Broad scale variation in seagrass benthic macrofaunal assemblages along the coast of Japan

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Abstract

Broad scale studies in seagrass benthic macrofauna are important for future regional marine conservation. We examined spatial variation in the community structure of seagrass-associated benthic macroinvertebrates collected by sediment coring in 2010 at six seagrass sites of Japan covering the latitudinal range of 24° to 43°N. Total species richness and ES(50) at site level did not show clear site variations and relationship with latitude. At core level, site variations of mean species richness, ES(50), Simpson diversity and abundance showed inconsistent pattern, but with more cases of statistically significant association with latitude. Variations were generally influenced by the seagrass species, often among subtropical species, among temperate *Zostera* species, and between *Zostera* and subtropical species. Finally, the community composition differed significantly across all sites and community similarity decreased rapidly with geographic distance, with only 5% similarity retained at the distance of 400 km. The dissimilarity among sites was higher with the similar distance compared to other types of coastal communities such as rocky intertidal assemblages, which is associated with minor occurrence of species with broad distributional range.

Keywords: Abundance and species diversity, Latitudinal gradient, Macroecology, Regional variation, Similarity-distance relationship

Introduction

There is a growing interest in broad-scale studies or macroecology of marine organisms to understand how and why assemblages of interacting species vary over wide areas (Underwood et al. 2000; Nakaoka and Noda 2004; Nakaoka et al. 2006; Sanford 2014). Variation in faunal structures across areas that are widely distant of each other or located at different latitudes, and with different environmental conditions, could be driven by differences in oceanographic current systems and climatic patterns acting in the system (Nishimura 1974; Asakura and Suzuki 1987; Nakaoka et al. 2006; Kurihara 2007; Kurihara et al. 2011; Sanford 2014). Moreover, similarity in species composition most likely decreases with distance (i.e., similarity-distance relationship) since variation in environmental conditions often increases with distance between areas (*sensu* Nekola and White 1999; Soininen et al. 2007). Macroecology can provide broader understanding on biodiversity pattern and the dynamics and processes that influence it. In the context of environmental management amidst rapidly increasing natural and human-induced disturbances, it is a growing field of research that is both urgent and important.

Seagrasses have a wide geographical distribution (with a latitudinal range between 72 N and 54 S; Hemminga and Duarte 2000; Green and Short 2003); thus, the associated population and community processes on broad spatial scales can be compared (Duffy et al. 2015, Ruesink et al. in press). The highest seagrass species diversity is mostly found in coastal areas of Asia (Green and Short 2003; Short et al. 2007), yet seagrass management in this region lags far behind (*sensu* Duarte 2002). In Japan, seagrass monitoring had been conducted in the past years (e.g., Tamaki et al. 2002; Nakaoka et al. 2013; Muraoka et al., 2016). However, the quantitative estimation of population and community variables over broad spatial scales is lacking for seagrass-associated animals except for that on fish community (Fukuta et al. 2017). While Japanese studies on seagrass-associated marine invertebrate were done mostly on small spatial scales (e.g., Nakaoka et al. 2001; Yamada et al. 2007; Yamada et al. 2010, Momota and Nakaoka 2017), broad-scale studies on marine benthic macrofauna were mostly conducted on rocky shores (Asakura and Suzuki 1987; Okuda et al. 2004; Nakaoka et al. 2006; Kurihara 2007; Kurihara et al. 2011), which consist of macroinvertebrate assemblages different from those thriving in soft-bottom seagrass beds (Larkum et al. 2006; Bertness et al. 2014). Meanwhile, seagrass beds are declining globally at an unprecedented rate (Short and Wyllie-

Echeverria 1996; Duarte 2002; Orth et al. 2006; Waycott et al. 2009) and seagrass management in Asia remains marginalized within many conservation agendas (Kirkman and Kirkman 2002; Unsworth and Cullen 2010). Seagrass beds and the associated macroinvertebrates provide various valuable services (Nakaoka et al. 2014). Filling in the identified knowledge-gap will contribute significantly to foray regional biodiversity conservation.

The Ministry of Environment of Japan has established a long-term monitoring program, called Monitoring Sites 1000, centering on surveys of various types of ecosystems including seagrass beds in Japan (Ishihara et al. 2011). Surveys in seagrass beds by the Monitoring Sites 1000 Coastal Area Survey started since 2008 at six sites covering the whole coast of Japan from northeast part of Hokkaido (43°N, 145°E) to southwest part of Okinawa (24°N, 124°E). The collection of benthic macrofaunal associates in seagrass beds was conducted in 2010 at those sites, giving an opportunity to analyze the broad scale data.

The main objective of this study was to determine broad scale biodiversity patterns of seagrass benthic macrofaunal associates using the collected data by the Monitoring Sites 1000 project. Specifically, the aim was to describe the large scale patterns of seagrass macrofaunal species richness (species number), ES(50) (expected number of species in a random sample of 50 individuals; Rosenberg et al. 2004), Simpson diversity (effective numbers or numbers equivalent of common species; *sensu* Jost 2006, 2007), abundance (the number of individuals), and community assemblage (species composition pattern) between the six sites of Japan. Specific focuses were placed to test (1) whether there were sites and latitudinal variations in those univariate variables, (2) the factors that could drive any of the variations; and (3) whether similarity of macrofaunal community decreased with geographic distance.

Materials and methods

Study sites

Collection of macrobenthic animals was conducted in 2010 at six seagrass beds registered for Monitoring Sites 1000 during the season of highest productivity (**Table 1**). These sites were Ishigaki (Okinawa Prefecture), Ibusuki (Kagoshima Prefecture), Ikunoshima (Hiroshima Prefecture), Futtsu (Chiba Prefecture), Otsuchi (Iwate Prefecture) and Akkeshi (Hokkaido Prefecture) (**Fig. 1**). The distance between two sites ranged from 410 km to 2619 km. At each

site, benthic macroinvertebrate samples were collected at one to three tidal elevations, that is, intertidal, shallow subtidal, and deep subtidal, depending on the zonation of seagrasses. Each tidal elevation per site was dominated by a specific seagrass species (**Table 1**) except for Otsuchi where *Zostera caulescens* dominated at both the shallow subtidal and deep subtidal elevations. The sampled intertidal beds had a water depth range of 0.1 m to 0.8 m, shallow subtidal of 0.25 m to 6.0 m, and deep subtidal of 0.5 m to 11.0 m. The annual average water temperature during the sampling periods varied between 8°C (Akkeshi) and 27°C (Ishigaki) (**Table 1**).

Collection and laboratory processing of macrofauna

Five replicate core samples for benthic macrofauna were randomly collected from each tidal elevation of the six study sites, resulting in a total of 60 core samples (**Table 1**). The samples were collected using a cylindrical PVC corer with an inner diameter of 15 cm (0.02 m^{-2} surface area per cored sediment). The corer was buried to the sediment at a depth of 10 cm. Core samples were initially sieved in the field using mesh box with 1.0 mm opening and then kept in labelled sample bags for laboratory processing.

Samples were cleaned with seawater in the laboratory, separating the fauna from the associated seagrasses. The faunal samples were fixed with 10% seawater-formalin solution, sorted and identified into lowest taxonomic level, whenever possible, based on taxonomic references (Nishimura 1992; Nishimura 1995; Okutani 2000) and the World Register of Marine Species online database (WoRMS Editorial Board 2015). The count for each sample or species in each core sample was also recorded. All samples were preserved with 70% ethanol. The seagrass shoots per core were counted before oven-drying them under 60°C to 80°C until constant weight. The data for dry weight and shoot density of seagrasses in Otsuchi were extracted from Nakaoka et al. (2001) since the original 2010 data and samples were destroyed during the 2011 tsunami in Japan.

Data management and statistical analyses

Seagrass biomass and shoot density across sites

In order to first provide an idea of the seagrass bed structures where the benthic macroinvertebrate samples had been collected, the structures of seagrasses from the cored samples were analyzed and compared across sites. Latitudinal cline was also explored. Site and latitudinal variations in seagrass biomass (above-ground and below-ground) and shoot density, expressed as g DW 0.02 m^{-2} and shoots 0.02 m^{-2}, respectively, were analyzed using the Generalized Linear Model (GLM) under negative binomial distribution (Zuur et al. 2009) that is known to address overdispersion (which was the case for our seagrass data). Two (nested) models were selected using likelihood ratio tests: M0 – model with intercept only, M1 – model with intercept and site (or latitude) as explanatory variable. Tukey tests were conducted with Bonferroni corrections (Hothorn et al. 2008) if there was any variation across sites. Further analyses were conducted to determine variables affecting any site and latitude pattern (i.e., testing for collinearity and automated model selection; see details below).

Macrofauna

To provide an overall insight of the macrofaunal samples collected from the six study sites, the total number of benthic macroinvertebrates individuals, phyla, classes, orders (including unassigned), families (including unknown), and species of the 60 core samples were recorded. The most common (frequently occurring) species was also identified. Finally, the number of species (or species richness) restricted to a single site relative to the total number of species collected and identified was also noted.

At site level, total values of species richness was obtained by counting the total number of species collected and identified for all core samples in each site and then descriptively compared across sites. The ES(50) was calculated using the *rarefraction* function of PRIMER version 6.1.13 (Clarke and Gorley 2006). Because each site is associated with specific latitude (**Table 1**), the latitudinal gradient for ES(50) values was tested using the simple linear regression.

The species richness, ES(50) and Simpson diversity values in each site were divided to total sample size per site (regardless of presence of different tidal elevation) in order to obtain the averages (e.g., mean of 15 species 0.02 m^{-2}). Each Simpson value was directly comparable to species richness (biased on rare species) per core since it is not an index (from Simpson diversity index) but a 'true diversity' (effective numbers) of common species (Jost 2006, 2007).

All response variables were tested for effect of site and latitude using GLM with negative binomial distribution. If there was a significant site variation (i.e., P value < 0.05) for each analysis, Tukey tests were conducted with Bonferroni corrections (Hothorn et al. 2008) in order to determine which among the means varied significantly at 95% level of confidence.

An automated model-selection was conducted in order to select the best model that explains the site (or latitudinal) variation for each variable. Prior to that, some explanatory variables showed collinearity. (i.e., the generalized variance inflation factor or GVIF was > 3; Fox and Monette 1992). The tidal elevation, seagrass species, water depth, shoot density and seagrass biomass had 9.98, 2.13, 5.67, 1.94 and 4.62 GVIF (obtained from taking the square of GVIF^(1/(2*Df))), respectively. We set 3 as the cut off (Zuur et al. 2009); any variable with > 3 GVIF was excluded from the proceeding automated model selection. As a result, four models were selected; M1 – model with intercept only, M2 – model with intercept and shoot density as explanatory variable, M3 – model with intercept and seagrass species, and M4 – model with intercept, shoot density and seagrass species.

Most of above analyses used several packages and were conducted under the RStudio version 1.0.143 (RStudio Team 2016). The statistical packages were the (i) *MASS* (Venables and Ripley 2002) for GLM; (ii) *multcomp* (Hothorn et al. 2008) for pairwise tests; (iii) *stats* (R Core Team 2016) for linear modelling; (iv) *vegetarian* (Charney and Record 2012) for computing Simpson diversity; (v) *car* (Fox and Weisberg 2011) for testing collinearity; and (vi) *MuMIn* (Barton 2016) for the *dredge* function to run the automated model selection.

Multivariate analyses

The presence/absence data of all species in 60 core samples was used for the multivariate analyses of macrofaunal community structure. First, the Canonical Analysis of Principal Coordinates (CAP; Anderson and Willis 2003), a constrained ordination, was conducted to visualize the distinctiveness of the sites through the multivariate cloud of points.

The ordination pattern was statistically tested using the Permutational Multivariate Analysis of Variance (PERMANOVA: Anderson 2001) with Jaccard resemblance index and 999 permutations using the PRIMER version 6.1.13 (Clarke and Gorley 2006) and PERMANOVA+ package version 1.0.3. We also conducted pairwise tests whenever site was found having significant effect on the community assemblage pattern to determine among

which of the sites differ significantly in terms of species composition of benthic macrofauna. Finally, to determine whether the similarity of seagrass benthic macrofaunal assemblages was related to geographic distance, a Mantel test (Legendre and Legendre 1998) was done based on Spearman correlation (*vegan* package of Oksanen et al. 2013) using the RStudio version 1.0.143 (RStudio Team 2016). The geographical distances among geographic coordinates (expressed as UTM values: easting, northing) were obtained from great circle distance matrix using the *fields* package (Nychka et al. 2015) of RStudio, while the community dissimilarity was obtained from Jaccard distance (using the presence-absence data).

Results

Regional pattern in seagrass biomass and shoot density

Seven species of seagrasses were recorded in this study, including the *Zostera marina*, *Zostera japonica*, *Zostera caulescens*, *Zostera asiatica*, *Halodule uninervis*, *Enhalus acoroides* and *Thalassia hemprichii*. The average seagrass biomass across sites ranged from 3.89 g DW 0.02 m^{-2} (Ibusuki) to 21.55 g DW 0.02 m^{-2} (Akkeshi). Seagrass biomass varied across sites (LRT of GLM models: test = null vs full model, LR stat = 12.723, $P = 0.026$), and Bonferroni-corrected Tukey tests showed difference only between Ibusuki and Akkeshi (**Fig. 2a**). We also found a significant effect of latitude on biomass (GLM: Estimate ± SE = 0.05 ± 0.02, z = 2.22, $P = 0.027$). Variations were influenced by seagrass species (automated model selection), particularly among some of the subtropical seagrass, across *Zostera* and subtropical seagrass , and among *Zostera* species.

The average seagrass shoot density across sites ranged from 3 shoots 0.02 m^{-2} (Akkeshi) to 20 shoots 0.02 m^{-2} (Ikunoshima). There was a statistically significant effect of site in shoot density (LRT of GLM models: test = null vs full model, *LR* stat = 19.604, $P = 0.001$), of which variations were found from Ikunoshima towards Akkeshi (Bonferroni-corrected Tukey tests: $P < 0.05$; **Fig. 2b**). The site variation was also influenced by seagrass species, which showed similar pattern as above for biomass. We did not find a latitudinal effect on shoot density (GLM: Estimate ± SE = -0.01 ± 0.02, $z = -0.617$, $P = 0.538$).

Macrofauna

A total of 4,520 benthic macrofaunal individuals were recorded from the 60 core samples (with 0.02 m^2 surface area per core). The samples comprised of 10 phyla, 19 classes, 54 orders (with 10 unassigned), 115 families (with 9 unknown), and 192 species (**Table S1**). The *Ampithoe lacertosa* (Order Amphipoda) was the only species found in the entire study sites. Conversely, a total of 133 species, representing 69% of the total number of species, were found restricted to a single site (**Table S2**).

Species richness and ES50 at site level

The species richness of benthic macrofauna per site was highly variable; Ishigaki (44), Ibusuki (30), Ikunoshima (35), Futtsu (86), Otsuchi (58) and Akkeshi (24). Furthermore, the ES50 of macrofauna ranged from 5 (Ishigaki) to 17 (Futtsu). ES(50) did not vary with latitude (**Fig. S1**) (LM: Estimate ± SE = -0.57 ± 0.30, t = -1.90, P = 0.13).

Species richness, ES50 and Simpson diversity at core level

The average species richness of benthic macrofauna ranged from 6.0 ± 1.0 species 0.02 m^{-2} (Ishigaki) to 17.0 (Futtsu) species 0.02 m^{-2} and varied significantly among sites (GLM model selection from nested models, **Table 2**), in which Futtsu, Otsuchi, Akkeshi and Ibusuki had significantly higher species richness compared to Ishigaki and Ikunoshima (Bonferroni-corrected Tukey tests, **Fig**. **3a**). In addition, although the variation across sites was highly variable (i.e., no consistent pattern), further analysis showed that the species richness and latitude had highly significant positive relationship (GLM: Estimate ± SE = 0.051 ± 0.012, z = 4.34, P < 0.001; **Table 2**). The variations in site and latitude were found attributed to seagrass species (automated model selection, **Table 3**), which specific variations were across some *Zostera* species and subtropical species *(*i.e., *Z. asiatica, Z. caulesce*ns, *Z. marina* > *E. acoroides; Z. asiatica, Z. caulescens, Z. marina* > *Halodule uninervis; and, Z. caulescens* > *T. hemprichii*), and among some *Zostera* species (i.e., *Z. caulescens* > *Z. japonica*; and *Z. marina* > *Z. japonica*).

The average ES(50) of macrofauna ranged from 5.0 0.02 m^{-2} (Ishigaki) to 13.0 (Ibusuki) 0.02 m^{-2} and varied significantly across sites (**Table 2**). Pairwise comparisons of means showed that variations were on Ibusuki, Futtsu and Otsuchi all having significantly higher mean ES(50)

compared to Ishigaki (**Fig. 3b**). Despite variable site pattern, we found a statistically positive relationship of ES(50) to latitude (GLM: Estimate ± SE = 0.03 ± 0.01, z = 2.46, P = 0.014; **Table 2**). Further analyses showed that the variation was influenced by the seagrass species (**Table 3**), and Bonferroni-corrected Tukey tests showed significant variation among some subtropical species (i.e., *T. hemprichii* > *E. acoroides),* and across some *Zostera* species and subtropical species *(*i.e., *Z. caulesce*ns, *Z. japonica*, *Z. marina* > *E. acoroides;* and *Z. caulescens, Z. marina* > *H. uninervis*).

The average Simpson diversity of macrofauna ranged from 2.0 0.02 m^{-2} (Akkeshi) to 6.0 0.02 m^{-2} (Futtsu) and varied significantly across sites (**Table 2**). All sites, except for Otsuchi, had significantly higher Simpson diversity compared to that of Akkeshi (Bonferroni-corrected Tukey tests, **Fig. 3c**). In addition, Simpson diversity of Futtsu was found significantly higher compared to Otsuchi. Diversity also appeared decreasing towards higher latitude but not statistically significant (GLM: Estimate ± SE = -0.02 ± 0.01, z = -1.26, P = 0.208; **Table 2**). Variations in Simpson diversity across sites were influenced by seagrass species (**Table 3**), specifically among some subtropical seagrasses (i.e., *T. hemprichii* > *E. acoroides*), across some of the *Zostera* and subtropical species (i.e., *Z. marina* > *E. acoroides*, *Z. asiatica* < *T. hemprichii*), and among some of the *Zostera* species (i.e., *Z. marina* > *Z. asiatica*).

Abundance

The average abundance ranged from 13.0 individuals 0.02 m^{-2} (Ishigaki) to 225.0 individuals 0.02 m^{-2} (Akkeshi) and statistically differed across sites (**Table 2**). Results of the pairwise tests showed that Ibusuki, Futtsu, Otsuchi and Akkeshi had significantly higher abundance compared to Ishigaki and Ikunoshima (**Fig. 3d**). Abundance also showed statistically significant relationship with latitude (GLM: Estimate ± SE = 0.05 ± 0.02, z = .31, P < 0.001; **Table 2**). Variation across sites and latitudes was attributed to seagrass species (**Table 3**), which differences were observed on similar pattern to that of species richness, ES(50), and Simpson diversity.

Community assemblage pattern and similarity-distance relationship

The result of Canonical Analysis of Principal Coordinates (CAP) showed the distinctiveness of each site, wherein all sampled species of benthic macrofauna was closely grouped together per site regardless of tidal elevation (**Fig. 4**). The observed pattern in the multivariate data cloud of CAP was supported by the result of permutational multivariate analysis of variance (PERMANOVA) wherein the species composition was found to vary significantly among sites (**Table 4**). In addition, further analysis showed that all sites had highly significant variation in composition (pairwise tests in PERMANOVA, $P < 0.01$). Based on the square root of the estimated component of variations (**Table 4**), the macrofaunal communities in different sites were 38% dissimilar in their composition, while communities in different depths has an additional of 18% dissimilarity.

Finally, we observed that the macrofaunal community dissimilarity (Jaccard distance), significantly increased with geographic distance (Mantel test: $r = 0.55$, $P = 0.034$) (**Fig. 5**). At a distance of 410 km, which was the shortest distance between two sites (Ibusuki and Ikunoshima), the community dissimilarity already reached 0.95. This was about the same level with the dissimilarity between Ishigaki and Akkeshi (0.92) which had the longest distance (2619 km).

Discussion

Our study analyzed the data of benthic macroinvertebrates in the six seagrass beds ranging from the southernmost (24^{o}N) to the northernmost (43^{o}N) part of Japan, spanning more than 2600 km distance. We explored the univariate and multivariate characteristics of the samples for a wide scale comparison which marked the first attempt on broad scale ecological research of seagrass beds in Japan.

The aboveground biomass of seagrasses from the macrofaunal cores showed significant increase with latitude, which could be explained by the fact that the seagrass beds of the study sites were dominated by different seagrass species. The tropical species in Ishigaki (*Halodule uninervis*, *Thalassia hemprichii*, *Enhalus acoroides*) were replaced by *Zostera* species towards the north (*Zostera japonica* and *Zostera marina* in Ibusuki, Ikunoshima and Futtsu, *Zostera caulescens* in Futtsu and Otsuchi, and *Zostera asiatica* in Akkeshi). Seagrass biomass is known

to vary significantly with latitude, with a tendency for large-sized species to occur in higher latitudes (Green and Short 2003). For instance, *Z. caulescens* and *Z. asiatica* allocate more resources to enlarging shoot size compared to *Z. marina* which apportions more to increasing shoot density by clonal propagation of rhizomes (Nakaoka et al. 2003; Watanabe et al. 2005). On the other hand, the seagrass shoot density decreased significantly from Ikunoshima and Futtsu to Otsuchi and Akkeshi (**Fig. 2b**), although with weak association to latitude. Since all seagrass variation across sites (and latitude for biomass) was attributed to seagrass species, it could then be highly expected that the species difference from south to north drives difference in seagrass associated macrofauna.

The macrofaunal species richness per site was highly variable across the study sites. This result generally conformed to previous reports on different taxa. For instance, the total species richness of bryozoans in the North Atlantic (Lidgard 1990) and benthic invertebrate assemblages in the Norwegian continental shelf (Ellingsen and Gray 2002) showed no clear latitudinal cline. Other previous works opposed to our result. The bivalve molluscs on global scale (Stehli et al. 1967), coastal fishes in the Indo-Pacific and Atlantic Oceans (Rohde 1992), bivalves and gastropods in the Atlantic deep-sea (Rex et al. 1993), and benthic invertebrates of rocky intertidal in North-western Pacific (Okuda et al. 2004) showed decreasing total regional species richness with increasing latitude. Caution must be applied for any result comparisons because of the differences in the studied taxa, and even on the investigative approach, such as spatial scales, locations, and data analyses (*sensu* Warwick 1997).

The average species richness 0.02 m^{-2} also showed variability across sites (**Fig. 3a**) and this result conformed to findings of prior studies (e.g., Coates 1998; Okuda et al. 2004; Clarke and Lidgard 2010). Nonetheless, the species richness was found significantly increasing towards higher latitude, implying that the number of rare macrobenthic species (i.e., species that are distributed in spatially restricted area) in seagrass beds seemed higher at higher latitude. On the other hand, our observation did not support findings of other studies. For instance, Okuda et al. (2004) found a decreasing number of rare rocky intertidal macrobenthic species towards higher latitude. Witman et al. (2004) also reported a decline of shallow-water benthic epifaunal local species richness from the tropics towards higher latitudes. Finally, Virnstein et al. (1984) did not find clear latitudinal gradient of seagrass epifaunal local species richness. The ES(50) at site level appeared lowest in Ishigaki, which was also the case at the core level (**Fig. 3b**). We also found a positive significant relationship between ES(50) and latitude. The

most tolerant individuals of a species are likely to be associated with the lowest ES(50) values (Rosenberg et al. 2004).

The Simpson diversity 0.02 m^{-2} significantly varied between sites and appeared decreasing with latitude but not statistically significant (**Table 2**). This observation possibly indicates that the relative abundance of common species does not have clear site variation and latitudinal cline. This result conformed to that of Okuda et al. (2004) who reported no clear latitudinal pattern of common rocky intertidal macrobenthic species in Japan. Buzas and Culver (1999) also found that the benthic foraminifera around the continental margins of North America have nearly equal proportions for abundant species.

The macrofaunal abundance also significantly varied across sites and has a significant positive relationship with latitude. While we suspect that the presence of higher seagrass biomass or productivity in the north (Nakaoka and Aioi 2001; Watanabe et al. 2005) may have provided more resources and habitats for the associated benthic macroinvertebrates in the high latitude (bottom-up control; see also Boström et al. 2006), our findings support that variations were explained by seagrass species. In addition, biotic interactions and dispersal limitation could also depressed abundance in other suitable areas (Bell and Westoby 1986; VanDerWal et al. 2009). Also, the cold Oyashio current in the north could have influenced the abundance pattern favoring the northern latitudes just as the case in Kurihara (2007) in which boreal regions of Japan (northern coasts) was reported having higher number of molluscs compared to those regions under the influence of warm Kuroshio Current. Conversely, Virnstein et al. (1984) did not find significant relationship of seagrass epifaunal density to latitude and suggested that habitat complexity may have more relevance to abundance pattern than latitudinal difference.

Analysis on the multivariate data showed a significant among-site variation in species composition across all sites. This result was not surprising because more than half of the species in the macrofaunal community were found only on a single site. The restrictedness of the species to a single area was unlikely a sampling error or sample identification error since similar pattern has been reported elsewhere (e.g., Ellingsen and Gray 2002; Clarke and Lidgard 2010). Furthermore, the variation was great only across sites, regardless of tidal elevation which differed from that of the univariate structures of macrofauna wherein site and latitudinal variations were attributed by seagrass species.

The Pacific coast of Japan, where the six sites are located, are influenced by the two major current systems: the Kuroshio and Oyashio currents. The warm Kuroshio is an extension of the North Equatorial Current arising from the Indo-Malayan Current that brings tropical species to southern islands of Japan. In contrast, the cold Oyashio current coming from Okhotsk and Bering Seas transports boreal species to the northern islands (Briggs 1966; Briggs 1995). Previous studies on biogeographical patterns of rocky intertidal community in Japan observed substantial changes in community structure between regions affected differently by these two current systems (Asakura and Suzuki 1987; Nakaoka 2005; Kurihara 2007). In our study, four sites in the south (Ishigaki, Ibusuki, Ikunoshima and Futtsu) are under the influence of Kuroshio current, while the two northern sites (Akkeshi and Otsuchi) are influenced by the Oyashio current. If Kuroshio and Oyashio currents play more important influence in the species composition pattern of seagrass macrofauna, Otsuchi and Akkeshi should be more similar. This assumption was confirmed from the result of constrained ordination of CAP in which samples from Akkeshi and Otsuchi lie closer together from the rest of the samples of other sites (**Fig. 4**). In contrast, among four sites affected by Kuroshio current, it was noticeable that that Ibusuki samples lie farther away. We could not cite direct explanation to the latter observation, but it could be worth to note that Ibusuki has an annual eelgrass habit compared to other sites which usually had perennial seagrasses (Shimabukuro et al. 2012). The variation in species composition of seagrass macrofauna is likely influenced by both regional current system and local environmental condition.

Finally, we found that the community similarity of macrofauna did not only decay with increasing geographic distance, but also showed surprisingly high values of community distances (**Fig. 5**). A decreasing similarity of communities with distance between sites is supported by previous studies (e.g., Nekola and White 1999; Soininen et al. 2007). More importantly, it is interesting to note that the degree of community dissimilarity in our study was higher even at the shorter distance compared to other published findings. For instance, within a distance of ≤ 500 km, the seagrass macrofauna between two points in our study already lost 95% similarity (two sites only had 5% similarity), whereas the benthic components on the rocky shore (i.e., algae, sessile and mobile animals) in Nakaoka et al. (2006) only lost similarity by at least 40% (two areas had ~60% similarity) on similar geographic distance. Much lower dissimilarity within the same range of geographic distance (i.e., ≤ 500 km) was also observed in other studies on other benthic communities (e. g., Ellingsen and Gray 2002; Blanchette et al.

2008). Similarity-distance relationship is influenced by the species geographic range limit that is determined by niche-breath, dispersal, and the similarity of environmental variables that is expected to decrease with distance along various environmental gradients (Nekola and White 1999). The high levels of dissimilarity within short distances in our study could somehow be associated with the presence of >50% of the total number of macrofaunal species occurring only in a single site. While the underlying factors responsible for the steep pattern of distance-decay relationship remained largely unknown in this study, we suspect that the dispersal ability and niche partitioning patterns of benthic macrofauna may be different between macrofaunal communities associated among seagrass beds and those of rocky intertidal areas.

In conclusion, our study revealed broad scale variability in species richness, ES(50), Simpson diversity, abundance, and species composition of seagrass-associated benthic macrofauna in Japan. The observed variability was influenced by seagrass species, which composition varied greatly across sites and latitude. While such patterns could be related to ecological and biological factors such as habitat heterogeneity and traits of component species, regional, biogeographic factors such as climate and oceanographic current regimes may also be important although these variables were not tested directly in this study. The results of our study can be used as baseline data for planning the broad scale conservation of biodiversity in seagrass beds of Japan which remain suffering from multiple human-induced threats such as coastal developments and climate changes.

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622

Figure captions

Fig. 1 Map showing the locations of the six seagrass beds in Japan under the Monitoring Sites 1000 Coastal Area Survey

Fig. 2 Boxplots of seagrass biomass (a) and shoot density (b) conditional on the nominal variable site. The median is represented by a white horizontal bar in the box. The boxes define the hinge (25–75% quartile, and the line is 1.5 times the hinge). Lines extending vertically from the boxes are called whiskers, indicating variability (maximum and minimum value represented by bars) outside the upper and lower quartiles. Dots represent the points outside this interval. The width of each box is proportional to the sample size per site (Table 1). Different lowercase letters represent statistically significant differences ($P < 0.05$; Tukey tests with Bonferroni corrections). Sites are A-Ishigaki, B-Ibusuki, C-Ikunoshima, D-Futtsu, E-Otsuchi and F-Akkeshi

Fig. 3 Boxplots of species richness (a), ES(50) (b), Simpson diversity (c), and abundance (d) of benthic macrofauna conditional on the nominal variable site. The median is represented by a white horizontal bar in the box. The boxes define the hinge (25–75% quartile, and the line is 1.5 times the hinge). Lines extending vertically from the boxes are called whiskers, indicating variability (maximum and minimum value represented by bars) outside the upper and lower quartiles. Dots represent the points outside this interval. The width of each box is proportional to the sample size per site (Table 1). Different lowercase letters represent statistically significant differences ($P < 0.05$; Tukey tests with Bonferroni corrections). Sites are A-Ishigaki, B-Ibusuki, C-Ikunoshima, D-Futtsu, E-Otsuchi and F-Akkeshi

Fig. 4 Canonical Analysis of Principal Coordinates (CAP) ordination showing the variation in benthic macrofaunal species. Data used was the presence/absence of all macrofaunal species analyzed via the Jaccard resemblance index

Fig. 5 Distance-decay plot showing increasing benthic macrofaunal dissimilarity as geographical distance increased. Data used was the presence/absence of all macrofaunal species analyzed via the Jaccard resemblance index.

Table 1 Details of the 6 sampling sites for seagrass benthic macrofauna in 2010 by Monitoring Sites 1000 Coastal Area Survey

Site	Prefecture	Latitude (°N)	Longitude (°E)	Temperature (°C)[a]	Depth (m)[b]	Tidal elevation	Seagrass species	Period of survey	No. of replicates
Ishigaki	Okinawa	24.49	124.23	27	0.7	Intertidal	*Halodule uninervis*	September	5
					1.0	Shallow subtidal	*Thalassia hemprichii*		5
					1.6	Deep subtidal	*Enhalus acoroides*		5
Ibusuki	Kagoshima	31.17	130.59	22	2.9	Shallow subtidal	*Zostera marina*	April	5
Ikunoshima	Hiroshima	34.30	132.91	18	1.0	Intertidal	*Zostera japonica*	June	5
					2.4	Shallow subtidal	*Z. marina*		5
Futtsu	Chiba	35.32	139.8	18	0.9	Intertidal	*Z. japonica*	May	5
					1.9	Shallow subtidal	*Z. marina*		5
					3.5	Deep subtidal	*Zostera caulescens*		5
Otsuchi	Iwate	39.37	141.95	12	6.0	Shallow subtidal	*Z. caulescens*	July	5
					10.5	Deep subtidal			5
Akkeshi	Hokkaido	43.00	144.86	8	1.7	Shallow subtidal	*Zostera asiatica*	August	5

[a] Data on annual average temperature from the nearest oceanographic station (http://www.jodc.go.jp/data/coastal/obs_data_index2.html)
[b] Water depth from mean tide level

Table 2 Summary results of the likelihood ratio tests of negative binomial models (M0 = null model, with intercept only; M1 = full model, with site and latitude as explanatory variables tested separately) for each response variable of benthic macrofaunal samples based on automated model

Model	AIC[a]	2 × Loglik[b]	LR stat[c]	*P* (Chi[d])
Site				
Species richness				
M0	392.05	−388.05		
M1	361.50	−347.50	40.55	< 0.001
ES(50)[e]				
M0	352.90	−348.90		
M1	332.30	−318.30	30.61	< 0.001
Simpson diversity				
M0	283.45	−279.45		
M1	267.42	−253.42	26.03	< 0.001
Abundance				
M0	639.59	−635.59		
M1	592.02	−578.02	57.57	< 0.001
Latitude				
Species richness				
M0	392.05	−388.05		
M1	379.28	−373.28	14.77	< 0.001
ES(50)[e]				
M0	352.9	−348.90		
M1	349.78	−343.78	5.13	0.023
Simpson diversity				
M0	283.45	−279.45		
M1	284.03	−278.03	1.43	0.232
Abundance				
M0	639.59	−635.59		
M1	605.25	−599.25	36.33	< 0.001

[a] Akaike Information Criterion measures goodness of fit and model complexity; the lower the AIC the better the fit

[b] The log-likelihood is used for model fitted by maximum likelihood; lower values imply a better fit

[c] When the logarithm of the likelihood ratio is used, the statistic is known as the log-likelihood ratio statistic or likelihood ratio statistic

[d] Chi-square test is used to test the significance of likelihood ratios

[e] The expected number of species from a random samples of 50 individuals

Table 3 Set of generalized linear models (GLMs) generated by automated model selection with combinations (subsets) of terms in the global model. Models are ranked by the small sample-size corrected version of Akaike information criterion (AICc). All GLMs are under negative binomial distribution, except for Simpson diversity (i.e., Poisson). The coefficient values for each category or level of seagrass species cannot be necessarily shown in this summary table, thus marked with + symbol. Blank rows means no applicable estimated parameters. DENS = shoot density, SEAG = seagrass species

Variables	Model	Intrc[a]	DENS	SEAG	*df*	Loglik[b]	AICc	Delta[c]	Weight[d]
Species richness	3	1.526		+	8	−167.70	354.2	0.00	0.797
	4	1.539	−0.001	+	9	−167.68	357.0	2.73	0.203
	2	2.526	−0.008		3	−192.73	391.9	37.66	0.000
	1	2.432			2	−194.03	392.3	38.04	0.000
ES(50)[e]	3	1.526		+	8	−155.02	328.9	0.00	0.793
	4	1.103	−0.001	+	9	−154.98	331.6	2.69	0.207
	1	2.197			2	−174.45	353.1	24.24	0.000
	2	2.235	−0.003		3	−174.19	354.8	25.95	0.000
Simpson diversity	3	1.482		+	7	−125.50	267.2	0.00	0.781
	4	1.505	−0.002	+	8	−125.44	269.7	2.54	0.219
	1	1.489			1	−142.81	286.7	20.53	0.000
	2	1.456	0.003		2	−142.53	289.3	22.11	0.000
Abundance	3	1.649		+	8	−279.83	578.5	0.00	0.787
	4	1.689	−0.003	+	9	−279.75	581.1	2.61	0.213
	2	4.597	−0.030		3	−313.28	633.0	54.50	0.000
	1	4.322			2	−317.79	639.8	61.32	0.000

[a] The intercept which is the predicted value of the dependent variable when all the independent variables are 0
[b] Used for a model fitted by maximum likelihood; lower values imply a better fit
[c] Indicates the difference between its AICc and the lowest of all the AICc values
[d] Can be used in model averaging as they represent the relative likelihood of a model
[e] Refers to expected number of species from a random samples of 50 individuals

Table 4 Summary results of the permutational multivariate analysis of variance (PERMANOVA) and the estimates of components of variation with square root equivalents

PERMANOVA					
Source	*df*	SS[a]	MS[b]	Pseudo-F	*P* (perm)[c]
Site	5	79932	15986	5.18	0.001
Depth	2	15935	7967.4	2.58	0.001
Residual	52	1.61×10^5	3089.2		
Total	59	2.61×10^5			

Estimates of components of variation		
Source	Estimate	Sq. root
Sum of square (Site)	1433.0	37.86
Variation (Depth)	325.2	18.03
Variation (Residual)	3089.2	55.58

[a] Sum of square, a measure of deviation from the mean
[b] Mean of square, the estimate of variance across groups
[c] *P* values after permutation procedure

Fig 1

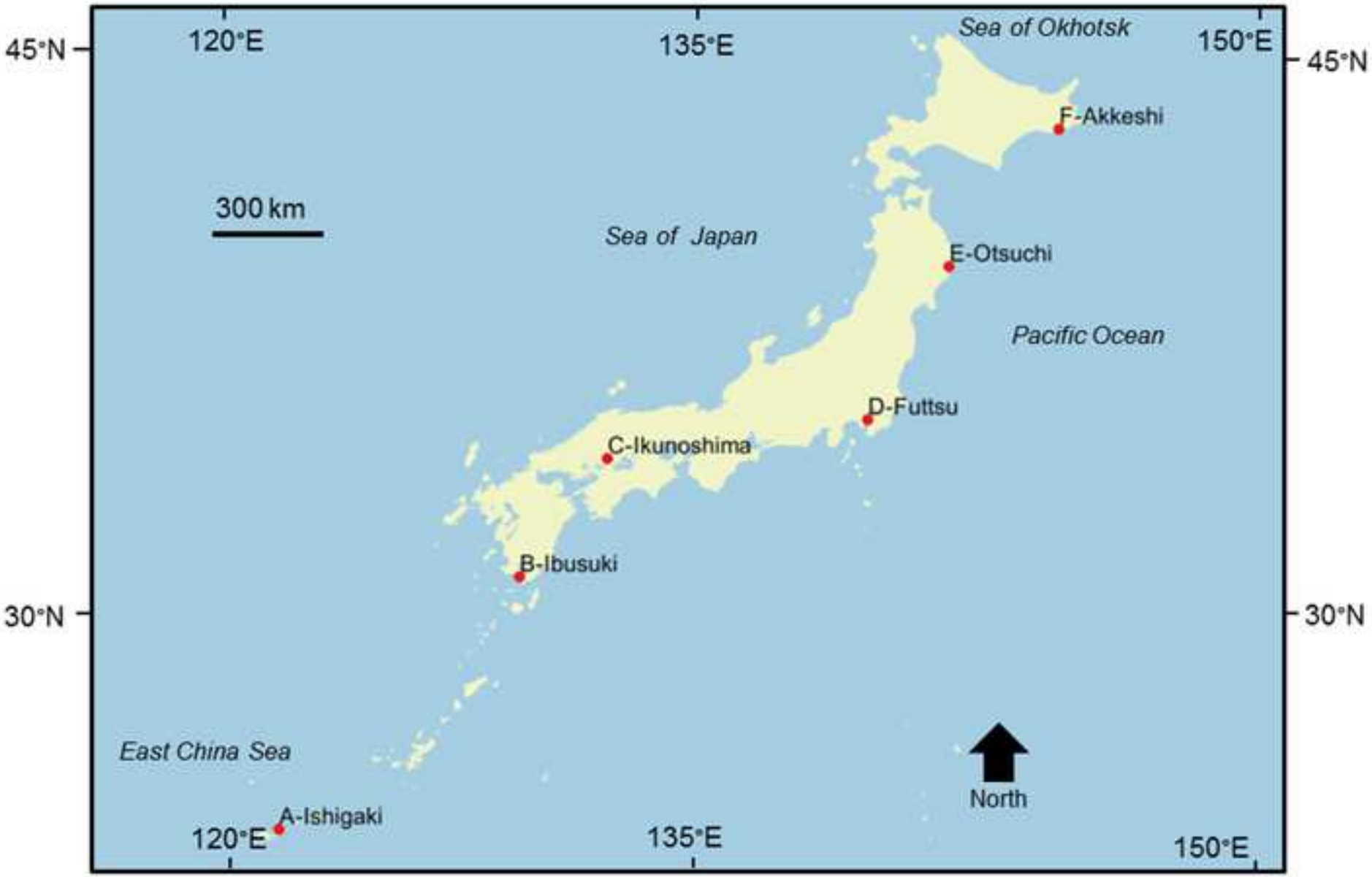

45°N
120°E
135°E
150°E
Sea of Okhotsk
F-Akkeshi
300 km
Sea of Japan
E-Otsuchi
Pacific Ocean
D-Futtsu
C-Ikunoshima
B-Ibusuki
30°N
East China Sea
North
A-Ishigaki

Fig 2

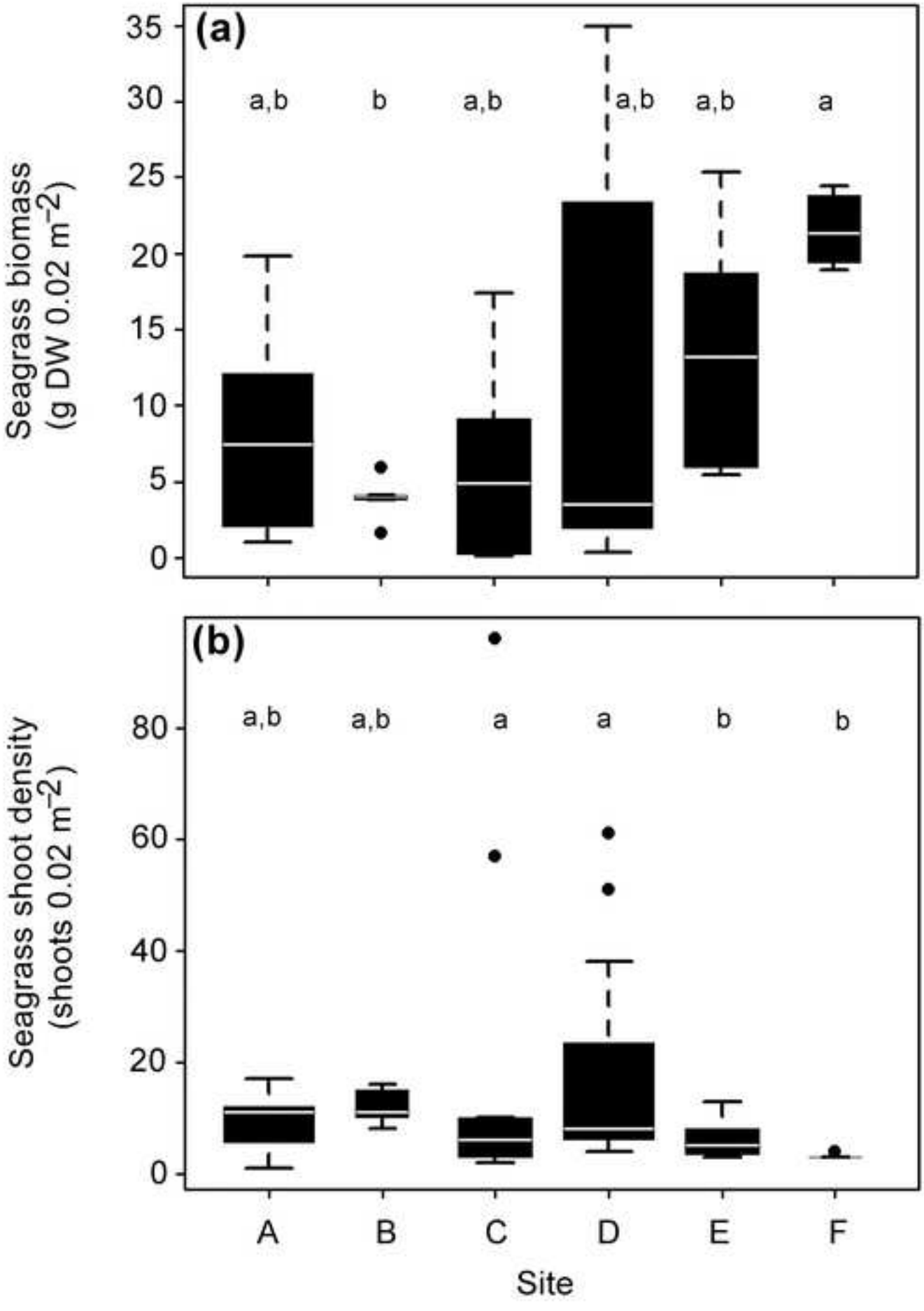

(a)
Seagrass biomass
(g DW 0.02 m^{-2})
35
30
25
20
15
10
5
0
a,b
b
a,b
a,b
a,b
a
(b)
Seagrass shoot density
(shoots 0.02 m^{-2})
80
60
40
20
0
a,b
a,b
a
a
b
b
A
B
C
D
E
F
Site

Fig 3

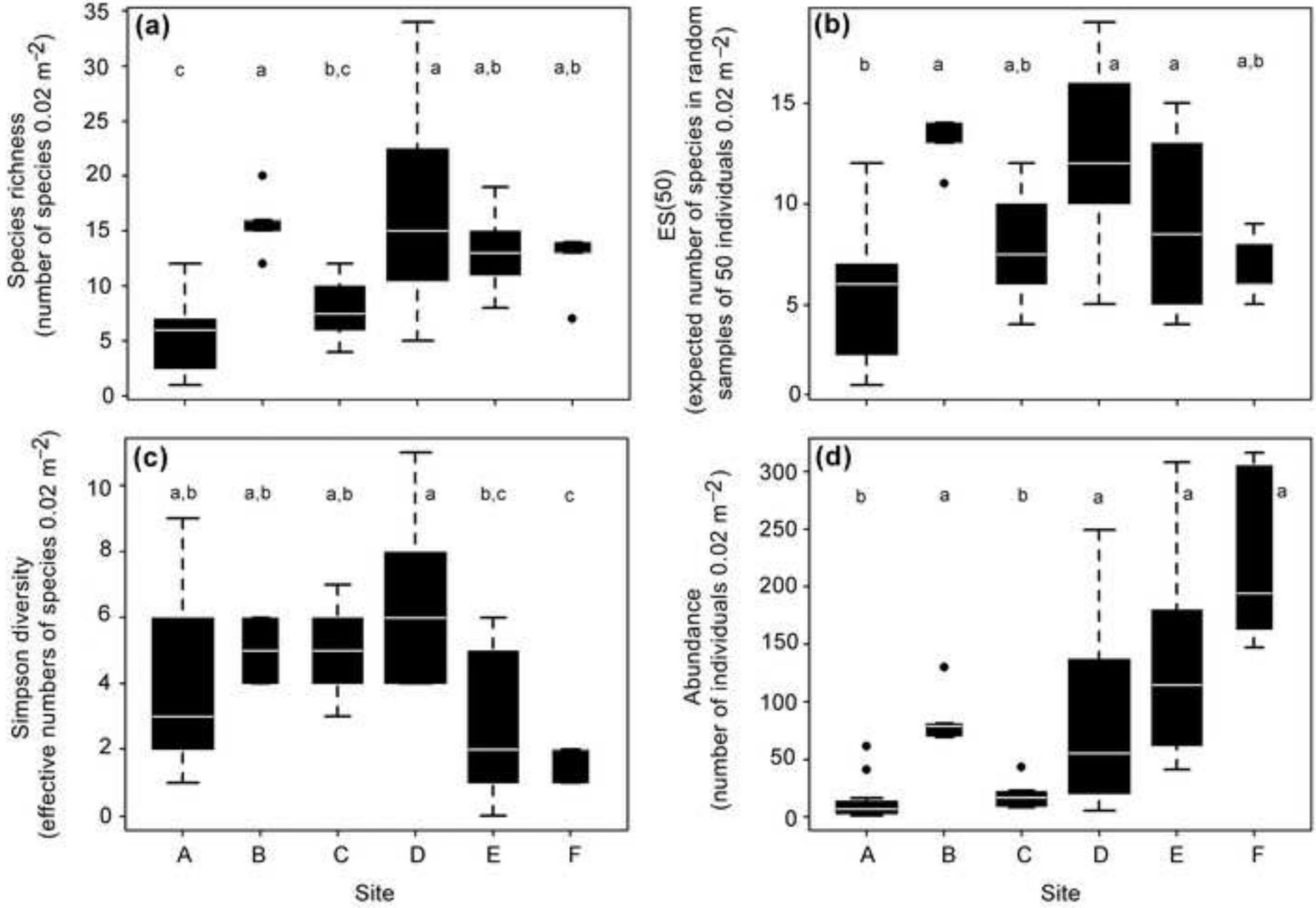

(a)
Species richness
(number of species 0.02 m^{-2})
c
a
b,c
a
a,b
a,b
(b)
ES(50)
(expected number of species in random samples of 50 individuals 0.02 m^{-2})
b
a
a,b
a
a
a,b
(c)
Simpson diversity
(effective numbers of species 0.02 m^{-2})
a,b
a,b
a,b
a
b,c
c
(d)
Abundance
(number of individuals 0.02 m^{-2})
b
a
b
a
a
a
A
B
C
D
E
F
Site

Fig 4

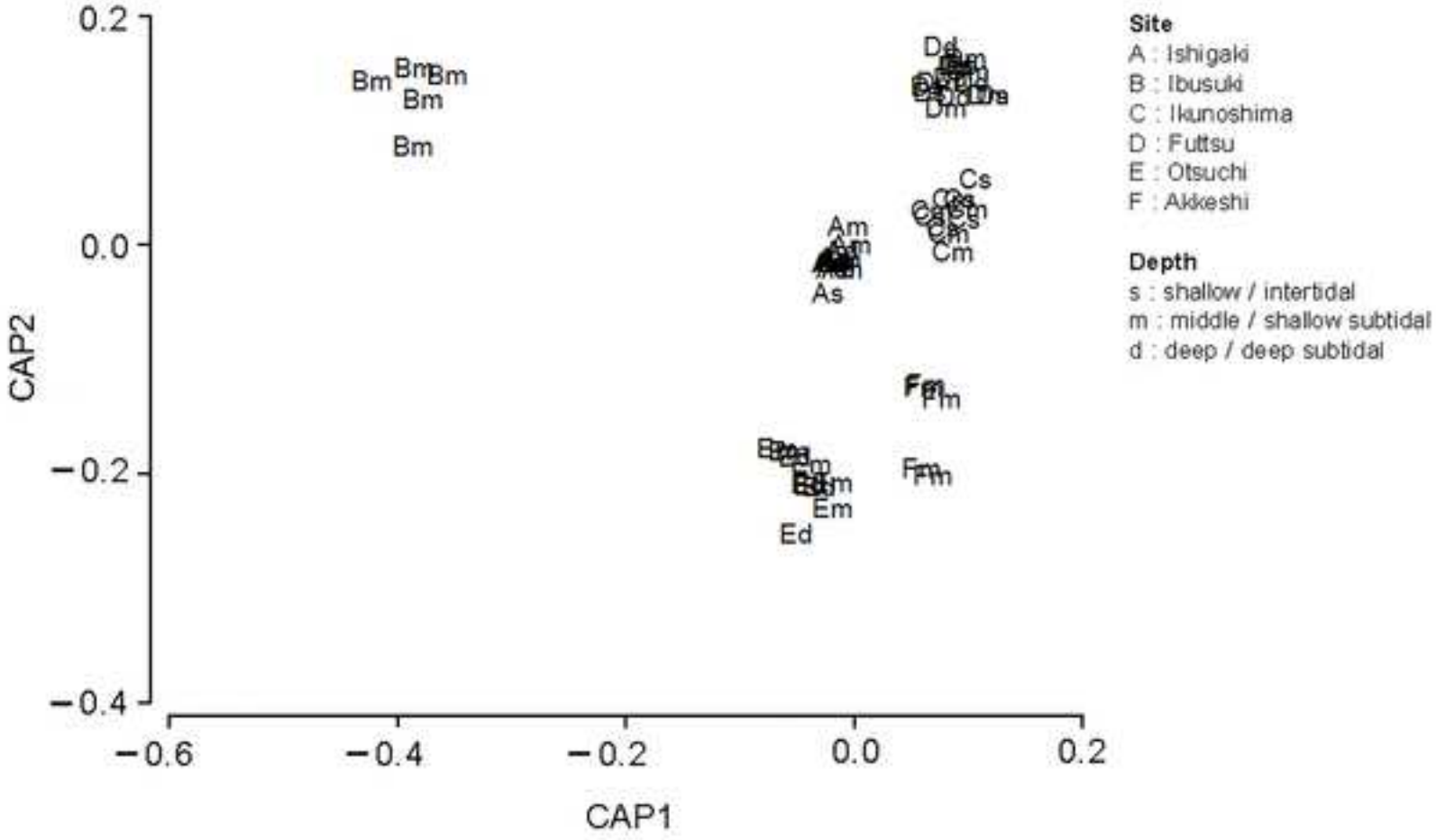
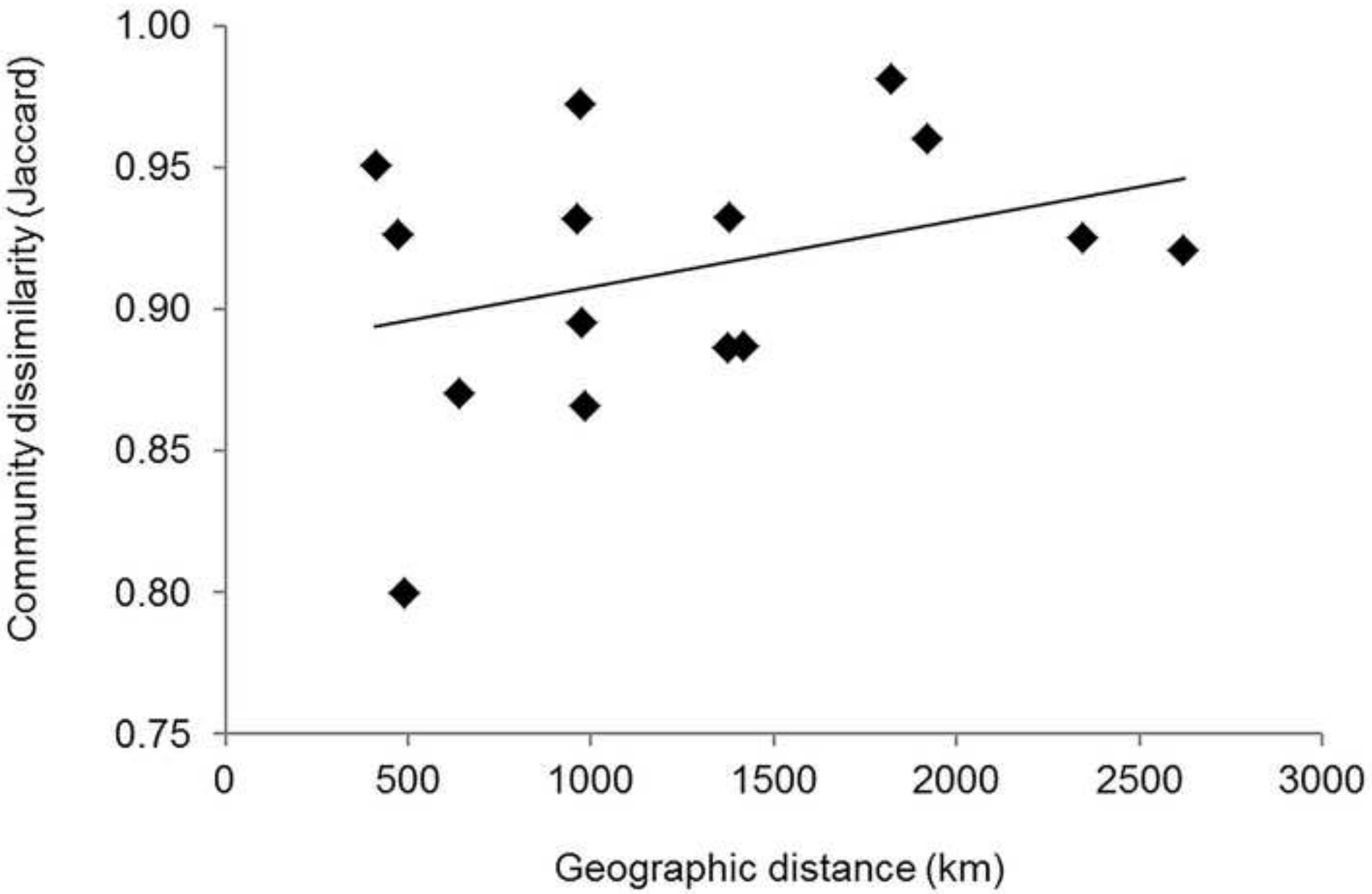

0.2
0.0
−0.2
−0.4
CAP2
−0.6
−0.4
−0.2
0.0
0.2
CAP1
Bm
Am
As
Cs
Cm
Em
Ed
Site
A : Ishigaki
B : Ibusuki
C : Ikunoshima
D : Futtsu
E : Otsuchi
F : Akkeshi
Depth
s : shallow / intertidal
m : middle / shallow subtidal
d : deep / deep subtidal

Fig 5


1.00
0.95
0.90
0.85
0.80
0.75
Community dissimilarity (Jaccard)
0
500
1000
1500
2000
2500
3000
Geographic distance (km)

Title: Broad scale variation in seagrass benthic macrofaunal assemblages along the coast of Japan
Journal: Ecological Research
Authors: Venus Leopardas, Masakazu Hori, Hiroshi Mukai, Yoshiyuki Tanaka, Ken-ichi Yokoi, Misuzu Aoki, Naoto Sato, Napakhwan Whanpetch, Masahiro Nakaoka*

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Table S1 List of macrofauna species collected in the six sites of Japan last 2010 under the Monitoring Sites 1000 project of the Ministry of Environment-Government of Japan

Phylum	Class	Order	Family	Binomial name/Species code
Annelida	Polychaeta	*	Arenicolidae	*Abarenicola pacifica*
			Capitellidae	*Capitella* sp.
		Echiuroidea	Echiuridae	Echiuridae gen. sp.
		Eunicida	Dorvilleidae	Dorvilleidae gen. sp. 1
				Dorvilleidae gen. sp. 2
			Lumbrineridae	*Lumbrineris* sp. 1
				Lumbrineris sp. 2
			Onuphidae	*Onuphis shirikishinaiensis*
		*	Opheliidae	*Ophelia* sp.
		*	Paraonidae	Paraonidae gen. sp.
		Phyllodocida	Dorvilleidae	Dorvilleidae gen. sp. 1
			Glyceridae	*Glycera* sp.
			Goniadidae	*Glycinde nipponica*
			Nephtyidae	*Nephtys* sp. 1
				Nephtys sp. 2
			Nereididae	*Neanthes* sp.
				Nectoneanthes sp.
				Nereididae gen. sp. 1
				Nereididae gen. sp. 2
				Nereis sp.
				Nereis zonata
			Phyllodicidae	*Eulalia viridis*
				Phyllodoce sp.
			Pilargidae	*Sigambra* sp.
			Polynoidae	*Lepidasthenia ocellata*
				Polynoidae gen. sp. 1
				Polynoidae gen. sp. 2
			Syllidae	Syllidae gen. sp. 1
				Syllidae gen. sp. 2
				Syllidae gen. sp. 3
		Sabellida	Maldanidae	*Maldane* sp.
			Oweniidae	*Owenia* sp.
			Sabellidae	*Bispira* sp.
				Sabellidae gen. sp.
			Serpulidae	*Spirorbis* sp.
		Spionida	Spionidae	*Prionospio* sp.
				Pseudopolydora sp.
		Terebellida	Cirratulidae	*Chaetozone setosa*
				Cirriformia sp.
			Terebellidae	Terebellidae gen. sp. 1
				Terebellidae gen. sp. 2
				Terebellidae gen. sp. 3
Arthropoda	Leptostraca	Nebaliacea	Nebaliidae	*Nebalia* sp.
	Malacostraca	Amphipoda	Ampeliscidae	*Ampelisca brevicornis*

			Byblis japonicus
		Ampithoidae	*Ampithoe lacertosa*
		Aoridae	*Aoroides* sp. 1
			Aoroides sp. 2
			Grandidierella japonica
		Atylidae	*Kamehatylus japonicus*
		Caprellidae	*Caprella* sp. 1
			Caprella sp. 2
			Caprella sp. 3
		Dexaminidae	*Paradexamine* sp.
		Hyalidae	*Ptilohyale barbicornis*
		Isaeidae	Isaeidae gen. sp.
		Ischyroceridae	*Cerapus tubularis*
			Ericthonius pugnax
			Jassa sp.
		Liljeborgiidae	*Liljeborgia* sp.
		Lysianassidae	*Orchomene* sp.
		Melitidae	*Melita* sp. 1
			Melita sp. 2
			Melitidae gen. sp.
		Oedicerotidae	*Eochelidium lenorostralum*
		Phoxocephalidae	Phoxocephalidae gen. sp.
		Pleustidae	*Pleustes* sp.
		Pontogeneiidae	*Pontogeneia rostrata*
	Cumacea	Diastylidae	*Diastylis* sp.
	Decapoda	Alpheidae	*Alpheus* sp.
		Callianassidae	*Callianassa* sp.
			Nihonotrypaea japonica
		Camptandriidae	Camptandriidae gen. sp
		Crangonidae	*Crangon* sp.
		Diogenidae	*Calcinus* sp.
		Dotillidae	*Tmethypocoelis choreutes*
		Hippolytidae	Hippolytidae gen. sp.
		Majidae	Majidae gen. sp.
		Mysidae	*Nipponomysis* sp.
		Paguridae	*Pagurus* sp.
		Pinnotheridae	*Pinnixa tumida*
			Pinnotheres sp.
		Portunidae	Portunidae gen. sp.
			Pseudomicippe nipponica
		Sesarmidae	*Nanosesarma gordoni*
		Upogebiidae	*Upogebia major*
			Upogebia sp.
		Varunidae	*Gaetice depressus*
	Isopoda	Idoteidae	*Idotea ochotensis*
			Idoteidae gen. sp.
			Synidotea laevidorsalis
		Paranthuridae	*Paranthura* sp.
			Paranthuridae gen. sp.
		Sphaeromatidae	Sphaeromatidae gen. sp.
	Mysida	Mysidae	*Nipponomysis* sp.
	Nebaliacea	Nebaliidae	*Nebalia* sp.
	Tanaidacea	Apseudidae	*Apseudes spectabilis*
		Tanaidae	*Sinelobus* sp.
Maxillopoda	Sessilia	Ballanidae	*Amphibalanus improvisus*

				Balanus sp.
	Ostracoda	Myodocopida	*unknown 1*	Myodocopida fam. gen. sp.
		Podocopida	*unknown 2*	Podocopida fam. gen. sp.
Cnidaria	Anthozoa	Actiniaria	Edwardsiidae	Edwardsiidae gen. sp.
			unknown 3	Actiniaria fam. gen. sp. 1
				Actiniaria fam. gen. sp. 2
		Ceriantharia	Halcampidae	Halcampidae gen. sp. 1
				Halcampidae gen. sp. 2
		Actiniaria	*unknown 4*	Actiniaria fam. gen. sp. 1
	Staurozoa	Stauromedusae	Lucernariidae	*Stenoscyphus inabai*
Echinodermata	Asteroidea	Forcipulatida	Asteriidae	Asteriidae gen. sp.
	Holothuroidea	Apodida	Myriotrochidae	Myriotrochidae gen. sp.
		Dendrochirotida	Sclerodactylidae	*Eupentacta chronhjelmi*
	Ophiuroidea	Ophiurida	Amphiuridae	*Amphiodia* sp.
				Amphiura sp.
			unknown 5	Ophiurida fam. gen. sp.
Foraminifera	Tubothalamea	Miliolida	Soritidae	*Marginopora zeniishi*
Hemichordata	Enteropneusta	Enteropneusta**	Ptychoderidae	*Balanoglossus* sp.
Mollusca	Bivalvia	Euheterodonta**	Hiatellidae	*Hiatella orientalis*
		Limoida	Limidae	*Limalatula* sp.
		Lucinoida	Lucinidae	Lucinidae gen. sp.
				Pillucina pisidium
			Thyasiridae	*Thyasira* sp.
		Myoida	Myidae	*Mya arenaria*
		Mytiloida	Mytilidae	*Arcuatula senhousia*
				Musculus cupreus
				Mytilus galloprovincialis
		Nuculida	Nuculidae	*Acila divaricata*
		Pectinoida	Propeamussiidae	*Propeamussium* sp.
		Solemyoida	Solemyidae	*Solemya pusilla*
		Veneroida	Veneridae	Veneridae gen. sp.
				Callithaca adamsi
				Lioconcha fastigiata
				Ruditapes philippinarum
				Veremolpa micra
			Cardiidae	*Fulvia australis*
			unknown 6	Veneroida fam. gen. sp.
			Galeommatidae	*Scintilla* sp.
			Galeommatoidea	Galeommatoidea gen. sp.
			Kelliidae	*Nesobornia* sp.
			Mactridae	Mactridae gen. sp.
			Mesodesmatidae	Mesodesmatidae gen. sp.
			Tellinidae	*Macoma incongrua*
				Tellina hokkaidoensis
				Tellina sp.
				Tellinidae gen. sp.
	Gastropoda	Caenogastropoda**	Batillariidae	*Batillaria* sp.
			Cerithiidae	*Cerithidium* sp.
				Cerithiidae gen. sp.
				Ittibittium parcum
			Dialidae	*Diala albugo*
				Diala semistriata
			Litiopidae	*Diaffalaba picta*
		*	Acmaeidae	*Erginus moskalevi*
				Siphonacmea oblongata

		Cephalaspidea	*unknown 7*	Cephalaspidea fam. gen. sp.
			Philinidae	*Philine* sp.
			Retusidae	*Retusa* sp.
				Retusidae gen. sp.
		Cycloneritimorpha	Neritidae	*Smaragdia* sp. 1
				Smaragdia sp. 2
		Discopoda	Scaliolidae	*Scaliola glareosa*
		Heterostropha	Pyramidellidae	*Orinella pulchella*
		Littorinimorpha	Littorinidae	*Lacuna decorata*
			Naticidae	*Tectonatica venustula*
			Rissoidae	*Lucidestea* sp.
				Rissoidae gen. sp.
			Rissoinidae	*Rissoina* sp.
		Neogastropoda	Columbellidae	*Zafra* sp.
			Costellariidae	*Vexillum* (*Costellaria*) *michaui*
				Vexillum exasperatum
			Nassariidae	*Nassarius albescens*
				Nassarius festivus
				Nassarius limnaeiformis
				Nassarius multigranosus
				Nassarius nodifer
				Nassarius sp. 1
				Nassarius sp. 2
			Olivellidae	*Olivella fulgurata*
		Nudibranchia	*unknown 8*	Nudibranchia fam. gen. sp.
		*	Pyramidellidae	*Turbonilla edoensis*
		Vetigastropoda	Fissurellidae	Fissurellidae gen. sp.
			Trochidae	*Cantharidus callichroa*
				Cantharidus japonicus
				Ethminolia sp.
				Komaitrochus pulcher
				Lirularia iridescens
				Trochidae gen. sp.
Nemertea	Anopla	*	Lineidae	*Lineus* sp.
	Enopla	Monostilifera	Amphiporidae	*Amphiporus* sp.
	Palaeonemertea	*	Carinomidae	Carinomidae gen. sp.
Platyhelminthes	Rhabditophora	Polycladida	Notoplanidae	*Notoplana* sp. 1
				Notoplana sp. 2
Sipuncula	Sipunculidea	Golfingiida	*unknown 9*	Golfingiida fam. gen. sp.
			Golfingiidae	*Golfingia* (*Golfingia*) *margaritacea*
			Sipunculidae	*Siphonosoma cumanense*
				Sipunculidae gen. sp.

*Unassigned; Order rank not documented in World Register of Marine Species (WoRMS Editorial Board, 2015)

** Order rank is stated but set as ‘Unassigned’ in WoRMS

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Table S2 Site-by-species abundance matrix of benthic macrofaunal samples collected and identified in the six sites of Japan last 2010 under the Monitoring Sites 1000 project of the Ministry of Environment-Government of Japan

Macrofaunal sample	Total number of individuals per site						Total abundance
	Ishigaki (A)	Ibusuki (B)	Ikunoshima (C)	Futtsu (D)	Otsuchi (E)	Akkeshi (F)	
Abarenicola pacifica					1		1
Acila divaricata					1		1
Actiniaria fam. gen. sp. 1				11			11
Actiniaria fam. gen. sp. 2					1		1
Alpheus sp.	11						11
Ampelisca brevicornis		1					1
Amphibalanus improvisus				4			4
Amphiodia sp.	2				1	2	5
Amphiporus sp.					2		2
Amphiura sp.		18					18
Ampithoe lacertosa	3	75	25	250	5	2	360
Aoroides sp. 1				5			5
Aoroides sp. 2				4	4		8
Apseudes spectabilis	1						1
Arcuatula senhousia		1		180			181
Asteriiidae gen. sp.					1		1
Balanoglossus sp.	2						2
Balanus sp.				4			4
Batillaria sp.			1				1
Bispira sp.					1		1
Byblis japonicus			1	3	2		6
Calcinus sp.	2						2
Callianassa sp.		4					4
Callithaca adamsi					2		2
Camptandriidae gen. sp			3				3
Cantharidus callichroa					4		4
Cantharidus japonicus				51			51
Capitella sp.		24			2		26
Caprella sp. 1		12		61	1		74
Caprella sp. 2		1		220	1		222
Caprella sp. 3						1	1
Carinomidae gen. sp.				23	2		25
Cephalaspidea fam. gen. sp.	2						2
Cerapus tubularis					63		63

Cerithidium sp.	1						1
Cerithiidae gen. sp.			2				2
Chaetozone setosa					1		1
Cirriformia sp.			5	43		32	80
Crangon sp.						1	1
Diaffalaba picta		15		8			23
Diala albugo	3						3
Diala semistriata			8				8
Diastylis sp.				19	2		21
Dorvilleidae gen. sp. 1				2	1		3
Dorvilleidae gen. sp. 2					1		1
Echiuridae gen. sp.	1						1
Edwardsiidae gen. sp.				1			1
Eochelidium lenorostralum		1		4			5
Erginus moskalevi					1	866	867
Ericthonius pugnax		154		1			155
Ethminolia sp.	2						2
Eulalia viridis				4			4
Eupentacta chronhjelmi				1			1
Fissurellidae gen. sp.				2			2
Fulvia australis	1						1
Gaetice depressus				1			1
Galeommatoidea gen. sp.				1			1
Glycera sp.	4	2		4	4		14
Glycinde nipponica						7	7
Golfingia (*Golfingia*) *margaritacea*				1			1
Golfingiida fam. gen. sp.	33		2				35
Grandidierella japonica	1		1			53	55
Halcampidae gen. sp. 1			5				5
Halcampidae gen. sp. 2				1	3		4
Hiatella orientalis				1			1
Hippolytidae gen. sp.			3				3
Idotea ochotensis						1	1
Idoteidae gen. sp.				2			2
Isaeidae gen. sp.				1			1
Ittibittium parcum	3			1			4
Jassa sp.				39	1		40
Kamehatylus japonicus						1	1
Komaitrochus pulcher				1			1
Lacuna decorata					51	5	56
Lepidasthenia ocellata		3			1		4
Liljeborgia sp.		2	4				6
Limalatula sp.	2						2
Lineus sp.			1				1
Lioconcha fastigiata	1						1
Lirularia iridescens					1019	1	1020

Lucidestea sp.			6				6
Lucinidae gen. sp.	15						15
Lumbrineris sp. 1	1						1
Lumbrineris sp. 2			8		9		17
Macoma incongrua				5			5
Mactridae gen. sp.				1			1
Majidae gen. sp.			1				1
Maldane sp.			1		5	1	7
Marginopora zeniishi	60						60
Melita sp. 1				2			2
Melita sp. 2					2		2
Melitidae gen. sp.			2	17			19
Mesodesmatidae gen. sp.				2			2
Musculus cupreus		1					1
Mya arenaria					3	63	66
Myodocopida fam. gen. sp.			2	1		13	16
Myriotrochidae gen. sp.			4				4
Mytilus galloprovincialis				2	2		4
Nanosesarma gordoni				1			1
Nassarius albescens	1						1
Nassarius festivus			8	16	1		25
Nassarius limnaeiformis	1						1
Nassarius multigranosus				5	5		10
Nassarius nodifer	1						1
Nassarius sp. 1		3	9				12
Nassarius sp. 2					5		5
Neanthes sp.			23	20			43
Nebalia sp.			1	3	1	1	6
Nectoneanthes sp.			1	11	1		13
Nephtys sp. 1		9					9
Nephtys sp. 2			25	12	25		62
Nereididae gen. sp. 1			2				2
Nereididae gen. sp. 2				5			5
Nereis sp.		14		46			60
Nereis zonata				5			5
Nesobornia sp.				1			1
Nihonotrypaea japonica		14		2			16
Nipponomysis sp.	10				2		12
Notoplana sp. 1				1			1
Notoplana sp. 2						3	3
Nudibranchia fam. gen. sp.		3					3
Olivella fulgurata		1			1		2
Onuphis shirikishinaiensis	2					18	20
Ophelia sp.		4			5		9
Ophiurida fam. gen. sp.	1		1		1		3
Orchomene sp.			1	10	21		32

Orinella pulchella				2			2
Owenia sp.				17	2		19
Pagurus sp.				2	8		10
Paradexamine sp.			9	3			12
Paranthura sp.				8			8
Paranthuridae gen. sp.		4					4
Paraonidae gen. sp.		18					18
Philine sp.				2			2
Phoxocephalidae gen. sp.						6	6
Phyllodoce sp.				1			1
Pillucina pisidium	1		3		13		17
Pinnixa tumida				1			1
Pinnotheres sp.		1					1
Pleustes sp.					1		1
Podocopida fam. gen. sp.				1	2	23	26
Polynoidae gen. sp. 1		10		2	28		40
Polynoidae gen. sp. 2		1					1
Pontogeneia rostrata				14		17	31
Portunidae gen. sp.	2			1			3
Prionospio sp.					12		12
Propeamussium sp.					1		1
Pseudomicippe nipponica				2			2
Pseudopolydora sp.				7			7
Ptilohyale barbicornis					10		10
Retusa sp.				1			1
Retusidae gen. sp.	1						1
Rissoidae gen. sp.		1					1
Rissoina sp.	1						1
Ruditapes philippinarum			1	7			8
Sabellidae gen. sp.			5				5
Scaliola glareosa	1						1
Scintilla sp.	1						1
Sigambra sp.		1					1
Sinelobus sp.				4	1		5
Siphonacmea oblongata					1		1
Siphonosoma cumanense			1	4			5
Sipunculidae gen. sp.				1			1
Smaragdia sp. 1	2						2
Smaragdia sp. 2	3						3
Solemya pusilla					1		1
Sphaeromatidae gen. sp.	1			3	2	2	8
Spirorbis sp.		31					31
Stenoscyphus inabai				11			11
Syllidae gen. sp. 1				10			10
Syllidae gen. sp. 2				5			5
Syllidae gen. sp. 3				2			2

Synidotea laevidorsalis					1		1
Tectonatica venustula	1						1
Tellina hokkaidoensis	1						1
Tellina sp.	1						1
Tellinidae gen. sp.				1			1
Terebellidae gen. sp. 1	1						1
Terebellidae gen. sp. 2			1				1
Terebellidae gen. sp. 3				1			1
Thyasira sp.				1			1
Tmethypocoelis choreutes	1						1
Trochidae gen. sp.				1			1
Turbonilla edoensis				5			5
Upogebia major						4	4
Upogebia sp.	2						2
Veneridae gen. sp.						2	2
Veneroida fam. gen. sp.				2			2
Veremolpa micra				1			1
Vexillum (*Costellaria*) *michaui*	1						1
Vexillum exasperatum	4						4
Zafra sp.				2			2
Total abundance	192	429	176	1247	1351	1125	4520

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* Corresponding author. E-mail: nakaoka@fsc.hokudai.ac.jp

Fig. S1 Relationship of ES(50) (expected number of species obtained from a subsample of 50 individuals) at site level to latitude ranging from 24°N to 43°N along the coast of Japan