 significant changes occurred. Sand microflora became increasingly depleted in ^{15}N, whereas

294 epiphytes and the red alga showed enrichment in ^{15}N as a function of time.

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296   **Stable isotope ratios of main mesograzer species**

297 We found significant effects of seasonality (MS = 35.2, F = 57.8, P < 0.001) and grazer

298 species (MS = 80.5, F = 132.3, P < 0.001) on stable carbon isotope ratios. The interaction of

299 the two factors was also significant (MS = 22.1, F = 36.3, P < 0.001).

300 The development of stable carbon isotope ratios of small and big *I. baltica* showed an even

301 pattern for both size classes with low values in April and July and for small specimen also in

302 winter (Fig. 5). The δ^{13}C signals of small *I. baltica* were significantly lower than found in the

303 larger specimen (P < 0.001), suggesting a larger amount of red algae in their diet. The δ^{13}C

304 values of *G. oceanicus* had a larger range than *I. baltica*. There were significant differences

305 between size classes (P = 0.006). The δ^{13}C values indicated that large *G. oceanicus* depended

306 more on red algae-derived carbon in summer than smaller ones, whereas the situation was

307 reversed in winter.

308   The δ^{13}C values of *R. membranacea* remained at the same level from April to July,

309 decreased to a minimum in August and increased again in autumn (Fig. 5). The range of δ^{13}C

310   values in *L. littorea* was generally lower than in *R. membranacea* with exception of the month

311   May suggesting that this gastropod feeds mainly on epiphytes in this month.

312   Stable nitrogen isotope ratios had a significant seasonal variation (MS = 3.5, F = 32.7, P <

313   0.001), but a weaker one than the δ^{13}C values, and depended on the mesograzer species (MS

314   = 14.4, F = 135.7, P < 0.001). The interaction of the two factors was also significant (MS =

315   0.9, F = 8.1, P < 0.001).

316   The stable nitrogen isotope ratios of the omnivorous isopod *I. baltica* ranged from 8.3 to

317   10.65‰ (Fig. 5). Larger specimen generally had significantly higher δ^{15}N values than smaller

318   ones indicating a more carnivorous diet (P < 0.001). Both size classes had significantly lower

319   ^{15}N values in summer pointing to a more herbivorous diet in this season (Fig. 5). The seasonal

320   variation in stable nitrogen values of the likewise omnivorous amphipod *G. oceanicus* showed

321   a similar, but less strongly pronounced pattern than *I. baltica*. The δ^{15}N values were higher in

322   the larger specimen; the difference was significant (P < 0.001). Significant lower stable

323   nitrogen ratios in both amphipod size classes were found in summer (Fig. 5).

324   The gastropod *R. membranacea* had distinctly lower δ^{15}N values than the crustacean species

325   in correspondence with its herbivorous feeding mode. The δ^{15}N signal of *L. littorea* had the

326   highest variation among individuals. The enriched δ^{15}N values compared to *R. membranacea*

327   were no indication of a higher trophic position, but resulted probably from a different

328   fractionation of stable nitrogen isotopes.

329

330   **Carbon sources of mesograzers according to the feasible source mixture model**

331   In all seasons epiphytes and sand microflora were most likely to substantially contribute to

332   mesograzer nutrition (mean overall seasons and species 49% and 37%, respectively), while

333   the red alga *D. sanguinea* had a lower contribution (mean 14%) (Fig. 6). Epiphytes were the

334   most important contributors to *I. baltica* carbon (45% in small specimen, 47% in larger ones).

335   The dependence on epiphyte derived carbon had a peak in May, was lowest in July (33% and

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336   30%, respectively) and increased again in autumn (Fig. 6). The diet of *G. oceanicus* consisted

337   of about 30% epiphytes in spring and summer. The contribution of this carbon source

338   increased in autumn up to 64% for small and 68% for larger specimen, respectively.

339   Throughout the growing season epiphytes were the most important contributors to *R.*

340   *membranacea* nutrition (Fig. 6). In summer the relative contribution of this primary producer

341   decreased to 45%. *L. littorea* obtained about 40% of its carbon from epiphytes with exception

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

343   Sand microflora increased as carbon source when epiphytes became less important (Fig. 6).

344   Red algae seem to be of minor importance for most of the studied mesograzers (Fig. 6), only

345   large *G. oceanicus* had a high contribution of this carbon source to their nutrition in summer

346   (45%).

347

348   **Trophic position**

349   The trophic position of main mesograzer species in the studied eelgrass system could only be

350   calculated between April and October, when δ^{15}N values of primary producers remained

351   relatively similar. In winter, strong changes in primary producer δ^{15}N values in combination

352   with reduced growth rate and lower metabolic activity of animals prevented the computing of

353   realistic results at this time of the year. The isopod *I. baltica* showed a significant seasonal

354   variation in trophic position (MS = 3.6, F = 104.3, P <0.0001) changing from nearly

355   exclusively carnivorous in spring to herbivorous in summer and back to a more carnivorous

356   feeding mode again in winter (Fig. 7). Smaller individuals generally hold a significant lower

357   trophic position than larger ones (MS = 2.7, F = 78.6, P <0.0001), suggesting a more

358   carnivorous diet with growing size.

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362 **Stable carbon isotope ratios and carbon sources of small predators**

363 All studied small predator species the green crab (*Carcinus maenas*), the shrimp (*Crangon*

364 *crangon*), the sea stickleback (*Spinachia spinachia*), the straightnose pipefish (*Nerophis*

365 *ophidion*) and the broad-nosed pipefish (*Syngnathus typhle*) had a significant seasonal

366 variation in their $\delta^{13}C$ values (Fig. 8).

367 All predators depended mainly on epiphyte carbon ranging from 39% annual mean for the

368 broad-nosed pipefish to 53% for the green crab (Fig. 9). Sand microflora was the second

369 important carbon source ranging from 35 to 45% annual mean contribution. Red algae were

370 less crucial as carbon source (annual mean 12-16%). The seasonal variation in the dependence

371 on epiphyte carbon, we found in mesograzers with a summer minimum, was also found for

372 the green crab and the straightnose pipefish. The broad-nosed pipefish had a more balanced

373 contribution of this carbon source to its diet and the stickleback showed an increase in

374 dependence on epiphyte carbon in autumn, in spring we didn't find this species in our study

375 area. In general, the seasonal variation of carbon sources was less pronounced in predators

376 than in mesograzers.

377

378 **DISCUSSION**

379 This study suggests that epiphytes were the most important carbon source of common

380 mesograzers and small predators in a subtidal eelgrass community throughout the year.

381 However, the dependence on this food source showed clear seasonal and species-specific

382 variability. The trophic importance of microalgae in benthic macrophyte systems was

383 confirmed in recent stable isotope studies (Créach et al. 1997, Connolly et al. 2005, Douglass

384 2008, Jaschinski et al. 2008). Our results support the assumption that fresh macrophyte leaves

385 are of minor relevance for the nutrition of mesograzers in temperate seagrass systems

386 (Lepoint et al. 2000, Moncreiff & Sullivan 2001, Douglass 2008, Jephson et al. 2008).

387 Throughout the year, the fatty acid 18:4(n-3), which is characteristic for living eelgrass

388 (Khotimchenko 1993), was only present in negligible amounts in *Idotea baltica*, *Littorina*

389 *littorea* and *Rissoa membranacea*.

390 All studied mesograzer species had the smallest contribution of epiphytes to their diet in early

391 summer, corresponding with a minimum in epiphyte biomass (Jaschinski & Sommer 2008b).

392 This indicates the capacity of mesograzers to control epiphyte biomass on eelgrass (Hughes et

393 al. 2004, Jaschinski & Sommer 2008a). The seasonal pattern was most pronounced in the

394 small gastropod *R. membranacea* with a reduction from total dependence on epiphytes in

395 spring and autumn to about 45% in summer according to the model of Phillips and Gregg

396 (2003). The decrease in epiphyte abundance caused this herbivorous species to switch to sand

397 microflora as alternative food source, a behaviour that we also observed in laboratory

398 experiments (Jaschinski & Sommer 2008a). The fatty acid composition of *R. membranacea*

399 confirmed the conclusions from the stable carbon signatures. The biomarker fatty acids for

400 diatoms 20:5(n-3) and 16:1(n-7) decreased in summer. These fatty acids are found in

401 epiphytes and sand microflora communities, which consist both mainly of diatoms, but the

402 absolute content is much lower in sand microflora than in epiphytes (Jaschinski et al. in

403 prep.). The increase of 18:1(n-7), a fatty acid characteristic for bacteria, starting in June, could

404 indicate detrital material as additional food source. The biomarker fatty acids for eelgrass

405 18:4(-n-3) and red algae 20:4(n-6) were only found in low amount throughout the year.

406 The periwinkle *L. littorea* had a strong dependence on epiphyte carbon in May. The rest of

407 the year, this species mostly fed on sand microflora according to stable isotope data. Red

408 algae seem to be of minor importance, but in December, when *Delesseria sanguinea*, which

409 grows mainly below the eelgrass meadows, is more often found in the eelgrass meadow (own

410 observations), the biomarker fatty acid for red algae in *L. littorea* increased.

411 Stable isotope data and biomarker fatty acids indicated a seasonally varying importance of

412 epiphyte carbon for both size classes of the omnivorous isopod *I. baltica* with high values in

413 spring and autumn and a summer depression in accordance with epiphyte development. Red

414   algae also seem to be more important from October to December. Small *I. baltica* had very

415   high values of 18:1(n-7) in April indicating detritus as an important food source in early

416   spring, when the biomass of primary producers is low. Large specimens seem to have a

417   different strategy for this period, when fresh herbal food was limited. The fatty acid 18:1(n-9),

418   which suggests a carnivorous nutrition, was especially high in April (32% of fatty acids).

419   Seasonally, the trophic position of *I. baltica* changed with a more herbivorous diet in summer.

420   Smaller individuals were always less carnivorous than the larger ones according to stable

421   isotope and fatty acid data. Gut content analyses support the omnivorous nutrition of *I. baltica*

422   (Douglass 2008).

423   The amphipod *Gammarus oceanicus* had the strongest contribution of epiphytes to its diet in

424   autumn. Nutrient input caused by storm events promoted the growth of diatom chains in this

425   season (Jaschinski, own observations), which are selectively eaten by this species, probably

426   because the uptake of smaller, more strongly adhering diatom species is not possible for *G.*

427   *oceanicus* (Jaschinski et al. 2010a). The stable isotope data indicated that smaller individuals

428   compensated for the decrease in epiphytes in summer by consuming more sand microflora

429   and larger individuals compensated by consuming more red algae. Unfortunately, most of the

430   fatty acid data on *G. oceanicus* were lost during analyses, therefore only an increase in red

431   algae as food in winter can be deduced from biomarker fatty acids. The trophic position of

432   this potentially omnivorous species increased in small specimens in winter, the larger

433   individuals were more carnivorous than the smaller ones in summer. Gut content analyses of

434   other *Gammarus* species support the assumption of the omnivorous nutrition of this genus

435   (Douglass 2008, Moksnes et al. 2008).

436   The total fatty acid composition of the four mesograzers supported the species-specific and

437   seasonal variability in nutrition in the studied eelgrass system.

438   The four studied mesograzers are common consumers in temperate shallow marine benthic

439   ecosystems. These species have a broad range of possible food sources, but distinct

440   preferences, if several food items are offered (Norton 1990, Warén 1996, Franke & Jahnke

441   1998, Orav-Kotta & Kotta 2004, Gohse-Reimann 2007). Our study indicated that the studied

442   mesograzer species and size classes used different strategies to compensate for the limitation

443   of the main food source epiphytes in early spring, summer and winter. A shift or switching of

444   prey, when preferred food sources are limited, has also been found for other invertebrate

445   species in eelgrass systems, e.g. the omnivorous crustacean *Palaemon* ssp. switched to the

446   qualitatively inferior macroalgae, if the preferred amphipods became scarce (Jephson et al.

447   2008, Persson et al. 2008). The cause for the limitation of preferred food sources can be

448   natural prey-consumer-cycles as in our study, seasonal nutrient limitation, overfishing, which

449   can promote competitors via a trophic cascade or even hypoxia as consequence of

450   eutrophication, which reduces small invertebrates as potential prey for omnivores (Jephson et

451   al. 2008, Fox et al. 2009).

452   In the studied eelgrass meadow, the summer minimum of epiphyte biomass was probably

453   caused by a combination of nitrogen limitation and an intense grazing pressure. In autumn

454   there was also a yet smaller increase in mesograzer abundance, but no strong negative effect

455   on epiphyte biomass. Increasing values of dissolved nutrients and especially a strong decrease

456   in epiphyte C:N values suggested a break-up of the nitrogen limitation (Jaschinski & Sommer

457   2008a). Experiments support strong interactions of bottom-up and top-down effects on

458   epiphytes (Douglass et al. 2007, Jaschinski & Sommer 2008b). The biomass of sand

459   microflora followed the same pattern as epiphyte biomass, but the summer minimum was less

460   pronounced (Jaschinski, unpubl. data). The cause could be bioturbation during the grazing

461   process, which can promote primary production via nutrient release from the sediment

462   (Lohrer et al. 2004). We also found a positive effect of mesograzers on epiphyte productivity

463   in this system (Jaschinski & Sommer 2010b) emphasising the complex interactions of abiotic

464   and biotic factors, which determine biomass and probably species composition even if only

465   the primary producer and herbivore level was considered.

466   The seasonal fluctuations in carbon sources at the mesograzer level were also present at the

467   next trophic level, but less pronounced. The main second order consumers in the studied

468   eelgrass system are the green crab (*Carcinus maenas*), a shrimp (*Crangon crangon*), the sea

469   stickleback (*Spinachia spinachia*) and two pipefish species (*Syngnathus typhle* and *Nerophis*

470   *ophidion*). The contributions of the different primary producers as carbon source of these

471   small predators had a summer minimum, with the exception of *S. typhle*. Epiphytes (47%,

472   annual mean, all predators) again provided the most likely carbon source, followed by sand

473   microflora (39%) and red algae (14%). Gut content analyses showed that amphipods and

474   isopods are important parts of the nutrition of the studied predators (Bobsien 2006, Moksnes

475   et al. 2008). Nordström et al. (2009) found that seasonal fluctuations of carbon contribution at

476   the herbivore level were strongly diminished at the predator level, but they sampled only from

477   June to September, a period of time, when our data suggested similarly stable carbon sources.

478   As the importance of seasonal variation in basic δ^{13}C values depends on the growth rates of

479   the species, the observed low variability in carbon sources of small predators is in accordance

480   with their lower turn-over rate compared to the smaller fast-growing mesograzers.

481   Nevertheless, there are significant changes in carbon sources detectable on the predator level

482   in the course of the year. This is supported by Nordström et al. (2009), who found that δ^{15}N

483   values, respectively trophic position changed significantly from month to month.

484   We show that trophic interactions at the mesograzer level can be dynamic at a temporal scale

485   during the growth season in a temperate eelgrass community. Earlier studies with lower

486   temporal resolution than ours indicated that these processes are relevant in macrophyte

487   systems, where epiphytes and/or annual macroalgae are of importance as food source (Orav-

488   Kotta & Kotta 2003, Vizzini & Mazzola 2003, Connolly et al. 2005, Nordström et al. 2009).

489   Trophic interactions in benthic food webs that depend more on macrophyte detritus seem to

490   be characterized by rather stable dynamics (Akin & Winemiller 2006, Douglass 2008). The

491   strong seasonality of epiphytic and annual algae biomass and the high selectivity of many

492   mesograzer species for food items that are of high quality concerning nutrient content or

493   digestibility result in a seasonally varying limitation of optimal food sources. This seems to

494   promote a community of generalist mesograzers, which are able to react with great flexibility

495   to the changing food conditions and thus contribute to the stability of the whole system.

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668   Table 1 Results of the pairwise comparison of the fatty acid composition of the studied

669   mesograzers (ANOSIM). Is = *I. baltica* 8 mm, Ib = *I. baltica* 15 mm, Gs = *G. oceanicus* 10

670   mm, R = *R. membranacea,* L = *L. littorea*

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

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689   Figure legends

690   Fig. 1. Study area in the outer Kiel Fjord, Baltic Sea, Germany (Bobsien 2006)

691   Fig. 2. Seasonal variation in biomarker fatty acids in main mesograzers species

692   Fig. 3. Multi-dimensional scaling (MDS) plots of the total fatty acid composition of

693   mesograzers. Stress = 0.1 gives a good representation in a MDA analysis. (○ = *I. baltica* 8

694   mm, ● = *I. baltica* 15 mm, ♦ = *G. oceanicus* 10 mm, ■ = *R. membranacea,* □ = *L. littorea*, the

695   number represents the month)

696   Fig. 4. Seasonal variation of stable isotopes in *Z. marina*, epiphytes, sand microflora and *D.*

697   *sanguinea* (mean±SD). A = $\delta^{13}C$, B = $\delta^{15}N$. Letters indicate significant differences between

698   sampling dates ($P \leq 0.05$)

699   Fig. 5. Seasonal variation of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of the most abundant

700   mesograzers (mean±SD). Letters indicate significant differences ($P \leq 0.05$) between sampling

701   dates (above = $\delta^{13}C$, below = δ15N)

702   Fig. 6. Seasonal variation in ultimate carbon sources (epiphytes, sand microflora, red algae)

703   for main mesograzers species. Shown are the mean contributions calculated with IsoSource

704   according to the model of Phillips & Gregg (2003), which could only be calculated from April

705   to October.

706   Fig. 7. Seasonal variation in trophic position of omnivorous mesograzers species (mean±SD).

707   Letters indicate significant differences between sampling dates ($P \leq 0.05$)

708   Fig. 8. Seasonal variation of $\delta^{13}C$ of the most abundant small predator species (mean±SD).

709   Letters indicate significant differences between sampling dates ($P \leq 0.05$)
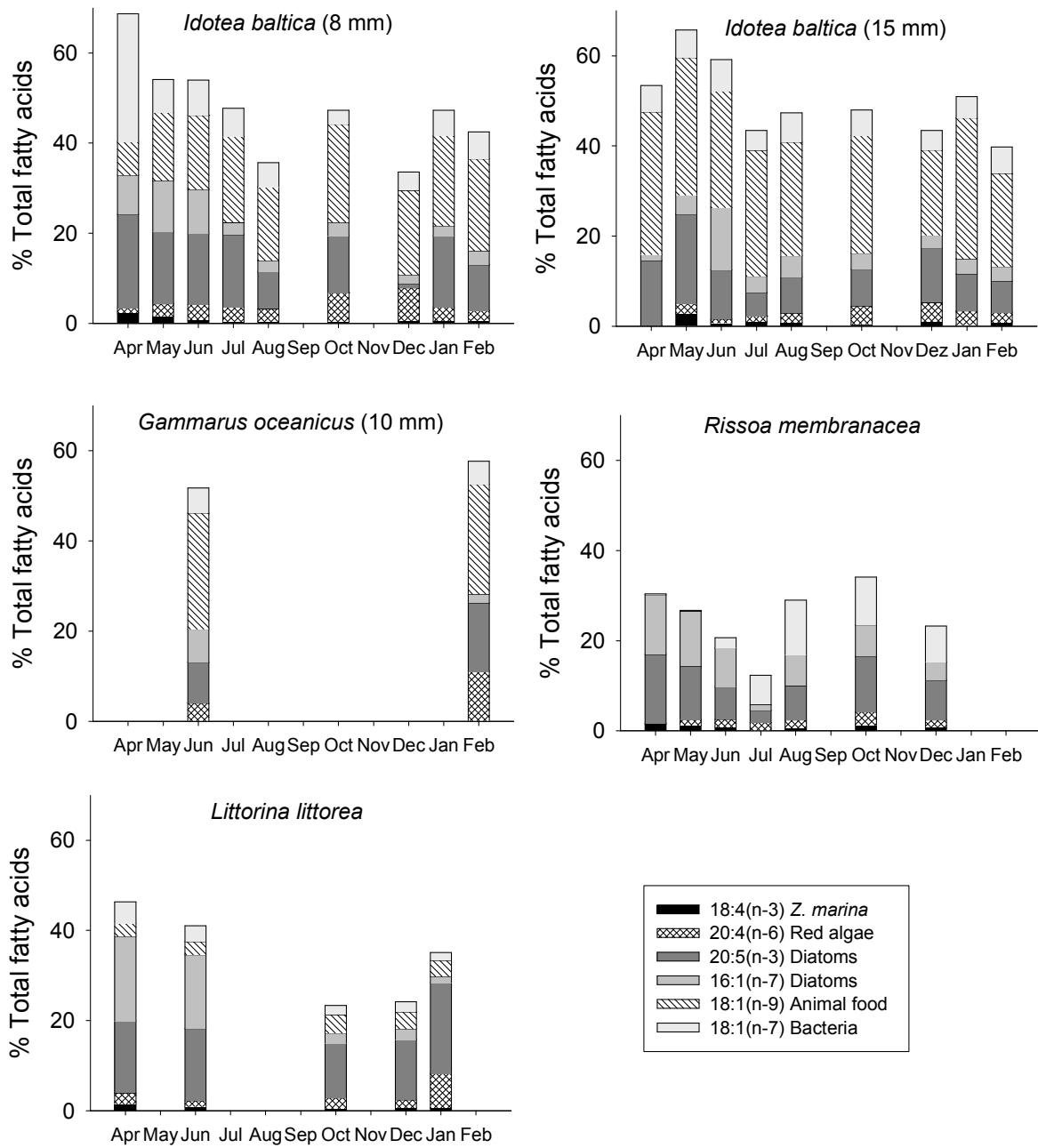
710   Fig. 9. Seasonal variation in ultimate carbon sources (epiphytes, sand microflora, red algae)

711   for main predator species. Shown are the mean contributions calculated with IsoSource

712   according to the model of Phillips & Gregg (2003), which could only be calculated from April
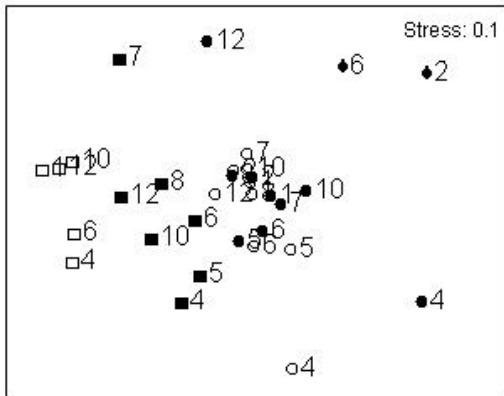
713   to October.

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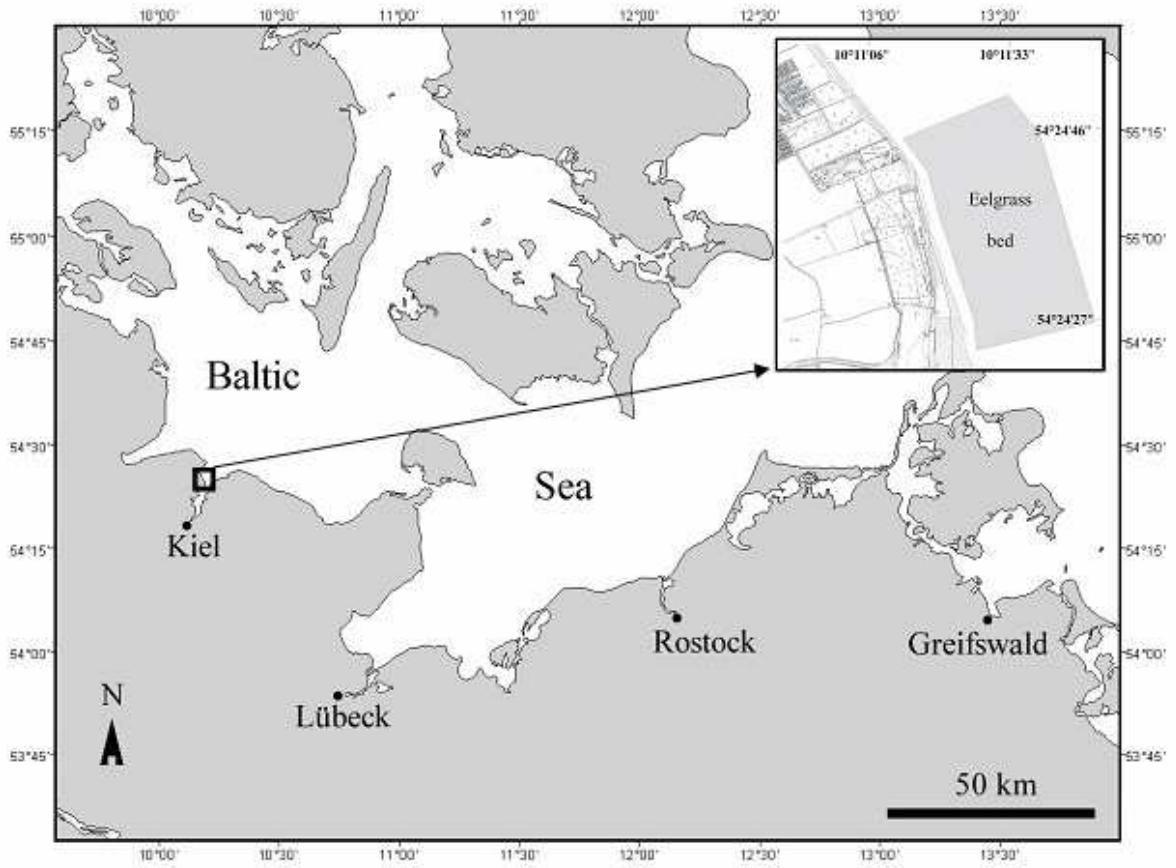
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743   Figure 2

Idotea baltica (8 mm)

Idotea baltica (15 mm)

Gammarus oceanicus (10 mm)

Rissoa membranacea

Littorina littorea

Legend:
- 18:4(n-3) *Z. marina*
- 20:4(n-6) Red algae
- 20:5(n-3) Diatoms
- 16:1(n-7) Diatoms
- 18:1(n-9) Animal food
- 18:1(n-7) Bacteria

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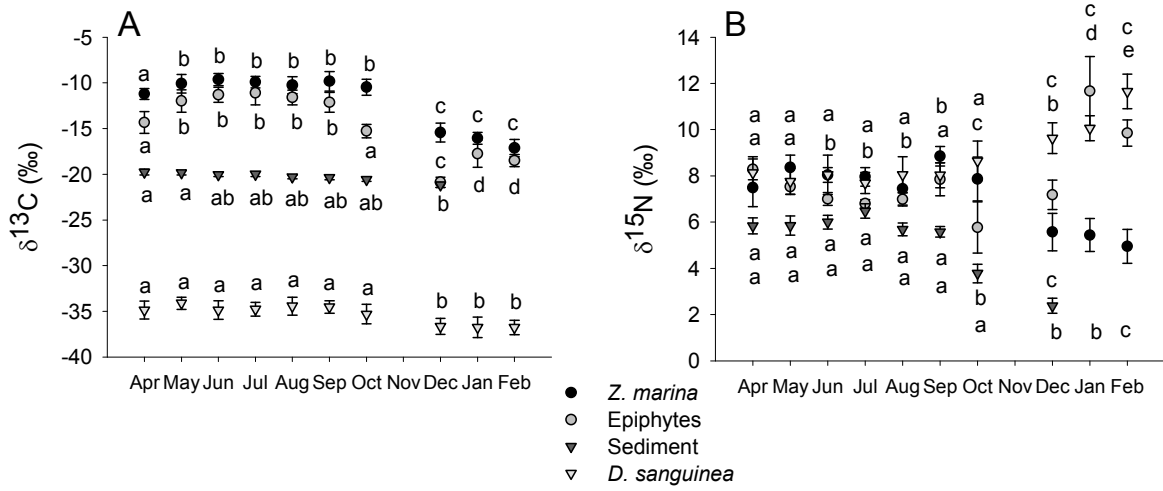
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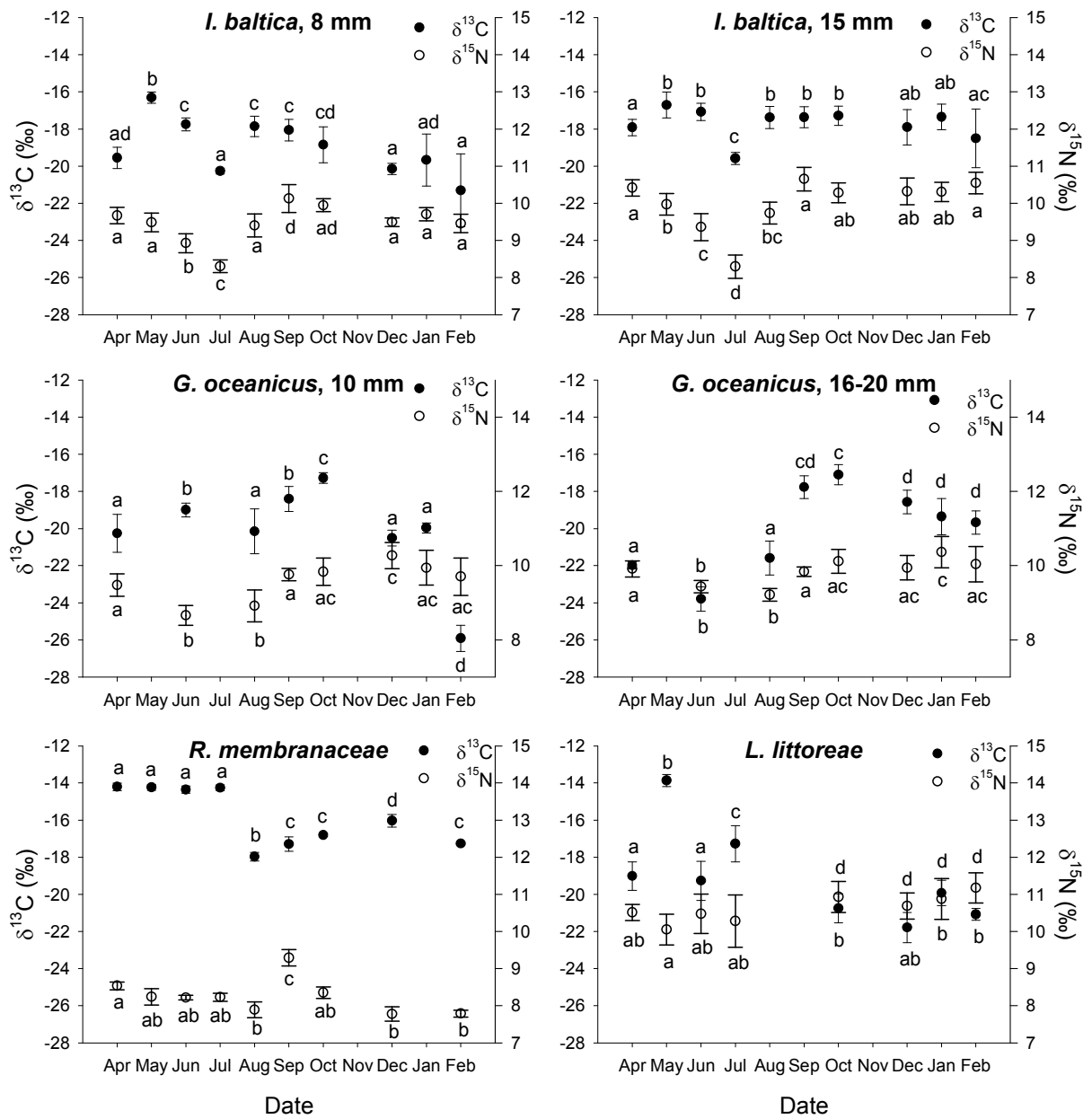
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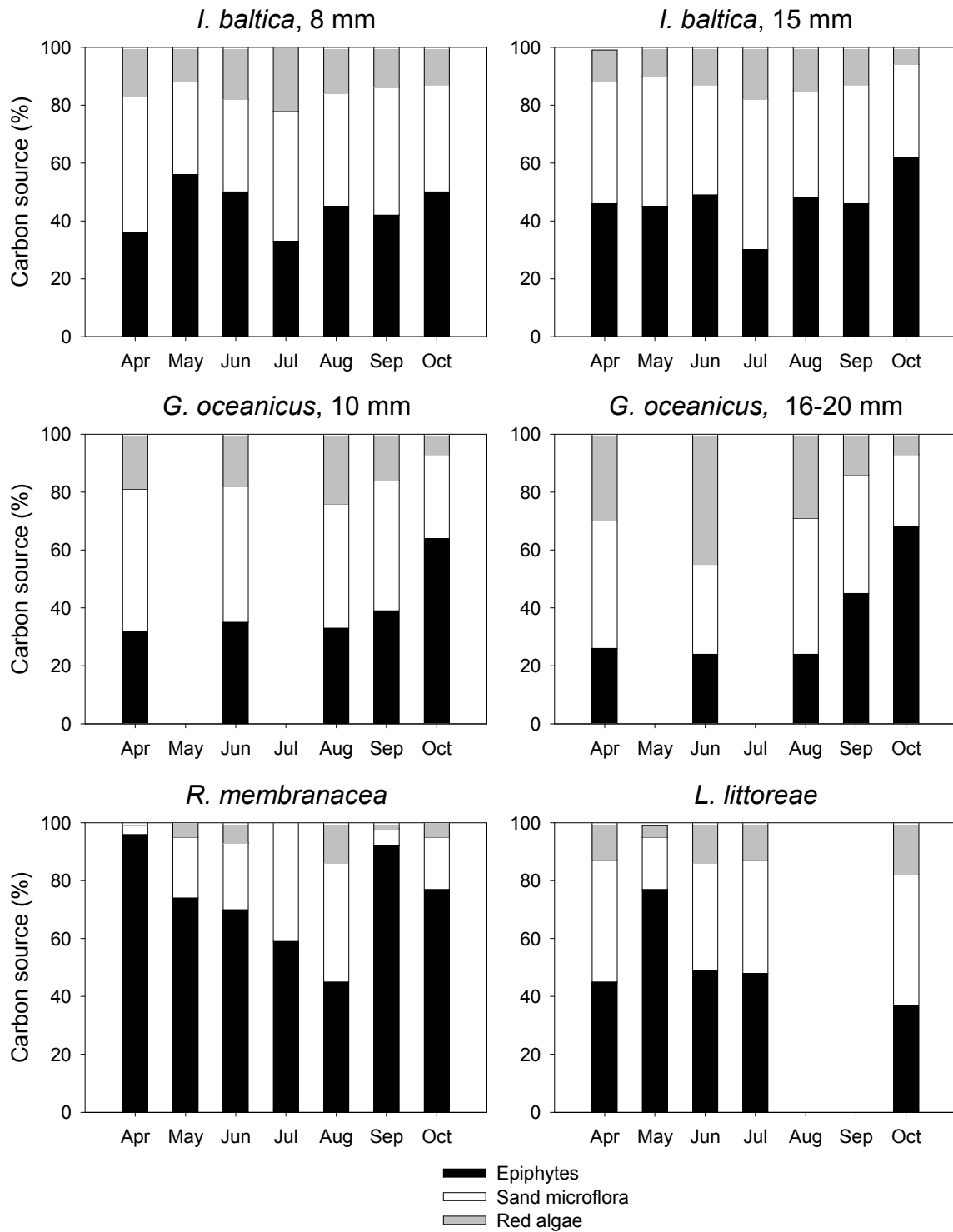
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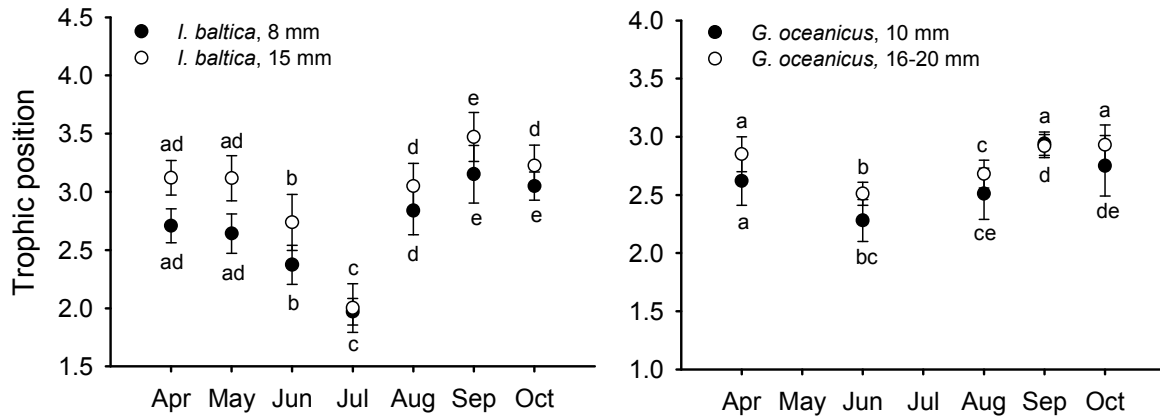
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853   Figure 7

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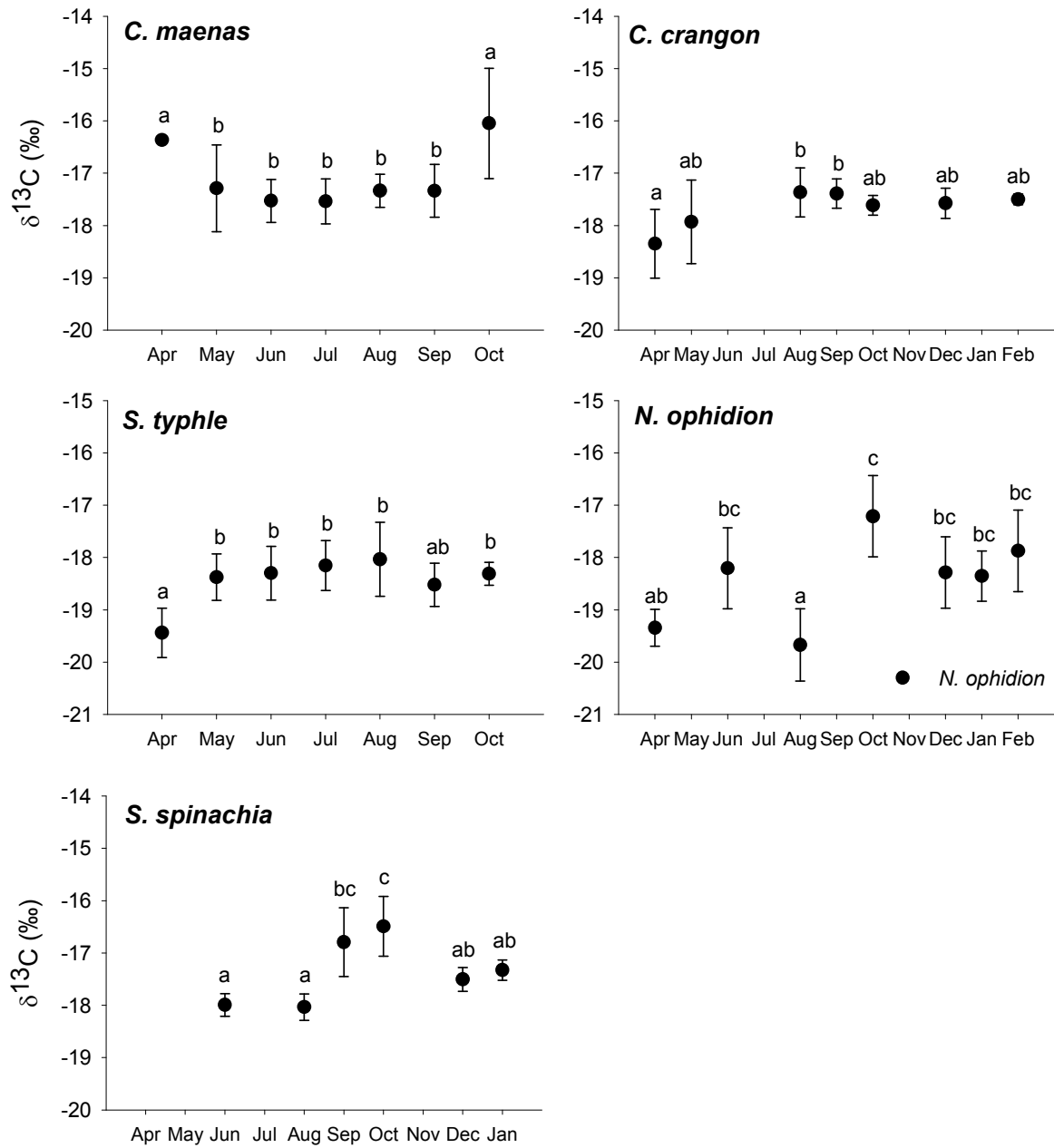
Figure 8

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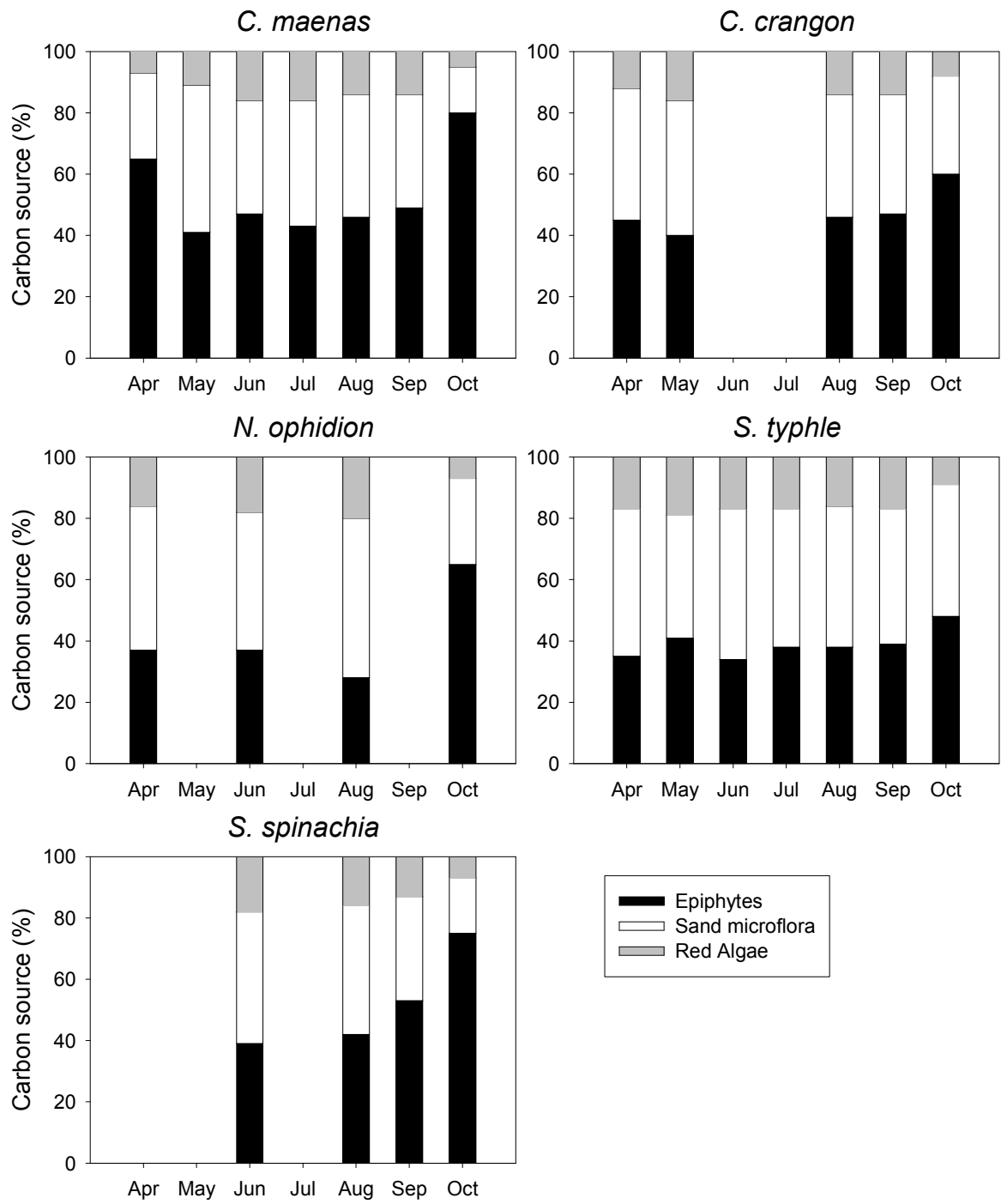880
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