1 **How do nutrient conditions and species identity influence the impact of mesograzers in**

2 **eelgrass-epiphyte systems?**

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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.

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1. Introduction

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

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

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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 homogenously ("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?

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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 µmol m^{-2} 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 °C $\pm$ 0.3). Sand-filtered brackish

192   deep water from the Kiel Fjord (salinity: 14.1 PSU $\pm$ 2.2) was used and additionally filtered

193   with a 0.8 µm 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 µmol l^{-1} nitrate and 0.75 µmol l^{-1} phosphate,

196   three-fold the concentrations used in the experiments under ambient nutrient conditions: about

197   5 µmol l^{-1} nitrate and 0.25 µmol l^{-1} phosphate. Silicate levels were high (12.3 µmol l^{-1} in the

198   enriched experiments, 14.7 µmol 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 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 ($54^o21'N/10^o9'E$). 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.

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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 ($450\ ^o$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 ^{o}C and subsequently combusted for 8 h at 540 ^{o}C to determine

225   the ash-free dry mass (AFDM). The eelgrass surface area was calculated using the formula

226   surface (mm^2) = AFDM (g) x 588.88 (R^2=0.97), determined by measuring and weighing 100

227   eelgrass shoots. All epiphytic chlorophyll concentrations were normalized to unit eelgrass

228   surface area.

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230   2.3. Eelgrass and epiphyte productivity

231   Primary productivity estimates, based on ^{14}C-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 ^{14}C-Na$_2$CO$_3$,

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 $^{\circ}$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 CO$_2$ 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:

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$$\mu g\ C\ (mg\ dry\ wt)^{-1}\ h^{-1} = \frac{dpm_1 * {}^{12}CO_2 * 1.06}{dpm^2 * wt * t},$$

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255   where dpm$_1$ is the activity (decay per minute) of the samples minus the activity in the dark

256   incubation as correction for non-photosynthetic uptake of ^{14}C, dmp$_2$ the activity of the isotope

257   added to the bottles and ^{12}CO$_2$ the mg available inorganic carbon. The factor 1.06 is a

258   correction for isotope discrimination. Wt is the dry weight of the epiphyte or eelgrass sample

259   and t the length of the incubation period in hours (Penhale 1977).

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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 mg AFDM m^{-2}, Tab. 1).

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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.

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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}).

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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.

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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 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.

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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

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

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

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

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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 servicesand 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 photosythesis. 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, Necola 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

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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
Idotea	128	**256**	512	0.48	**0.96**	1.92
Littorina	**64**	128	256	**0.96**	1.92	3.84
Rissoa	320	640	**1280**	0.24	0.48	**0.96**

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

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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	
Idotea	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
Littorina	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
Rissoa	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

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

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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).

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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 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.

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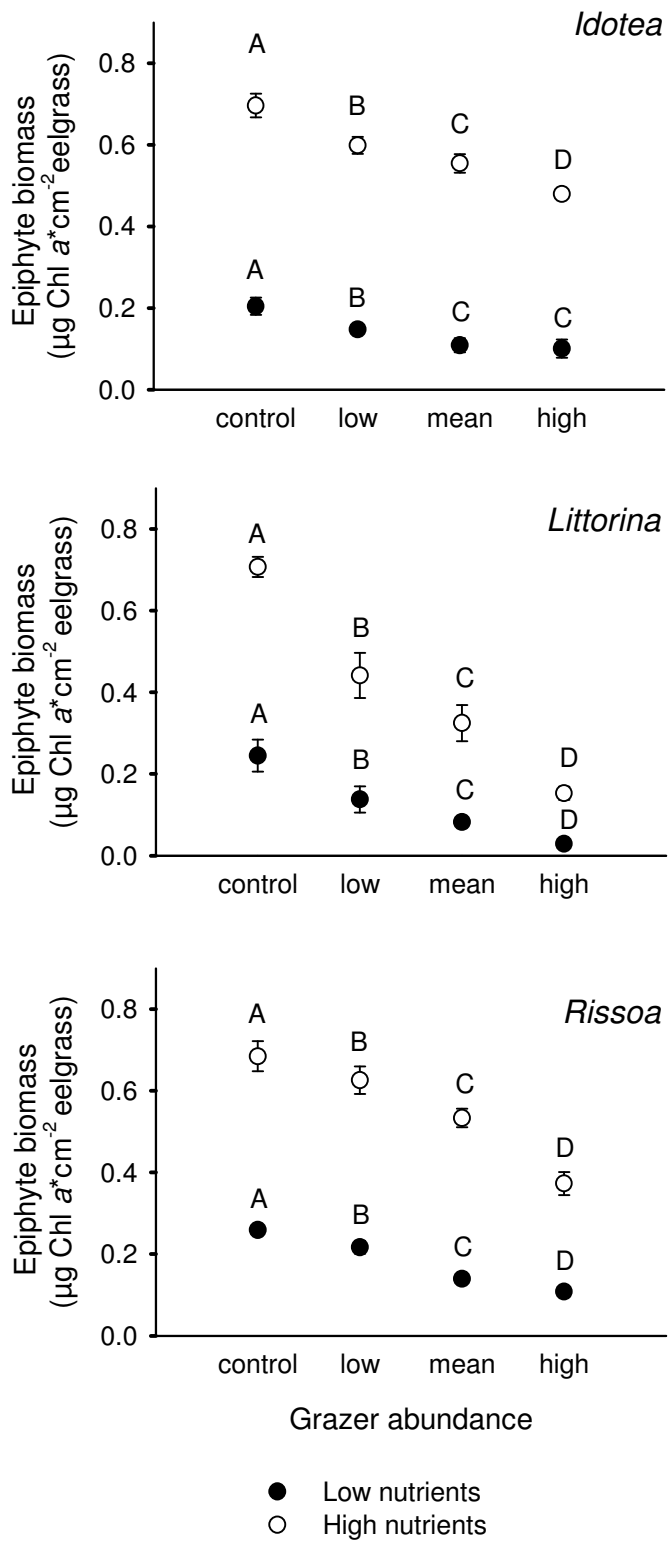
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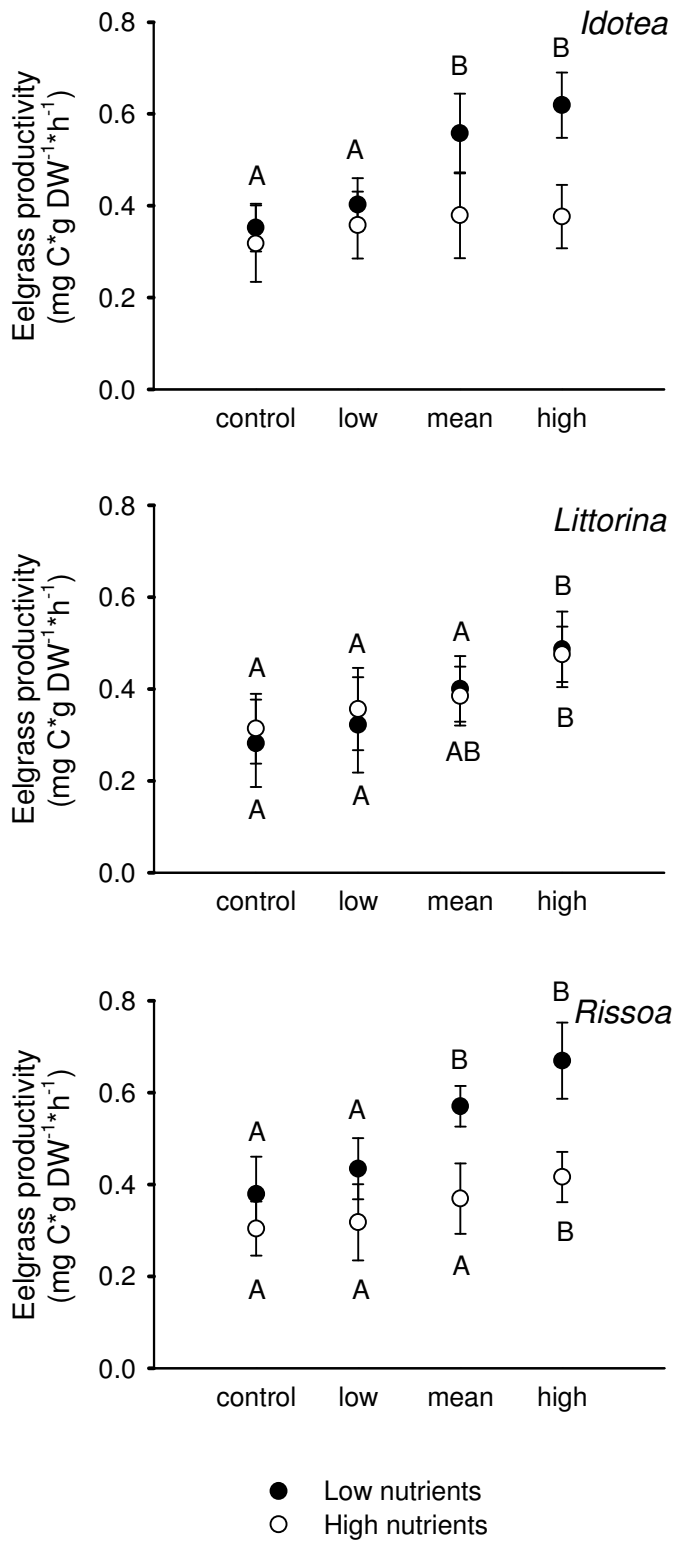
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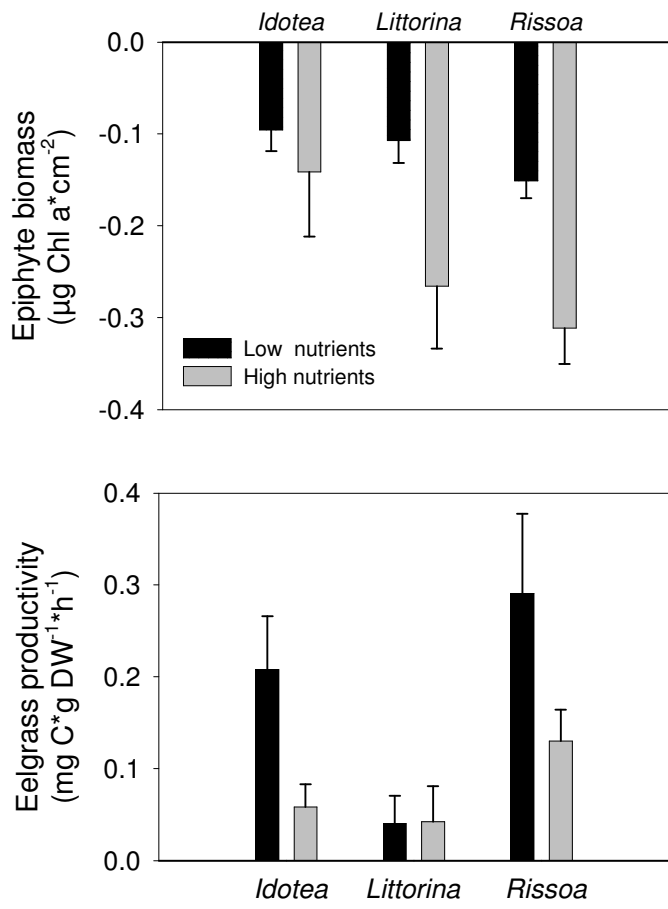679

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681   Figure 1

Epiphyte biomass (μg Chl a*cm^{-2} eelgrass)

Idotea

Littorina

Rissoa

Grazer abundance

● Low nutrients
○ High nutrients

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685   Figure 2

689   Figure 3

702   Figure 4

Figure 4

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