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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.

TITLE

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

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25 ABSTRACT

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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; Tittersor 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

distributed along the coast of the northern hemisphere (Hughes et al., 2009). The complex physical structures created by eelgrass provide a habitat for many organisms (Heck & Orth, 1980; Orth, Heck & van Montfrans, 1984; Edgar et al., 1994; Jernakoff, Brearley & Nielsen, 1996; Heck, Hays & Orth, 2003), which leads to an enhanced biodiversity and secondary production (Hemminga & Duarte, 2000; Duffy, 2006; Valentine & Duffy, 2006). A benthic invertebrate community in the above-ground parts of seagrass beds mainly consists of small crustaceans, gastropod mollusks and polychaetes, most of which are herbivores and detritivores (Valentine & Heck, 1999; Heck et al., 2000). These invertebrates play an important role in mediating the energy flow in the eelgrass bed ecosystem (Duffy & Hay, 2000; Duffy, Richardson & France, 2005). To explore plant-animal interactions in eelgrass bed communities, many studies have investigated the relationship between animal abundance and various eelgrass traits, such as biomass, shoot density, leaf length, habitat patch structure, and epiphytic microalgal biomass of (e.g. diatoms) that serve as food resources (Webster Rowden & Attrill, 1998; Attrill, Strong & Rowden, 2000; Healey & Hovel, 2004; Gartner et al., 2013; Whalen, Duffy & Grace, 2013). However, large epibiotic organisms, such as macroalgae and sessile animals (e.g. spirorbid polychaetes, tunicates, bryozoans, hydrozoans), attached to eelgrass blades can also affect the mobile invertebrate community through resource provisioning and/or habitat modification. Despite some studies noting that the role of macroalgae on seagrass blades as food resource and habitat provider can be one of the determinants of the abundance of mobile invertebrates (Hall & Bell, 1988; Bologna & Heck, 1999; Valentine & Duffy, 2006; Gartner et al., 2013; Whalen, Duffy & Grace, 2013), most studies have focused only on the importance of seagrass and/or microalgae (e.g. Orth, 1977; van Montfrans, Wetzel & Orth, 1984; Irlandi & Peterson, 1991; Klumpp, Salita-Espinosa & Fortes, 1992; Jernakoff, Brearley & Nielsen, 1996;

Heck & Valentine, 2006). Regarding the effect of sessile animals on mobile invertebrates, although the relevant studies remain few in seagrass systems, the relationships with tunicates and scallops attached to eelgrass blades have been reported (Duffy & Harvilicz, 2001; Duffy, Richardson & Canuel, 2003; Lefcheck et al., 2014; Long & Grosholz, 2015). Interpreting variations in the mobile invertebrate community in relation to various functional groups of epibiotic organisms is thus necessary to deepen our understanding of the organization of animal assemblages in eelgrass beds and of the influences these organisms have on each other and eelgrass.

An extensive eelgrass meadow, consisting mostly of *Zostera marina* and partly of *Z. japonica* and *Ruppia maritima*, is located in the Akkeshi-ko estuary and the Akkeshi Bay in eastern Hokkaido, Japan (Hasegawa, Hori & Mukai, 2007). From early summer to late fall, a massive variety of algae and sessile animals (epibiotic species), which attach to eelgrass blades, are observed, including microalgae, branched red algae, *Neosiphonia* sp., *Chondria dasyphylla*, filamentous green algae, *Cladophora* sp., calcareous algae, *Circeis spirillum*, and spirorbid polychaetes, such as *Neodexiospira brasiliensis*, bryozoans, hydrozoans, and tunicates. Among them, microalgae, the branched red algae and the spirorbid polychaetes are dominant in eelgrass beds for a long term, between early summer and late fall, with the peak of abundance between August and September (Hamamoto & Mukai, 1999; Kasim & Mukai, 2006; Hasegawa, Hori & Mukai, 2007; Momota, unpublished data). Previous studies on benthic invertebrate assemblages in the Akkeshi-ko estuary and Akkeshi Bay have focused on their variability in relation to the salinity gradient (Yamada et al., 2007a; Yamada et al., 2007b). In addition to salinity, the spatial heterogeneity of other abiotic/biotic factors (e.g. water temperature, microalgal biomass and eelgrass biomass) is also high in estuarine systems, such as the Akkeshi-ko estuary (Iizumi et al.,

1996; Kashim & Mukai, 2006; Hasegawa, Hori & Mukai, 2007). Nevertheless, no previous study has investigated the mobile invertebrate community structure using an approach that simultaneously accounts for the details of sessile epibionts and environmental control by abiotic factors in the seagrass beds in Akkeshi.

In the present study, we investigated how multiple abiotic and biotic factors are related to the variation in the community structure (total mobile invertebrate biomass, species richness and species diversity) of the mobile invertebrates and the population biomass of the dominant species in the eelgrass beds in Akkeshi. Our specific focus was to test the relationship between various sessile epibionts on eelgrass blades and the mobile invertebrates that live on eelgrass blades. Including these factors in the multivariate model, this analysis expands the classical models that consider only abiotic factors, eelgrass and microalgae as the explanatory variables.

MATERIALS AND METHODS

Study area

The Akkeshi-ko estuary (locally called Akkeshi Lake) and Akkeshi Bay are located in Northeastern Hokkaido, Japan (Fig. 1) and are connected to each other through a narrow channel (width: approximately 500 m, depth: approximately 10 m). The Akkeshi-ko is a brackish estuary, shallow water (depth range in most of the lake: 0.8-1.7 m with the maximum difference in tide levels of up to approximately ± 0.6 m), with an area of approximately 32 km². Most bottom areas of the Akkeshi-ko estuary are muddy and covered with eelgrass (*Z. marina*) except for the

aquaculture farms of the clam *Venerupis philippinarum* in the intertidal zone near the channel (Kashim & Mukai, 2006; Hasegawa, Hori & Mukai, 2007; Yamada et al., 2007a, Yamada et al., 2007b). Here, freshwater input from the Beganbeushi River, which accounts for 98.8% of all of the flow volume (Iizumi et al., 1996), and tidal seawater input from the Akkeshi Bay cause steep physical and chemical environmental gradients (Iizumi et al., 1996; Yamada et al., 2007a).

Akkeshi Bay has an area of approximately 110 km² and opens to the Pacific Ocean at the south end. Two seagrass species *Z. marina* and *Zostera asiatica*, are present from the intertidal zone to the subtidal zone (5 m below mean low water); the former occurs at depths shallower than 2 m and the latter dominates in deeper water (Watanabe, Nakaoka & Mukai, 2005). The influence of the freshwater discharge is observed near the channel connecting the bay to the Akkeshi-ko estuary (Yamada et al., 2007a).

In this study, we established seven study stations consisting of six stations in the Akkeshi-ko estuary, (BK: river mouth of the Beganbeushi River, HN: Horonitai, TB: Toubai, SL: the southern lakeside, CL: the central lake and CK: Chikarakotan) and one station in Akkeshi Bay (SR: Shinryu) (Fig. 1). BK (mean sea level, MSL hereafter: 0.9 m) is located at the mouth of the Beganbeushi River and is strongly affected by freshwater inflow. The vegetation is dense with small-sized *Z. marina* (average shoot length of 1.0 m in August). HN (MSL: 1.1 m) is in a location with a high water temperature and medium salinity relative to the other stations. In addition to *Z. marina*, *Rupia maritima*, a seagrass species that is more tolerant to low-saline water, occurs at HN. The eelgrass beds at HN are mostly continuous but have some gaps, and the average shoot length in August is 1.3 m. TB (MSL: 1.1 m) and SL (MSL: 1.0 m) have a relatively low salinity compared to that of the other stations and are the furthest stations from the Akkeshi Bay. Although these two stations are in a similar environment, the water is often more

turbid and the eelgrass bed is patchier at TB than SL. SL has a higher seagrass biomass and shoot density than TB. The average shoot length of eelgrass is approximately 1.3 m in August at both of these stations. CL (MSL: 1.4 m) and CK (MSL: 1.5 m) are deeper stations with a higher salinity and are dominated by longer eelgrass (shoot length: 1.5-3.5 m at the peak season). The eelgrass at SR (MSL: 1.5 m) in the Akkeshi Bay, has a similar shoot size to that in CL and CK. Here, the dominant seagrass species changes from *Z. marina* bed to *Z. asiatica* at a depth of approximately 2 m, as mentioned above.

According to Yamada et al. (2007a), salinity varies significantly among stations but does not very greatly among seasons. During the summer (from July to August), eelgrass biomass, microalgal biomass and mobile invertebrates reach their peak (Hasegawa, Hori & Mukai, 2007; Yamada et al., 2007b). Seasonal changes in the mobile invertebrate species richness are not clearly understood (Yamada et al., 2007b).

Field sampling

We conducted a field survey in August 2012. Sample collection was performed when the tidal current was slow. We collected mobile invertebrates on eelgrass blades when the water level was deeper than the average sheath length of the eelgrass at each station (BK: 20 cm; HN, TB, SL: 30 cm; CL, CK, SR: 40 cm). Because the eelgrass at our study stations is tall (> 1 m) compared to the average water depth of each station, the canopy usually reaches the water surface (except for at extremely high tides). All sampling was performed under these conditions. We targeted mobile invertebrates and excluded some species with remarkably higher mobility and less dependency on eelgrass habitat, such as mysids and decapods (Yamada et al., 2007b), which

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

We measured water temperature and salinity once at each station using a memory sensor (AAQ-175 RINKO, JFE Advantech Co. Ltd., Japan). To obtain the representative values, the sensor was carefully placed approximately 50 cm from the bottom to accurately reflect the environment inside of the seagrass meadow.

We collected three replicate samples (a total of 21 samples from all stations) of mobile invertebrates, spirorbid polychaetes and epiphytic macroalgae together with the above-ground parts of the eelgrass using a mesh bag (bore diameter: 20 cm, mesh size: 0.1 mm) based on the mouth area of the mesh bag (314 cm²). Upon collection, we counted the number of eelgrass shoots to determine shoot density. For microalgae, five replicate samples were collected per station together with one eelgrass shoot using a plastic zip bag, because microalgae easily fell off from eelgrass blades when collected with the mesh bag.

Laboratory procedures

Immediately after being transported to the laboratory, the microalgae were scraped from the eelgrass blades using a glass slide; separated from other organisms such as macroalgae and spirorbid polychaetes; and then filtered using glass fiber filters (Whatman GF/F filter ϕ 47 mm, Whatman International Ltd., Maidstone, UK). If other organisms were present in the microalgal samples, we carefully removed them from the filters with forceps. Other epibiotic organisms collected using mesh bags were separated from the eelgrass by scraping them off with a glass slide; these organisms were classified as red algae, green algae, spirorbid polychaetes and mobile invertebrates. To obtain dry mass, eelgrass shoots, red algae, green algae, spirorbid polychaetes

and filtered microalgae were dried at 60°C for 4 days in small aluminum foil bags, and then weighed. We counted and identified the mobile invertebrates after extraction with a sieve (500 μm) and fixation with 70% ethanol. Identification of mobile invertebrates was made to the lowest taxonomical unit possible (mostly to species) using detailed guides from the literature (Gammarid amphipod: Nishimura, 1995; Carlton, 2007; Ogawa, 2011; Caprella amphipod, Isopod, Copepod, Cumacea: Nishimura, 1995; Carlton, 2007; Gastropod: Okutani, 2000; Polychaeta: Nishimura, 1992; Imajima, 1996; Imajima, 2001; Turbellaria: Nishimura, 1992; Carlton, 2007; Hirunoidea: Nishimura, 1992) and the World Register of Marine Species online database (WoRMS: <http://www.marinespecies.org>).

Statistical analysis

We used, as predictors, two abiotic factors (water temperature and salinity) and six biotic factors (eelgrass biomass [g dry weight per unit area: g DW m^{-2}], eelgrass shoot density [shoots m^{-2}], microalgal biomass [g DW m^{-2}], red algal biomass [g DW m^{-2}], green algal biomass [g DW m^{-2}] and spirorbid polychaete biomass of [g DW m^{-2}]). For eelgrass biomass, we used the dry weight data collected using mesh bags. Because microalgal biomass was collected by a different sampling procedure from other biotic variables, we used the mean value of five replicates. All invertebrate biomass (mg ash-free dry weight per unit area, mg AFDW m^{-2}) was estimated from the abundance and the size fraction using the empirical equations in Edgar (1990).

To test which of the eight biotic/abiotic factors was a likely predictor of the variation in the mobile invertebrate community, we fit linear mixed models (LMMs) with a Gaussian distribution. The station was used as a random variable. As response variables, we used the

biomass of 11 dominant species for the population-level analyses, and total invertebrate biomass, species richness and species diversity (Shannon-Wiener diversity index) for community-level analyses. The 11 most dominant species were selected by a threshold whereby the biomass proportion accounted for more than 1% of the total invertebrate biomass (see Table S1). *Ostreobdella kakibir* (Hirudinoidea) was omitted from the analysis because it occurred only at one station (SR), even though they satisfied the requirement. R software (version 3.1.3) was used for all of the analyses (R Development Core Team, 2015).

Prior to the LMM fit, all of the variables excluding species diversity were square root transformed to improve homoscedasticity and meet the assumptions of normality of the LMMs after checking for normality with the Shapiro-Wilk test. To test for collinearity between the eight environmental variables, we calculated Pearson's correlation coefficients for all pairs. If the absolute value of the coefficient (r) was greater than 0.7, the level where collinearity does not affect model predictions (Dormann et al., 2013), we removed the relevant predictor as necessary. Because it followed that water temperature and microalgal biomass were highly correlated (Pearson's $r = -0.82$, $P < 0.01$), we removed microalgal biomass from the models. After this removal, we tested potential multicollinearity among the remaining predictors using the variance inflation factor (VIF) analysis with a cutoff of 10 (e.g. Dormann et al., 2013). VIF values were calculated using the *vif.mer* function developed by Frank (<https://raw.githubusercontent.com/aufrank/R-hacks/master/mer-utils.R>). However, all seven predictors were below the VIF value of 10 and remained. We therefore defined a reduced model with the seven predictors as the full model.

We fit the LMMs using the *lmer* function in the lme4 package (Bates et al., 2014). To obtain P -values of the LMMs, we used the *lmerTest* package (Kuznetsova et al., 2014). We selected the

optimal model comparing the candidate models on all combinations of the predictors by the Akaike information criterion as corrected for the small sample size (AIC_c ; Burnham & Anderson, 2002). We obtained AIC_c based on the maximum likelihood (ML) for comparisons among the LMMs because the restricted maximum likelihood (REML) is inappropriate in the case when the fixed structure is different between the candidate models (Zuur et al., 2009), but the parameters were estimated by REML. We used the *AIC_ctab* function in the *bbmle* library (Bolker & R Core Team, 2013) to compare the AIC_c . After setting the optimal models, we obtained the standardized coefficients as effect sizes by re-fitting using standardized variables that were scaled by the sample standard deviation and centered by sample mean values.

Additionally, when the effect of water temperature was detected, we tested the relationship between mobile invertebrates and microalgal biomass which was omitted from the LMM because of the multi-collinearity with water temperature.

RESULTS

Environmental factors

Water temperature was lower at the four stations (BK, CL, CK and SR) near the channel than at the other three stations in the inner parts of the estuary (HN, TB and SL) (Table 1). Salinity was lower at the lakeside stations (BK, HN, TB, SL and CK) that were influenced by freshwater inputs. For these stations, the inter-annual variation was also higher (Table 1).

Eelgrass biomass varied between 140 and 278 g DW m⁻² among the stations. It was the

lowest at TB, followed by HN and BK (Table 1). Eelgrass shoot density ranged between 85 and 234 shoot m⁻². It was highest at BK and second highest at SL. The mean densities were not largely different among other stations. Microalgal biomass varied by more than ten-fold between the lowest station (SL) and the highest station (CL). In the latter, the microalgal biomass exceeded the biomass of the eelgrass. Macroalgae were not present at TB and SR. Red algae were dominated by *Neosiphonia* sp. and *Chondria dasyphylla*, and green algae were dominated by *Cladophora* sp. The mean biomass of red algae was highest at HN and that of green algae was highest at CL, although their biomasses were less than 15 % that of eelgrass. Spirorbid polychaetes were not present at SL and CL. They were highly abundant at SR where their biomass was more than three-fold greater than the eelgrass biomass.

Mobile invertebrate community

A total of 32 mobile invertebrate species were collected in this study (Table S1). At taxonomic levels, polychaete worms made up 32.2% of the total biomass, followed by gastropods (31.3%), gammarid amphipods (23.0%), and isopods (8.8%). At the species level, a polychaete *Nereis* sp. was most dominant (24.6%), followed by gastropods *Lacuna* spp. (23.4%) and the gammarid amphipod *Ampithoe lacertosa* (18.0%). For an additional eight species including two gammarid amphipod (*Monocorophium* spp. and *Pontogeneia rostrata*), two isopods (*Cymodoce japonica* and *Paranthura japonica*), two gastropods (*Ansola angustata* and *Siphonacmea oblongata*) and two polychaetes (*Exogone naidina* and *Syllis* sp.), their proportions were less than 5% at most.

The mean value of the total mobile invertebrate biomass was the highest at CK and much lower at stations along the coastline (HN, TB and SL). Species richness was the highest at

CL, followed by CK and was approximately the same level at the other stations (Fig. 2). The mean value of species diversity was the highest at CL and the lowest at SR (Fig. 2).

Population level analyses

We found that each of the nine invertebrate populations belonging to gammaridae, gastropoda and polychaeta was predicted by a different combination of environmental factors in the optimal models (Table 2). For two isopods, no environmental factor correlated with their biomass.

Water temperature was selected as the responsible factor for the variation of *A. lacertosa*, *Lacuna* spp. and all three polychaetes. Among them, only *Syllis* sp. showed a significant correlation (positive). The significant effect of the salinity gradient was detected for *A. angustata* (negative) and *S. oblongata* (positive).

For the two predictors relevant to the characteristics of the eelgrass bed, the above-ground biomass showed a significantly positive relationship only with *Syllis* sp., whereas shoot density was significantly correlated with *Monocorophium* spp. (positive), *P. rostrata* (negative) and *E. naidina* (negative).

The biomasses of sessile epibionts (red algae, green algae and spirorbid polychaetes) on eelgrass blades were correlated with many invertebrate populations excluding *A. lacertosa*, *Monocorophium* spp., two isopods and *E. naidina* in different manners. Red algal biomass was positively correlated with *P. rostrata*, *Lacuna* spp. and *Nereis* sp., but negatively correlated with *Syllis* sp. and tended to be negatively correlated with *A. angustata*. Green algal biomasses were positively correlated with *P. rostrate* and *Lacuna* spp. The biomass of spirorbid polychaetes was 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

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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).

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338

339 DISCUSSION

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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,

the observed relationships between these functional groups and mobile invertebrate populations varied greatly among the species.

In the optimal models, the effects of biomass of epibiotic organisms on the gammarid amphipod *P. rostrata*, all three gastropod species (*A. angustata*, *Lacuna* sp. and *S. oblongata*) and two polychaetes (*Nereis* sp. and *Syllis* sp.) were detected. For those species, the sessile epibionts were positively related to mobile invertebrate biomasses except for *Syllis* sp. and *P. rostrata*, which showed a positive correlation with both red and green algae. The algae are considered to be used as a temporal shelter (habitat) rather than as a food resource because these animals do not firmly attach to the eelgrass blades but rather drift among shoots (Suh & Yu, 1997; Yamada et al., 2007b; Yu, Jeong & Suh, 2008), and because they have a preference for feeding on phytoplankton and detritus (Yu & Suh, 2011). High predation risk for swimming amphipods with low self-defense abilities, such as *P. rostrata*, has been reported in several studies (Sudo & Azeta, 1992; Beare & Moore, 1998). In fact, gammarid amphipods are a major source of prey for blennoid fish in the eelgrass beds of Northern Japan (Watanabe et al., 1996; Sawamura, 1999; Yamada et al., 2010). Therefore, the complex micro-habitat created by macroalgae allows them to escape these predators.

All three gastropods increased in correlation with spirorbid polychaetes, whereas the responses to the other factors were different (Table 2). The positive effect of spirorbid polychaetes appears to result from habitat-formation in terms of our assumption, but should not be oversimplified because of the overlap in the life cycle and because of the functional mismatch. Because the gastropods adhere to flat seagrass blades, the flat (simple) structure created by seagrass blades can be better than the rough structure of spirorbid polychaetes. Therefore, competition for space (negative effect) appears to be more expected than facilitation. Although

we do not have a good answer for the positive relationships, one possibility for this unexpected result is that the rough structure acts as a shelter because small-sized individuals (< 3 mm) are frequent in gastropod populations during the summer season (*A. angustata*: Momota, personal observation; *Lacuna* spp.: Kanamori, Goshima & Mukai, 2004; *S. oblongata*: Toyohara, Nakaoka & Tsuchida, 2001).

Red algae are considered to positively affect *Nereis* sp. by provisioning habitat because polychaetes build tubes both on eelgrass blades and in red algal canopies in Akkeshi (Momota, unpublished data). The negative effect of red algae and spirorbid polychaetes on *Syllis* sp. may suggest that this mobile polychaete prefers a simple structured habitat without a complex micro-habitat created by eelgrass blades with sessile epibionts.

In addition to the effects of sessile epibionts, the significant effects of water temperature, salinity, eelgrass biomass and shoot density were detected for a majority of the dominant species, although the patterns and directions of the effects were different among them. Surprisingly, eelgrass biomass was not correlated with most species except for *Syllis* sp., and the direction (positive/negative) of the effect of eelgrass shoot density was different among the species. The same response of syllid polychaetes was reported in previous studies (e.g. Bone & San Martín, 2003). For eelgrass shoot density, the result suggests that it indirectly affects mobile invertebrates through interfering with multiple physical and biological processes (e.g. water current and flux, detritus and drifting algae trapping, recruitment, and predation intensity: Gambi, Nowell & Jumars, 1990; Robbins & Bell, 1994; Attrill, Strong & Rowden 2000; Boström & Bonsdorff, 2000; Lee et al. 2001; Hovel et al., 2002). Notably, the contrasting relationships of *P. rostrate* with eelgrass shoot density and macroalgae imply that the shelter effect is different depending on the spatial scale (i.e. blade scale, shoot/patch scale).

The isopods *C. japonica* and *P. japonica* were not correlated with any abiotic or biotic factors because of the low dependency on the seagrass habitat; they can utilize other numerous habitats created by both natural and artificial materials (e.g. mussel beds, oyster reefs: Marchini et al., [2014]; Nakamachi, Ishida & Hirohashi, [2015]; gravel, litter layer of macrophytes, *Sargassum* meadow: Momota, personal observation). Additionally, their uniform appearance throughout all of the stations indicates that they have a wide tolerance to a broad range of environmental stress, which leads to a lack of correlation with any of the abiotic factors. Additionally, the gammarid amphipod *A. lacertosa* was not significantly correlated with any factors. This species is widely distributed along the Pacific-rim coast of the northern hemisphere and utilizes a variety of plant habitats by building tubes (Hiebert, 2013), which may explain why it did not show any relationship with the environmental gradients.

Although the discussion on underlying drivers that generate apparent correlations (i.e. the causalities) between epibionts and mobile invertebrates is not our main focus, the indirect effects and the top-down control of mobile invertebrates should also be taken into account to interpret present findings. For example, we can give an alternative possibility for the positive relationship between gastropods and spirorbid polychaetes such that high grazing of the gastropods facilitates the recruitment of spirorbid polychaetes through the removal of the microalgal cover.

Total biomass, species richness and species diversity were differentially correlated with abiotic/biotic factors, and varied in a complex manner although processes were unclear. The optimal model of the three community variables contains one or two variables of sessile epibionts. The positive correlation of total biomass with red algae reflects a result pulled by the biomass proportions of highly dominant species, such as *Lacuna* spp. and *Nereis* sp. The negative interaction of spirorbid polychaetes with species richness and diversity suggest that

spirorbid polychaetes can decrease the homogeneity of the biomasses of component species within a community by allowing some competitive species to dominate. Because no effect on species diversity, total biomass, species richness, or most of the dominant species populations was detected by green algae, it may work on species evenness.

CONCLUSIONS

The present study suggests that macroalgae and sessile animals on eelgrass blades can affect the biomass and diversity of mobile invertebrates and that incorporating these biotic factors can improve the prediction of the variability of the mobile invertebrate community in the eelgrass bed. However, the underlying causal relationships appear to be complex and vary greatly from species to species. Our findings were based on data collected over one sampling period when the eelgrass bed was most productive and when the abundance and/or diversity of algae and mobile invertebrates typically reached their maximum. A more comprehensive investigation of the functional relationships among the various types of organisms and of the temporal changes should be conducted in future studies on eelgrass bed communities.

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REFERENCES

- Aguilera MA, Navarrete SA. 2012.** Functional identity and functional structure change through succession in a rocky intertidal marine herbivore assemblage. *Ecology* **93**:75–89 DOI 10.1890/11-0434.1.
- Attrill MJ, Strong JA, Rowden AA. 2000.** Are macroinvertebrate communities influenced by seagrass structural complexity? *Ecography* **23**:114–121 DOI 10.1111/j.1600-0587.2000.tb00266.x.
- Bates D, Maechler M, Bolker B, Walker S. 2014.** lme4: linear mixed-effects models using Eigen and S4. <http://CRAN.R-project.org/package=lme4>
- Beare DJ, Moore PG. 1998.** Aspects of the life histories of *Perioculodes longimanus*, *Pontocrates arcticus* and *Synchelidium maculatum* (Crustacea: Amphipoda) at Millport, Scotland. *Journal of the Marine Biological Association of the United Kingdom* **78**:193-209 DOI 10.1017/S0025315400040029.
- Blake RE, Duffy JE. 2010.** Grazer diversity affects resistance to multiple stressors in an experimental seagrass ecosystem. *Oikos* **119**:1625–1635 DOI 10.1111/j.1600-0706.2010.18419.x.

- 461 **Bolker BM, R Development Core Team. 2013.** bbmle: tools for general maximum likelihood
462 estimation. <http://CRAN.R-project.org/package=bbmle>.
- 463 **Bologna PAX, Heck Jr KL. 1999.** Macrofaunal associations with seagrass epiphytes relative
464 importance of trophic and structural characteristics. *Journal of Experimental Marine*
465 *Biology and Ecology* **242**:21–39 DOI 10.1016/S0022-0981(99)00092-1.
- 466 **Bone D, San Martín G. 2003.** Ecological aspects of syllids (Annelida: Polychaeta: Syllidae) on
467 *Thalassia testudinum* beds in Venezuela. *Hydrobiologia* **496**:289–298 DOI
468 10.1023/A:1026117503709.
- 469 **Boström C, Bonsdorff E. 2000.** Zoobenthic community establishment and habitat complexity -
470 The importance of seagrass shoot-density, morphology and physical disturbance for faunal
471 recruitment. *Marine Ecology Progress Series* **205**:123–138 DOI 10.3354/meps205123.
- 472 **Burnham KP, Anderson DR. 2002.** *Model selection and multimodel inference: a practical*
473 *Information-Theoretic approach*. New York: Springer.
- 474 **Carlton JT, ed. 2007.** *The Light and Smith manual: intertidal invertebrates from Central*
475 *California to Oregon, 4th edition*. Berkeley, CA: University of California Press. ISBN
476 9780520239395.
- 477 **Dean RL, Connell JH. 1987.** Marine invertebrates in an algal succession. III. Mechanisms
478 linking habitat complexity with diversity. *Journal of Experimental Marine Biology and*
479 *Ecology* **109**:249–273 DOI 10.1016/0022-0981(87)90057-8.
- 480 **Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B,**
481 **Lafourcade B, Leitão PJ, Münkemüller T, McClean C, Osborne PE, Reineking B,**
482 **Schröder B, Skidmore AK, Zurell D, Lautenbach S. 2013.** Collinearity: A review of
483 methods to deal with it and a simulation study evaluating their performance. *Ecography*

- 484 **36:27–46** DOI 10.1111/j.1600-0587.2012.07348.x.
- 485 **Duffy JE, Harvilicz AM. 2001.** Species-specific impacts of grazing amphipods in an eelgrass-
486 bed community. *Marine Ecology Progress Series* **223**:201–211 DOI 10.3354/meps223201.
- 487 **Duffy JE, Hay ME. 1991.** Food and shelter as determinants of food choice by an herbivorous
488 marine amphipod. *Ecology* **72**:1286–1298 DOI 10.2307/1941102.
- 489 **Duffy JE, Richardson JP, Canuel EA. 2003.** Grazer diversity effects on ecosystem functioning
490 in seagrass beds. *Ecology Letters* **6**:637–645 DOI 10.1046/j.1461-0248.2003.00474.x.
- 491 **Duffy JE. 2006.** Biodiversity and the functioning of seagrass ecosystems. *Marine Ecology*
492 *Progress Series* **311**:233–250 DOI 10.3354/meps311233.
- 493 **Duffy JE, Hay ME. 2000.** Strong impacts of grazing amphipods on the organization of a benthic
494 community. *Ecological Monographs* **70**:237–263 DOI 10.1890/0012-
495 9615(2000)070[0237:SIOGAO]2.0.CO;2.
- 496 **Duffy JE, Richardson JP, France KE. 2005.** Ecosystem consequences of diversity depend on
497 food chain length in estuarine vegetation. *Ecology Letters* **8**:301–309 DOI 10.1111/j.1461-
498 0248.2005.00725.x.
- 499 **Douglass JG, France KE, Richardson JP, Duffy JE. 2010.** Seasonal and interannual change in
500 a Chesapeake Bay eelgrass community: Insights into biotic and abiotic control of
501 community structure. *Limnology and Oceanography* **55**:1499–1520 DOI
502 10.4319/lo.2010.55.4.1499.
- 503 **Edgar GJ. 1990.** The use of the size structure of benthic macrofaunal communities to estimate
504 faunal biomass and secondary production. *Journal of Experimental Marine Biology and*
505 *Ecology* **137**:195–214 DOI 10.1016/0022-0981(90)90185-F.
- 506 **Edgar GJ, Shaw C, Watsona GF, Hammond LS. 1994.** Comparisons of species richness, size-

- structure and production of benthos in vegetated and unvegetated habitats in Western Port, Victoria. *Journal of Experimental Marine Biology and Ecology* **176**:201–226 DOI 10.1016/0022-0981(94)90185-6.
- Gambi MC, Nowell ARM, Jumars PA. 1990.** Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Marine Ecology Progress Series* **61**:159–169 DOI 10.3354/meps061159.
- Garrity SD. 1984.** Some adaptations of gastropods to physical stress on a tropical rocky shore. *Ecology* **65**:559–574 DOI 10.2307/1941418.
- Gartner A, Tuya F, Lavery PS, McMahon K. 2013.** Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. *Journal of Experimental Marine Biology and Ecology* **439**:143–151 DOI 10.1016/j.jembe.2012.11.009.
- Gutow L, Long JD, Cerda O, Hinojosa IA, Rothäusler E, Tala F, Thiel M. 2012.** Herbivorous amphipods inhabit protective microhabitats within thalli of giant kelp *Macrocystis pyrifera*. *Marine Biology* **159**:141–149 DOI 10.1007/s00227-011-1794-4.
- Hall MO, Bell SS. 1988.** Response of small motile epifauna to complexity of epiphytic algae on seagrass blades. *Journal of Marine Research* **46**:613–630 DOI 10.1357/002224088785113531.
- Hamamoto K., Mukai H. 1999.** Effects of larval settlement and post-settlement mortality on the distribution pattern and abundance of the spirorbid tube worm *Neodexiospira brasiliensis* (Grube) (Polychaeta) living on seagrass leaves. *Marine Ecology* **20**:251–272 DOI 10.1046/j.1439-0485.1999.2034075.x.
- Harley CDG. 2006.** Effects of physical ecosystem engineering and herbivory on intertidal community structure. *Marine Ecology Progress Series* **317**:29–39 DOI 10.3354/meps317029.

- 530 **Harley CDG, Hughes AR, Hulgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez**
- 531 **LF, Tomanek L, Williams SL. 2006.** The impacts of climate change in coastal marine
- 532 systems. *Ecology Letters* **9**:228–241 DOI 10.1111/j.1461-0248.2005.00871.x.
- 533 **Hasegawa N, Hori M, Mukai H. 2007.** Seasonal shifts in seagrass bed primary producers in a
- 534 cold-temperate estuary: Dynamics of eelgrass *Zostera marina* and associated epiphytic algae.
- 535 *Aquatic Botany* **86**:337–345 DOI 10.1016/j.aquabot.2006.12.002.
- 536 **Healey D, Hovel KA. 2004.** Seagrass bed patchiness: effects on epifaunal communities in San
- 537 Diego Bay, USA. *Journal of Experimental Marine Biology and Ecology* **313**:155–174 DOI
- 538 10.1016/j.jembe.2004.08.002.
- 539 **Heck Jr KL, Hays G, Orth RJ. 2003.** Critical evaluation of nursery hypothesis for seagrasses.
- 540 *Marine Ecology Progress Series* **253**:123–136 DOI 10.3354/meps253123.
- 541 **Heck Jr. K.L., Orth R.J. 1980.** Seagrass habitats: the role of habitat complexity, competition
- 542 and predation in structuring associated fish and motile macroinvertebrate assemblages. In:
- 543 Kennedy VS, ed. *Estuarine Perspectives*. New York: Academic Press, 449–464.
- 544 **Heck Jr KL, Pennock JR, Valentine JF, Coen LD, Sklenar SA. 2000.** Effects of nutrient
- 545 enrichment and small predator density on seagrass ecosystems: An experimental assessment.
- 546 *Limnology and Oceanography* **45**:1041–1057 DOI 10.4319/lo.2000.45.5.1041.
- 547 **Heck Jr KL, Valentine JF 2006.** Plant–herbivore interactions in seagrass meadows. *Journal of*
- 548 *Experimental Marine Biology and Ecology* **330**:420–436 DOI 10.1016/j.jembe.2005.12.044.
- 549 **Hemminga MA, Duarte CM. 2000.** *Seagrass Ecology*. Cambridge: Cambridge University Press.
- 550 **Hiebert, TC. 2015.** *Ampithoe lacertosa*. In: Hiebert TC, Butler BA, Shanks AL, eds. *Oregon*
- 551 *Estuarine Invertebrates: Rudys' Illustrated Guide to Common Species, 3rd edition*. Charleston,
- 552 OR: University of Oregon Libraries and Oregon Institute of Marine Biology.

- 553 **Hoegh-Guldberg O, Bruno JF. 2010.** The impact of climate change on the world's marine
554 ecosystems. *Science* **328**:1523–1528 DOI 10.1126/science.1189930 .
- 555 **Hovel KA, Fonseca MS, Myer DL, Kenworthy WJ, Whitfield PE. 2002.** Effects of seagrass
556 landscape structure, structural complexity and hydrodynamic regime on macrofaunal
557 densities in North Carolina seagrass beds. *Marine Ecology Progress Series* **243**:11–24 DOI
558 10.3354/meps243011.
- 559 **Hughes AR, Williams SL, Duarte CM, Heck KL, Waycott M. 2009.** Associations of concern:
560 Declining seagrasses and threatened dependent species. *Frontiers in Ecology and Evolution*
561 **7**:242–246 DOI 10.1890/080041.
- 562 **Iizumi H, Taguchi S, Minami T, Mukai H, Maekawa S. 1996.** Distribution and variability of
563 nutrients, chlorophyll a, particulate organic matters, and their carbon and nitrogen contents,
564 in Akkeshi-ko, an estuary in northern Japan. *Bulletin of the Hokkaido National Fisheries*
565 *Research Institute* **59**:43–67.
- 566 **Imajima M, ed. 1996.** *Polychaetous Annelids*. Tokyo: AQUABIOLOGY. ISBN
567 9784915342103.
- 568 **Imajima M, ed. 2001.** *Polychaetous Annelids II*. Tokyo: AQUABIOLOGY. ISBN
569 9784915342257.
- 570 **Irlandi EA, Peterson CH. 1991.** Modification of animal habitat by large plants: mechanisms by
571 which seagrasses influence clam growth. *Oecologia* **87**:307–318 DOI 10.1007/BF00634584.
- 572 **Jernakoff P, Brearley A, Nielsen J. 1996.** Factors affecting grazer-epiphyte interactions in
573 temperate seagrass meadows. *Oceanography and Marine Biology: An Annual Review*
574 **34**:109–162.
- 575 **Kanamori M, Goshima S, Mukai H. 2004.** Seasonal variation in host utilization of epiphytic

- Lacuna species in mixed algal and surfgrass stands in Japan. *Marine Ecology* **25**:51–69 DOI 10.1111/j.1439-0485.2004.00014.x.
- Kasim M, Mukai H. 2006.** Contribution of benthic and epiphytic diatoms to clam and oyster production in the Akkeshi-ko estuary. *Journal of Oceanography* **62**:267–281 DOI 10.1007/s10872-006-0051-9.
- Klumpp DW, Salita-Espinosa JS, Fortes MD. 1992.** The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. *Aquatic Botany* **43**:327–349 DOI 10.1016/0304-3770(92)90046-L.
- Kuznetsova A, Brockhoff PB, Christensen RHB. 2014.** lmerTest: Tests for random and fixed effects for linear mixed effect models (*lmer* objects of lme4 package). R package version 2.0–11.
- Lee SY, Fong CW, Wu RSS. 2001.** The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: a study using artificial seagrass units and sampling of natural beds. *Journal of Experimental Marine Biology and Ecology* **259**:23–50 DOI 10.1016/S0022-0981(01)00221-0.
- Lefcheck JS, van Montfrans J, Orth RJ, Schmitt EL, Duffy JE, Luckenbach MW. 2014.** Epifaunal invertebrates as predators of juvenile bay scallops (*Argopecten irradians*). *Journal of Experimental Marine Biology and Ecology* **454**:18–25 DOI 10.1016/j.jembe.2014.01.014.
- Long HA, Grosholz ED. 2015.** Overgrowth of eelgrass by the invasive colonial tunicate *Didemnum vexillum*: Consequences for tunicate and eelgrass growth and epifauna abundance. *Journal of Experimental Marine Biology and Ecology* **473**:188–194 DOI 10.1016/j.jembe.2015.08.014.

- 599 **Mannino A, Montagna PA. 1997** Small-scale spatial variation of macrobenthic community
600 structure. *Estuaries* **20**:159–173.
- 601 **Marchini A, Sorbe J, Torelli F, LodolaA. 2014** The non-indigenous *Paranthura japonica*
602 Richardson, 1909 in the Mediterranean Sea : travelling with shellfish? *Mediterranean*
603 *Marine Science*. **15**:545–553 DOI 10.12681/mms.779.
- 604 **Meager JJ, Schlacher TA, Green M. 2011.** Topographic complexity and landscape
605 temperature patterns create a dynamic habitat structure on a rocky intertidal shore. *Marine*
606 *Ecology Progress Series* **428**:1–12 DOI 10.3354/meps09124.
- 607 **Nakamachi T, Ishida H, Hirohashi N. 2015.** Sound Production in the Aquatic Isopod
608 *Cymodoce japonica* (Crustacea : Peracarida). *The Biological Bulletin* **229**:167–172 DOI
609 10.1086/BBLv229n2p167.
- 610 **Montagna PA, Kalke RD. 1992.** The Effect of Freshwater Inflow on Meiofaunal and
611 Macrofaunal Populations in the Guadalupe and Nueces Estuaries, Texas. *Estuaries* **15**:307–
612 326 DOI 10.2307/1352779.
- 613 **Nishimura S, ed. 1992.** *Guide to seashore animals of Japan with color pictures and keys, vol*
614 *1(in Japanese)*. Osaka: Hoikusha. ISBN 978-4586302017.
- 615 **Nishimura S, ed. 1995.** *Guide to seashore animals of Japan with color pictures and keys, vol*
616 *2(in Japanese)*. Osaka: Hoikusha. ISBN 978-4586302024.
- 617 **Ogawa H, ed. 2011.** *A Guidebook of Gammarids in Tokyo Bay. Open edition ver.1.2.* Toho
618 University, Japan: Furota Lab, Laboratory of Environmental Ecology, Department of
619 Environmental Science, Faculty of Science.
- 620 **Okutani T, ed. 2000.** *Marine Mollusks in Japan.* Tokyo: University of Tokyo Press.
- 621 **Orth RJ. 1977.** Effect of nutrient enrichment on growth of eelgrass *Zostera marina* in

- 622 Chesapeake Bay, Virginia, USA. *Marine Biology* **44**:187–194 DOI 10.1007/BF00386958.
- 623 **Orth RJ, Heck Jr KL, van Montfrans J. 1984.** Faunal communities in seagrass beds: a review
- 624 of the influence of plant structure and prey characteristics on predator-prey relationships.
- 625 *Estuaries* **7**:339–350 DOI: 10.2307/1351618.
- 626 **Poore AGB, Campbell AH, Coleman RA, Edgar GJ, Jormalainen V, Reynolds PL, Sotka**
- 627 **EE, Stachowicz JJ, Taylor RB, Vanderklift MA, Duffy JE. 2012.** Global patterns in the
- 628 impact of marine herbivores on benthic primary producers. *Ecology Letters* **15**:912–922
- 629 DOI 10.1111/j.1461-0248.2012.01804.x.
- 630 **R Development Core Team 2015.** R: A language and environment for statistical computing.
- 631 Vienna, Austria: R Foundation for Statistical Computing.
- 632 **Remane A, Schlieper C. 1971.** *Biology of Brackish Waters*. New York: Wiley Interscience
- 633 Division, 1–373.
- 634 **Robbins BD, Bell SS. 1994.** Seagrass landscapes: A terrestrial approach to the marine subtidal
- 635 environment. *Trends in Ecology and Evolution* **9**:301–304 DOI 10.1016/0169-
- 636 5347(94)90041-8.
- 637 **Robertson AI, Lucas JS. 1983.** Food choice, feeding rates, and the turnover of macrophyte
- 638 biomass by a surf-zone inhabiting amphipod. *Journal of Experimental Marine Biology and*
- 639 *Ecology* **72**:99–124 DOI 10.1016/0022-0981(83)90138-7.
- 640 **Sawamura M. 1999** One-year Comparison of Stomach Contents among Demersal Fishes off the
- 641 Coast of Usujiri, Hokkaido (in Japanese with English abstract). *Japanese Journal of*
- 642 *Benthology* **54**:14-23.
- 643 **Somero GN. 2002.** Thermal physiology and vertical zonation of intertidal animals: optima,
- 644 limits, and costs of living. *Integrative and Comparative Biology* **42**:780–789 DOI

- 645 10.1093/icb/42.4.780.
- 646 **Sudo H, Azeta M. 1992.** Selective predation on mature male *Byblis japonicas* (Amphipoda:
647 Gammaridea) by the barface cardinalfish, *Apogon semilineatus*. *Marine Biology* **114**:211–
648 217 DOI 10.1007/BF00349521.
- 649 **Suh HL, Yu OH. 1997.** Winter zonation of the benthic amphipods in the sandy shore surf zone
650 of Dolsando, southern Korea (in Korean with English abstract). *Korean Journal of Fisheries*
651 *and Aquatic Sciences* **30**:340–348.
- 652 **Thomsen MS. 2010.** Experimental evidence for positive effects of invasive seaweed on native
653 invertebrates via habitat-formation in a seagrass bed. *Aquatic Invasions* **5**:341–346 DOI
654 10.3391/ai.2010.5.4.02.
- 655 **Tittensor DP, Mora C, Jetz W, Lotze HK, Ricard D, Berghe EV, Worm B. 2010.** Global
656 patterns and predictors of marine biodiversity across taxa. *Nature* **466**:1098–101 DOI
657 10.1038/nature09329.
- 658 **Toyohara T, Nakaoka M, Tsuchida E. 2001.** Population dynamics and life history traits of
659 *Siphonacmea oblongata* Yokohama on seagrass leaf in Otsuchi Bay (Siphonariidae,
660 Pulamonata). *Venus (Jap. J. Malaco)* **60**:27–36.
- 661 **Valentine JF, Duffy JE. 2006.** The Central Role of Grazing in Seagrass Ecology. In: Larkum
662 AWD, Orth RJ, Duarte CM, eds. *Seagrasses: Biology, Ecology, and Conservation*. Springer,
663 463–501.
- 664 **Valentine JF, Heck Jr KL. 1999.** Seagrass herbivory: Evidence for the continued grazing of
665 marine grasses. *Marine Ecology Progress Series* **176**:291–302 DOI 10.3354/meps176291.
- 666 **van Montfrans J, Wetzel RL, Orth RJ. 1984.** Epiphyte-grazer relationships in seagrass
667 meadows: consequences for seagrass growth and production. *Estuaries* **7**:289–309 DOI

- 668 10.2307/1351615.
- 669 **Watanabe K, Minami T, Iizumi H, Imamura S. 1996.** Interspecific relationship by
670 composition of stomach contents of fish at Akkeshi-ko, an estuary at eastern Hokkaido,
671 Japan (in Japanese with English abstract). *Bulletin of the Hokkaido National Fisheries*
672 *Research Institute* **60**:239-276
- 673 **Watanabe M, Nakaoka M, Mukai H. 2005.** Seasonal variation in vegetative growth and
674 production of the endemic Japanese seagrass *Zostera asiatica*: A comparison with
675 sympatric *Zostera marina*. *Botanica Marina* **48**:266–273 DOI 10.1515/BOT.2005.036.
- 676 **Webster PJ, Rowden AA, Attrill MJ. 1998.** Effect of shoot density on the infaunal macro-
677 invertebrate community within a *Zostera marina* seagrass Bed. *Estuarine, Coastal and Shelf*
678 *Science* **47**:351–357 DOI 10.1006/ecss.1998.0358.
- 679 **Whalen MA, Duffy JE, Grace JB. 2013.** Temporal shifts in top-down vs. bottom-up control of
680 epiphytic algae in a seagrass ecosystem. *Ecology* **94**:510–520 DOI 10.1890/12-0156.1.
- 681 **Williams GA, Morritt D. 1995.** Habitat partitioning and thermal tolerance in a tropical limpet,
682 *Cellana grata*. *Marine Ecology Progress Series* **124**:89–103 DOI 10.3354/meps124089 .
- 683 **Yamada K, Hori M, Tanaka Y, Hasegawa N, Nakaoka M. 2007b.** Temporal and spatial
684 macrofaunal community changes along a salinity gradient in seagrass meadows of Akkeshi-
685 ko estuary and Akkeshi Bay, northern Japan. *Hydrobiologia*, **592**, 345–358 DOI
686 10.1007/s10750-007-0767-6.
- 687 **Yamada K, Hori M, Tanaka Y, Hasegawa N, Nakaoka M. 2010.** Contribution of different
688 functional groups to the diet of major predatory fishes at a seagrass meadow in northeastern
689 Japan. *Estuarine, Coastal and Shelf Science* **86**:71–82 DOI 10.1016/j.ecss.2009.10.015.
- 690 **Yamada K, Takahashi K, Vallet C, Taguchi S, Toda T. 2007a.** Distribution, life history, and

production of three species of *Neomysis* in Akkeshi-ko estuary, northern Japan. *Marine Biology* **150**:905–917 doi:10.1007/s00227-006-0403-4.

Ysebaert T, Herman PMJ, Meire P, Craeymeersch J, Verbeek H, Heip CHR. 2003. Large-scale spatial patterns in estuaries: Estuarine macrobenthic communities in the Schelde estuary, NW Europe. *Estuarine, Coastal and Shelf Science* **57**:335–355 DOI 10.1016/S0272-7714(02)00359-1.

Ysebaert T, Meire P, Coosen J, Essink K. 1998. Zonation of intertidal macrobenthos in the estuaries of Schelde and Ems. *Aquatic Ecology* **32**:53–71 DOI 10.1023/A:1009912103505 .

Yu OH, Jeong SJ, Suh HL. 2008. Reproductive Pattern of the Epifaunal Amphipod *Pontogeneia rostrata* (Crustacea) on Dolsando Sandy Shore in Korea. *Ocean Science Journal* **43**:127–133 DOI 10.1007/BF03020693.

Yu OH, Suh HL. 2011. Secondary production of the eusirid amphipod *Pontogeneia rostrata* Gurjanova, 1938 (Crustacea: Peracarida) on a sandy shore in Korea. *Ocean Science Journal* **46**:211–217 DOI 10.1007/s12601-011-0017-8.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. *Mixed effects models and extensions in ecology with R*. New York, USA: Springer.

List of figures

Fig. 1. Location of the study sites in the Akkeshi-ko estuary and the Akkeshi Bay in Northeastern Japan. The area enclosed by a dashed circle is the Akkeshi-ko estuary. Most of the clam aquaculture grounds are located in the western part of the estuary (indicated by a dotted

circle).

Fig. 2. The total invertebrate biomass, species richness and Shannon-Wiener diversity index at the seven stations in the Akkeshi-ko estuary and Akkeshi Bay. The bars indicate the mean values with SDs. The order of the stations is lined up based on relative size of the impact of freshwater inflow or seawater from Akkeshi Bay.

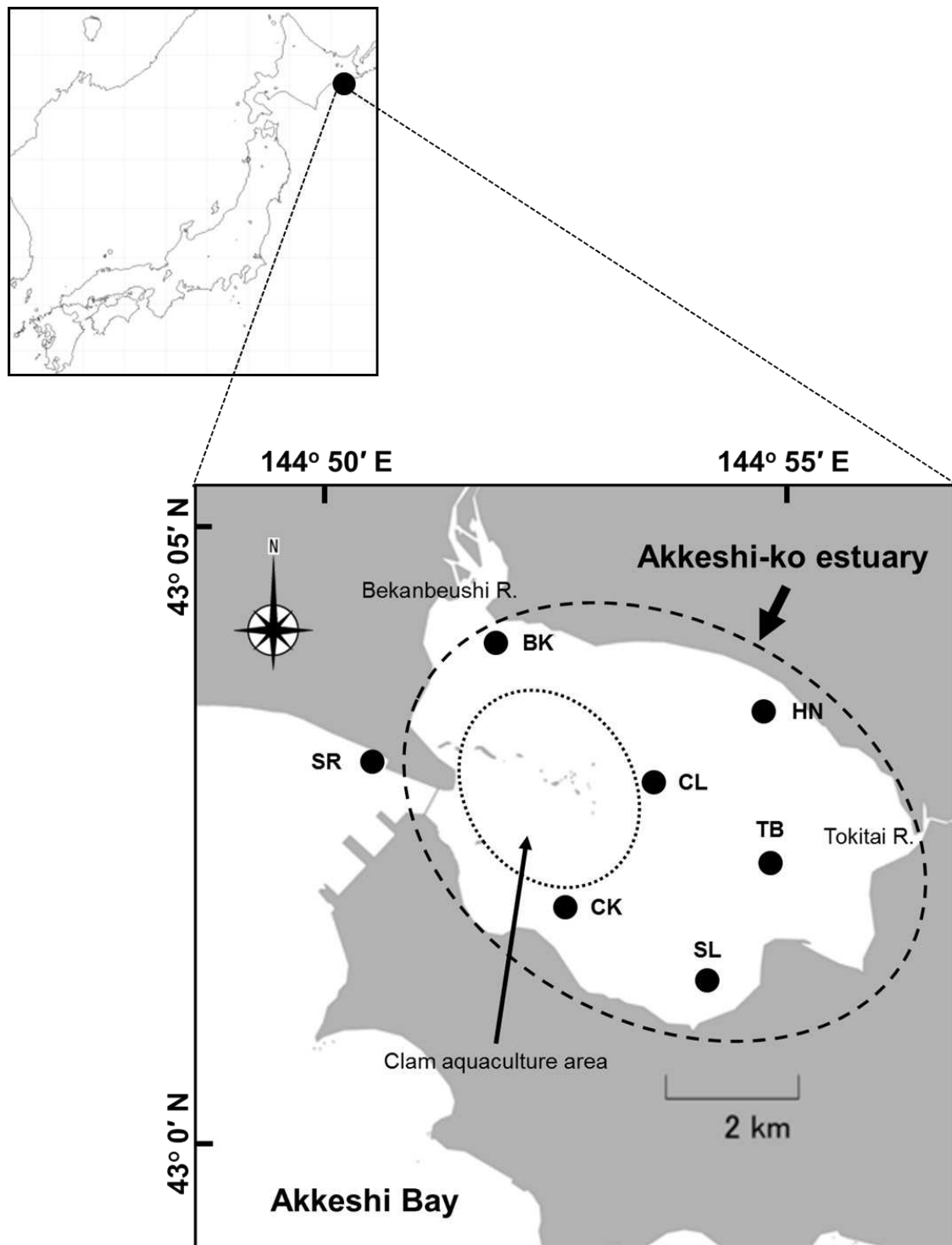
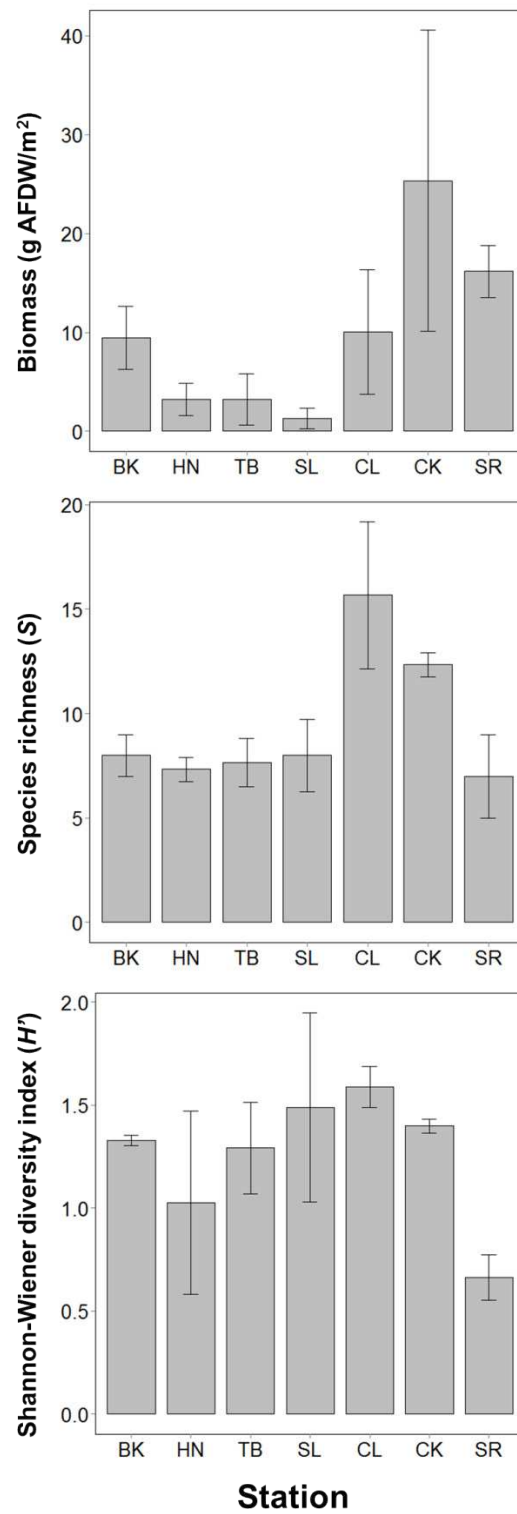


Fig. 1. Momota & Nakaoka

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Fig. 2. Momota & Nakaoka

Table

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	Δ AIC _c
		(Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm		
Population											
Gammarid amphipoda											
<i>Ampithoe lacertosa</i>	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	—
	<i>Std.coef</i>	< -0.01	-0.601	-0.790						—	—
<i>Monocorophium</i> 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	—
	<i>Std.coef</i>	< -0.01				0.249				—	—
<i>Pontogeneia rostrata</i>	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	—
	<i>Std.coef</i>	< -0.01				-0.541	0.775	0.611		—	—

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Table 2. (continued 1)

Response	Model	Predictor								AIC _c	Δ AIC _c
		(Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm		
Isopoda											
<i>Cymodoce japonica</i>	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	—
	<i>Std.coef</i>	< -0.01								—	—
<i>Paranthura japonica</i>	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	—
	<i>Std.coef</i>	< 0.01								—	—
Gastropoda											
<i>Ansola angustata</i>	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	—
	<i>Std.coef</i>	< -0.01		-1.137			-0.217	0.658	0.767	—	—
<i>Lacuna</i> 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	—
	<i>Std.coef</i>	< 0.01	-0.522				0.351		0.723	—	—

Table 2. (continued 2)

Response	Model	Predictor								AIC _c	ΔAIC _c
		(Intercept)	WT	Sal	ZM.bm	ZM.den	Red.alg	Grn.alg	SP.bm		
<i>Siphonacmea oblongata</i>	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	—
	<i>Std.coef</i>	< 0.01		0.287					0.768	—	—
Polychaeta <i>Exogone naidina</i>	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	—
	<i>Std.coef</i>	< -0.01	-0.728	-0.825		-0.607				—	—
<i>Nereis</i> 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	—
	<i>Std.coef</i>	< -0.01	-0.741				0.494			—	—
<i>Syllis</i> 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	—
	<i>Std.coef</i>	< -0.01	0.650		0.905		-0.636		-0.710	—	—

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	—	—