Influence of different types of sessile epibionts on the community structure of mobile invertebrates in an eelgrass bed

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The variation in the biomass, abundance and diversity of mobile invertebrates in eelgrass (*Zostera marina*) beds has been examined in relation to various abiotic and biotic factors, such as water temperature, salinity, eelgrass biomass and epiphytic microalgae presence. However, the importance of sessile epibionts, such as macroalgae and calcific spirorbid polychaetes attached to eelgrass blades, has not been the focus of previous studies. In the present study, we examined the effects of three different sessile epibionts, namely, branched red algae, filamentous green algae, and calcific spirorbid polychaetes, on the biomass and diversity of mobile invertebrates in the eelgrass beds of Akkeshi in Northeastern Japan. The relationships of the dominant mobile invertebrate biomass, the total biomass of mobile invertebrates and the species diversity to multiple abiotic and biotic variables were analyzed using a linear mixed model. Our results show that large-sized epibiotic organisms can be good predictors of the variation in the total biomass, species richness and species diversity of mobile invertebrates and the biomass of major dominant species, especially for species that have a relatively high dependency on eelgrass blades. These results suggest that the different functional groups of sessile epibionts have significant roles in determining the biomass and diversity of mobile invertebrates in eelgrass beds.

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25 **ABSTRACT**

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29 water temperature, salinity, eelgrass biomass and epiphytic microalgae presence. However, the

30 importance of sessile epibionts, such as macroalgae and calcific spirorbid polychaetes attached to

31 eelgrass blades, has not been the focus of previous studies. In the present study, we examined the

32 effects of three different sessile epibionts, namely, branched red algae, filamentous green algae,

33 and calcific spirorbid polychaetes, on the biomass and diversity of mobile invertebrates in the

34 eelgrass beds of Akkeshi in Northeastern Japan. The relationships of the dominant mobile

35 invertebrate biomass, the total biomass of mobile invertebrates and the species diversity to

36 multiple abiotic and biotic variables were analyzed using a linear mixed model. Our results show

37 that large-sized epibiotic organisms can be good predictors of the variation in the total biomass,

38 species richness and species diversity of mobile invertebrates and the biomass of major dominant

39 species, especially for species that have a relatively high dependency on eelgrass blades. These

40 results suggest that the different functional groups of sessile epibionts have significant roles in

41 determining the biomass and diversity of mobile invertebrates in eelgrass beds.

42

43 **KEYWORDS**

44 Biomass; Community structure; Environmental gradient; Epibiotic organisms; Macroalgae;

45 Marine invertebrates; Linear mixed model; Species diversity; Spirorbid polychaetes; *Zostera*

46 *marina*

INTRODUCTION

The abundance, biomass and species diversity of marine benthic invertebrate communities vary greatly with multiple abiotic/biotic factors. The effects of temperature and salinity as environmental filters have been known to be critical factors that regulate population/community patterns and processes in coastal habitats, especially in estuaries where strong environmental gradients are generated by tidal fluctuation and freshwater inflow (e.g. Remane & Schlieper, 1971; Garrity, 1984; Montagna & Kalke, 1992; Williams & Morritt, 1995; Mannino & Montagna, 1997; Ysebaert et al., 1998; Ysebaert et al., 2003; Yamada et al., 2007b; Douglass et al., 2010; Titternsor et al., 2010; Blake & Duffy, 2010). Water temperature can either increase or decrease the abundance and diversity of component species (e.g. Somero, 2002; Harley et al., 2006; Hoegh-Guldberg & Bruno, 2010; Meager, Schlacher & Green, 2011), whereas a decrease in salinity generally leads to a lower species diversity and higher dominance by tolerant species (e.g. Remane & Schlieper, 1971; Montagna & Kalke, 1992; Mannino & Montagna, 1997; Ysebaert et al., 1998; Ysebaert et al., 2003; Yamada et al., 2007b). For the biotic factors, the effect of various macrophytes (e.g. kelps, sargassum, seagrasses) on mobile invertebrates has been investigated. Marine plants act as both a food resource because plant resource utilizers dominate in marine benthic invertebrate communities (e.g. Robertson & Lucas, 1983; Duffy & Hay, 1991; Duffy, Richardson & Canuel, 2003; Valentine & Heck, 1999; Harley, 2006; Aguilera & Navarrete, 2012; Poore et al., 2012) and as habitat-former (e.g. Dean & Connell, 1987; Lee, Fong & Wu, 2001; Attrill, Strong & Rowden, 2000; Thomsen, 2010; Gutow et al., 2012; Gartner et al., 2013).

Eelgrass (*Zostera marina*) is an important marine foundation species that is widely

70   distributed along the coast of the northern hemisphere (Hughes et al., 2009). The complex

71   physical structures created by eelgrass provide a habitat for many organisms (Heck & Orth,

72   1980; Orth, Heck & van Monfrans, 1984; Edgar et al., 1994; Jernakoff, Brearley & Nielsen,

73   1996; Heck, Hays & Orth, 2003), which leads to an enhanced biodiversity and secondary

74   production (Hemminga & Duarte, 2000; Duffy, 2006; Valentine & Duffy, 2006). A benthic

75   invertebrate community in the above-ground parts of seagrass beds mainly consists of small

76   crustaceans, gastropod mollusks and polychaetes, most of which are herbivores and detritivores

77   (Valentine & Heck, 1999; Heck et al., 2000). These invertebrates play an important role in

78   mediating the energy flow in the eelgrass bed ecosystem (Duffy & Hay, 2000; Duffy, Richardson

79   & France, 2005). To explore plant-animal interactions in eelgrass bed communities, many

80   studies have investigated the relationship between animal abundance and various eelgrass traits,

81   such as biomass, shoot density, leaf length, habitat patch structure, and epiphytic microalgal

82   biomass of (e.g. diatoms) that serve as food resources (Webster Rowden & Attrill, 1998; Attrill,

83   Strong & Rowden, 2000; Healey & Hovel, 2004; Gartner et al., 2013; Whalen, Duffy & Grace,

84   2013). However, large epibiotic organisms, such as macroalgae and sessile animals (e.g.

85   spirorbid polychaetes, tunicates, bryozoans, hydrozoans), attached to eelgrass blades can also

86   affect the mobile invertebrate community through resource provisioning and/or habitat

87   modification. Despite some studies noting that the role of macroalgae on seagrass blades as food

88   resource and habitat provider can be one of the determinants of the abundance of mobile

89   invertebrates (Hall & Bell, 1988; Bologna & Heck, 1999; Valentine & Duffy, 2006; Gartner et

90   al., 2013; Whalen, Duffy & Grace, 2013), most studies have focused only on the importance of

91   seagrass and/or microalgae (e.g. Orth, 1977; van Montfrans, Wetzel & Orth, 1984; Irlandi &

92   Peterson, 1991; Klumpp, Salita-Espinosa & Fortes, 1992; Jernakoff, Brearley & Nielsen, 1996;

93 Heck & Valentine, 2006). Regarding the effect of sessile animals on mobile invertebrates,

94 although the relevant studies remain few in seagrass systems, the relationships with tunicates and

95 scallops attached to eelgrass blades have been reported (Duffy & Harvilicz, 2001; Duffy,

96 Richardson & Canuel, 2003; Lefcheck et al., 2014; Long & Grosholz, 2015). Interpreting

97 variations in the mobile invertebrate community in relation to various functional groups of

98 epibiotic organisms is thus necessary to deepen our understanding of the organization of animal

99 assemblages in eelgrass beds and of the influences these organisms have on each other and

100 eelgrass.

101 An extensive eelgrass meadow, consisting mostly of *Zostera marina* and partly of *Z.*

102 *japonica* and *Ruppia maritima*, is located in the Akkeshi-ko estuary and the Akkeshi Bay in

103 eastern Hokkaido, Japan (Hasegawa, Hori & Mukai, 2007). From early summer to late fall, a

104 massive variety of algae and sessile animals (epibiotic species), which attach to eelgrass blades,

105 are observed, including microalgae, branched red algae, *Neosiphonia* sp., *Chondria dasyphylla*,

106 filamentous green algae, *Cladophora* sp., calcareous algae, *Circeis spirillum*, and spirorbid

107 polychaetes, such as *Neodexiospira brasiliensis*, bryozoans, hydrozoans, and tunicates. Among

108 them, microalgae, the branched red algae and the spirorbid polychaetes are dominant in eelgrass

109 beds for a long term, between early summer and late fall, with the peak of abundance between

110 August and September (Hamamoto & Mukai, 1999; Kasim & Mukai, 2006; Hasegawa, Hori &

111 Mukai, 2007; Momota, unpublished data). Previous studies on benthic invertebrate assemblages

112 in the Akkeshi-ko estuary and Akkeshi Bay have focused on their variability in relation to the

113 salinity gradient (Yamada et al., 2007a; Yamada et al., 2007b). In addition to salinity, the spatial

114 heterogeneity of other abiotic/biotic factors (e.g. water temperature, microalgal biomass and

115 eelgrass biomass) is also high in estuarine systems, such as the Akkeshi-ko estuary (Iizumi et al.,

116   1996; Kashim & Mukai, 2006; Hasegawa, Hori & Mukai, 2007). Nevertheless, no previous study

117   has investigated the mobile invertebrate community structure using an approach that

118   simultaneously accounts for the details of sessile epibionts and environmental control by abiotic

119   factors in the seagrass beds in Akkeshi.

120   In the present study, we investigated how multiple abiotic and biotic factors are related

121   to the variation in the community structure (total mobile invertebrate biomass, species richness

122   and species diversity) of the mobile invertebrates and the population biomass of the dominant

123   species in the eelgrass beds in Akkeshi. Our specific focus was to test the relationship between

124   various sessile epibionts on eelgrass blades and the mobile invertebrates that live on eelgrass

125   blades. Including these factors in the multivariate model, this analysis expands the classical

126   models that consider only abiotic factors, eelgrass and microalgae as the explanatory variables.

127

128

129   **MATERIALS AND METHODS**

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131   **Study area**

132

133   The Akkeshi-ko estuary (locally called Akkeshi Lake) and Akkeshi Bay are located in

134   Northeastern Hokkaido, Japan (Fig. 1) and are connected to each other through a narrow channel

135   (width: approximately 500 m, depth: approximately 10 m). The Akkeshi-ko is a brackish estuary,

136   shallow water (depth range in most of the lake: 0.8-1.7 m with the maximum difference in tide

137   levels of up to approximately ± 0.6 m), with an area of approximately 32 km^2. Most bottom areas

138   of the Akkeshi-ko estuary are muddy and covered with eelgrass (*Z. marina*) except for the

139 aquaculture farms of the clam *Venerupis philippinarum* in the intertidal zone near the channel

140 (Kashim & Mukai, 2006; Hasegawa, Hori & Mukai, 2007; Yamada et al., 2007a, Yamada et al.,

141 2007b). Here, freshwater input from the Bekanbeushi River, which accounts for 98.8% of all of

142 the flow volume (Iizumi et al., 1996), and tidal seawater input from the Akkeshi Bay cause steep

143 physical and chemical environmental gradients (Iizumi et al., 1996; Yamada et al., 2007a).

144 Akkeshi Bay has an area of approximately 110 km^2 and opens to the Pacific Ocean at the

145 south end. Two seagrass species *Z. marina* and *Zostera asiatica*, are present from the intertidal

146 zone to the subtidal zone (5 m below mean low water); the former occurs at depths shallower

147 than 2 m and the latter dominates in deeper water (Watanabe, Nakaoka & Mukai, 2005). The

148 influence of the freshwater discharge is observed near the channel connecting the bay to the

149 Akkeshi-ko estuary (Yamada et al., 2007a).

150 In this study, we established seven study stations consisting of six stations in the Akkeshi-ko

151 estuary, (BK: river mouth of the Bekanbeushi River, HN: Horonitai, TB: Toubai, SL: the

152 southern lakeside, CL: the central lake and CK: Chikarakotan) and one station in Akkeshi Bay

153 (SR: Shinryu) (Fig. 1). BK (mean sea level, MSL hereafter: 0.9 m) is located at the mouth of the

154 Bekanbeushi River and is strongly affected by freshwater inflow. The vegetation is dense with

155 small-sized *Z. marina* (average shoot length of 1.0 m in August). HN (MSL: 1.1 m) is in a

156 location with a high water temperature and medium salinity relative to the other stations. In

157 addition to *Z. marina*, *Rupia maritima*, a seagrass species that is more tolerant to low-saline

158 water, occurs at HN. The eelgrass beds at HN are mostly continuous but have some gaps, and the

159 average shoot length in August is 1.3 m. TB (MSL: 1.1 m) and SL (MSL: 1.0 m) have a

160 relatively low salinity compared to that of the other stations and are the furthest stations from the

161 Akkeshi Bay. Although these two stations are in a similar environment, the water is often more

162   turbid and the eelgrass bed is patchier at TB than SL. SL has a higher seagrass biomass and shoot

163   density than TB. The average shoot length of eelgrass is approximately 1.3 m in August at both

164   of these stations. CL (MSL: 1.4 m) and CK (MSL: 1.5 m) are deeper stations with a higher

165   salinity and are dominated by longer eelgrass (shoot length: 1.5-3.5 m at the peak season). The

166   eelgrass at SR (MSL: 1.5 m) in the Akkeshi Bay, has a similar shoot size to that in CL and CK.

167   Here, the dominant seagrass species changes from *Z. marina* bed to *Z. asiatica* at a depth of

168   approximately 2 m, as mentioned above.

169   According to Yamada et al. (2007a), salinity varies significantly among stations but does not

170   very greatly among seasons. During the summer (from July to August), eelgrass biomass,

171   microalgal biomass and mobile invertebrates reach their peak (Hasegawa, Hori & Mukai, 2007;

172   Yamada et al., 2007b). Seasonal changes in the mobile invertebrate species richness are not

173   clearly understood (Yamada et al., 2007b).

174

175   **Field sampling**

176

177   We conducted a field survey in August 2012. Sample collection was performed when the tidal

178   current was slow. We collected mobile invertebrates on eelgrass blades when the water level was

179   deeper than the average sheath length of the eelgrass at each station (BK: 20 cm; HN, TB, SL: 30

180   cm; CL, CK, SR: 40 cm). Because the eelgrass at our study stations is tall (> 1 m) compared to

181   the average water depth of each station, the canopy usually reaches the water surface (except for

182   at extremely high tides). All sampling was performed under these conditions. We targeted

183   mobile invertebrates and excluded some species with remarkably higher mobility and less

184   dependency on eelgrass habitat, such as mysids and decapods (Yamada et al., 2007b), which

185 were not quantitatively collected by our method (see below).

186 We measured water temperature and salinity once at each station using a memory sensor

187 (AAQ-175 RINKO, JFE Advantech Co. Ltd., Japan). To obtain the representative values, the

188 sensor was carefully placed approximately 50 cm from the bottom to accurately reflect the

189 environment inside of the seagrass meadow.

190 We collected three replicate samples (a total of 21 samples from all stations) of mobile

191 invertebrates, spirorbid polychaetes and epiphytic macroalgae together with the above-ground

192 parts of the eelgrass using a mesh bag (bore diameter: 20 cm, mesh size: 0.1 mm) based on the

193 mouth area of the mesh bag (314 cm^2). Upon collection, we counted the number of eelgrass

194 shoots to determine shoot density. For microalgae, five replicate samples were collected per

195 station together with one eelgrass shoot using a plastic zip bag, because microalgae easily fell off

196 from eelgrass blades when collected with the mesh bag.

197

198 **Laboratory procedures**

199

200 Immediately after being transported to the laboratory, the microalgae were scraped from the

201 eelgrass blades using a glass slide; separated from other organisms such as macroalgae and

202 spirorbid polychaetes; and then filtered using glass fiber filters (Whatman GF/F filter φ 47 mm,

203 Whatman International Ltd., Maidstone, UK). If other organisms were present in the microalgal

204 samples, we carefully removed them from the filters with forceps. Other epibiotic organisms

205 collected using mesh bags were separated from the eelgrass by scraping them off with a glass

206 slide; these organisms were classified as red algae, green algae, spirorbid polychaetes and mobile

207 invertebrates. To obtain dry mass, eelgrass shoots, red algae, green algae, spirorbid polychaetes

208   and filtered microalgae were dried at 60°C for 4 days in small aluminum foil bags, and then

209   weighed. We counted and identified the mobile invertebrates after extraction with a sieve (500

210   μm) and fixation with 70% ethanol. Identification of mobile invertebrates was made to the lowest

211   taxonomical unit possible (mostly to species) using detailed guides from the literature

212   (Gammarid amphipod: Nishimura, 1995; Carlton, 2007; Ogawa, 2011; Caprella amphipod,

213   Isopod, Copepod, Cumacea: Nishimura, 1995; Carlton, 2007; Gastropod: Okutani, 2000;

214   Polychaeta: Nishimura, 1992; Imajima, 1996; Imajima, 2001; Turbellaria: Nishimura, 1992;

215   Carlton, 2007; Hirunoidea: Nishimura, 1992) and the World Register of Marine Species online

216   database (WoRMS: http://www.marinespecies.org).

217

218   **Statistical analysis**

219

220   We used, as predictors, two abiotic factors (water temperature and salinity) and six biotic factors

221   (eelgrass biomass [g dry weight per unit area: g DW m^{-2}], eelgrass shoot density [shoots m^{-2}],

222   microalgal biomass [g DW m^{-2}], red algal biomass [g DW m^{-2}], green algal biomass [g DW m^{-2}]

223   and spirorbid polychaete biomass of [g DW m^{-2}]). For eelgrass biomass, we used the dry weight

224   data collected using mesh bags. Because microalgal biomass was collected by a different

225   sampling procedure from other biotic variables, we used the mean value of five replicates. All

226   invertebrate biomass (mg ash-free dry weight per unit area, mg AFDW m^{-2}) was estimated from

227   the abundance and the size fraction using the empirical equations in Edgar (1990).

228   To test which of the eight biotic/abiotic factors was a likely predictor of the variation in the

229   mobile invertebrate community, we fit linear mixed models (LMMs) with a Gaussian

230   distribution. The station was used as a random variable. As response variables, we used the

231   biomass of 11 dominant species for the population-level analyses, and total invertebrate biomass,

232   species richness and species diversity (Shannon-Wiener diversity index) for community-level

233   analyses. The 11 most dominant species were selected by a threshold whereby the biomass

234   proportion accounted for more than 1% of the total invertebrate biomass (see Table S1).

235   *Ostreobdella kakibir* (Hirudinoidea) was omitted from the analysis because it occurred only at

236   one station (SR), even though they satisfied the requirement. R software (version 3.1.3) was used

237   for all of the analyses (R Development Core Team, 2015).

238   Prior to the LMM fit, all of the variables excluding species diversity were square root

239   transformed to improve homoscedasticity and meet the assumptions of normality of the LMMs

240   after checking for normality with the Shapiro-Wilk test. To test for collinearity between the eight

241   environmental variables, we calculated Pearson's correlation coefficients for all pairs. If the

242   absolute value of the coefficient (r) was greater than 0.7, the level where collinearity does not

243   affect model predictions (Dormann et al., 2013), we removed the relevant predictor as necessary.

244   Because it followed that water temperature and microalgal biomass were highly correlated

245   (Pearson's r = -0.82, *P* < 0.01), we removed microalgal biomass from the models. After this

246   removal, we tested potential multicollinearity among the remaining predictors using the variance

247   inflation factor (VIF) analysis with a cutoff of 10 (e.g. Dormann et al., 2013). VIF values were

248   calculated   using   the   *vif.mer*   function   developed   by   Frank

249   (https://raw.githubusercontent.com/aufrank/R-hacks/master/mer-utils.R).   However,   all   seven

250   predictors were below the VIF value of 10 and remained. We therefore defined a reduced model

251   with the seven predictors as the full model.

252   We fit the LMMs using the *lmer* function in the lme4 package (Bates et al., 2014). To obtain

253   *P*-values of the LMMs, we used the *lmerTest* package (Kuznetsova et al., 2014). We selected the

254 optimal model comparing the candidate models on all combinations of the predictors by the

255 Akaike information criterion as corrected for the small sample size (AIC$_c$: Burnham & Anderson,

256 2002). We obtained AIC$_c$ based on the maximum likelihood (ML) for comparisons among the

257 LMMs because the restricted maximum likelihood (REML) is inappropriate in the case when the

258 fixed structure is different between the candidate models (Zuur et al., 2009), but the parameters

259 were estimated by REML. We used the *AIC$_c$tab* function in the *bbmle* library (Bolker & R Core

260 Team, 2013) to compare the AIC$_c$. After setting the optimal models, we obtained the

261 standardized coefficients as effect sizes by re-fitting using standardized variables that were

262 scaled by the sample standard deviation and centered by sample mean values.

263 Additionally, when the effect of water temperature was detected, we tested the relationship

264 between mobile invertebrates and microalgal biomass which was omitted from the LMM

265 because of the multi-collinearity with water temperature.

266

267

268 **RESULTS**

269

270 **Environmental factors**

271

272 Water temperature was lower at the four stations (BK, CL, CK and SR) near the channel than at

273 the other three stations in the inner parts of the estuary (HN, TB and SL) (Table 1). Salinity was

274 lower at the lakeside stations (BK, HN, TB, SL and CK) that were influenced by freshwater

275 inputs. For these stations, the inter-annual variation was also higher (Table 1).

276 Eelgrass biomass varied between 140 and 278 g DW m^{-2} among the stations. It was the

277   lowest at TB, followed by HN and BK (Table 1). Eelgrass shoot density ranged between 85 and

278   234 shoot m^{-2}. It was highest at BK and second highest at SL. The mean densities were not

279   largely different among other stations. Microalgal biomass varied by more than ten-fold between

280   the lowest station (SL) and the highest station (CL). In the latter, the microalgal biomass

281   exceeded the biomass of the eelgrass. Macroalgae were not present at TB and SR. Red algae

282   were dominated by *Neosiphonia* sp. and *Chondria dasyphylla*, and green algae were dominated

283   by *Cladophora* sp. The mean biomass of red algae was highest at HN and that of green algae was

284   highest at CL, although their biomasses were less than 15 % that of eelgrass. Spirorbid

285   polychaetes were not present at SL and CL. They were highly abundant at SR where their

286   biomass was more than three-fold greater than the eelgrass biomass.

287

288   **Mobile invertebrate community**

289

290   A total of 32 mobile invertebrate species were collected in this study (Table S1). At taxonomic

291   levels, polychaete worms made up 32.2% of the total biomass, followed by gastropods (31.3%),

292   gammarid amphipods (23.0%), and isopods (8.8%). At the species level, a polychaete *Nereis* sp.

293   was most dominant (24.6%), followed by gastropods *Lacuna* spp. (23.4%) and the gammarid

294   amphipod *Ampithoe lacertosa* (18.0%). For an additional eight species including two gammarid

295   amphipod (*Monocorophium* spp. and *Pontogeneia rostrata*), two isopods (*Cymodoce japonica*

296   and *Paranthura japonica*), two gastropods (*Ansola angustata* and *Siphonacmea oblongata*) and

297   two polychaetes (*Exogone naidina* and *Syllis* sp.), their proportions were less than 5% at most.

298   The mean value of the total mobile invertebrate biomass was the highest at CK and

299   much lower at stations along the coastline (HN, TB and SL). Species richness was the highest at

300 CL, followed by CK and was approximately the same level at the other stations (Fig. 2). The

301 mean value of species diversity was the highest at CL and the lowest at SR (Fig. 2).

302

303 **Population level analyses**

304

305 We found that each of the nine invertebrate populations belonging to gammaridae, gastropoda

306 and polychaeta was predicted by a different combination of environmental factors in the optimal

307 models (Table 2). For two isopods, no environmental factor correlated with their biomass.

308   Water temperature was selected as the responsible factor for the variation of *A.*

309 *lacertosa*, *Lacuna* spp. and all three polychaetes. Among them, only *Syllis* sp. showed a

310 significant correlation (positive). The significant effect of the salinity gradient was detected for *A.*

311 *angustata* (negative) and *S. oblongata* (positive).

312   For the two predictors relevant to the characteristics of the eelgrass bed, the above-ground

313 biomass showed a significantly positive relationship only with *Syllis* sp., whereas shoot density

314 was significantly correlated with *Monocorophium* spp. (positive), *P. rostrata* (negative) and *E.*

315 *naidina* (negative).

316   The biomasses of sessile epibionts (red algae, green algae and spirorbid polychaetes) on

317 eelgrass blades were correlated with many invertebrate populations excluding *A. lacertosa*,

318 *Monocorophium* spp., two isopods and *E. naidina* in different manners. Red algal biomass was

319 positively correlated with *P. rostrata*, *Lacuna* spp. and *Nereis* sp., but negatively correlated with

320 *Syllis* sp. and tended to be negatively correlated with *A. angustata*. Green algal biomasses were

321 positively correlated with *P. rostrate* and *Lacuna* spp. The biomass of spirorbid polychaetes was

322 positively correlated with all three species of gastropods and was negatively correlated with

323   *Syllis* sp.

324   Although epiphytic microalgae were removed from our analysis because of the

325   collinearity with water temperature, no significant correlation was found for species that were

326   correlated with water temperature (*A. lacertosa*: Pearson's r = 4.05, *P* = 0.25; *Lacuna* spp.: r = -

327   3.42, *P* = 0.69; *E. naidina*: r = 0.31, *P* = 0.92; *Nereis* sp.: r = 10.87, *P* = 0.34; *Syllis* sp.: r = 0.21,

328   *P* = 0.86).

329

330   **Community level analyses**

331

332   The total invertebrate biomass tended to decrease with water temperature, and significantly

333   increased with increasing eelgrass biomass and red algal biomass (Table 2). Species richness

334   showed a negative correlation with water temperature and spirorbid polychaetes. Species

335   diversity was positively correlated with green algal biomass, but was negatively correlated with

336   spirorbid polychaetes (Table 2).

337

338

339   **DISCUSSION**

340

341   The present study demonstrated that the biomass gradient of large-sized epibiotic organisms (e.g.

342   macroalgae and spirorbid polychaetes) was a good predictor of the variation in the mobile

343   invertebrate community in the eelgrass bed and the population biomass of some dominant

344   component species. The effect of the macroalgae is notable because these sessile epibionts have

345   a much lower biomass compared to the biomass of eelgrass and epiphytic microalgae. However,

346 the observed relationships between these functional groups and mobile invertebrate populations

347 varied greatly among the species.

348   In the optimal models, the effects of biomass of epibiotic organisms on the gammarid

349 amphipod *P. rostrata*, all three gastropod species (*A. angustata*, *Lacuna* sp. and *S. oblongata*)

350 and two polychaetes (*Nereis* sp. and *Syllis* sp.) were detected. For those species, the sessile

351 epibionts were positively related to mobile invertebrate biomasses except for *Syllis* sp. and *P.*

352 *rostrata*, which showed a positive correlation with both red and green algae. The algae are

353 considered to be used as a temporal shelter (habitat) rather than as a food resource because these

354 animals do not firmly attach to the eelgrass blades but rather drift among shoots (Suh & Yu,

355 1997; Yamada et al., 2007b; Yu, Jeong & Suh, 2008), and because they have a preference for

356 feeding on phytoplankton and detritus (Yu & Suh, 2011). High predation risk for swimming

357 amphipods with low self-defense abilities, such as *P. rostrata*, has been reported in several

358 studies (Sudo & Azeta, 1992; Beare & Moore, 1998). In fact, gammarid amphipods are a major

359 source of prey for blennoid fish in the eelgrass beds of Northern Japan (Watanabe et al., 1996;

360 Sawamura, 1999; Yamada et al., 2010). Therefore, the complex micro-habitat created by

361 macroalgae allows them to escape these predators.

362   All three gastropods increased in correlation with spirorbid polychaetes, whereas the

363 responses to the other factors were different (Table 2). The positive effect of spirorbid

364 polychaetes appears to result from habitat-formation in terms of our assumption, but should not

365 be oversimplified because of the overlap in the life cycle and because of the functional mismatch.

366 Because the gastropods adhere to flat seagrass blades, the flat (simple) structure created by

367 seagrass blades can be better than the rough structure of spirorbid polychaetes. Therefore,

368 competition for space (negative effect) appears to be more expected than facilitation. Although

369   we do not have a good answer for the positive relationships, one possibility for this unexpected

370   result is that the rough structure acts as a shelter because small-sized individuals (< 3 mm) are

371   frequent in gastropod populations during the summer season (*A. angustata*: Momota, personal

372   observation; *Lacuna* spp.: Kanamori, Goshima & Mukai, 2004; S. oblongata: Toyohara,

373   Nakaoka & Tsuchida, 2001).

374   Red algae are considered to positively affect *Nereis* sp. by provisioning habitat because

375   polychaetes build tubes both on eelgrass blades and in red algal canopies in Akkeshi (Momota,

376   unpublished data). The negative effect of red algae and spirorbid polychaetes on *Syllis* sp. may

377   suggest that this mobile polychaete prefers a simple structured habitat without a complex micro-

378   habitat created by eelgrass blades with sessile epibionts.

379   In addition to the effects of sessile epibionts, the significant effects of water temperature,

380   salinity, eelgrass biomass and shoot density were detected for a majority of the dominant species,

381   although the patterns and directions of the effects were different among them. Surprisingly,

382   eelgrass biomass was not correlated with most species except for *Syllis* sp., and the direction

383   (positive/negative) of the effect of eelgrass shoot density was different among the species. The

384   same response of syllid polychaetes was reported in previous studies (e.g. Bone & San Martín,

385   2003). For eelgrass shoot density, the result suggests that it indirectly affects mobile

386   invertebrates through interfering with multiple physical and biological processes (e.g. water

387   current and flux, detritus and drifting algae trapping, recruitment, and predation intensity: Gambi,

388   Nowell & Jumars, 1990; Robbins & Bell, 1994; Attrill, Strong & Rowden 2000; Boström &

389   Bonsdorff, 2000; Lee et al. 2001; Hovel et al., 2002). Notably, the contrasting relationships of *P.*

390   *rostrate* with eelgrass shoot density and macroalgae imply that the shelter effect is different

391   depending on the spatial scale (i.e. blade scale, shoot/patch scale).

392   The isopods *C. japonica* and *P. japonica* were not correlated with any abiotic or biotic

393   factors because of the low dependency on the seagrass habitat; they can utilize other numerous

394   habitats created by both natural and artificial materials (e.g. mussel beds, oyster reefs: Marchini

395   et al., [2014]; Nakamachi, Ishida & Hirohashi, [2015]; gravel, litter layer of macrophytes,

396   *Sargassum* meadow: Momota, personal observation). Additionally, their uniform appearance

397   throughout all of the stations indicates that they have a wide tolerance to a broad range of

398   environmental stress, which leads to a lack of correlation with any of the abiotic factors.

399   Additionally, the gammarid amphipod *A. lacertosa* was not significantly correlated with any

400   factors. This species is widely distributed along the Pacific-rim coast of the northern hemisphere

401   and utilizes a variety of plant habitats by building tubes (Hiebert, 2013), which may explain why

402   it did not show any relationship with the environmental gradients.

403   Although the discussion on underlying drivers that generate apparent correlations (i.e. the

404   causalities) between epibionts and mobile invertebrates is not our main focus, the indirect effects

405   and the top-down control of mobile invertebrates should also be taken into account to interpret

406   present findings. For example, we can give an alternative possibility for the positive relationship

407   between gastropods and spirorbid polychaetes such that high grazing of the gastropods facilitates

408   the recruitment of spirorbid polychaetes through the removal of the microalgal cover.

409   Total biomass, species richness and species diversity were differentially correlated with

410   abiotic/biotic factors, and varied in a complex manner although processes were unclear. The

411   optimal model of the three community variables contains one or two variables of sessile

412   epibionts. The positive correlation of total biomass with red algae reflects a result pulled by the

413   biomass proportions of highly dominant species, such as *Lacuna* spp. and *Nereis* sp. The

414   negative interaction of spirorbid polychaetes with species richness and diversity suggest that

415   spirorbid polychaetes can decrease the homogeneity of the biomasses of component species

416   within a community by allowing some competitive species to dominate. Because no effect on

417   species diversity, total biomass, species richness, or most of the dominant species populations

418   was detected by green algae, it may work on species evenness.

419

420

421   **CONCLUSIONS**

422

423   The present study suggests that macroalgae and sessile animals on eelgrass blades can affect the

424   biomass and diversity of mobile invertebrates and that incorporating these biotic factors can

425   improve the prediction of the variability of the mobile invertebrate community in the eelgrass

426   bed. However, the underlying causal relationships appear to be complex and vary greatly from

427   species to species. Our findings were based on data collected over one sampling period when the

428   eelgrass bed was most productive and when the abundance and/or diversity of algae and mobile

429   invertebrates typically reached their maximum. A more comprehensive investigation of the

430   functional relationships among the various types of organisms and of the temporal changes

431   should be conducted in future studies on eelgrass bed communities.

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433

434   **ACKNOWLEDGEMENTS**

435

436   We wish to thank S. Hamano, H. Katsuragawa and other members in Akkeshi Marine Station,

437   N.M. Kollars in UC Davis, Dr. K. Abe and other staff in National Research Institute of Fisheries

438   and Environment of Inland Sea, and T. Maezawa in Hokkaido University. This study is partly

439   supported by JST/CREST (Establishment of core technology for the preservation and

440   regeneration of marine biodiversity and ecosystems), and by JSPS/ KAKENHI (no. 21241055) to

441   M.N.

442

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709   **List of figures**

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711   **Fig. 1.** Location of the study sites in the Akkeshi-ko estuary and the Akkeshi Bay in

712   Northeastern Japan. The area enclosed by a dashed circle is the Akkeshi-ko estuary. Most of the

713   clam aquaculture grounds are located in the western part of the estuary (indicated by a dotted

714   circle).

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716   **Fig. 2.** The total invertebrate biomass, species richness and Shannon-Wiener diversity index at

717   the seven stations in the Akkeshi-ko estuary and Akkeshi Bay. The bars indicate the mean values

718   with SDs. The order of the stations is lined up based on relative size of the impact of freshwater

719   inflow or seawater from Akkeshi Bay.

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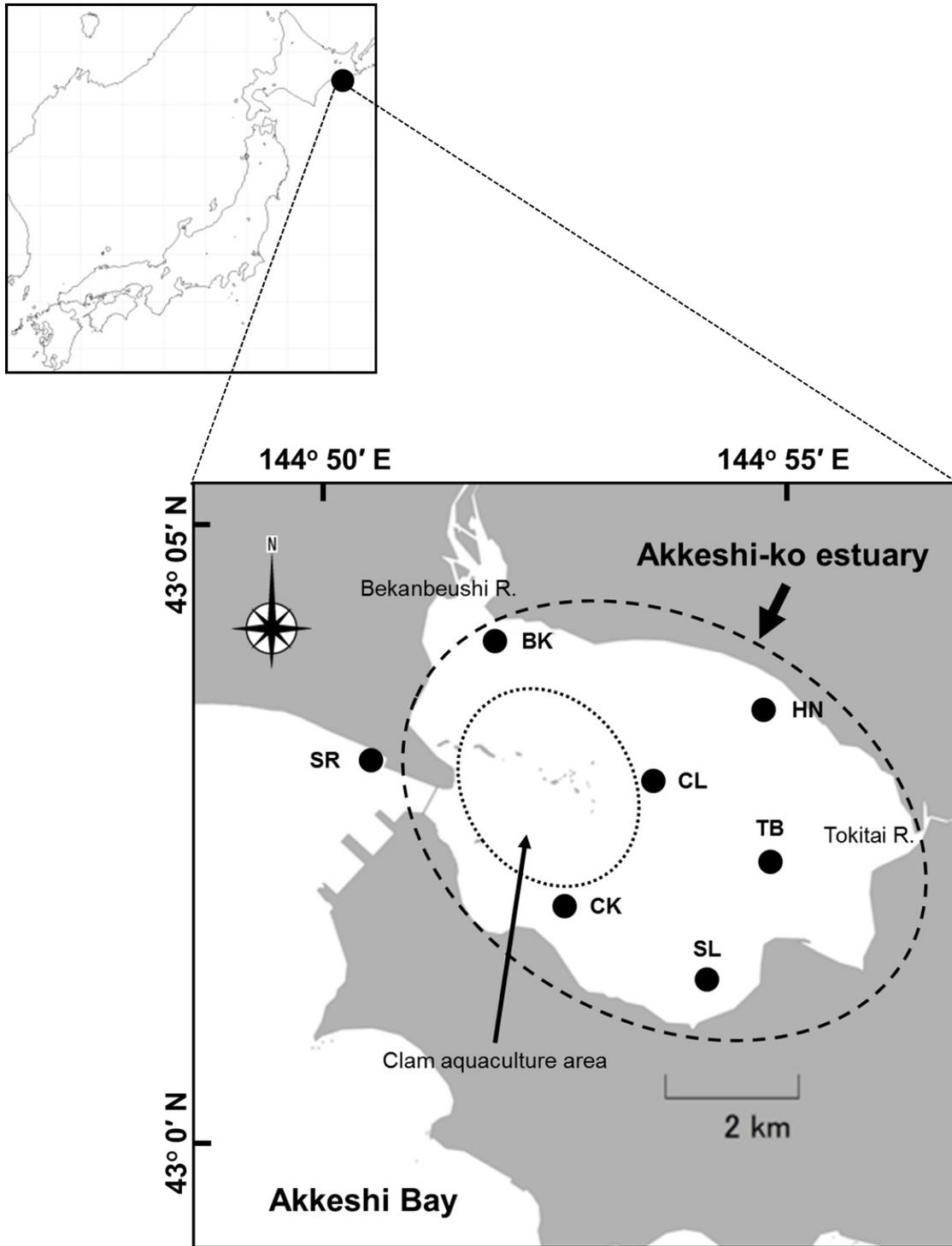
734   **Fig. 1.** Momota & Nakaoka

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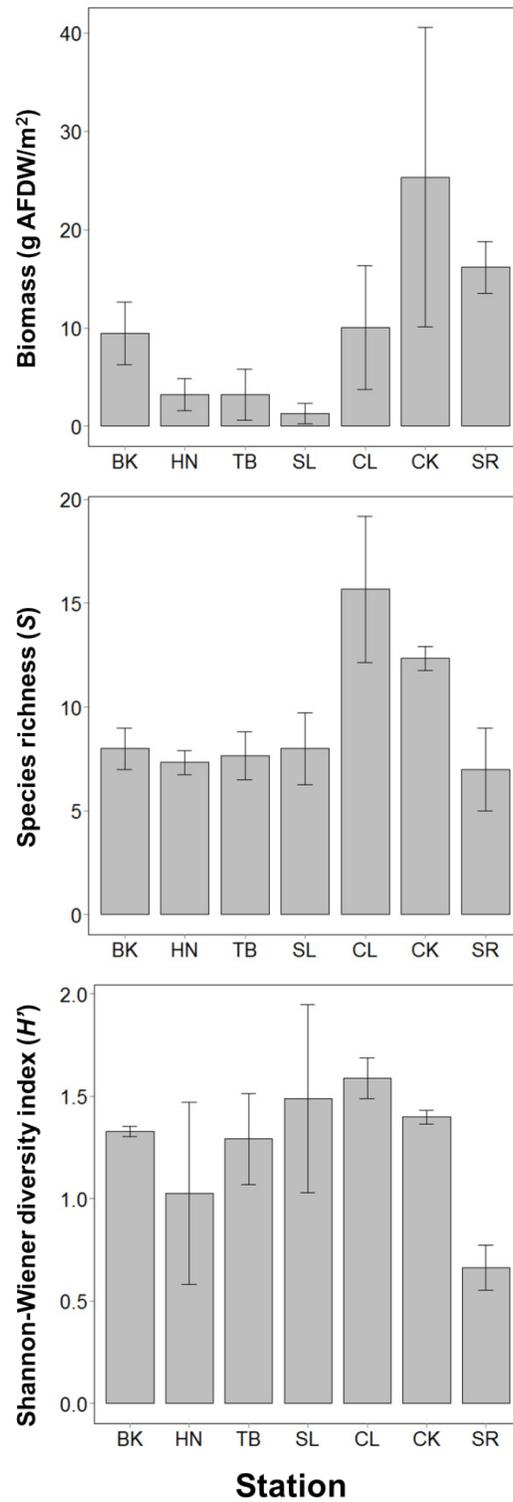
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738   **Fig. 2.** Momota & Nakaoka

739   **Table**

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Table 1. Environmental conditions at seven stations in the Akkeshi-ko estuary and Akkeshi Bay. Abiotic factors in this study are indicated by **boldface**. For water temperature and salinity, we also presented data in August reported by the other studies.

Factors		Stations							Ref.
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	
Abiotic									
Water temperature (°C)		**23.8**	**26.1**	**25.9**	**25.5**	**21.0**	**22.6**	**22.5**	a
		21.4	22.4	22.9	—	22.5	20.0	18.8	b
		18.1	20.3	20.3	21.0	18.5	17.3	16.6	c
		21.7	24.1	23.8	23.9	21.9	22.7	18.8	d
Salinity		**25.0**	**26.4**	**27.0**	**27.1**	**29.2**	**26.3**	**29.9**	a
		16.8	28.1	28.4	—	29.6	32.0	28.6	b
		16.1	—	—	23.9	26.0	26.5	29.6	e
		26.7	25.0	13.6	22.4	27.4	28.4	29.9	c
		21.2	23.6	26.0	26.2	26.8	26.7	29.9	d
Biotic									
Eelgrass factor									
Dry mass (g m^{-2})	Mean	152.2	140.4	119.5	216.3	216.8	190.3	277.9	a
	SD	25.8	37.3	30.8	30.9	26.8	65.0	68.5	
Shoot density (m^{-2})	Mean	233.7	85.3	74.7	159.0	85.3	85.3	96.0	a
	SD	18.5	18.5	18.5	18.5	0.0	18.5	18.5	
Epibiont dry mass									
Microalgae (g m^{-2})	Mean	73.2	25.6	77.9	19.2	384.5	113.4	76.3	a
	SD	63.9	6.5	46.6	5.0	119.8	58.9	26.2	
Red algae (g m^{-2})	Mean	0.1	9.0	0.0	4.1	0.0	4.6	0.0	a
	SD	0.1	6.0	—	2.2	0.0	7.6	—	
Green algae (g m^{-2})	Mean	7.5	0.0	0.0	8.2	28.0	0.1	0.0	a
	SD	7.4	—	—	4.3	16.2	0.0	—	
Spirorbid shell (g m^{-2})	Mean	53.5	21.8	6.8	0.0	0.0	1.9	944.3	a
	SD	28.0	18.7	7.6	—	—	3.2	190.6	

[a] This study; [b] Iizumi et al., (1996); [c] Nakaoka et al., (unpubl.); [d] Momota, (unpubl.); [e] Kashim and Mukai, (2006)

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Table 2. Results of LMMs for explaining responsible environmental factors on variation in mobile invertebrate populations and community components. AIC_c scores and delta AIC_c are also reported. Significant coefficients (*P*-values < 0.05 level) and the lowest AIC_c scores are in **bold face**. Standardized coefficients (*Std.coef*) of the optimal models are reported right below each optimal model in *italic face*. Abbreviations as follows; WT: water temperature, Sal: salinity, ZM.bm: eelgrass biomass, ZM.den: eelgrass shoot density, Red.alg: red algal biomass, Grn.alg: green algal biomass, SP.bm: spirorbid polychaete biomass.

| Response | Model | Predictor | | | | | | | | AIC_c | ΔAIC_c |
		(Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm		
Population											
Gammarid amphipoda											
Ampithoe lacertosa	Null	**33.978**	—	—	—	—	—	—	—	193.9	1.0
	Full	673.282	-87.465	-48.498	-0.482	4.427	7.243	-0.761	-0.736	216.7	23.8
	Optimal	**1097.930**	-79.180	-129.700						**192.9**	—
	Std.coef	*< -0.01*	*-0.601*	*-0.790*						—	—
Monocorophium spp.	Null	8.669	—	—	—	—	—	—	—	137.7	2.0
	Full	582.409	-42.288	-72.767	0.079	0.986	0.265	0.428	0.059	160.1	24.4
	Optimal	-6.824				**1.469**				**135.7**	—
	Std.coef	*< -0.01*				*0.249*				—	—
Pontogeneia rostrata	Null	**10.068**	—	—	—	—	—	—	—	147.2	19.8
	Full	247.381	-11.576	-32.023	0.407	**-2.751**	**4.934**	**2.878**	0.111	139.5	12.1
	Optimal	**20.728**				**-1.842**	**5.023**	**2.523**		**127.4**	—
	Std.coef	*< -0.01*				*-0.541*	*0.775*	*0.611*		—	—

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Table 2. (continued 1)

Response	Model	Predictor (Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm	AIC$_c$	Δ AIC$_c$
Isopoda											
Cymodoce japonica	Null	**11.630**	—	—	—	—	—	—	—	**187.0**	0.0
	Full	605.245	-15.501	-100.306	4.532	-5.585	0.308	2.552	-0.113	211.1	24.1
	Optimal	**11.630**								**187.0**	—
	Std.coef	*< -0.01*								—	—
Paranthura japonica	Null	**14.077**	—	—	—	—	—	—	—	**173.4**	0.0
	Full	132.132	8.691	-32.148	0.349	-0.924	2.623	4.411	0.463	200.0	26.6
	Optimal	**14.077**								**173.4**	—
	Std.coef	*< 0.01*								—	—
Gastropoda											
Ansola angustata	Null	6.014	—	—	—	—	—	—	—	159.6	10.1
	Full	555.710	-12.507	**-96.596**	-0.524	1.180	-1.487	3.730	**0.923**	167.3	17.8
	Optimal	**600.167**		**-116.259**			-2.645	**5.102**	**1.149**	**149.5**	—
	Std.coef	*< -0.01*		*-1.137*			*-0.217*	*0.658*	*0.767*	—	—
Lacuna spp.	Null	28.820	—	—	—	—	—	—	—	197.0	11.2
	Full	880.106	-129.988	-43.910	0.940	-2.094	**10.442**	-2.761	**2.607**	203.9	18.1
	Optimal	522.161	-106.591				**10.634**		**2.696**	**185.8**	—
	Std.coef	*< 0.01*	*-0.522*				*0.351*		*0.723*	—	—

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Table 2. (continued 2)

Response	Model	Predictor (Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm	AIC$_c$	Δ AIC$_c$
Siphonacmea oblongata	Null	8.003	—	—	—	—	—	—	—	166.6	17.2
	Full	-350.288	-16.668	**82.450**	-1.654	2.567	1.664	-2.471	1.063	172.7	23.3
	Optimal	**-190.996**		36.374					**1.426**	**149.4**	—
	Std.coef	*< 0.01*		*0.287*					*0.768*	—	—
Polychaeta											
Exogone naidina	Null	8.274	—	—	—	—	—	—	—	182.1	> 0.1
	Full	1003.724	-66.607	-119.185	1.236	-5.958	-1.510	0.675	-0.184	203.4	21.3
	Optimal	**988.365**	-75.725	-106.916		**-4.922**				**182.1**	—
	Std.coef	*< -0.01*	*-0.728*	*-0.825*		*-0.607*				—	—
Nereis sp.	Null	23.110	—	—	—	—	—	—	—	211.7	6.4
	Full	1994.677	-171.788	-216.463	5.651	-9.760	**13.928**	7.192	0.017	221.1	15.8
	Optimal	844.824	-171.482				**16.967**			**205.3**	—
	Std.coef	*< -0.01*	*-0.741*				*0.494*			—	—
Syllis sp.	Null	**6.678**	—	—	—	—	—	—	—	175.2	1.3
	Full	-342.880	**45.302**	14.699	**5.108**	-0.141	**-6.889**	-1.247	-1.140	191.5	17.6
	Optimal	**-269.866**	**45.615**		**4.886**		**-6.616**		**-0.908**	**173.9**	—
	Std.coef	*< -0.01*	*0.650*		*0.905*		*-0.636*		*-0.710*	—	—

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Table 2. (continued 3)

Response	Model	Predictor								AIC$_c$	Δ AIC$_c$
		(Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm		
Community component											
Total invertebrate biomass	Null	**2.785**	—	—	—	—	—	—	—	72.0	10.3
	Full	60.096	-5.936	-5.985	**0.214**	-0.097	**0.456**	0.184	0.056	81.0	19.3
	Optimal	23.569	-4.937		**0.219**		**0.401**			**61.7**	—
	Std.coef	*< 0.01*	*-0.645*		*0.372*		*0.354*			—	—
Species richness	Null	**3.027**	—	—	—	—	—	—	—	28.0	14.7
	Full	**16.581**	**-1.485**	-1.211	0.080	-0.094	-0.036	0.080	-0.023	33.6	20.3
	Optimal	**13.909**	**-2.185**						**-0.031**	**13.3**	—
	Std.coef	*< 0.01*	*-0.798*						*-0.619*	—	—
Species diversity	Null	**1.255**	—	—	—	—	—	—	—	19.9	8.6
	Full	2.056	-0.126	-0.049	0.010	0.012	-0.068	0.034	-0.026	36.7	25.4
	Optimal	**1.288**						**0.065**	**-0.020**	**11.3**	—
	Std.coef	*< 0.01*						*0.354*	*-0.578*	—	—

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