Title	Variation in community structure of seagrass macrofauna across multiple spatial scales
Author(s)	Leopardas, Venus Empron
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第11988号
Issue Date	2015-09-25
DOI	https://doi.org/10.14943/doctoral.k11988
Doc URL	https://hdl.handle.net/2115/82590
Type	doctoral thesis
File Information	Venus_Empron.pdf

Variation in community structure of seagrass macrofauna across multiple spatial scales

Venus Empron Leopardas

Doctoral Dissertation

Graduate School of Environmental Science

Division of Biosphere Science

HOKKAIDO UNIVERSITY FOUNDED 1876

Sapporo, Japan

August 2015

TABLE OF CONTENTS

CHAPTER 4 Broad scale variation in seagrass benthic macrofaunal assemblages along the coast of Japan **89**

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

This dissertation examined the role of multiple environmental scales to the diversity and abundance of macrofauna in seagrass beds. This thesis was motivated by two research questions: (1) How biological communities are structured in seagrass ecosystems? (2) How biological communities in seagrass beds are structured from fine to large spatial scales? To examine these questions, this dissertation offered one main hypothesis: the structure of population and community dynamics of macrofauna in seagrass beds is influenced by multiple factors of different spatial scale. This thesis has three specific goals: (1) At a fine scale, to clarify the role of spatial structure of seagrass beds affecting the macrofauna, (2) At a local scale, to elucidate the impacts of human-induced disturbance to macrofauna, and (3) At broad spatial scale, to understand patterns of macrofaunal community structures confronted by broad-scale environmental factors. The most important contribution of this thesis is that it advances our understanding on small to broad-scale community patterns, and on how to predict patterns necessary for management strategies of seagrass beds habitat in response to small and large-scale disturbances.

I conducted three independent studies to test the main hypothesis, as well as to address the following three hypotheses specific for each study: (1) variation in seagrass macrofaunal structures is influence by seagrass taxonomic identity which has different morphology across taxa (Chapter 2); (2) organic pollution in seagrass beds alters structures of associated macrofauna (Chapter 3); and (3) variation in climate and oceanographic current system has important role on macrofauna in seagrass beds across broad spatial scale (Chapter 4). Sediment coring (depth of 10 cm, diameter range: 15-20 cm) was employed in all of the studies Temporal sampling was limited (e.g., two months within same year, sampling only during highest productivity), but did not significantly affected temporal patterns, so most of the data analyses were based on pooled data.

First, I investigated the role of different seagrass species to macrofauna in multispecific seagrass beds of southern Philippines to clarify whether seagrass spatial structure has influence to associated macrofaunal animals (Chapter 2). Three target seagrass species

included are most common and abundant in the tropics: *Enhalus acoroides*, *Thalassia hemprichii*, and *Cymodocea rotundata*. I did not find a variation in macrofaunal diversity and abundance between the three vegetation types dominated those seagrass species despite the striking difference in seagrass shoot density and biomass between *Enhalus*-dominated vegetation to other vegetation types. This suggest that seagrass taxonomic identity is not a good determinant of taxon diversity and abundance of macrofaunal assemblages. Nonetheless, I also observed the significant influence of aboveground biomass to epifaunal macrofauna. Hence, seagrass bed conservation should not be focused on one specific species, but on multiple factors interacting into the system.

Secondly, I clarified the impact of nutrient pollution to benthic animals in seagrass beds lying along a pollution gradient in Bolinao, northwestern Philippines to elucidate how human-induced disturbance control local variation in macrofaunal structures (Chapter 3). Results species diversity (in terms of total number of species per site) and abundance decreased and increased, respectively, towards the polluted sites. All other measures of species diversity showed a non-linear pattern as a possible response to variation in habitat productivity and on varying life strategies. The community composition was dominated by an opportunistic species, *Capitella capitata* and a bivalve *Gafrarium pectinatum*, resulting to significant changed in species composition across sites. More importantly, the species heterogeneity (variability in species composition among cores within station) was reduced towards polluted stations. Reduction of species heterogeneity suppresses biodiversity and hence alter ecosystem functioning. Knowledge on this aspect should be incorporated into environmental impact assessment and management of coastal ecosystems facing organic pollution issues.

Finally, I examined the broad-scale patterns of the diversity and abundance of macrofauna in six seagrass beds of Japan, ranging from 24° to 43°N (Chapter 4). In core level, results revealed that species richness, expected number of species from 50 subsample individuals (ES50) and abundance increased with latitude. Variation in species composition was found across sites, indicating that multiple factors influenced broad-scale pattern, and not just the current and climatic variation, The community similarity decayed more rapidly compared to other previous reports on rocky intertidal, indicating possible difference in dispersal ability and

niche breath between seagrass associated macrofauna and those in the rocky shore. Overall, the findings suggest the seagrass macrofauna are influenced by multiple factors that operates from small to large spatial scales. Knowledge from this study advances our understanding on how to manage seagrass beds confronted with local and regional disturbances.

The main findings of this dissertation illustrate how responses of macrofauna in seagrass beds vary across multiple environmental factors that differ at different spatial scales. Results from this fine to broad scale studies are complementary; integration of the findings show that the influence of different environmental factors is more complex than previously assumed. Hence, the findings of this thesis support the prediction that macrofauna in seagrass beds are controlled by local and regional processes/variables. The results, implications for managers, and future research directions are discussed in this dissertation.

主 論 文 要 旨

"How biological communities are structured in seagrass ecosystems?" is a fundamental question in seagrass ecology. I suggest that factors influencing patterns in seagrass beds operates on various scales and that a new generation of research in this area needs to address the extended question: How biological communities in seagrass beds are structured from fine to large spatial scales? To examine these questions, this dissertation examined the role of multiple environmental gradients operating at different spatial scales to the diversity and abundance of macrofauna in seagrass beds. The thesis hypothesized that the structure of population and community dynamics of macrofauna in seagrass beds is influenced by local and regional factors. This thesis has three specific goals: (1) At a fine scale, clarify the role of spatial structure of seagrass beds to associated macrofauna, (2) At a local scale, elucidate the impacts of human-induced disturbance to seagrass macrofauna, and (3) At broad spatial scale, determine the influence of regional processes/variables to seagrass macrofauna. These were tested independently through three different studies. In Chapter 2 of this thesis, I revealed that seagrass taxonomic identity was not a good determinant of macrofaunal taxon diversity and abundance in multispecific seagrass beds. However, seagrass aboveground biomass was found influential to epifaunal assemblage pattern. The results suggest that multiple factors should be considered for seagrass conservation and not just focus on conservation of single seagrass species. In Chapter 3, results revealed that organic pollution from fish farms altered structures of macrofauna and more importantly suppressed species heterogeneity which could have potential impact on biodiversity and ecosystem functioning. In Chapter 4, I showed that species richness (including that from ES50) and abundance at core level had a clear latitudinal cline which was increasing with latitude. The species composition significantly varied across sites, indicating influence of multiple factors and not only the variation in current system and climatic condition. The community similarity decayed with geographic distance at a relatively higher rate compared to reported values for rocky shore assemblages, indicating possible role of varying dispersal ability and on niche breadth between seagrass macrofauna and those from rocky shores. In conclusion, macrofauna in seagrass beds are influenced by multiple factors operating on various spatial scales. Overall, this dissertation illustrates a complex responses of seagrass macrofauna across multiple spatial scales. Complementarity in the patterns associated from fine scale to broad scale analysis contributes to wide scale perspectives in designing management strategies of seagrass beds.

ACKNOWLEDGEMENTS

I am indebted to my supervisor, Dr. Masahiro Nakaoka, for his support throughout the years of my PhD, keeping me going when times were tough, asking insightful questions, and offering invaluable advices.

I would also like to thank the committee members of my dissertation, Dr. Helena Fortunato Martins, Dr. Takashi Noda, and Dr. Kasuzhi Miyashita, for spending a part of their time and expertise to help review and improve this thesis.

I am also grateful to Dr. Wilfredo Uy, my former supervisor in Mindanao State University-Naawan (MSU-N), Philippines, for his encouragement to further my study and for being the first one to provide me knowledge regarding marine ecology.

Special thanks are also due to my former professors in MSU-N; Dr. Asuncion de Guzman, Jaime Jimenez, MSc, Cesaria Jimenez, MSc, and Dr. Jessie Gorospe, for the encouragement and for their invaluable contribution of knowledge during my college and master course years.

I thank all former and current members of Akkeshi Marine Station, including the visiting students, for sharing their knowledge to me, supporting some of my laboratory works and technical concerns, and for providing a great company with lots of fun and excitement.

I am also grateful to all of my Filipino friends for their encouragement and appreciation in everything I do. Special thanks are also due to some of my former colleagues in MSU-N and students of UP-MSI, for the motivation and friendship. I also thank Dr. Napakhwan Whanpetch, for not only helping me in species identification, but also for being a good friend and role model.

Special gratitude to my family and relatives for serving as my inspiration to struggle despite the odds. I am also very grateful to Mark and his family, for the unconditional love and support all of them selflessly shared to me.

All studies I conducted in Philippines were supported by Japan Science and Technology (JST) and Japan International Cooperation Agency (JICA) under the Coastal Ecosystem Conservation and Adaptive Management under Local and Global Environmental Impacts in the

Philippines (CECAM) project. I am grateful to key members of CECAM, especially to Dr. Miguel Fortes and Dr. Kazuo Nadaoka, for their continuous support.

The regional study in Japan was under the Ministry of Environment-Government of Japan. I am thankful to Dr. Masahiro Hori, Dr. Yoshiyuki Tanaka, Dr. Hiroshi Mukai, Dr. Misuzu Aoki, Dr. Naoto Sato, and all others who were involved in this research.

My PhD program is financially supported by the MONBUKAGAKUSHO:MEXT scholarship of the Government of Japan. I am indebted to Dr. Nakaoka for recommending me to enter this scholarship grant. I am also grateful to the CECAM project for without it I would never make it to this scholarship.

All praise and gratitude to the Lord Almighty, for giving me the strength I need to pursue this study and for all the wonderful people He had blessed me along the w

CHAPTER 1

General Introduction

Biotic and abiotic factors that influence the structures and dynamics of biological communities operates on various spatial and temporal scales (*sensu* Nakaoka and Noda 2004; Nakaoka et al. 2006), but this is poorly emphasized in seagrass-faunal system. This dissertation aimed to examine the control of various environmental variables to the diversity and abundance of macrofauna in seagrass beds. My interest is on the macrofauna, defined in this thesis as a group of benthic invertebrates that can retained on 1.0 mm mesh, because they are often more diverse and abundant in seagrass beds and they provide several ecological goods and services (Nakaoka et al. 2014).

This dissertation is composed mainly of three independent studies, dealing on different aspect of environmental parameters operating on various scales. First, I studied role of seagrass species to macrofaunal patterns. While information on the biodiversity status of seagrass species is important in managing seagrass species conservation (Short et al. 2011), conservation strategies should be coupled with knowledge regarding ecological roles of the seagrass species or by the biodiversity effects (Walker 1992; Duffy 2006) because seagrasses are not taxonomic structures, but are plants that form ecological group (den Hartog and Kuo 2006). Secondly, I examined the impact of organic pollution to macrofauna in seagrass beds lying along a pollution gradient. Despite the pervasive knowledge of pollution effects to seagrass beds (Duarte 2002; Orth et al. 2006), the impact to associated macrofauna is poorly studied. The last study dealt on the influence of regional factors/processes to macrofauna in seagrass beds lying over large areas. There is little information regarding broad spatial scale patterns of macrofauna in seagrass beds in Asia despite the importance of this analysis to understand community/ecosystem response to regional factors (e.g., variation in climate, current system). In this section, I explain the detailed background of the thesis, as well as the objectives and description of the various chapters.

1.1 Multiple threats to marine ecosystems and their biodiversity

Alteration in the complexity of marine ecosystems is a result of multiple drivers, comprising of natural and/or man-made disturbances (e.g., storms, invasion, pollution) and biological events (e.g., growth dynamics, herbivory). Combination of these disturbances will have synergistic effects to coastal ecosystems (Nyström et al. 2000). Overexploitation can lead to reconfiguration of established habitats or worst, to collapse of coastal ecosystems (e.g., overfishing; Jackson et al. 2001). Human activities directly or indirectly affecting coastal areas, such as coastal development and tourism, bring forth mechanical damage to coastal habitats including seagrass beds (Short and Wyllie-Echeverria 1996) and coral reefs. Upland agriculture activities could also enhance sedimentation which is particularly critical for seagrass beds and coral reefs (Rogers 1990; Short and Wyllie-Echeverria 1996). Mangroves are also cleared for development of aquaculture and coastal development, which will have devastating economic and environmental consequences for coastal communities (Alongi 2002; Polidoro et al. 2010).

Pollution is considered one of the biggest environmental threats to marine ecosystems as it depletes water quality which a basic important requirement for coastal habitats (e.g., seagrass, corals) to survive. But even so, most developing nations are still producing huge pollution loads, with the trends are expected to increase (Islam and Tanaka 2004).Pollution changes the sediment and water chemistry, reduces biodiversity leading to degradation and loss of coastal ecosystems (Tanaka et al. 2014). This has serious threat to sustainability of ecosystem functioning. Conservation of biodiversity should be strengthened on areas affected by urbanization which activities often leads to anthropogenic inputs into the coastal waters.

Seagrass beds are highly impacted by local disturbances through natural and/or man-made impacts. At the same time, disturbances acting on broad spatial scales may also be important (*sensu* Nakaoka and Noda 2004; Nakaoka et al. 2006). Aside from highlighting the control of biological traits of species to marine biogeographical patterns, modern macroecological studies generally demonstrate the potential role of regional physical factors in the large scale pattern of faunal communities (Sanford 2014 and references therein). These factors often include influence of oceanographic current system dominating a particular range and the variations in climate condition (also influencing sea-surface water temperature). With ongoing changes in world's climate that can potentially alters the seagrass dynamics (Short and Neckles 1999; Short et al. 1999; Fraser et al. 2014), and with the predicted rise of extreme climate events in the future, it is rightly to ask ecologically, "How biological communities in declining seagrass beds respond to regional disturbances?". Broad-scale analysis of biological patterns in faunal communities of seagrass beds is challenging and yet an important step for the conservation of biodiversity that maintains ecosystem functioning (*sensu* Gamfeldt et al. 2014).

1.2 Information needed for biodiversity conservation in marine coastal ecosystems

There is a growing concern over impairment of ecosystem functioning by biodiversity loss (Chapin et al. 2000; Duarte 2000; Hooper et al. 2005; Worm et al. 2006). Hence, advancing strategies on biodiversity conservation is now becoming a popular platform in ecology (Loreau 2000). Investigations on biodiversity and ecosystem functioning is however, a daunting and challenging task. A number of related studies are often hampered by confounding factors, including difficulties in experimental design (Ieno et al., 2006), and by problems in the unequivocal interpretation of data (Mikola et al. 2002). Nonetheless, most of the results from previous investigations are information-rich. For instance, Ieno et al. (2006) reported clear effects of increased infaunal species diversity on nutrient generation, while Covich et al. (2004) suggested that changes in biodiversity (equivalent to local species richness) are highly variable over space and time, with frequent dependency on species-specific traits or functional roles of individual species. Major future challenge is to determine how biodiversity dynamics, ecosystem processes, and abiotic factors interact (Loreau 2000; Loreau et al. 2001; Hooper et al. 2005).

As changing biodiversity alters ecosystem processes and change the resilience of ecosystems to environmental change, its societal and ecological consequences should be minimized (Chapin et al. 2000). At the same time, conservation managers should be able to easily grasp the ecological implications of several empirical knowledge on faunal community patterns. Ecological data sets are often analyzed through diversity and abundance. Species diversity is the most widespread biological diversity measure, and information on its offshoot, the species richness, is mostly utilized in the understanding of biodiversity effects to ecosystem functioning (Chapin et al. 2000). Moreover, knowledge on pattern of community composition is important in biodiversity conservation because it provide information on the general identity of flora or fauna making up the community and implicates on how the community work and how important organisms are to the community. Some research recently emphasized knowledge on species variability (beta diversity) as it indicates species turnover rate along an environmental gradient. The applicability of any of these ecological measures will depend on what aspect of faunal community dynamics that a conservation initiative has been pre-identified as a priority. For instance, managers are more likely incline to use knowledge on species richness if they are more interested in the conservation of rare species.

Biodiversity conservation in the marine system is often concerned over the synergistic effects of habitat loss (including destruction and/or degradation), and the accelerating loss of important habitats has many ecological impacts. Clearly, the most recognized one is the change in the abundance and diversity of habitat's associated fauna as negative changes in the habitat take place. Therefore, understanding faunal community patterns in important yet threatened ecosystems should advance biodiversity conservation strategies.

1.3 General description of seagrass beds and seagrass macrofaunal communities

1.3.1 Community structure in seagrass beds

Seagrasses are submerged flowering plants found in all marine waters except in Antarctic. Despite low taxonomic diversity, with approximately 60 species, seagrasses forms a habitat from patches of several centimeters to meadows/beds stretching several kilometers into the coastline. The spatial structure of the beds differ between tropical and temperate areas (Green and Short 2003; Short et al. 2007). The tropical-Indo Pacific is the bioregion of seagrass that have the highest assemblages in the world (Short et al. 2007). The seagrass beds in this bioregion are often composed of more than one dominant seagrass species. Some of the most common and abundant species are *Enhalus acoroides*, *Thalassia hemprichii* and *Cymodocea rotundata*. The range of these species can still be found in the southernmost part of East Asia, but are replaced mostly by temperate seagrass species such as *Zostera* towards the north. The beds in temperate areas are considered monospecific, with single species dominating the entire vegetation.

Seagrass beds are some of most valuable key ecosystems in the world (Costanza et al. 1998; Spalding et al. 2003), providing several ecosystem services including maintenance of high faunal species diversity and abundance. The associated animals represent various kinds of marine organisms, including microorganisms like plankton, small to large invertebrates, fish, and large herbivores, which contribute significantly to ecosystem functioning (Nakaoka et al. 2014). The faunal productivity in the beds is primarily enhanced by the epiphytic algae attached in seagrass aboveground parts, particularly on the shoots. Despite the importance of seagrass beds, their worldwide status is declining at a fast rate. Threats to seagrass beds include natural and/human disturbances (Short and Wyllie-Echeverria 1996). Impact of seagrass degradation or loss is devastating for the associated fauna and other ecosystem services (Duarte 2002; Orth et al. 2006; Hughes et al. 2009).

1.3.2 Macrofauna in seagrass beds

Macrofauna, defined in this study as a group of benthic invertebrates retained on a 1.0 mm mesh (but other authors used 3 μm, 5 μm; Snelgrove 1998, Lenihan & Micheli 2001), are naturally some of the most abundant assemblage in soft sediment (Snelgrove 1998). They are common inhabitants of soft-sediment (Lenihan and Micheli 2001) across several depths: intertidal, subtidal and deep zones. Macrofauna are generally represented by benthic molluscs (Mollusca), crustaceans (Arthropoda), polychaete worms (Annelida) and spiny-skinned animals (Echinodermata). Some other faunal taxa, such as peanut worms (Sipuncula), flatworms (Platyhelminthes), lamp shells (Brachipoda), anthozoans (Cnidaria), and ribbon worms (Nemertea), are also part of the bulk of macrofauna in the marine sediments. Marine sediment macrofauna provide several ecosystem goods and services, including the nutrient regulation (Snelgrove 1998; Lenihan and Micheli 2001; Norling et al. 2007; Queirós et al. 2013) like that of carbon, sulfur and nitrogen cycling, water column processes, detoxification and distribution of pollutants. The macrobenthic animals also provide important energy pathways in the marine coastal ecosystems (Marcus and Boero 1998), secondary production (Warwick et al. 1979; Oliver and Slattery 1985), and in fishery (Smaal 1991). Because macrofauna generally spend their entire life in their habitat, many species, like mussels and polychaetes are also used as indicators of ecosystem health (Bilyard 1987; Bustos-Baez and Frid 2003; Nicholson and Lam 2005). Perhaps, one of the most outstanding feature of macrofauna is that of its biological diversity structures, such as abundance, biomass, species diversity and composition, which vary greatly with both space and time (Lenihan and Micheli 2001). Nonetheless, only 1% of the macrofaunal communities is documented globally, with 500 thousand to 10 million species representing only a small portion of sampled areas (Snelgrove 1998).

Comparison of patterns of macrofauna between bare sand and seagrass beds suggest the critical role of vegetation to macrobenthic animals (Orth et al. 1984; Connolly 1997; Nakaoka 2005; Boström et al. 2006). Macrofauna benefits much from sheltering on seagrass beds because the (i) sediment are more stabilized in the beds, (ii) food is more abundant through higher presence of epiphytes, seagrass detritus and plankton communities in the beds, and (iii) there is more biogenic habitats to use for environmental and predator protection. Changes in the complexity of seagrass beds alters the above-and-belowground structures for associated benthic fauna (Boström et al. 2006). Despite many possible drivers influencing population and community patterns, it remains necessary to advance our knowledge on how assemblages respond to various accelerating environmental stressors affecting seagrass beds.

1.4 Some drivers of faunal community patterns in seagrass beds

1.4.1 Habitat complexity

Investigations on the effect of seagrass structures (e.g., shoot density, biomass, cover) have implications on how disturbances in seagrass beds will alter associated fauna (e.g., Stoner

1980; Schneider and Mann 1991; Attrill et al. 2000). At the same time, results of these studies emphasized close association of animals to seagrass beds. For instance, Heck and Wetstone (1977) reported that aboveground biomass of seagrass is an adequate indicator of benthic macroinvertebrate species richness and abundance. They explained that more cryptic species are attractive to dense seagrass habitats resulting to higher species richness of macroinvertebrates, and that higher macroinvertebrate abundance is either due to protection from predators offered by high biomass seagrass beds, or to higher biogenic habitat from the greater plant surface area. Stoner (1980) suggested that biomass, independent of sediment type and hydrodynamic conditions, is an important regulator of species abundance, dominance, diversity and trophic organization in macrofaunal assemblages. Specifically, he found that the relative abundances of epifaunal amphipods and epifaunal polychaetes were directly related to seagrass biomass, and the abundance of deposit feeding and omnivorous polychaetes decreased as a function of seagrass standing crop, while that of suspension feeding and carnivorous polychaetes increased with vegetation. Macroinvertebrate size and species composition is also reported to be dependent on the amount of plant available (Attrill et al. 2000). Lee et al. (2001) showed that epibenthos abundance increases in high biomass *Zostera japonica* beds, and they explained the pattern through seagrass provision of canopy (shelter) and indirectly trapping of marine detritus that serves as food for many species.

Our knowledge regarding the influence of seagrass structures effects to associated macrofauna is not limited to biomass. Webster et al. (1998) reported that increase in seagrass shoot density lead to increase in infaunal diversity. Moreover, Heck and Wetstone (1977) found that number of seagrass species is not an adequate representative of macroinvertebrates species richness and abundance. Abundance and biomass of small motile epifauna is also reduced with reduction in seagrass cover (Connoly and Butler 1996). Further analyses also showed that community patterns traits are driven by complex processes. For instance, Orth et al. (1984) suggested that predation in seagrass beds, which determines the abundance of many seagrass benthic species, both epifauna and infauna, is positively correlated to seagrass root-rhizome and plant canopy. Bologna and Heck (2000) also reported that settlement of bivalves is determined by multiple processes operating over many spatial scales, such as microstructures provided by epiphytes, small-scale patch size and shape, and large scale within habitat difference.

All seagrass species vary in morphological structures (Duarte 1991; Kuo and den Hartog 2006; Hori et al. 2009), and seagrass beds are usually composed of more than one species. Will the spatial structure driven by different seagrass species influence community patterns? The seagrass leaves vary among species in the amount of surface area per unit biomass (Orth et al. 1984). Thus, plants with foliose leaves, and therefore with greater surface area per unit weight, should provide more protection than plants with simpler leaves and lower surface area per weight (Heck and Orth, 1980). Moreover, different seagrass species have different ability in stabilizing the sediment and in the development of canopy frictions (Fonseca and Fisher 1986). These are both important in structuring benthic faunal communities. Most seagrass beds in the world are composed of two or more seagrass structurally different species, and yet, we have little understanding on the role of the species to associated fauna (Gillanders 2006).

1.4.2 Local pollution

One of the well-documented human-induced disturbances to seagrass beds is the organic enrichment (Short and Wyllie-Echeverria 1996). This form of man-made disturbance operates mostly in local scale, affecting population and community structures of benthic communities. It is well understood that organic pollution alters the abundance, biomass and species richness of sediment fauna (Pearson and Rosenberg 1978; Heip 1995). Pollution changes species composition (Warwick and Clarke 1994) and hypoxic condition received numerous discussion already regarding its impact to benthic structures (e.g., Diaz and Rosenberg 1995; Heip 1995; Nilsson and Rosenberg 2000; Rosenberg 2001). Among the various marine fauna, fish are the most sensitive to hypoxia, whereas the least sensitive were molluscs, followed by polychaetes (Gray et al. 2002). Many benthic species react to a pollution-stressed environment with various behavioral responses before they eventually die (Diaz and Rosenberg 1995). The seagrass beds supports diverse and abundant benthic faunal communities. With pollution confronting the complexity of seagrass beds at a local scale, it is important to understand how we will manage these important ecological habitats.

Many of the introduced mariculture structures from fishponds to shallow coastal waters are constructed nearby seagrass beds. Habitat loss or changes in the structure of seagrass beds is an alarming response of community tolerance and resilience to coastal degradation (Fortes et al. 2012; Tanaka et al. 2014). Coastal pollution poses many serious threats especially to benthic communities (Baird et al. 2010); however, there is little understanding on how seagrass macrofauna respond to organic pollution.

1.4.3 Regional factors

There is growing recognition that community patterns are further understood when viewed through larger spatial scales (Nakaoka and Noda 2004; Okuda et al. 2004; Nakaoka et al. 2006). Community patterns at local scale is not necessarily same at regional scale (e.g., species interaction at local scale vs broad-scale climate variation). Configuration on the structures of seagrass beds (e.g., whether it is monospecific or multispecific) is largely based on the geographical range of seagrass species, climate and oceanographic system (Short et al. 2007). These factors operate on a broad spatial scale. How about for the macrobenthic animals of seagrass beds over large areas?

Differences in the oceanographic processes driven by different current systems, as well as that of geographical factors (e.g., climatic condition), are expected to influence the seagrass macrofaunal community dynamics across broad spatial scale (*sensu* Soininen et al. 2007, Sanford 2014). Nonetheless, this requires further hypothesis-testing because we have paucity of literature regarding this aspect. There are at least three major reasons why investigation at broad spatial scale for seagrass macrofauna is often overlooked. First, at all ecological research, large scale studies are hampered with many constraints, including manpower, time, funding, and even on common interest. Second, the broad scale structure of seagrass bed itself is rarely documented. Finally, most of the studies of seagrass macrofaunal communities were analyzed using fragmented approaches and small spatial scale (Nakaoka 2005), making both scaling up of results and its interpretation difficult (Warwick 1997). Broad-scale studies on community patterns and processes are necessary to predict or evaluate community/ecosystem responses against ongoing changes in world's climate, hence providing advances to knowledge on seagrass-faunal system across wide spatial scale is both timely and important.

1.5 Objectives and chapter outline

The general objective of this dissertation is to elucidate the community dynamics of seagrass macrofauna across major biotic/abiotic environmental gradients operating at various spatial/temporal scales; which are (1) different seagrass species as a function of habitat heterogeneity, (2) the level of pollutants that causes degradation in habitats, and (3) climate and oceanographic gradient driven by regional processes.

The main body of this thesis is organized into three independent studies. Each chapter addresses one main objective, but all analysing biological diversity patterns of seagrass macrofauna. After the general introduction given here (Chap. 1), Chapter 2 examines whether or not the seagrass species variation in tropical seagrass beds influences abundance, species richness and species composition of macrofauna. It also try to determine if associated seagrass aboveground and belowground biomass and shoot density have influence on the faunal community structures. This chapter emphasizes the importance of understanding role of seagrass species in faunal community dynamics.

Chapter 3 investigates the effect of organic pollution to seagrass associated macrofaunal abundance, species diversity, turnover, and species composition in Bolinao, northwestern Philippines. The source of pollution in the study area was the mariculture of milkfish (*Chanos chanos*) farming. The farming of milkfish was introduced from nearby fishponds to shallow coastal areas since 1995. This chapter highlights the negative effects of pollution to macrofaunal community in seagrass beds thriving within the pollution gradient.

Chapter 4 determines the large scale patterns of diversity and abundance of macrofaunal associates of seagrass beds in Japan. These beds represent six sites, covering the latitudinal range of 24° to 43°N. This chapter highlights the importance of managing seagrass habitats considering small to large-scale processes.

The thesis concludes with a General Discussion (Chapter 5), summarizing and integrating the overall results of the three studies, discussing the implication of results in seagrass management and suggesting important directions for future research.

1.6 References

Alongi DM (2002) Present state and future of the world's mangrove forests. Environ Conserv 29:331–349. doi: 10.1017/S0376892902000231.

Attrill MJ, Strong JA, Rowden AA (2000) Are macroinvertebrate communities influenced by seagrass structural complexity? Ecography (Cop) 23:114–121. doi: 10.1111/j.1600-0587.2000.tb00266.x.

Baird D, Christian RR, Peterson CH, Johnson G a (2010) Consequences of hypoxia on estuarine ecosystem function : Energy diversion from consumers to microbes. Ecol Soc Am 14:805–822.

Bilyard GR (1987) The value of benthic infauna in marine pollution monitoring studies. Mar Pollut Bull 18:581–585. doi: 10.1016/0025-326X(87)90277-3.

Bologna P a. X, Heck KL (2000) Impacts of seagrass habitat architecture on bivalve settlement. Estuaries 23:449. doi: 10.2307/1353138.

Boström C, Jackson EL, Simenstad CA (2006) Seagrass landscapes and their effects on associated fauna: A review. Estuar Coast Shelf Sci 68:383–403. doi: 10.1016/j.ecss.2006.01.026.

Bustos-Baez S, Frid C (2003) Using indicator species to assess the state of macrobenthic communities. Hydrobiologia 496:299–309. doi: 10.1023/A:1026169520547.

Chapin FS, Zavaleta ES, Eviner VT, et al (2000) Consequences of changing biodiversity. Nature 405:234–242.

Connolly RM (1997) Differences in composition of small, motile invertebrate assemblages from seagrass and unvegetated habitats in a southern Australian estuary. Hydrobiologia 346:137–148. doi: 10.1023/A:1002970100662.

Connoly RM, Butler AJ (1996) The effects of altering seagrass canopy height on small, motile, invertebrates of shallow Mediterranean embayments. Mar Ecol 17:637–652. doi: 10.1111/j.1439-0485.1996.tb00422.x.

Costanza R, D’Arge R, De Groot R, et al (1998) The value of ecosystem services: Putting the issues in perspective. Ecol Econ 25:67–72. doi: 10.1016/S0921-8009(98)00019-6.

Covich AP, Austen MC, Bärlocher F, et al (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. Bioscience 54:767. doi: 10.1641/0006-3568(2004)054[0767:TROBIT]2.0.CO;2.

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia : A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol an Annu Rev 33:245–303.

den Hartog C, Kuo J (2006) Taxonomy and Biogeography of Seagrasses. Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, pp 1–23.

Duarte CM (2000) Marine biodiversity and ecosystem services: an elusive link. J Exp Mar Bio Ecol 250:117–131.

Duarte CM (1991) Allometric scaling of seagrass form and productivity. Mar Ecol Prog Ser 77:289–300. doi: 10.3354/meps077289.

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192–206. doi: 10.1017/S0376892902000127.

Duarte CM, Terrados J, Agawin NSR, et al (1997) Response of a mixed Philippine seagrass meadow to experimental burial. Mar Ecol Prog Ser 147:285–294. doi: 10.3354/meps147285.

Duffy JE (2006) Biodiversity and the fuctioning of seagrass ecosystems. 233:233–250. doi: 10.3354/meps311233.

Fonseca M, Fisher J (1986) A comparison of canopy friction and sediment movement between four species of seagrass with reference to their ecology and restoration . Mar Ecol Prog Ser 29:15–22. doi: 10.3354/meps029015.

Fortes MD, Go GA, Bolisay K, et al (2012) Seagrass response to mariculture-induced physico-chemical gradients in Bolinao, northwestern Philippines. Procedings 12th Int Coral Reef Symp 9–13 Sept.

Fraser MW, Kendrick GA Statton J, et al (2014) Extreme climate events lower resilience of foundation seagrass at edge of biogeographical range. J Ecol 1528–1536. doi: 10.1111/1365-2745.12300.

Gamfeldt L, Lefcheck JS, Byrnes JEK, et al (2014) Marine biodiversity and ecosystem functioning: what’s known and what's next? Oikos 000:001–014. doi: 10.1111/oik.01549.

Gillanders BM (2006) Seagrasses, Fish, and Fisheries. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, pp 503–536.

Gray JS (1992) Eutrophication in the sea. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine Eutrophication and Population Dynamics. Olsen and Olsen, Fredensborg, pp 3–15.

Green EP, Short FT (2003) World atlas of seagrasses. University of California Press.

Heck KL, Orth RJ (1980) Seagrass habitats: the roles of habitat complexity, competition and predation in structuring associated fish and motile macroinvertebrate assemblages. In: Kennedy VS (ed) Estuarine Perspectives. Academic Press, Inc., New York, pp 449–464.

Heck Jr. KL, Wetstone GS (1977) Habitat complexity and invertebrate species richness and abundance in tropical seagrass meadows. J Biogeogr 4:135–142.

Heip C (1995) Eutrophication and zoobenthos dynamics. Ophelia 41:113–136.

Hooper DU, Chapin ES, Ewel JJ, et al (2005) Effects of biodiversity on ecosystem functioning: A concensus of current knowledge. Ecol Monogr 75:3–35.

Hori M, Suzuki T, Monthum Y, et al (2009) High seagrass diversity and canopy-height increase associated fish diversity and abundance. Mar Biol 156:1447–1458. doi: 10.1007/s00227-009-1184-3.

Hughes AR, Williams SL, Duarte CM, et al (2009) Associations of concern: Declining seagrasses and threatened dependent species. Front Ecol Environ 7:242–246. doi: 10.1890/080041.

Ieno EN, Solan M, Batty P, Pierce GJ (2006) How biodiversity affects ecosystem functioning: Roles of infaunal species richness, identity and density in the marine benthos. Mar Ecol Prog Ser 311:263–271. doi: 10.3354/meps311263

Islam MS, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Mar Pollut Bull 48:624–649. doi: 10.1016/j.marpolbul.2003.12.004.

Jackson JB, Kirby MX, Berger WH, et al (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–637. doi: 10.1126/science.1059199.

Kuo J, den Hartog C (2006) Seagrass Morphology, Anatomy, and Ultrastructure. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands.

Lee SY, Fong CW, Wu RSS (2001) The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: A study using artificial seagrass units and sampling of natural beds. J Exp Mar Bio Ecol 259:23–50. doi: 10.1016/S0022-0981(01)00221-0.

Lenihan HS, Micheli F (2001) Soft-sediment communities. In: Bertness, M. D. Gaines, S. D. Hay ME (ed) Marine community ecology. Sinauer Associates, Inc., Sunderland, pp 253–287

Loreau M (2000) Biodiversity and ecosystem functioning: Recent theoretical advances. Oikos 91:3–17. doi: 10.1034/j.1600-0706.2000.910101.x.

Loreau M, Naeem S, Inchausti P, et al (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science 294:804–808. doi: 10.1126/science.1064088.

Marcus NH, Boero F (1998) Minireview: The importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. Limnol Oceanogr 43:763–768. doi: 10.4319/lo.1998.43.5.0763.

Mikola J, Salonen V, Setälä H (2002) Studying the effects of plant species richness on ecosystem functioning: Does the choice of experimental design matter? Oecologia 133:594–598. doi: 10.1007/s00442-002-1077-3.

Nakaoka M (2005) Plant-animal interactions in seagrass beds: Ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177. doi: 10.1007/s10144-005-0226-z.

Nakaoka M, Ito N, Yamamoto T, et al (2006) Similarity of rocky intertidal assemblages along the Pacific coast of Japan: Effects of spatial scales and geographic distance. Ecol Res 21:425–435. doi: 10.1007/s11284-005-0138-6.

Nakaoka M, Lee K-S, Huang X, et al (2014) Regional Comparison of the Ecosystem Services from Seagrass Beds in Asia. In: Nakano S, Yahara T, Nakashizuka T (eds) Integrative Observations and Assessments. Springer, pp 367–393.

Nakaoka M, Noda T (2004) Special feature: Multiple spatial scale approaches in population and community ecology. Popul Ecol 46:103–104. doi: 10.1007/s10144-004-0186-8.

Nicholson S, Lam PKS (2005) Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). Environ Int 31:121–132. doi: 10.1016/j.envint.2004.05.007.

Nilsson HC, Rosenberg R (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: Analysed by sediment profile-imaging and by grab samples. Mar Ecol Prog Ser 197:139–149. doi: 10.3354/meps197139.

Norling K, Rosenberg R, Hulth S, et al (2007) Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. Mar Ecol Prog Ser 332:11–23. doi: 10.3354/meps332011.

Nyström M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment. Trends Ecol Evol 15:413–417. doi: 10.1016/S0169-5347(00)01948-0.

Okuda T, Noda T, Yamamoto T, et al (2004) Latitudinal gradient of species diversity: Multi-scale variability in rocky intertidal sessile assemblages along the Northwestern Pacific coast. Popul Ecol 46:159–170. doi: 10.1007/s10144-004-0185-9.

Oliver JS, Slattery PN (1985) Destruction and opportunity on the sea floor: Effects of gray whale feeding. Ecology 66:1965–1975.

Orth RJ, Carruthers TJB, Dennison WC, et al (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Orth RJ, Heck KL, van Montfrans J (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350. doi: 10.2307/1351618.

Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Annu. Rev. 16:229–311.

Polidoro BA, Carpenter KE, Collins L, et al (2010) The loss of species: Mangrove extinction risk and geographic areas of global concern. PLoS One. doi: 10.1371/journal.pone.0010095.

Queirós AM, Birchenough SNR, Bremner J, et al (2013) A bioturbation classification of European marine infaunal invertebrates. Ecol Evol 3:3958–3985. doi: 10.1002/ece3.769.

Rasheed MA, Unsworth RKF (2011) Long-term climate-associated dynamics of a tropical seagrass meadow: Implications for the future. Mar Ecol Prog Ser 422:93–103. doi: 10.3354/meps08925.

Rogers C (1990) Responses of coral reefs and reef organisms to sedimentation . Mar Ecol Prog Ser 62:185–202. doi: 10.3354/meps062185.

Rosenberg R (2001) Marine benthic faunal successional stages and related sedimentary activity. Sci Mar 65:107–119.

Sanford E (2014) The Biogeography of Marine Communities. In: Bertness MD, Bruno JF, Silliamn BR, Stachowicz JJ (eds) Marine Ecology and Conservation. Sinauer Associates, Suntherland.

Schneider FI, Mann KH (1991) Species specific relationships of invertebrates to vegetation in a seagrass bed. I. Correlational studies. J Exp Mar Bio Ecol 145:101–117.

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. J Exp Mar Bio Ecol 350:3–20. doi: 10.1016/j.jembe.2007.06.012.

Short FT, Polidoro B, Livingstone SR, et al (2011) Extinction risk assessment of the world ' s seagrass species. Biol Conserv 144:1961–1971. doi: 10.1016/j.biocon.2011.04.010.

Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. Aquat Ecol 63: 169-196.

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. Environ Conserv 23:17–27. doi: 10.1017/S0376892900038212.

Smaal AC (1991) The ecology and cultivation of mussels: new advances. Aquaculture 94:245–261. doi: 10.1016/0044-8486(91)90121-M.

Snelgrove PVR (1998) The biodiversity of macrofaunal organisms in marine sediments. Biodivers Conserv 7:1123–1132. doi: 10.1023/A:1008867313340.

Soininen J, Lennon JJ, Hillebrand H, Lennon J (2007) A multivariate analysis of beta diversity across organisms and environments. Ecology 88:2830–2838.

Spalding M, Taylor M, Ravilious C, et al (2003) Global overview: The distribution and status of seagrasses. In: Green EP, Short FT (eds) World Atlas of Seagrasses: Present Status and Future Conservation. University of California Press, Berkely, pp 5–26.

Stoner AW (1980) The role of seagrass biomass in the organization of benthic macrofaunal assemblages. Bull Mar Sci 30:537–551.

Tanaka Y, Go GA, Watanabe A, et al (2014) 17-year change in species composition of mixed seagrass beds around Santiago Island, Bolinao, the northwestern Philippines. Mar Pollut Bull 88:81–85. doi: 10.1016/j.marpolbul.2014.09.024.

Walker BH (1992) Biodiversity and ecological redundancy. Biol. Conserv. 6:18–23.

Warwick RM (1997) The ecology of soft- bottom habitats: Matching spatial patterns with dynamic processes. J Exp Mar Bio Ecol 2:216:ix.

Warwick RM, Clarke KR (1994) Relearning the ABC: Taxonomic changes and abundance/biomass relationships in disturbed benthic communities. Mar Biol 118:739–744. doi: 10.1007/BF00347523.

Warwick RM, Joint IR, Radford PJ (1979) Secondary production of the benthos in an estuarine environment. In: Jefferies RL, Davy AJ (eds) Ecological processes in coastal environments. Blackwell Scientific, Oxford, pp 429–450.

Webster PJ, Rowden AA, Attrill MJ (1998) Effect of shoot density on the infaunal macro-invertebrate community within a Zostera marina seagrass Bed. Estuar Coast Shelf Sci 47:351–357. doi: 10.1006/ecss.1998.0358.

Worm B, Barbier EB, Beaumont N, et al (2006) Impacts of biodiversity loss on ocean ecosystem services. Science (80-) 314:787–790. doi: 10.1126/science.1132294.

CHAPTER 2

Benthic macrofaunal assemblages in multispecific seagrass meadows of the southern Philippines: Variation among vegetation dominated by different seagrass species

Abstract

Although the influence of seagrasses on the diversity and abundance of associated fauna is generally well understood, the effects of seagrass specific identity are not. To determine whether benthic epifaunal and infaunal assemblage patterns vary by the identity of dominant seagrass species in shallow water meadows, we compared quantitative measures of faunal species richness, abundance, and assemblage structure among beds dominated by *Cymodocea rotundata*, *Enhalus acoroides*, and *Thalassia hemprichii*; the study site was at Lopez Jaena, Misamis Occidental Province in the southern Philippines. The *Cymodocea*-dominated vegetation had higher seagrass shoot density and lower seagrass biomass than those dominated by *Enhalus* and *Thalassia*. Across vegetation types, we encountered 30 and 15 species of epifauna and infauna at average densities of 1.73 and 0.82 animals/core (0.0314 m^2), respectively. Both densities and species richnesses of epifauna and infauna nor species composition varied significantly by vegetation type. Multivariate analyses of macrofaunal assemblage structure and abiotic/biotic environmental factors demonstrated that seagrass aboveground biomass explained a significant proportion of the multivariate variation in epifaunal species composition (39%); none of the potential explanatory variables was related to variation in ecological parameters of the infauna. Thus, seagrass specific identity is not a good predictor of either macrofaunal abundance or diversity patterns. Although the ecological parameters of the benthic macrofauna may be influenced by seagrass biomass and structure, responses differ between epifauna and infauna.

2.1 Introduction

Seagrasses are marine flowering plants that grow on sedimentary substrata in the shallow waters of tropical, temperate, and boreal regions (den Hartog and Kuo 2006). Compared to the striking numbers of other marine taxa (e.g., fishes, corals), seagrass diversity is relatively low, with only about 60 species worldwide. Nevertheless, the extent of individual seagrass meadows can be very large; some occupy kilometers of coastline, although others occur in small fragmented patches (Orth et al. 2006). Seagrass beds provide physical structure for complex assemblages of associated species, and along with phytoplankton, periphyton, and macroalgae, they provide many essential ecosystem services. Seagrass meadows are among the most productive ecosystems on the planet (Costanza et al. 1998; Short et al. 2007). While the meadows function as major natural sinks of blue carbon in the ocean (Fourqurean et al. 2012), they are also important in carbon production and export (Ziegler and Benner 1999; Eyre and Ferguson 2002), nutrient cycling (Hemminga et al. 1991; Flindt et al. 1999), stabilization (Fonseca 1989), and trophic transfers (Heck et al. 2008). Finally, seagrass beds enhance biodiversity. For example, more faunal elements are attracted to seagrass vegetated areas than to bare sand or non-vegetated estuarine seabed plots (Ferrell and Bell 1991; Lee et al. 2001; Hosack et al. 2006). Despite the many ecosystem services that seagrass beds provide, a growing realization indicates that seagrasses are declining worldwide due to increases in natural and human-induced disturbances (Short and Wyllie-Echeverria 1996; Duarte 2002; Orth et al. 2006; Waycott et al. 2009). The link between biodiversity and ecosystem functions in seagrass ecosystems (Duffy 2006) implies that accelerating the decline of seagrass beds will threaten the sustainability of their ecosystem services (Duarte 2000). Efforts to maintain, protect, and conserve coastal biodiversity should be especially focused on the conservation and management of seagrasses.

The effect of seagrasses on associated fauna is generally well understood; seagrass beds enhance faunal diversity by providing structural habitat, food, nursery grounds, protection from predators, and a much larger range of available niches than areas devoid of vegetation. Much of the previous works on plant–animal interactions in seagrass beds has focused on epifauna, including fish and epibenthic invertebrates (e.g., Pollard 1984; Jenkins et al. 1997; Gillanders 2006); however, it is becoming increasingly clear that infaunal assemblages of seagrass beds have important roles in the ecological processes operating in the meadows (e.g., Peterson and Heck 2001; Johnson et al. 2002; van der Heide et al. 2012; Blackburn and Orth 2013). Because the emergent structures of seagrass beds are readily observable, the aboveground benthic epifauna has been subjected to more intense investigation (e.g., Bell and Westoby 1986; Edgar 1990; Nakaoka et al. 2001) than infaunal assemblages associated with seagrass belowground structures (root–rhizome complex; e.g., Harrison 1987; Berkenbusch et al. 2007; see also the review of Orth et al. 1984 and references therein). Are infaunal elements as dependent on seagrass structures as epifaunal taxa; do positive and negative interactions belowground depend on diverse interacting factors, including environmental conditions and biological traits of component species (see review by Nakaoka 2005)? The current knowledge base is insufficient to provide comprehensive answers to these questions. One approach to resolving the issues is a comparative analysis of epifaunal and infaunal diversity structures in seagrass beds. In particular, it is essential to determine the ecological responses of epifauna and infauna in mixed meadows dominated by different seagrass species. Different seagrass species in mixed meadows have diverse above- and belowground structures (Duarte 1991; Kuo and den Hartog 2006; Hori et al. 2009). If the responses of associated faunas differ among seagrass species, then seagrass identity within a meadow may directly or indirectly determine the structure of the benthic faunal communities, and may be a good predictor of faunal abundance and biodiversity patterns (see Gillanders 2006).

I examined effects of the identities of different seagrass species on associated benthic macrofauna through quantitative comparative studies of meadow epifaunas and infaunas. My premise was that the benthic epifaunal assemblage depends more strongly on seagrass structure than the infaunal assemblage (see Orth et al. 1984 and Nakaoka 2005 for extensive reviews). Thus, I postulated that interspecific differences in the aboveground structures of seagrass beds will affect the assemblage structure of the benthic epifauna, but not the assemblage structure of the benthic infauna. To test this postulate, I made quantitative comparisons among benthic epifaunas and infaunas in tropical seagrass vegetation types dominated by three different seagrass species: *Cymodocea rotundata* Ehrenb. & Hempr. ex Aschers., *Enhalus acoroides* (L.f.) Royle, and *Thalassia hemprichii* (Ehrenb) Aschers. The study was conducted in the southern Philippines.

2.2 Materials and methods

2.2.1 Study area

I sampled in the months of September and December 2010 in Lopez Jaena municipality, Misamis Occidental Province (southern Philippines; Fig. 2-1). This municipality was previously reported to have 526.2 ha of aggregated seagrass meadows (de Guzman et al. 2009). We sampled three sites in these meadows (Mansabay Bajo, Danlugan, and Capayas; Figure 2.1). The sites differed by topography. Mansabay was located in a beach area where the fine sandy sediments were mostly dominated by pioneer seagrasses such as *Halodule* sp. and *C. rotundata*. In contrast, Danlugan had a muddy seagrass area dominated by *E. acoroides* and *T. hemprichii*; this site was located in the vicinity of mangrove stands and an old wharf left standing in an abandoned fishing port. Capayas, a marine protected area near Capayas Island, was located approximately 0.32 km offshore; the water depth ranged from about 20 cm at low tide to about 200 cm at high tide. The substratum comprised coarse sediments and supported at least five seagrass species dominated (in no particular order) by *T. hemprichii*, *C. rotundata*, and *E. acoroides*. Although all of the locations had heterogeneous seagrass beds, I limited the sampling to the three chosen sites due to logistical constraints, including the lack of a nearby laboratory facility, and limited budget and time for sampling.

2.2.2 Target seagrass species

C. rotundata, *E. acoroides*, and *T. hemprichii* were targeted for this study because they are the most common species in seagrass meadows of Southeast Asia (Meñez et al. 1983; Fortes 1986; Vermaat et al. 1995), and because they were the most abundant in the study area (Rollon et al. 1999; de Guzman et al. 2009). *C. rotundata* has a monopodial rhizome; it is herbaceous and has leaf blades that shed before the leaf-sheaths (den Hartog and Kuo 2006). The species occurs widely along the coasts of the tropical Indo–West Pacific region, and is considered to be a pioneer seagrass with rapid rhizome elongation rates, low reproductive output, and limited seed dispersal (Olesen et al. 2004). Although *C. rotundata* is relatively small compared to the other two targeted seagrasses, it frequently has the highest shoot density, albeit with reduced biomass. *E. acoroides*, a very coarse plant with a thick rhizome and strap-shaped leaves, is the largest tropical seagrass species; *T. hemprichii*, a medium-sized species, is moderately coarse. *E. acoroides* and *T. hemprichii* are slow-growing, but produce large, widely dispersed seeds that dominate seagrass sexual colonization; they are capable of forming climax communities (Olesen et al. 2004). *E. acoroides* and *T. hemprichii* are widely distributed in the coastal waters of the Indian Ocean and the western Pacific (den Hartog and Kuo 2006).

2.2.3 Field sampling

I established duplicate sites for each of three seagrass vegetation types: *C. rotundata-, T. hemprichii-*, and *E. acoroides*-dominated types (hereafter referred to as Cr, Th, and Ea vegetation, respectively). We collected samples from Th and Ea vegetation in the Danlugan and Capayas sites; Cr vegetation was sampled in Capayas and Mansabay. Each of the duplicate sites comprised a multispecific seagrass meadow with an estimated area of ≥100 m^{-2}. The parameters of the vegetation types were based on shoot density because core samples sometimes contained more than one seagrass species. In each replicate core, the target seagrass species had a shoot density >50% greater than those of other seagrass species found in the same core. We used a stainless steel cylindrical sediment sampler (20 cm diameter) to simultaneously collect the benthic epifauna, infauna, and seagrasses down to a depth of 10 cm (0.0314 m^2 surface area for each core sample). Five core samples were obtained from within each seagrass vegetation type in each of the duplicate sites. I sampled without regard to edge effects or the presence of patches; instead, we collected the cores haphazardly in the selected seagrass areas. Core samples were initially sieved in the field using a wooden mesh box (1.0-mm mesh size) and then taken to the laboratory for processing. I also haphazardly collected five additional sediment samples per vegetation unit in each site using a smaller PVC core ($0.0026\ m^2$ surface area) to 10 cm depth and took them to the laboratory to determine the silt–clay content of the surrounding sediment. I sampled twice in the months of September and December 2010.

2.2.4 Laboratory analyses

All benthic macrofaunal samples were preserved in 75% ethanol. They were sorted to lowest taxonomic level using appropriate literature (Hinton 1975; Okutani 2000; Schoppe 2000) and the World Register of Marine Species online database (WoRMS Editorial Board 2013). The density of each taxon was recorded. Epifaunal and infaunal animals were distinguished in the samples based on knowledge of their biology or their predominant mode of life (Lee et al. 2001).

The seagrass samples were cleaned with seawater. They were identified to species, after which shoot density was recorded. The shoots and sheaths were then separated from the roots and rhizomes and dried for ≥2 days to constant weight.

Samples for sediment analyses were first air-dried. We collected 100.0 g of dried sediment (free of organic debris) from each replicate core for dry-sieving (Buchanan 1984) through six sets of sieves (2 mm, 850 µm, 710 µm, 180 µm, 150 µm, 45 µm). Following the Udden–Wentworth scale (Wenthworth 1922), we set the size cutoff for the silt–clay fraction at 45 µm.

2.2.5 Statistical analyses

All of the statistical analyses were performed with R v.3.0 software (R Development Core Team 2013). The variation among seagrass vegetation types was my primary interest; we replicated sampling in different sites in each of 2 months to account for random variation in space and time. Thus, the data set contained one fixed effect (seagrass vegetation types) and two random effects (site, month). In addition, we had a partially crossed sampling design because while the design had three sites levels, collections from all three vegetation types were conducted only at the Capayas site; collections from two vegetation types (Th and Ea) were made at Danlugan, and collections from one vegetation type (Cr) were made at Mansabay. Therefore, the design was asymmetrical.

2.2.5.1 Univariate analyses

I first tested among-vegetation type variation in seagrass shoot density, aboveground biomass, belowground biomass, and surrounding sediment silt–clay content to clearly determine the structure of the seagrass vegetation sampled. I used generalized linear mixed models (GLMMs) to analyze the seagrass vegetation structure by species. Untransformed core data from the vegetation structures were analyzed [assuming Poisson distribution and penalized quasi-likelihood (PQL) approximation] using the *glmmPQL* function of the *MASS* library (Venables and Ripley 2002). We used the PQL estimation since it allows overdispersion, and the mean numbers of all measured structures per seagrass vegetation type within site and month groups usually exceeded 5 (Bolker et al. 2009). Model selection is not easily implemented in *glmmPQL* (e.g., AIC and log-likelihood are apparently meaningless in the PQL approximation), but a likelihood-based test can be used for model selection. I performed the likelihood ratio test (LRT) using the *anova* function in the *lmer* (linear mixed-effects model) routine of the *lme4* library (Bates et al. 2014) to find the difference between the null model and the reduced model (full-model) after excluding an ‘effect’ term. The null-intercept model did not contain the fixed effect (seagrass), but varied among two crossed random effects (random group intercepts), i.e., site and month; the full model included seagrass and intercepts varying among sites and months. When the full model was not significant in the LRT, the null model was chosen as the best selection, although by default, LRT does not compute χ^2 degrees of freedom, or χ^2 or p values for the null model. I did not test the significance of the random effects to avoid the pitfall of pseudoreplication (Hurlbert 1984). I subsequently used the Tukey contrasts in the *multcomp* library (Hothorn et al. 2008) for multiple comparisons between the levels of seagrass species (fixed effect) whenever the effect itself was found significant at $\alpha = 0.05$.

After determining the structures of the sampled seagrass vegetation types, I also used the GLMM to examine variation in overall abundance and species richness of both epifaunal and infaunal assemblages using zero-inflation models. The core data (n = 60) was analyzed using the *glmmADMB* library (Skaug et al. 2012); this has been built with the open-source *AD Model Builder* (Fournier et al. 2012), which is able to handle zero-inflated data (many of my core data had zero values) and has a negative binomial distribution to allow for overdispersion (Martin et al. 2005). I fitted five candidate models (that were mostly non-nested and suitable for a crossed-factor design) based on multilevel model specifications of the *lme4* library (Bates and Sarkar 2006; Bates 2010; Bates 2011). In addition, to account for differences in seagrass vegetation structures, log shoot density and aboveground biomass were used as offsets in the linear predictor for epifauna; the log of belowground biomass was used as the offset for infauna. An offset is used to specify as *a priori* known component that is to be included in the linear predictor during fitting (Bolker et al. 2012). That is, for any given vegetation type, the expected abundances or species richnesses of the epifauna or infauna were assumed to be proportional to the structure of seagrass species in that vegetation type. Silt–clay content data were excluded as offsets as they did not show a clear pattern across vegetation types (based on the prior analysis of the seagrass vegetation structures). Other models other than the null model had offset terms. The candidate models were generally built as follows: (i) null model, only the levels were defined, contained only site and month (random group intercepts); (ii) full model 1, with seagrass plus the offset term and intercept varying among sites and months; (iii) full model 2, with seagrass plus the offset and random slope of seagrass within sites and within months with the correlated intercept; (iv) full model 3, with seagrass plus the offset and random slope of seagrass within site and within month, no variation in intercept; and (v) full model 4, with seagrass plus the offset and uncorrelated random intercept and random slope within seagrass and within month. I used the Akaike information criterion (AIC; Akaike 1973) for model selection; the preferred model had the lowest AIC value, the smallest difference between its AIC value and the maximum AIC (dAIC), and the maximum weight. I used the *AICtab* function in the *bbmle* library (Bolker and R Development Core Team 2013) to obtain the dAIC, degrees of freedom (df), and weights.

2.2.5.2 Multivariate analyses

For graphic visualisation of variation in benthic species composition of epifauna and infauna, I performed nonmetric multidimensional scaling (NMDS) on a matrix of Bray-Curtis distances (Bray and Curtis 1957) the *metaMDS* function in *vegan* library (Oksanen et al. 2013). Because zero or empty rows are undefined (meaningless) in method "Bray" and since my core data had many zero-occurrences, I pooled the data for the five replicate cores for each vegetation type, site, and month. The pooled replicate measure was coded by species-site-month, e.g., CrCS: *Cymodocea rotundata*-dominated vegetation at Capayas in September. I analyzed data from 12 sampled locations for epifauna, and 10 sampled locations for infauna (2 missing data points to exclude those replicate cores without any infaunal occurrence). I then performed non-parametric multivariate analysis of variance (PERMANOVA; Anderson 2001) on same data set and distance measure using the *adonis* function in *vegan*. The PERMANOVA calculated using *adonis* is able to handle an unbalanced sampling design. We consistently used 9999 permutations for NMDS ordination and PERMANOVA.

Finally, to determine the relationships between species compositions of epifauna and infauna and the environmental variables (seagrass vegetation structures), I conducted a direct gradient analysis using canonical correspondence analysis (CCA; Ter Braak 1986, 1987; Ter Braak and Verdonschot 1995) using the *cca* function of *vegan* (Oksanen et al. 2013). We separately analyzed data from 12 and 10 sampled locations for epifauna and infauna, respectively. The data for each sampled location were mean values of both the response and explanatory environmental variables. The response variables were mean density (n = 5) of each macrofaunal species log-transformed to reduce the skewness in the data set (Ter Braak 1986). Some of the species had a mean of less than one individual per observation; we added a constant value of 1 to circumvent the impossibility of finding the log of zero [log(x + 1)] (Ter Braak and Verdonschot 1995; Underwood 1997). Mean data (n = 5) for structures of seagrass vegetation were used as environmental variables and all were log-transformed, except for the percentage data of silt–clay proportions, which were arcsine-transformed (Snedecor and Cochran 1967). I decided to match every environmental variable to macrofaunal type and we limited the number of macrofaunal species in the plots to obtain a clear interpretation of the results. Thus, I used shoot density, aboveground biomass, and silt–clay content of sediment as environmental variables for epifauna; for infauna, we used belowground biomass instead of aboveground biomass. I also plotted the scores for the top ranking four and three species of epifauna and infauna, respectively. The CCA ordination diagram displayed the species, sampling locations, and classes of environmental variables in coordinate space. The weighted mean (WA) species and site scores were used for plots of species and sites since WA is unaffected by noisy environmental variables (Ter Braak 1986). For easy interpretation, I limited the ordinations to two-dimensional plots for convenience in visualization (Ter Braak 1986). The eigenvalues of the CCA were tested through 9999 permutations to statistically determine whether any set or single dominating environmental variable had significant effects on the faunal assemblage, or alternatively, had none. I focused on testing the significance of eigenvalues in the framework of canonical axes and environmental constraints (variables) to corroborate patterns emerging from the CCA diagram. The variances explained (%) by any constrained axis and the environmental variables were also computed as the proportion of the (the constrained axis) eigenvalue in total eigenvalues multiplied by 100.

2.3 Results

2.3.1 Seagrass habitat structure

Seagrass density ranged from 4.8 ± 2.8 to 62.0 ± 8.9 shoots/0.0314 m^2 (Table 2-1), and was significantly higher in Cr and Th vegetation than in Ea vegetation (GLMM, $\chi^2 = 15.42$, $P < 0.001$; Table 2-2). Aboveground and belowground biomasses were within the following ranges: 2.4 ± 0.6–12.8 ± 3.6 g dw/0.0314 m^2 and 7.5 ± 4.9–64.4 ± 38.1 g dw/0.0314 m^2, respectively (Table 2.1). Seagrass biomasses (both above ground and below ground) were higher in Ea vegetation than in Th and Cr vegetation (GLMM, $P < 0.0001$; Table 2-2). The silt–clay proportion in the surrounding sediment was within the range of 6.4 ± 0.6–33.4 ± 1.3% (Table 2-1). The GLMM demonstrated that the full model (with seagrass as a fixed effect) was not significant (Table 2.2).

2.3.2 Macrofaunal assemblage structure

Sixty core samples collected in 2 months yielded 153 individuals, most of which were molluscs and polychaete annelids. The 45 species collected belonged to five invertebrate phyla and 41 families (Table 2-3). Epifaunal species belonged to 3 phyla and 26 families; infaunal collections fell into 3 phyla but only 15 families. The epifauna (30 species) was more speciose than the infauna (15 species). The average densities of benthic epifauna and infauna were 1.73 ind./0.0314 m^2 and 0.82 ind./0.0314 m^2, respectively.

Overall benthic macrofaunal density and species richness did not vary significantly among the three vegetation types (Fig. 2-2A & B; GLMM in Table 2-4) even after taking into account the seagrass vegetation structures as offset terms. The species composition structures of epifaunal and infaunal assemblages did not vary consistently across the three seagrass vegetation types (NMDS, Fig. 2-3A & B). PERMANOVA detected no significant overall effects of vegetation type on either epifauna or infauna, although the effect on epifauna was significant among sites (Table 2-5).

2.3.3 Relationships among macrofaunal assemblage structure and environmental variables

The CCA for the epifaunal assemblage explained 41% of the total variation (Table 2-6). The first canonical axis (horizontal CCA1) was significant ($F = 2.409$, $P = 0.015$) and explained 43% of the captured total constrained variation (Fig. 2-4A). This axis represented silt–clay content, and decreasing gradients of seagrass shoot densities and aboveground biomass. The second axis in the two-dimensional plot was not significant, but all environmental variables tracked decreasing gradients along its length, with the steepest decreases in silt–clay content. Among the three environmental variables tested, aboveground biomass of seagrass significantly explained 39% of the multivariate variation on epifaunal species composition (F = 2.200, P = 0.010; Table 2-6).

Epifaunal assemblage structure was not clearly differentiated among different vegetation types; instead, assemblage structure varied among different sites (Fig. 2-4A). For example, the locations Capayas and Mansabay Bajo (for Cr vegetation) were widely separated in the coordinate space of the CCA diagram, and so were the coordinate locations of Capayas and Danlugan (for Th vegetation).

The relationship between seagrass vegetation type and epifaunal assemblage structure was also ambiguous when focusing only on dominant taxa (Fig. 2-4A). For example, *Bulla ampulla* was a dominant component of the epifauna in Th vegetation at Danlugan, but this was not the case at Capayas, where the dominant component was *Brissus latecarinatus*. Similarly, *Canarium urceus* characterized the assemblages in Cr vegetation at Capayas, but not at Mansabay Bajo. The variation in epifaunal assemblage structure in Ea vegetation was difficult to interpret because all of the components were located near the centroid of the CCA plot.

The CCA plot for the infaunal assemblage explained 35% of the constrained variation, and 71% of this proportion was explained by CCA1 (Table 2-6). The first axis was significant and correlated positively with seagrass belowground biomass and shoot density (Fig. 2-4B). CCA2 represented an increasing proportion of silt–clay and a decreasing gradient of seagrass belowground biomass and shoot density, but the axis itself was not significant (Table 2.6). None of the environmental variables significantly influenced infaunal community variation ($P > 0.05$; Table 2-6). Although infaunal assemblage structure tended to vary along the silt–clay gradient, it was not clearly related to either vegetation type or site. The contributions of the dominant infaunal elements to the observed assemblage pattern were neither consistent nor clear (Fig. 2-4B). For example, *Golfingia* sp. was located in the coordinate space of the CCA diagram closest to in assemblage in Th vegetation at Capayas, but not at Danlugan.

2.4 Discussion

Numerous investigations of seagrass–faunal interactions have shown that the presence of seagrass is a strong determinant of faunal diversity. However, effects of differences among seagrass species with different morphological characters on faunal abundance and diversity are much less well understood (Ansari et al. 1991; Nakaoka et al. 2001; Paula et al. 2001; Somerfield et al. 2002). Importantly, we found no significant variation in epifaunal and infaunal assemblage abundance or taxon diversity across different seagrass vegetation types. Nevertheless, my multivariate analyses of relationships between assemblage structures and environmental variables (using CCA) demonstrated that a significant epifaunal variation was best explained by seagrass aboveground biomass, while I was unable to detect an equivalent determinant for infauna. The different responses of epifauna and infauna are in partial agreement on my premise that epifauna would be more dependent on seagrass structure than infaunal elements, which do not necessarily depend on the presence of seagrass belowground biomass as a key habitat component.

The overall species diversity of benthic macrofauna I recorded at Lopez Jaena (45 species) was less than half of that reported previously for other areas (e.g., Lewis and Stoner 1983; Nacorda and Yap 1997; Webster et al. 1998; Nakaoka et al. 2001; Klumpp & Kwak 2005). These earlier reports also describe higher macrofaunal densities than at Lopez Jaena, where I found approximately 1–2 individuals per core (0.0314 m^2). When I previously used the same sampling methodology at Bolinao Pangasinan in the northern Philippines, I recorded higher average densities of epifaunal (12/0.0314 m^2) and infaunal (22/0.0314 m^2) individuals than at Lopez Jaena (V.E. Leopardas, unpublished data). Currently, I do not have an explanation for the strikingly low abundance in my samples collected at Lopez Jaena. A comprehensive analysis of benthic population dynamics across broader spatial and temporal scales would be a first step to better understanding this issue.

The rather depauperate epifaunal assemblage at Lopez Jaena was nevertheless more speciose and more abundant than the infaunal assemblage. I attribute this difference to the association between epifauna and seagrass shoots, which I believe is closer than the interaction between infauna and belowground roots and rhizomes. Previous studies have also reported that epifaunal assemblages are more speciose than those in the sediments (e.g., cf. Stoner 1980; Peterson et al. 1984; Bell and Westoby 1986; Lee et al. 2001; Parker et al. 2001; Vonk et al. 2010).

Although I found that shoot density and above-and-belowground biomass varied greatly among the three dominant seagrass species, I detected no significant effects of these parameters on the abundance and diversity of either epifauna or infauna. This outcome is congruent with my predictions for seagrass–infaunal interactions but not for seagrass–epifaunal associations. A lack of significant seagrass species-specific effects on macrofauna has been previously documented (e.g., Nienhuis et al. 1989; Edgar 1990; Nakaoka et al. 2001; Brito et al. 2005), although not universally (e.g. Virnstein and Howard 1987; Somerfield et al. 2002; Hamilton et al. 2012; Gartner et al. 2013). Thus, Nakaoka et al. (2001) detected inconsistent patterns in epifaunal assemblages among *Zostera marina*, *Zostera caulescens*, and *Zostera caespitosa*, and Hamilton et al (2012) suggested that individual seagrass species cannot act as surrogates for one another in the provision of habitat for epifaunal taxa. Edgar (1990) reported that epifaunal assemblages on different seagrass species subjected to the same environmental conditions are more often similar than epifaunal assemblages associated with the same seagrass species in different sites. Somerfield et al. (2002) demonstrated that the presence of several seagrass species promotes structural complexity of a seagrass meadow, which influences the range of habitats provided for associated benthic epifauna and infauna. Finally, the influence of seagrass species on associated fauna may be nonsignificant because of the very much larger swamping effects of other parameters, such as sediment chemistry (e.g., lack of oxygen; Brito et al. 2005), biological traits of the fauna (e.g., larvae of benthic decapods are unable to discriminate between beds with different structural complexities; Bell and Westoby 1986), or combinations of several interacting factors (Edgar 1990; Nakaoka et al. 2001), which were not assessed in this study.

Although variation in seagrass species identity did not have a significant influence on macrofaunal assemblages in this study, I did find that seagrass aboveground biomass explained a proportion of epifaunal variation among the sampled sites. This effect is not unexpected and is congruent with earlier works (e.g., Stoner 1980; Lewis and Stoner 1983; Ansari et al. 1991; Attrill et al. 2000; Klumpp and Kwak 2005).

The high degree of association between epifauna and seagrass standing biomass may be attributable to increased food availability, physical protection (Schneider and Mann 1991; Edgar and Robertson 1992), or the habitat complexity provided by elevated seagrass biomass; the long life spans of seagrass modular units promote higher population stability among epifaunal elements (Ansari et al. 1991). Attrill et al. (2000) emphasized that increased seagrass biomass does not reflect increased structural complexity, but does provide a larger surface areas for occupancy by macroinvertebrates. Nevertheless, contradictory views have been published (e.g., Virnstein and Howard 1987; Nienhuis et al. 1989). Lee et al (2001) suggests that detritus accumulation enhances faunal species richness and abundance in seagrass habitats, and that the benefits of aboveground seagrass biomass are expressed indirectly through increased detrital accumulation, which is beneficial for both epifauna and infauna.

Finally, I did not detect a significant effect of belowground seagrass biomass on the associated infauna, a finding that was consistent with my expectations. This outcome is congruent with some previous investigations, but not all. Webster et al (1998) found that root–rhizome measures are unimportant for infaunal assemblages, but some reported that the dense root–rhizome mat of seagrass beds provides protection from predators (e.g., Lee et al. 2001; Paula et al. 2001). Dense seagrass roots may also inhibit burrowing and tube-building activities, thereby exposing infauna to digging predators (e.g., Skilleter 1994). However, the extent of the effects of seagrass belowground complexity on infauna depends on the behaviors of individual species. For example, juvenile infaunal elements are more susceptible to predators than adults, which are able to burrow deeper to escape digging carnivores (see review by Orth et al. 1984). Stoner (1980) demonstrated that increased belowground seagrass biomass decreases the abundance of deposit-feeding and omnivorous polychaetes, but enhances the abundances of suspension-feeding and carnivorous polychaetes. Increased total seagrass plant biomass reportedly increases the abundance of polychaetes and bivalves (Ogden and Ogden 1982), although this work did not specifically report effects of the belowground vegetation.

This study demonstrated that taxonomic identity of seagrass species is not a major determinant of variation in the abundance and taxon diversity of benthic macrofaunal assemblages at my study sites in the southern Philippines. Nevertheless, we found that aboveground seagrass biomass did significantly influence variation in epifaunal assemblage structures. Thus, within multispecific meadows, seagrass species redundancy may exist according to the perceptions of macrobenthic invertebrates, even though morphological structures differ strikingly among seagrass taxa. Moreover, Hughes et al. (2009) demonstrated interdependence and facilitation among seagrass species. The effect of the global decline in seagrass biodiversity on the associated fauna has recently received detailed attention (e.g., Duarte 2000; Duffy 2006; Waycott et al. 2009), which has emphasized the importance of conserving seagrass ecosystems wherever possible (see also Hughes et al. 2009). This study has significant implications for planning the conservation and management of biodiversity in seagrass meadows, which are rapidly declining in extent worldwide (Short and Wyllie-Echeverria 1996; Duarte 2002; Orth et al. 2006; Waycott et al. 2009).

2.5 Acknowledgements

Special thanks to F. Tomarong and staff of Local Government Unit- Municipal Agriculture Office in Lopez Jaena for the support during the implementation of the study. I also thank the Institute of Fisheries Research and Development of Mindanao State University at Naawan for the laboratory space and equipment. Special thanks are also due to A.B. de Guzman, J. Jimenez, C. Jimenez, J. Gorospe for providing earlier inputs while developing the research, A. Pantallano, C. de los Reyes, J. Mingoc, R. Lasagas, R. Neri for field assistance, A. Laurden, N. Whanpetch, T. Genovia for help in the laboratory, R. Eballe and K. Watanabe for technical assistance in the figures, and M. Sato for help with statistical analyses. I appreciate the comments by anonymous reviewers that improved the quality of this paper. This study was supported by Japan Science and Technology Agency/Japan International Cooperation Agency, Science and Technology Research Partnership for Sustainable Development, for the project Coastal Ecosystem Conservation and Adaptive Management under Local and Global

Environmental Impacts in the Philippines (CECAM). I am grateful to key members of CECAM, especially to M.D. Fortes and K. Nadaoka, for their continuous support.

2.6 References

Akaike H (1973) Information theory and an extension of themaximum likelihood principle. In: Petrov BN, CzAki F (eds) 2nd International Symposium on Information Theory. Akademiai Kiadó, Budapest, pp 267–281.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust Ecol 26:32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x.

Ansari ZA, Rivonker CU, Ramani P, Parulekar AH (1991) Seagrass habitat complexity and macroinvertebrate abundance in Lakshadweep coral reef lagoons, Arabian Sea. Coral Reefs 10:127–131.

Attrill MJ, Strong JA, Rowden AA (2000) Are macroinvertebrate communities influenced by seagrass structural complexity? Ecography (Cop) 23:114–121. doi: 10.1111/j.1600-0587.2000.tb00266.x.

Bates D (2010) lme4: Mixed-effects modeling with R. http://lme4.r-forge.r-project.org/lMMwR/lrgprt.pdf. Accessed 1 Dec 2013.

Bates D (2011) Linear mixed model implementation in lme4. http://www.icesi.edu.co/CRAN/web/packages/lme4/vignettes/Implementation. Accessed 1 Dec 2013.

Bates D, Maechler M, Bolker BM, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4.

Bates D, Sarkar S (2006) The lme4 package. http://lmqesalquspbr/CRAN/doc/packages/lme4pdf. Accessed 1 Dec 2013.

Bell JD, Westoby M (1986) Variation in seagrass height and density over a wide spatial scale: Effects on common fish and decapods. J Exp Mar Bio Ecol 104:275–295. doi: 10.1016/0022-0981(86)90110-3.

Berkenbusch K, Rowden AA, Myers TE (2007) Interactions between seagrasses and burrowing ghost shrimps and their influence on infaunal assemblages. J Exp Mar Bio Ecol 341:70–84. doi: 10.1016/j.jembe.2006.10.026.

Blackburn NJ, Orth RJ (2013) Seed burial in eelgrass *Zostera marina*: the role of infauna. Mar Ecol Prog Ser 474:135–145. doi: 10.3354/meps10103.

Bolker BM, Brooks ME, Clark CJ, et al (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135.

Bolker BM, R Development Core Team (2013) bbmle: tools for general maximum likelihood estimation.

Bolker BM, Skaug H, Magnusson A, Nielsen A (2012) Getting started with the glmmADMB package. https://r-forge.r-project.org/. Accessed 1 Dec 2013.

Bray J, Curtis J (1957) An ordination of the upland forest communities of souther Wisconsin. Ecol Monogr 27:325–349.

Brito MC, Martin D, Núñez J (2005) Polychaetes associated to a *Cymodocea nodosa* meadow in the Canary Islands: Assemblage structure, temporal variability and vertical distribution compared to other Mediterranean seagrass meadows. Mar Biol 146:467–481. doi: 10.1007/s00227-004-1460-1.

Buchanan JB (1984) Sediment analyses. In: Holme NA, McIntyre AD (eds) Methods for the study of marine benthos, 2nd editio. Blackwell, Oxford, pp 41–65.

Costanza R, D'Arge R, De Groot R, et al (1998) The value of ecosystem services: Putting the issues in perspective. Ecol Econ 25:67–72. doi: 10.1016/S0921-8009(98)00019-6.

de Guzman AB, Quiñones MB, Eballe RC, Israel MB (2009) Coastal Environment Profile of Lopez Jaena , Misamis Occidental Keywords : Participatory coastal resource assessment , coral reefs , seagrasses , mangroves , coastal fisheries and socio-economic profile . 1:43–61.

den Hartog C, Kuo J (2006) Taxonomy and Biogeography of Seagrasses. Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, pp 1–23.

Duarte CM (2000) Marine biodiversity and ecosystem services: an elusive link. J Exp Mar Bio Ecol 250:117–131.

Duarte CM (1991) Allometric scaling of seagrass form and productivity. Mar Ecol Prog Ser 77:289–300. doi: 10.3354/meps077289.

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192–206. doi: 10.1017/S0376892902000127.

Duffy JE (2006) Biodiversity and the fuctioning of seagrass ecosystems. 233:233–250. doi: 10.3354/meps311233.

Edgar GJ (1990) The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with Western Australian seagrass beds. Deep Sea Res Part B Oceanogr Lit Rev 37:1120. doi: 10.1016/S0198-0254(06)80446-2.

Edgar GJ, Robertson AI (1992) The influence of seagrass structure on the distribution and abundance of mobile epifauna: pattern and process in a Western Australian Amphibolis bed. J Exp Mar Bio Ecol 160:13–31. doi: 10.1016/0022- 0981(92)90107-L.

Eyre BD, Ferguson a. JP (2002) Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Mar Ecol Prog Ser 229:43–59. doi: 10.3354/meps229043.

Ferrell DJ, Bell JD (1991) Differences among assemblages of fish associated with *Zostera capricorni* and bare sand over a large spatial scale. Mar Ecol Prog Ser 72:15–24. doi: 10.3354/meps072015.

Flindt MR, Pardal MÂ, Lillebø AI, et al (1999) Nutrient cycling and plant dynamics in estuaries: A brief review. Acta Oecologica 20:237–248. doi: 10.1016/S1146-609X(99)00142-3

Fonseca MS (1989) Sediment stabilization by Halophila decipiens in comparison to other seagrasses. Estuar Coast Shelf Sci 29:501–507. doi: 10.1016/0272-7714(89)90083-8.

Fortes MD (1986) Taxonomy and ecology of Philippine seagrasses. University of the Philippines.

Fournier DA, Skaug HJ, Ancheta J, et al (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim Methods Softw 27:233–249. doi: 10.1080/10556788.2011.597854.

Fourqurean JW, Duarte CM, Kennedy H, et al (2012) Seagrass ecosystems as a globally significant carbon stock. Nat Geosci 5:505–509. doi: 10.1038/ngeo1477.

Gacia E, Duarte CM, Middelburg JJ (2002) Carbon and nutrient deposition in a Mediterranean seagrass (Posidonia oceanica) meadow. Limnol Oceanogr 47:23–32. doi: 10.4319/lo.2002.47.1.0023.

Gartner a., Tuya F, Lavery PS, McMahon K (2013) Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. J Exp Mar Bio Ecol 439:143–151. doi: 10.1016/j.jembe.2012.11.009.

Gillanders BM (2006) Seagrasses, Fish, and Fisheries. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, pp 503–536.

Hamilton BM, Fairweather PG, McDonald B (2012) One species of seagrass cannot act as a surrogate for others in relation to providing habitat for other taxa. Mar Ecol Prog Ser 456:43–51. doi: 10.3354/meps09647.

Harrison PG (1987) Natural expansion and experimental manipulation of seagrass (*Zostera* spp.) abundance and the response of infaunal invertebrates. Estuar Coast Shelf Sci 24:799–812. doi: 10.1016/0272-7714(87)90153-3.

Heck KL, Carruthers TJB, Duarte CM, et al (2008) Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11:1198–1210. doi: 10.1007/s10021-008-9155-y.

Hemminga M a, Harrison PG, van Lent F (1991) The balance of nutrient losses and gains in seaccrass meadows. Mar Ecol Prog Ser 71:85–96. doi: 10.3354/meps071085.

Hinton A (1975) Shells of New Guinea and the Central Indo-Pacific. Charles E. Turtle Corporation, Incorporated, Ruthland, Vermont

Hori M, Suzuki T, Monthum Y, et al (2009) High seagrass diversity and canopy-height increase associated fish diversity and abundance. Mar Biol 156:1447–1458. doi: 10.1007/s00227-009-1184-3.

Hosack GR, Dumbauldt BR, Ruesink JL, Armstrong D a (2006) Habitat associations of estuarine species: Comparisons of intertidal mudflat, seagrass (*Zostera marina*), and oyster (*Crassostrea gigas*) habitats. Estuaries & Coasts 29:1150. doi: 10.1007/BF02781816.

Hothorn T, Bretz F, Heilberger R, Schuetzenmeister A (2008) Simltaneous interference in general parametric models. Biometric J 50:346–363.

Hughes a. R, Williams SL, Duarte CM, et al (2009) Associations of concern: Declining seagrasses and threatened dependent species. Front Ecol Environ 7:242–246. doi: 10.1890/080041

Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54:187–211.

Jenkins GP, May HM a., Wheatley MJ, Holloway MG (1997) Comparison of Fish Assemblages Associated with Seagrass and Adjacent Unvegetated Habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with Emphasis on Commercial Species. Estuar Coast Shelf Sci 44:569–588. doi: 10.1006/ecss.1996.0131.

Johnson M a, Fernandez C, Pergent G (2002) The ecological importance of an invertebrate chemoautotropfic symbiosis to phanerogam seagrass beds. Bull Mar Sci 71:1343–1351.

Klumpp DW, Kwak SN (2005) Composition and abundance of benthic macrofauna of a tropical sea-grass bed in North Queensland, Australia. Pacific Sci 59:541–560. doi: 10.1353/psc.2005.0046.

Kuo J, den Hartog C (2006) Seagrass Morphology, Anatomy, and Ultrastructure. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, p 51.87.

Lee SY, Fong CW, Wu RSS (2001) The effects of seagrass (*Zostera japonica*) canopy structure on associated fauna: A study using artificial seagrass units and sampling of natural beds. J Exp Mar Bio Ecol 259:23–50. doi: 10.1016/S0022-0981(01)00221-0

Lewis FG, Stoner AW (1983) Distribution of macrofauna within seagrass beds: an exlanation for patterns of abundance. Bull Mar Sci Sci 33:296–304.

Martin TG, Wintle B a., Rhodes JR, et al (2005) Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. Ecol Lett 8:1235–1246. doi: 10.1111/j.1461-0248.2005.00826.x.

Meñez EG, Phillips RC, Calumpong HP (1983) Seagrasses from the Philippines. Smithson Contrib to Mar Sci 21:40.

Nacorda HME, Yap HT (1997) Structure and temporal dynamics of macroinfaunal communities of a sandy reef flat in the northwestern Philippines. Hydrobiologia 353:91–106. doi: 10.1023/A:1003002810410.

Nakaoka M (2005) Plant-animal interactions in seagrass beds: Ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177. doi: 10.1007/s10144-005-0226-z.

Nakaoka M, Toyohara T, Matsumasa M (2001) Seasonal and between-substrate variation in mobile epifaunal community in a multispecific seagrass bed of Otsuchi bay, Japan. Mar Ecol 22:379–395. doi: 10.1046/j.1439-0485.2001.01726.x.

Nienhuis PH, Coosen J, Kiswara J (1989) Community structure and biomass distribution of seagrasses andmacrofauna in the Flores Sea, Indonesia. Netherlands J Sea Res 23:197–214.

Ogden JC, Ogden NB (1982) A preliminary study of two representative seagrass communities in Palau, Western Caroline Island (Micronesia). Aquat Bot 12:229–244.

Oksanen J, Blanchet FG, Kindt R, et al (2013) vegan: Community Ecology Package.

Okutani T (ed) (2000) Marine Mollusks in Japan. University of Tokyo Press, Tokyo.

Olesen B, Marba N, Duarte CM, et al (2004) Recolonization dynamics in a mixed seagrass meadow: The role of clonal versus sexual processes. Estuaries 27:770–780. doi: 10.1007/BF02912039.

Orth RJ, Carruthers TJB, Dennison WC, et al (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Orth RJ, Heck KL, van Montfrans J (1984) Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350. doi: 10.2307/1351618.

Parker JD, Duffy JE, Orth RJ (2001) Plant species diversity and composition: Experimental effects on marine epifaunal assemblages. Mar Ecol Prog Ser 224:55–67. doi: 10.3354/meps224055.

Paula J, Fidalgo e Costa P, Martins A, Gove D (2001) Patterns of abundance of seagrasses and associated infaunal communities at Inhaca Island, Mozambique. Estuar Coast Shelf Sci 53:307–318. doi: 10.1006/ecss.2001.0809.

Peterson BJ, Heck KL (2001) Positive interactions between suspension-feeding bivalves and seagrass-A facultative mutualism. Mar Ecol Prog Ser 213:143–155. doi: 10.3354/meps213143.

Peterson CH, Summerson HC, Duncan PB (1984) The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, *Mercenaria mercenaria*. J Mar Res 42:123–138. doi: 10.1357/002224084788506194.

Pollard DA (1984) A review of ecological studies on seagrass-fish communities, with particular reference to recent studies in Australia. Aquat Bot 18:3–42.

R Development Core Team (2013) R: a language and environment for statistical computing.

Rollon RN, Quiaoit HAR, Beyer GG, Zamora PM (1999) Coastal resources profile of Lopez Jaena, Misamis Occidental: a participatory assessment.

Schneider FI, Mann KH (1991) Species specific relationships of invertebrates to vegetation in a seagrass bed. I. Correlational studies. J Exp Mar Bio Ecol 145:101–117.

Schoppe S (2000) A guide to common shallow water sea stars, brittle stars, sea urchins, sea cucumbers, and feather stars (Echinoderms) of the Philippines. Times Media Private Limited, Singapore.

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. J Exp Mar Bio Ecol 350:3–20. doi: 10.1016/j.jembe.2007.06.012.

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. Environ Conserv 23:17–27. doi: 10.1017/S0376892900038212.

Skaug HJ, Fournier DA, Nielsen A, et al (2012) Generalized linear mixed models using AD Model Builder.

Skilleter G a. (1994) Refuges from predation and the persistence of estuarine clam populations. Mar Ecol Prog Ser 109:29–42. doi: 10.3354/meps109029.

Snedecor GW, Cochran WG (1967) Statistical Methods, 6th edn. Ames, Iowa.

Somerfield PJ, Yodnarasri S, Aryuthaka C (2002) Relationships between seagrass biodiversity and infaunal communities: Implications for studies of biodiversity effects. Mar Ecol Prog Ser 237:97–109. doi: 10.3354/meps237097.

Stoner AW (1980) The role of seagrass biomass in the organization of benthic macrofaunal assemblages. Bull Mar Sci 30:537–551.

ter Braak CJF (1987) The analysis of vegetation-environment relationships by canonical correspondence analysis. Vegetatio 69:69–77. doi: 10.1007/BF00038688.

ter Braak CJF (1986) Canonical Correspondence Analysis : A new eigenvector technique for multivariate direct gradient analysis. Ecol Soc Am 67:1167–1179. doi: 10.2307/1938672.

ter Braak CJF, Verdonschot PF (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquat Sci 57:255–289. doi: 10.1007/BF00877430.

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

van der Heide T, Govers LL, de Fouw J, et al (2012) A three-stage symbiosis forms the foundation of seagrass scosystems. Science 336:1432–1434. doi: 10.1126/science.1219973.

Venables WN, Ripley BD (2002) Modern Applied Statistics with S, 4th edn. Springer US, New York.

Vermaat JE, Agawin NSR, Duarte CM, et al (1995) Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. Mar Ecol Prog Ser 124:215–225. doi: 10.3354/meps124215.

Virnstein RW, Howard RK (1987) Motile epifauna of marine macrophytes in the Indian River lagoon. Florida. I. Comparisons among three species of seagrasses from adjacent beds. 41:1–12.

Vonk JA, Christianen MJ a., Stapel J (2010) Abundance, edge effect, and seasonality of fauna in mixed-species seagrass meadows in southwest Sulawesi, Indonesia. Mar Biol Res 6:282–291. doi: 10.1080/17451000903233789.

Waycott M, Duarte CM, Carruthers TJB, et al (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci U S A 106:12377–12381. doi: 10.1073/pnas.0905620106.

Webster PJ, Rowden AA, Attrill MJ (1998) Effect of shoot density on the infaunal macro-invertebrate community within a *Zostera marina* seagrass Bed. Estuar Coast Shelf Sci 47:351–357. doi: 10.1006/ecss.1998.0358.

Wenthworth CK (1922) A scale of grade and class terms for clastic sediments. J Geol 30:377–392.

WoRMS Editorial Board (2013) World Register of Marine Species. In: VLIZ. http://www.marinespecies.org/.

Ziegler S, Benner R (1999) Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Mar Ecol 180:149–160. doi: 10.3354/meps180149.

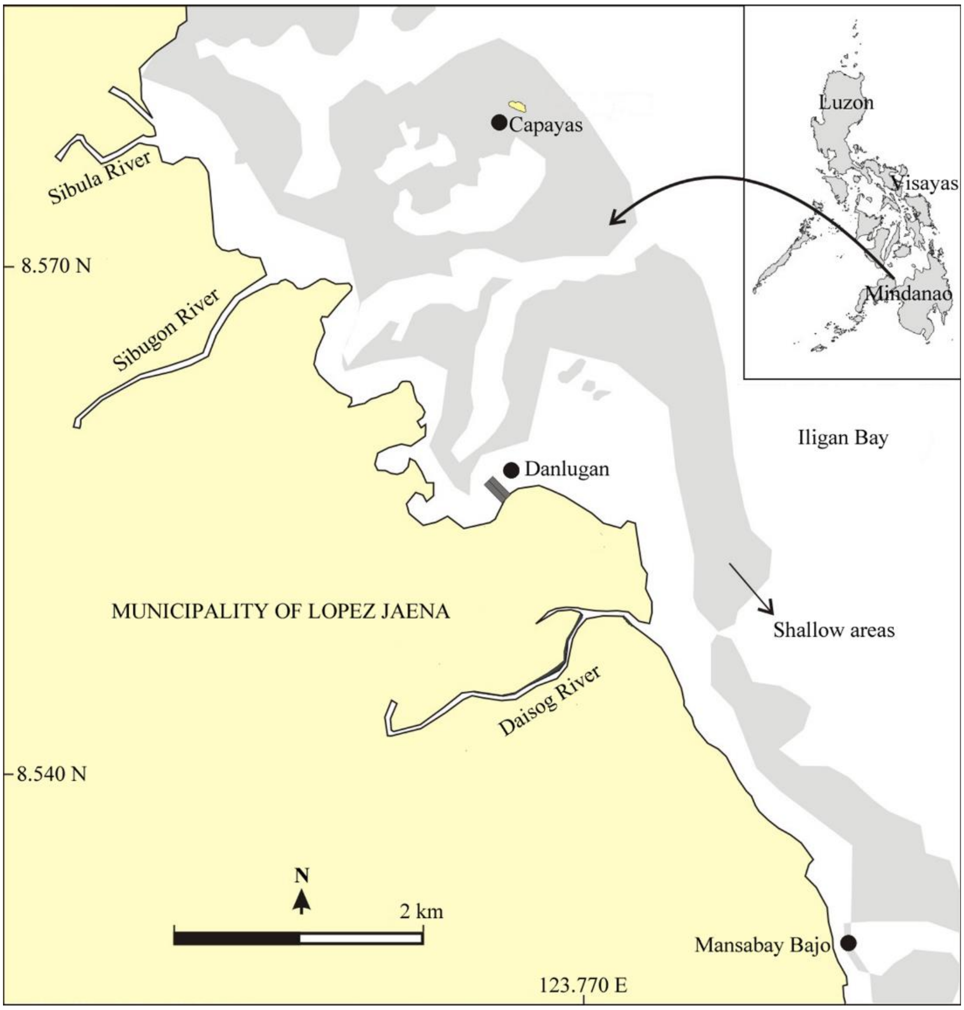

Fig. 2-1 Map of the study sites at Lopez Jaena, Misamis Occidental Province, southern Philippines. The base map was provided by de Guzman et al (2009).

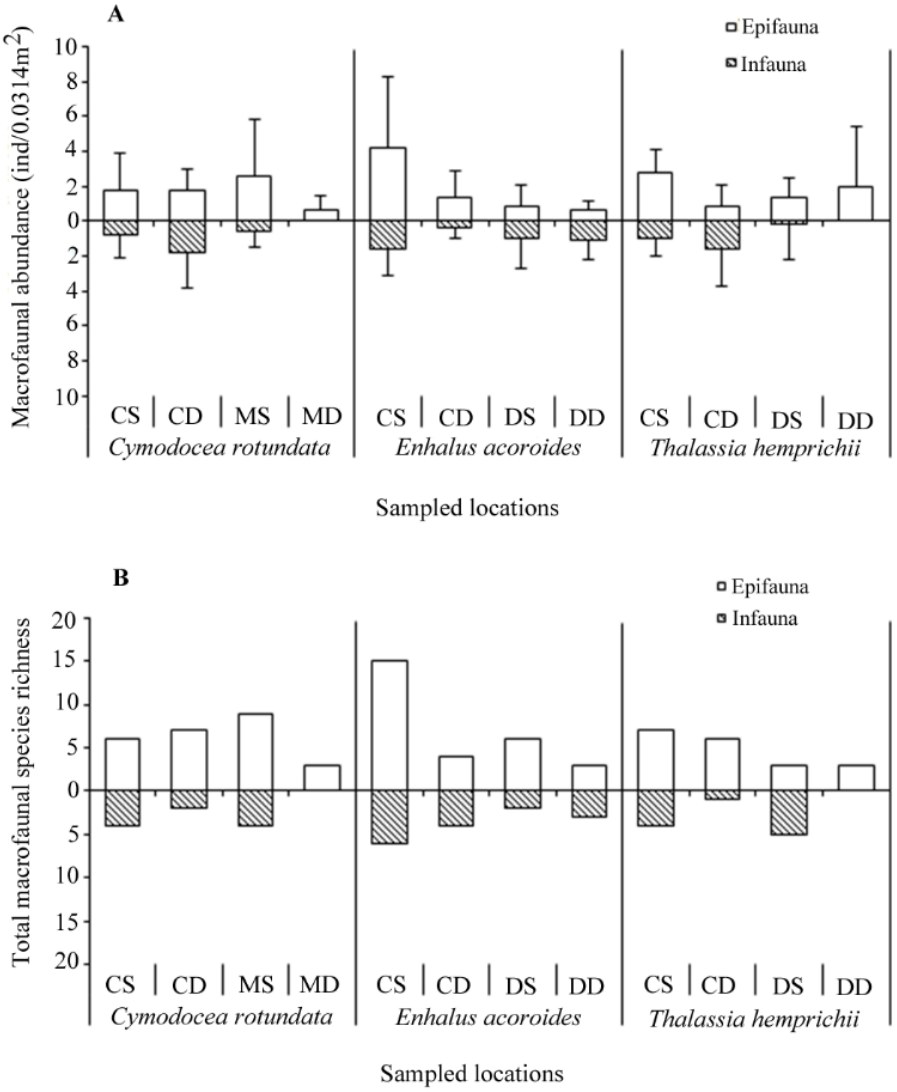

Fig. 2-2 (A) Mean benthic macrofaunal density (±SD, n = 5) and (B) total species richness of benthic macrofauna (n = 5) recorded in three seagrass vegetation types (dominated by *Cymodocea rotundata*, *Enhalus acoroides*, and *Thalassia hemprichii*) located in Capayas (C), Danlugan (D), and Mansabay (M) within the Lopez Jaena municipality, Misamis Occidental Province, southern Philippines; collections were made in September (S) and December (D) 2010. Abbreviations are configured as follows: CD, Capayas collection in December.

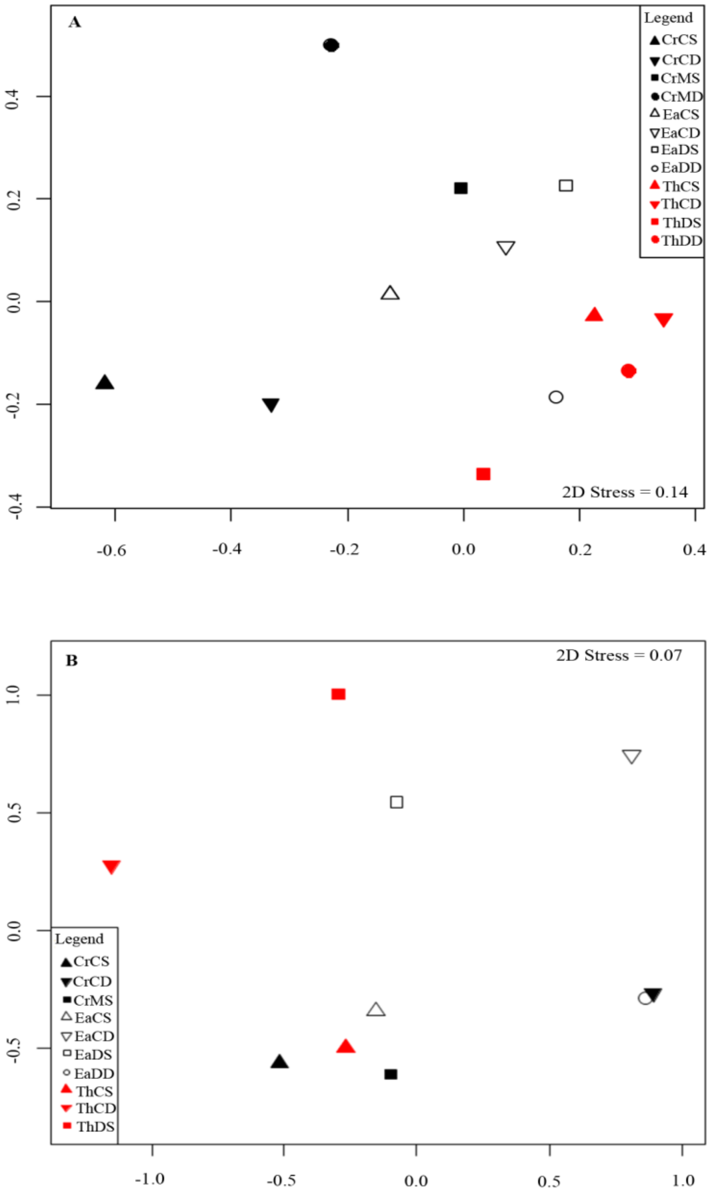

Fig. 2-3 Nonparametric multidimensional scaling (NMDS) plot based on a matrix of Bray–Curtis distance measures for (A) epifauna and (B) infauna based on total density (n = 5) in three vegetation types (Cr: *Cymodocea rotundata*, Ea: *Enhalus acoroides*, Th: *Thalassia hemprichii*) sampled at Capayas (C), Danlugan (D), and Mansabay (M) in Lopez Jaena, Misamis Occidental, southern Philippines, during September (S) and December (D) 2010. Sample code abbreviations are configured as follows: CrCS, *Cymodocea rotundata*-dominated vegetation at Capayas in September.

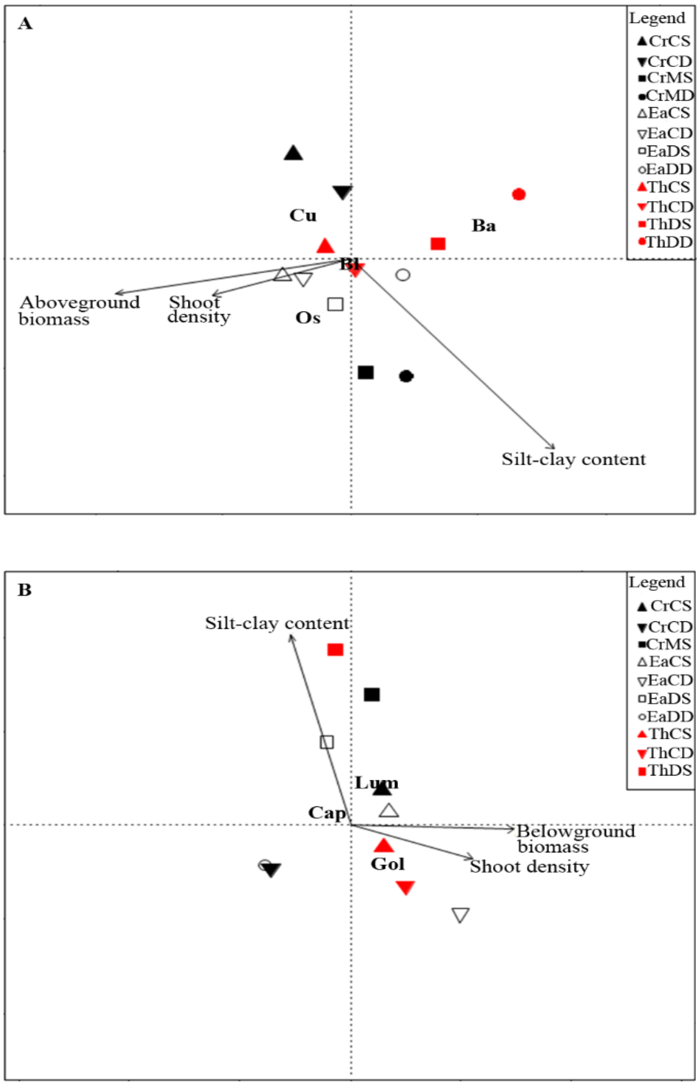

Fig. 2-4 Canonical correspondence analysis (CCA) ordination plots based on mean densities of each (A) epifaunal and (B) infaunal species in three vegetation types (Cr: *Cymodocea rotundata*, Ea: *Enhalus acoroides*, Th: *Thalassia hemprichii*) sampled in Capayas (C), Danlugan (D), and Mansabay (M) within Lopez Jaena municipality, Misamis Occidental Province, southern Philippines, during September (S) and December (D) 2010. CCA1, horizontal axis; CCA2, vertical axis. Environmental variables for epifauna were seagrass shoot density, aboveground seagrass biomass, and proportion of silt–clay; for infauna, they were seagrass shoot density, belowground seagrass biomass, and proportion of silt–clay. Plotted species are the four and three most abundant elements in the epifauna (Cu: *Canarium urceus*, Ba: *Bulla ampulla*, Bl: *Brissus latecarinatus*, Os: *Ophiactis savignyi*), and infauna (Lum: *Lumbrineris* sp., Cap: *Capitella* sp., Gol: *Golfingia* sp.), respectively. Other species are not included for graphical clarity. Sample code abbreviations are configured as follows: CrCS: *Cymodocea rotundata*-dominated vegetation at Capayas in September.

Table 2-1 Means and standard deviations (n=5) for seagrass vegetation structures measured in Lopez Jaena, Misamis Occidental, southern Philippines during September (S) and December (D) 2010 sample collection. nd = no data.

		Cymodocea rotundata		*Enhalus acoroides*		*Thalassia hemprichii*	
		S	D	S	D	S	D
Shoot density (shoot/0.0314m^2)	Capayas	62.0±8.9	31.6±10.8	19.4±7.8	31.2±11.9	36.6±14.3	46.2±20.7
	Danlugan	not used	not used	10.0±2.9	4.8±2.8	17.6±4.8	10.6±3.4
	Mansabay	53.2±9.1	59.4±18.1	not used	not used	not used	not used
Aboveground biomass (g dw/0.034m^2)	Capayas	6.1±12.6	3.7±1.6	12.8±3.6	10.7±2.0	6.7±2.1	6.2±2.5
	Danlugan	not used	not used	11.3±6.3	6.3±3.5	6.8±2.1	3.3±1.5
	Mansabay	4.4±2.6	2.4±0.6	not used	not used	not used	not used
Belowground biomass (g dw/0.034m^2)	Capayas	18.8±12.0	11.9±9.8	40.1±10.7	64.6±38.1	28.7±6.5	28.0±15.6
	Danlugan	not used	not used	30.4±14.9	23.0±27.4	15.5±8.0	8.9±3.7
	Mansabay	17.0±8.4	7.5±4.9	not used	not used	not used	not used
Sediment silt-clay content (%/0.034m^2)	Capayas	6.4±0.6	7.6±1.2	6.8±0.6	7.2±0.9	7.0±0.9	7.2±0.8
	Danlugan	not used	not used	18.9±1.1	17.1±2.4	18.8±1.0	18.6±1.0
	Mansabay	32.9±1.3	33.4±1.3	not used	not used	not used	not used

Table 2-2 Summary results of Generalized Linear Mixed Models (GLMMs) via Penalized Quasi-Likelihood (PQL) estimation for testing variation in seagrass vegetation structures among three vegetation types (Cr: *Cymodocea rotundata*, Th: *Thalassia hemprichii*, Ea: *Enhalus acoroides*), wherein seagrass is set as fixed effect, and site and month as crossed random effects under Poisson distribution. The AIC, Chi-square df, χ^2, and P values were obtained from testing the deviance using likelihood-ratio test to select the better model between null (reduced) and full model.

Response variable	AIC	χ^2 df	χ^2	P(> χ^2)	Post-hoc[a]
Shoot density	498.37	2.0	15.42	<0.001	Cr Th Ea
Aboveground biomass	313.14	2.0	34.09	<0.001	Ea Th Cr
Belowground biomass	523.07	2.0	22.85	<0.001	Ea Th Cr
Sediment silt-clay content	224.87	2.0	0.44	0.801	

[a] Vegetation types are arranged from highest to lowest. Non-significant pair is connected by an underline.

Table 2-3 Taxonomic list of benthic macrofauna found and identified in Lopez Jaena, Misamis Occidental, southern Philippines during the September and December 2010 sampling.

EPIFAUNA		INFAUNA	
Phylum/Family	Binomial name or species code	Phylum/Family	Binomial name or species code
Arthropoda		**Annelida**	
Alpheidae	*Alpheus* sp.	Capitellidae	*Capitella* sp.
Calappidae	Unknown box crab	Eunicidae	*Eunice* sp.
Callianassidae	Unknown ghost shrimp	Glyceridae	*Glycera alba*
Corystidae	*Corystes* sp.	Hesionidae	Unknown hesionid
Epialtidae	*Menaethius* sp. 1	Lumbrineridae	*Lumbrineris* sp.
	Menaethius sp. 2	Nereididae	*Nereis* sp.
Paguridae	Unknown hermit crab	Orbiniidae	Unknown orbiniid
Penaiedae	Unknown shrimp	Sphaerodoridae	Unknown sphaerodorid
Pilumnidae	*Pilumnus* sp.	Terebellidae	*Terebella* sp.
Portunidae	Unknown crab	**Mollusca**	
Echinodermata		Arcidae	*Anadara antiquata*
Archasteridae	*Archaster typicus*	Lucinidae	*Codakia tigerina*
Brissidae	*Brissus latecarinatus*	Pinnidae	*Pinna* sp.
Holothuriidae	*Holothuria* sp.	Veneridae	*Gafrarium dispar*
	Holothuria scabra	Sipuncula	
Ophiactidae	*Ophiactis savignyi*	Golfingiidae	*Golfingia* sp.
Oreasteridae	*Protoreaster nodosus*	Sipunculidae	*Sipunculus nudus*
Mollusca			
Angariidae	*Angaria delphinus*		
Buccinidae	*Pollia* sp.		
Bullidae	*Bulla ampulla*		
Cerithiidae	*Cerithium atratum*		
Columbellidae	*Euplica scripta*		
Conidae	*Conus eburneus*		
Cypraeidae	*Monetaria annulus*		
	Monetaria moneta		
Mitridae	*Mitra luctuosa*		
Nassariidae	*Nassarius limnaeformis*		
Family 1	Unknown nudibranch		
Strombidae	*Canarium* sp.		
	Canarium urceus		
Turridae	*Turris crispa*		

Table 2-4 AIC, difference in AIC ($AIC_i - AIC_{min}$), df and Akaike weight values of each candidate model as summary results of zero-inflated Generalized Linear Mixed Models (GLMMs) using negative binomial distribution for macrofaunal abundance and species richness accounting the effect of seagrass vegetation structures.

Response variable	Model	AIC	dAIC	df	weight
Epifaunal abundance	Null-model	234.7	0.0	4.0	0.996
	Full-model 1	246.0	11.3	6.0	0.004
	Full-model 2	251.6	16.9	10.0	<0.001
	Full-model 3	251.6	16.9	10.0	<0.001
	Full-model 4	255.6	20.9	12.0	<0.001
Epifaunal species richness	Null-model	187.7	0.0	4.0	0.861
	Full-model 1	191.4	3.7	6.0	0.133
	Full-model 2	199.1	11.4	10.0	0.003
	Full-model 3	199.1	11.4	10.0	0.003
Infaunal abundance	Null-model	156.6	0.0	4.0	0.851
	Full-model 1	160.3	3.8	6.0	0.129
	Full-model 2	167.0	8.3	10.0	0.014
	Full-model 3	164.8	10.4	10.0	0.005
	Full-model 4	168.8	12.3	12.0	0.002
Infaunal species richness	Null-model	134.1	0.0	4.0	0.547
	Full-model 1	134.6	0.5	6.0	0.423
	Full-model 2	142.2	6.8	10.0	0.019
	Full-model 3	140.9	8.1	10.0	0.010
	Full-model 4	144.9	10.8	12.0	0.003

Table 2-5 Results of permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distance matrix for multivariate species composition of benthic macrofaunal assemblage based on 9999 permutations.

Response variable	Source	df	SS	MS	F	R^2	P(>F)
Epifaunal abundance	Seagrass	2	0.581	0.291	1.528	0.200	0.062
	Site	2	0.906	0.453	2.382	0.311	0.001
	Month	1	0.282	0.282	1.482	0.097	0.111
	Residuals	6	1.141	0.190		0.392	
	Total	11	2.910			1.000	
Infaunal abundance	Seagrass	2	0.634	0.317	0.950	0.192	0.524
	Site	2	0.842	0.421	1.262	0.255	0.247
	Month	1	0.497	0.497	1.489	0.150	0.183
	Residuals	4	1.334	0.333		0.404	
	Total	9	3.306			1.000	

Table 2-6 Summary results of the Canonical Correspondence Analysis (CCA) in assessing the importance of seagrass vegetation structures to benthic epifauna- and infaunal assemblages. Response variables are average values of epifauna and infaunal abundance for each vegetation types, sites, and months (log x+1 transformed), while environmental variables include average values of shoot density, aboveground biomass, belowground biomass (all log-transformed), and surrounding sediment silt-clay content (arcsin-transformed).

CCA measures	Epifauna				Infauna			
	Eigenvalues	Variance (%)	F	P(>F)	Eigenvalues	Variance (%)	F	P(>F)
General								
Total	2.9	100			2.97	100		
Constrained	1.2	41.37			1.02	34.51		
Unconstrained	1.7	58.63			1.94	65.49		
Canonical axes								
CCA1	0.51	42.68	2.41	0.02	0.73	71.27	2.254	0.03
CCA2	0.35	29.43	1.66	0.13	0.18	17.12	0.541	0.75
CCA3	0.33	27.89	1.58	0.19	0.12	11.61	0.367	0.92
Residual	1.7				1.94			
Constraints								
Shoot density	0.37	31.07	1.75	0.11	0.29	28.07	0.884	0.64
Above-ground biomass	0.47	38.98	2.2	0.01	nd			
Below-ground biomass	nd				0.55	54.28	1.709	0.14
Silt-clay	0.36	29.95	1.69	0.13	0.18	18.04	0.57	0.85
Residual	1.2				1.94			

CHAPTER 3

Organic pollution alters the macrofaunal structure of seagrass beds in Bolinao, northwestern Philippines

Abstract

This study examined the impact of marine fish farming in Bolinao, northwestern Philippines on macrofauna in seagrass beds along a pollution gradient. I compared the diversity and abundance of macrofauna at four stations at different distances from fish farming areas. The Shannon diversity per station and macrofauna abundance decreased and increased, respectively, toward polluted stations, whereas there were inconsistent patterns of species richness per station and sample core. The species composition varied between polluted and clean stations, with the difference explained largely by the dominance of the filter-feeding bivalve, *Gafrarium pectinatum*, and opportunistic polychaete, *Capitella capitata,* at polluted stations. Species heterogeneity also decreased toward polluted stations. The results demonstrated that organic pollution alters the macrofaunal structure of seagrass beds. The species heterogeneity has important implication in alteration of ecosystem functioning, hence it should be considered in environmental impact assessments and in the management of coastal areas confronted with organic pollution.

3.1 Introduction

The worldwide decline in seagrass beds due to human-induced pollution, including pollution from marine fish farming, has been documented (Hemminga and Duarte 2000; Larkum et al. 2006; Waycott et al. 2009). Although most studies have focused on the effects of pollution on seagrasses, there have been few analyses of its effects on seagrass-associated fauna, including fish and marine benthic invertebrates (e.g., Kalantzi and Karakassis 2006; Watai et al. 2015; Katavic and Antolic 1999; Dimech et al. 2002; Eklöf et al. 2005; Apostolaki et al. 2007; Papageorgiou et al. 2009). Many marine sediment fauna are dependent on seagrass beds as a habitat as they provide protection from predators and more food than the adjacent bare sand (Orth et al. 1984; Hemminga and Duarte 2000). These fauna also have several important ecological roles, such as global carbon and geochemical cycling, secondary production, pollutant metabolism and burial, filtration, and sediment stability and transport (Snelgrove 1999; Lenihan and Micheli 2001; Nakaoka et al. 2014). Understanding the impacts of organic pollution on the diversity and abundance of the benthic faunal components of seagrass beds is an important step toward the effective conservation management of seagrass ecosystems that are at risk of deterioration due to pollution and other human effects.

A qualitative description of the possible effects of organic pollution on benthic dynamics was provided by Pearson and Rosenberg (1978), who reported that species richness falls, whereas biomass and abundance initially rise and then fall, as the pollution load increases. The dominance of opportunistic species in a pollution-stressed environment (i.e., primarily some species of polychaetes) has the potential to change the species composition of benthic communities (Warwick and Clarke 1994). Studies involving a predictive analysis have highlighted the role of hypoxia in the alteration of benthic structures (e.g., Diaz and Rosenberg 1995; Heip 1995; Nilsson and Rosenberg 2000; Rosenberg 2001). Gray et al. (2002) suggested that, among the various marine fauna, fish are the most sensitive to hypoxia, whereas the least sensitive were molluscs, followed by polychaetes. Many benthic species react to a pollution-stressed environment with various behavioral responses before they eventually die (Diaz and

Rosenberg 1995), and this leads to a wide variation in assemblage structures along the pollution gradient.

Univariate measures, such as the number of species, abundance, and biomass, and multivariate measures, such as species composition and similarities, are often used to describe the population 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 more 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 can vary 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.

One of the best ways to understand the effects of organic pollution on seagrass fauna is to investigate seagrass beds along a pollution gradient. This is also applicable in the case of Bolinao in the northwestern Philippines, in which part of the 22,500 ha of extensive seagrass beds lies along a gradient of dissolved nutrients (nitrogen, silicate, phosphate) and chlorophyll-a concentrations (Fortes et al. 2012). The pollution gradients are the result of the extensive and intensive mariculture of milkfish (*Chanos chanos*) that started in 1995 and have been aggravated by the south to north siltation gradient from the Alaminos River (Le Jeune 1995). The intensification of fish farming activities in this area has led to an increase in the nutrient flux from the unconsumed feed items and fecal matter that accumulates in the sediment. As a result, the surrounding water can become eutrophic (San Diego-McGlone et al. 2008), and the sediment quality deteriorates (Nacorda et al. 2012). The status of seagrasses in Bolinao has changed markedly due to mariculture, that is, a reduction in the species richness in polluted beds (Fortes et al. 2012) and the smothering of some seagrass species at mariculture sites

(Tanaka et al. 2014). I expected that pollution from mariculture would also have an impact on seagrass macrofauna.

The aim of this study was to investigate the community structure of macrofauna in seagrass beds along a pollution gradient in Bolinao. I expected that species diversity and abundance of macrofauna would be suppressed toward the polluted areas. I also hypothesized that the community assemblage would vary along the pollution gradient. The likely dominance of some opportunistic species and species tolerant to pollution stress was also expected in the more polluted areas. Finally, I hypothesized that organic pollution would reduce species heterogeneity (i.e., the variation in species composition among samples within a given area).

3.2 Materials and methods

3.2.1 Study area

The study was conducted in seagrass beds at Bolinao in northwestern Philippines (Fig. 3-1) that are located along a gradient of dissolved nutrients and chlorophyll-a concentration (Table 3-1, Fortes et al. 2012). This area has the most extensive reef system in the northwestern part of the country (Mcmannus et al. 1992), with 22,500 ha of seagrass coverage (Fortes et al. 2012). The seagrass beds are located along the reef flat on the eastern side of Santiago Island, the largest island of Bolinao, which is separated from the mainland by a deep channel. The area has a wind-driven circulation that creates a clockwise circulation pattern (southward flow) mediated by an abrupt bathymetric change, irregular coastal geometry, and the prevailing wind direction (north-northwest; NNW) (Rivera 1997). Its tide-driven circulation also moves southward during flood currents (<10 cm/s) and northward during ebb tides (≈10 cm/s). The water movement is dominated by a semidiurnal tide, with current magnitudes of >0.2 m/s during a peak flood or ebb flow in the narrow passages (San Diego-McGlone et al. 2008). Due to mariculture in this area, the sediment and water quality has deteriorated (Holmer et al. 2002; Holmer et al. 2003; San Diego-McGlone et al. 2008; Santander et al. 2008; David et al. 2009; Nacorda et al. 2012), resulting in harmful algal blooms (HABs) and fish kills (Yap et al. 2004;

Azanza et al. 2005; Escobar et al. 2013). Mariculture has also led to a reduction in δ^{13}C values and the growth rate of seagrass fish (Watai et al. 2015).

Four stations were established in the study area (Fig. 3-1) along the subtidal zone, with a sampling depth of 0.5 m–1.0 m (near the low tide water mark). All of these stations were located in the eastern reef flat of Santiago Island. Station 1 was closest to the source of pollution, <1 km from the mariculture areas. Station 2 was <2 km northeast of station 1, and station 3 was <4 km north of station 2. Station 4 was <4 km northwest of station 3 and was located within the Bolinao seagrass reserve. Due to their proximity to the mariculture areas, we considered stations 1 and 2 to be polluted stations, whereas stations 3 and 4 were regarded as clean or pristine stations. A 15-month seagrass monitoring program (September 2010–December 2011) conducted by Fortes et al. (2012) at the same stations found that the seagrass species richness from two species (*Enhalus acoroides*, *Thalassia hemprichii*) increased at station 1 to seven species (*E*. *acoroides*, *T*. *hemprichii*, *Halophila ovalis*, *Cymodocea rotundata*, *Cymodocea serrulata*, *Halodule uninervis*, *Syringodium isoetifolium*) at station 4. They also observed that, the most dominant and common seagrass species (*E. acoroides* and *T. hemprichii*) at station 1 had a smaller belowground biomass compared with their aboveground biomass. Investing in shoots is a typical strategy of plants exposed to reduced light conditions. Tanaka et al. (2014) compared the status of seagrasses before and after the mariculture activities in Bolinao and found that *E. acoroides* and *C. serrulata* disappeared in St. Barbara, on the south coast, where both siltation and nutrient loads are heavy.

A recent snapshot of the variation in the environmental parameters from stations 1 to 4 showed that the levels of dissolved nutrients, nitrogen, phosphate, and silicate as well as the chlorophyll-a concentration increased toward the polluted stations (Table 3-1, Fortes et al. 2012). The silt-clay component of the sediment at the stations was measured by Terrados et al. (1998) before mariculture was fully established and was found to be 6.7-9.8%. This was much lower than in the south of the channel between Santiago Island and the mainland (27.4%) (Fig. 3-1).

3.2.2 Sample collection and laboratory work

Macrofauna were collected by sediment coring to a depth of 10 cm. The aluminum corer had an inner diameter of 20 cm, resulting in a surface area of 0.03 m^2 per core sample. The samples were haphazardly sampled within a ca. 50-m diameter of seagrass 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 sampling was conducted twice; 10 replicated samples were collected at each site in September 2010, and an additional 5 samples were collected in March 2011.

The collected samples were placed into pre-labelled 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. All macrofaunal individuals were sorted to the lowest taxonomic level, where possible, using taxonomic references for polychaetes (Fauchald 1977; Fauchald and Jumars 1979), molluscs (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.

3.2.3 Data analyses

I conducted univariate and multivariate analyses on my data set. The data from two sampling periods were pooled, resulting in 15 replicated samples per station, because a preliminary analysis did not show variation between sampling periods. All univariate analyses were conducted in R version 3.1.2 (R Core Team 2014), whereas most 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.

3.2.3.1 Univariate analyses

Macrofaunal species diversity values were obtained using species richness which is known to be sensitive to rare species, and the Shannon diversity that does not disproportionately favor rare and common species. Rather than using the conventional Shannon Diversity Index, I employed an equivalent statistic that was converted into a true diversity value (Jost 2006, 2007) using the *vegetarian* package (Charney and Record 2012). This measure refers to true diversity values or effective numbers of species based on the Shannon Diversity Index, which weights species frequencies (Jost 2006, 2007). The resulting values are then directly comparable to values of species richness, which are already true diversity values by default. Both the species richness and Shannon diversity were calculated for each station and core level and are hereafter referred to as species richness per station, Shannon diversity per station, species richness per core, and Shannon diversity per core, respectively.

The formulas for obtaining the Shannon diversity for each station and core are shown in equations 1 and 2, respectively, (slightly modified from equations 17b and 11b in Jost 2007):

Equation 1

$$D_{\gamma} = \exp\left[\sum_{i=1}^{S} -(W_1 P_{i1} + W_2 P_{i2} + \cdots) \text{ x } \ln(W_1 P_{i1} + W_2 P_{i2} + \cdots)\right]$$

Equation 2

$$D_{\alpha} = \exp\left[-W_1 \sum_{i=1}^{S} (P_{i1} ln P_{i1}) + \ -W_2 \sum_{i=1}^{S} (P_{i2} ln P_{i2}) + \cdots\right],$$

where, *D* is ‘diversity’, γ is gamma (station level), α is alpha (core level), W_i is the statistical weightings of the communities or samples *i*, *S* is the number of species, and P_i is the proportion of species *i* relative to the total number of species.

To statistically test if species diversity measures at core level differed between stations, I used a one-way analysis of variance (ANOVA) in the *stats* package (R Core Team 2014). The Shapiro–Wilk test was performed through *stats* to initially check the normality of the distribution and Levene’s test was performed via the *car* package (Fox and Weisberg 2011) to determine the homogeneity of the variance before performing the ANOVA. I used a Tukey's honest significant difference (HSD) test in the *car* package for multiple pairwise comparisons when the effect of the station was found to be significant at the 95% confidence level. The species diversity measures at station level were not statistically tested because they represented only one value per station.

The macrofaunal abundance data, which contained the total number of individuals per core (0.03 m^2), were log-transformed after adding a constant value of 1 (Underwood 1997) to achieve a normal distribution and variance homogeneity before conducting the ANOVA. I also used multiple pairwise comparison when a station was found to have a significant effect on abundance.

3.2.3.2 Multivariate analyses

To determine if between-station variation explained a significant portion of the total variation in the macrofaunal community, I conducted a permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) for the presence–absence data of all macrofaunal species from the Jaccard resemblance index. I applied pairwise tests when a station was found to be significant and obtained the P-values from Monte-Carlo tests. PERMANOVA is sensitive to dispersion in the data set (Anderson 2001) only when the data had an unbalanced design (Anderson and Walsh 2013), which was not the case for my data set. Using the same data set and resemblance index, I also conducted a canonical analysis of principal coordinates (CAP; Anderson and Willis 2003) to visualize the distinctiveness of the stations in multivariate space (Anderson et al. 2008). To determine which macrofaunal species explained the variation in species composition between stations, the abundance data for each of the species per station pairs (e.g., stations 1 and 2) were entered into univariate negative binomial regressions (Warton et al. 2012) using the *mvabund* package of R (Wang et al. 2014). The resulting likelihood ratio (LR) statistic is a measure of the strength of between-group effects. To determine the percentage of the total effect due to a species, I divided the species LR statistic (deviance) by the sum of the LR or total deviance of the multivariate data (personal communication, D. Warton).

To analyze the species heterogeneity pattern between stations, I performed the permutation of multivariate dispersion (PERMDISP; Anderson 2006; Anderson et al. 2006) on the same presence–absence data and resemblance index. I also applied pairwise tests when a station was found to be significant, and the P-values were estimated using the Monte-Carlo simulation. The use of presence–absence data in this study is ideal for straightforward interpretation. A multivariate dispersion pattern based on a binary data set has ecological implications that differ from those of an analysis incorporating abundance information (Anderson et al. 2011).

3.3 Results

A total of 86 species and 975 individual macrofauna were recorded from 60 core samples (0.03 m^2 per core) taken from the four stations at Bolinao, northwestern Philippines (Appendix 3-1). The communities were classified into 7 phyla, 11 classes, ca. 27 orders, and 53 families of macrobenthic invertebrates. The phylum Mollusca contained most species, followed by Arthropoda and Annelida. The phylum Mollusca also had the highest value in terms of abundance, followed by Annelida and Arthropoda. The most abundant and frequently-occurring species, which was found in all four stations, was the polychaete worm, *Capitella capitata*. This species represented 39% of the total number of individuals, and 90% of its total abundance was recorded from stations 1 and 2. In contrast, the bivalve, *Gafrarium pectinatum*, was the only species present at one station (station 1), where it had a high abundance (27 individuals).

3.3.1 Univariate community structure

The species richness per station ranged from 33 to 50 species, with the lowest and highest values recorded at stations 1 and 2, respectively (Fig. 3-2A). In contrast, the Shannon diversity per station ranged from 9 to 27, with values increasing from station 1 to station 4 (Fig. 3-2B).

The species richness per core (0.03 m^2) yielded mean values ranging from four to seven species (Fig. 3-2C) and differed significantly across stations (Table 3-2). The results of pairwise comparisons revealed that the variation was significant between stations 2 and 4. Furthermore, the Shannon diversity per core had mean values ranging from 3 to 5 (Fig. 3-2D) and varied significantly between stations 1 and 3 (Table 3-2).

The mean abundance of macrofauna ranged from 6 to 25 individuals per core (0.03 m^2), with the maximum at station 1 and a decreasing trend toward station 4 (Fig. 3-3). The variation was significant across stations (Table 3-2). The results of Tukey-HSD tests showed that the values for stations 1 to 3 were significantly higher than that for station 4 (Fig. 3-3).

3.3.2 Multivariate community structure

The PERMANOVA results showed that station had a significant effect on between-station variations in species composition (Table 3-3). The variation was significant for all station comparisons except that between stations 2 and 3 and between stations 3 and 4; this was confirmed by the pattern found in the multivariate space by the CAP (Fig. 3-4).

The results of univariate tests to identify the most influential macrofaunal species showed that the variation of species composition across some stations was explained by the dominance of several species (Table 3-4). *Capitella capitata* made a significant contribution to the variation between all pairs of stations except that between stations 1 and 2, where *G. pectinatum* solely explained the observed variation. This bivalve mollusc species generally made the greatest and most consistent contribution to the variation in species composition, except that between stations 2 and 4. The other influential species (i.e., *Tectus* sp. and *Nassarius elegantissimus*) were also molluscs.

Finally, the multivariate dispersion also significantly varied across stations (PERMDISP: $F = 4.99$, $df1 = 3$, $df2 = 56$, $P = 0.004$). The results of pairwise tests showed that stations 1 to 3 had significantly lower values (in terms of distance from the centroid) compared with station 4 (Fig. 3-5).

3.4 Discussion

This study investigated the influence of organic pollution on the associated macrofauna in seagrass beds in Bolinao, northwestern Philippines. I found a reduction in the Shannon diversity per station, an increase in macrofauna abundance, changes in species composition between polluted and clean stations, and a reduction in species heterogeneity along a mariculture-induced pollution gradient.

This study recorded a total of 86 species and 975 individuals. Both values are much lower than those reported in previous studies of seagrass macrofauna and pollution (e.g., Apostolaki et al. 2007), but they cannot be compared directly due to differences in the regions studied, the spatial scale sampled, and methodological approaches. The scale of the sampling in Eklöf et al. (2005) was slightly smaller than that in my study (30 x 30-m area, 12-cm diameter core, same depth; i.e., 10 cm), but a higher abundance of macrofauna was reported. The most abundant phylum in my study was Mollusca, whereas Arthropoda often dominate in other studies (e.g., Eklöf et al. 2005; Apostolaki et al. 2007).

The Shannon diversity per station clearly decreased toward the polluted stations. Although no statistical inference was made regarding this observation, the clear pattern indicates the negative impact of organic pollution on species diversity at the station level. However, other diversity measures, (i.e., species richness per station and core and Shannon diversity per core) did not display a clear pattern, which may be the result of an increase in productivity in the affected seagrass beds, leading to a localized increase in the species richness of the associated macroinvertebrates (Dimech et al. 2002). The inconsistent pattern could also be influenced by the varying life strategies of the benthic animals (Gray 1979; Gray 1992). A qualitative model of the responses of benthos to organic enrichment (Pearson and Rosenberg 1978) also showed this non-linear pattern of species richness (along with abundance and biomass), which tends to peak in the intermediate stage of organic pollution and then drops as pollution escalates. 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 lack of detection in that study could be due to the limited analysis of the data, little variation in the pollution gradient across sites, or unobserved biological traits of the constituent species.

The increase in macrofauna abundance toward polluted stations did not conform to my expectation. The reduction in the abundance of associated fauna is an expected indirect effect of any alteration of the habitat function of seagrass beds (e.g., predator refugia, food availability) by organic pollution (Eklöf et al. 2005). However, my study found the opposite pattern. The increase in the abundance of macrofauna toward the polluted stations may be because more food was available in stations affected by pollution, which could be beneficial for some species. This could also be part of the benthic abundance pattern along a pollution gradient (i.e., abundance increases as pollution escalates until the entire faunal population eventually disappears in the azoic sediments) (Pearson and Rosenberg 1978; Warwick 1986; 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. Inside the pen of a fish farm, in the bare sediments of a lagoon impacted by organic pollution, Soares et al. (2004) reported an 86% drop in macrobenthic faunal abundance after just 21 days of culture. Eklöf et al. (2005) also reported a lower macrofaunal abundance in seagrass beds underneath seaweed farms compared with in unfarmed seagrass beds. However, Apostolaki et al. (2007) found little variation in the macrofaunal abundance between control and highly impacted areas in *P. oceanica* meadows. The increase in macrobenthic abundance toward the source of pollution in my study (stations 1 and 2) indicates that the polluted stations were not severely damaged.

A difference in species composition was generally observed between the polluted and clean stations. A shift in species composition with pollution has been documented in several previous studies (e.g., Ryu et al. 2011 and references therein), although not specifically on seagrass beds. Such a change in species composition could be influenced by the dominance of specific phyla tolerant to stress, such as the polychaetes (Warwick and Clarke 1994). Differences in the tolerance or behavioral responses of many macrobenthic species to organic pollution is also 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; although see Heip 1995).

The variation in species composition between some stations was explained largely by the molluscs, particularly *G. pectinatum*, and then by the opportunistic polychaete, *C. capitata*. Among benthic taxa, the molluscs are the least sensitive to hypoxia, followed by polychaetes, then crustaceans and echinoderms (Gray et al. 2002). This could explain why mollusc species, with the exception of *Tectus* sp., whose abundance was actually highest in station 3, were more dominant in polluted areas. The enhanced primary productivity in the polluted stations may have been beneficial to benthic grazers, whereas increased sedimentation may benefit some filter-feeding and deposit-feeding macrobenthos (Grall and Chauvaud 2002; but see Heip 1995). *Capitella capitata* is well known as a dominant pioneering species in stressed areas (Tsutsumi 1987; Tsutsumi et al. 1991; Karakassis et al. 2000); therefore, its dominance in polluted stations was not surprising. 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 dominance of *C*. *capitata* in my study is consistent with its known potential as an indicator of environmental pollution (Gray et al. 1979; Tsutsumi et al. 1991).

Finally, the finding that the distance from the centroid (multivariate dispersion) was significantly lower in stations 1 to 3 than in station 4 indicates that the species heterogeneity of macrofauna in seagrass beds was negatively impacted by organic pollution. This conformed to my expectation, but this issue has been given little attention in previous studies. 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 the observation. The suppressed species heterogeneity suggests a reduction in species turnover (Heip 1995; Papageorgiou et al. 2009). The dominance of certain opportunistic species in a pollution-stressed environment generally results in the homogenization of the community (Warwick 1986). However, the mechanisms driving the pattern of species heterogeneity along an environmental gradient are still poorly understood. However, the role of the dominant species in the reduction 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 *G. pectinatum*, which belongs to a molluscan taxa that is generally considered to be very tolerant to less oxygenated environments (Gray et al. 2002).

In conclusion, environmental degradation due to mariculture activities macrofauna in seagrass beds of Bolinao, northwestern Philippines, altered the community structures of associated macrofauna through negative impacting its diversity and enhancing its abundance. The most significant finding of this research is the reduction of species heterogeneity due to organic pollution. The homogenization of species composition (suppression of β-diversity) has a serious negative impact because reduction in species complexity results to alteration in ecosystem functioning (Gamfeldt et al. 2014b). This study advances our understanding of how local organic pollution affects macrobenthic animals in the seagrass ecosystem. Aspects of species heterogeneity should be considered one of the major response variables in environmental impact assessments and should be considered in the management of coastal areas confronted with organic pollution, such as that produced by mariculture activities.

3.5 Acknowledgements

I thank J. Leriorato, M. Watai, N. Whanpetch, T. Genovia, M. Sarceda, A. Lampad and F. Pacencia for field and lab assistance. Special thanks also to K. Sudo for making the study map, and to Prof. D. Warton for the statistical advices regarding implementation of *mvabund* package. This study was supported by Science and Technology Research Partnership for Sustainable Development program (SATREPS) co-funded by Japan Science and Technology (JST) and Japan International Cooperation Agency (JICA), for the project “Coastal Ecosystem Conservation and Adaptive Management under Local and Global Environmental Impacts in the Philippines (CECAM)”.

3.6 References

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust Ecol 26:32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x.

Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62:245–253. doi: 10.1111/j.1541-0420.2005.00440.x.

Anderson MJ, Crist TO, Chase JM, et al (2011) Navigating the multiple meanings of β diversity: A roadmap for the practicing ecologist. Ecol Lett 14:19–28. doi: 10.1111/j.1461-0248.2010.01552.x.

Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. Ecol Lett 9:683–693. doi: 10.1111/j.1461-0248.2006.00926.x.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: Guide to Software and Statistical Methods. 199 pp.

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecol Monogr 83:557–574.

Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. Ecology 84:511–525.

Apostolaki ET, Tsagaraki T, Tsapakis M, Karakassis I (2007) Fish farming impact on sediments and macrofauna associated with seagrass meadows in the Mediterranean. Estuar Coast Shelf Sci 75:408–416. doi: 10.1016/j.ecss.2007.05.024.

Azanza R V., Fukuyo Y, Yap LG, Takayama H (2005) *Prorocentrum minimum* bloom and its possible link to a massive fish kill in Bolinao, Pangasinan, Northern Philippines. Harmful Algae 4:519–524. doi: 10.1016/j.hal.2004.08.006.

Becking LE, Cleary DFR, Voogd NJ, et al (2006) Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Mar Ecol 27:76–88. doi: 10.1111/j.1439-0485.2005.00051.x.

Botter-Carvalho ML, Carvalho PVVC, Valença APMC, Santos PJP (2014) Estuarine macrofauna responses to continuous in situ nutrient addition on a tropical mudflat. Mar Pollut Bull 83:214–223. doi: 10.1016/j.marpolbul.2014.03.049.

Brooks KM, Stierns AR, Mahnken CVW, Blackburn DB (2003) Chemical and biological remediation of the benthos near Atlantic salmon farms. Aquaculture 219:355–377. doi: 10.1016/S0044-8486(02)00528-8.

Charney N, Record S (2012) vegetarian: Jost Diversity Measures for Community Data.

David CPC, Maria YYS, Siringan FP, et al (2009) Coastal pollution due to increasing nutrient flux in aquaculture sites. Environ Geol 58:447–454. doi: 10.1007/s00254-008-1516-5.

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia : A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol an Annu Rev 33:245–303.

Dimech M, Borg JA, Schembri PJ (2002) Changes in the structure of a *Posidonia oceanica* meadow and in the diversity of associated decapod , mollusc and echinoderm assemblages , resulting from inputs of waste from a marine fish farm (Malta, Central Mediterranean). Bull Mar Sci 71:1309–1321.

Eklöf JS, De La Torre Castro M, Adelsköld L, et al (2005) Differences in macrofaunal and seagrass assemblages in seagrass beds with and without seaweed farms. Estuar Coast Shelf Sci 63:385–396. doi: 10.1016/j.ecss.2004.11.014.

Ellingsen KE (2001) Biodiversity of a continental shelf soft-sediment macrobenthos community. Mar Ecol Prog Ser 218:1–15. doi: 10.3354/meps218001.

Ellingsen KE (2002) Soft-sediment benthic biodiversity on the continental shelf in relation to environmental variability. Mar Ecol Prog Ser 232:15–27. doi: 10.3354/meps232015.

Ellingsen KE, Gray JS (2002) Spatial patterns of benthic diversity : Is there a latitudinal gradient along the Norwegian continental shelf ? J Anim Ecol 71:373–389.

Escobar MTL, Sotto LPA, Jacinto GS, et al (2013) Eutrophic conditions during the 2010 fish kill in Bolinao and Anda, Pangasinan, Philippines. J Environ Sci Manag 35:29–35.

Fauchald K (1977) The polychaete worms: Definitions and keys to the orders, families and genera.

Fauchald K, Jumars PA (1979) The diet of worms: a study of polychaete feeding guilds. Oceanogr Mar Biol 17:193–284.

Fortes MD, Go GA, Bolisay K, et al (2012) Seagrass response to mariculture-induced physico-chemical gradients in Bolinao, northwestern Philippines. Procedings 12th Int Coral Reef Symp 9–13 Sept.

Fox J, Weisberg S (2011) An {R} Companion to Applied Regression.

Gamfeldt L, Lefcheck JS, Byrnes JEK, et al (2014) Marine biodiversity and ecosystem functioning: what’s known and what's next? Oikos 000:001–014. doi: 10.1111/oik.01549.

Grall J, Chauvaud L (2002) Marine eutrophication and benthos: The need for new approaches and concepts. Glob Chang Biol 8:813–830. doi: 10.1046/j.1365-2486.2002.00519.x

Gray JS (1979) Pollution-induced changes in populations. Philos Trans R Soc London 286:545–561. doi: 10.1098/rstb.1979.0045.

Gray JS (1992) Eutrophication in the sea. In: Colombo G, Ferrari I, Ceccherelli VU, Rossi R (eds) Marine Eutrophication and Population Dynamics. Olsen and Olsen, Fredensborg, pp 3–15.

Gray JS, Wu RS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. Mar Ecol Prog Ser 238:249–279.

Heck Jr. KL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. Mar Ecol Prog Ser 253:123–136.

Heip C (1995) Eutrophication and zoobenthos dynamics. Ophelia 41:113–136.

Hemminga M, Duarte CM (2000) Seagrass Ecology. Cambridge University Press, Cambridge

Hinton A (1975) Shells of New Guinea and the Central Indo-Pacific. Charles E. Turtle Corporation, Incorporated, Ruthland, Vermont.

Holmer M, Duarte CM, Heilskov A, et al (2003) Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar Pollut Bull 46:1470–1479. doi: 10.1016/S0025-326X(03)00281-9.

Holmer M, Marbá N, Terrados J, et al (2002) Impacts of milkfish (*Chanos chanos)* aquaculture on carbon and nutrient fluxes in the Bolinao area, Philippines. Mar Pollut Bull 44:685–696. doi: 10.1016/S0025-326X(02)00048-6.

Jost L (2006) Entropy and diversity. Oikos 113:363–375. doi: 10.1111/j.2006.0030-1299.14714.x.

Jost L (2007) Partitioning diversity into independent alpha and beta components. Ecology 88:2427–2439.

Kalantzi I, Karakassis I (2006) Benthic impacts of fish farming: Meta-analysis of community and geochemical data. Mar Pollut Bull 52:484–493. doi: 10.1016/j.marpolbul.2005.09.034.

Karakassis I, Tsapakis M, Hatziyanni E, et al (2000) Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES J Mar Sci 57:1462–1471. doi: 10.1006/jmsc.2000.0925.

Larkum WD, Orth RJ, Duarte CM (eds) (2006) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht (The Netherlands).

Le Jeune EL (1995) Causes of siltation in the Santiago Island reef system, Philippines. no. 030/95.

Lenihan HS, Micheli F (2001) Soft-sediment communities. In: Bertness, M. D. Gaines, S. D. Hay ME (ed) Marine community ecology. Sinauer Associates, Inc., Sunderland, pp 253–287.

Lohrer AM, Rodil IF, Townsend M, et al (2013) Biogenic habitat transitions influence facilitation in a marine soft-sediment ecosystem. Ecology 94:136–145. doi: 10.1890/11-1779.1.

Mcmannus JW, Nañola CL, Reyes RB, Kesner KN (1992) Resource ecology of the Bolinao coral reef system. 117.

Nacorda HME, Obliosca JM, Tentia MCL, et al (2012) Deterioration of Soft Bottom Macroinfaunal Communities in a Milkfish Mariculture Zone off Bolinao-Anda , Pangasinan (NW Philippines). In: Kawaguchi M, Misaki K, Sato H, et al (eds) Interdisciplinary Studies on Environmental Chemistry—Environmental Pollution and Ecotoxicology. TERRAPUB, pp 387–395.

Nakaoka M (2005) Plant-animal interactions in seagrass beds: Ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177. doi: 10.1007/s10144-005-0226-z.

Nakaoka M, Lee K-S, Huang X, et al (2014) Regional Comparison of the Ecosystem Services from Seagrass Beds in Asia. In: Nakano S, Yahara T, Nakashizuka T (eds) Integrative Observations and Assessments. Springer, pp 367–393.

Nilsson HC, Rosenberg R (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: Analysed by sediment profile-imaging and by grab samples. Mar Ecol Prog Ser 197:139–149. doi: 10.3354/meps197139.

Okuda T, Noda T, Yamamoto T, et al (2004) Latitudinal gradient of species diversity: Multi-scale variability in rocky intertidal sessile assemblages along the Northwestern Pacific coast. Popul Ecol 46:159–170. doi: 10.1007/s10144-004-0185-9.

Okutani T (ed) (2000) Marine Mollusks in Japan. University of Tokyo Press, Tokyo.

Oliver JS, Slattery PN (1985) Destruction and opportunity on the sea floor: Effects of gray whale feeding. Ecology 66:1965–1975.

Orth RJ, Heck KL, van Montfrans J (1984) Faunal communities in seagrass beds: A review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350. doi: 10.2307/1351618.

Papageorgiou N, Sigala K, Karakassis I (2009) Changes of macrofaunal functional composition at sedimentary habitats in the vicinity of fish farms. Estuar Coast Shelf Sci 83:561–568. doi: 10.1016/j.ecss.2009.05.002.

Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Annu. Rev. 16:229–311.

R Core Team (2014) R: A language and environment for statistical computing.

Rivera PC (1997) Hydrodynamics, sediment transport and light extinction off Cape Bolinao, Philippines. Wageningen Agricultural University.

Rosenberg R (2001) Marine benthic faunal successional stages and related sedimentary activity. Sci Mar 65:107–119.

Ryu J, Khim JS, Kang SG, et al (2011) The impact of heavy metal pollution gradients in sediments on benthic macrofauna at population and community levels. Environ Pollut 159:2622–2629. doi: 10.1016/j.envpol.2011.05.034.

San Diego-McGlone ML, Azanza R V., Villanoy CL, Jacinto GS (2008) Eutrophic waters, algal bloom and fish kill in fish farming areas in Bolinao, Pangasinan, Philippines. Mar Pollut Bull 57:295–301. doi: 10.1016/j.marpolbul.2008.03.028.

Santander SMS, San Diego-McGlone ML, Reichardt W (2008) Indicators of diminished organic matter degradation potential of polychaete burrows in Philippine mariculture areas. Philipp Agric Sci 91:295–300.

Schoppe S (2000) A guide to common shallow water sea stars, brittle stars, sea urchins, sea cucumbers, and feather stars (Echinoderms) of the Philippines. Times Media Private Limited, Singapore.

Simboura N, Zenetos A, Panayotidis P, Makra A (1995) Changes in benthic community structure along an environmental pollution gradient. Mar Pollut Bull 30:470–474. doi: 10.1016/0025-326X(95)00237-H.

Snelgrove PVR (1999) Getting to the bottom of marine biodiversity : Sedimentary habitats. Bioscience 49:129–138. doi: 10.2307/1313538.

Soares R, Peixoto S, Bemvenuti C, et al (2004) Composition and abundance of invertebrate benthic fauna in *Farfantepenaeus paulensis* culture pens (Patos Lagoon estuary, Southern Brazil). Aquaculture 239:199–215. doi: 10.1016/j.aquaculture.2004.05.041.

Tanaka Y, Go GA, Watanabe A, et al (2014) 17-year change in species composition of mixed seagrass beds around Santiago Island, Bolinao, the northwestern Philippines. Mar Pollut Bull 88:81–85. doi: 10.1016/j.marpolbul.2014.09.024.

Terrados J, Duarte CM, Fortes MD, et al (1998) Changes in community structure and biomass of seagrass communities along gradients of siltation in SE Asia. Estuar Coast Shelf Sci 46:757–768. doi: 10.1006/ecss.1997.0304.

Tsutsumi H (1987) Population dynamics of *Capitella capitata (*Polychaeta; Capitellidae) in an organically polluted cove. Mar Ecol Prog Ser 36:139–149. doi: 10.3354/meps036139.

Tsutsumi H, Kikuchi T, Tanaka M, et al (1991) Benthic faunal succession in a cove organically polluted by fish farming. Mar Pollut Bull 23:233–238. doi: 10.1016/0025-326X(91)90680-Q.

Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge

Wang Y, Naumann Y, Stephen W, Warton D (2014) mvabund: Statistical Methods for Analysing Multivariate Abundance Data.

Warton DI, Wright ST, Wang Y (2012) Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol Evol 3:89–101. doi: 10.1111/j.2041-210X.2011.00127.x.

Warwick RM (1986) A new method for detecting pollution effects on marine macrobenthic communities. Mar Biol 92:557–562. doi: 10.1007/BF00392515.

Warwick RM, Clarke KR (1994) Relearning the ABC: Taxonomic changes and abundance/biomass relationships in disturbed benthic communities. Mar Biol 118:739–744. doi: 10.1007/BF00347523.

Warwick RM, Pearson TH, Ruswahyuni (1987) Detection of pollution effects on marine macrobenthos: further evaluation of the species abundance/biomass method. Mar Biol 95:193–200. doi: 10.1007/BF00409005.

Watai M, Nakamura Y, Honda K, et al (2015) Diet, growth, and abundance of two seagrass bed fishes along a pollution gradient caused by milkfish farming in Bolinao, northwestern Philippines. Fish Sci 81:43–51. doi: 10.1007/s12562-014-0824-9.

Waycott M, Duarte CM, Carruthers TJB, et al (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci U S A 106:12377–12381. doi: 10.1073/pnas.0905620106.

WoRMS Editorial Board (2015) World Register of Marine Species. In: VLIZ. http: Accessed 31 Mar 2015.

Yap LG, Azanza R V., Talaue-McManus L (2004) The community composition and production of phytoplankton in fish pens of Cape Bolinao, Pangasinan: A field study. Mar Pollut Bull 49:819–832. doi: 10.1016/j.marpolbul.2004.06.030.

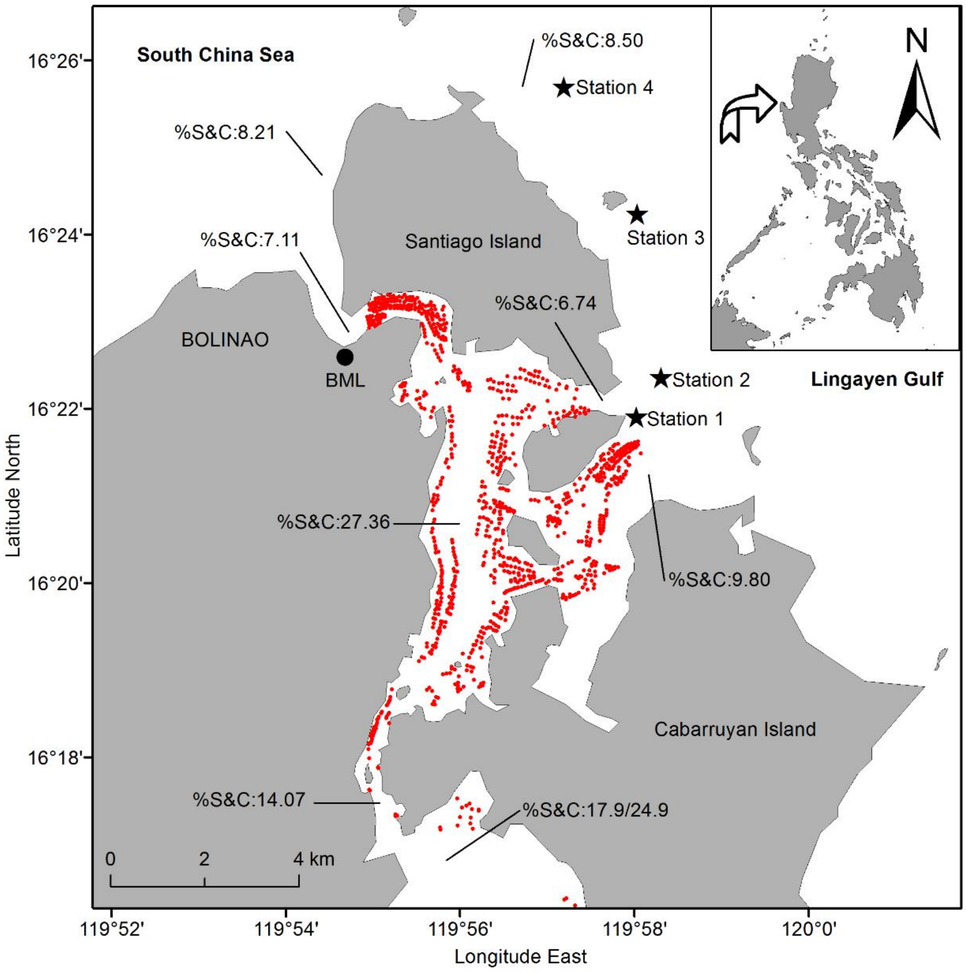

Fig. 3-1 Map of the study area showing the locations of the four sampling stations in Bolinao, northwestern Philippines. The mariculture structures (fish pens and cages) in red dots were determined from a satellite image (Google Earth, checked on June 23, 2015) using Arc-GIS. Values of the silt-clay content of the sediment were based on Terrados et al. (1998).

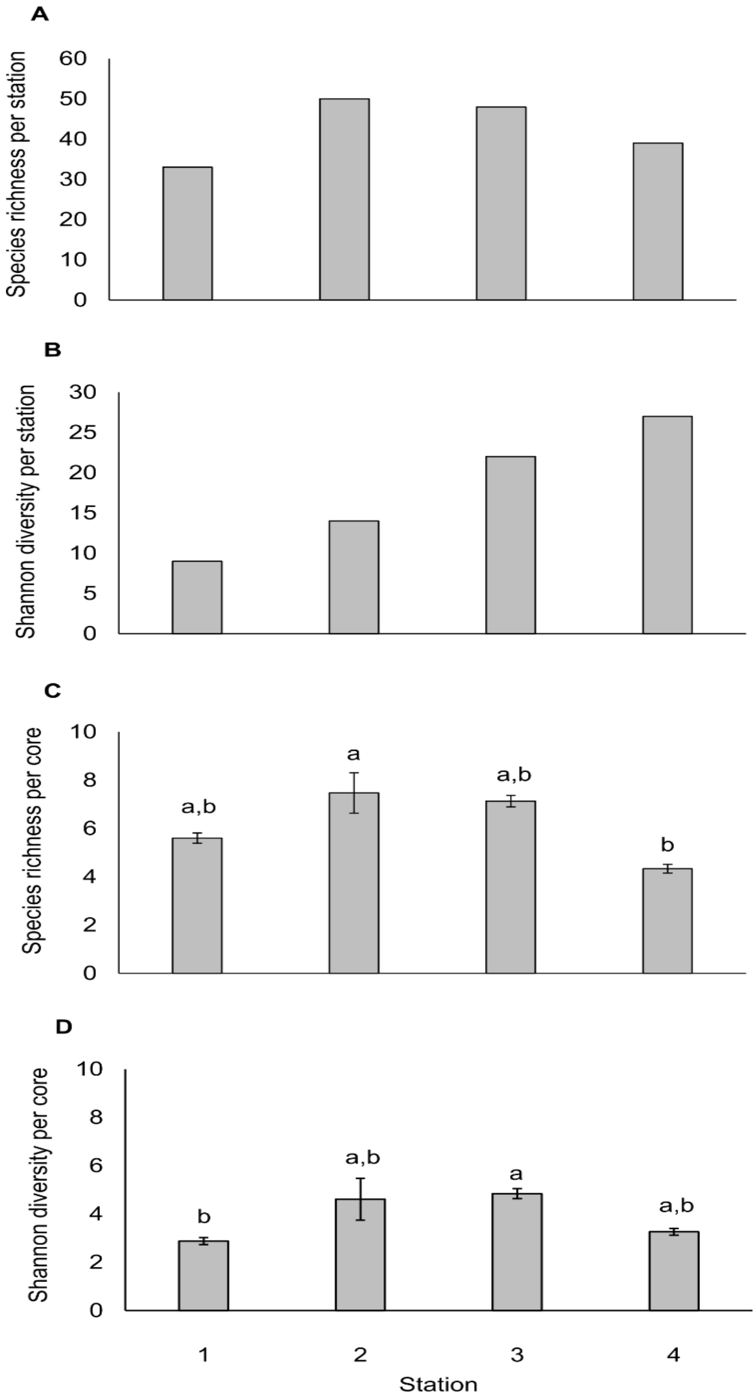

Fig. 3-2 (A-B) Univariate measurements of macrofauna, species richness, and the Shannon Diversity Index at station level and (C–D) mean values of species richness and the Shannon diversity index at core level (0.03 m^2). Each error bar (±) is a standard error of the mean. Horizontal bars with the same letter are not significantly different, with a 95% confidence level.

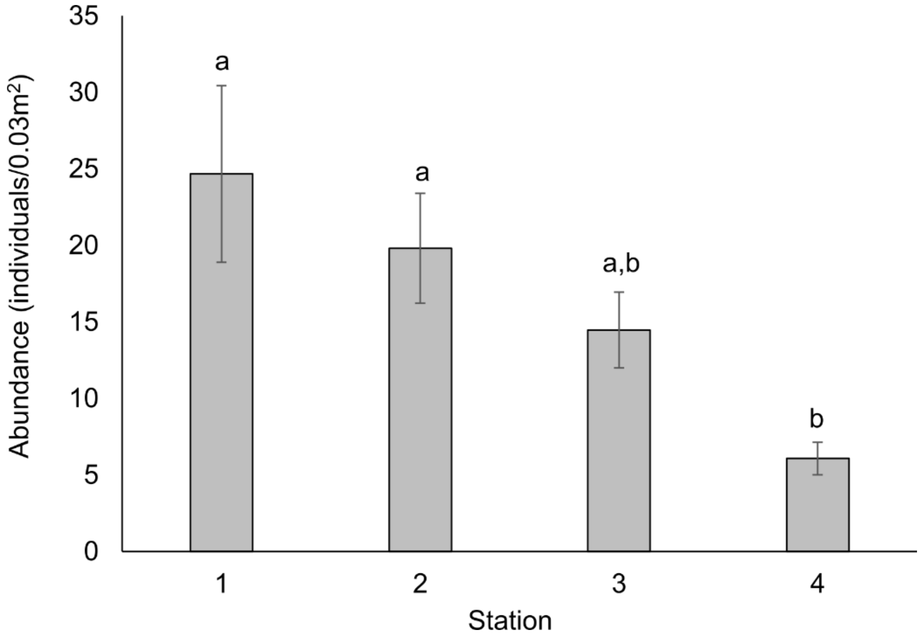

Fig. 3-3 Mean macrofaunal abundance at four stations (n = 15). Each error bar (±) is a standard error of the mean. Horizontal bars with the same letter are not significantly different, with a 95% confidence level.

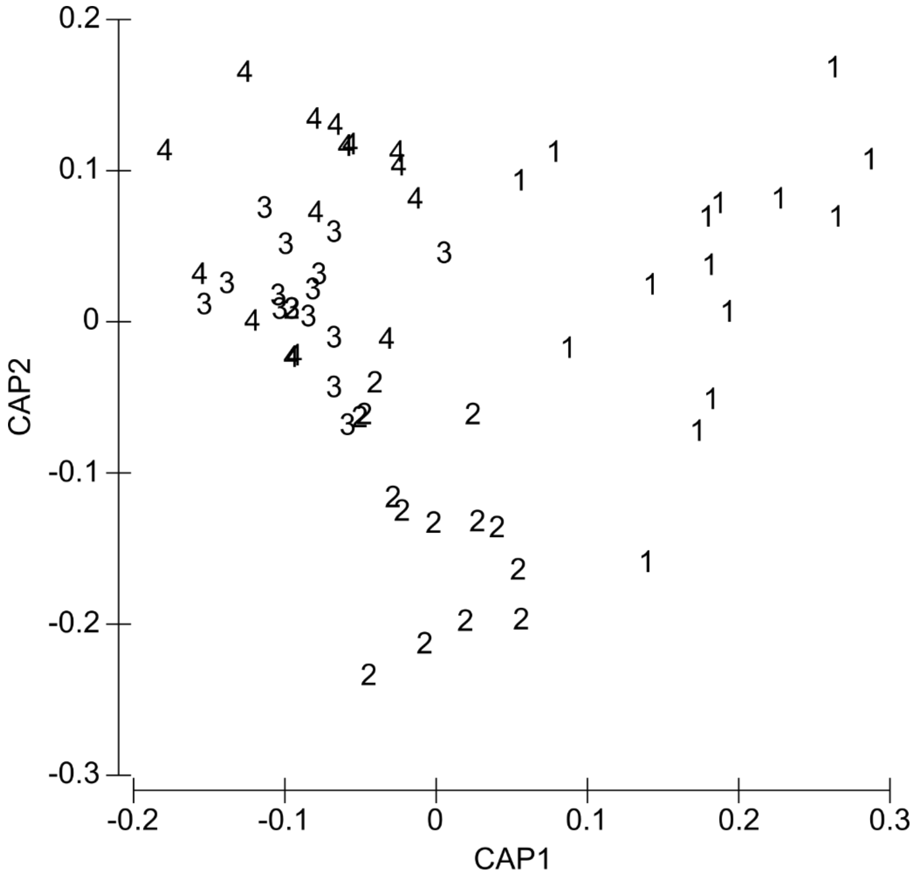

Fig. 3-4 Species composition of macrofauna at the four stations shown in multivariate space through the canonical analysis of principal coordinates (CAP) of presence/absence data based on the Jaccard resemblance index.

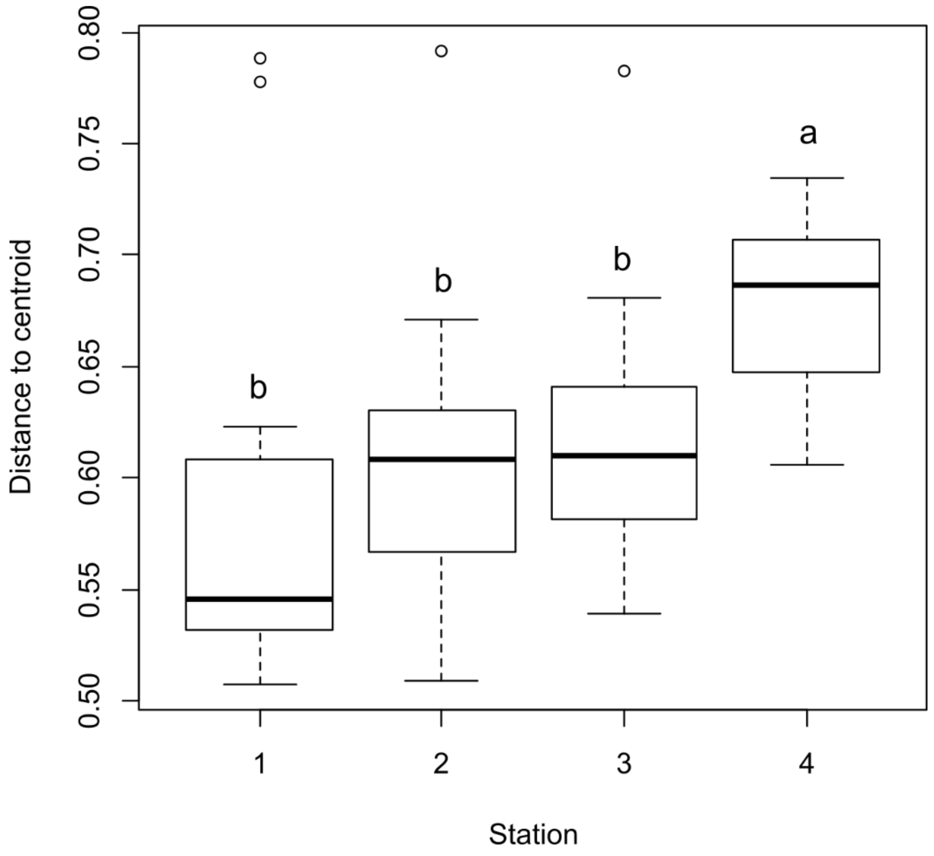

Fig. 3-5 Boxplot of the species heterogeneity pattern across four stations along a pollution gradient, analyzed through a distance-based test for homogeneity of multivariate dispersions (PERMDISP) using presence–absence data and the Jaccard resemblance index. Stations with the same letters are not significantly different, with a 95% confidence level.

Table 3-1 Six-day temporal average concentration of dissolved nutrients (nitrogen, phosphate, and silicate) and chlorophyll-a from water samples collected in Bolinao, northern Philippines, based on Fortes et al. (2012).

	Station			
	1	2	3	4
Dissolved nitrogen (μmol/l)	19.0	7.5	2.0	2.3
Dissolved phosphate (μmol/l)	2.3	1.0	<1.0	<1.0
Dissolved silicate (μmol/l)	17.00	14.00	10.00	10.00
Chlorophyll-a (μg/l)	~10	~3	<1.0	<0.005

Table 3-2 Results of a one-way analysis of variance (ANOVA) for univariate analyses between the four stations along a pollution gradient.

	Source of variation	Df	Sum Sq	Mean Sq	F	Pr(>F)
Species richness per core	Station	3	94.5	31.5	3.01	0.038
	Residuals	56	586.4	10.5		
Shannon diversity per core	Station	3	52.4	17.5	3.64	0.018
	Residuals	56	268.7	4.8		
Abundance	Station	3	1.9	0.6	4.472	0.007
	Residuals	56	8.1	0.1		

Table 3-3 Results of a permutational multivariate analysis of variance (PERMANOVA) using presence–absence data of macrofauna and the Jaccard resemblance index.

Source	df	SS	MS	Pseudo-F	P(perm)
Station	3	28420	9473.4	2.4	**0.001**
Residual	56	220000	3933.2		
Total	59	249000			

Pair-wise tests

Stations	t	P(MC)
1, 2	1.70	**0.001**
1, 3	1.89	**0.001**
1, 4	1.76	**0.001**
2, 3	1.25	0.065
2, 4	1.42	**0.008**
3, 4	1.21	0.097

Table 3-4 Summary of the results of univariate tests in *mvabund* to determine which macrofaunal species influenced the observed variation in species composition at some stations.

Station contrast	Species	Deviance	% Contribution	Pr (<Dev)
1_2	*Gafrarium pectinatum*	23.97	18.2	**0.001**
1_3	*Gafrarium pectinatum*	23.97	13.3	**0.001**
	Tectus sp.	12.29	6.0	**0.012**
	Capitella capitata	8.875	4.9	**0.022**
1_4	*Gafrarium pectinatum*	23.97	15.3	**0.001**
	Capitella capitata	12.88	8.2	**0.001**
	Nassarius elegantissimus	10.49	6.7	**0.012**
2_4	*Capitella capitata*	22.89	14.7	**0.010**

Appendix 3-1 List of macrobenthic invertebrates collected on September 2010 and March 2011 (N=60 cores, 0.03 m^2 per core) in Bolinao, northwestern Philippines.

ANNELIDA
Amphinomidae gen. sp.
Capitella capitata
Eunicidae gen. sp.
Glycera alba
Lumbrineris sp.
Nereis sp.
Psamathe sp.
Terebellidae gen. sp.

ARTHROPODA
Alpheus sp.
Calappa sp.
Diogenidae gen. sp. 1
Diogenidae gen. sp. 2
Leucosia sp.
Majidae gen. sp.
Penaeidae gen. sp.
Pilumnidae gen. sp.
Scylla serrata
Squilla sp.
Xanthidae gen. sp.

BRACHIOPODA
Lingula sp.

ECHINODERMATA
Actinopyga echinites
Amphiura filiformis
Archaster typicus
Holothuria (*Metriatyla*) *scabra*
Ophiactis savignyi
Synapta sp.
Tripneustes gratilla

MOLLUSCA
Anadara oceanica
Canarium sp. 1
Canarium sp. 2
Canarium urceus
Cerithium punctatum
Cerithium rostratum
Chione sp.
Clithon oualaniense
Clypeomorus batillariaeformis
Clypeomorus sp.
Codakia tigerina
Conus sp.
Cymatium sp.
Drupella margariticola

MOLLUSCA-cont.
Euchelus atratus
Euplica scripta
Euplica varians
Gafrarium pectinatum
Galeommatidae gen. sp.
Hebra subspinosa
Katylesia sp.
Lasaeidae gen. sp.
Loripes sp.
Mactra sp.
Mactridae gen. sp.
Milda ventricosa
Modiolus philippinarum
Monetaria moneta
Nassarius coronatus
Nassarius elegantissimus
Nassarius limnaeiformis
Naticidae gen. sp.
Neritina violacea
Neverita didyma
Paphia sp.
Peristernia sp.
Phasianella solida
Pitar sp. 1
Pitar sp. 2
Placamen lamellatum
Polinices mammilla
Pseudamycla formosa
Pseudamycla sp.
Pterygia undulosa
Pyramidella sp.
Seminella peasei
Smaragdia paulucciana
Smaragdia rangiana
Tapes literatus
Tapes sulcarius
Tectus sp.
Trochus sp.
Umbonium costatum
Vasticardium flavum subrugosum
Veneridae gen. sp.
Vexillum sinuosum
Vexillum virgo

NEMERTEA
Tetrastemmatidae gen. sp.

SIPUNCULA
Golfingia sp.

CHAPTER 4

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

Abstract

There is little information on the faunal communities in seagrass beds of Asia, despite that its seagrass species diversity is the highest in the world. I examined spatial variation in the community structure of seagrass-associated benthic macroinvertebrates collected by sediment coring in 2010 at six seagrass sites of Japan (south to north: Ishigaki, Ibusuki, Ikunoshima, Futtsu, Otsuchi, Akkeshi) covering the latitudinal range of 24° to 43°N. Both the species richness and ES50 (expected number of species from a subsample of 50 individuals at a given area) at core level significantly increased with latitude, indicating that the number of rare species increased towards high latitudes. The macrofaunal abundance also has clear site latitudinal cline which increased towards higher latitude. The community composition differed significantly across all sites, indicating influence of multiple factors, and not just the variation in current systems and climatic condition. A fast-decaying community similarity relative to geographic distance was also observed. The results of this study have important implications in the management of seagrass beds as important ecological habitats affected by small to broad-scale processes.

4.1 Introduction

Seagrass beds are some of most productive ecosystems on Earth (Costanza et al. 1998) that support high faunal diversity. Asia has the highest seagrass species diversity in the world (Green and Short 2003; Short et al. 2007); however, there is little knowledge on seagrass faunal communities in this region. Over the past years, patterns of faunal communities in seagrass beds received numerous empirical discussions, but most of the analyses were based on data from temperate seagrass beds or on other areas except those in Asia (e.g., Orth et al. 1984; Pollard 1984; Eggleston et al. 1999; Bell et al. 2001; Connolly and Hindell 2006; Boström et al. 2006). Moreover, Asian seagrass beds in general are less monitored (Duarte 2002; Kirkman and Kirkman 2002; Unsworth and Cullen 2010; Ooi et al. 2011), and some leading to gaps in empirical knowledge regarding seagrass community dynamics in this region (e.g., Waycott et al. 2009). I believe that the lack of monitoring efforts on seagrass beds will influence our level of understanding towards seagrass faunal associates. Another reason for the paucity of literature on Asian seagrass community dynamics is that most of the previous studies were conducted on various scales and methods (Thrush et al. 1997; Nakaoka 2005) resulting to difficulty in standardizing the results and scaling them up into broad scale context (Warwick 1997; Duarte 1999). Regrettably, seagrasses are declining in extent worldwide (Short and Wyllie-Echeverria 1996; Duarte 2002; Orth et al. 2006; Waycott et al. 2009) and the seagrass conservation in Asia is lagging far behind (Duarte 2002). Because seagrass beds and animals inhabiting the beds provide various valuable ecosystem services (Nakaoka et al. 2014), it is imperative to fill in the identified knowledge-gap and make use of the data to foray regional biodiversity conservation.

There is a growing interest in broad scale analyses of marine faunal structures to understand how and why assemblages of interacting marine species vary over wide areas (Underwood et al. 2000; Nakaoka and Noda 2004; Nakaoka 2005; Nakaoka et al. 2006; Sanford 2014). Large scale variations are driven not only by local processes (Orth et al. 1984; Boström et al. 2006) but also of several regional processes (Nakaoka et al. 2006; Sanford 2014). For instance, the distribution and abundance of faunal species both influence the composition and structure of local assemblages (Sanford 2014), and patterns also often corresponds to climatic variation and oceanographic current system (Nishimura 1974; Asakura and Suzuki 1987; Kurihara 2007; Kurihara et al. 2011; Nakaoka et al. 2006; Sanford 2014). This kind of investigative approach is, however, rarely done in seagrass beds despite the fact that the beds have naturally wide distribution (i.e., forming patches to several kilometers stretches of meadows in the coastal areas). Providing important faunal habitats, the seagrass beds are ideal targets for studies across broad spatial scale (defined in this study as >2,000 km maximum distance between two sites). With this, I expected that univariate community structure of seagrass fauna will vary between areas, assuming further that such variation will follow a latitudinal gradient. I also expected that the species composition will vary and such variation will correspond to the major oceanographic current system and/or climatic differences in the system (*sensu* Nakaoka and Noda 2004; Nakaoka et al. 2006). Similarity-distance relationship is also a common variable being measured to understand how species composition vary spatially (Soininen et al. 2007), and I assumed that community similarity in seagrass beds will also decay with geographic distance. I also expected that the distance-decay rate is faster in extent when seagrass beds separated at different geographic locations differed in physical complexity.

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 project 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 in those sites, giving an opportunity to analyse the broad scale data.

The main objective of this study was to determine broad scale biodiversity patterns of seagrass benthic macrofaunal associates using some of the collected data by the Monitoring Sites 1000 project. Specifically, my aim was to describe the large scale patterns of seagrass macrofaunal species richness, abundance, and community assemblage between the six seagrass bed sites of Japan under that project. Specific focuses were placed to test (1) whether there were site variation and latitudinal gradient in these variables, and (2) whether similarity of macrofaunal community decreased with geographic distance.

4.2 Materials and methods

4.2.1 Collection seagrass-associated macrofauna by Monitoring Sites 1000 project

Collection of macrobenthic animals was conducted in 2010 at six seagrass beds sites during the highest productivity (Table 4-1). These sites were Ishigaki (Okinawa Prefecture), Ibusuki (Kagoshima Prefecture), Ikunoshima, (Hiroshima Prefecture) Futtsu (Chiba Prefecture), Otsuchi (Iwate Prefecture) and Akkeshi (Hokkaido Prefecture) (Fig. 4-1). The distance between two sites ranged from 407 km to >2000 km. During each collection, benthic samples were collected at one to three depth zones, that is, intertidal, shallow subtidal, deep subtidal, depending on the zonation of seagrasses. Each sampled depth zone per site is dominated by different seagrass species (Table 4-1), but the three depth zones of seagrass beds were often not completely found in some sites. This led to asymmetrical design in my data set. The sampled intertidal beds has a water depth range of 0.1 to 0.8 m, shallow subtidal has 0.25 to 6.0 m, and deep subtidal has 0.5 to 11 m. The six sites had an annual average water temperature of 8 to 27°C (Table 4-1).

4.2.2 Collection and laboratory processing of macrofauna

Five replicate core samples for benthic macrofauna were randomly collected from each depth zone of the six seagrass beds, resulting in a total of sixty core samples (Table 4-1). The samples were collected using a cylindrical sediment with an inner diameter of 15 cm (resulting to 0.02 m^2 area per core). The corer was buried to the sediment at a depth of 10 cm. Collection was supported by scuba diving for seagrass beds with high water depth. Core samples were initially sieved in the field using mesh box with 1.0 mm opening and then kept in labelled sample bags for later processing.

In the laboratory, samples were cleaned with seawater, 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 identified sample or species was also recorded. All samples were then preserved with 70% ethanol. The seagrass shoots per core were counted before oven-drying them until constant weight under 60 to 80°C. 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.

4.2.3 Data analyses

Although samples were collected from different depth zones, the interest of this study was on the site variation while accounting for depth-related variability. Thus, in all proceeding analyses, site (with six levels representing the six sites) was set as fixed factor/effect, while depth (with three levels, i.e., intertidal, shallow subtidal and deep subtidal) was assigned as random factor.

4.2.3.1 Univariate analyses

Most of the proceeding univariate analyses were conducted in R version 3.1.2 (R Core Team 2014) and all data from 60 core samples were used. At site and core level, I used the ES50 to obtain the number of species expected from a subsample of 50 individuals taken from the population of all individuals present at a given site and core, respectively (*sensu* Rosenberg et al. 2004) through rarefaction in the DIVERSE function of PRIMER version 6.1.13 (Clarke and Gorley 2006). I then conducted a simple linear regression to carry out regression between the ES50 values expected and latitude via the *stats* package (R Core Team 2014).

At core level, the species diversity of macrofauna was analyzed using species richness (number of species) and Simpson diversity (effective numbers of species converted from

Simpson diversity index) (Jost 2006, 2007). I used the *vegetarian* package (Charney and Record 2012) to convert the index values to effective numbers of species. I conducted all univariate analyses for macrofauna (i.e., species richness, Simpson diversity, and abundance) using the Generalized Linear Mixed Model (GLMM) capability of the *lme4* package (Bates et al. 2014) with negative binomial distribution to account for overdispersion in the data (Bates & Sarkar 2006, Bates 2010). I also used the analysis of deviance from *car* package (Fox and Weisberg 2011) to determine the preferred model among null (M0: without fixed effect) and full (M1: with site as fixed effect) models. Whenever site was found having significant effect, I checked which sites had significant variation in the means by conducting multiple pairwise comparisons through the Tukey-contrast function in the *multcomp* package (Hothorn et al. 2008). I also used the GLMM approach to determine the structures of associated seagrasses and to determine if macrofaunal species richness, Simpson diversity, abundance, and seagrass biomass and shoot density have significant relationship with latitude.

4.2.3.2 Multivariate analyses

I used the presence/absence data of all species in 60 core samples for the multivariate analyses of macrofaunal community structure. To determine if species composition varied between sites, I conducted a Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson 2001) with Jaccard resemblance index and 999 permutations using PRIMER version 6.1.13 (Clarke and Gorley 2006) with the aid of PERMANOVA+ package version 1.0.3. Preliminary analysis of the multivariate data showed statistically significant dispersion (PERMDISP: F = 24.88, P = 0.001). However, PERMANOVA is only sensitive to dispersion when the data is unbalanced (i.e., with unequal number of replicates) (Anderson and Walsh 2013), which was not the case in my study. Whenever site was found having significant effect on the community assemblage pattern, I did a pairwise tests to determine which among of the sites had significantly different species composition of benthic macrofauna. I also used the Canonical Analysis of Principal Coordinates (CAP; Anderson and Willis, 2003), a constrained ordination, to visualize the distinctiveness of the sites through the multivariate cloud of points with reference to *a priori* hypothesis that samples influenced by different oceanographic current system and/or climatic condition will also vary as such.

Finally, to determine whether the similarity of seagrass benthic macrofaunal assemblages was related to geographic distance, I conducted a Mantel test (Legendre and Legendre 1998) based on Spearman correlation via the *vegan* package (Oksanen et al. 2013) of R. The geographical distances were obtained from Euclidean distance while the dissimilarity values were from Jaccard resemblance index.

4.3 Results

4.3.1 Site variation in seagrass biomass and shoot density

Results on the analyses on the seagrass structures showed that the seagrass biomass significantly varied among sites (GLMM: deviance = 340.76, $P < 0.001$, Fig. 4-2A), while shoot density did not (GLMM: deviance = 379.28, $P > 0.05$, Fig. 4-2B). Results of multiple pairwise comparisons showed high variability in biomass between sites, in which the significant difference was only found between Ibusuki (lowest biomass) and the three northern sites (Futtsu, Otsuchi and Akkeshi) (Fig. 4-2A). The seagrass biomass was found increasing with latitude and the relationship was statistically significant (GLMM: Estimate = 0.03 ± 0.1, $t = 2.31$, $P = 0.02$). The shoot density was not significant related to latitude (GLMM: Estimate = -0.008 ± 0.02, $t = 0.47$, $P = 0.64$).

4.3.2 Species diversity of benthic macrofauna

From 60 core samples (with 0.02 m^2 surface area per core), I recorded a total of 198 species (Appendix 4-1) and 4,513 benthic macrofaunal individuals (with size ≥1.0 mm). The macrofaunal samples comprised of 10 invertebrate phyla, 17 classes, 52 orders (with four orders unclassified), and 116 families. There were four species found present in more than three sites and they represented only 2% of the total number of species in this study. One of those species, the *Ampithoe lacertosa* (Amphipoda), spanned the entire study sites.

Conversely, 134 species, comprising of 68% of the total number of species, were found restricted to a single site.

The species richness per site (gamma diversity) was highly variable, with a range from 24 (Akkeshi) to 90 (Futtsu). Further analysis using ES50 showed that the expected number of species (obtained from a subsample of 50 individuals per site) ranged from 5 (Akkeshi) to 21 (Ikunoshima). The ES50 at site level appeared decreasing towards high latitude (Fig. 4-3A), but result of simple linear regression showed no significant relationship (LM: $R^2 = 0.37$, $F = 3.93$, $P=0.12$).

The mean of species richness of benthic macrofauna ranged from 5.5±1.0 to 15.4±1.1 per core (0.02 m^2) (Fig. 4-4A) and varied significantly between sites (Table 4-2). Results of multiple pairwise comparisons showed inconsistent pattern in site variation, in which the significant difference was only found between Ishigaki (lower richness) and both Futtsu and Akkeshi (higher richness) (Fig. 4-4A). Further analysis showed that the species richness increased with latitude and this was found statistically significant (Table 4-3). Furthermore, the ES50 at core level appeared increasing towards high latitude (Fig. 4-3B) and the relationship was also found statistically significant (LM: $R^2 = 0.06$, $F = 4.87$, $P = 0.03$).

The mean of Simpson diversity ranged from 1.7±1.0 to 5.6±0.3 per core (0.02 m^2) (Fig. 4-4B) and significantly varied between sites (Table 4-2). Results of pairwise tests showed that variability between sites, particularly between Ibusuki and two northern sites, Otsuchi and Akkeshi (Fig. 4-4B). It also appeared decreasing towards high latitude, but the relationship was found not statistically significant (Table 4-3).

4.3.3 Abundance of benthic macrofauna

The mean abundance of benthic macrofauna ranged from12.9±4.3 to 225.4±36.0 individuals per core (0.02 m^2) (Fig. 4-4C) and statistically differed between sites (Table 4-2). Results of the pairwise tests also showed high variability between sites; however, further analysis showed that abundance significantly increased with latitude (Table 4-3).

4.3.4 Community assemblage and similarity-distance relationship

Result of PERMANOVA showed that the species composition of benthic macrofauna varied significantly between sites (Table 4-4). According to the square root of the estimated component of variations, the macrofaunal community in different sites were around 37% dissimilar in their composition, while communities in different depths has an additional of 18% dissimilarity (Table 4-4). Further analysis showed that all sites had highly significant variation in composition (pairwise tests in PERMANOVA, $P<0.01$) and this was supported by the pattern observed in a multivariate data cloud of CAP where all samples were grouped together per site (Fig. 4-5).

Finally, I found that the macrofaunal dissimilarity increased with geographic distance (Fig. 4-6) and such relationship was statistically significant (Mantel test, $r=0.55$, $P=0.032$). The lowest decay in similarity of macrofauna, which was 15% similarity (85% dissimilarity), was observed in less than 100 km distance between two points (Fig. 4-6). The highest was 1% similarity (~99% dissimilarity) in 1025 - 1500 km distance between two points.

4.4 Discussion

There is little information regarding seagrass faunal communities in Asia, despite that is harbor highest species diversity of seagrass in the world (Green and Short 2003; Short et al. 2007). This study analysed a l data of benthic macroinvertebrates in seagrass beds across broad spatial scale along the coast of Japan. The results has important implications on the management of seagrass beds subjected to small-scale and broad-scale processes.

Firstly, the biomass of seagrasses from the macrofaunal cores was found significantly increasing with latitude, which is not surprising considering the possible role of different growth strategies of the seagrasses sampled in this study. 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 varies 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). This explanation, however, may not be directly applied to shoot density of seagrasses in my study since it did not show clear site variation and latitudinal cline.

The species richness per site (gamma diversity) was highly variable between the study sites and the ES50 values did not show significant relationship with latitude, although it tend to decrease towards high latitude. 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 my 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 Northwestern Pacific (Okuda et al. 2004) showed decreasing total 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). For instance, in this study I found no latitudinal cline of species richness at site level even when using E50 values, while other studies did not use the same analytical approach (e.g., Ellingsen and Gray 2002).

The species richness per core (0.02 m^2 area) also showed high variability between sites and this result conformed to findings of prior studies (e.g., Coates 1998; Okuda et al. 2004; Clarke and Lidgard 2010). Even with high site variability, both the species richness and ES50 at core level were found significantly increasing towards higher latitude. This indicates that the number of rare macrobenthic species in seagrass beds at high latitude is higher compared to lower latitude. On the other hand, this 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 Simpson diversity per core significantly varied between sites and appeared decreasing with latitude but not statistically significant. This 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.

Like species richness, the macrofaunal abundance also significantly varied between sites and significantly increased with latitude. Ecological interpretation regarding this positive, significant relationship between abundance and latitude should be interpreted with caution because site variation was highly variable (i.e., no consistent south-or north-wise pattern). The increased of abundance with latitude may suggests that there were more number of seagrass macrofaunal individuals towards high latitudes. I suspect that the presence of higher seagrass productivity in the north directly/indirectly driven by higher seagrass biomass (Nakaoka & Aioi 2001, Watanabe et al. 2005) may have provided more heterogeneous habitat for the associated benthic macroinvertebrates in high latitude (bottom-up control). However, biotic interactions and dispersal limitation could also depressed abundance in other suitable areas (Bell & Westoby 1986, VanDerWal et al. 2009). Also, the cold Oyashio current in the north may have influenced the abundance pattern favoring the northern latitudes if based on Kurihara (2007) report that the boreal regions of Japan (northern coasts) have higher number of molluscan taxa 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 site variation of species composition between all sites. This result was not surprising since 68% of the total number of species was found only on a single site. The restrictedness of a species to a single area is unlikely a sampling or sample identification error since similar pattern has been reported elsewhere (e.g., Clarke and Lidgard 2010; Ellingsen and Gray 2002).

The Pacific coast of Japan, where the six sites are located, are influenced by two major current systems: the Kuroshio and Oyashio current. 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). In my 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 were expected to 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. Eventhough pattern shown in CAP showed separation of samples between Oyashio and Kuroshio current system, it also clearly complemented the result of PERMANOVA in which all sites varied significantly in species composition. This suggest that local environmental condition within each site also has influence to the observed pattern of community assemblage of seagrass macrofauna. In addition to that, for sites affected by Kuroshio current, it was noticeable that Ibusuki samples lie farther away, and this could be due to annual eelgrass habit in this site compared to other sites which usually had perennial seagrasses. This observation in species composition of seagrass macrofauna being likely influenced by both regional current system and local environmental condition is, nonetheless, not a universal pattern. For instance, in a 500-km study on the benthic infaunal assemblages on the mainland shelf of southern California, Bergen et al (2001) did not find latitudinal/regional variation in infaunal community assemblage.

Climate variation is another major factor affecting marine biogeography (Briggs 1995). The Hokkaido Island of the north, where Akkeshi is located, has subarctic climate, the Honshu Island has temperate climate, while Ishigaki, the southernmost site, have subtropical climate.

Otsuchi, located south, below the Hokkaido Prefecture, also has a subarctic type of climate. With this variation in climate, I particularly expected that Ishigaki would also be separated from the rest of the sites especially from those in the north (Otsuchi, Akkeshi), and result of constrained ordination (CAP) supported that. I suspect that the variation in annual sea water temperature, which is also influenced by the current system in the coast of Japan, also have important influence to the observed ecological pattern of community assemblage in seagrass macrofauna.

Previous geographic works in Japan have highlighted the influence of oceanic and biogeographic variations to faunal biogeography (e.g., Asakura and Suzuki 1987; Nishimura 1974; Nakaoka et al. 2006; Kurihara 2007; Kurihara et al. 2011). In Japan, there are tropical and temperate rocky intertidal molluscs (Asakura and Suzuki 1987; Kurihara 2007) and invertebrate communities (Nishimura 1974). They also reported boreal or subarctic distribution of benthic species. Although results of my multivariate analyses indicates importance of local environmental conditions such as productivity habit of seagrasses, it also suggest the potential influence of regional factors, such as current system and climatic condition. Specific differences between my results to other previous works (e.g., difference in species composition even across sites within same current system or climatic condition in my study) was possibly because of difference in the extent of studied fauna and type of habitat. Most of the prior biogeographical studies conducted in Japanese waters were done on rocky intertidal and/or targeting on specific taxa (e.g., molluscs), while I conducted my study on seagrass beds and on a multiphyletic community of benthic macrofauna.

Finally, I found that the community similarity of macrofauna decayed with increasing geographic distance. This result was not surprising and had been discussed in abundance on previous reports (e.g., Nekola and White 1999; Parmesan and Yohe 2003; Gilman 2006; Martínez et al. 2012). More importantly, it was interesting to note that the rate of decay in the similarity of macrofaunal community in my study was generally higher compared to other published findings. For instance, within a distance of ≤ 500 km, the seagrass macrofauna between two points in my study already lost up to 80% 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% on similar geographic distance. The same observation was observed in Blanchette et al. (2008) where similarity on the benthic components in the rocky intertidal along the Pacific coast of North America decayed up to ~30%. Within same range of geographic distance (i.e., ≤ 500 km), Ellingsen and Gray (2002) reported a decay of up to 57% similarity of soft sediment macrobenthos in Norwegian continental shelf. I suspect that the the higher distance-decay rates reported in my study was influenced by the differ extent of seagrass habitat heterogeneity driven by variation in environmental factors dominating each site. Similarity-distance relationship is influenced by the species geographic range limit reflecting niche-breath (Parmesan and Yohe 2003; Martínez et al. 2012) and dispersal ability (Gilman 2006; Kuo and Sanford 2013), as well as on the similarity of environmental variables that is expected to decrease with distance along various environmental gradients (Nekola and White 1999).

4.5 Acknowledgements

Special thanks to N. Whanpetch, M. Ito, and other Hokkaido University students who provided laboratory assistance. I also thank K. Sudo, for providing additional information regarding environmental data; K. Momota, T. Takuya and M. Hashimoto for support in the identification of amphipod samples; and K. Watanabe for making the study map. The data set was obtained from the Monitoring Sites 1000 project funded by the Ministry of Environment-Government of Japan.

4.6 References

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Aust Ecol 26:32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x.

Anderson MJ, Willis TJ (2003) Canonical analysis of principal coordinates: A useful method of constrained ordination for ecology. Ecology 84:511–525.

Asakura A, Suzuki H (1987) Zoogeographical aspects of rocky-intertidal molluscan fauna of the Pacific coasts of Japan. Mar Biol 95:75–81.

Bates D (2010) lme4: Mixed-effects modeling with R. http://lme4.r-forge.r-project.org/lMMwR/lrgprt.pdf.

Bates D, Maechler M, Bolker BM, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4.

Bates D, Sarkar S (2006) The lme4 package. http://lmqesalqusp.br/CRAN/doc/packages/lme4pdf.

Bell JD, Westoby M (1986) Variation in seagrass height and density over a wide spatial scale: Effects on common fish and decapods. J Exp Mar Bio Ecol 104:275–295. doi: 10.1016/0022-0981(86)90110-3.

Bell SS, Brooks R a., Robbins BD, et al (2001) Faunal response to fragmentation in seagrass habitats: Implications for seagrass conservation. Biol Conserv 100:115–123. doi: 10.1016/S0006-3207(00)00212-3.

Bergen M, Weisberg B, Smith RW, et al (2001) Relationship between depth , sediment , latitude , and the structure of benthic infaunal assemblages on the mainland shelf of southern California. Mar Biol 138:637–647.

Blanchette C a., Melissa Miner C, Raimondi PT, et al (2008) Biogeographical patterns of rocky intertidal communities along the Pacific coast of North America. J Biogeogr 35:1593–1607. doi: 10.1111/j.1365-2699.2008.01913.x.

Boström C, Jackson EL, Simenstad CA (2006) Seagrass landscapes and their effects on associated fauna: A review. Estuar Coast Shelf Sci 68:383–403. doi: 10.1016/j.ecss.2006.01.026.

Briggs JC (1966) Zoogeography and evolution. Evolution (N Y) 20:282–289.

Briggs JC (1995) Global biogeography. Elsevier, Amsterdam.

Buzas MA, Culver SJ (1999) Understanding regional species diversity through the log series distribution of occurrences. Divers Distrib 5:187–195.

Charney N, Record S (2012) vegetarian: Jost Diversity Measures for Community Data.

Clarke A, Lidgard S (2010) Spatial patterns of diversity in the sea: Bryozoan species richness in the North Atlantic. J Anim Ecol 69:799–814.

Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial.

Coates M (1998) A comparison of intertidal assemblages on exposed and sheltered tropical and temperate rocky shores. Glob Ecol Biogeogr Lett 1:115–124.

Connolly RM, Hindell JS (2006) Review of nekton patterns and ecological processes in seagrass landscapes. Estuar Coast Shelf Sci 68:433–444. doi: 10.1016/j.ecss.2006.01.023.

Costanza R, D’Arge R, De Groot R, et al (1998) The value of ecosystem services: Putting the issues in perspective. Ecol Econ 25:67–72. doi: 10.1016/S0921-8009(98)00019-6.

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192–206. doi: 10.1017/S0376892902000127.

Duarte CM (1999) Seagrass ecology at the turn of the millennium: Challenges for the new century. Aquat Bot 65:7–20. doi: 10.1016/S0304-3770(99)00027-3.

Eggleston DB, Elis WE, Etherington LL, et al (1999) Organism responses to habitat fragmentation and diversity: Habitat colonization by estuarine macrofauna. J Exp Mar Bio Ecol 236:107–132. doi: 10.1016/S0022-0981(98)00192-0.

Ellingsen KE, Gray JS (2002) Spatial patterns of benthic diversity : Is there a latitudinal gradient along the Norwegian continental shelf ? J Anim Ecol 71:373–389.

Fox J, Weisberg S (2011) An {R} Companion to Applied Regression.

Gilman, SE (2006) The northern geographic range limit of the intertidal limpet *Collisella scabra*: a test of performance, recruitment, and temperature hypotheses. Ecography, 29, 709-720.

Green EP, Short FT (2003) World atlas of seagrasses. University of California Press.

Hothorn T, Bretz F, Heilberger R, Schuetzenmeister A (2008) Simltaneous interference in general parametric models. Biometric J 50:346–363.

Ishihara MI, Suzuki SN, Nakamura M, et al (2011) Forest stand structure, composition, and dynamics in 34 sites over Japan. Ecol Res 26:1007–1008. doi: 10.1007/s11284-011-0847-y

Jost L (2006) Entropy and diversity. Oikos 113:363–375. doi: 10.1111/j.2006.0030-1299.14714.x.

Jost L (2007) Partitioning diversity into independent alpha and beta components. Ecology 88:2427–2439.

Kirkman H, Kirkman JA. (2002) The management of seagrasses in Southeast Asia. Bull Mar Sci 71:1379–1390.

Kuo EL, Sanford E (2013) Geographic variation in the upper thermal limits of an intertidal snail: 634 Implications for climate envelope models. Mar Ecol Prog Ser 388* 303-313.

Kurihara T (2007) Spatiotemporal variations in rocky intertidal malacofauna throughout Japan in the 1970s and 1980s. Mar Biol 153:61–70. doi: 10.1007/s00227-007-0784-z.

Kurihara T, Takami H, Kosuge T, et al (2011) Area-specific temporal changes of species composition and species-specific range shifts in rocky-shore mollusks associated with warming Kuroshio Current. Mar Biol 158:2095–2107. doi: 10.1007/s00227-011-1717-4.

Legendre P, Legendre L (1998) Numerical Ecology, 2nd Englis. Elsevier.

Lidgard S (1990) Growth in encrusting cheilostone bryozoan. II. Circum-Atlantic distribution pattern. Palaeobiology 16:304–321.

Martínez B, Viejo RM, Carreno F, Aranda SC (2012) Habitat distribution models for 654 intertidal seaweeds: responses to climatic and non-climatic drivers. J Biogeo 655:1877–1890. doi:10.1111/j.1365-2699.2012.02741.x.

Nakaoka M (2005) Plant-animal interactions in seagrass beds: Ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177. doi: 10.1007/s10144-005-0226-z.

Nakaoka M, Aioi K (2001) Ecology of seagrasses *Zostera* spp. in japanese water, a review. Otsuchi Mar. Sci. 26:7–22.

Nakaoka M, Ito N, Yamamoto T, et al (2006) Similarity of rocky intertidal assemblages along the Pacific coast of Japan: Effects of spatial scales and geographic distance. Ecol Res 21:425–435. doi: 10.1007/s11284-005-0138-6

Nakaoka M, Kouchi N, Aioi K (2003) Seasonal dynamics of *Zostera caulescens*: Relative importance of flowering shoots to net production. Aquat Bot 77:277–293. doi: 10.1016/j.aquabot.2003.08.002.

Nakaoka M, Lee K-S, Huang X, et al (2014) Regional Comparison of the Ecosystem Services from Seagrass Beds in Asia. In: Nakano S, Yahara T, Nakashizuka T (eds) Integrative Observations and Assessments. Springer, pp 367–393.

Nakaoka M, Noda T (2004) Special feature: Multiple spatial scale approaches in population and community ecology. Popul Ecol 46:103–104. doi: 10.1007/s10144-004-0186-8.

Nakaoka M, Toyohara T, Matsumasa M (2001) Seasonal and between-substrate variation in mobile epifaunal community in a multispecific seagrass bed of Otsuchi bay, Japan. Mar Ecol 22:379–395. doi: 10.1046/j.1439-0485.2001.01726.x.

Nekola JC, White PS (1999) The distance decay of similarity in biogeography and ecology. J Biogeogr 26:867–878. doi: 10.1046/j.1365-2699.1999.00305.x.

Nishimura S (1974) Biogeographical Aspects of The Sea Shore Animals. In: Nishimura S, Yamamoto T (eds) The Sea Shore Life. HOIKUSHA Publishing Co., Ltd., Tokyo, pp 126–131.

Nishimura S (ed) (1992) Guide to Seashore Animals of Japan with Color Pictures and Keys, Vol. I. HOIKUSHA Publishing Co., Ltd.

Nishimura S (ed) (1995) Guide to Seashore Animals of Japan with Color Pictures and Keys, Vol. II. HOIKUSHA Publishing Co., Ltd., Osaka.

Oksanen J, Blanchet FG, Kindt R, et al (2013) vegan: Community Ecology Package.

Okuda T, Noda T, Yamamoto T, et al (2004) Latitudinal gradient of species diversity: Multi-scale variability in rocky intertidal sessile assemblages along the Northwestern Pacific coast. Popul Ecol 46:159–170. doi: 10.1007/s10144-004-0185-9.

Okutani T (ed) (2000) Marine Mollusks in Japan. University of Tokyo Press, Tokyo.

Ooi JLS, Kendrick GA, Van Niel KP, Affendi YA (2011) Knowledge gaps in tropical Southeast Asian seagrass systems. Estuar Coast Shelf Sci 92:118–131. doi: 10.1016/j.ecss.2010.12.021.

Orth RJ, Carruthers TJB, Dennison WC, et al (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Orth RJ, Heck KL, van Montfrans J (1984) Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. Estuaries 7:339–350. doi: 10.2307/1351618.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37-42.

Pollard DA (1984) A review of ecological studies on seagrass-fish communities, with particular reference to recent studies in Australia. Aquat Bot 18:3–42.

R Core Team (2014) R: A language and environment for statistical computing.

Rex MA, Stuart CT, Hessler RR, et al (1993) Global-scale latitudinal patterns of species diversity in the deep-sea benthos. Nature 365:636–639. doi: 10.1038/365636a0.

Rohde K (1992) Latitudinal gradients in species diversity: the search for the primary cause. Oikos 65:514–527.

Rosenberg R, Blomqvist M, Nilsson HC, et al (2004) Marine quality assessment by use of benthic species-abundance distributions: A proposed new protocol within the European

Union Water Framework Directive. Mar Pollut Bull 49:728–739. doi: 10.1016/j.marpolbul.2004.05.013.

Sanford E (2014) The Biogeography of Marine Communities. In: Bertness MD, Bruno JF, Silliamn BR, Stachowicz JJ (eds) Marine Ecology and Conservation. Sinauer Associates, Suntherland.

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. J Exp Mar Bio Ecol 350:3–20. doi: 10.1016/j.jembe.2007.06.012.

Short FT, Wyllie-Echeverria S (1996) Natural and human-induced disturbance of seagrasses. Environ Conserv 23:17–27. doi: 10.1017/S0376892900038212.

Soininen J, Lennon JJ, Hillebrand H, Lennon J (2007) A multivariate analysis of beta diversity across organisms and environments. Ecology 88:2830–2838.

Stehli FG, McAlester AL, Helsley CE (1967) Taxonomic diversity of recent bivalves and some implications for geology. Geol Soc Am Bull 78:455–466.

Takyu M, Kubota Y, Aiba S, et al (2005) Pattern of changes in species diversity, structure and dynamics of forest ecosystems along latitudinal gradients in East Asia. Ecol Res 20:287–296. doi: 10.1007/s11284-005-0044-y.

Thrush SF, Schneider DC, Legendre P, et al (1997) Scaling-up from experiments to complex ecological systems: Where to next? J. Exp. Mar. Bio. Ecol. 216:243–254.

Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: You can’t make progress on processes without understanding the patterns. J Exp Mar Bio Ecol 250:97–115. doi: 10.1016/S0022-0981(00)00181-7.

Unsworth RKF, Cullen LC (2010) Recognising the necessity for Indo-Pacific seagrass conservation. Conserv Lett 3:63–73. doi: 10.1111/j.1755-263X.2010.00101.x.

VanDerWal J, Shoo LP, Johnson CN, Williams SE (2009) Abundance and the environmental niche: environmental suitability estimated from niche models predicts the upper limit of local abundance. Am Nat 174:282–291. doi: 10.1086/600087.

Virnstein RW, Nelson WG, Lewis FG, Howard RK (1984) Latitudinal patterns in seagrass epifauna: Do patterns exist, and can they be explained? Estuaries 7:310. doi: 10.2307/1351616.

Warwick RM (1997) The ecology of soft- bottom habitats: Matching spatial patterns with dynamic processes. J Exp Mar Bio Ecol 2:216:ix.

Watanabe M, Nakaoka M, Mukai H (2005) Seasonal variation in vegetative growth and production of the endemic Japanese seagrass *Zostera asiatica*: A comparison with sympatric *Zostera marina*. Bot Mar 48:266–273. doi: 10.1515/BOT.2005.036.

Waycott M, Duarte CM, Carruthers TJB, et al (2009) Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc Natl Acad Sci U S A 106:12377–12381. doi: 10.1073/pnas.0905620106.

Witman JD, Etter RJ, Smith F (2004) The relationship between regional and local species diversity in marine benthic communities: A global perspective. Proc Natl Acad Sci U S A 101:15664–15669. doi: 10.1073/pnas.0404300101.

WoRMS Editorial Board (2015) World Register of Marine Species. In: VLIZ. http: Accessed 31 Mar 2015.

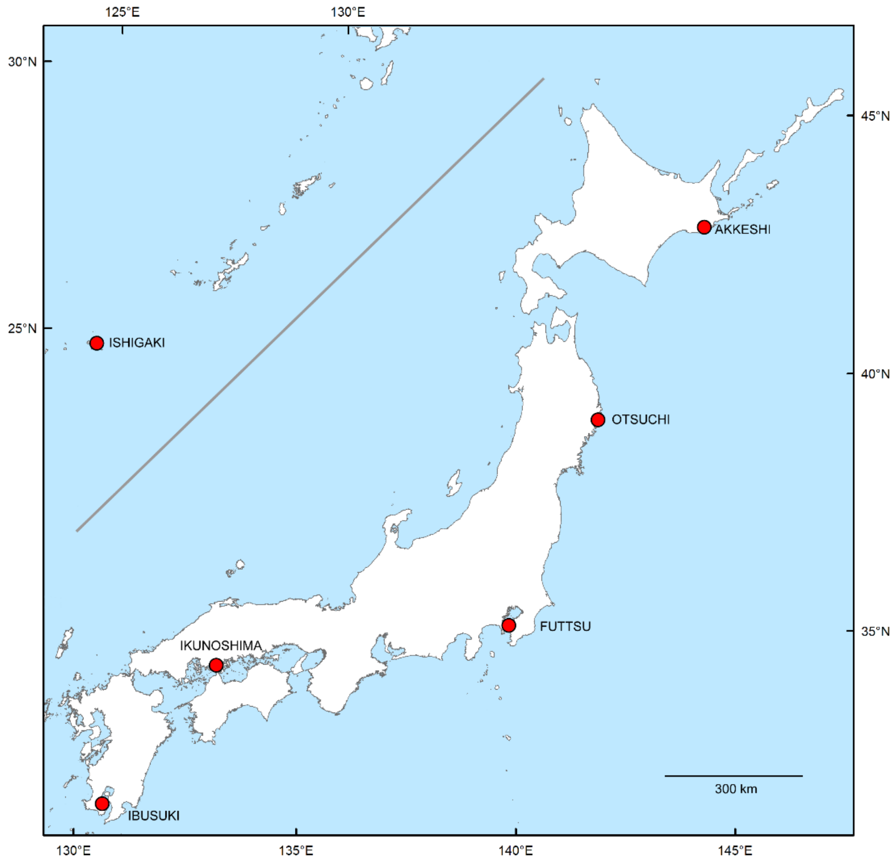

Fig. 4-1 Map showing the locations of the six study sites in Japan under the Monitoring Sites 1000 project of the Ministry of Environment-Government of Japan.

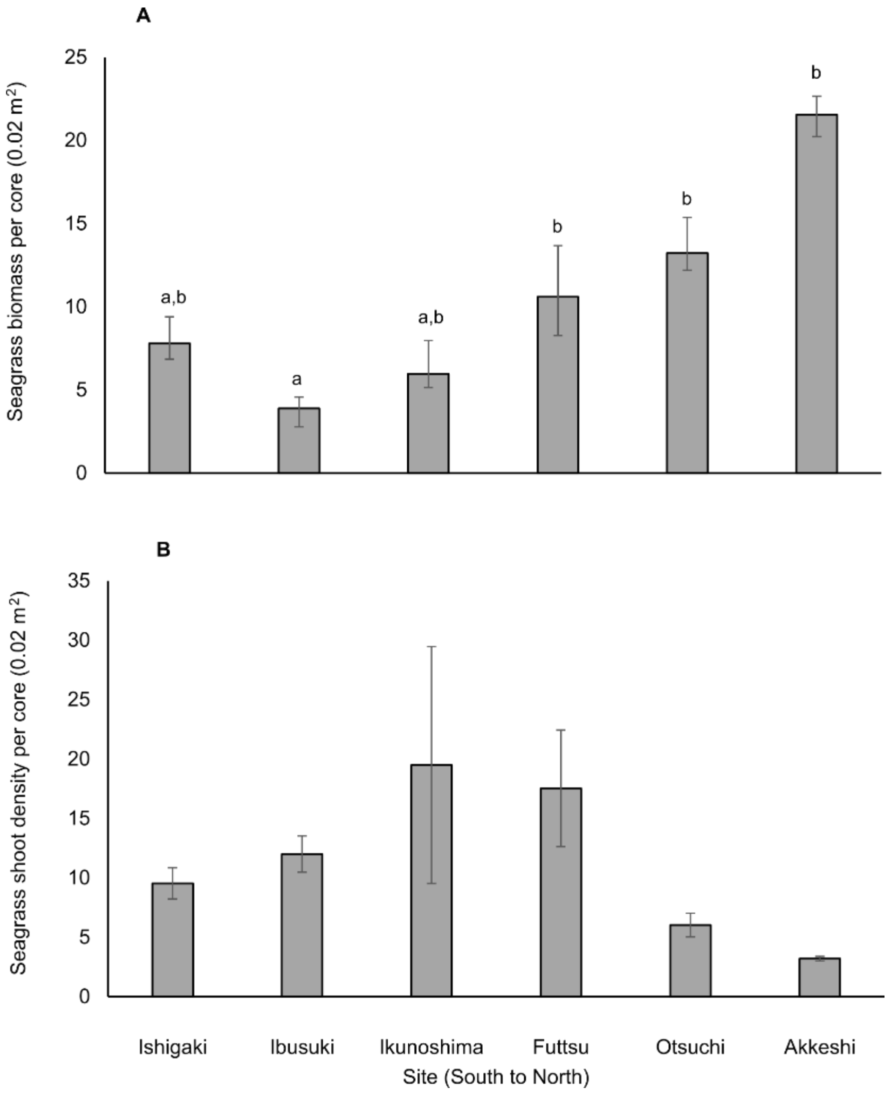

Fig. 4-2 Mean values for (A) seagrass biomass and (B) shoot density in six sites. Non-significant values were marked with same letter. Error bars are standard error of the mean (with N ranging from 5 to 15 core samples).

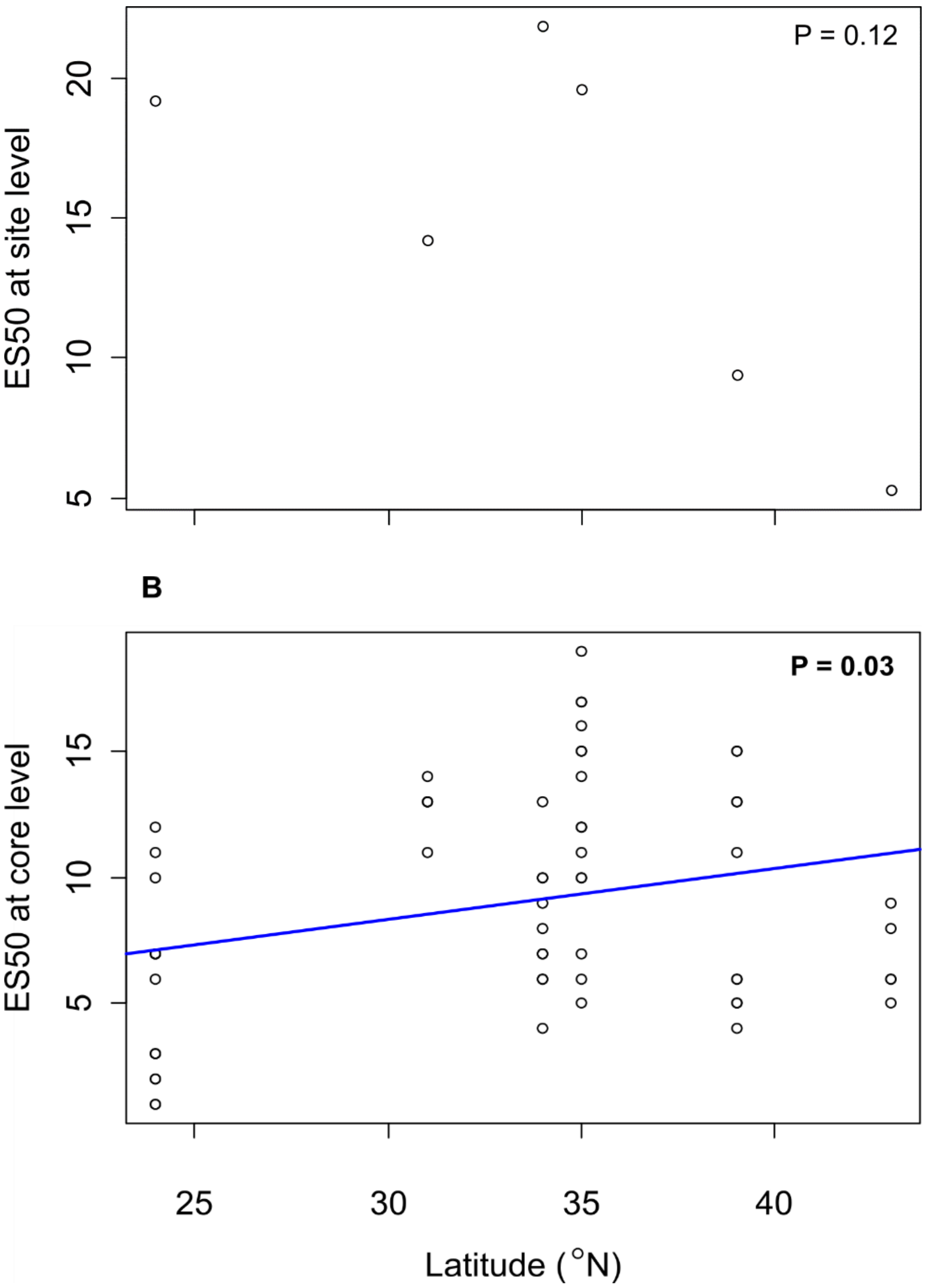

Fig. 4-3 Relationship of ES50 (expected number of species obtained from a subsample of 50 individuals) at (A) site and (B) core level to latitude ranging from 24° to 43°N.

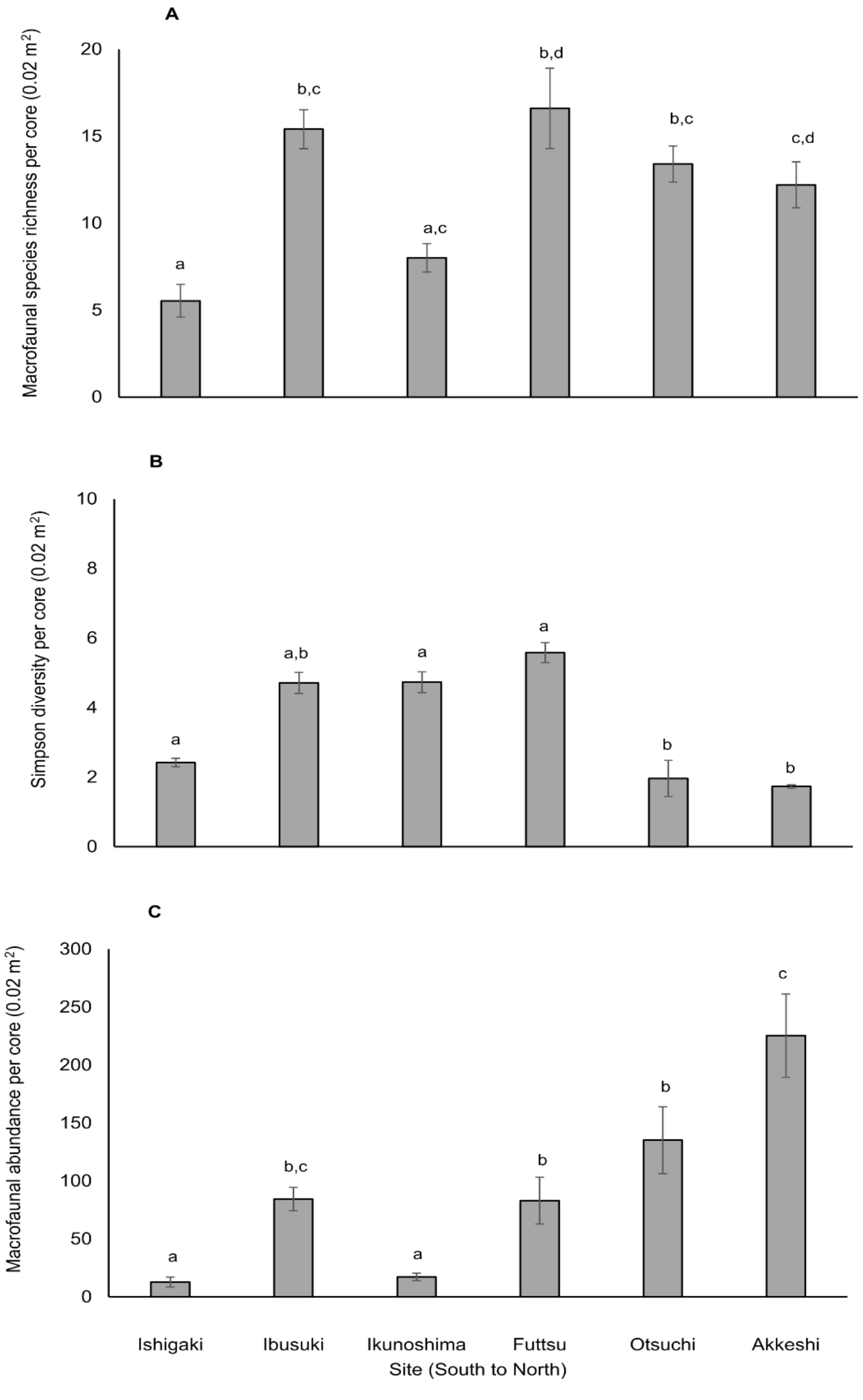

Fig. 4-4 Mean values at core level for (A) species richness, (B) Simpson diversity, and (C) abundance of benthic macrofauna collected in six sites. Non-significant values were marked with same letter. Error bars are standard error of the mean (with N ranging from 5 to 15 cores).

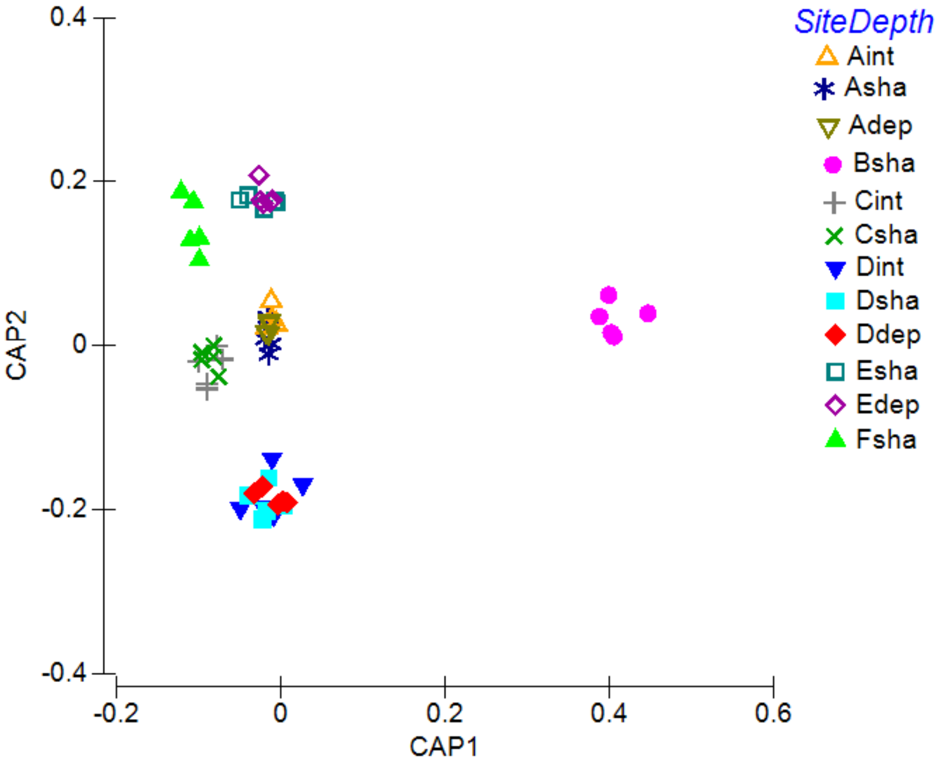

Fig. 4-5 Canonical Analysis of Principal Coordinates (CAP) ordination of benthic macrofaunal species using presence/absence data via Jaccard resemblance index. Site symbols: A = Ishigaki, B = Ibusuki, C = Ikunoshima, D = Futtsu, E = Otsuchi, F = Akkeshi. Depth symbols: int = intertidal, sha = shallow subtidal, dep = deep subtidal.

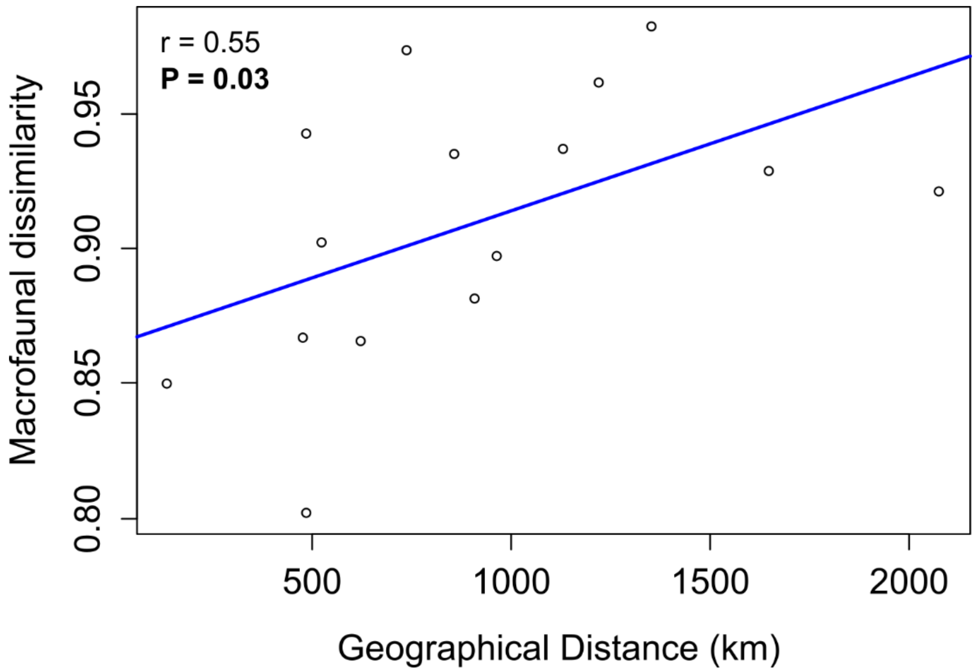

Fig. 4-6 Distance-decay plot showing an increasing benthic macrofaunal dissimilarity as geographical distance increased.

Table 4-1 Summary of the location, water depth during sampling, depth zones, annual average water temperature, and dominant seagrass species in six sampled sites of Japan sampled on 2010 under the Monitoring Sites 1000 project of the Ministry of the Environment-Government of Japan.

Site	Prefecture	Latitude (°N)	Longitude (°E)	Temperature (° C)[*1]	Depth zone	Depth (m)	N	Seagrass	Month
Ishigaki	Okinawa	24	124	27	Intertidal	0.10	5	*Halodule uninervis*	September
					Shallow subtidal	0.25	5	*Thalassia hemprichii*	
					Deep subtidal	0.50	5	*Enhalus acoroides*	
Ibusuki	Kagoshima	31	130	22	Shallow subtidal	1.40	5	*Zostera marina*	April
Ikunoshima	Hiroshima	34	132	18	Intertidal	0.80	5	*Zostera japonica*	June
					Shallow subtidal	0.80	5	*Zostera marina*	
Futtsu	Chiba	35	139	18	Intertidal	0.80	5	*Z. japonica*	June
					Shallow subtidal	0.25	5	*Z. marina*	
					Deep subtidal	0.50	5	*Zostera caulescens*	
Otsuchi	Iwate	39	141	12	Shallow subtidal	6.00	5	*Z. caulescens*	July
					Deep subtidal	11.00	5		
Akkeshi	Hokkaido	43	144	8	Shallow subtidal	2.50	5	*Zostera asiatica*	August

[*1] Data from the nearest oceanographic station (http://www.jodc.go.jp/data/coastal/obs_data_index2.html)

Table 4-2 Summary results of the analysis of deviance under the Generalized Linear Mixed Model (GLMM) analysis of site variation in macrofaunal species richness, Simpson diversity and abundance at core level (0.02 m^2). M0 is the null model where there is no fixed effect (site), while M1 is the full model where site and depth were set as fixed and random effect, respectively.

	AIC	loglik	deviance	Chisq	Chi Df	Pr (>Chisq)
Species richness						
M0	389.76	-191.88	383.76			
M1	360.85	-172.43	344.85	38.9	5	<0.001
Simpson diversity						
M0	283.04	-138.52	277.04			
M1	253.3	-118.81	237.63	39.41	5	<0.001
Abundance						
M0	624.65	-309.33	618.65			
M1	577.62	-280.81	561.62	57.03	5	<0.001

Table 4-3 Summary results on the estimates of the effect of latitude (ranging from of 24° to 43°N) to macrofaunal univariate measures at core level (0.02 m^2) analyzed under the Generalized Linear Mixed Model (GLMM) where latitude and depth were set as fixed and random factor, respectively.

	Estimate	Std. error	t	Pr(>|z|)
Species richness	0.04	0.01	3.77	<0.001
Simpson diversity	-0.02	0.01	-1.75	0.079
Abundance	0.13	0.02	7.57	<0.001

Table 4-4 Summary results of the permutational multivariate analysis of variance (PERMANOVA) to determine community assemblage pattern across sites using the presence/absence data of macrofaunal species and Jaccard resemblance index. Site and depth were set as fixed and random

Source	df	SS	MS	Pseudo-F	P(perm)
Site	5	77585	15517.0	4.91	0.001
Depth	2	16085	8042.4	2.54	0.001
Residual	52	1.64E+05	3160.3		
Total	59	2.62E+05			

Estimates of components of variation

Source	Estimate	Sq.root
S(Site)	1373.00	37.054
V(Depth)	325.48	18.041
V(Residual)	3160.30	56.216

Appendix 4.1 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.

ANNELIDA
Abarenicola sp.
Anelassorhynchus porcellus
Bispira sp.
Capitella sp.
Chaetozone sp.
Cirratulidae gen. sp.
Cirriformia sp.
Dorvilleidae gen. sp. 1
Dorvilleidae gen. sp. 2
Eulalia viridis
Glycera sp.
Glycinde nipponica
Lepidasthenia ocellata
Lumbrineris sp. 1
Lumbrineris sp. 2
Maldane sp.
Neanthes sp.
Nectoneanthes sp.
Nephtys sp. 1
Nephtys sp. 2
Nereididae gen. sp. 1
Nereididae gen. sp. 2
Nereis sp.
Nereis zonata
Onuphis shirikishinaiensis
Ophelia sp.
Owenia sp.
Paraonidae gen. sp.
Phyllodoce sp.
Polynoidae gen. sp. 1
Polynoidae gen. sp. 2
Polynoidae gen. sp. 3
Prionospio sp.
Pseudopolydora sp.
Sabellidae gen. sp.
Sigambra sp.
Spirorbis sp.
Syllidae gen. sp. 1
Syllidae gen. sp. 2
Syllidae gen. sp. 3
Terebellidae gen. sp. 1
Terebellidae gen. sp. 2
Terebellidae gen. sp. 3
ARTHROPODA
Alpheus sp.
Ampelisca brevicornis
Amphibalanus improvisus
Ampithoe lacertosa
Aoroides sp. 1
Aoroides sp. 2
Apseudes spectabilis
Balanus sp.
Byblis japonicus
Calcinus sp.
Callianassa sp.
Camptandriidae gen. sp
Caprella sp. 1
Caprella sp. 2
Caprella sp. 3
Cerapus tubularis
Charybdis sp.
Crangon sp.
Decapoda fam. gen. sp.
Diastylis sp.
Eochelidium lenorostralum
Ericthonius pugnax
Gaetice depressus
Grandidierella japonica
Harpinia sp.
Hippolytidae gen. sp.
Idotea ochotensis
Idoteidae gen. sp.

Appendix 4.1 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 (Continued).

ARTHROPODA-cont.
Isaeidae gen. sp.
Jassa sp.
Kamehatylus japonicus
Liljeborgia sp.
Liljeborgiidae gen. sp.
Majidae gen. sp.
Melita sp. 1
Melita sp. 2
Melitidae gen. sp.
Myodocopida fam. gen. sp.
Nanosesarma gordoni
Nebalia sp.
Nihonotrypaea sp.
Nipponomysis sp.
Orchomene sp.
Pagurus sp.
Paradexamine sp.
Paranthura sp.
Paranthuridae gen. sp.
Pinnixa tumida
Pinnotheres sp.
Pleustes sp.
Podocopida fam. gen. sp.
Pontogeneia rostrata
Ptilohyale barbicornis
Sinelobus sp.
Sphaeromatidae gen. sp.
Synidotea laevidorsalis
Talitridae gen. sp.
Tmethypocoelis choreutes
Upogebia major
Upogebia sp.
CNIDARIA
Actiniaria fam. gen. sp. 1
Actiniaria fam. gen. sp. 2
Edwardsiidae gen. sp.
Halcampidae gen. sp. 1
Halcampidae gen. sp. 2
Stenoscyphus inabai
ECHINODERMATA
Amphiodia sp.
Amphiura sp.
Asteriiidae gen. sp.
Eupentacta chronhjelmi
Myriotrochidae gen. sp.
Ophiurida fam. gen. sp.
FORAMINIFERA
Marginopora zeniishi
HEMICHORDATA
Ptychoderidae gen. sp.
MULLUSCA
Acila sp.
Anodontia edentula
Arcuatula senhousia
Batillaria sp.
Callithaca adamsi
Cantharidus callichroa
Cantharidus japonicus
Cephalaspidea fam. gen. sp.
Cerithidium sp.
Cerithiidae gen. sp.
Diaffalaba picta
Diala albugo
Diala sp.
Diffalaba picta
Erginus moskalevi
Ethminolia sp.
Fissurellidae gen. sp.
Fulvia australis
Galeommatidae gen. sp.
Hiatella orientalis
Ittibittium parcum
Komaitrochus pulcher

Appendix 4.1 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 (Continued).

MULLUSCA-cont.
Lacuna decorata
Limalatula sp.
Lioconcha fastigiata
Lirularia iridescens
Lucidestea sp.
Macoma incongrua
Mactridae gen. sp.
Mesodesmatidae gen. sp.
Musculus cupreus
Mya arenaria
Mytilus galloprovincialis
Nassarius albescens
Nassarius festivus
Nassarius limnaeiformis
Nassarius multigranosus
Nassarius nodifer
Nassarius sp. 1
Nassarius sp. 2
Nassarius sp. 3
Nesobornia sp.
Nudibranchia fam. gen. sp.
Olivella fulgurata
Orinella pulchella
Philine sp.
Pillucina pisidium
Propeamussium sp.
Retusa sp.
Retusidae gen. sp.
Rissoidae gen. sp.
Rissoina sp.
Ruditapes philippinarum
Scaliola glareosa
Scintilla sp.
Siphonacmea oblongata
Smaragdia sp. 1
Smaragdia sp. 2
Solemya pusilla
Tectonatica venustula
Tellina sp.
Tellinidae gen. sp.
Thyasira sp. 1
Thyasira sp. 2
Trochidae gen. sp.
Turbonilla edoensis
Veneridae gen. sp.
Veneroida fam. gen. sp.
Veremolpa micra
Vexillum (*Costellaria*) *michaui*
Vexillum exasperatum
Zafra sp.
NEMERTEA
Carinoma sp.
Carinomidae gen. sp.
PLATYHELMINTHES
Notoplana sp. 1
Notoplana sp. 2
SIPUNCULA
Golfingia sp. 1
Golfingia sp. 2
Siphonosoma cumanense
Sipunculidae gen. sp.

CHAPTER 5

General Discussion

The results of this dissertation confirmed that the diversity and abundance of macrofauna in seagrass beds are influenced by local and regional factors. In Chapter 2, seagrass taxonomic identity was found not a good determinant of small-scale variation in macrofauna in a multispecific seagrass beds. Whereas, in Chapter 3, local organic pollution showed significant role in the alteration of macrofaunal patterns in seagrass beds lying along a pollution gradient. Moreover, in Chapter 4, broad-scale analysis showed that variations in the species richness (including ES50, expected number of species obtained from 50 individual subsamples) and abundance at core level followed a statistically significant latitudinal clines, which were both increasing towards higher latitude. These indicates that diversity of rare species and abundance were both higher towards the north. More importantly, I found in this chapter the potential influence not only of local environmental condition but also of regional factors, such as current and climate variations, in the community assemblage pattern of seagrass macrofauna across sites. Also, I found that the community similarity decayed in higher extent compared to those previously reported, particularly in the rocky intertidal. The overall contribution of this dissertation is that it provides advancement in the ecological understanding regarding control of various environmental factors to macrofaunal communities of seagrass beds, ranging from small (local) to broad-scale (regional). This section highlights each of the main findings, discusses their implications, and provides directions for future research.

5.1 Control of local and regional factors on macrofauna in seagrass beds

5.1.1 Macrofaunal patterns across different seagrass species in multispecific seagrass beds

Understanding the role of seagrass species to the faunal communities received little attention (Gillanders 2006). This happens despite the fact that spatial structure of seagrass beds is influenced by morphological architectures of individual seagrasses which differ among seagrass taxa (Kuo and den Hartog 2006). Variation in community patterns can occur at very small spatial scale; changes in seagrass shoot density, cover, canopy, and biomass are known to influence structures of associated fauna. But most of these measures differed across many seagrass species (Vermaat et al. 1995), for instance, large-sized seagrasses have less shoot density but high biomass (*sensu* Duarte & Chiscano 1999), while small-sized seagrasses has a life strategies favoring density over biomass. With ongoing disturbances in seagrass beds, it is important to clarify the role of seagrass species within the bed (Gillanders 2006).

In Chapter 2 of this dissertation, I clarified the role of seagrass species to associated fauna by investigating the diversity and abundance of macrofauna between *Enhalus acoroides*, *Thalassia hemprichii*, and *Cymodocea rotundata* – dominated vegetation in multispecific seagrass beds of Lopez Jaena, southern Philippines. The main finding revealed that despite difference in shoot density and biomass between the vegetation, there was no significant variation in the macrofaunal patterns. This suggests that seagrass taxonomic identity is not a good determinant of macrofaunal diversity and abundance in a multispecific seagrass bed. This was congruent to other studies (e.g., Nienhuis et al. 1989; Edgar 1990; Nakaoka et al. 2001; Brito et al. 2005), although not universally (e.g., Virnstein and Howard 1987; Somerfield et al. 2002; Hamilton et al. 2012; Gartner et al. 2013). Large swamping effects of other parameters, such as sediment quality (e.g., lack of oxygen; Brito et al. 2005), biological traits of the fauna (e.g., larvae of benthic decapods are unable to discriminate between beds with different structural complexities; Bell and Westoby 1986), or combinations of several interacting factors (Edgar 1990; Nakaoka et al. 2001) could have influenced the lack of significant seagrass species-specific effects on macrofauna. Thus, within multispecific meadows, seagrass species redundancy may exist according to the perceptions of macrobenthic invertebrates, even though morphological structures differ strikingly among seagrass taxa.

5.1.2 Macrofauna in seagrass beds lying along a pollution gradient

Human-anthropogenic activities are some of the local disturbances to seagrass ecosystems (Duarte 2002; Orth et al. 2006), but there is little knowledge on how these impact the associated benthic animals. Fish farming are often introduced in shallow coastal waters where important ecosystems such as seagrass bed are found. This often result to degradation or loss of seagrass beds which are important habitat for wide variety of marine fauna including macrobenthic animals. Hence, I expect that the impact of organic pollution to macrofauna in seagrass beds can be mediated through the degradation of seagrass beds. One way to clarify this is to investigate macrofaunal patterns in seagrass beds lying along a pollution gradient. With increasing anthropogenic inputs into our coastal areas, knowledge of how seagrass macrofauna respond to organic pollution has important implication in the management of mariculture activities in shallow coastal waters.

The seagrass beds in shallow coastal waters of Bolinao, northwestern Philippines are negatively impacted organic pollution from farming of milkfish, *Chanos chanos* (Fortes et al. 2012; Tanaka et al. 2014). In Chapter 3 of this dissertation, I showed that the patterns of diversity and abundance of macrofauna in seagrass beds lying along a pollution gradient of that area were altered by organic pollution through reduced species diversity (Shannon diversity), enhanced abundance, changed in community assemblage pattern, and suppression of species heterogeneity. Localized enhancement of species richness and abundance could be due to increased productivity of an impacted seagrass bed (Dimech et al. 2002), or of varying life strategies (Gray 1979). The change in species composition is often influenced by the dominance of specific phyla tolerant to stress, such as the polychaetes (Warwick and Clarke 1994) and molluscs (Diaz and Rosenberg 1995). Patterns of univariate structures (i.e., richness, abundance) were supported by previous reports (e.g., Gray et al. 2002). Nonetheless, the aspect of species heterogeneity that I found in this study is often overlooked in pollution studies, despite its potential impact on ecosystem functioning. I found that organic pollution in seagrass beds reduced the macrofaunal species variability towards the polluted sites (sites near mariculture areas). The suppressed variability indicates reduction in species turnover (beta diversity) that affects ecosystem functioning (Heip 1995; Papageorgiou et al. 2009). Our knowledge on the impact of organic pollution to species variability is currently limited. The results of this study should advance our understanding on the importance of mitigating anthropogenic inputs into shallow coastal waters and the inclusion of aspects of species heterogeneity to environmental impact assessments and the management of seagrass beds confronted by organic pollution.

5.1.3 Macrofauna in seagrass beds across broad spatial scale

There is a growing recognition that community patterns can be clearly explained by broad scale studies (Nakaoka 2005; Nakaoka et al. 2006; Boström et al. 2006). This is because difference in community patterns over large areas are influenced by regional processes or factors (e.g., climate, current) which are quite different to those operating on local scale (e.g., species interaction, habitat heterogeneity) (Nakaoka and Noda 2004). However, this aspect is poorly studied is seagrass-faunal system. More importantly, there is no known broad scale data of macrofauna in seagrass beds of Asia, despite that this region has the highest seagrass species diversity in the world (Green and Short 2003; Short et al. 2007). With the ongoing changes in world’s climate and increasing human-induced disturbances to seagrass ecosystems (Orth et al. 2006), knowledge on the control of regional processes to variation of seagrass macrofauna over large spatial scale is necessary to foray regional biodiversity conservation.

In Chapter 4 of this dissertation, I clarified the broad scale pattern on the diversity and abundance of macrofauna in seagrass beds lying along the coast of Japan, ranging from 24 to 43°N. Some of the main findings showed the increased of species richness and abundance both at core level with latitude, indicating that the number of rare species and abundance of seagrass macrofauna increased towards high latitude. Another important result in this chapter was the variation of species composition across all sites, suspected to be influenced by variations in oceanographic current system and climatic condition, as well as that of localized environmental conditions. It was also expected since more than half of the population of the species (68%) were found restricted on a single site. I also found that while community similarity decayed with distance, the values reported in my study were generally higher compared to other reports, for instance, those from rocky intertidal studies (e.g., Nakaoka et al. 2006). The results in Chapter 4 has important implications in the management of seagrass beds as ecological habitats subjected to local and regional processes. This advances our understanding on the large-scale variation in seagrass faunal associates, which is a necessary step to predict or evaluate community/ecosystem responses against local and regional disturbances.

5.2 Complementarity in the understanding of the influence of multiple environmental scales to macrofauna in seagrass beds

Factors that influence community patterns operates on various scales (Nakaoka and Noda 2004; Nakaoka et al. 2006). Although the studies in this dissertation were not conducted on hierarchical design (*sensu* Nakaoka & Noda 2004; Okuda et al. 2004; Nakaoka et al. 2006), the variation in the responses of macrofaunal patterns across the studies deserves an explanation.

In Chapter 2, the spatial structure in seagrass beds, though difference in seagrass species, did not show important role in macrofaunal patterns. Apart from the main indication of this result, it can further suggest that small-scale alteration in seagrass bed may not necessarily alter or change the macrofaunal patterns. In the large scale meta-analysis of Boström et al. (2006), it was shown that seagrass beds 'spatial patterns/fragmentation on organisms are not likely to be important if habitat patches are abundant and well connected, edge effects are not central to the process/species under study, and movement between suitable habitats is relatively unlimited.' Hence, there are other factors that can be considered as to why difference in seagrass species within a bed appeared to be unimportant.

In Chapter 3, however, the beds showed variation in macrofaunal patterns in response to pollution. This study has a wider spatial scale compared to the first one (Chapter 2), which conforms to Nakaoka and Noda (2004) and Nakaoka (2005) claim that variation patterns can be further explained through expanding the scales of the study. The observation further implies that human-anthropogenic inputs has more serious impact on macrofauna because it can affect many seagrass beds compared to alteration in seagrass spatial structure that is limited within the bed.

In Chapter 4, broad-scale analysis of community patterns by macrofauna that is highly known more diverse and abundant in seagrass beds, has shed light to wider perspectives of ecological patterns which were otherwise difficult to untangle when just dealing on local scale studies (*sensu* Nakaoka and Noda 2004; Nakaoka et al. 2006). Compared to studies in Chapter 2 and 3, this broad-scale study of seagrass macrofauna allowed us to try finding any consistent regional variation and/or latitudinal cline. Or on relating regional processes (i.e., climate, temperature, current system) to observed ecological patterns such that it could provide a cue on what to expect should there be significant changes in the physical factors acting within the marine system.

The importance of the environmental factors examined clearly operates on different scale. Nonetheless, the community patterns observed from the three independent studies (Chapter 2 to 4) are unique responses to the environmental variable being associated to it, accompanied with varying ecological interpretation that complements one from another. Thus, knowledge on the control of local and regional factors to the diversity and abundance of macrofauna in seagrass beds are complementary. Understanding patterns at different scale is indeed a necessary investigative approach in modern ecology (Underwood et al. 2000).

5.3 Conclusion and implications

Based on the main results of this dissertation, I conclude that the patterns of diversity and abundance of macrofauna in seagrass beds are influenced by various factors operating from local to regional scales. The following are my specific implications for management of our seagrass beds:

(1) Seagrasses function as a whole bed and not as an individual seagrass species (Chapter 2). Moreover, in seagrass bed, interdependencies and facilitation among seagrass species occur (Hughes et al. 2009). Hence, to manage faunal biodiversity in seagrass beds at a local scale, it is imperative to consider multiple factors operating on the seagrass beds (*sensu* Hughes et al. 2009) rather than just conserving a specific seagrass species (*sensu* Short et al. 2011).

(2) Organic pollution reduced species heterogeneity, the variability in species composition among cores within station (Chapter 3), but this aspect is poorly understood. Change in species heterogeneity as a result of pollution should be incorporated into environmental impact assessment and management of coastal areas confronted with organic pollution issues.

(3) Regional factors like climate and current system are difficult to control, but these factors could potentially disrupt/alter the local diversity and abundance in seagrass ecosystem. Hence, conservation managers should focus on doable type of coastal management, like managing local disturbances that lead to seagrass degradation or loss (e.g., pollution, overexploitation), so that any ecological consequences resulting from regional disturbances will be minimized.

5.4 Future research directions

Finally, there are five major research directions I identified for future research:

(1) Determine the influence of interacting factors to seagrass macrofaunal community structures. It is interesting to understand, for instance, the influence of organic pollution at broad spatial scale, or to determine if the influence of organic pollution differed across different type of seagrass vegetation.

(2) Consider to evaluate macrofaunal community patterns between specialist and generalist, and between other forms of functional groups (based e.g. on feeding habit, living position, size).

(3) Determine role of different temperate seagrass species (with varying morphological features) to associated benthic fauna through its many range of properties (i.e., density, biomass, cover, leaf architecture, epiphyte biomass).

(4) Use of species heterogeneity as a method to measure the impact of human-induced disturbances to seagrass communities.

(5) Consider variation of seagrass macrofaunal community structures across broad temporal scale to also evaluate emerging seasonality patterns.

5.5 References

Bell JD, Westoby M (1986) Variation in seagrass height and density over a wide spatial scale: Effects on common fish and decapods. J Exp Mar Bio Ecol 104:275–295. doi: 10.1016/0022-0981(86)90110-3.

Boström C, Jackson EL, Simenstad CA (2006) Seagrass landscapes and their effects on associated fauna: A review. Estuar Coast Shelf Sci 68:383–403. doi: 10.1016/j.ecss.2006.01.026.

Brito MC, Martin D, Núñez J (2005) Polychaetes associated to a *Cymodocea nodosa* meadow in the Canary Islands: Assemblage structure, temporal variability and vertical distribution compared to other Mediterranean seagrass meadows. Mar Biol 146:467–481. doi: 10.1007/s00227-004-1460-1.

Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia : A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol an Annu Rev 33:245–303.

Dimech M, Borg JA, Schembri PJ (2002) Changes in the structure of a *Posidonia oceanica* meadow and in the diversity of associated decapod , mollusc and echinoderm assemblages , resulting from inputs of waste from a marine fish farm (Malta, Central Mediterranean). Bull Mar Sci 71:1309–1321.

Duarte CM (2002) The future of seagrass meadows. Environ Conserv 29:192–206. doi: 10.1017/S0376892902000127

Duarte CM, Chiscano CL (1999) Seagrass biomass and production: A reassessment. Aquat Bot 65:159–174.

Edgar GJ (1990) The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with Western Australian seagrass beds. Deep Sea Res Part B Oceanogr Lit Rev 37:1120. doi: 10.1016/S0198-0254(06)80446-2

Fortes MD, Go GA, Bolisay K, et al (2012) Seagrass response to mariculture-induced physico-chemical gradients in Bolinao, northwestern Philippines. Procedings 12th Int Coral Reef Symp 9–13.

Gartner A, Tuya F, Lavery PS, McMahon K (2013) Habitat preferences of macroinvertebrate fauna among seagrasses with varying structural forms. J Exp Mar Bio Ecol 439:143–151. doi: 10.1016/j.jembe.2012.11.009.

Gillanders BM (2006) Seagrasses, Fish, and Fisheries. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands, pp 503–536.

Gray JS (1979) Pollution-induced changes in populations. Philos Trans R Soc London 286:545–561. doi: 10.1098/rstb.1979.0045.

Green EP, Short FT (2003) World atlas of seagrasses. University of California Press.

Hamilton BM, Fairweather PG, McDonald B (2012) One species of seagrass cannot act as a surrogate for others in relation to providing habitat for other taxa. Mar Ecol Prog Ser 456:43–51. doi: 10.3354/meps09647.

Heip C (1995) Eutrophication and zoobenthos dynamics. Ophelia 41:113–136.

Hughes a. R, Williams SL, Duarte CM, et al (2009) Associations of concern: Declining seagrasses and threatened dependent species. Front Ecol Environ 7:242–246. doi: 10.1890/080041.

Kuo J, den Hartog C (2006) Seagrass Morphology, Anatomy, and Ultrastructure. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer Netherlands.

Nakaoka M (2005) Plant-animal interactions in seagrass beds: Ongoing and future challenges for understanding population and community dynamics. Popul Ecol 47:167–177. doi: 10.1007/s10144-005-0226-z.

Nakaoka M, Ito N, Yamamoto T, et al (2006) Similarity of rocky intertidal assemblages along the Pacific coast of Japan: Effects of spatial scales and geographic distance. Ecol Res 21:425–435. doi: 10.1007/s11284-005-0138-6.

Nakaoka M, Noda T (2004) Special feature: Multiple spatial scale approaches in population and community ecology. Popul Ecol 46:103–104. doi: 10.1007/s10144-004-0186-8.

Nakaoka M, Toyohara T, Matsumasa M (2001) Seasonal and between-substrate variation in mobile epifaunal community in a multispecific seagrass bed of Otsuchi bay, Japan. Mar Ecol 22:379–395. doi: 10.1046/j.1439-0485.2001.01726.x.

Nienhuis PH, Coosen J, Kiswara J (1989) Community structure and biomass distribution of seagrasses andmacrofauna in the Flores Sea, Indonesia. Netherlands J Sea Res 23:197–214.

Okuda T, Noda T, Yamamoto T, et al (2004) Latitudinal gradient of species diversity: Multi-scale variability in rocky intertidal sessile assemblages along the Northwestern Pacific coast. Popul Ecol 46:159–170. doi: 10.1007/s10144-004-0185-9.

Orth RJ, Carruthers TJB, Dennison WC, et al (2006) A global crisis for seagrass ecosystems. Bioscience 56:987–996. doi: 10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Papageorgiou N, Sigala K, Karakassis I (2009) Changes of macrofaunal functional composition at sedimentary habitats in the vicinity of fish farms. Estuar Coast Shelf Sci 83:561–568. doi: 10.1016/j.ecss.2009.05.002.

Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Annu. Rev. 16:229–311.

Short F, Carruthers T, Dennison W, Waycott M (2007) Global seagrass distribution and diversity: A bioregional model. J Exp Mar Bio Ecol 350:3–20. doi: 10.1016/j.jembe.2007.06.012

Short FT, Polidoro B, Livingstone SR, et al (2011) Extinction risk assessment of the world's seagrass species. Biol Conserv 144:1961–1971. doi: 10.1016/j.biocon.2011.04.010

Somerfield PJ, Yodnarasri S, Aryuthaka C (2002) Relationships between seagrass biodiversity and infaunal communities: Implications for studies of biodiversity effects. Mar Ecol Prog Ser 237:97–109. doi: 10.3354/meps237097

Tanaka Y, Go GA, Watanabe A, et al (2014) 17-year change in species composition of mixed seagrass beds around Santiago Island, Bolinao, the northwestern Philippines. Mar Pollut Bull 88:81–85. doi: 10.1016/j.marpolbul.2014.09.024

Underwood AJ, Chapman MG, Connell SD (2000) Observations in ecology: You can't make progress on processes without understanding the patterns. J Exp Mar Bio Ecol 250:97–115. doi: 10.1016/S0022-0981(00)00181-7

Vermaat JE, Agawin NSR, Duarte CM, et al (1995) Meadow maintenance, growth and productivity of a mixed Philippine seagrass bed. Mar Ecol Prog Ser 124:215–225. doi: 10.3354/meps124215.

Virnstein RW, Howard RK (1987) Motile epifauna of marine macrophytes in the Indian River lagoon. Florida. I. Comparisons among three species of seagrasses from adjacent beds. 41:1–12.

Warwick RM, Clarke KR (1994) Relearning the ABC: Taxonomic changes and abundance/biomass relationships in disturbed benthic communities. Mar Biol 118:739–744. doi: 10.1007/BF00347523.