1 **Variation in macrofaunal communities of sea grass beds along a pollution gradient in**
2 **Bolinao, northwestern Philippines**

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ABSTRACT

This study examined the variation of macrofaunal communities in sea grass beds along a pollution gradient in Bolinao, northwestern Philippines. We established four stations and compared the diversity and abundance of macrofauna between them. The Shannon diversity index in the least polluted station was more than twice higher than that in the most polluted one. Abundance was more than thrice higher in the most polluted station. The species composition generally varied, with community difference explained largely by the predominance of the filter-feeding bivalve *Gafrarium pectinatum* and polychaete *Capitella capitata*. Species heterogeneity was reduced along the pollution gradient by approximately 19% from the least polluted to the most polluted station. This reduction indicates biodiversity alteration, which has a significant impact on ecosystem functioning. Aspects of species heterogeneity should be considered in environmental impact assessments and the management of coastal areas encountered with anthropogenic disturbances.

Keywords: Abundance, Macrobenthos, Mariculture-induced pollution, Sea grass bed, Species diversity, the Philippines

1. Introduction

The worldwide decline in sea grass beds due to human-induced pollution, including marine fish farming, has been previously reported (Hemminga and Duarte 2000; Larkum et al. 2006; Waycott et al. 2009; Unsworth et al. 2014). However, as the majority of the studies have focused on the effects of pollution on sea grasses, there have been only few reports on the impact of fish farming on sea grass-associated fauna, including benthic macrofauna or macroinvertebrates (e.g., Kalantzi and Karakassis 2006; Katavic and Antolic 1999; Dimech et al. 2002; Eklöf et al. 2005; Apostolaki et al. 2007; Papageorgiou et al. 2009). Hence, we lack valid information on mariculture-induced effects on sea grass macrofaunal communities despite their significant importance in maintaining multiple functions

60 of sea grass ecosystems and on the sustainable use of provisioning services by sea grass beds
61 (Cullen-Unsworth et al. 2013; Nakaoka et al. 2014; Unsworth et al. 2014; Duffy et al. 2015).

62 Sea grass beds are highly valuable ecosystems on Earth (Costanza et al. 1998; Moberg and
63 Rönnbäck 2003; Nakaoka et al. 2014) and serve as a valid example of coupled social–ecological
64 system (Cullen-Unsworth et al. 2013; Unsworth et al. 2014). The declining status of sea grass
65 resource, due to local- and/or global-scale environmental change, has significant effects on the
66 resilience of the society and coupled social–ecological system (Cullen-Unsworth et al. 2014). Many
67 macrobenthic animals, together with fish, associated with sea grass beds, such as crustaceans,
68 mollusks, and echinoderms, have been exploited by humans for their income generation and/or food
69 (see also Nakaoka et al. 2014). Dramatic decrease or even nonexistence of sea grass invertebrates
70 threatens an important food and income source, resulting in negative impacts on the well-being and
71 livelihood of the society that is dependent on sea grass fisheries (*sensu* Cullen-Unsworth et al. 2014).
72 Despite their importance, sea grass beds have been experiencing unprecedented rates of loss and
73 increasing exploitations (Duarte 2002; Waycott et al. 2009). Considering the role of sea grass
74 macrofaunal community in achieving environmental change is an important step to protect the society
75 and maintain ecological resilience in sea grass system.

76 A qualitative description of the effects of organic pollution on benthic dynamics was described by
77 Pearson and Rosenberg (1978): species richness declines, whereas biomass and abundance initially
78 increase and then decrease as the pollution load increases. The predominance of opportunistic
79 species in a polluted environment (i.e., primarily some species of polychaetes) changes the species
80 composition of benthic communities (Warwick and Clarke 1994). Studies involving a predictive
81 analysis have highlighted the role of hypoxia in the alteration of benthic structures (e.g., Diaz and
82 Rosenberg 1995; Heip 1995; Nilsson and Rosenberg 2000; Rosenberg 2001). Gray et al. (2002)
83 suggested that, among the various marine fauna, fish are the most sensitive to hypoxia, whereas
84 mollusks are the least sensitive, followed by polychaetes. Many benthic species exhibit different
85 behaviors to a polluted environment before they eventually die (Diaz and Rosenberg 1995), which
86 leads to a wide variation in assemblage structures along the pollution gradient.

87 Univariate measures, such as the number of species, abundance, and biomass, and multivariate
88 measures, such as species composition and similarities, are often used to describe the population
89 and community responses to natural and/or human-induced disturbances (e.g., Warwick 1986;

Warwick et al. 1987; Tsutsumi et al. 1991; Simboura et al. 1995; Botter-Carvalho et al. 2014). While a diversity index is commonly used to analyze most field data, the index values can be converted to true diversity values or effective numbers of species, making it directly comparable to species richness (Jost 2006, 2007). Although recent studies have considered scale dependency in the measures of community composition by partitioning the variability in diversity into alpha and beta components (Ellingsen and Gray 2002; Okuda et al. 2004), there is little information on whether species composition varies at the within-site scale or whether such within-site variability or heterogeneity changes along environmental gradients (Ellingsen 2001; Ellingsen 2002; Becking et al. 2006; Lohrer et al. 2013). Understanding several aspects of macrobenthic community structures based on field data may lead to more informed management decisions regarding pollution-related environmental concerns.

The aim of this study was to investigate the variation in community structure of macrobenthic invertebrates in sea grass beds along a pollution gradient. The study was conducted in a mariculture-impacted area of Bolinao, northwestern Philippines, with four sampling stations established along a known gradient of dissolved nutrients and chlorophyll-a concentrations. Our general hypothesis was that the macrofaunal community structures would be negatively altered; in particular, species diversity and abundance of macrofauna would be suppressed toward the polluted stations. We also hypothesized that the community assemblages would vary across stations. The likely predominance of some opportunistic species and species tolerance to pollution were also expected in the stations. Finally, we hypothesized that the species heterogeneity (i.e., variation in species composition among samples within a given area) would be reduced toward polluted stations.

2. Materials and methods

2.1 Study area

The study was conducted in September 2010 and March 2011 in Bolinao, northwestern Philippines (**Fig. 1**). The area has the most extensive reef system in the northwestern part of the country (Mcmannus et al. 1992), with approximately 22,500 ha of sea grass coverage. The unregulated establishment of milkfish (*Chanos chanos*)-farming pens and cages on its shallow coastal waters since 1995 has dramatically produced negative effects in water and sediment qualities (Holmer et al.

2002; Holmer et al. 2003; San Diego-McGlone et al. 2008; Santander et al. 2008; David et al. 2009) such as fish kills and occurrences of harmful algal blooms (Yap et al. 2004; Azanza et al. 2005; Escobar et al. 2013). The environmental condition in this poorly flushed area remains serious (*sensu* Ferrera et al. unpublished).

Four stations located along the subtidal zone, with a sampling depth of 0.5–1.0 m (near the low-tide watermark), were established in the study area (**Fig. 1**). All these stations were located in the eastern reef flat of Santiago Island, the largest island of Bolinao, which is separated from the mainland by a deep channel. Station 1 was the closest to mariculture areas (i.e., <1 km away from the fish pens and cages). Station 2 was located <2 km northeast of station 1, station 3 was <4 km north of station 2, and station 4 was located <4 km northwest of station 3 within the Bolinao sea grass reserve. As a point of reference, we considered stations 1 and 4 as the most and least polluted stations, respectively (Watai et al. 2015). The silt-clay component of the sediment near the sampling stations, before mariculture was fully established in Bolinao, did not show a huge difference (**Fig. 1**, 6.7–9.8%, Terrados et al. 1998). The range was found to be much lower than that in the south of the channel between Santiago Island and the mainland (27.4%).

A short-term temporal survey on these sampling stations regarding the dissolved nutrients and chlorophyll-a concentrations (**Table 1**, Fortes et al. 2012) showed some variation in the water quality parameters between them (Ashikawa et al. 2008 in Watai et al. 2015). In addition, hydrodynamic simulation by the members of our research group in the “Coastal Ecosystem Conservation and Adaptive Management under Local and Global Environmental Impacts in the Philippines (CECAM)” project showed a monotonic decline in nitrogen load from the most polluted station (1) to the less polluted station (4) (Nadaoka 2015). Other colleagues of the project, Ferrera et al. (unpublished), whose study locations were close to our sampling stations, reported that Bolinao coastal waters remained eutrophic, with high dissolved inorganic phosphate (up to 4 μ M during the dry season) being exacerbated by the increase in fish farm structures.

Tanaka et al. (2014) reported that the mariculture-induced pollution negatively alters the composition and/or richness of the species of the sea grass vegetation in Bolinao. Within our sampling stations, the species richness starts from at least two species at station 1 (*Enhalus acoroides* and *Thalassia hemprichii*) to seven species at station 4 (*E. acoroides*, *T. hemprichii*, *Halophila ovalis*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule uninervis*, and *Syringodium*

isoetifolium) (Fortes et al. (2012)). On the contrary, the difference in gleaning/fishing access across some stations could possibly influence the variation of macrofaunal community structure. The sea grass beds in stations 1–3 can be freely gleaned by coastal villagers, whereas those in station 4 were not (as it is located within the sea grass sanctuary). This and other possible confounding factors were beyond the scope of this study.

2.2 Sample collection and laboratory work

Macrofauna were collected by sediment coring to a depth of 10 cm (2010: $n = 10$, 2011: $n = 5$). The aluminum core had an inner diameter of 20 cm, resulting in a surface area of 0.03 m² per core sample. The samples were haphazardly sampled within approximately 50-m diameter of sea grass bed at each station (i.e., the same area used by Fortes et al. 2012). A sieve with a 1.0-mm mesh opening was used to partially sieve core samples in the field, thereby removing unwanted items such as stones, sand, and debris.

The collected samples were placed in prelabeled sample bags and then brought to the laboratory after each sampling period for processing. In the laboratory, the core samples were cleaned using seawater, and the macrofaunal individuals were collected and sorted to the lowest taxonomic level, where possible, using taxonomic references for polychaetes (Fauchald 1977; Fauchald and Jumars 1979), mollusks (Hinton 1975; Okutani 2000), echinoderms (Schoppe 2000), and a general updated taxonomy of all marine fauna (WoRMS Editorial Board 2015). The count for each species per core sample was also recorded. Macrofaunal samples were preserved with 70% ethanol.

2.3 Data analyses

Majority of the results of our univariate and multivariate analyses did not show variation across sampling time/period. Our data set then composed of 15 replicate samples per station and all proceeding statistical analyses were compared between stations.

2.3.1 Univariate analyses

We counted the total number of species per station and computed the effective number of species per station based on the Shannon diversity index (Jost 2006, 2007). These values were set as the

species richness and Shannon diversity per station, respectively. We also counted the average number of species per core (0.03 m^2 , $n = 15$) across all stations and computed the effective number of species per core based on the Shannon diversity index. These values were set as the species richness and Shannon diversity per core, respectively. The latter two univariate entities were statistically tested using one-way analysis of variance (ANOVA) for station-wise comparison. We conducted pairwise comparisons when the station effect was found statistically significant (95% confidence interval). We also applied ANOVA to test whether macrofaunal abundance varied significantly between stations. Of all the tested univariate data, only the abundance data set was transformed (logarithmic; with addition of a constant value of 1) to achieve normality in distribution and homogeneity in variance before conducting the parametric test. All univariate analyses were conducted using RStudio version 0.99.467 (RStudio Team 2015).

2.3.2 Multivariate analyses

We used the presence–absence data of all species based on the Jaccard resemblance index in the canonical analysis of principal coordinates (CAP; Anderson and Willis 2003) to visualize the distinctiveness of community assemblages across the four stations in multivariate space (Anderson et al. 2008). Using the same data set and index, we conducted the permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to determine whether between-station variation (reflected in CAP ordination) significantly contributes to the total variation in the macrofaunal community. The pairwise tests were conducted when the effect of the stations was found statistically significant and the p values were obtained from Monte Carlo tests.

In order to determine the macrofaunal species which explained the variation in species composition between stations, the abundance data for all species per station contrast (i.e., those stations that were statistically different in PERMANOVA) were used for the univariate tests in the *mvabund* program (Warton et al. 2012; Wang et al. 2014). The contribution of a species to the total effect was determined by dividing the species likelihood ratio (LR) statistic (deviance) by the sum of the LR or total deviance of the multivariate data (personal communication, D. Warton). This analysis was specifically conducted using RStudio version 0.99.467 (RStudio Team, 2015).

In order to analyze the species heterogeneity within each station, we performed the permutation of multivariate dispersion (PERMDISP; Anderson 2006; Anderson et al. 2006) on the same data set and

resemblance index. We also applied pairwise tests when the effect of station was found significant. Dispersion in the data set could influence the patterns observed in PERMANOVA (*sensu* Anderson 2001), but only when the data have an unbalanced design (Anderson and Walsh 2013), which was not the case with our data set. All of the multivariate analyses were conducted using PRIMER 6 version 6.1.13 (PRIMER-E Ltd) with the aid of the PERMANOVA+ version 1.0.3 statistical package.

3. Results

3.1 Macrofaunal community in sea grass beds

A total of 81 species and 975 individuals were obtained from the 60 core samples (0.0314 m²) collected in this study. The communities comprised seven phyla (Annelida, Arthropoda, Brachiopoda, Echinodermata, Mollusca, Nemertea, and Sipuncula), 11 classes, approximately 26 orders, 51 families, and 81 species (**Table 2**). The phylum Mollusca contained the majority of the species (51, 63% of the total number of species), followed by Arthropoda (12, 15%) and Annelida (8, 10%). Phylum Mollusca contained the highest number of individuals (430, 44% of the total sample macrofaunal population), slightly higher than phylum Annelida (427 individuals, 43.8%).

In station 1, *Capitella capitata* (Annelida: Capitellidae) was the most abundant species, followed by *Gafrarium pectinatum* (Mollusca: Bivalvia) and *Clypeomorus batillariaeformis* (Mollusca: Gastropoda). Also in station 2, *C. capitata* was found to comprise the highest number of individuals, followed by *Lingula* sp. (Brachiopoda: Lingulidae) and *Canarium urceus* (Mollusca: Gastropoda). In station 3, both *C. capitata* and *Euchelus atratus* (Mollusca: Gastropoda) were found to be the most predominant species, followed by *Neritina* sp. (Mollusca: Gastropoda), whereas in station 4, *Modiolus* sp. (Mollusca: Bivalvia) was found to be the most predominant species, followed by *C. capitata* and *Cerithium punctatum*. Overall, *C. capitata* was determined as the most predominant and frequently occurring species.

A total of 10 species (12% of the total number of species) was found in all the four stations: Amphinomidae gen. sp., *Glycera* sp., and *C. capitata* (Annelida); *Scylla serrata* (Arthropoda); *Lingula* sp. (Brachiopoda); and *Anadara* sp., *Modiolus* sp., *C. batillariaeformis*, *Neretina* sp., and *C. urceus* (Mollusca). By contrast, 33 species (41%) were found restricted in one station, of which 21 (64%) belonged to phylum Mollusca.

3.2 Species richness and abundance

The species richness (total number of species) per station ranged from 30 to 50, with the lowest and highest values recorded at stations 1 and 2, respectively (**Fig. 2A**). By contrast, the Shannon diversity per station ranged from 9 to 27, which was found to increase from station 1 to station 4 (**Fig. 2B**).

The average species richness per core (0.03 m^2) ranged from four to eight species (**Fig. 2C**) and differed significantly across stations (**Table 3**). The results of pairwise comparisons revealed that the variation was statistically significant between stations 2 and 4. The Shannon diversity per core (0.03 m^2), whose average values ranged from 3 to 6 (**Fig. 2D**), also varied significantly across stations (**Table 3**). Results of Tukey's test showed that the variation was between stations 1 and 3 (**Fig. 2D**).

The average abundance ranged from six to 25 individuals per core (0.03 m^2) (**Fig. 3**) and significantly differed across stations (**Table 3**). Results of multiple pairwise comparison showed that the difference was between stations 1 and 4 and stations 2 and 4 (**Fig. 3**).

3.3 Assemblage pattern

The ordination result of CAP appeared to show a slight station-wise grouping of benthic macrofaunal assemblages (**Fig. 4**). The benthic assemblages of station 1 appeared to be grouped together, similar to station 2. By contrast, the assemblages of stations 3 and 4 were mixed-up, suggesting no possible difference in their species composition. Finally, the assemblages of stations 2 and 3 appeared to be slightly mixed-up, indicating no possible difference in their community assemblage.

In order to statistically test the patterns observed from the CAP (**Fig. 4**), we conducted the PERMANOVA. Result of the analysis indicated a statistical variation in the community assemblage pattern across stations (**Table 4**). Findings of pairwise tests showed that all stations varied significantly in species composition pattern, except between stations 2 and 3 and stations 3 and 4 (**Table 4**).

Further analyses showed the predominant contributions of *G. pectinatum* (Mollusca: Bivalvia) and capitellid worm, *C. capitata*, to the variation of community assemblage pattern (**Table 5**). The bivalve

G. pectinatum explained most of the variation in community assemblage between stations 1 and 2–4, with contribution ranging from 14% to 19% (**Table 5**). *C. capitata* was also found to be significantly influential in the community assemblage variation between stations 1 and 3–4 and stations 2 and 4, with contribution in the range of 5–14%. The other influential species, that is, *Tectus* sp. and *Nassarius elegantissimus*, generally contributed 7% of the community variation between stations 1 and 3 and stations 1 and 4, respectively (**Table 5**).

We also conducted the PERMDISP, which can be related to the beta diversity (variability of species composition between samples within a given area) pattern across stations. Results of the analysis showed a statistically significant variation in multivariate dispersion across stations (PERMDISP: $F = 5.05$, $df_1 = 3$, $df_2 = 56$, p (perm) = 0.009). Pairwise tests showed significantly lower values for stations 1–3 (i.e., distance from the centroid) than station 4 (**Fig. 5**).

4. Discussion

This study investigated the variation of macrofaunal community structure in sea grass beds along a pollution gradient in Bolinao, northwestern Philippines. We acknowledge that our research was constrained by at least two factors: (1) limited data set, that is, lack of critical data on water quality or organic pollution-related measures and (2) influence of other confounding variables, which were beyond the scope of this study. First, the water quality information is obtained from previous studies (e.g., Ferrera et al. unpublished; Ashikawa et al. 2008; San Diego-McGlone et al. 2008; Fortes et al. 2012; Nadaoka 2015; Watai et al. 2015). This justifies our reliance on previous data. Second, even without the effects of mariculture, we initially suspect that the difference in gleaning/fishing access across the stations, particularly between stations 1–3 (free to access) and 4 (reserved; no-take zone) would have influenced the observed variation in community structure. Regardless of these considerations, factors affecting important benthic macrofaunal habitats such as the sea grass beds in Bolinao are mostly in the form of anthropogenic disturbances such as siltation (Terrados et al. 1998) and mariculture-induced pollution (Fortes et al. 2012; Tanaka et al. 2014). On the basis of this fact, we strongly expect that alteration in invertebrate assemblages in sea grass beds of Bolinao was driven by the impact of pollution from marine fish farms. It was also unlikely that physical aspects (e.g., wave exposure, depth difference) played confounding roles, because all the four stations were

located inside the reef where waves are not strong and they were sampled in the same depth zone (i.e., subtidal). Nonetheless, the findings of our study can serve as a pilot information intended to lay the foundation for a more complete investigation in the future.

In this study, we found out that the Shannon diversity (true diversity based on the Shannon index) per station of sea grass benthic macrofauna increases toward the least polluted station (station 4), which conformed to our expectation. However, the abundance of macrofauna showed an opposite trend, in contrast to our assumption regarding pattern on the number of individuals. The community assemblages varied significantly across some stations, and some of the macrofauna that influenced the variation has opportunistic characteristics. Both these findings were in agreement with our expectations regarding assemblage pattern across stations and the possible dominance of opportunistic species. Finally, we found that the species heterogeneity of benthic macrofauna reduced significantly toward polluted stations (1 and 2), which conformed to our hypothesis.

We recorded a total of 81 species and 975 individuals ($n = 60$) of benthic macroinvertebrates in sea grass beds of Bolinao. Both values are much lower than those reported in the previous studies of sea grass macrofauna and pollution (e.g., Apostolaki et al. 2007); however, we cannot compare them directly because of the difference in the study area, sampled spatial scale, and methodological approaches. For instance, the scale of sampling in Eklöf et al. (2005) was slightly smaller than that in our study (area, 30×30 m; diameter core, 12 cm; same depth, i.e., 10 cm), but the abundance of macrofauna was relatively higher. The most abundant phylum in this study was Mollusca, whereas Arthropoda was often found to predominate in previous studies (e.g., Eklöf et al. 2005; Apostolaki et al. 2007).

The Shannon diversity per station was more than twice higher in the least polluted station (station 4) than the most polluted station (station 1). This pattern was not clearly shown by species richness per station. Because the former measure is more comprehensive than the latter as it was taken from a diversity index, which favors neither rare nor common species (*sensu* Jost 2006, 2007), the clear pattern could indicate that total species richness was altered along the pollution gradient. Nonetheless, this observation should be carefully interpreted, because we did not statistically infer the data. On the contrary, other diversity measures (i.e., species richness per station, species richness per core, and the Shannon diversity per core) generally showed nonlinear patterns. This could be explained by the increase of productivity in the polluted sea grass beds, leading to a localized increase in species

richness of the associated macroinvertebrates (*sensu* Dimech et al. 2002). Apostolaki et al. (2007) also reported little variability in the total number of species of macrobenthic animals associated with *Posidonia oceanica* as a function of distance from fish cages at sample sites in the Mediterranean. The varying life strategies of the benthic animals (Gray 1979; Gray 1992) could also influence nonlinear responses of fauna along a pollution gradient (Pearson and Rosenberg 1978).

The macrofaunal abundance was more than thrice higher in the most polluted station (station 1) than the least polluted station (station 4). Such an increase in abundance could be due to higher availability of food in highly polluted areas. If pollution continually increases in an area, there is high possibility of the azoic sediment being defaunated as described by Pearson and Rosenberg (1978), Warwick (1986), and Gray (1992). Nacorda et al. (2012) reported that the slightly enriched sediment in some parts of Bolinao in 2003 became impoverished of macrofauna by 2008 as mariculture activities intensified. Soares et al. (2004) even reported an 86% decrease in macrobenthic faunal abundance inside the pen of a fish farm just after 21 days of culture. Eklöf et al. (2005) showed a lower macrofaunal abundance in sea grass beds underneath seaweed farms than that in unfarmed sea grass beds. On the contrary, Apostolaki et al. (2007) found little variation in the macrofaunal abundance between control and highly impacted areas in *P. oceanica* meadows. This variation in abundance responses indicates the importance of species biological traits used in adapting to environmental changes (*sensu* Nakaoka et al. 2005).

Another finding is on the variation of community assemblages across some stations explained largely by the predominance of the filter-feeding bivalve *G. pectinatum* and polychaete *C. capitata* at polluted stations. Difference in biological tolerance, response, or behavior of many macrobenthic species to environmental disturbance such as pollution is an important factor in the differentiation of community composition along a pollution gradient (Gray et al. 1979; Diaz and Rosenberg 1995; Gray et al. 2002). In addition, the presence of even one or two dominant taxa that are tolerant to stress is known to significantly influence community variation along a gradient of disturbance. Examples of these taxa are the bivalves and polychaetes (Warwick and Clarke 1994; Ryu et al. 2011), which, in our study, were the bivalve *G. pectinatum* and opportunistic polychaete *C. capitata*, respectively. Among the benthic taxa, mollusks are known to be the least sensitive to hypoxia, followed by polychaetes, crustaceans, and echinoderms (Gray et al. 2002). The enhanced primary productivity in the polluted stations may also provide benefits to benthic grazers, whereas increased sedimentation

could benefit some filter-feeding and deposit-feeding macrobenthos (Grall and Chauvaud 2002; but see Heip 1995). *C. capitata* is a well-known predominant pioneering species in stressed areas (Tsutsumi 1987; Tsutsumi et al. 1991; Karakassis et al. 2000), and hence one of the most influential species of pollution. Polychaete taxa other than *C. capitata* (e.g., *Ophryotrocha vivipara* (Brooks et al. 2003), Spionidae (Nacorda et al. 2012), and *Heteromastus filiformis* (Ryu et al. 2011)) are also abundant in polluted areas. The predominance of *C. capitata* in our study is consistent with its known potential as an indicator of environmental pollution (Gray et al. 1979; Tsutsumi et al. 1991).

Finally, we generally found a significant decrease of species heterogeneity along the pollution gradient by approximately 19% from the least polluted station (4) to the most pollution one (1). This finding indicates that the species heterogeneity of macrofauna in sea grass beds was negatively altered in polluted areas. However, such kind of community response to environmental disturbance (e.g., pollution) is seldom overlooked. For instance, Dimech et al. (2002) found a decreasing similarity of macrobenthic organisms in replicated samples at sites closer to fish farms, but they did not thoroughly discuss such an observation. Decrease of species heterogeneity indicates homogenization of community assemblages or suppresses species production (Heip 1995; Papageorgiou et al. 2009). The predominance of opportunistic species in a pollution-stressed environment could also influence the homogenization of community (Warwick 1986). However, the mechanisms driving the pattern of species heterogeneity along an environmental gradient are still poorly understood. The role of the predominant species in the decrease of species heterogeneity may have been important in this study, where the species composition at the polluted stations was characterized by the abundance of *C. capitata*, an indicator of organic pollution (Tsutsumi 1987; Tsutsumi et al. 1991), and bivalve *G. pectinatum*, which is generally considered to be very tolerant to less-oxygenated environments (*sensu* Gray et al. 2002).

5. Conclusion

In conclusion, the community structure of benthic macrofauna associated with sea grass beds was altered by mariculture-induced pollution. However, it is suggested only by the results of this study, given the limitations in our data set. Regardless of this consideration, the most significant aspect of our research was the decreased species heterogeneity (reduced variability of species composition among cores within the station) in the most disturbed areas, because it indicates homogenization of

species composition that alters biodiversity and ecosystem functioning (*sensu* Gamfeldt et al. 2014). Despite some constraints in our data, this study improves the understanding of the significant impact of local anthropogenic disturbance on macrofaunal communities in sea grass beds. In particular, aspects of species heterogeneity should be considered one of the major response variables in environmental impact assessments and the management of coastal areas encountered with organic pollution resulting from mariculture activities.

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Figures with captions

Fig. 1 Map of the study area showing the locations of the four sampling stations in Bolinao, northwestern Philippines. The mariculture structures (fish pens and cages) in red dots were determined from a satellite image (Google Earth, checked on June 23, 2015) using Arc-GIS. Values of the silt-clay content of the sediment were taken from Terrados et al. (1998).

(2-column fitting image)

Fig. 2 Benthic macrofaunal (A-B) species richness and the Shannon diversity at station level and (C-D) mean values ($n = 15$) of species richness and Shannon diversity at core level (0.03 m^2). Each error bar ($\pm$) is a standard error of the mean. Horizontal bars with the same letter are not significantly different (with 95% confidence level).

(1 to 1.5 column)

Fig. 3 Mean benthic macrofaunal abundance at four stations ($n = 15$). Each error bar ($\pm$) is a standard error of the mean. Horizontal bars with the same letter are not significantly different (with 95% confidence level).

(1 to 1.5 column)

Fig. 4 Species composition of benthic macrofauna at the four sampling stations shown in multivariate space through the canonical analysis of principal coordinates (CAP) using the presence–absence data and Jaccard resemblance index.

(1 to 1.5 column)

Fig. 5 Boxplot of the multivariate dispersion pattern of benthic macrofaunal assemblage across sampling stations using the presence-absence data and Jaccard resemblance index. Increasing values of distance to centroid indicate higher dispersion. Stations with the same letters are not significantly different (with a 95% confidence level).

(1 to 1.5 column)

704 **Tables with captions**

705 **Table 1** Six-day temporal average concentrations of dissolved nutrients (nitrogen, phosphate,
706 and silicate) and chlorophyll-a from water samples collected in Bolinao, northern Philippines,
based on the data from Fortes et al. (2012).

	Station			
	1	2	3	4
Dissolved nitrogen (µmol/l)	20.2	6.9	2.0	2.3
Dissolved phosphate (µmol/l)	2.5	1.0	<1.0	<1.0
Dissolved silicate (µmol/l)	18.5	14.00	10.00	10.02
Chlorophyll-a (µg/l)	10.5	2.11	0.11	0.03

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Table 2 Checklist and total density (total number of individuals per core (0.03 m²), *N* = 15) of macrofaunal species collected in September 2010 and March 2011 in four sampling stations of Bolinao, northwestern Philippines.

Phylum	Class	Order	Family	Binomial name/Species code	1	2	3	4		
Annelida	Polychaeta	Amphinomida	Amphinomidae	Amphinomidae gen. sp.	5	1	3	2		
		Eunicida	Eunicidae	Eunicidae gen. sp.	0	0	1	1		
			Lumbrineridae	<i>Lumbrineris</i> sp.	1	0	3	3		
			Phyllodocida	Glyceridae	<i>Glycera</i> sp.	3	4	1	2	
		Hesionidae		Hesionidae gen. sp.	4	2	0	0		
		Nereididae		Nereididae gen. sp.	4	3	0	3		
		Terebellida	Terebellidae	Terebellidae gen. sp.	0	1	0	1		
		Capitellida*	Capitellidae	<i>Capitella capitata</i>	219	123	28	9		
		Arthropoda	Malacostraca	Decapoda	Leucosiidae	<i>Leucosia</i> sp.	0	1	0	0
Alpheidae	<i>Alpheus</i> sp.			0	0	2	1			
	Calappidae			Calappidae gen. sp. 1	0	1	0	0		
Calappidae gen. sp. 2				0	0	0	1			
Diogenidae	Diogenidae gen. sp. 1			0	0	18	2			
	Diogenidae gen. sp. 2			2	0	0	0			
fam. (unknown)	Decapoda fam. gen. sp.			0	1	0	0			
Majidae	Majidae gen. sp.			0	1	0	0			
Penaeidae	Penaeidae gen. sp.			0	1	2	1			
Pilumnidae	Pilumnidae gen. sp.			0	1	0	0			
Portunidae	<i>Scylla serrata</i>			2	1	3	6			
Squillidae	Squillidae gen. sp.			0	2	0	0			
Brachiopoda	Lingulata			Lingulida	Lingulidae	<i>Lingula</i> sp.	10	29	1	1

Table 2 Checklist and total density (total number of individuals per core (0.03 m²), *N* = 15) of macrofaunal species collected in September 2010 and March 2011 in four sampling stations of Bolinao, northwestern Philippines (Continued).

Phylum	Class	Order	Family	Binomial name/Species code	1	2	3	4				
Echinodermata	Asteroidea	Valvatida	Archasteridae	<i>Archaster typicus</i>	4	1	2	0				
	Echinoidea	Camarodonta	Toxopneustidae	<i>Tripneustes gratilla</i>	0	1	0	0				
	Holothuroidea	Apodida	Synaptidae	<i>Synapta maculata</i>	0	0	1	0				
		Aspidochirotida	Holothuriidae	<i>Holothuria (Metriatyla) scabra</i>	0	0	3	3				
	Ophiuroidea			<i>Holothuria</i> sp.	0	1	3	2				
			Ophiurida	Ophiotrichidae	<i>Ophiothrix</i> sp.	0	1	1	0			
Ophiurina			Amphiuridae	<i>Amphioplus</i> sp.	0	2	0	0				
Mollusca	Bivalvia	Arcoida	Arcidae	<i>Anadara</i> sp.	1	13	10	1				
		Lucinoida	Lucinidae	<i>Codakia tigerina</i>	0	0	0	1				
				<i>Loripes</i> sp.	0	2	0	0				
				Lucinidae gen. sp.	0	5	1	0				
		Myoida	Myidae	<i>Mya</i> sp.	2	7	0	0				
		Mytiloida	Mytilidae	<i>Modiolus</i> sp.	1	12	7	11				
		Nuculida	Sareptidae	Sareptidae gen. sp.	1	0	0	0				
		Veneroida	Cardiidae	<i>Vasticardium flavum subrugosum</i>	3	3	2	0				
			Veneridae	<i>Gafrarium pectinatum</i>	27	0	0	0				
								<i>Mercenaria mercenaria</i>	1	0	2	0
								<i>Paphia</i> sp.	5	2	0	0
								<i>Pitar</i> sp.	0	2	0	0
								<i>Placamen</i> sp.	2	2	0	0
								<i>Tapes literatus</i>	0	1	0	0
								<i>Tapes</i> sp.	4	1	1	0

Table 2 Checklist and total density (total number of individuals per core (0.03 m²), *N* = 15) of macrofaunal species collected in September 2010 and March 2011 in four sampling stations of Bolinao, northwestern Philippines (Continued).

Phylum	Class	Order	Family	Binomial name/Species code	1	2	3	4
Mollusca	Gastropoda	[unassigned] Caenogastropoda	Cerithiidae	<i>Cerithium punctatum</i>	0	0	2	8
				<i>Cerithium rostratum</i>	1	0	0	1
				<i>Clypeomorus batillariaeformis</i>	24	1	2	2
		Cycloneritimorpha	Neritidae	<i>Neritina</i> sp.	10	6	24	1
				<i>Smaragdia paulucciana</i>	0	1	2	1
				<i>Smaragdia rangiana</i>	0	0	1	1
		Littorinimorpha	Cypraeidae	<i>Monetaria annulus</i>	0	2	2	0
			Naticidae	Naticidae gen. sp.	0	1	0	0
				<i>Neverita didyma</i>	0	3	1	0
				<i>Polinices</i> sp.	0	1	0	0
			Ranellidae	<i>Gutturnium muricinum</i>	0	1	0	0
			Strombidae	<i>Canarium</i> sp. 1	0	10	5	0
				<i>Canarium</i> sp. 2	0	0	2	0
				<i>Canarium urceus</i>	3	16	18	2
		Neogastropoda	Columbellidae	Columbellidae gen. sp.	0	0	0	1
				<i>Euplica scripta</i>	0	3	7	2
				<i>Euplica</i> sp.	0	3	3	2
				<i>Pseudamycla formosa</i>	0	0	1	0
				<i>Seminella peasei</i>	0	0	1	0
			Conidae	<i>Conus</i> sp.	0	0	0	1
			Costellariidae	<i>Vexillum sinuosum</i>	1	0	0	0
				<i>Vexillum virgo</i>	1	1	1	0

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Table 2 Checklist and total density (total number of individuals per core (0.03 m²), *N* = 15) of macrofaunal species collected in September 2010 and March 2011 in four sampling stations of Bolinao, northwestern Philippines (Continued).

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Phylum	Class	Order	Family	Binomial name/Species code	1	2	3	4	
Mollusca	Gastropoda	Neogastropoda	Fasciariidae	Fasciariidae gen. sp.	0	0	1	0	
			Mitridae	<i>Pterygia undulosa</i>	0	1	1	0	
			Muricidae	<i>Drupella margariticola</i>	1	0	0	0	
			Nassariidae	<i>Hebra subspinosa</i>	0	0	1	0	
				<i>Nassarius coronatus</i>	0	0	1	0	
				<i>Nassarius elegantissimus</i>	23	3	1	0	
				<i>Nassarius limnaeiformis</i>	1	2	0	0	
			order 1 (unspecified)**	Chilodontidae	<i>Euchelus atratus</i>	0	8	28	2
			order 2 (unspecified)**	Phasianellidae	<i>Phasianella solida</i>	0	0	0	4
			order 3 (unspecified)**	Pyramidellidae	<i>Milda ventricosa</i>	4	4	0	1
					<i>Pyramidella maculosa</i>	0	0	1	0
			order 4 (unspecified)**	Trochidae	<i>Tectus</i> sp.	0	3	11	1
					<i>Trochus</i> sp.	0	0	3	3
					<i>Umbonium costatum</i>	0	0	1	4
Nemertea	Enopla	Monostilifera	Tetrastemmatidae	Tetrastemmatidae gen. sp.	0	0	0	1	
Sipuncula	Sipunculidea	Golfingiida	Golfingiidae	<i>Golfingia</i> sp.	0	0	1	1	

Number of phylum = 7

Number of class = 11

Number of orders = ca. 26 (with one *nomen dubium* and four unspecified orders)

Number of families = 51 (with one unidentified family)

Number of species = 81

Table 3 Summary results of the one-way analysis of variance (ANOVA) analyzing the station-wise pattern of benthic macrofaunal species richness, Shannon diversity, and abundance at core level (0.03 m²).

	Source of variation	Df	Sum Sq	Mean Sq	F	Pr (>F)
Species richness per core	Station	3	97.20	32.40	2.99	0.039
	Residuals	56	606.40	10.83		
Shannon diversity per core	Station	3	52.64	17.55	3.83	0.015
	Residuals	56	256.57	4.58		
Abundance per core	Station	3	1.95	0.65	4.54	0.006
	Residuals	56	8.04	0.14		

Table 4 Results of a permutational multivariate analysis of variance (PERMANOVA) using the presence–absence data of macrofauna and Jaccard resemblance index. *P* values for pairwise tests were obtained from Monte Carlo simulations.

Source	df	SS	MS	Pseudo-F	P(perm)
Station	3	28465	9488.3	2.42	0.001
Residual	56	2.20E+05	3925		
Total	59	2.48E+05			
Pairwise tests					
Groups	t	P (MC)			
1,2	1.70	0.001			
1,3	1.91	0.001			
1,4	1.76	0.001			
2,3	1.24	0.082			
2,4	1.42	0.008			
3,4	1.22	0.104			

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Table 5 Summary of the results of univariate tests in *mvabund* to determine the macrofaunal species which influenced the observed variation in species composition pattern across some stations.

Station contrast	Most influential species	Deviance	% Contribution	Pr (>Dev)
1_2	<i>Gafrarium pectinatum</i>	23.97	18.8	0.001
1_3	<i>G. pectinatum</i>	23.97	13.6	0.001
	<i>Tectus</i> sp.	12.294	7.0	0.012
	<i>Capitella capitata</i>	8.875	5.0	0.027
1_4	<i>G. pectinatum</i>	23.97	15.6	0.001
	<i>C. capitata</i>	12.88	8.4	0.003
	<i>Nassarius elegantissimus</i>	10.491	6.8	0.008
2_4	<i>C. capitata</i>	22.886	14.4	0.001

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