

1 **How do nutrient conditions and species identity influence the impact of mesograzers in**
2 **eelgrass-epiphyte systems?**

3 Sybill Jaschinski*, Ulrich Sommer

4 IFM-GEOMAR Leibniz Institute of Marine Sciences, Düsternbrooker Weg 20, 24105 Kiel,
5 Germany

6 * Corresponding author, email: sjaschinski@ifm-geomar.de

7 Tel: +49 431-600-4409

8 Fax: +49 431-600-1515

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26 **Abstract**

27 Coastal eutrophication is thought to cause excessive growth of epiphytes in eelgrass beds,
28 threatening the health and survival of these ecologically and economically valuable
29 ecosystems worldwide. Mesograzers, small crustacean and gastropod grazers, have the
30 potential to prevent seagrass loss by grazing preferentially and efficiently on epiphytes. We
31 tested the impact of three mesograzers on epiphyte biomass and eelgrass productivity under
32 three fold enriched nutrient concentrations in experimental indoor mesocosm systems under
33 summer conditions. We compared the results with earlier identical experiments that were
34 performed under ambient nutrient supply. The isopod *Idotea baltica*, the periwinkle *Littorina*
35 *littorea*, and the small gastropod *Rissoa membranacea* significantly reduced epiphyte load
36 under high nutrient supply with *Rissoa* being the most efficient grazer, but only high densities
37 of *Littorina* and *Rissoa* had a significant positive effect on eelgrass productivity. Although all
38 mesograzers increased epiphyte ingestion with higher nutrient load, most likely as a
39 functional response to the quantitatively and qualitatively better food supply, the promotion of
40 eelgrass growth by *Idotea* and *Rissoa* was diminished compared to the study performed under
41 ambient nutrient supply. *Littorina* maintained the level of its positive impact on eelgrass
42 productivity regardless of nutrient concentrations.

43

44

45

46

47

48

49

50

51 **Keywords:** *Zostera marina*, eutrophication, grazing, *Idotea*, *Littorina*, *Rissoa*,

52 **1. Introduction**

53 Seagrass meadows are ecologically and economically important ecosystems that provide
54 numerous crucial services for society (Constanza et al. 1997; Waycott et al. 2009). Seagrasses
55 stabilize sediments and reduce the water flow, thus contributing to coastal protection (Orth et
56 al. 2006). They recycle nutrients and produce a large amount of organic carbon, providing a
57 critical supply of organic matter to the deep sea and significantly adding to the sequestration
58 of carbon in the biosphere (Duarte et al. 2005). Furthermore, they provide the habitat for a
59 diverse community of plants and animals and serve as nursery ground for many important
60 finfish and shellfish species (Heck et al. 2003). Seagrass beds also have important linkages to
61 other habitats like coral reefs or mangroves, facilitating trophic transfer and cross-habitat
62 utilization of invertebrates and fish (Beck et al. 2001; Heck et al. 2008).

63 Coastal development, growing human population and climate change threaten these valuable
64 ecosystems (Harley et al. 2006; Orth et al. 2006). One third of the known seagrass meadows
65 has disappeared since the first records in 1879 and the rate of loss has accelerated in recent
66 decades (Waycott et al. 2009). One of the most severe anthropogenic stressors of coastal
67 submerged vegetation is eutrophication (Howarth et al. 2000). This process has the potential
68 to initiate shifts in coastal and freshwater ecosystems from high-diversity to low-diversity
69 status that reduce the ecological and economical functioning and value of these systems
70 (Smith et al. 1999; Howarth et al. 2000). Excessive nutrient inputs have been linked to
71 increasing occurrence of harmful algae blooms, fish kills caused by toxins or hypoxia, and the
72 destruction or degradation of highly productive valuable coral reefs, kelp beds and seagrass
73 ecosystems (Anderson et al. 2002; Kemp et al. 2005). Higher nutrient supply promotes the
74 growth of epiphytes, fast-growing annual algae and phytoplankton. Epiphytic assemblages
75 reduce the light reaching the seagrasses (Brush and Nixon 2002), impede carbon uptake, and
76 thus, decrease seagrass productivity (Sand-Jensen 1977). Additionally, epiphytes are superior
77 competitors for water column nutrients, reducing the availability to their host plant. Despite

78 the ability of eelgrass to use sediment porewater nutrients, 30-90 % of the nitrogen uptake
79 occurs through the leaves (Touchette and Burkholder 2000 and references therein). A meta-
80 analysis of 35 published seagrass studies revealed a strong negative effect of water column
81 enrichment on seagrass biomass and productivity (Hughes et al. 2004).

82 Epiphyte grazers, mostly small invertebrates i.e. amphipods, isopods and gastropods
83 (mesograzers), were shown to have a positive effect on seagrasses (Hughes et al. 2004).

84 Mesograzers mainly feed on the epiphytic assemblages and thus, have the potential to lessen
85 the impact of high nutrient supply by reducing competition for light, carbon and nutrients;
86 although the effect is species-specific (Duffy et al. 2001; Jaschinski and Sommer 2008a) and
87 mesograzers can be destructive for seagrasses under extremely high grazing pressure by
88 switching to seagrass as food source (Fredriksen et al. 2004).

89 The effects of nutrient enrichment and grazing are of comparable magnitude (Jernakoff et al.
90 1996; Hughes et al. 2004), although the positive impact of mesograzers depends on their
91 density and on nutrient levels (Neckles et al. 1993; Jaschinski and Sommer 2008a; Jaschinski
92 and Sommer 2008b; Jephson et al. 2008). Nevertheless, we can assume that mesograzers are a
93 key determinant in structuring marine seagrass systems and in mediating the negative impact
94 of eutrophication. Recent studies demonstrated the importance of species identity for positive
95 effects in seagrass communities under ambient nutrient conditions because of the different
96 traits of the studied mesograzers (Duffy et al. 2001; Duffy et al. 2003; Hays 2005; Jaschinski
97 and Sommer 2008a). Species-specific differences concerning food selectivity, feeding
98 capacity and intraspecific interactions may also influence mesograzer impacts under higher
99 nutrient supply. Eutrophication does not only increase epiphyte biomass, but can also have an
100 influence on epiphyte composition. Epiphytes on eelgrass mostly consist of diatoms and small
101 filamentous algae. Prostrate and stalked diatom species dominate the epiphyte assemblage
102 under nutrient poor conditions. Nutrient enrichment supports the growth of diatom chains and
103 filamentous algae, which are preferentially consumed by some grazers (Hillebrand et al. 2000;

104 Jaschinski and Sommer 2008b; Jaschinski et al. 2010). Grazer effects can be enhanced by
105 nutrient enrichment (Hillebrand et al. 2000; Hillebrand 2002 and references therein;
106 Jaschinski and Sommer 2008b), because of the higher availability of algal biomass and
107 suitable algae species. Furthermore, grazers may consume larger amounts of algae per capita,
108 when these are enriched with nitrogen (Russell and Connell 2007).

109 We studied the impact of natural densities of three potentially dominant mesograzers in
110 experimental eelgrass communities under enriched nutrient supply and compared their effect
111 with results from experiments conducted under ambient nutrient supply (Jaschinski and
112 Sommer 2008a). The isopod *Idotea baltica* (hereafter, *Idotea*) is an important mesograzer in
113 submerged aquatic vegetation in the Baltic Sea living in eelgrass meadows, but also occurring
114 in macroalgae systems. *Idotea* is omnivorous and can feed on a wide array of food sources,
115 i.e. epiphytes, macroalgae, eelgrass, detritus, small invertebrates, and even its conspecifics.
116 Nevertheless, if several food sources are available, *Idotea* is rather selective in its dietary
117 choice (Schaffelke et al. 1995; Orav-Kotta and Kotta 2004). This actively swimming
118 crustacean species removes epiphytes from eelgrass rather homogeneously (“lawn-mover” type
119 of grazer, Sommer 1999). Under low nutrient concentrations and accordingly low food
120 supply, *Idotea* reduces the three-dimensional structure of the epiphyte assemblage to a
121 monolayer of strongly adhering diatoms. Under higher nutrient and food supply, *Idotea*
122 becomes more selective and favours diatom chains and filamentous algae (Jaschinski and
123 Sommer, 2008b).

124 The periwinkle *Littorina littorea* (hereafter, *Littorina*) is predominantly herbivorous and can
125 feed on microalgae growing on rock, sand and mud, epiphytes, filamentous algae and
126 macroalgae. The periwinkle shows, however, clear preferences, i.e. it prefers ephemeral green
127 algae, but rejects coralline red algae and decaying algae (Norton et al. 1990). Gastropods
128 produce a feeding trail by scraping the eelgrass surface with their radula (“bulldozer” type of
129 grazer, Sommer 1999). *Littorina* ingests the epiphyte assemblages on eelgrass unselectively

130 under low nutrient supply; even the basic layer of strongly adhering diatoms is removed
131 (Jaschinski et al. 2010). Under higher nutrient concentrations and epiphyte biomass, the
132 periwinkle becomes more selective, feeding preferentially on stalked diatoms and facilitates
133 the growth of diatom chains (Jaschinski et al. 2009).

134 The small gastropod *Rissoa membranacea* (hereafter *Rissoa*) lives in shallow, sheltered
135 environments, especially eelgrass beds and algae belts. This species predominantly grazes on
136 microalgae and filamentous algae (Warén 1995), but it can also feed on eelgrass, when other
137 food is scarce (Fredriksen et al. 2004). Under low nutrient supply, *Rissoa* is a rather
138 unselective grazer feeding on everything aside from strongly adhering diatom species, but this
139 gastropod also becomes more selective when more epiphyte biomass is available (Jaschinski
140 et al. 2010).

141 As benthic small herbivores are thought to be highly prone to qualitative or quantitative food
142 limitation (Lamberti 1996), we expected that an increase in available epiphyte biomass under
143 higher nutrient conditions will promote the grazing rates of the studied mesograzers. *Idotea*
144 may additionally profit by the compositional changes of epiphytes, because this mesograzer
145 mainly feeds on diatom chains (Jaschinski & Sommer 2008a). We expected that the
146 anticipated change to an epiphyte community dominated by diatom chains under high nutrient
147 supply would particularly enhance the grazing efficiency of this mesograzer.

148 Here we report the results of three mesocosm experiments conducted to test the effect of
149 mesograzer species identity and high nutrient supply on epiphyte biomass and eelgrass
150 productivity. We compared the results with already published experiments conducted under
151 ambient nutrient supply, but otherwise exactly the same conditions (Jaschinski & Sommer
152 2008a). In this previous study we tested the relevance of species-specific impacts of common
153 mesograzers on epiphyte and eelgrass biomass and productivity. Now, we used part of the
154 data as a basis to assess changes in grazing impact in a more eutrophic situation.

155 With this approach, we wanted to answer the following questions:

156 (1) Can mesograzers reduce the increase in epiphyte biomass under high nutrient supply?

157 (2) Can they consequently promote eelgrass productivity in eutrophic situations?

158 (3) Are all studied mesograzers functionally redundant or is their impact species-specific?

159

160 **2. Methods**

161 2.1. Experimental design

162 We conducted three mesocosm experiments to test the impact of *Idotea*, *Littorina* and *Rissoa*

163 under high nutrient supply in an eelgrass-epiphyte system and to compare the results with

164 grazing experiments performed under ambient nutrient conditions (Jaschinski & Sommer

165 2008a). A planned experiment with *Gammarus oceanicus* under high nutrient conditions

166 could not be accomplished because of the early onset of the storm season in that year. The

167 experimental conditions – with exception of the nutrient supply- were exactly the same in all

168 experiments. All experiments took place from June to August 2002 in the sequence: *Idotea*-

169 ambient nutrient supply, *Idotea*-high nutrient supply, *Littorina*-ambient nutrient supply,

170 *Littorina*-high nutrient supply, *Rissoa*-ambient nutrient supply and *Rissoa*-high nutrient

171 supply. A preliminary field study had shown that the qualitative and quantitative composition

172 of epiphytes remains relatively similar during this period. This was further proven by the

173 analyses of epiphyte composition in the experiments (Jaschinski et al. 2010). Each experiment

174 included four treatments: a grazer-free control and low, mean and high abundances of one

175 grazer species (Table 1). Mesograzer abundances were chosen based on field densities in

176 summer according to monitoring data for eelgrass associated macrofauna in the Kiel Bight (4

177 stations, 1997-2001). The average of all stations and years was used as the mean density for

178 the four consumer species. Half of this abundance represented the low density treatment and

179 we doubled the average in the high density treatment. Thus the total range of naturally

180 occurring densities was tested in our study (Table 1). Each treatment was replicated in six

181 independent aquaria in a randomized block-design. All treatments in one aquarium (control,
182 low, mean, high density; randomly distributed) were regarded as one block.

183 The experiments took place in a constant temperature chamber. Six 125 l aquaria (50 cm x 50
184 cm x 50 cm) were divided into four compartments with 1 mm metal mesh resulting in 24
185 mesocosm units (25 cm x 25 cm x 50 cm). This corresponds to the minimum size
186 recommended for experiments with seagrass (Short et al. 2001). Summer conditions found in
187 eelgrass systems in the western Baltic Sea were established with respect to light and
188 temperature. The aquaria were illuminated by HQI-lamps with a 16 h day and 8 h night cycle.
189 The light intensity was $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the water surface. The temperature in the constant
190 temperature chamber was set to 17 °C. However, due to a warming-effect of the lamps the
191 water temperature in the aquaria was slightly higher ($18.6 \text{ °C} \pm 0.3$). Sand-filtered brackish
192 deep water from the Kiel Fjord (salinity: $14.1 \text{ PSU} \pm 2.2$) was used and additionally filtered
193 with a $0.8 \mu\text{m}$ membrane filter to avoid contamination with plankton species. The filtered
194 water was kept in reservoirs, enriched with NaNO_3 and NaH_2PO_4 and used for the daily water
195 exchange. Nutrient concentrations were about $15 \mu\text{mol l}^{-1}$ nitrate and $0.75 \mu\text{mol l}^{-1}$ phosphate,
196 three-fold the concentrations used in the experiments under ambient nutrient conditions: about
197 $5 \mu\text{mol l}^{-1}$ nitrate and $0.25 \mu\text{mol l}^{-1}$ phosphate. Silicate levels were high ($12.3 \mu\text{mol l}^{-1}$ in the
198 enriched experiments, $14.7 \mu\text{mol l}^{-1}$ under ambient nutrient conditions).

199 Continuous water circulation was created using pumps and the water was exchanged (up to
200 90% of the total volume) every day. Periphyton growing on the walls of the aquaria was
201 removed every day before the water exchange.

202 The mesocosms were filled with 1 mm-sieved homogenized sediment (5 cm depth), which
203 consisted mainly of fine sand with low organic content. After 24 h, 20 freshly harvested
204 eelgrass shoots were planted in each mesocosm ($320 \text{ shoots m}^{-2}$, average abundance in the
205 Kiel Fjord in summer). Only shoots with at least four leaves were selected and the average
206 length of shoots was 40 cm. We measured the initial biomass of epiphytes (chl a) on 10

207 eelgrass shoots in each experiment. There was no significant difference in initial epiphyte
208 biomass between experiments. On the following day, the mesocosms were stocked with
209 grazers. All experimental material was collected at Falkenstein Beach in the inner Kiel Fjord,
210 Germany (54°21'N/10°9'E). The experiment was terminated after ten days. At this time, the
211 eelgrass was harvested, placed in plastic bags and stored frozen until further processing.
212 A preliminary experiment had shown that the optimal experimental duration was ten days,
213 because overgrazing, cannibalism and reproduction occurred soon after 10 days in the
214 crustacean treatments.

215

216 2.2. Epiphyte and eelgrass biomass

217 Epiphyte biomass was measured using chlorophyll *a* as proxy. Six eelgrass shoots were
218 randomly selected from each mesocosm. Epiphytes were carefully scraped from the eelgrass
219 blades using a special plastic scraper and a scalpel and transferred to small amounts of filtered
220 sea water. This suspension was filtered on precombusted (450 °C, 24 h) Whatman GF/F
221 filters. Pigment analyses with HPLC, carried out on scraped eelgrass blades and epiphytes,
222 indicated that removal efficiency by scraping was up to 99%. Chlorophyll *a* concentration
223 was calculated according to Lorenzen (1967). The cleaned eelgrass blades were dried to a
224 constant weight for 48 h at 60 °C and subsequently combusted for 8 h at 540 °C to determine
225 the ash-free dry mass (AFDM). The eelgrass surface area was calculated using the formula
226 $\text{surface (mm}^2\text{)} = \text{AFDM (g)} \times 588.88 \text{ (R}^2=0.97\text{)}$, determined by measuring and weighing 100
227 eelgrass shoots. All epiphytic chlorophyll concentrations were normalized to unit eelgrass
228 surface area.

229

230 2.3. Eelgrass and epiphyte productivity

231 Primary productivity estimates, based on ¹⁴C-measurements, were carried out on the last day
232 of the experiment. Four eelgrass shoots were randomly selected from each mesocosm and the

233 mid section of each shoot (10 cm) was transferred into a transparent Nalgene plastic bottle
 234 containing 250 ml seawater (0,2 µm filtrated). After inoculation with 26.4 µCi ¹⁴C-Na₂CO₃,
 235 three hour incubations (between 10.00 and 14.00 h) were carried out under experimental
 236 conditions. One bottle out of each mesocosm was wrapped up in aluminium foil and used as
 237 dark incubation. After incubation all eelgrass shoots were placed in plastic bags and stored
 238 frozen until further processing. Epiphytes were separated from the eelgrass blades by
 239 carefully scraping the blades using a special plastic scraper and a scalpel and then transferred
 240 into small amounts of filtered sea water. This suspension was filtered on preweighted
 241 membrane filters. The filters and the eelgrass blades were dried for 48 h at 60 °C and
 242 weighted to calculate dry weight. Then the filters were transferred into scintivials containing
 243 10 ml Lumagel. Radioactivity was measured in a Liquid Scintillation Counter. The dried
 244 eelgrass was wrapped up in Whatman ashless filter paper with a small amount of starch to
 245 promote combustion and compressed into pellets. Combustion took place in a Carbon
 246 Oxidiser where the CO₂ was trapped in a scintillating solution. All counts were corrected for
 247 background, recovery efficiency after combustion, and counting efficiency.
 248 Productivity was calculated as follows:

249
 250

$$251 \quad \text{dpm}_1 * ^{12}\text{CO}_2 * 1.06$$

$$252 \quad \mu\text{g C (mg dry wt)}^{-1} \text{ h}^{-1} = \frac{\text{dpm}_1 * ^{12}\text{CO}_2 * 1.06}{\text{dpm}_2 * \text{wt} * t},$$

$$253 \quad \text{dpm}_2 * \text{wt} * t$$

254

255 where dpm₁ is the activity (decay per minute) of the samples minus the activity in the dark
 256 incubation as correction for non-photosynthetic uptake of ¹⁴C, dpm₂ the activity of the isotope
 257 added to the bottles and ¹²CO₂ the mg available inorganic carbon. The factor 1.06 is a

258 correction for isotope discrimination. W_t is the dry weight of the epiphyte or eelgrass sample
259 and t the length of the incubation period in hours (Penhale 1977).

260

261 2.4. Per biomass effects

262 To compare the per biomass impact of the four studied mesograzer species on processes in the
263 epiphyte-eelgrass system, mesograzer effects on epiphytes and eelgrass were calculated as the
264 raw difference between controls and grazer treatments with the same biomass level per
265 bottom surface ($0.96 \text{ mg AFDM m}^{-2}$, Tab. 1).

266

267 2.5. Grazer net effects (GNE)

268 An estimate of the grazing rate was calculated by dividing the difference in epiphyte biomass
269 between control and treatment by 10 days (duration of the experiment). This “total GNE” per
270 treatment was used to calculate the GNE per capita and per g AFDW (ash free dry weight) of
271 mesograzers.

272

273 2.5. Statistics

274 We performed one-way ANOVAs to analyse the influence of mesograzer abundance on
275 epiphytes biomass, eelgrass productivity and grazer net effects (GNE) for each independent
276 experiment. Initially we analysed the data using randomized block ANOVAs, in which the
277 different abundances were considered fixed factors. The block effect was non-significant in
278 all analyses, therefore the block factor was ignored and the data were reanalysed with a one-
279 way ANOVA. Differences between treatments were tested with Tukey’s test.

280 We calculated the effect size (Hedges’ d) of the mesograzers’ impact on epiphyte biomass and
281 eelgrass productivity. This effect measure represents the standardized difference between
282 treatment and control means divided by the combined SD of both treatments (Gurevitch and
283 Hedges 1993) and can be used to compare the magnitude of effects of different experiments.

284 A statistical comparison of nutrient levels or the effect of species identity was not possible as
285 the data derived from six different experiments.

286

287 **3. Results**

288 3.1. Epiphyte biomass

289 All three mesograzers had significant negative effects on epiphyte biomass under low and
290 high nutrient supply (Fig. 1, Table 2), but the effect size depended on species identity and
291 nutrient level (Table 3). The isopod *Idotea* had the smallest impact on the epiphyte
292 assemblage regardless of nutrient concentrations. Under ambient nutrient supply, the negative
293 effect of this mesograzer on epiphyte biomass did not significantly increase from mean to
294 high density suggesting a threshold level for the capacity of this species to remove epiphytes
295 (Fig. 2). *Idotea* (512 individuals m⁻²) reduced epiphyte biomass by about 51% (low nutrients)
296 and 31% (high nutrients) compared to control treatments. The small gastropod *Rissoa*
297 maximally decreased the epiphyte assemblage by about 58% and 46%, respectively (1280
298 individuals m⁻²), and the periwinkle *Littorina* exerted the strongest negative effect on epiphyte
299 biomass with a decrease of about 88% and 78%, respectively (256 individuals m⁻²).

300

301 3.2. Eelgrass productivity

302 All mesograzers increased eelgrass productivity significantly at low nutrient concentrations
303 (Fig. 2, Table 2). *Idotea* enhanced eelgrass growth up to 76% at high densities; *Rissoa* caused
304 a rise of about 77% and *Littorina* boosted eelgrass productivity by about 72%. This relatively
305 similar pattern changed with increasing nutrient supply. Only the two gastropod species were
306 able to significantly enhance eelgrass productivity under these conditions. At high densities
307 *Rissoa* increased this parameter about 32%, *Littorina* even induced a rise of 52%. The small
308 gain in growth (about 19%) caused by *Idotea* was not significant.

309

310 3.3. Mesograzer per biomass effects on epiphyte biomass and eelgrass productivity
311 One treatment in all experiments had a constant mesograzer biomass ($0.96 \text{ mg AFDM m}^{-2}$,
312 Tab. 1) to compare the effects of the three studied species. All studied mesograzers had a
313 stronger per biomass effect on epiphyte biomass under higher nutrient supply (Fig. 3). *Rissoa*
314 was always the best-performing species. *Littorina* nearly reached the same impact as *Rissoa*
315 in the experiments with higher nutrient conditions, whereas *Littorina* and *Idotea* had
316 approximately the same per biomass effect under ambient nutrient supply. *Rissoa* as most
317 efficient mesograzer had the strongest positive effect on eelgrass productivity followed by
318 *Idotea* and *Littorina* (Fig. 3). The promotion of eelgrass growth was strongly reduced in
319 *Rissoa* and *Idotea* under high nutrient supply. *Littorina* was the only species, which
320 maintained its impact on eelgrass productivity despite the increase in nutrients.

321

322 3.4. Grazer net effects (GNE)

323 The total GNE, the collective impact of all mesograzers in a treatment, increased significantly
324 with mesograzer density in all experiments (Fig. 4, Table 4). We also found an increase in
325 grazing effect with higher nutrient supply. *Littorina* had the strongest effect for both aspects.
326 As the grazing effect depends on the size and biomass of the mesograzers, we calculated the
327 GNE per individual and per weight to make the grazing impact more comparable. In the case
328 of *Idotea* and *Littorina* the GNE per individual mesograzer decreased significantly with
329 mesograzer density indicating food limitation or intraspecific negative effects, whereas *Rissoa*
330 did not show such a trend (Fig. 4, Table 4). *Littorina* had the strongest negative effect on
331 epiphyte biomass, followed by *Idotea* and *Rissoa*, which was in accordance with the size and
332 biomass of the mesograzers (approximate weight: *Littorina* = 15 mg, shell diameter ~10 mm,
333 *Idotea* = 4 mg, length ~10 mm, *Rissoa* = 1 mg AFDW, length ~6 mm).

334 The GNE per weight showed that *Rissoa* was the most efficient mesograzer regardless of
335 nutrient concentration. When mesograzer biomass was considered, *Littorina* had a stronger
336 negative effect on epiphyte biomass than *Idotea* in the high nutrient experiments.

337

338 **4. Discussion**

339 Eutrophication is thought to be one of the major determinants of negative changes in aquatic
340 ecosystems. The overgrowth of seagrasses by fast-growing algae, which are promoted by high
341 nutrient supply, is thought to contribute significantly to the deterioration of seagrass beds in
342 many coastal areas worldwide (Hauxwell et al. 2001; Orth et al. 2006; Waycott et al. 2009). A
343 recent meta-analysis, however, ascertained that the impact of small crustacean and gastropod
344 grazers (“mesograzers”) feeding mainly on epiphytes can potentially mediate this negative
345 effect of eutrophication, and thus contribute substantially to the protection and conservation
346 of seagrass communities (Hughes et al. 2004).

347 To verify their capacity to counterbalance increasing epiphyte burden on eelgrass caused by
348 eutrophication, we studied the effect of three mesograzer species in an experimental eelgrass-
349 epiphyte system under nutrient enrichment and compared the results with earlier experiments
350 under ambient nutrient supply (Jaschinski and Sommer 2008a). All studied mesograzers
351 significantly reduced epiphyte biomass under high nutrient concentrations although the
352 impact varied with mesograzer identity. *Littorina* had the strongest effect in the high
353 abundance treatment, but *Rissoa* had the highest per biomass impact. This is in accordance
354 with the small size of this species and the resulting high metabolism activity. *Littorina* had a
355 stronger negative per biomass effect on epiphyte accumulation than *Idotea* despite being the
356 largest studied mesograzer. The different feeding modes probably make *Littorina* the more
357 efficient mesograzer.

358 For all species, the total ingestions rate increased with growing epiphyte biomass,
359 demonstrating the capacity of these mesograzers to reduce the epiphyte cover in eutrophic

360 situations. The decrease in per capita grazing effect with growing mesograzer densities for
361 *Idotea* and *Littorina* indicates that the stimulation of consumption under high food supply is at
362 least partially a functional response to food limitation. Lamberti (1996) claimed that benthic
363 mesograzers are likely to be quantitatively or qualitatively food-limited, a hypothesis that was
364 confirmed for mobile epifauna in seagrass and *Sargassum* systems (Edgar 1990; Edgar and
365 Aoki 1993). Furthermore, increasing nitrogen content of epiphytes may promote the grazing
366 rate of mesograzers (Heck et al. 2006; Russell and Connell 2007) and may additionally help
367 to counteract the negative effects of eutrophication.

368 Despite the significant reduction of epiphyte accumulation found for all mesograzers, only
369 high abundances of the two gastropod species had a significant positive effect on eelgrass
370 productivity. Under higher nutrient supply, *Littorina* had the most positive impact on eelgrass
371 growth, boosting eelgrass productivity up to the same level as under lower nutrient supply.
372 *Rissoa*'s effect on eelgrass was reduced compared to ambient nutrient conditions.

373 Furthermore this species had the most positive per biomass impact.

374 *Idotea* had no positive effect on eelgrass productivity under high nutrient supply despite the
375 fact that the threshold level of this mesograzer's capacity to remove epiphytes found under
376 ambient nutrient conditions disappeared with higher nutrient supply. *Idotea* is only able to
377 remove larger erectly growing epiphytes, and thus profited from the compositional change to
378 a epiphyte assemblage dominated by diatom chains under high nutrient supply (Jaschinski &
379 Sommer 2008b). We found that the selectivity of *Idotea* increased with increasing nutrient
380 supply, and thus, epiphyte biomass. At high nutrient supply only diatom chains were
381 significantly grazed upon (Jaschinski et al. 2010). The reduction of this growth form is
382 probably not essential for the amount of light that reaches the eelgrass surface (Brush and
383 Nixon 2002), so that the negative impact of *Idotea* on the epiphyte load did not result in a
384 positive effect on eelgrass growth.

385 Increased nutrient supply alone did not decrease eelgrass growth, because under low ambient
386 nutrient supply eelgrass is nitrogen-limited in our study area. The reduction in light caused by
387 growing epiphyte accumulation seems to be compensated by the better supply with nitrogen
388 (Jaschinski and Sommer 2008b). Nitrogen enrichment of eelgrass may stimulate herbivory on
389 the macrophyte as found for small fish and other seagrass species (Heck and Valentine 2007
390 and references therein). We could not confirm that the higher food quality of eelgrass under
391 nutrient enrichment enhanced direct grazing on eelgrass as we only found very few grazing
392 scars in the high abundance treatment of *Idotea* regardless of nutrient supply.

393 There is growing evidence that the effect of mesograzer species in eelgrass systems is not
394 functionally redundant, but depends on the different traits even of superficially similar
395 mesograzers (Duffy et al. 2001; Duffy et al. 2003, Hays 2005, Råberg and Kautsky 2007;
396 Jaschinski and Sommer 2008a). Our results support the assumption that, depending on the
397 species, mesograzers can at least partially mediate the negative effect of eutrophication in
398 macrophyte systems. Under the condition of short-term laboratory experiments both
399 gastropods seemed to be capable of restricting epiphyte cover under high nutrient supply. In a
400 longer experiment (3 weeks) with similar nutrient conditions, however, *Idotea* had the
401 strongest negative effect on epiphyte biomass. This was due to the high reproduction rate of
402 this species under unlimited food supply, summer temperatures and the lack of predation
403 pressure (Jaschinski et al. 2009). Under favourable conditions, this crustacean species is able
404 to quickly increase in numbers. The compositional change in the epiphyte assemblage under
405 high nutrient supply to a dominance of diatom chains may also be beneficial for *Idotea*,
406 because these algal growth forms are preferentially eaten by the isopod (Jaschinski et al.
407 2010). This mechanism, however, may not always be positive for macrophytes in the field. A
408 mass occurrence of *Idotea* probably caused by the increasing availability of filamentous algae
409 during high nutrient supply destroyed the vegetation of the macroalgae *Fucus vesiculosus* in
410 large areas of the Baltic Sea (Svensson et al. 2004). An identical phenomenon was found for

411 *Rissoa* and eelgrass, but the reasons for the high gastropod densities are unknown in this case
412 (Fredriksen et al. 2004). Thus, the abundance of mesograzers can change their effect on
413 eelgrass from insignificant at low density levels to supporting at intermediate and high density
414 and to detrimental at very high mesograzer densities.

415 Currently, there is growing evidence that indirect consumer effects can have the same
416 negative influence in shallow benthic systems as eutrophication (Heck and Valentine 2007
417 and references therein, Douglass et al. 2008). E.g. the strong reduction of piscivorous fish
418 species can reduce via a trophic cascade, which favours smaller predatory fish, the
419 mesograzer populations to such low numbers that their function as controllers of epiphytes
420 and ephemeral macroalgae is threatened (Moksnes et al. 2008, Eriksson et al. 2009). Our
421 study supports the importance of top-down effects via mesograzers in controlling and
422 stabilising benthic macrophyte systems, promoting the assumption that not only
423 eutrophication must be regulated to maintain the health and functioning of these important
424 ecosystems, but that the sustainment of mesograzer populations is just as crucial.

425 In conclusion, mesograzers may diminish or counteract effects of increasing nutrient loads on
426 seagrass communities in coastal areas, but their impact depends on grazer identity. Higher
427 epiphyte consumption per capita may result from a functional response to release from food
428 limitation or from a preferential feeding of nitrogen enriched algae. Strong population
429 responses to higher quantity and quality of food may additionally promote the capacity of
430 mesograzers to reduce negative effects of eutrophication in coastal benthic macrophyte
431 systems.

432

433 **Acknowledgements**

434 We are grateful to S. Flöder for critical reading of the MS and helpful comments. Financial
435 support was provided by the German Research Foundation (So 145 /20).

436

437 **References**

- 438 Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication:
439 nutrient sources, composition, and consequences. *Estuaries* 25: 704-726
- 440 Beck MW, Heck JKL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern BS,
441 Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP (2001) The
442 identification, conservation, and management of estuarine and marine nurseries for
443 fish and invertebrates. *BioScience* 51: 633-641
- 444 Brush MJ, Nixon SW (2002) Direct measurements of light attenuation by epiphytes on
445 eelgrass *Zostera marina*. *Mar Ecol Prog Ser* 238: 73-79
- 446 Constanza R, d' Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S,
447 O'Neill RV, Paruelo J, Raskin RG, Sutton P, van der Belt M (1997) The value of the
448 world's ecosystem services and natural capital. *Nature* 387: 253-260
- 449 Douglass JG, Duffy JE, Spivak AC, Richardson AJ (2007) Nutrient versus consumer control
450 of community structure in a Chesapeake Bay eelgrass habitat. *Mar Ecol Prog Ser* 348:
451 71-83
- 452 Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic
453 carbon cycle. *Biogeoscience* 1: 1-8
- 454 Duffy JE, MacDonald KS, Rhode JM, Parker JD (2001) Grazer diversity, functional
455 redundancy, and productivity in seagrass beds: an experimental test. *Ecology* 82:
456 2417-2434
- 457 Duffy JE, Richardson JP, Canuel EA (2003) Grazer diversity effects on ecosystem
458 functioning in seagrass beds. *Ecol Lett* 6: 637-645
- 459 Edgar GJ (1990) Population regulation, population dynamics and competition amongst
460 mobile epifauna associated with seagrass. *J Exp Mar Biol Ecol* 144: 205-234
- 461 Edgar GJ, Aoki M (1993) Resource limitation and fish predation: their importance to mobile
462 epifauna associated with Japanese *Sargassum*. *Oecologia* 95: 122-133

463 Eriksson BK, Ljunggren L, Sandström A, Johansson G, Mattila J, Rubach A, Raberg S,
464 Snickars M (2009) Declines in predatory fish promote bloom-forming macroalgae.
465 Ecol. Appl. 19: 1975-1988

466 Fredriksen S, Christie H, Boström C (2004) Deterioration of eelgrass (*Zostera marina* L.)
467 through destructive grazing by the gastropod *Rissoa membranacea* (J. Adams). Sarsia
468 89: 218-222

469 Gurevitch J, Hedges LV (1993) Meta-analysis: combining the results of independent
470 experiments. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological
471 experiments. Chapman and Hall, London, pp 378-398

472 Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF,
473 Tomanek L, Williams SL (2006) The impact of climate change in coastal marine
474 systems. Ecol Lett 9: 228-241

475 Hauxwell J, Cebrián J, Furlong C, Valiela I (2001) Macroalgal canopies contribute to
476 eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82:
477 1007-1022

478 Hays CG (2005) Effect of nutrient availability, grazer assemblage and seagrass source
479 population on the interaction between *Thalassia testudinum* (turtle grass) and its algal
480 epiphytes. J Exp Mar Biol Ecol 314: 53-68

481 Heck Jr. KL, Valentine JF (2007) The primacy of top-down effects in shallow benthic
482 ecosystems. Estuaries Coasts 30: 371-381

483 Heck Jr. KL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for
484 seagrass meadows. Mar Ecol Prog Ser 253: 123-136

485 Heck Jr. KL, Valentine JF, Pennock JR, Chaplin G, Spitzer PM (2006) Effects of nutrient
486 enrichment and grazing on shoalgrass *Halodule wrightii* and its epiphytes: results of a
487 field experiment. Mar Ecol Prog Ser 326: 145-156

488 Heck Jr. KL, Carruthers TJB, Duarte CM, Hughes AR, Kendrick GA, Orth RJ, Williams SL
489 (2008) Trophic transfers from seagrass meadows subsidize diverse marine and
490 terrestrial consumers. *Ecosystems* 11: 1198-1210

491 Hillebrand H, Worm B, Lotze HK (2000) Marine microphytobenthic community structure
492 regulated by nitrogen loading and grazing pressure. *Mar Ecol Prog Ser* 204: 27-38

493 Howarth RW, Anderson D, Cloern J, Elfring C, Hopkinson C, Lapointe B, Malone T, Marcus
494 N, McGlathery K, Sharpley A, Walker D (2000) Nutrient pollution of coastal rivers,
495 bays, and seas. *Issues Ecol* 7: 1-15

496 Hughes AR, Jun Bando K, Rodriguez LF, Williams SL (2004) Relative effects of grazers and
497 nutrients on seagrasses: a meta-analysis approach. *Mar Ecol Prog Ser* 282: 87-99

498 Jaschinski S, Sommer U (2008a) Functional diversity of mesograzers in an eelgrass-epiphyte
499 system. *Mar Biol* 154: 475-482

500 Jaschinski S, Sommer U (2008b) Top-down and bottom-up control in an epiphyte-eelgrass
501 (*Zostera marina* L.) system. *Oikos* 117: 754-762

502 Jaschinski S, Aberle N, Gohse-Reimann S, Brendelberger H, Wiltshire KH, Sommer U
503 (2009) Grazer diversity effects in an eelgrass-epiphyte-microphytobenthos system.
504 *Oecologia* 159: 607-615

505 Jaschinski S, Flöder S, Sommer U (2010) Consumer identity, abundance, and nutrient
506 concentration affect epiphyte diversity in an experimental eelgrass system. *Oikos*
507 accepted

508 Jephson T, Nyström P, Moksnes P-O, Baden S (2008) Trophic interactions in *Zostera marina*
509 beds along the Swedish coast. *Mar Ecol Prog Ser* 369: 63-76

510 Jernakoff P, Brearley A, Nielsen J (1996) Factors affecting grazer-epiphyte interactions in
511 temperate seagrass meadows. *Oceanogr Mar Biol Ann Rev* 34: 109-162

512 Lamberti GA (1996) The role of periphyton in benthic food webs. In: Stevenson RJ, Bothwell
513 ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic Press,
514 San Diego, pp 533-572

515 Moksnes P-O, Gullström M, Tryman K, Baden S (2008) Trophic cascade in a temperate
516 seagrass community. *Oikos* 117: 763-777

517 Neckles HA, Wetzel RL, Orth RJ (1993) Relative effects of nutrient enrichment and grazing
518 on epiphyte-macrophyte (*Zostera marina* L.) dynamics. *Oecologia* 93: 285-295

519 Norton TA, Hawkins SJ, Manley NL, Williams GA, Watson DC (1990) Scraping a living: a
520 review of littorinid grazing. *Hydrobiol* 193: 117-138

521 Orav-Kotta H, Kotta J (2004) Food and habitat choice of the isopod *Idotea baltica* in the
522 northeastern Baltic Sea. *Hydrobiol* 514: 79-85

523 Orth RJ, Carruthers TJB, Dennison WC, Duarte CM, Fourqurean JW, Heck Jr. KL, Hughes
524 AR, Kendrick GA, Kenworthy WJ, Olyarnik S, Short FT, Waycott M, Williams SL
525 (2006) A global crisis for seagrass ecosystems. *BioScience* 56: 987-996

526 Råberg S, Kautsky L (2008) Grazer identity is crucial for facilitating growth of the perennial
527 brown algae *Fucus vesiculosus*. *Mar Ecol Prog Ser* 361: 111-118

528 Russell BD, Connell SD (2007) Response of grazers to sudden nutrient pulses in oligotrophic
529 versus eutrophic conditions. *Mar Ecol Prog Ser* 349: 73-80

530 Sand-Jensen K (1977) Effects of epiphytes on eelgrass photosynthesis. *Aquat Bot* 3: 55-63

531 Schaffelke B, Evers D, Walhorn A (1995) Selective grazing of the isopod *Idotea baltica*
532 between *Fucus evanescens* and *F. vesiculosus* from Kiel Fjord (Western Baltic). *Mar*
533 *Biol* 124: 215-218

534 Smith VH, Tilman GD, Nocola JC (1999) Eutrophication: impacts of excess nutrient inputs
535 on freshwater, marine, and terrestrial ecosystems. *Environ Pollut* 100: Sommer U
536 (1999) The impact of herbivore type and grazing pressure on benthic microalgae
537 diversity. *Ecol Lett* 2: 65-69

538 Svensson PA, Malm T, Engkvist R (2004) Distribution and host plant preference of *Idotea*
539 *baltica* (Pallas) (Crustacea: Isopoda) on shallow rocky shores in the central Baltic Sea.
540 *Sarsia* 89: 1-7

541 Touchette BW, Burkholder JM (2000) Review of nitrogen and phosphorus metabolism in
542 seagrass. *J Exp Mar Biol Ecol* 250: 133-167

543 Warén A (1996) Ecology and systematics of the north european species of *Rissoa* and
544 *Pusillina* (Prosobranchia: Rissoidae). *J mar biol Ass UK* 76: 1013-1059

545 Waycott M, Duarte CM, Carruthers TJB, Orth RJ, Dennison WC, Olyarnik S, Calladine A,
546 Fourqurean JW, Heck Jr. KL, Hughes AR, Kendrick GA, Kenworthy WJ, Short FT,
547 Williams SL (2009) Accelerating loss of seagrasses across the globe threatens coastal
548 ecosystems. *PNAS* 106: 12377-12381

549
550
551
552
553
554
555
556
557
558
559
560
561
562

563 Table 1. Grazer density and biomass per bottom surface in all experiments. Treatments with
564 the same biomass are shown in bold.

Grazer abundances	Density (m ⁻²)			Biomass (g AFDM*m ⁻²)		
	low	mean	high	low	mean	high
<i>Idotea</i>	128	256	512	0.48	0.96	1.92
<i>Littorina</i>	64	128	256	0.96	1.92	3.84
<i>Rissoa</i>	320	640	1280	0.24	0.48	0.96

565

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582

583

584

585 Table 2. ANOVA results for the effects of mesograzer abundance (ambient and high nutrient
 586 concentration) on epiphyte biomass and eelgrass productivity. Degree of freedom for the
 587 tested factor mesograzer abundance was 3 in all analyses; degree of freedom in the error term
 588 was 20.

Epiphyte biomass	MS-error	MS	F-ratio	p-level
Low nutrients				
<i>Idotea</i>	0.0003	0.014	41.10	<0.0001
<i>Littorina</i>	0.0338	4.912	145.36	<0.0001
<i>Rissoa</i>	0.0002	0.029	185.32	<0.0001
High nutrients				
<i>Idotea</i>	0.0005	0.049	108.77	<0.0001
<i>Littorina</i>	0.0015	0.326	222.33	<0.0001
<i>Rissoa</i>	0.0010	0.111	116.01	<0.0001
Eelgrass productivity				
Low nutrients				
<i>Idotea</i>	0.0043	0.096	22.41	<0.0001
<i>Littorina</i>	0.0045	0.071	15.82	<0.0001
<i>Rissoa</i>	0.0050	0.104	20.85	<0.0001
High nutrients				
<i>Idotea</i>	0.0064	0.005	0.76	0.5322
<i>Littorina</i>	0.0054	0.028	5.23	0.0079
<i>Rissoa</i>	0.0048	0.016	3.30	0.0416

589

590

591

592

593

594

595

596

597

598

599

600 Table 3. Effect size (Hedges' *d*) for the effects of the three mesograzers on epiphyte biomass
 601 and eelgrass productivity under low and high nutrient supply. Treatments with the same
 602 biomass are shown in bold.

603

Grazer	Density	Epiphyte biomass		Eelgrass productivity	
<i>Idotea</i>	low	-3.25	-1.80	0.84	0.48
	mean	-4.61	-2.61	2.65	0.64
	high	-4.44	-4.20	3.96	0.71
<i>Littorina</i>	low	-2.75	-5.73	0.37	0.47
	mean	-5.12	-9.83	1.30	0.93
	high	-7.16	-24.31	2.13	2.18
<i>Rissoa</i>	low	-2.68	-1.52	5.49	0.18
	mean	-7.77	-4.55	8.17	0.88
	high	-10.65	-8.73	8.44	1.82
Nutrients		low	high	low	high

604

605

606

607

608

609

610

611

612

613

614

615

616

617

618

619

620 Table 4. ANOVA results for grazer net effects (GNE) of three potentially dominant
 621 mesograzers in Baltic Sea eelgrass meadows under ambient and enriched nutrient conditions.
 622 Degree of freedom for the tested factor mesograzer abundance was 2 in all analyses; degree of
 623 freedom in the error term was 15.

Total GNE	MS-error	MS	F-ratio	p-level
Low nutrients				
<i>Idotea</i>	0.000005	0.000037	7.23	0.006
<i>Littorina</i>	0.000013	0.000178	13.30	<0.001
<i>Rissoa</i>	0.000002	0.000188	105.81	<0.001
High nutrients				
<i>Idotea</i>	0.000011	0.000220	20.84	<0.001
<i>Littorina</i>	0.000024	0.001270	52.48	<0.001
<i>Rissoa</i>	0.000014	0.000988	3028.96	<0.001
GNE per animal				
Low nutrients				
<i>Idotea</i>	0.000000025	0.000000243	9.59	0.002
<i>Littorina</i>	0.000000241	0.000002640	10.97	0.001
<i>Rissoa</i>	0.000000001	0.000000020	22.35	<0.001
High nutrients				
<i>Idotea</i>	0.00000007	0.00000044	5.95	0.013
<i>Littorina</i>	0.00000063	0.00001530	24.19	<0.001
<i>Rissoa</i>	0.00000001	0.00000002	1.38	0.281
GNE per weight				
Low nutrients				
<i>Idotea</i>	0.0018	0.0173	9.59	0.002
<i>Littorina</i>	0.0011	0.0117	10.97	0.001
<i>Rissoa</i>	0.0016	0.0359	22.35	<0.001
High nutrients				
<i>Idotea</i>	0.0053	0.0314	5.95	0.013
<i>Littorina</i>	0.0028	0.0678	24.19	<0.001
<i>Rissoa</i>	0.0218	0.0301	1.38	0.281

624

625

626

627

628

629

630 **Figure legends**

631 Figure 1. The effect of three potentially dominant mesograzers in Baltic Sea eelgrass
632 meadows on epiphyte biomass under ambient and enriched nutrient conditions (mean±SD, n
633 = 6). Filled circles represent low nutrient concentration and unfilled circles high nutrient
634 concentration. Capital letters indicate significant differences between treatments of increasing
635 mesograzer abundance (low nutrients: $p \leq 0.007$, high nutrients: $p \leq 0.009$). There was no
636 comparison between nutrient levels, because the data derived from different experiments.
637 Data under ambient nutrient conditions originate from the experiments described in Jaschinski
638 and Sommer (2008a).

639

640 Figure 2. The effect of three potentially dominant mesograzers in Baltic Sea eelgrass
641 meadows on eelgrass productivity under ambient and enriched nutrient conditions (mean±SD,
642 n = 6). Filled circles represent low nutrient concentration and unfilled circles high nutrient
643 concentration. Capital letters indicate significant differences between treatments of increasing
644 mesograzer abundance (low nutrients: $p \leq 0.016$, high nutrients: $p \leq 0.048$). There was no
645 comparison between nutrient levels, because the data derived from different experiments.
646 Data under ambient nutrient conditions originate from the experiments described in Jaschinski
647 and Sommer (2008a).

648

649 Figure 3. Per biomass effects of mesograzer species on epiphyte biomass and eelgrass
650 productivity. Shown are the raw, arithmetic differences between grazer-free controls and the
651 grazer treatments with the same biomass ($0.96 \text{ mg AFDM m}^{-2}$, Tab. 1). A statistical
652 comparison of the data was not possible as the data derived from 6 different experiments.
653 Data source under ambient nutrient conditions Jaschinski and Sommer (2008a).

654

655 Figure 4. Grazer net effects (GNE) of three potentially dominant mesograzers in Baltic Sea
656 eelgrass meadows under ambient and enriched nutrient conditions. Shown are the total GNE
657 in the different treatments, the GNE per animal and GNE per grazer biomass. Filled circles
658 represent low nutrient concentration and unfilled circles high nutrient concentration. Capital
659 letters indicate significant differences between treatments of increasing mesograzer
660 abundance. There was no comparison between nutrient levels, because the data derived from
661 different experiments.

662

663

664

665

666

667

668

669

670

671

672

673

674

675

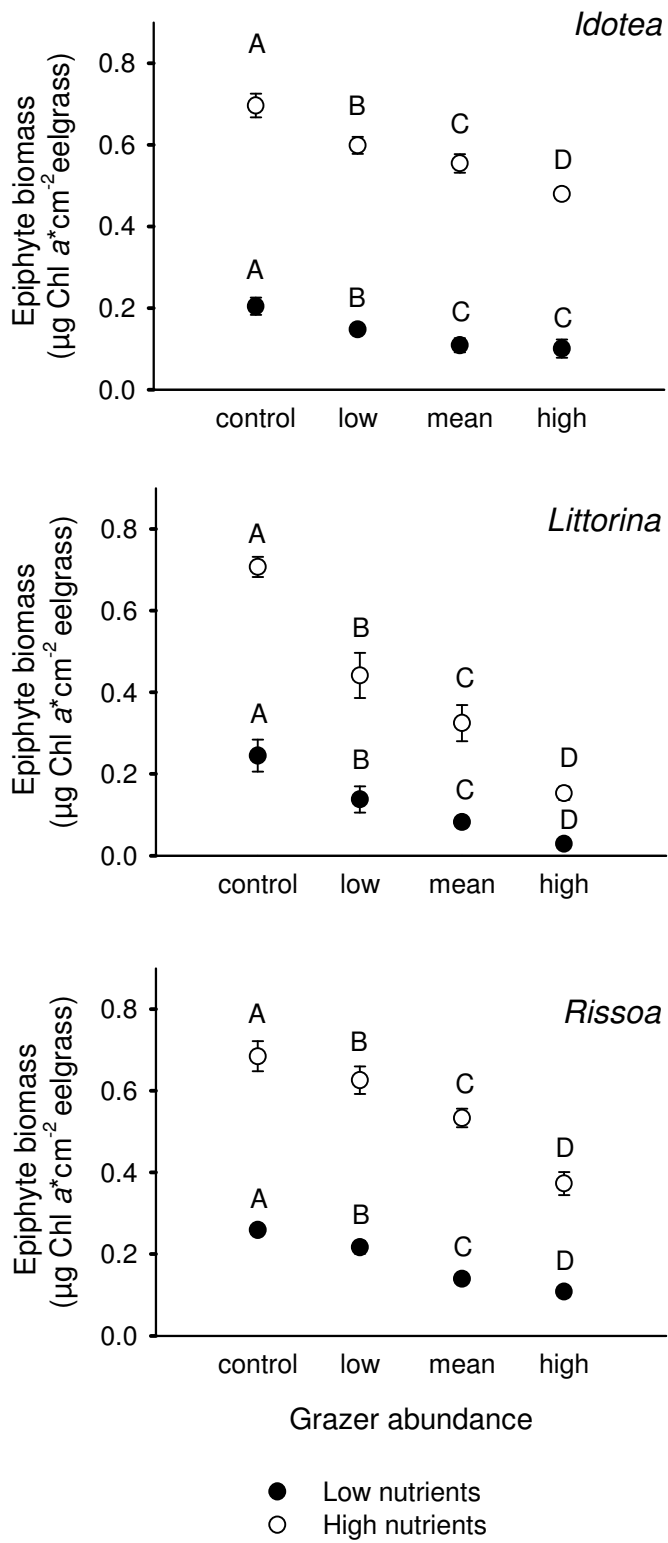
676

677

678

679

680

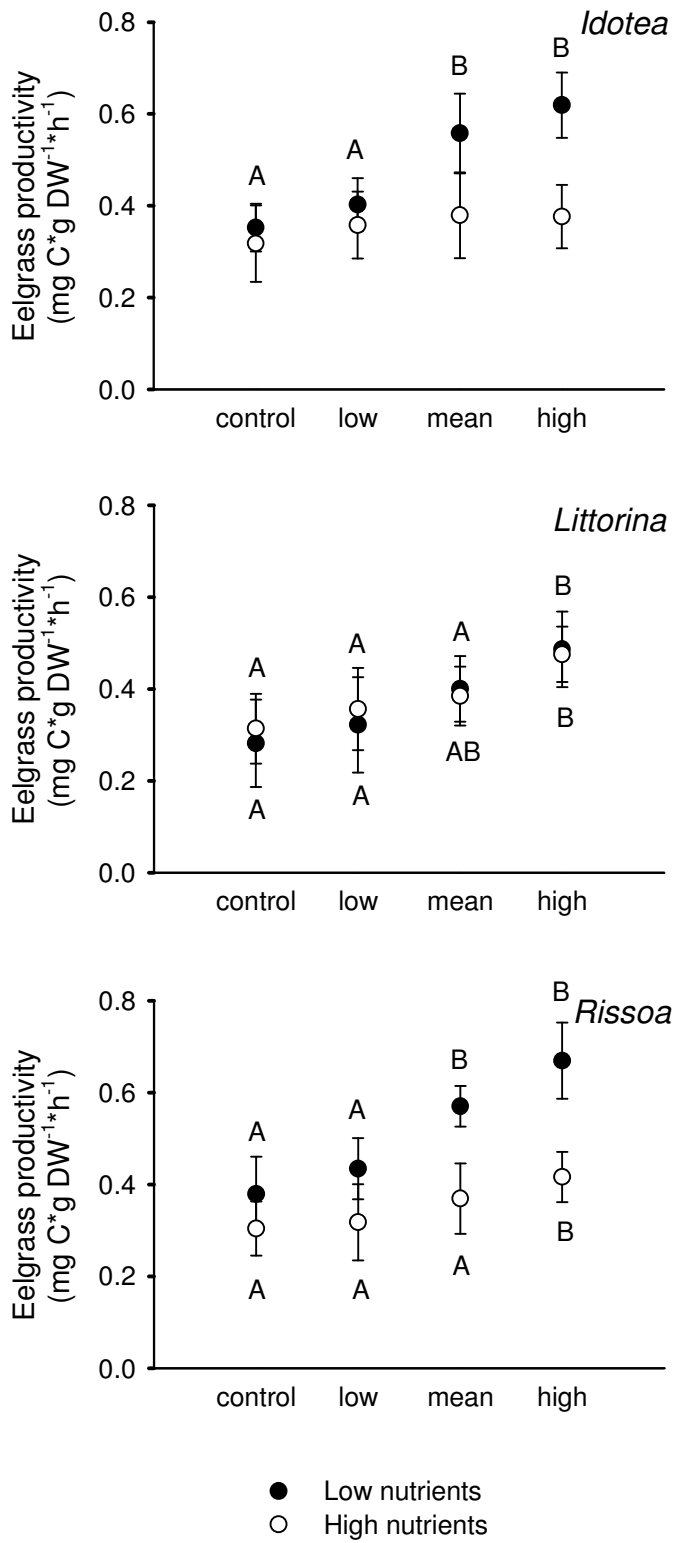


682

683

684

685 Figure 2

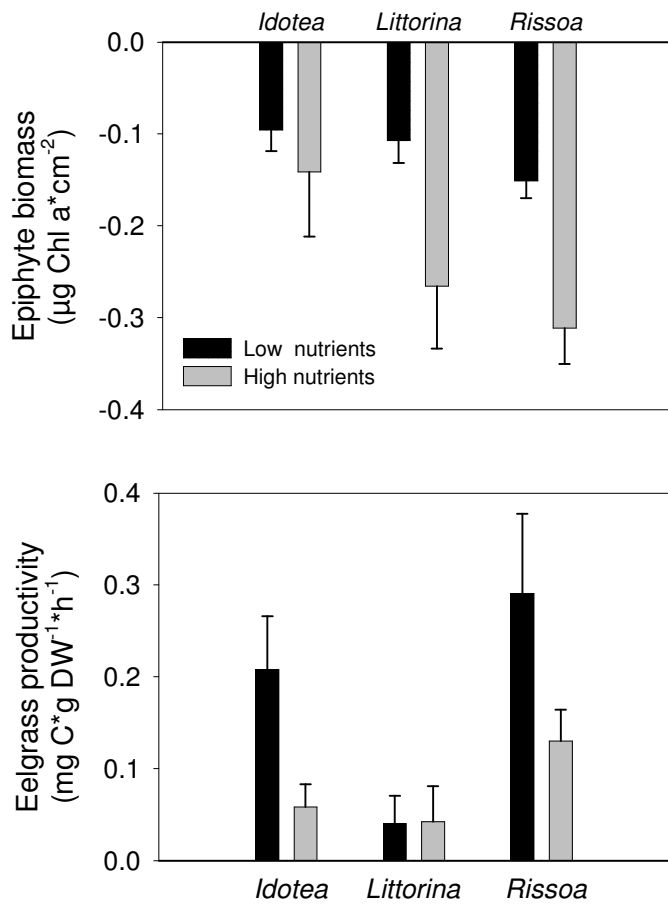


686

687

688

689 Figure 3



690

691

692

693

694

695

696

697

698

699

700

701

