THE EFFECT OF SALINITY GRADIENT ON ECOSYSTEM FUNCTIONS AND BIODIVERSITY OF EELGRASS BED COMMUNITIES

By

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To my family and my deceased grandpa, who are always my greatest supporters

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SUMMARY

CHAPTER 1

Marine ecosystem hosts diverse organisms and provides important ecosystem functions. Eelgrass beds, formed by an angiosperm seagrass *Zostera marina*, in coastal environments are among the most ubiquitous and the best-studied marine ecosystems in terms of functions and services in the northern hemisphere including Japan. In this chapter, I reviewed the current knowledge on biotic and abiotic factors affecting biodiversity and ecosystem functions of eelgrass beds, the importance of stress gradients shaped by these factors in eelgrass ecosystems, and the effects of alteration of stress levels in eelgrass beds followed by increasing human impacts and climate change. To investigate the effects of ambient environmental conditions and salinity gradients on primary and secondary productions and biodiversity and to examine the response of eelgrass ecosystems to salinity decline, I outlined the direction of the thesis based on the following two hypotheses; (1) there are differences in the influence of abiotic and biotic factors on productions and biodiversity among eelgrass bed organisms of different functional groups and of lagoons with different salinity gradient steepness, and (2) there are difference in responses to salinity decline and fluctuation among functional groups and organisms from different salinity gradient steepness.

CHAPTER 2

Eelgrass (*Zostera marina*) forms extensive beds in coastal and estuarine environments and provides various ecosystem functions. The aboveground part of eelgrass provides habitats for other types of primary producers such as epiphytic microalgae and for epifaunal invertebrate grazers. Because of the different sizes, generation times and resource requirements, these different types of producers and consumers may be affected by different sets of biotic/abiotic factors over multiple spatial scales. I examined the spatial variations in three functional groups of eelgrass beds (eelgrass, epiphytic microalgae and epifaunal invertebrates) and the abiotic/biotic factors responsible for these variations in three lagoons with different environmental properties at the eastern region of Hokkaido Island, Japan. The spatial scale responsible for the variation in the biomasses of the three functional groups varied, where within-lagoon variation was important for eelgrass and epifauna but among-lagoon variation was important for microalgae. The environmental predictors for the observed spatial variations also differed among the different functional groups, with variation in eelgrass biomass related to depth, nutrient and salinity, epiphytes to water temperature, eelgrass biomass and water column chlorophyll and epifauna mainly to eelgrass biomass. These results revealed that the level of importance of among- and within-lagoon environmental gradients vary in the different functional groups of the eelgrass bed community. The large-scale variation in pelagic productivity, which is tightly related to the ocean current regimes, is likely responsible for the great among-lagoon variation in microalgae. The local variations in environmental factors such as salinity and nutrients, which change with alterations in terrestrial river inputs, are likely related to the great variations in eelgrass and epifauna within the ecosystem. The observed relationship of epifauna with eelgrass biomass indicates the importance of non-trophic plant-animal interactions because epifauna utilize eelgrass as habitat. I therefore emphasize the importance of evaluating spatial variations at multiple scales to further understand the functions of coastal and estuarine ecosystems.

CHAPTER 3

Environmental filtering and dispersal limitation are important processes within the metacommunity concept. Non-random species turnover occurs in places where environmental filtering plays the key role in determining local community structure, whereas dispersal limitation causes nested patterns of species assemblages organized by non-random colonization processes. However, factors that modify the relative importance of these processes remain unclear for many ecosystems. I tested whether salinity gradient affect the relative importance of environmental filtering and dispersal limitation for structuring epifaunal and infaunal communities in three lagoons in Hokkaido, Japan, that have different salinity gradients. Specifically, I compared patterns of species diversity and similarity of eelgrass-associated invertebrate assemblages across space. Beta diversity (i.e., species turnover among different sites in each lagoon) was highest in Akkeshi, the lagoon with the salinity gradients. Variation partitioning of similarity components showed that spatial variation in the community assemblage pattern was mostly explained by environmental filtering in Akkeshi, but that it was explained more by species dispersal patterns and the difference in eelgrass biomass and shoot density in Notoro and Saroma, the lagoons without clear salinity gradient. Redundancy analysis showed that spatial variation in community structure was related to salinity and eelgrass biomass in Akkeshi, and to eelgrass aboveground biomass in Notoro and Saroma. My findings highlight the effects of environmental heterogeneity on beta diversity and community structure and indicate that environmental gradients can be a key factor causing a shift in the relative importance of different metacommunity processes and the role of the foundation species in provisioning habitat.

CHAPTER 4

Understanding factors influencing spatio-temporal variations of productions by inhabiting invertebrates is one of the important goals in coastal marine ecosystems. In addition to spatial and temporal factors, environmental variables such as water temperature and salinity as well as the biomass of primary producers can affect fluctuation of the biomass of invertebrates which can be used as a proxy for production. Identifying the dominant factors as well as the relative importance of multiple factors operated simultaneously to cause the variations is necessary to quantify the productions. In this study, I studied multiple functional groups of inhabiting epifaunal invertebrates classified by their feeding mode; i.e. grazers, predators, and suspension feeders in eelgrass (*Zostera marina*) ecosystems along salinity gradients to 1) evaluate variations in the relative importance of environmental (salinity and water temperature), spatial (difference in salinity measured as distance from river mouth), temporal (months within a year) factors and primary producer biomass (plant biomass factor) on spatio-temporal variations in biomass of grazers, predators, and suspension feeders, and to 2) compare the differences in the patterns between Akkeshi and Notoro, lagoons with and without a strong gradient respectively, for examining how the presence of gradients affects the variations. I used multiple regressions on distance matrices (MRM) followed by variation partitioning to identify the relative importance of each factor on each functional group. The results indicated that the variations of the grazer biomass were most affected by difference in salinity among sites, followed by the temporal factor and biomass of epiphytic microalgae in Akkeshi but not in Notoro. Variation of the predator biomass was explained by water temperature in Notoro but not in Akkeshi. Variation of the suspension feeder biomass was most affected by salinity and microphytobenthos biomass in Akkeshi and by eelgrass biomass in Notoro. I conclude that the relative importance of each factor varied among the functional groups of invertebrates. The influence of the gradients and primary producer biomass indicate the importance of environmental similarity and resource availability on the spatio-temporal variations of secondary productions in coastal marine ecosystems.

CHAPTER 5

Understanding biological community responses to disturbances under climate change is crucial when predicting ecosystem changes and functions. However, it is still unclear how species interaction changes with increasing disturbances, and how disturbance susceptibility varies among organisms with different habitat stress levels is unclear. By focusing on macrophyte (eelgrass *Zostera marina*), epiphytic microalgae, and grazer (the gastropod *Lacuna decorata*) interactions across salinity gradients, I examined whether 1) higher frequency and longer duration of salinity change affected the primary producers, animals, and their interactions to a greater extent, and whether 2) organisms from naturally stressful and unstable sites were more tolerant to the change in salinity. Using mesocosm experiments, I manipulated salinity levels, grazer presence, and organism origin in two sites per each of the two lagoons; Akkeshi with strong salinity gradient, and Notoro with weak gradients. I compared the variations in eelgrass growth, amount of epiphytic microalgae, and grazer survival as well as grazer growth and feeding rates. Eelgrass growth rate was higher with the presence of grazers, varying across salinity gradients only in Notoro. Epiphytic algae abundance was higher with the absence of grazers and with higher salinity stress across all sites. Grazer survival decreased with higher salinity stress, but less so in unstable saline environments. Grazer growth rates also decreased with increased salinity stress. Their grazing rate on eelgrass decreased with higher salinity stress, but grazing on epiphytic microalgae was highest at intermediate stress levels across all sites. These findings suggest the presence of a top-down control of epiphytic microalgae by grazers, which indirectly promotes eelgrass growth that decreases with salinity changes. Hence, increasing stress levels that are expected to occur with ongoing climate change can affect coastal ecosystems such as eelgrass beds through changes in plant-animal interactions, and the stress levels of ambient environments are the keys to understand the potential effects of the disturbances on ecosystem functions.

CHAPTER 6

Based on the findings from foregoing chapters, I conclude that the spatial and temporal variation in the primary and secondary productions and biodiversity of organisms of different functional groups and/or lagoons with different salinity gradient steepness are affected and shaped by different sets of abiotic and biotic factors in eelgrass ecosystems. I also conclude that the variation of stress responses among functional groups and among sites with different ambient stress levels affect the resistance of the ecosystems against potential regime shifts induced by anthropogenic disturbances and climate change. I discuss the findings in this thesis based on how variation in biodiversity and functions of eelgrass ecosystems is shaped by stress gradients and the responses of marine ecosystems to increasing stress levels and potential regime shifts. I also mention how the results of this thesis can be applied to management and conservation practices and to local fisheries activities. In the end, I show the directions of further research by indicating the importance of combining techniques and knowledge such as *in situ* experiments, long-term monitoring, numerical modeling and remote sensing to examine the resilience of eelgrass and other marine ecosystems against regime shifts.

LIST OF ABBREVIATIONS USED

µg	Microgram
mg	Milligram
g	Gram
µm	Micrometer
mm	Millimeter
cm	Centimeter
m	Meter
km	Kilometer
l	Liter
ml	Milliliter
min	Minute
h	Hour
°C	Degrees Celsius
n.d.	No data
Log	Logistic
n	Sample size
ind	Individual(s)
SE	Standard error
SD	Standard deviation
LMM	Linear mixed model
ANOVA	Analysis of Variance
NMDS	Non-metric multidimentional scaling
MRM	Multiple regressions on distance matrices
r	Pearson’s correlation
P	P-value
R^2	Coefficient of determination
df	Degrees of freedom
F	Fischer statistic
AIC	Akaike information criterion
Lat	Latitude
Long	Longitude
°	Degrees
‘	Minutes
“	Seconds
N	North
S	South
E	East
W	West
SOM	Sediment organic matter
Chl-*a*	Chlorophyll a

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CHAPTER 1

GENERAL INTRODUCTION

1.1. Biodiversity and functions of marine ecosystems

Marine environments cover approximately 70% of the Earth's surface and are home to diverse organisms (Pinet 2010). More than 242,000 species have been recorded so far (up to 2018) in The World Register of Marine Species, a major database specialized for marine species (Vandepitte et al. 2018), and the number of newly identified species is increasing to this day. Many of these species are found in specific habitats including coastal to deep-sea and pelagic ecosystems. High biodiversity in the marine ecosystems is highly associated with the provisioning of diverse functions (Duffy et al. 2001; Hewitt et al. 2008). For example, high marine macrophyte diversity is linked to high primary production and nutrient uptakes from the environments (Gustafsson and Norkko 2019). Moreover, diversity in animals such as grazers is known to enhance secondary production (Duffy et al. 2003), which is important for fish yield and indirectly linked to human society by supporting fishery activities.

Coastal marine ecosystems are one of the most studied examples among marine ecosystems in terms of biodiversity and ecosystem functions. The ability of coastal marine ecosystems to support biodiversity and provide various functions is comparable to terrestrial systems such as tropical rain forests (Costanza et al. 1997; Tittensor et al. 2010). For example, Costanza et al. (1997) showed that the economic values of ecosystem services, the term used for ecosystem functions that are useful for human society (Nordlund et al. 2017), of coastal seagrass ecosystems and wetlands are approximately ten times more than that of tropical forest. After the publication of this study, ecosystem functions and services provided by coastal marine ecosystems are increasingly recognized internationally and gained attentions by people from various sectors (Costanza et al. 2020). Functions and services include support of high primary and secondary productions, carbon burial, and protections against coastal erosions, and the needs for accurate evaluation of these functions and services are increasing with their degradation due to coastal development which is associated with increasing human activities along the coastlines of the world (Millennium Ecosystem Assessment 2005; Martínez et al. 2007). The legacy of the study by Costanza et al. (1997) is that it bridged the ecological perspectives to the economic activities and connected ecosystem functions to services. After 20 years of its publication, the synthesis led by Costanza et al. (2020) points out that many nations and management practices are still focused on narrow economic goals such as GDP growth despite the increasing number of studies on ecosystem services and that more integrated systems for connecting the natural ecosystems and human society are essential for the coexistence of the two in the 21st century and for the achievement of UN's sustainable development goals (SDGs).

1.2. Eelgrass bed ecosystems

Seagrass beds are among the most productive ecosystems in the coastal environments (Costanza et al. 1997; Nordlund et al. 2017). Seagrass is a collective noun for angiosperm plants found in marine to brackish environments, and approximately 59 species of seagrass have been identified around the world (Hemminga and Duarte 2000;

Green and Short 2003). Seagrass beds are formed when monospecific or multi-specific seagrass populations occur in intertidal and/or subtidal zones. They are found in both the northern and southern hemispheres and in various climate zones ranging from sub-polar to tropical regions (Green and Short 2003). They are inhabited by various organisms from prokaryotes to large mammals such as dugongs (Hemminga and Duarte 2000).

Eelgrass (*Zostera marina*) bed ecosystem is one of the most ubiquitous and best-studied seagrass ecosystems for evaluating biodiversity and functions they support and provide. Eelgrass beds are found in muddy to sandy seafloor in intertidal to shallow subtidal zones of coasts, estuaries, and lagoons in the temperate northern hemisphere, including North America, Europe, and Japan (Waycott et al. 2009; Duffy et al. 2015). In Japan, they occur widely from Kagoshima to Hokkaido (Nakaoka and Aioi 2001). Eelgrass is an important foundation species of the coastal ecosystem and hosts diverse animals and primary producers by providing habitats and other resources (Duffy 2006). Its ability to maintain biodiversity and provide various ecosystem functions such as support of primary and secondary production has been recognized (Duffy 2006). Moreover, complex plant-animal interactions, such as those among eelgrass, epiphytic microalgae attached to eelgrass, and grazers, have been identified and studied by field observations and *in situ* experiments (e.g. Whalen et al. 2013; Tomas et al. 2015).

1.3. Factors affecting biodiversity and ecosystem functions in eelgrass beds

In eelgrass beds, various abiotic and biotic factors affect spatial and temporal variations in biodiversity and ecosystem functions of primary producers. Beside eelgrass, the ecosystem hosts various primary producers such as epiphytic microalgae and macroalgae on eelgrass blades and microphytobenthos on sediment surface. Seasonal changes in water temperature affect phenology and temporal variations in biomass of eelgrass and associated primary producers, which usually peaks in the summer (Hasegawa et al. 2007; Douglass et al. 2010; Clausen et al. 2014). Moreover, eelgrass biomass as well as community compositions and biomass of epiphytes and microphytobenthos are affected by abiotic factors such as light, nutrients, sediment types, and salinity (Bengtsson et al. 2017; Lefcheck et al. 2017; Schmidt et al. 2017; Whippo et al. 2018; Virta et al. 2019). Besides these bottom-up controls, biotic factors such as top-down controls by grazing organisms including waterfowls and invertebrates, and competition for resources such as light and nutrients among primary producers regulate the amount of production and biodiversity (Nakaoka 2005; Thomsen et al. 2012).

In addition to primary producers, eelgrass beds are inhibited by diverse populations and communities of consumers. In this thesis, consumers refer to heterotrophic organisms that consume primary producers or other heterotrophs (Stites 1999). These consumers are mostly invertebrates, and various abiotic and biotic factors determining the biodiversity and productivity have been studied for decades. For example, abiotic factors such as water temperature and salinity cause variations in community compositions and productions of eelgrass beds in various spatial and temporal scales, ranging from latitudinal to lagoon or estuarine-level variations (Yamada et al. 2007; Momota and Nakaoka 2017; Leopardas et al. 2018) and from diurnal to decadal variations (Howard 1987; Douglass et al. 2010). Moreover, biotic factors such as habitat complexity and plant-animal and/or animal-animal interactions affect the diversity and productions. Higher habitat complexity attributed to eelgrass bed structures such as shoot density and canopy height is linked to more habitats and refugia for invertebrates, resulting in higher biodiversity and production (Orth et al. 1984; Voigt and Hovel 2019). Habitat complexity is also linked to availability of food sources such as epiphytic algae for grazers, and removal of these algae promotes growth of eelgrass, thus affecting direct and indirect plant-animal interactions (Duffy et al. 2015). Animal-animal interactions such as predation are also recognized as factors affecting community compositions and biomass of consumers in eelgrass beds (Moksnes et al. 2008; Baden et al. 2010).

1.4. Importance of stress gradients in eelgrass bed ecosystems

Eelgrass beds are found in estuarine, lagoonal, and coastal environments where various environmental gradients occur naturally, and some of the environmental factors act as stressors to the inhabiting organisms (Hitchcock et al. 2017). Moreover, various types of anthropogenic disturbances and stressors are present in eelgrass beds because of close vicinity to the center of human population growth and development (Unsworth et al. 2015). The presence of spatial and temporal variations in natural and anthropogenic environmental stressors produces stress gradients in ecosystems (Steudel et al. 2012). For example, depth gradient affects distribution of organisms, and the effects of light attenuation along the gradient axis are the main interests of past studies on dynamics and functions of seagrass beds (Krause-Jensen et al. 2003; Schories et al. 2009). The effects of nutrient gradient found in water column and seafloor sediments have been studied extensively for eelgrass beds impacted by eutrophication (Underwood et al. 1998; Ferdie and Forqurean 2004; Hitchcock et al. 2017). Increase in nutrient attributed to excess input of nitrogen and phosphorous induces overgrowing of turf algae and epiphytic microalgae over eelgrass (Baden et al. 2010; Whalen et al. 2013), and as a result, shifts of species compositions in sites with high nutrient stress have been observed along the gradients (Baden et al. 2010; Leopardas et al. 2016).

Fluctuation of stress levels along these gradients affects individuals, populations, and communities as well as functioning of ecosystems (Gough and Grace 1998; Winder and Schindler 2004). At an individual level, environmental stress affects physiological responses of primary producers and consumers of eelgrass beds such as osmoregulation, metabolic processes and survival (Salo et al. 2014; Tanner et al. 2019), and the degree of the effects vary spatially along the gradients (Salo et al. 2014). At a population level, difference in stress levels along the gradient lead to changes in genetic diversity of eelgrass (Salo et al. 2014) and may result in intra-specific variability in the timing of reproduction and growth rate of consumers as observed in other aquatic ecosystems (e.g. Salo et al. 2018), which may ultimately affecting population dynamics. At a community level, stress gradients affect community assembly processes through multiple mechanisms. For example, sorting of species by its functional traits such as stress tolerance and food preference along stress gradient is common in eelgrass ecosystems (Boström et al. 2014). This process, known as environmental filtering, results in variation in community compositions along gradient axes of eelgrass beds (Whippo et al. 2018). Variation in resource availability causes difference in direction and strengths of interspecific interactions along stress gradients were observed in kelp forests and bare-sediment estuarine ecosystems (Bennett et al. 2015; Cheng and Grosholz 2016). Whether or not a similar process occurs in eelgrass beds is an important question to ask for comprehensive understanding of the link between stressors and communities in marine environments.

Salinity gradient is one of the most studied stress gradients in estuaries and coasts where eelgrass beds occur. It occurs in various spatial scales, ranging from several to hundreds of kilometers in estuarine, coastal, and lagoonal ecosystems (Telesh and

Khlebovich 2010) with difference in steepness (Figure 1.1). In addition, temporal variation in salinity gradients is also known. Tidal cycles cause diurnal variation in salinity, and variability in freshwater input from rivers and precipitation is related to fluctuation of salinity levels and alteration of the gradients at daily to yearly basis (van Diggelen and Montagna 2016). Eelgrass can withstand salinity from 5 to above 30 and expand habitats from brackish to marine environments (Boström et al. 2014). In Hokkaido, eelgrass beds are found in lagoons with steep salinity gradients whose salinity ranges from less than 10 to above 30 as well as in lagoons with subtle salinity gradients with salinity range limited to around 30 (Hokkaido Aquaculture Promotion Corporation 2015; Shinomiya et al. 2017; Momota and Nakaoka 2018).

Salinity is a major stressor for organisms inhabiting marine to brackish environments, and the variations in biodiversity such as species richness and abundance along salinity gradients have been studied since Remane (1934) proposed the species minimum concept, showing that species richness decreases from marine to brackish water and again increases at freshwater habitats. Most recent research shows that high β-diversity of ecosystem associated with the presence of salinity gradients (Medeiros et al. 2016), and replacement of species along gradient axes has been connected to the process of environmental filtering (Darr et al. 2014). However, it remains largely unsolved whether changes in species composition along salinity gradients trigger further effects on species interactions and production of ecosystems (Barnes and Ellwood 2012; Barnes and Hendy 2015). Additional studies are needed to reveal detailed effects of salinity gradients on biodiversity and functions of eelgrass ecosystems. This could be effectively done by comparing systems with differences in gradient steepness, where a system with a steep gradient has large differences in stress (i.e. salinity) levels and a system with a marginal gradient has relatively uniform stress levels.

1.5. The effects of alteration of stress levels in eelgrass bed ecosystems

Marine ecosystems including eelgrass beds face multiple stressors and disturbances, including eutrophication, heat waves, water turbidity, and heavy precipitation, which are caused by increasing human activities and climate change (Duarte 2002; Lotze et al. 2006; Duffy et al. 2019). Increase in stress levels affects ecosystems through loss of foundation species and shifts of communities and ecosystems to alternative stable states by both abrupt and persistent changes in environments known as regime shifts (Filbee-Dexter et al. 2014), and these changes have severe impacts on biodiversity and function of ecosystems (Rocha et al. 2018). Alteration of stress gradients in marine ecosystems has been documented through empirical studies and model predictions, and it is recognized as threats to functioning of ecosystems. For example, water turbidity due to sediment inputs from coastal development affects the light availability for photosynthesizing macrophytes along depth gradients, and the range shifts of macrophyte distribution to shallower parts of the gradients have been observed (Shepherd et al. 2009). Another example is the change of salinity gradients by heavy precipitation. Climate change models predict increase in precipitation in many regions of the world (IPCC 2014). Following freshwater discharge into coastal, estuarine, and lagoonal systems is expected to shift existing salinity gradients to be more brackish by decreasing salinity levels and increasing its fluctuation (Vuorinen et al. 2015). Several empirical studies have shown the occurrence of such alteration of the gradient in coastal marine ecosystems after sporadic heavy precipitation and flood (Cloern et al. 2017; Phan et al. 2018). Further effects of salinity change on biodiversity and ecosystem functions are concerned (Vuorinen et al. 2015), and investigations based on more empirical studies are necessary to understand the underlying processes.

Eelgrass ecosystem is threatened by increase in these various stressors and alteration of stress gradients at both local and global scales (Waycott et al. 2009; Duffy et al. 2019). Regime shifts of the ecosystem from macrophyte dominated systems to alternative states that are often dominated by epiphytic turf algae were reported which were due to increase in nutrient levels (Campbell et al. 2017; Schmidt et al. 2017), to increase in water temperature (Hammer et al. 2018) and to decline of salinity (Preen et al. 1995; Campbell and McKenzie 2004; M. Namba, *personal observation*). Salinity decline and fluctuation are concerned with ongoing climate change and potentially affect eelgrass ecosystems. Response of the ecosystem to increase in the stress and change in the stress gradients as well as the processes behind loss of ecosystem functions need to be investigated in the era of climate change and increasing anthropogenic disturbances in eelgrass beds. Moreover, potential resistance of the ecosystem to regime shifts should be addressed to provide insights into the functions of the ecosystems in the future.

1.6. Outline of the thesis

In this thesis, I investigate the effects of salinity gradients on biodiversity and ecosystem functions of eelgrass ecosystems. I specially study the responses of invertebrate consumers and primary producers to the ambient salinity regimes and the changes of salinity stress in eelgrass beds of lagoons with difference in gradient steepness in eastern Hokkaido, Japan. I hypothesize that (1) the factors affecting the spatial and temporal variations of primary and secondary productions as well as biodiversity patterns vary among lagoons with different salinity gradient steepness and among functional groups of organisms, and that (2) the stress responses vary among functional groups and populations of organisms from lagoons with and without a steep salinity gradient and affect species interaction and overall resistance of ecosystems against the stressor.

In Chapter 2, I identify the environmental factors explaining the spatial variations in biomass of three major groups of organisms, namely eelgrass, epiphytic microalgae on eelgrass, and invertebrates living on eelgrass (epifauna), in three lagoons with variable stress gradients. This chapter highlights the importance of incorporating multiple spatial scales into study designs and understanding the effects of environmental factors operated at each scale on primary and secondary productions.

In Chapter 3, I compare community structures and biodiversity of epifauna and infauna (i.e. invertebrates living below seafloor) of eelgrass beds between lagoons with a steep salinity gradient and with a subtle gradient, and I determine the community assembly processes and driving factors of the community variabilities. This chapter emphasizes the differences in underlying processes of community assembly and biodiversity among ecosystems with and without a strong salinity gradient and between epifauna and infauna, which are two functional groups of consumers.

In Chapter 4, I evaluate the relative importance of spatial, temporal, abiotic and biotic factors in explaining the spatio-temporal variations of the consumer biomass as a proxy for production. For the analysis, I use biomass data of three main functional groups of epifaunal consumers from eelgrass bed sites along salinity gradients in two lagoons with different gradient steepness. This chapter identifies the relative importance of simultaneously operating factors to understand what determines the system- and functional group-specific secondary productions at given spatial and temporal scales.

In Chapter 5, given that the previous chapters are based on in-situ field observations for understanding the effects of environmental regimes in the ambient condition, I experimentally measure the effects of increasing stress levels on eelgrass bed communities by manipulating salinity levels and its fluctuation patterns and examine the relationship between the responses of organisms to the stressors and the ambient stress levels of their habitats. This chapter specially examine whether the negative effects of salinity decline on top-down controls by hervibores on plants may trigger the regime shifts in eelgrass ecosystems.

By integrating my findings in foregoing chapters, I address the contribution of my thesis to our knowledge on variation in biodiversity and functions of eelgrass bed ecosystems shaped by stress gradients in Chapter 6. Here, I discuss the connection of the variation and the vulnerability and resistance of ecosystems to stressors and our understanding of responses of eelgrass ecosystems to increasing stress levels and potential regime shifts. Moreover, I suggest the application of the results to conservation and managements of eelgrass beds for the coexistence of coastal societies and the ecosystems.

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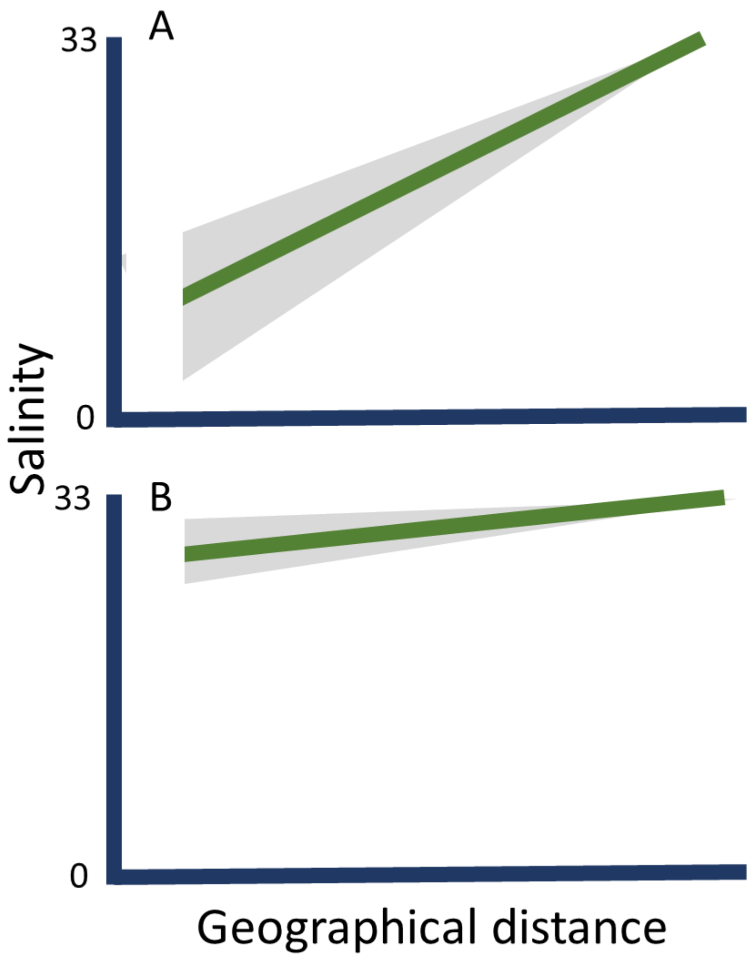

Figure 1.1. Schematic diagram for the concept of steepness in salinity gradients. Here, the geographical distance is equivalent to the distance from river mouth, and thus the zero point indicates the river mouth of each lagoon. (A) is the steep salinity gradient, which has a great temporal (including daily changes due to tides and multi-day changes due to freshwater inputs) average salinity change over geographical distance and (B) is the gentle salinity gradient, which has a small daily average salinity change over distance. Gray areas in the graphs indicate the ranges of the salinity change, and the green lines indicate the average salinity.

CHAPTER 2

SPATIAL PATTERNS AND PREDICTOR VARIABLES VARY AMONG DIFFERENT TYPES OF PRIMARY PRODUCERS AND CONSUMERS IN EELGRASS (*ZOSTERA MARINA*) BEDS

The work presented in Chapter 2 also appears in:
Namba, M., and M. Nakaoka. 2018. Spatial patterns and predictor variables vary among different types of primary producers and consumers in eelgrass (*Zostera marina*) beds. *PLOS ONE* 13:e0201791.

2.1. Introduction

Intertidal and subtidal meadows formed by submerged aquatic vegetation (SAV) are among the most common habitat types in coastal marine ecosystems (Barbier et al. 2010; Seitz et al. 2014), and these ecosystems are often compared to terrestrial forests and grasslands in terms of their ecosystem functions and services (Costanza et al. 1997). Seagrass beds are one of these ecosystems and are found in shallow coastal marine and estuarine environments around the world (Short and Neckles 1999). The ecosystem functions provided by seagrass beds include the provisioning of habitats for diverse fauna and flora through the addition of physical structures to the seafloor (de Groot et al. 2002; Seitz et al. 2014) and the support of high primary and secondary productivity (Duffy

2006), which makes them one of the most productive ecosystems in the world (Duarte and Chiscano 1999). Primary producers in seagrass beds consist of various plant functional groups that are separated in terms of their turnover rates and light and nutrient requirements and include groups such as seagrasses, epiphytic microalgae on seagrass blades, benthic algae and phytoplankton (Moncreiff and Sullivan 2001; Nakaoka 2005). The amount of production by various primary producers differs spatially and seasonally (Burkholder et al. 2007; Hasegawa et al. 2007). The amount of production by epiphytic and benthic algae sometimes exceeds the production by seagrass (Moncreiff and Sullivan 2001). Consumers in seagrass beds are also diverse and consist of small invertebrates such as gastropods, amphipods, shrimps, and annelids, and some vertebrates such as rabbitfish, green sea turtles, manatees and waterfowl (Fredette et al. 1990; Kollars et al. 2017). The invertebrates can be categorized as epifauna or infauna depending on where they inhabit the seagrass beds, and they utilize the food sources and habitats provided by the primary producers, as most of the invertebrates are grazers and detritivores (Duffy et al. 2005).

There have been many studies that have examined the variations in primary producers and consumers in seagrass beds (Hemminga and Duarte 2000; Duffy et al. 2013). Investigations have been conducted on plant-plant interactions, such as the competition for light and nutrients between seagrass and algae (Davis and Fourqurean 2001), and plant-animal interactions, such as the control of plant abundance by herbivores (Duffy et al. 2015; Kollars et al. 2017). For epifaunal consumers such as invertebrate herbivores, the bottom-up effects from plants on animals have been investigated, and these investigations have focused on the relationships among the abundances of associated animals, microalgae and seagrass (e.g., Boström and Bonsdorff 2000; Boström et al. 2006; Shinomiya et al. 2017). The investigations of the top-down effects have focused on the predation pressures on herbivores by predators such as fish (Orth et al. 1984). Such plant-plant and plant-animal interactions are affected by the variations in multiple environmental factors such as temperature, salinity and nutrients (Ferdie and Fourqurean 2004; Yamada et al. 2007).

The different environmental factors that affect the ecosystem functions of coastal habitats, including seagrass beds, operate at different spatial scales. The abiotic factors of ocean currents and associated oceanographic factors (such as water temperatures and nutrient concentrations) affect the community structures of primary producers and consumers over large spatial scales; e.g., 100 km scale (Schils and Coppejans 2003; Blanchette et al. 2006). At the meso scale (e.g., 10 km scale), the presence of temporal and spatial salinity gradients within lagoonal and estuarine ecosystems is common due to the changes in the tidal effects from the ocean and freshwater inputs from rivers (Damme et al. 2005). At this spatial scale, the differences in land-use along the watersheds of the rivers that flow into lagoons and estuaries also cause variations in the amounts of nutrient input (McIver et al. 2015), while variations in physical properties, such as water depth, salinity and hydrodynamic conditions, impact the community structures and the amount of production (Krause-Jensen et al. 2003; Katsuki et al. 2009; Palmer et al. 2011). At fine spatial scales (< 1 km, for example), it is commonly observed that microhabitat heterogeneity and species interactions affect the diversity and abundance of different components of organisms in a community, as mentioned above. Different functional groups of organisms likely respond differently to these environmental variables due to differences in functional traits. Microalgae within seagrass beds have much faster turnover rates than seagrasses (Maranon et al. 2000) and may have different relationships with nutrient concentrations and other environmental requirements, leading to different spatial variation patterns across multiple scales compared to seagrass. Therefore, a simultaneous investigation of different types of primary producers and consumers over multiple spatial scales is necessary to understand how these functional groups interact and structure the highly productive ecosystem. Additionally, how various environmental factors in seagrass beds affect these groups of organisms should be analyzed by comparing multiple seagrass bed ecosystems that are stretched over a large spatial scale, that are different in environmental properties at meso scales, and that environmental heterogeneity is present at fine scales for more precise evaluation of ecosystem functions.

This study aims to investigate the patterns in the spatial variation of each of the major functional groups of primary producers (eelgrass *Zostera marina* and epiphytic microalgae) and consumers (invertebrate epifauna) in eelgrass beds, the most widely distributed seagrass beds in the temperate northern hemisphere (Hemminga and Duarte 2000), and to determine how they are shaped by various environmental factors. First, I determine the most prominent spatial scale of the variability in the biomass of the two groups of primary producers and epifauna by comparing the among-lagoon (20 - 200 km; large scale) and within-lagoon (< 20 km; meso and fine scale, but hereafter we refer to this as fine scale) variation. I then explore the abiotic and biotic factors that are related to the observed variabilities in the biomasses of different functional groups. I study three lagoons on the eastern part of Hokkaido Island, Japan (hereafter eastern Hokkaido) which exhibit differences in the effect of ocean currents and the amount of river inputs. In these lagoons, eelgrass forms extensive beds and supports high primary and secondary production, which benefits the local communities through commercial fishing of seagrass-associated secondary producers such as *Pandalus* shrimp (Mizushima 1981;

Seto et al. 1996; Biodiversity Center of Japan 2008). Here, I hypothesized that the among-lagoon variation would be responsible for the variation in biomass if the organisms are affected more by large scale environmental factors such as the difference in water temperature, whereas within-lagoon variation would be more important if the organisms are affected by the factors associated with finer-scale nutrient or salinity gradients caused by freshwater and terrestrial inputs with river discharges.

2.2. Material and Method

2.2.1. Study area

Eastern Hokkaido faces two different oceans: the Pacific on the southeastern side and the Sea of Okhotsk on the northeastern side (Figure 2.1). The coastal areas are characterized by the presence of multiple semi-enclosed estuaries and lagoons with saline to brackish water. The Oyashio cold current flows in the southwest direction along the Kuril Islands and Hokkaido coast (Saito et al. 2002), strongly affecting the Pacific Ocean side of eastern Hokkaido. The seawater in the Oyashio-influenced area is characterized by cold temperatures reaching < 0 °C in the winter (Saito et al. 2002) and a high concentration of chlorophyll *a* (hereafter Chl-*a*) in the water column during the spring bloom from February to April (Saito et al. 2002; Kasai et al. 1997; T. Isada *personal observation*). In the coastal areas, an autumn bloom from August to October has also been observed (T. Isada *personal communication*). The water mass of the current is rich in NO_3 in the winter but is depleted in the spring when phytoplankton blooms (Kasai et al. 1997). The Soya warm current flows in the Sea of Okhotsk towards the southeast along eastern Hokkaido (Kasai et al. 2010; Kasai et al. 2017). The current dominates in May-October and is responsible for the higher salinities and temperatures than in the Oyashio current (Kasai et al. 2017). In November-April, the effects of the Soya current in the Sea of Okhotsk are weakened, and the East Sakhalin cold current becomes more influential and brings water masses with low salinities and low temperatures (Takizawa 1982). The timing of the spring bloom and nutrient depletion is similar to that in the Oyashio-influenced region, but the coastal areas of the Sea of Okhotsk have lower phytoplankton abundances and nutrient concentrations compared to the Pacific coastal areas (Kasai et al. 1997; Saito et al. 2002; Kasai et al. 2017). Moreover, seasonal sea ice usually covers the Okhotsk coast from January to April (Cavalieri and Parkinson 1987).

For this research, no permit was required for the field sampling as the method used for the study (use of mesh bags) was exempt from the list of fishing gears that need to be declared and used with fishing permits. Moreover, the sampled plants and invertebrates did not include the commercial species or protected species.

Akkeshi

The Akkeshi-ko Lagoon and Akkeshi Bay are located along the Pacific side of eastern Hokkaido (Table 2.1; Figure 2.1). They are connected to each other by a narrow channel that is approximately 500 m wide and 10 m deep. The surface areas of the Akkeshi-ko Lagoon and Akkeshi Bay are 32 km^2 and 110 km^2, respectively. The southern part of the bay is open to the Pacific Ocean. There are three major rivers (Bekanbeushi, Tokitai, and Tobai) that flow into the Akkeshi-ko Lagoon; 98.8 % of the total input to the lagoon is from the Bekanbeushi River (Yamada et al. 2007), and its average daily input accounts for 5.8 % of the total water volume of the lagoon (Iizumi et al. 1995). The watershed of the Bekanbeushi River is mostly covered by wetlands, agricultural lands, and forests. The combination of saline water from Akkeshi Bay that is influenced by the Oyashio current and the freshwater input from the rivers makes the water mass in the Akkeshi-ko Lagoon brackish and is responsible for the various physical and chemical gradients, including the presence of temporal and spatial salinity and nutrient gradients (Iizumi et al. 1995; Yamada et al. 2007).

The water depth of the Akkeshi-ko Lagoon ranges between 0.8 and 1.7 m, and its floor is muddy and covered by mostly eelgrass (*Zostera marina*) except in the intertidal areas where Manila clam (*Ruditapes philippinarum*) aquaculture grounds are present (Kasim and Mukai 2006; Yamada et al. 2007; Momota and Nakaoka 2017). Manila clam and Pacific oyster (*Crassostrea gigas*) farming are the main aquaculture activities in the lagoon (Abe 2016). In Akkeshi Bay, *Z. marina* occurs in the intertidal to shallow subtidal zones where the depth is less than 2 m, and *Z. asiatica* dominates in the deeper subtidal zone where the depth is limited to 5 m below the mean low water (Watanabe et al. 2005).

A total of 6 study sites were established on the shallow subtidal bottom of Akkeshi-ko Lagoon (BK: at the Bekanbeushi River mouth; CK: Chikarakotan; CL: Central Lagoon; HN: Horonitai; SL: South Lagoon; TB: Toubai), and 2 sites were established in Akkeshi Bay (AK: Aininkappu; SR: Shinryu) (Table 2.1; Figure 2.1). See Momota and Nakaoka (2017) for the detailed explanation of each site and Table 1 for the physical parameters.

Saroma

The Saroma-ko Lagoon is located on the northeastern part of Hokkaido and is connected to the Sea of Okhotsk by one channel in the east and another channel in the west (Table 2.1; Figure 2.1). The area of the lagoon is 152 km^2 and has an average depth of 14 m (Katsuki et al. 2009). There are 8 major rivers that flow into the lagoon, with seasonal differences in the freshwater discharges, and the Saromabetsu River in the eastern part of the lagoon is the largest in terms of catchment size (Sato et al. 2007). The watersheds of these rivers mainly consist of forests and agricultural lands. Like the Akkeshi-ko Lagoon, the mixture of seawater and freshwater, as well as the presence of water currents within the lagoon, creates a complex physical environment (Terasaki et al. 2013), yet the nutrient and salinity gradients within the lagoon are not as notable as the ones in Akkeshi-ko lagoon.

Eelgrass beds are found in the shallow subtidal areas near the coast of the Saroma-ko Lagoon, together with other seagrass species, *Z. caspitosa* and *Z. japonica* (Onishi et al. 2001; Katsuki et al. 2009; M. Namba *personal observation*). The bottom of the lagoon is mainly sand. Scallop (*Mizuhopecten yessoensis*) and oyster (*Crassostrea gigas*) farming are the major primary industries in the lagoon. However, hypoxic events due to the excessive inputs of organic matter from the scallop farming sites and the rivers have been occurring in recent years (Terasaki et al. 2013) and are considered to be responsible for the decline of the eelgrass beds over the past decade (Katsuki et al. 2009).

A total of 7 study sites (SA1, 2, 3, 5, 6, 7, and 8) were established in the subtidal part of the Saroma-ko Lagoon (Table 2.1; Figure 2.1), and these sites corresponded to the periodical monitoring sites by the Hokkaido Aquaculture Promotion Corporation (2015).

Notoro

The Notoro-ko Lagoon is located in the south of Saroma-ko lagoon on the Sea of Okhotsk (Figure 2.1) and has a maximum depth of 20 m (Nishino et al. 2014a) and an area of 58.4 km^2 (Nishino et al. 2014b). It is connected to the ocean through a channel that is 324 m side and 13 m deep, and the water exchange rate between the lagoon and the ocean is influenced by tides (Nishino et al. 2014a). Although there are 11 rivers that run into the lagoon, the outfall from Ubaranai River, which is the largest, constitutes only 0.01 % of the total water volume of the lagoon due to the small catchment area (Nishino et al. 2014b). Thus, the effects of the rivers are much smaller than that of the ocean, and this makes both salinity and nutrient concentrations almost uniform within the lagoon (S. Chiba *personal communication*). The salinity of the lagoon is approximately 33, which is similar to the salinity of the Sea of Okhotsk (Imada et al. 1995). The watershed of the Notoro-ko Lagoon is smaller than those of the Akkeshi-ko and Saroma-ko Lagoons, and the environment surrounding the lagoon is mainly agricultural lands, with some forests present on the northeastern shore.

In the Notoro-ko Lagoon, two species of seagrasses, *Z. marina* and *Z. caespitosa*, form beds on the sandy bottoms of the shallow subtidal areas (Shinomiya et al. 2017), and *Z. japonica* occurs in the intertidal areas. The eelgrass beds provide habitat for the shrimp *Pandalus latirostris*, which is the main fishery resource in the lagoon (Shinomiya et al. 2017).

A total of 5 study sites were established in the shallow subtidal part of the lagoon (NO1, 2, 3, 4, 5, Table 2.1; Figure 2.1). A site was not established in the middle of the lagoon as it is deeper than the depth limit of seagrasses.

2.2.2. Field sampling

In the three lagoons, the first set of field sampling was carried out between June 23 and July 13, 2016 (hereafter 'summer') when the eelgrass beds are most productive in this region (Nakaoka and Aioi 2001; Hasegawa et al. 2007). The second set of field sampling was undertaken between August 16 and September 28, 2016 (hereafter 'fall'). Throughout the study, the data obtained from these two seasons were analyzed separately as temporal replicates to assess the reproducibility of the spatial variation. The sampling was conducted during the daytime neap tide so that the effects of tidal currents were minimized. In the summer, SA2 in the Saroma-ko Lagoon was not accessible due to the excess amount of freshwater discharge and subsequent turbidity. In the fall, samples were not collected at SA5 and SA7 in the Saroma-ko Lagoon because the eelgrass at these sites had been decimated by a storm. The water depth in Saroma was deeper than in the other two lagoons, and the depth at SA5 was approximately 3 m. Because of the lack of eelgrass beds in the fall at SA5 where the depth was the deepest, the differences among the average depths in Saroma, Notoro, and Akkeshi were smaller in the fall.

I used memory sensors (AAQ-1186s: JFE Advantech Co. Ltd., Japan, and RINKO-Profiler ASTD102-ALC-R02: JFE Advantech Co., Ltd., Japan) to measure the water temperature, salinity, and water depth at each site. For each site, water samples were collected using a plastic bucket that was washed three times prior to sampling. The water used for the Chl-*a* measurements was collected using a 138.5 ml darkened polyethylene bottle (one sample per site). For nutrient analysis, 200 ml opaque polyethylene bottles were used to collect water (one sample per site). All the bottles containing water samples were brought back to the laboratory in a darkened cooler box filled with ice.

At each site, I haphazardly collected five replicate samples of mobile invertebrates (hereafter 'epifauna') from the aboveground parts of eelgrass using a mesh bag (20 cm diameter, mesh size 0.1 mm) in a circular 0.0314 m^2 area in the middle of the eelgrass beds (Momota and Nakaoka 2017). From these samples, I obtained data on the biomass for both epifauna and eelgrass and then extrapolated the values for $1m^2$ area from the data. Microalgal samples were obtained by collecting five replicate samples of eelgrass shoots per site, and each sample was placed in a separate plastic zip bag (Momota and Nakaoka 2017) and stored in a darkened cooler box until analysis in the laboratory.

2.2.3. Laboratory procedure

The water sample used for nutrient analysis was filtered through a 0.45 μm nylon membrane filter (Millex - HN Filter Unit, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Ireland) into glass vials and frozen at - 20 °C until analysis. The nutrient concentrations, including the amounts of total nitrogen (TN, the sum of NO_2-N, NO_3-N, and NH_4-N) and PO_4-P in the water filtrates, were measured simultaneously using an AutoAnalyzer (QuAAtro 39, BL tec, Osaka, Japan).

The non-acidification method of Welschmeyer (1994) was used to analyze the Chl-*a* concentrations. For the water column Chl-*a* analysis, each water sample was filtered through GF/F glass-fiber filters (Whatman International Ltd., Maidstone, UK). To determine the abundance of epiphytic microalgae from the Chl-*a* concentration, microalgae on each eelgrass sample was scraped off using a glass slide and filtered through the GF/F filters. The filters were then extracted in 6 ml N,N-dimethylformamide for more than one day and stored at −20 °C in the dark until analysis (Suzuki and Ishimaru 1990). The Chl-*a* concentration within each extract was then measured by using a fluorometer (10-AU-005-CE Fluorometer, Turner Designs, Sunnyvale, CA, USA).

The epifaunal invertebrates collected in the mesh bags were scraped from the eelgrass and filtered through a 0.5 mm sieve. The epifaunal samples retained on the sieve were then fixed with 70 % ethanol, and only those with the sizes between 0.5 mm and 8 mm were counted, identified to the lowest possible taxonomic group with a dissecting microscope, and grouped into different size groups for the estimation of the ash-free dry weight using the formulas provided by Edgar (1990b). In total, 19 groups of epifauna were identified to the order, class, or phylum level based on the taxonomic knowledge and information available. All eelgrass shoots were then put in small aluminum foil bags and dried at 60 °C for 48 hr or until completely dried in an oven, and they were weighed to obtain the dry mass.

2.2.4. Statistical analysis

The statistical program R (2018) was used for all statistical analyses. To assess the spatial variation in the biomasses of the different functional groups in the eelgrass bed community and determine the effects of different spatial scales, a one-factor nested analysis of variance with the sites nested in the lagoons was performed for each season and for each of the following variables; eelgrass (g dry weight per unit area: g DW m^{-2}), microalgae (g Chl-*a* per unit area: g Chl-*a* m^{-2}) and epifauna (g ash-free dry weight per unit area: g AFDW m^{-2}). All variables were log-transformed to meet the assumptions of normality and homogeneity. The test was followed by partitioning the variance components using *VCAinference* code in the VCA package (Schuetzenmeister 2016).

To determine the abiotic and biotic factors responsible for the variation in the three variables, linear mixed models (LMMs) with Gaussian distributions were used (Bolker et al. 2009). For the fixed factors, a list of abiotic and biotic candidate variables (water temperature, PO_4-P, TN, depth, salinity, microalgae Chl-*a*, water Chl-*a*, eelgrass biomass) that could affect the eelgrass biomass, abundance of microalgae and epifaunal biomass was made based on previous observations and the literature (e.g. Yamada et al.

2007; Momota and Nakaoka 2017). Collinearity among the variables was then checked by calculating Pearson's correlation coefficients for all pairs. Any variables that had absolute values of the coefficient greater than 0.7 (Dormann et al. 2013) were removed from the list, which resulted in a list of five explanatory variables (TN, depth, water temperature, salinity, and water Chl-*a*) used for the assessment of eelgrass biomass. Microalgae Chl-*a* and PO_4-P were removed from the list due to observed collinearity between salinity and TN, respectively. Different variable combinations were used for the microalgae biomass (TN, depth, water temperature, and water Chl-*a*, eelgrass biomass) and the epifaunal biomass (TN, depth, water temperature, water Chl-*a*, eelgrass biomass, microalgae Chl-*a*) after checking for collinearity among the variables by the abovementioned method. LMM analyses for each of the three functional groups were first carried out to examine all lagoons together (with three random factors: sites nested in the lagoon, lagoon, and season) and then for each lagoon separately (with two random factors: site and season).

The LMMs were fit with the *lmer* function in the lme4 package (Bates et al. 2016), and their P-values were obtained by using the lmerTest package (Kuznetsova et al. 2015). The best model for each of the three producers was chosen based on the AICc, which is Akaike's information criterion (AIC) adjusted for small sample sizes, where AIC and AICc will be equal at large sample sizes (Burnham and Anderson 2004). The AICc was based on restricted maximum likelihood (REML), as the selection was made among the models with nested random factors (Zuur et al. 2009). To determine the components of the variances explained (R^2) by the fixed factors (marginal R^2) and the combination of the fixed and random factors (conditional R^2) (Nakagawa and Schielzeth 2013), the *r.squaredGLMM* function in the MuMIn package (Bartoń 2013) was used. The variables chosen by the best models were then plotted with linear regression lines shown only if there was a significant relationship between the two variables (linear models, $P \leq 0.05$).

2.3. Results

2.3.1. Environmental variables

I expected to see the differences in environmental gradients among the lagoons due to water currents at larger spatial scales and the amount of river discharges at finer spatial scales. The results showed that the difference in water temperature was observed among the lagoons, and that variations in salinity and nutrient concentration were most notable within Akkeshi where the amount of river discharge was largest among the three lagoons.

The water temperature in Akkeshi was lower overall than in Saroma and Notoro in the summer, but it was warmer than in the two lagoons on the Okhotsk coast in the fall (Table 2.1). The average salinity was lower in Akkeshi than in Saroma and Notoro. The salinity varied from 12.3 to 30.9 among the sites in Akkeshi, but it was homogeneous among the sites in Saroma and Notoro (Table 2.1). Four components of the nutrient concentrations varied among ecosystems and among sites within each ecosystem, and the water nutrient concentrations were higher in Akkeshi than Saroma and Notoro (Figure 2.2). In addition, the measured concentrations were more heterogeneous among the sites in Akkeshi than in Saroma or Notoro (Table A1). The water-column Chl-*a* concentration was 2 to 3-fold higher in Akkeshi than in the two lagoons on the Okhotsk coast during both sampling times (Figure 2.2), and the concentrations in Saroma and Notoro were relatively homogeneous compared to those in Akkeshi.

2.3.2. Eelgrass and microalgae

The above-ground dry weight of eelgrass differed among the lagoons (two-way nested ANOVA: summer $F_{2, 74} = 9.49$, $P < 0.001$; fall $F_{2, 72} = 11.16$, $P < 0.001$) and among the sites within the lagoons (summer $F_{16, 74} = 6.38$, $P < 0.001$; fall $F_{15, 72} = 4.88$, $P < 0.001$) in both the summer and fall (Table 2.2; Figure 2.3). The partitioning of the variance components for the nested ANOVA results showed that 50 and 39 % of the variances were attributed to the within-lagoon differences, 45 and 50 % were attributed to the within-site differences, and only 5 and 10 % of the variances were attributed to the among-lagoon differences in the summer and fall, respectively (Table 2.3)

The amount of microalgae biomass differed significantly among the lagoons (two-way nested ANOVA: summer $F_{2, 74} = 80.62$, $P < 0.001$; fall $F_{2, 71} = 233.74$, $P < 0.001$), showing that the abundance of microalgae was much higher in Akkeshi (> 100-fold) than in the two Okhotsk lagoons (Table 2.2; Figure 2.3). The differences among the sites (two-way nested ANOVA: summer $F_{16, 74} = 7.84$, $P < 0.001$; fall $F_{15, 71} = 12.36$, $P < 0.001$) were also prominent, and the overall variations were higher in Akkeshi than in the other two lagoons. In contrast to the results for eelgrass, the partitioning of the variance components indicated that 50 and 70 % of the variances were attributed to the among-lagoon differences, 29 and 21 % of the variances were attributed to the within-lagoon differences, and 21 and 9 % of the variances were attributed to the within-site differences in the summer and fall, respectively (Table 2.3).

2.3.3. Epifauna

A total of 104 taxa belonging to 19 taxonomic groups were collected in this study. Gammaridea were most common in the summer in Akkeshi, followed by gastropoda. In the fall, gastropoda was dominant in most sites in Akkeshi. In the summer in Saroma, caprellidea, bivalva, and gastropoda were abundant, whereas gastropoda was the dominant group in the fall. In Notoro, gastropoda was the group that contributed most to the total biomass both in the summer and fall. See Figure A1 for the taxonomic composition of the epifauna for each lagoon and season.

The epifaunal biomass significantly differed among the lagoons (two-way nested ANOVA: summer $F_{2, 76} = 13.79$, $P < 0.001$; fall $F_{2, 72} = 35.02$, $P < 0.001$) for both seasons (Table 2.2; Figure 2.3). The lowest epifaunal biomass was observed at the sites of Saroma. There were also differences among the sites within the lagoons (two-way nested ANOVA: summer $F_{16, 76} = 5.00$, $P < 0.001$; fall $F_{15, 72} = 10.66$, $P < 0.001$). BK in Akkeshi had almost 30 times higher epifaunal biomass than the rest of the sites in Akkeshi and all sites in Notoro and Saroma. The partitioning of the variance components showed that 14 and 22 % of the variances were from the among-lagoon variations, 38 and 51 % were from the within-lagoon variations, and 48 and 27 % were from the within-site variations in the summer and fall, respectively (Table 2.3).

2.3.4. Factors responsible for the spatial variation

For the eelgrass biomass and microalgal abundance, the combinations of explanatory environmental factors differed in the best models (Table 2.4). Eelgrass was best described by depth (AICc 390.3) when all data from the three lagoons were combined, indicating that the biomass of eelgrass increases with water depth (Figure 2.4). The fixed factor explained 9 % of the total variance of the model, and 54 % of the variance was explained when the fixed factor was combined with the random factors (lagoon, site, and season). When the model was analyzed for each lagoon separately, the combinations of the fixed factors differed among lagoons. TN and salinity best described the eelgrass biomass in Akkeshi (AICc 156.5), the water Chl-*a* concentration and salinity best described the eelgrass biomass in Saroma (AICc 133.7), and depth and salinity best described the eelgrass biomass in Notoro (AICc 59.3).

The microalgae abundance was best described by the combination of water temperature and eelgrass biomass (AICc 468.1), where the microalgae abundance increased with water temperature and eelgrass biomass (Fig 2.5). The two fixed factors together explained 7 % of the total variance of the model, and 81 % of the variance was explained by the fixed factors and three random factors. The best models analyzed for each of the three lagoons selected eelgrass biomass and water Chl-*a* for Akkeshi (AICc 164.1), depth and water Chl-*a* for Saroma (AICc 144.3), and eelgrass biomass and water temperature for Notoro (AICc 141.4).

For epifauna, only the above-ground dry weight of eelgrass was selected as the explanatory variable of the best model (AICc 413.5; Table 2.4). This result showed that the epifaunal biomass increased with eelgrass biomass (Fig 2.6). The above-ground dry weight of eelgrass explained only 4 % of the variance, whereas 57 % of the total variance was explained by the three random factors and the fixed factor. Although not significant, the microalgae abundance was selected for the best model (AICc 170.4) for Akkeshi. TN and water temperature were selected for Saroma (AICc 150.4), and eelgrass biomass was selected for Notoro (AICc 78.3).

2.4. Discussion

In this study, I assessed 1) the spatial scale at which eelgrass, epiphytic microalgae and epifauna vary the most, and 2) the abiotic/biotic factors that affect the observed spatial variability in producers and consumers in the eelgrass beds of eastern Hokkaido based on our hypotheses that that susceptibility of each functional group to these large and fine scale gradients is responsible for the spatial scale in which the differences in the biomass are the largest. I found that the amount of variation assigned to different spatial scales varied greatly among the different functional groups. For eelgrass and epifaunal biomass, the within-lagoon variation was greater than the among-lagoon variation. The factors that were responsible for the variation were related to fine scale environmental variations observed within each lagoon. In contrast, the differences in microalgae abundance were more prominent among lagoons than within lagoon. Furthermore, the combinations of responsible factors also varied among functional groups, suggesting that the processes causing the observed variations are different.

2.4.1. Eelgrass

For eelgrass, the variables for explaining the variation in biomass are related to within-lagoon environmental gradients. Depth was selected as one of the predictor variables in the optimal models for each lagoon where eelgrass biomass was greater in deeper depths, which may be related to the taller canopy heights in deeper habitats. For example, SA5 was the deepest site in the Saroma-ko Lagoon, and the biomass was also highest at this site in the lagoon. For eelgrass, increased water depth limits light availability in the deeper parts of the bed, whereas eelgrass is subject to more intense stresses and disturbances in the shallower parts including the intertidal zone (Krause-

Jensen et al. 2000; Riis and Hawes 2003). Due to the constraints at both ends of the depth gradient, eelgrass biomass is generally maximized in the intermediate zone of its depth distribution (Chambers and Kaiff 1985; Krause-Jensen et al. 2000). The observed pattern in this study indicated that the increase in eelgrass biomass occurred from the shallower limits to the optimum depth because our study sites were in the shallower areas of the subtidal zones where it is unlikely that light availability limits plant growth. Nevertheless, depth had overall a weak contribution in the model for all lagoons combined, and this indicates that it is not the most influential factor to explain the observed spatial variations.

Other responsible variables explaining the variation in eelgrass biomass were nutrient concentrations, the amount of phytoplankton in the water column (water Chl-*a*), and salinity, and these are also related to the environmental gradients within the lagoons. TN was a significant factor only in Akkeshi where nutrient-rich terrestrial water flows from the Bekanbeushi River (Akabane et al. 2003) and also from the Oboro River and the Obetsu River (Nagao et al. 2016). In addition to the terrestrial sources, excess nutrients come from shellfish aquaculture (Abe 2016), and the intensive oyster farming in Akkeshi may somewhat contribute to the presence of the within-lagoon nutrient gradients. Moreover, increase in NH_4^+ flux from sediments in late summer at eelgrass beds in Akkeshi may be responsible for the observed result (Hasegawa et al. 2008). The depletion of marine-originated nutrients at the time of eelgrass growth (Kasai et al. 1997; Kasai et al. 2017) and the lack of both large river inflow and congregated aquacultural activities would explain why TN was not a significant factor in the two Okhotsk lagoons. The amount of water Chl-*a* is influenced by the water column nutrient concentration and can cause light attenuation in eelgrass bed ecosystems especially under eutrophic conditions (Olesen 1996). This competition for the light resources between eelgrass and water column phytoplankton could be related to our observed pattern, and we could expand it to understand indirect negative effects of nutrients on eelgrass in relation to increase in water Chl-*a* for further research on the eelgrass bed ecosystem.

Salinity was selected as a predictor variable that was related to the variation in eelgrass biomass in each of the three lagoons. Similar to the observed nutrient input pattern, the salinity gradient within lagoon was most prominent in Akkeshi due to the presence of the large freshwater input volume from the Bekanbeushi River (Yamada et al. 2007). In contrast, Saroma and Notoro exhibited less obvious salinity gradients because of the strong and uniform tidal influences across the lagoons. Eelgrass can withstand a wide salinity range (Touchette 2007), but the levels of tolerance vary among populations (Salo et al. 2014). More investigations are however required to understand the relationship between eelgrass biomass and salinity gradients.

2.4.2. Microalgae

Unlike eelgrass, the results showed that the difference between the microalgae abundance in Akkeshi and the two sites along the Okhotsk coast (Saroma and Notoro) was great (up to 100-fold higher in Akkeshi), suggesting that the among-lagoon variation was greater than the within-lagoon variation. This difference might be related to the effects of the different ocean currents flowing along the two coasts. The water mass from the Oyashio cold current contained higher nutrient concentrations than that from the Soya warm current during the sampling seasons (Saito et al. 2002; Kasai et al. 1997; Kasai et al. 2017), which was consistent with our observations in the eelgrass beds. Also, the difference in water temperature is created by these two water currents, which is possibly related to the relationship between microalgae and water temperature observed from our results. It is possible that the higher nutrient concentrations along the Oyashio-influenced Pacific coast sustain the higher abundances, or biomass, of microalgae than along the Soya-influenced Okhotsk coast through various mechanisms such as supporting higher turnover rates (Huete-Ortega et al. 2014). Nevertheless, the effect of water temperature on microalgae remains unclear from this study and that the nutrient content was not selected as a predictor in our models, possibly because the nutrients from the ocean were already depleted at the time of sampling, and the rate of nutrient uptake by microalgae is faster than that by eelgrass (Pedersen and Borum 1996).

Although microalgae biomass was best explained by the among-lagoon variation, there were still some effects of environmental variations observed within lagoons. Eelgrass biomass was one of the factors explaining the microalgal abundance in all lagoons except for Saroma. Aboveground eelgrass tissues provide substrata for the attachment of epiphytic microalgae (Gordon et al. 2008), and eelgrass leaf emergence rate is also related to epiphytic algae load (Ruesink 2016). In addition, the negative relationship between the microalgal abundance and water Chl-*a* in two of the studied lagoons were observed. As both functional groups have similar light and nutrient requirements (Sand-Jensen and Borum 1991), the observed pattern may reflect the competitions for these resources among epiphytic microalgae and water column phytoplankton. In Akkeshi, on the other hand, a large portion of the Chl-*a* in the water comes from benthic microalgae that are detached and resuspended in the water column and not from phytoplankton (Kasim and Mukai 2006), and this explain the slight difference in the trend.

2.4.3. Epifauna

The variation partitioning results suggest that scale-specific variation patterns of epifauna are similar to those of eelgrass, in which the spatial variation at the within-lagoon level was much more pronounced than that at the among-lagoon level. The LMM for all lagoons combined, as well as that for Notoro, showed a relationship between epifaunal biomass and eelgrass biomass, suggesting a bottom-up regulation of the abundance of the animals as recorded in previous studies (Momota and Nakaoka 2017; Edgar 1990a). Gustafsson and Boström (2011) reported that the increase in plant biomass that is followed by an increase in seagrass species richness is related to the increase in the associated faunal abundance. Leopardas et al. (2014) presented the effect of aboveground seagrass biomass on epifaunal species composition. The ecosystem functions of an eelgrass bed include both habitat provisioning and the supply of food resources to the epifauna (Barbier et al. 2010; Nakaoka 2005; Nordlund et al. 2017). The epifaunal invertebrate grazers studied here consisted mostly of gammaridea, caprellidea, and gastropoda that directly consume epiphytic microalgae on eelgrass blades (Valentine and Heck 1999; Valentine and Duffy 2006). For these species, it is likely that the eelgrass biomass provides important habitat space and foraging ground (Stoner 1980; Doi et al. 2009; Sturaro et al. 2015), which could explain the observed pattern regarding to eelgrass and epifaunal biomass and the similarity in the spatial variance pattern over multiple scales. As I see from the interaction between microalgae and eelgrass, and the fact that many epifauna feed on microalgae, there are possible implications for a trophic interaction in the studied eelgrass bed ecosystem. Nevertheless, my result does not show a clear link between microalgae biomass and epifauna biomass, and the observed pattern suggests that the plant-animal interactions in the studied eelgrass beds are more influenced by non-trophic interactions, such as habitat provisioning, than trophic interactions.

There were major differences in the taxonomic composition of epifauna within and among the lagoons, and among the temporal replicates. Although the results of the models for all lagoons combined did not show any effects of abiotic factors on the total epifaunal biomass, I found that TN and water temperature were affecting the epifauna in some degree, which may be explained by the differences in the compositions and functions of the epifaunal communities. For example, nutrient gradients caused by regional-scale eutrophication events (Schmidt et al. 2017) as well as regional differences in water temperature (Namba et al. 2018) are some of the known causes of variations in species composition.

2.5. Conclusion

This research fulfills the needs for a local-scale assessment of the functions and services provided by coastal marine ecosystems by examining the spatial variation in the biomasses of different functional groups of plants and animals in eelgrass beds found within three lagoons with unique environmental properties. As expected, differences in the amount of freshwater input and terrestrial runoff from the river input to the lagoons and the current regimes create both among- and within-lagoon environmental variations. Moreover, different patterns of spatial-scale dependency in the variation in the different functional groups, and different combinations of predictors explained the patterns of variation in these organisms were observed from this study. Specifically, I found that those functional groups that exhibit more within- than among-lagoon variations in biomass are influenced by finer scale environmental gradients, and the group that has higher among-lagoon variation is more affected by large scale environmental factors. Thus, it is important to consider and analyze abiotic/biotic variables at multiple spatial scales when assessing the ecosystem functions and services of coastal and estuarine ecosystems, including seagrass beds. Also, future studies should take into account of the differences in environmental properties among various lagoons and other study areas as a same ecosystem can be affected by different combinations of environmental factors based on the properties of the surrounding habitats.

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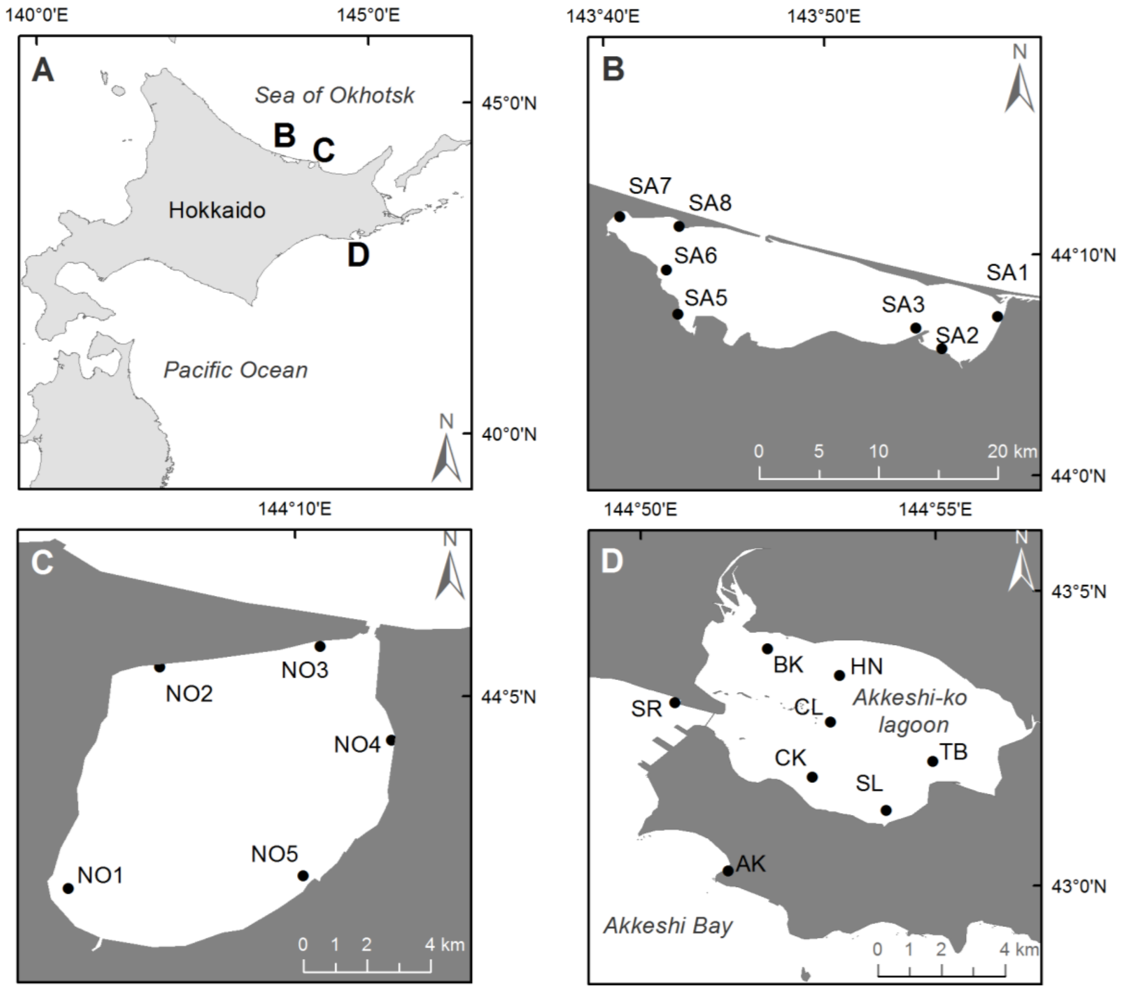

Figure 2.1. Study sites. (A) In eastern Hokkaido at the (B) Saroma-ko Lagoon, (C) Notoro-ko Lagoon, and (D) Akkeshi-ko Lagoon and Akkeshi Bay.

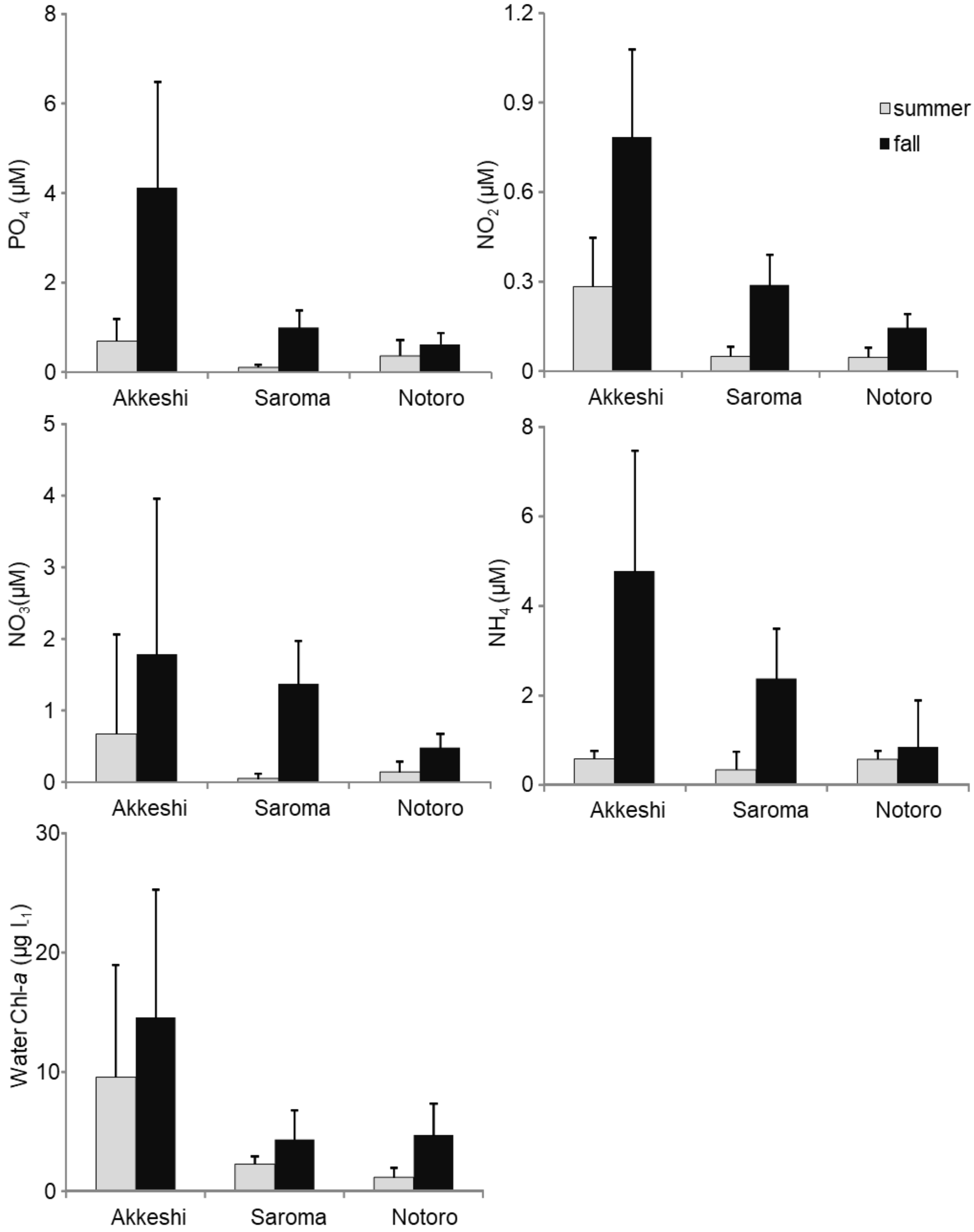

Figure 2.2. Mean (+ SD) concentrations of water nutrients and Chl-a concentrations in the water column. Nutrients include PO4, NO2, NO3, and NH4. n = 8 for Akkeshi in the summer and fall, n = 6 for Saroma in the summer, n = 5 for Saroma in the fall, n = 5 for Notoro in the summer and fall.

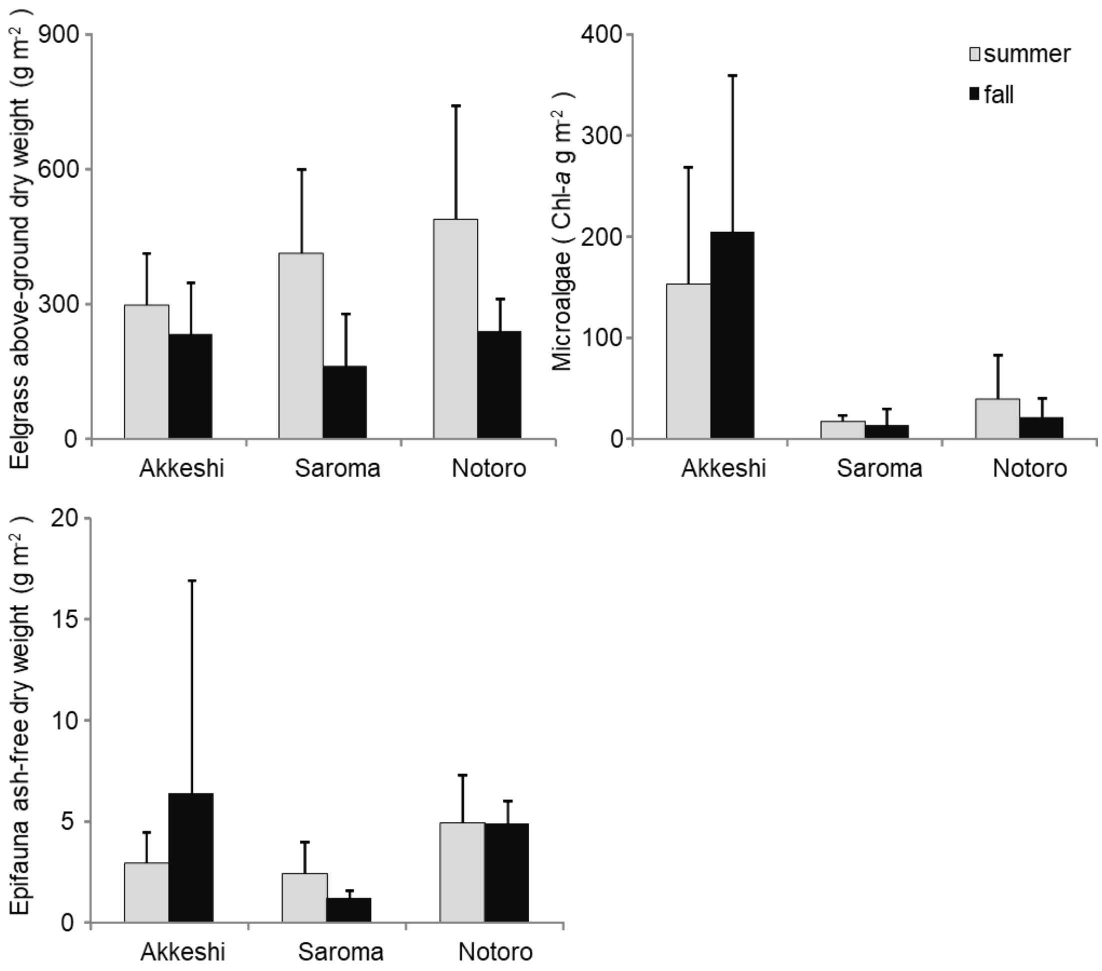

Figure 2.3. Mean (+ SD) biomass of eelgrass, microalgae, and epifauna. n = 8 for Akkeshi in the summer and fall, n = 6 for Saroma in the summer, n = 5 for Saroma in the fall, n = 5 for Notoro in the summer and fall of the above-ground dry weight of eelgrass (g m-2), microalgae weight (g Chl-a m-2), and ash-free dry weight of epifauna (g m-2) at Akkeshi, Saroma, and Notoro for the two temporal replicates (summer and fall). The error bars show the standard deviations.

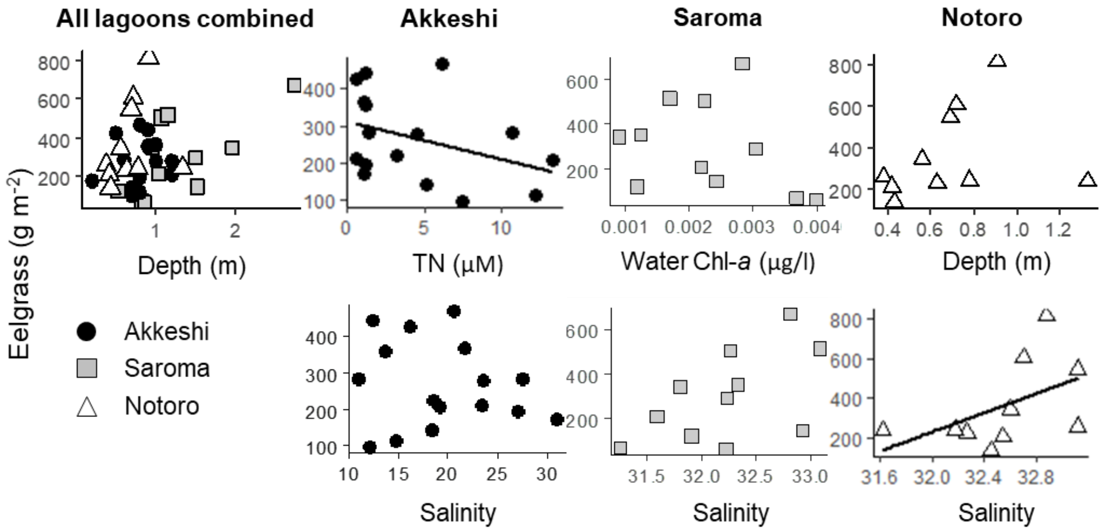

Figure 2.4. Scatterplots showing the relationships between eelgrass (above-ground dry weight, g m^{-2}) and the explanatory variables. The line was obtained from linear regression (only shown if the relationship between the two variables was significant; linear model, $P \leq 0.05$), and the values for the explanatory variables ($P \leq 0.05$) were chosen in the best LMM for all lagoons combined, Akkeshi, Saroma, and Notoro.

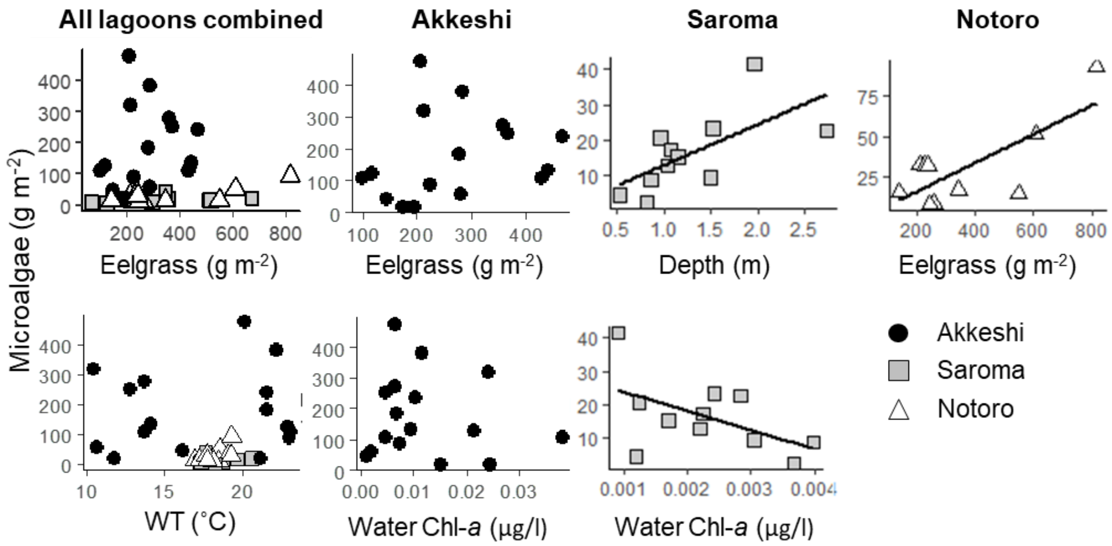

Figure 2.5. Scatterplots showing the relationships between microalgae weight (Chl-*a* g m^{-2}) and the explanatory variables. The line was obtained from linear regression (only shown if the relationship between the two variables was significant; linear model, $P \leq 0.05$), and the values for the explanatory variables ($P \leq 0.05$) were chosen in the best LMM for all lagoons combined, Akkeshi, Saroma, and Notoro.

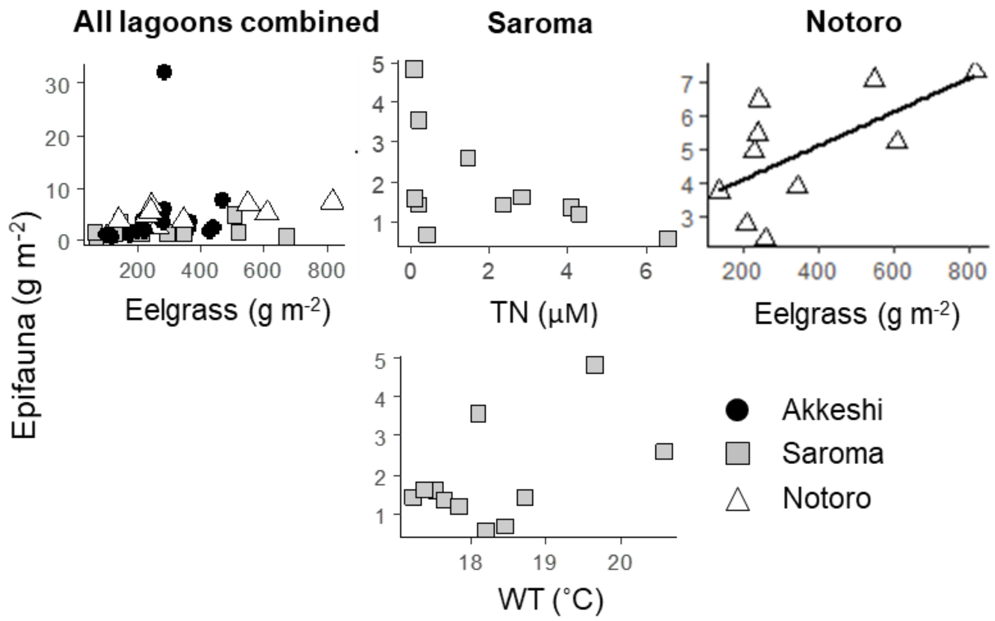

Figure 2.6. Scatterplots showing the relationships between epifauna (ash-free dry weight, g m^{-2}) and the explanatory variables. The line was obtained from linear regression (only shown if the relationship between the two variables was significant; linear model, $P \leq 0.05$), and the values for the explanatory variables ($P \leq 0.05$) were chosen in the best LMM for all lagoons combined, Akkeshi, Saroma, and Notoro.

Table 2.1. Environmental conditions in the summer (June to July) and fall (August to September) 2016 at the study sites in Akkeshi, Saroma and Notoro. n.d. indicates no data available. The water depth is calculated based on the mean lower low water.

Site	Water temperature (°C)		Salinity		Water Depth (m)	
	Summer	Fall	Summer	Fall	Summer	Fall
Akkeshi						
AK	11.8	21.1	30.9	27.0	0.2	0.8
BK	16.1	22.1	18.4	11.0	0.7	0.6
CK	12.8	21.5	21.7	23.5	1.0	1.2
CL	10.6	21.5	27.4	20.6	1.0	0.8
HN	14.1	23.0	12.3	12.0	0.9	0.7
SL	13.7	22.8	16.1	18.6	0.5	0.8
SR	10.4	20.0	23.4	19.2	0.8	1.2
TB	13.7	22.7	13.7	14.8	0.9	0.8
Mean±SD	12.9 ± 1.9	21.8 ± 1.0	20.5 ± 6.6	18.3 ± 5.5	0.8 ± 0.2	0.9 ± 0.2
Saroma						
SA1	18.7	17.6	32.2	31.8	1.5	2.0
SA2	n.d.	17.8	n.d.	31.6	n.d.	1.0
SA3	20.6	17.2	32.3	31.9	1.0	0.5
SA5	18.5	n.d.	32.8	n.d.	2.7	n.d.
SA6	19.7	18.2	32.3	31.3	1.1	0.8
SA7	18.1	n.d.	32.9	n.d.	1.6	n.d.
SA8	17.5	17.4	33.1	32.2	1.3	0.9
Mean±SD	18.8 ± 1.1	17.7 ± 0.4	32.6 ± 0.4	31.8 ± 0.4	1.4 ± 0.6	1.0 ± 0.5
Notoro						
NO1	18.5	17.2	32.7	32.5	0.7	0.4
NO2	19.3	17.7	32.9	32.3	0.9	0.6
NO3	17.0	18.1	33.1	32.2	0.7	0.8
NO4	18.5	19.3	33.1	31.6	0.4	1.3
NO5	19.1	17.7	32.5	32.6	0.4	0.6
Mean±SD	18.5 ± 0.9	18.0 ± 0.8	32.9 ± 0.3	32.2 ± 0.4	0.6 ± 0.2	0.7 ± 0.4

Table 2.2. Mean above-ground dry weight (n = 5) of eelgrass, microalgae, and epifauna. The results for eelgrass (Eelgrass, g m-2), microalgae abundance (Microalgae, Chl-a g m-2), and ash-free dry weight of epifauna (Epifauna, g m-2) in the summer and fall 2016 at the study sites in Akkeshi, Saroma, and Notoro are shown. n.d. indicates no data available.

Site	Eelgrass		Microalgae		Epifauna	
	Summer	Fall	Summer	Fall	Summer	Fall
Akkeshi						
AK	172.4	194.5	21.8	23.0	1.1	1.9
BK	142.5	283.3	47.4	384.0	2.4	31.8
CK	365.6	276.4	252.9	186.8	3.7	3.7
CL	280.2	465.4	61.0	240.8	5.9	7.7
HN	440.2	98.0	135.1	109.8	2.6	1.1
SL	425.6	221.6	108.9	90.0	1.8	1.9
SR	211.5	206.2	321.8	475.6	4.1	2.2
TB	355.3	115.2	276.7	128.1	2.2	0.7
Mean±SD	295.9 ± 157.4	232.6 ± 156.5	147.3 ± 120.0	204.8 ± 161.7	3.0 ± 2.1	6.4 ± 10.6
Saroma						
SA1	292.8	344.7	9.3	41.5	1.4	1.4
SA2	n.d.	209.3	n.d.	12.8	n.d.	1.2
SA3	352.2	122.9	20.7	4.5	2.6	1.4
SA5	670.0	n.d.	22.7	n.d.	0.7	n.d.
SA6	506.2	68.9	17.2	2.4	4.8	0.6
SA7	145.3	n.d.	23.3	n.d.	3.6	n.d.
SA8	516.9	64.4	15.3	8.6	1.6	1.6
Mean±SD	413.9 ± 258.8	162.0 ± 129.8	18.1 ± 13.6	14.0 ± 17.0	2.4 ± 2.0	1.2 ± 0.7
Notoro						
NO1	608.2	138.9	51.4	16.1	5.2	3.7
NO2	816.1	228.9	92.1	32.5	7.3	5.0
NO3	548.4	243.1	15.3	8.9	7.1	6.5
NO4	260.3	239.7	9.0	32.4	2.3	5.5
NO5	210.3	344.3	32.6	17.6	2.7	3.9
Mean±SD	488.7 ± 280.6	239.0 ± 99.6	40.1 ± 42.6	21.7 ± 19.0	4.9 ± 3.5	4.9 ± 2.3

Table 2.3. Variance components (in %) assigned to the different levels by the nested ANOVA. The effects of the lagoons and the sites nested in the lagoons for eelgrass, microalgae, and epifauna for two temporal replicates (summer and fall) are shown.

	Summer					Fall				
Source	df	MS	*F*	*P*	%	df	MS	*F*	*P*	%
Eelgrass										
Lagoon	2	1.78	9.49	> 0.001	4.55	2	3.48	11.16	> 0.001	10.83
Lagoon:Site	16	1.20	6.38	> 0.001	50.03	15	1.52	4.88	> 0.001	38.93
Error	74	0.19			45.43	72	0.31			50.24
Microalgae										
Lagoon	2	35.01	80.62	> 0.001	49.81	2	75.59	233.74	> 0.001	70.01
Lagoon:Site	16	3.41	7.84	> 0.001	29.28	15	4.00	12.36	> 0.001	20.89
Error	74	0.43			20.92	71	0.32			9.10
Epifauna										
Lagoon	2	5.60	13.79	> 0.001	13.60	2	13.86	35.02	> 0.001	22.18
Lagoon:Site	16	2.03	5.00	> 0.001	38.38	15	4.22	10.66	> 0.001	51.27
Error	76	0.41			48.02	72	0.40			26.55

Table 2.4. Results of the LMMs for the best combination of the environmental factors responsible for the variation in each producer group (eelgrass, microalgae, and epifauna). The table shows the results for all lagoons combined (All) and for each lagoon (Akkeshi, Saroma, and Notoro), which were chosen based on AICc. T values with significant *P*-values ($\alpha \leq 0.05$) are in bold.

	Response											
	Eelgrass				**Microalgae**				**Epifauna**			
	All	Akkeshi	Saroma	Notoro	All	Akkeshi	Saroma	Notoro	All	Akkeshi	Saroma	Notoro
T values for Fixed Factors												
(Intercepts)	**13.9**	**8.2**	**-0.7**	**-8.4**	**-1.7**	**4.6**	-2.1	-1.5	**-1.2**	0.4	**-2.8**	**-3.2**
TN		**-4.8**									**-3.9**	
Depth	**4.8**			**8.1**			**5.3**					
Water temperature					**3.2**			1.4			**2.8**	
Salinity		**-3.7**	**0.6**	**8.8**								
Water Chl-a			**-4.1**			**3.6**	**-3.7**					
Eelgrass					**6.7**	**5.1**		**3.1**	**3.8**			**5.7**
Microalgae										1.3		
Random Factors (Variance ± SD)												
Site:Lagoon	0.0 ± 0.2				0.4 ± 0.6				0.2 ± 0.5			
Lagoon	0.1 ± 0.4				1.7 ± 1.3				0.4 ± 0.5			

Site		0.3 ± 0.5	0.3 ± 0.5	0.0 ± 0.0		0.8 ± 0.9	0.0 ± 0.0	0.2 ± 0.5		0.2 ± 0.4	0.3 ± 0.5	0.0 ± 0.0
Season	0.2 ± 0.5	0.0 ± 0.0	0.5 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 1.8	0.1 ± 0.3
Residual	0.4 ± 0.6	0.2 ± 0.5	0.4 ± 0.6	0.0 ± 0.0	0.5 ± 0.7	0.2 ± 0.5	0.5 ± 0.7	0.7 ± 0.8	0.4 ± 0.6	0.3 ± 0.6	0.5 ± 0.7	0.2 ± 0.4
AICc												
Null	397.3	159.8	148.9	93.8	506.0	181.4	160.9	162.8	452.1	171.3	155.6	92.4
Full	403.0	159.0	149.0	81.3	498.9	186.9	166.0	147.9	424.7	199.6	173.6	114.8
Optimal	**390.3**	**156.5**	**133.7**	**59.3**	**468.1**	**164.1**	**144.3**	**141.4**	**413.5**	**170.4**	**150.4**	**78.3**
Marginal R^2	0.09	0.29	0.18	0.66	0.07	0.13	0.44	0.19	0.04	0.03	0.36	0.35
Conditional R^2	0.54	0.65	0.74	0.69	0.81	0.80	0.44	0.41	0.57	0.38	0.92	0.61

CHAPTER 3

THE EFFECT OF ENVIRONMENTAL GRADIENT ON BIODIVERSITY AND SIMILARITY OF INVERTEBRATE COMMUNITIES IN EELGRASS (*ZOSTERA MARINA*) BEDS

The work presented in Chapter 3 also appears in:
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3.1. Introduction

The recognition that biological communities are interconnected, not isolated, systems has led to a rise of investigations into how multiple communities interact with each other, and to the development of the metacommunity concept (Leibold et al. 2004). Metacommunity refers to a set of local communities that are linked by dispersal of interacting species (Wilson 1992; Leibold et al. 2004), and different paradigms of metacommunity have been studied both theoretically and empirically (e.g. Driscoll and Lindenmayer 2009; Logue et al. 2011; Shoemaker and Melbourne 2016). One idea is that community assemblage is explained solely by dispersal limitation as discussed in Hubbell's neutral theory (2001), with several assumptions such as the same dispersal rates

(Jabot et al. 2008). In addition, dispersal and subsequent colonization of species are influenced by habitat size and quality (Thomas 2000). However, dispersal limitation is often outweighed by the effect of habitat (e.g. patch size) or environmental filtering in many ecosystems (Carvajal-Endara et al. 2017). Environmental filtering is a process by which the tolerances of organisms to local abiotic conditions determines the survival, interactions, and successful establishment of a species' population in a given community (Leibold et al. 2004; Kraft et al. 2015; Datry et al. 2016).

Environmental filtering has been shown to play a major role in many ecosystems, especially systems with strong environmental gradients, by affecting community composition among sites (i.e., beta diversity, Anderson et al. 2011; Gascón et al. 2016). Characteristics of environmental gradients in the context of environmental filtering could be explained by various processes, including intermittency (Tornés and Ruhí 2013) and spatial distances in which gradients expand (e.g. Menegotto et al. 2019). Examination of the relative importance of dispersal limitation and environmental filtering on the formation of communities has been conducted in multiple ways, such as comparing the effects of environmental and geographical factors (Legendre et al. 2005; Dray et al. 2012), and by partitioning the component of species turnover and nestedness among local communities to explain community beta diversity (Baselga 2010; Baselga 2012). Here, turnover refers to species replacement of a site to another in a given metacommunity, and nestedness refers to loss of species from a site to another relative to a species pool in a given metacommunity (Baselga 2012; Menegotto et al. 2019). Nevertheless, the relative importance of the two processes may vary among ecosystems or even among different functional groups of organisms within the same ecosystem, each of which are influenced by different abiotic and biotic factors. In order to deepen our understanding of metacommunity processes, it is important to identify the factors that can induce relative shifts from one process to another.

The regulation of beta diversity by environmental factors rather than spatial factors (e.g. geographical distance between sites) is often discussed in relation to the process of environmental filtering (Jamoneau et al. 2018). Salinity is a major environmental filter in coastal ecosystems, such as lagoons and estuaries, and it has been recognized since Remane (1934) proposed the species minimum concept. For example, a decrease/increase in species richness, abundance, and diversity from a saline environment to a freshwater-influenced upstream environment has been observed in multiple aquatic ecosystems including seagrass beds (Palmer et al. 2011; Barnes and Ellwood 2012). In coastal ecosystems, both chemical and physical environments are highly spatially variable because they are located at the edge of marine and terrestrial ecosystems and receive resources delivered by tides, currents and river runoff. This creates different patterns of environmental gradients among systems such as lagoons, and this may cause variations in the spatial patterns of biodiversity among different metacommunities of these systems (Sigala et al. 2012; Prado et al. 2014). Here, local communities (hereafter communities) refer to the communities found in each site within a given system (e.g. lagoon), and a metacommunity refer to the set of these local communities organized in each system. We would expect that environmental filtering would influence beta diversity in a lagoon with environmental gradients, where species turnover, or species replacement, drives community organization (e.g. Yamada et al. 2007). In the absence of environmental gradients, however, spatial factors such as the size of meadows and geographical distances between sites may be a key factor associated with species loss, or nestedness, explained by dispersal processes. Previous studies on marine benthic ecosystems have examined the relative importance of environmental filtering and dispersal limitation on benthic macroinvertebrate communities on unvegetated seafloor (Josefson and Göke 2013) and in seagrass beds (Yamada et al. 2014). However, to my knowledge, studies examining the relationship between the presence/absence of environmental gradients and differences in the strengths of different metacommunity processes have been rarely done to explain the variation in community composition across multiple localities of the same habitats (e.g. eelgrass beds).

Eelgrass (*Zostera marina*) beds are one of the most ubiquitous habitats found in temperate coastal regions of Northern Hemisphere, including semi-enclosed lagoons and estuaries, and they support invertebrate biodiversity (Duffy 2006). In eelgrass beds, diversity and distribution of living organisms are affected by environmental gradients such as salinity (Yamada et al. 2007; Momota and Nakaoka 2018). Benthic invertebrates in eelgrass beds are classified into two functional groups based on their habitats; epifauna inhabit the aboveground parts of eelgrass and infauna inhabit the sediments around the rhizomes and roots of eelgrass. Variation in aboveground eelgrass structure (e.g., shoot biomass and density) greatly affects the abundance and diversity of epifauna, whereas infauna tend to be less affected by belowground structure (Webster et al. 1998; Leopardas et al. 2014). Such differences in habitat requirements and association with eelgrass structures between the two faunal groups may influence how their biodiversity is affected by eelgrass. Some abiotic factors such as salinity would affect both epifauna and infauna (e.g. Yamada et al. 2007; Menegotto et al. 2019), but there are potentials that differences in the physical environments occupied by infauna and epifauna could alter which abiotic factors influence the biodiversity of each group. For example, infauna may be affected by sediment properties such as the amount of sediment organic matter (SOM) as they live belowground (Menegotto et al. 2019). SOM is used as an indicator for carbon stored in sediments and food sources available for organisms (Greiner et al. 2013; Dias et al. 2016). Together, these may lead to the differences in the effect of gradient and processes causing spatial variation in community structure between epifauna and infauna.

This study examines differences in species diversity and community structure of benthic invertebrates in eelgrass beds from three lagoons in Hokkaido, Japan, that vary in terms of presence or absence of natural salinity gradients. I hypothesized that lagoons with salinity gradients would show greater differences in species composition along the gradient axis, and that the dissimilarity of invertebrate communities would be explained by species turnover rather than a pattern of nestedness as a result of environmental filtering. When the contribution of turnover is higher than nestedness, the interpretation is that the changes in community composition occurring within a given lagoon are due to replacement of the set of species among sites as a result of environmental filtering. I also hypothesized that the presence of different dominant species at different places along the gradient would also contribute to higher beta diversity of eelgrass-associated organisms in each lagoon. Finally, I hypothesized that lagoons without gradients would have similar species composition among sites explained by the nestedness, due to dispersal limitation and niche availability, in addition to weak filtering effects. I also expected that the effects of abiotic (salinity and sediment property represented by SOM) and biotic (eelgrass biomass, shoot density, and canopy height) factors would be different between epifauna and infauna.

3.2. Material and Method

3.2.1. Study sites and sampling procedures

To examine the effect of salinity gradient on community structures of epifauna and infauna in eelgrass beds, I sampled eelgrass-associated benthic organisms in three semi-enclosed lagoons (i.e., Akkeshi-ko Lagoon, Notoro-ko Lagoon, and Saroma-ko Lagoon) in eastern Hokkaido, Japan (Figure 3.1; the detailed descriptions can be found in Namba and Nakaoka 2018). In all the three lagoons, eelgrass beds are found in the shallow subtidal zone (0.2 – 1.6 m). Among the lagoons, Akkeshi had the steep salinity gradient, where salinity varied between 12 to 30. Salinity gradient was not detectable in Notoro and Saroma, where most studied sites had salinity of 31 to 33.

Field sampling in the three lagoons was conducted during daytime neap tides between June 23 and July 13, 2016, when the eelgrass beds are most productive at this time of the year (Hasegawa et al. 2007; Nakaoka and Aioi 2001). Eight sites in Akkeshi, five sites in Notoro, and five sites in Saroma were chosen to evenly space each site within each lagoon (Figure 3.1). Because Akkeshi had an eelgrass bed thoroughly in the lagoon, whereas that in Notoro and Saroma is limited to nearshore part of the lagoons, the number of sites was different among the lagoons. Salinity and water temperature data were obtained by conductivity-temperature-depth (CTD) sensors (RINKO Profiler ASTD102-ALC-R02: JFE Advantech Co., Ltd., Japan) at each site (Namba and Nakaoka, 2018). To measure SOM via the loss on ignition technique, I collected three replicate sediment cores at each site. For each sample, a 10 cm-diameter plastic core was inserted into the sediment to 20 cm depth, and the samples were cut into 5 cm intervals in the field and then frozen until analysis. For faunal sampling, I collected five replicate samples of epifauna and five samples of infauna from each site. Invertebrate epifauna attached to eelgrass blades were collected by surrounding the aboveground part of eelgrass (shoots and leaves) with a 20 cm-diameter mesh bag (0.1 mm mesh size). The infauna samples were collected using a 20 cm-diameter plastic sediment core sampler, which was inserted to the seafloor down to 20 cm depth to collect the belowground part of eelgrass (rhizomes and roots) and macroinvertebrate infauna (hereafter infauna). Both the mesh-bag and core samples were brought back to the laboratory and stored in a darkened cooler box until further procedures.

3.2.2. Laboratory procedure

The sediment samples for the loss of ignition test were first transferred into pre-combusted and weighed crucibles and dried at 60 °C for 48 hr. The dried samples were then weighed to measure dry weight and then weighed again after combustion in a muffle oven at 450 °C for 5 hr. The amount of SOM in each sample was calculated by subtracting the post-combustion weight from the dry weight, and an average SOM was calculated for each core across all depths. The percentage of SOM was then calculated.

The mesh-bag epifauna samples were first decanted in a plastic tub and sorted for the aboveground part of eelgrass and epifauna. The epifauna was scraped from the eelgrass using a microscope slide, sieved through a 0.5 mm-sieve and fixed with 70 % ethanol. Only epifauna between the sizes of 0.5 to 8 mm (Edgar 1990) were sorted and identified to the lowest possible taxa. After scraping off the epifauna from the eelgrass, the canopy height (from the tip of the longest leaf to the shoot meristem) of a randomly picked eelgrass shoot from each sample was recorded, and the total number of shoots in each bag was counted to calculate shoot density per 1 m^2 for each site. All the eelgrass shoots were then put into small aluminum bags, placed in a drying oven at 60 °C for 48 hr or until completely dried, and weighted to obtain the dry mass (i.e. above-ground biomass). The sediment core samples containing infauna were sieved through a 1mm-sieve and fixed with 70 % ethanol. For infauna, it was difficult to obtain animal samples using a 0.5 mm-sieve because the samples contained too fine sediment particles. Therefore, I followed the protocols in previous studies (e.g. Leopardas et al. 2018) using 1mm-sieve for infauna sampling. All the belowground parts found in the core samples were sorted, placed in small aluminum bags, and dried in a drying oven at 60 °C for 48 hr or until completely dried (i.e. below-ground biomass).

3.2.3. Statistical analysis

All the statistical analyses were carried out using the software R version 3.5.1 (R Core Team 2018). First, I examined the relationship between salinity gradient and the benthic invertebrate communities by calculating alpha, beta, and gamma diversity of the invertebrate communities based on the species presence-absence data to reflect the effect of rare species and based on Simpson's diversity index to examine the effect of dominant species in the dataset (Krebs 1994; Lande 1996; Miyashita and Noda 2003). In this paper, I defined alpha diversity as species richness and Simpson's diversity index at each sampling site and gamma diversity as those of all sites for each lagoon. Additive beta diversity was then calculated by subtracting the mean alpha diversity (calculated by the mean of the values at each site) of each lagoon from the gamma diversity (Crist et al. 2003; Miyashita and Noda 2003). The pattern of spatial differences in community composition for epifauna and infauna in each lagoon was assessed by using the R package *betapart* (Baselga 2010; Baselga and Orme 2012; Baselga et al. 2018) to calculate the turnover and nestedness components for each set of the community data from the spatial variation in the species assemblages (beta-diversity; but see Almeida-Neto et al. 2012; Baselga 2012). The Jaccard dissimilarity matrix from the presence-absence data and the Bray-Curtis dissimilarity matrix based on the abundance data were used for the analyses. Additionally, I applied redundancy analysis (RDA) followed by the forward step-wise test (Blanchet et al. 2008; Oksanen et al. 2019) for the community data based on Hellinger transformed abundance and presence-absence data (Legendre and Gallagher 2001) to choose environmental factors that may explain the community structures. I then examined if the factors chosen for each lagoon were related to salinity gradient (e.g. salinity) or other factors such as the structure of the eelgrass bed (e.g. shoot density) that might regulate niche availability. Explanatory variables for this analysis were salinity, water temperature, eelgrass above-ground and below-ground biomass, eelgrass canopy height and shoot density, and SOM. Multicollinearity among the variables was checked by pairwise correlations of the all variables prior to the analyses (threshold: $P < 0.7$; Dormann et al. 2013), and all the explanatory variables were normalized (a mean of 0 and standard deviation of 1) prior to analysis. RDA was carried out using the R package *vegan* (Oksanen et al. 2019).

3.3. Results

Environmental conditions and eelgrass bed properties for each site are summarized in Table 3.1 (also see Namba and Nakaoka 2018). Overall, 104 species of epifauna and 37 species of infauna were found across all samples (see Table S1 of the published version of the chapter). The total number of species (gamma diversity) of epifauna in each lagoon was highest in Akkeshi (72 species), followed by Notoro (54 species) and Saroma (44 species), and gamma diversity for infauna was the same between

Akkeshi and Notoro (21 species) and lowest in Saroma (14 species) (Table 3.2). Alpha diversity of epifauna, or the average number of species at each sampling site, was highest in Notoro followed by Akkeshi and Saroma based on species presence-absence data, and it was highest in Akkeshi based on Simpson's. For infauna, alpha diversity was highest in Akkeshi followed by Saroma and Notoro based on species presence-absence, and it was highest in Notoro based on Simpson's. Additive beta diversity was highest in Akkeshi, followed by Saroma and Notoro for epifauna, and highest in Notoro, followed by Akkeshi and Saroma for infauna.

Gamma diversity by Simpson's index for epifauna was higher in Akkeshi than Notoro and Saroma, but there were little differences among the three lagoons for infauna. For epifauna, alpha, gamma, and additive beta diversity calculated from Simpson's diversity showed that all three diversity measures were highest in Akkeshi, followed by Saroma and Notoro (Table 3.2). Negative beta diversity in Notoro is due to lower total diversity than average diversity within the communities, as mentioned in Lande (1996). For infauna, alpha diversity was highest in Saroma, followed by Akkeshi and Notoro, and gamma diversity and additive beta diversity were highest in Notoro, followed by Akkeshi and Saroma.

The percentage contribution of turnover and nestedness components differed among the three lagoons and between the two functional groups (Table 3.3). For both epifauna and infauna, the results based on the presence-absence data (Jaccard index) showed that the percentage of the turnover component was higher than the nestedness component in most of the cases. The results based on the abundance data (Bray-Curtis index) showed more lagoon-specific trends. For epifauna, the contribution of nestedness was lower than turnover in all the lagoons. Turnover was highest for Akkeshi, followed by Saroma and then Notoro. Notoro had equal contributions of nestedness and turnover. For infauna, the percentage contribution of turnover was highest for Akkeshi, followed by Notoro and then Saroma. In Saroma, the percentage of the nestedness component was much higher than that of the other two lagoons.

The results from RDA for the presence-absence data (Figure 3.2; Table 3.4) showed that different environmental variables explain the community composition of epifauna and infauna in each lagoon. For the presence-absence data, RDA showed associations between epifauna/infauna and factors related to salinity gradients, sediment properties, and eelgrass structures. Epifauna in Akkeshi was associated with salinity, SOM, and both eelgrass biomass and shoot density. Infauna in Akkeshi was associated with salinity and canopy height as well as below ground biomass and SOM. Epifaunal communities in Notoro and Saroma were explained by shoot density, canopy height, and biomass of eelgrass. Interestingly, RDA showed that infaunal communities in Notoro and Saroma were also associated with canopy height, shoot density, biomass, yet none of these was statistically significant in the best fit models. In most cases, except epifauna for Akkeshi, shoot density, above-ground biomass, and canopy height seem to be correlated (also in RDA for the abundance data). Although multicollinearity for these variables had been checked, we expected that there are connections among them as they all represent the characteristics of above-ground part of eelgrass.

RDA for the abundance data (Figure 3.3; Table 3.5) showed the results different from the results for the presence-absence data. Epifaunal communities in Akkeshi were associated with all of salinity, sediment properties, and eelgrass structures including biomass, shoot density, and canopy height. For infauna in Akkeshi, above-ground biomass, salinity, and SOM explained most of the variations. Both epifauna and infauna in Notoro and Saroma, eelgrass structures including shoot density, canopy height and biomass were associated with the community organization, yet no variable related to salinity or sediment properties was associated with them.

3.4. Discussion

This research explored the relationship between multiple environmental factors and within-lagoon variation in community structure and revealed that the presence or absence of environmental gradients can influence the relative importance of environmental filtering and dispersal limitation for structuring local assemblages within a metacommunity. In this study, I analyzed the community structure of epifauna and infauna, two functional groups with different habitat requirements in eelgrass beds, within three lagoons that differed with respect to salinity gradient, sediment properties, and eelgrass structures. The diversity values for epifauna and infauna were lagoon-specific, and the way the community changes over space differed between Akkeshi and the other two lagoons. Our results supported the hypothesis that the presence of salinity gradients correlate with larger variation in species composition of epifaunal communities with higher species turnover than lagoons without salinity gradients. In Akkeshi, the patterns observed for epifaunal communities were similar to that of infauna, and the results from the species presence-absence-based analysis with Jaccard dissimilarity matrix did not greatly differ from the results from the abundance-based analysis with Bray-Curtis dissimilarity matrix.

Higher environmental heterogeneity often has a positive relationship with biodiversity as it promotes niche differentiation, which allows coexistence of a wide range of species with diverse functions (Amarasekare 2003; Hewitt et al. 2008). I observed that, in a lagoon with a strong salinity gradient created by river input, sites near the river mouth have lower salinity and also higher daily salinity fluctuations, whereas sites that are far from the river mouth have lower salinity fluctuations (Yamada et al. 2007; Momota and Nakaoka 2018). This heterogeneity in terms of salinity fluctuation would make more niche space available to organisms with different salinity tolerances and therefore contribute to increased biodiversity (Van Diggelen and Montagna 2016). My results were congruent with this idea, showing higher beta diversity in Akkeshi for both species richness-based and Simpson's diversity index-based analyses versus the other two lagoons without salinity gradients. Moreover, different distribution patterns among organisms with different salinity tolerances leads to species turnover, making environmental filtering by salinity the most significant factor for community organization in lagoons with salinity gradients (Baden and Boström 2001; Barnes and Ellwood 2012; Prado et al. 2014).

For epifaunal community composition in Akkeshi, salinity was the only variable included in the best RDA model for the abundance data, and the community patterns were most strongly explained by the axis with a decreasing salinity gradient. This could be related to variation in tolerance to salinity levels among species with different functional traits. In the case of epifauna, highly mobile species such as amphipods can freely move between habitats (Yamada et al. 2007), yet they have optimal environmental conditions and thus differences in species composition occur along the gradient axis (Cloern et al. 2017). The distribution of less mobile species tends to be regulated by environmental factors including salinity, as it is more difficult for those species to move as adults among spatially distant habitats (Ushakova 2003). As organisms with different functional traits exhibit distribution patterns in lagoons with salinity gradients, beta-diversity in these systems becomes high.

I also saw that epifaunal species presence/absence is related to the abundance of eelgrass. Higher amount of eelgrass could host higher number of species as eelgrass beds provide habitats and foraging ground for the epifauna (Edgar and Robertson 1992; Duffy et al. 2001). Previous studies also showed some redundancy in presence/absence of the observed species among the study sites in Akkeshi (Yamada et al. 2007; Momota and Nakaoka 2018) and other places (e.g. Whippo et al. 2018) as patches of eelgrass meadows are interconnected within a given system. Site-specific community compositions can be determined not only by presence/absence of species but also by abundance of dominant species (Whippo et al. 2018). Therefore, the results for both abundance-based and presence-absence-based RDA should be considered and that both salinity gradient and the amount of eelgrass habitats are the important components for determining the identities of species and their relative abundance.

For infauna in Akkeshi, I found that the contribution of turnover was similar to that of epifauna. I saw that the infaunal communities from different ecosystems are affected by abiotic factors such as salinity (Josefson and Göke 2013) and nutrients (Borum 1985; Gascón et al. 2016) as well as by biotic factors such as quality of food sources (e.g. Fujii et al. 2018). In case of infauna in eelgrass bed, we would expect that greater influence of salinity on their distribution patterns along the gradient axis in a lagoon because of their limited mobility below seafloor as adults. This lack of movement may contribute to the greater selection for salinity tolerance in fluctuating salinity environment like lagoons. My results from RDA for the abundance data showed that salinity and SOM best explain the community composition. Previous studies (e.g.

Josefson and Göke 2013) have shown that high species turnover was observed along the salinity gradient axis, indicating that salinity variability not only influences water column fauna but also infauna. In coastal environments, pore water salinity is often correlated with water column salinity (Miklesh and Meile 2018). Therefore, epifaunal and infaunal communities may experience the same salinity gradient. SOM was another component explaining the community composition of epifauna and infauna in Akkeshi. This may be related to the fact that organic matter in sediment is a primary food source for some functional types of infauna, including detritus-eating polychaetes (Dias et al. 2016). The amount of SOM is positively associated with distance to the river mouth, as sites close to the river receive organic materials carried from terrestrial ecosystems (Watanabe and Kuwae 2015). Therefore, lagoons with salinity gradients may also have similar gradients of SOM, which will affect the infaunal communities. Further investigation is needed to disentangle the simultaneous effect of multiple environmental gradients as several studies show the combined effects of factors such as nutrients and salinity on community compositions and functions in marine benthic ecosystems (Borum 1985; Hitchcock et al. 2017). By contrast to previous studies (e.g. Webster et al. 1998), RDA for the presence-absence data indicated that infaunal diversity was associated with below-ground biomass of eelgrass. Rhizomes and roots of seagrass could retain sediments and potential food sources for infauna in the form of SOM more than bare sediments could (Leduc and Probert 2011). I saw that while the species richness of infauna was affected by the presence of habitats and potential food sources, the abundance and dominance were regulated by the salinity gradients as the ability of a species to tolerate and thrive in the specific conditions vary.

The results from Notoro and Saroma showed that alpha diversity calculated from species richness for Notoro was higher than Saroma, but the diversity calculated from Simpson's diversity index was higher in Saroma than Notoro for both epifauna and infauna. This difference may be explained by the presence of dominant species, such as several species of bivalves in these two lagoons (e.g. *Arcuatula senhousia* for epifauna, *Limecola contabulata* for infauna). Thus, there were smaller differences in the abundance of dominant species and rarer species at the local scale in Notoro than in Saroma. Higher additive beta diversity and gamma diversity in Notoro versus Saroma for epifauna and infauna could be related to the patterns of alpha diversity as mentioned above, where high local biodiversity affects the similarity between sites and the community within the lagoon. Higher gamma diversity of infauna in Saroma based on species richness indicated that more rare species are present in the lagoon, possibly related to its area as Saroma is more than twice as large as Notoro.

The components explained by species turnover were lower in Notoro and Saroma than Akkeshi for both epifauna and infauna based on species count and abundance. I observed that the contribution of nestedness varied between the lagoons, but interestingly, nestedness was higher in epifauna at Notoro and in infauna at Saroma. Nestedness is the outcome of the formation of a subset of species at a diversity-poor site including species from more diverse sites by limited dispersal (Ulrich et al. 2009), or variation in tolerance to gradients among species (Menegotto et al. 2019). Although species turnover led by environmental filtering is more commonly observed in aquatic ecosystems with heterogeneous environments (Medeiros et al. 2016; Jyrkänkallio-Mikkola et al. 2018), the effect of environmental filtering on community assemblages is weaker in these uniform lagoons than Akkeshi-ko lagoon, which has a salinity gradient.

Our RDA results did not include salinity as a significant determinant of community structure of either epifauna or infauna in Notoro or Saroma. This suggested that the distribution patterns of inhabiting organisms are not strongly correlated with individual tolerance to salinity stress. Instead, eelgrass above-ground biomass and eelgrass shoot density were the factors mainly explaining the community variations based on abundance. High dependency of epifauna on eelgrass above-ground parts as habitats makes them more sensitive to the variation in eelgrass abundance (Orth et al. 1984). The abundance and structure of eelgrass can thus affect the community structure of invertebrate assemblages as it is directly related to their carrying capacity (Thomas 2000; Mills and Berkenbusch 2009). The nestednesss of the communities in the two lagoons could be related to the dependency of epifauna on eelgrass bed structures, as spatial dispersal related to the habitat abundance of eelgrass beds is likely more influential than salinity to the epifauna. Therefore, biological structures, such as the abundance and biomass of plants, could be important factors for epifaunal community patterns in the absence of obvious abiotic gradients.

For infauna, variables related to eelgrass bed structure, such as biomass, canopy height, and shoot density were the strongest predictors of community composition based on presence-absence and abundance data in the full RDA, though none of these were statistically significant. Interestingly, variables related to sediment properties were not included in the results. Compared to Akkeshi, where various species of detritus-eating polychaetes were present and abundant, Notoro and Saroma tended to have more bivalve species (see Table S1 of the published version of this chapter). While detritus-eating polychaetes are known to feed on organic matter in the sediment and thus more depending on the amount of SOM (Dias et al. 2016), the filter-feeding bivalves feed on both benthic and water column phytoplankton (Kasim and Mukai 2006) and thus not only depending on SOM. This difference in feeding behavior and dependency on SOM between polychaetes and bivalves could explain why SOM was not a strong explanatory variable for the infaunal communities in Notoro and Saroma. Infauna communities living in the absence of strong abiotic gradients, spatial factors and other factors such as the size of species pool in the source population or the distance of dispersal would affect the community compositions, yet more investigations would be needed in future studies.

3.5. Conclusion

The present study highlights that the presence or absence of environmental gradient affects the community organization processes of eelgrass-associate invertebrate communities in lagoonal environments. Environmental filtering caused by variation in salinity plays a more important role in lagoons with salinity gradients, and results in higher species turnover among invertebrate communities within a lagoon by enhancing the beta species diversities. In contrast, the influence of environmental filtering is weakened in lagoons without salinity gradients as shown in relatively lower turnover rates and higher nestedness components. Here, variation in eelgrass structure has stronger influence on diversity of associated fauna. Elucidating which metacommunity processes are most influential in different environmental contexts across space and time will deepen our understanding of community organization in coastal ecosystems.

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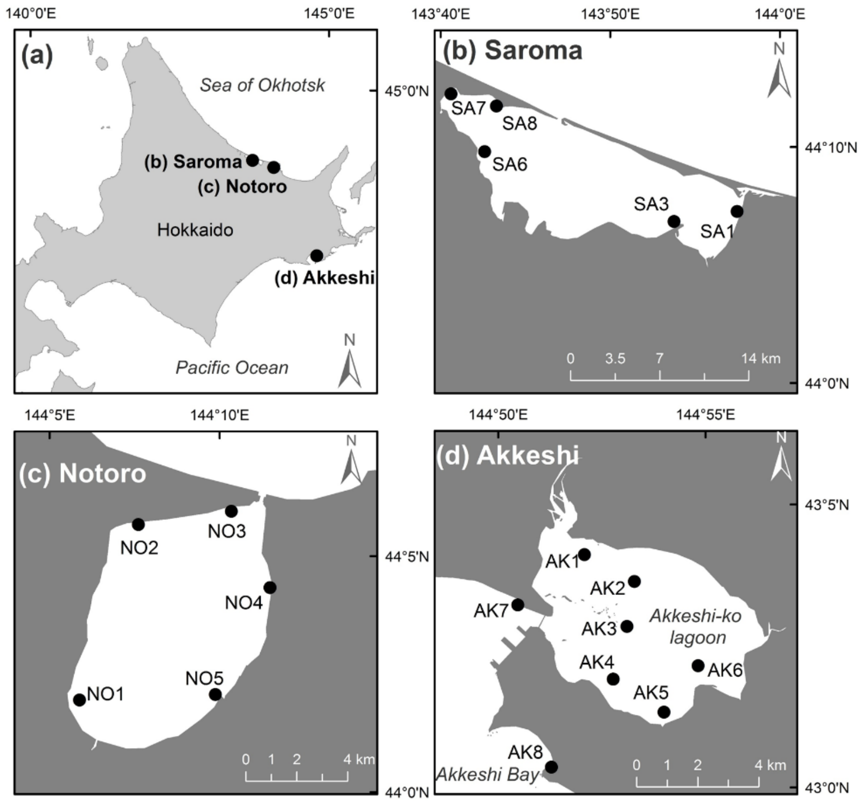

Figure 3.1. (a) Relative locations of the three lagoons and study sites in each of (b) Saroma, (c) Notoro, and (d) Akkeshi as indicated in the black dots.

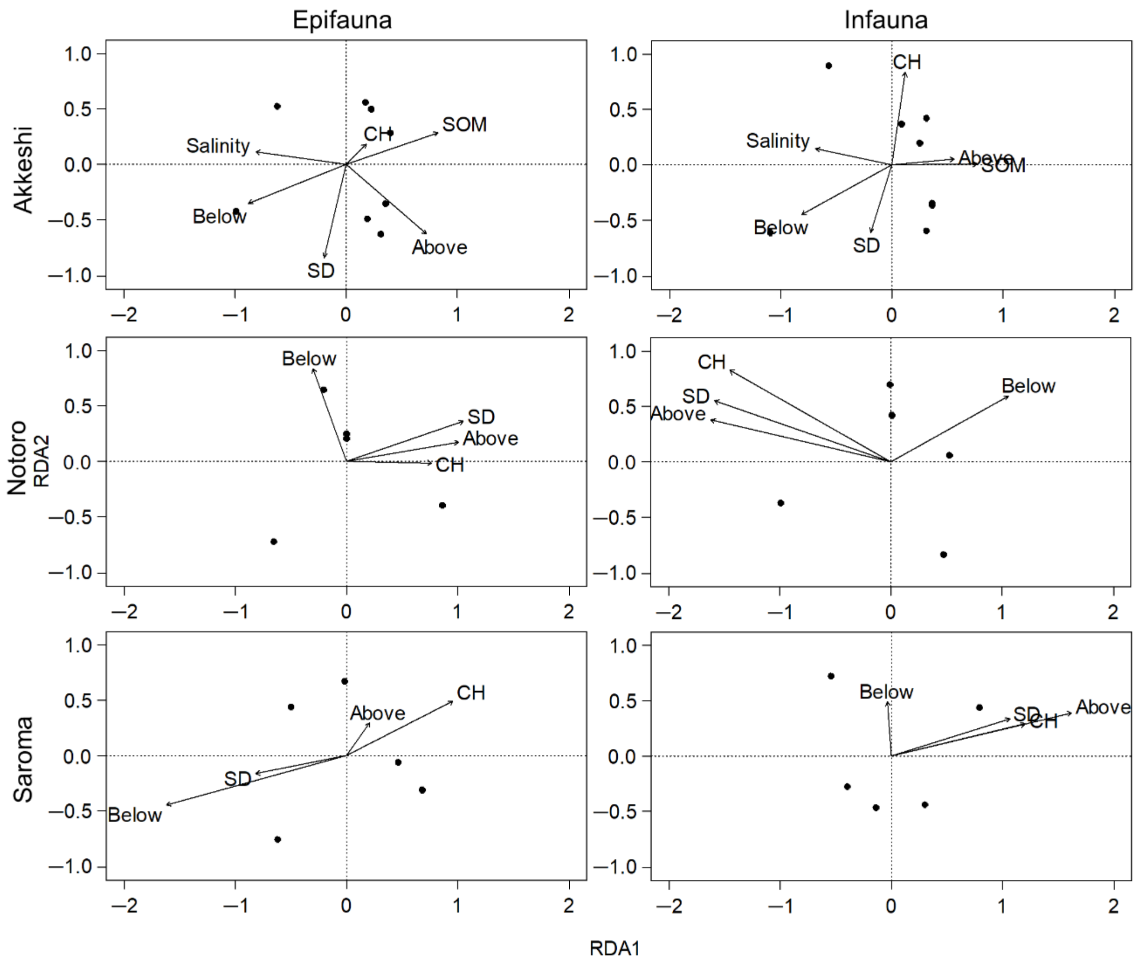

Figure 3.2. Results of RDA based on the presence-absence data showing the association of the epifaunal and infaunal communities with the measured environmental variables in the three lagoons. Each dot represents the value at each site. Above and below indicate above and below-ground biomass of eelgrass, SD indicates shoot density, CH indicates canopy height, and SOM indicates sediment organic matter.

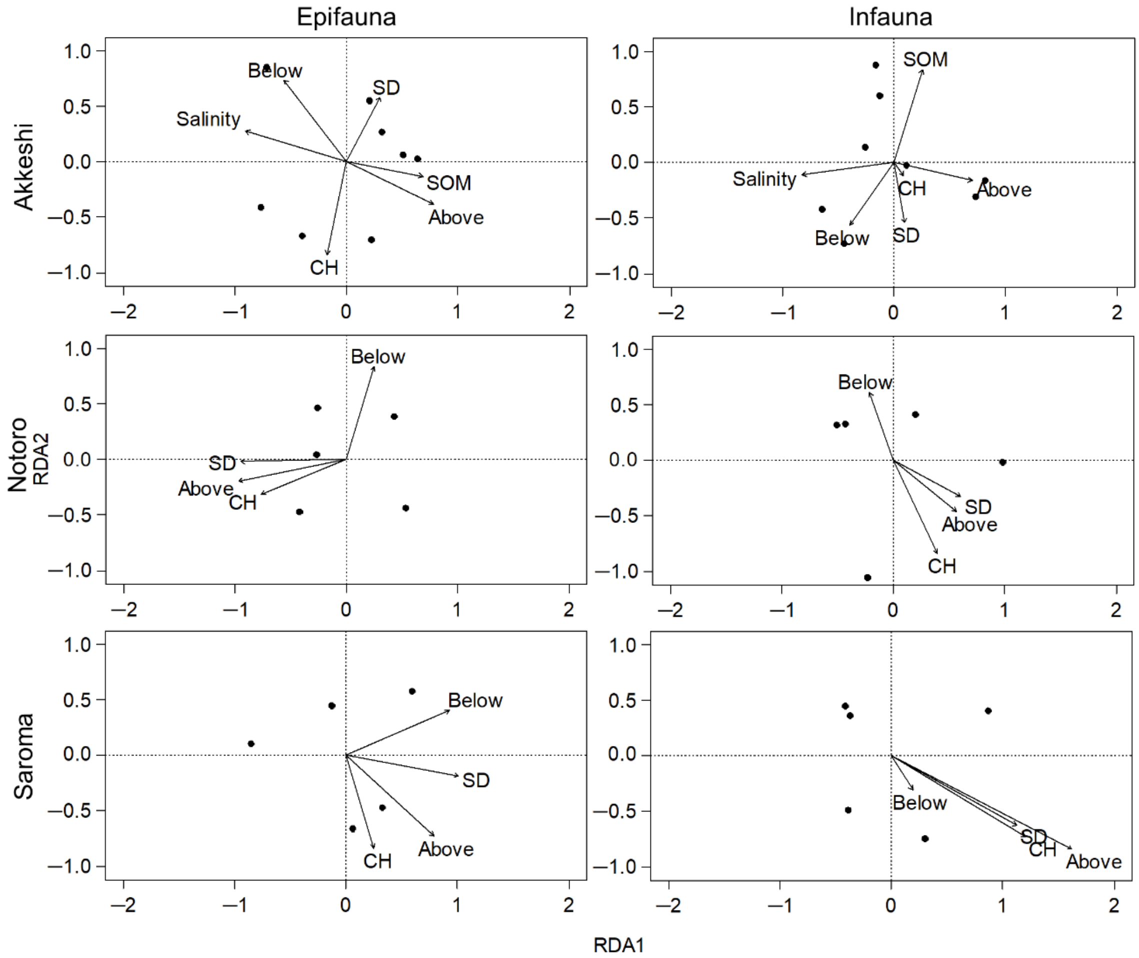

Figure 3.3. Results of RDA based on the abundance data showing the association of the epifaunal and infaunal communities with the measured environmental variables in the three lagoons. Each dot represents the value at each site. The abbreviations for the explanatory variables are the same as those in Figure 3.2.

Table 3.1. Environmental variables measured at each study site in Akkeshi, Saroma and Notoro. SOM indicates sediment organic matter, and above and below indicate above and below ground biomass of eelgrass. SD indicates standard deviation, and CV indicates coefficient of variation.

Site	Salinity	Water temp (℃)	SOM (%)	Shoot density (n m^{-2})	Canopy height (cm)	Above (g m^{-2})	Below (g m^{-2})
Akkeshi							
AK1	18.4	16.1	5.3	216.6	107.8	142.5	22.7
AK2	12.3	14.1	3.3	286.6	187.0	440.2	21.0
AK3	27.4	10.6	3.3	127.4	220.3	280.2	9.7
AK4	21.7	12.8	4.3	216.6	196.6	365.6	21.4
AK5	16.1	13.7	3.7	388.5	145.4	425.6	7.6
AK6	13.7	13.7	3.7	210.2	185.3	355.3	8.5
AK7	23.4	10.4	2.9	216.6	205.1	211.5	19.8
AK8	30.9	11.8	1.2	324.8	134.8	172.4	155.8
Mean ± SD	20.5 ± 6.6	12.9 ± 1.9	3.5 ± 1.2	248.4 ± 81.1	172.8 ± 39.0	299.1 ± 114.7	33.3 ± 49.9
CV	32.1	14.9	34.0	32.7	22.6	38.3	149.8
Notoro							
NO1	32.7	18.5	4.2	573.3	152.2	608.2	25.1
NO2	32.9	19.3	1.6	745.2	135.1	816.1	28.3
NO3	33.1	17	1.1	605.1	81.8	548.4	76.5
NO4	33.1	18.5	1.3	426.8	69.3	260.3	74.7

NO5	32.5	19.1	1.0	305.7	70.4	210.3	34.0
Mean ± SD	32.9 ± 0.3	18.5 ± 0.9	1.6 ± 1.4	531.2 ± 169.4	101.8 ± 39.0	488.7 ± 252.3	47.7 ± 25.7
CV	0.8	4.9	72.8	31.9	38.3	51.6	53.8
Saroma							
SA1	32.2	18.7	1.3	305.7	99.8	292.8	61.3
SA3	32.3	20.6	3.3	636.9	73.7	352.2	92.1
SA6	32.3	19.7	1.8	433.1	136.4	506.2	52.1
SA7	32.9	18.1	1.9	191.1	70.6	145.3	48.3
SA8	33.1	17.5	1.7	668.8	106.0	516.9	77.3
Mean ± SD	32.6 ± 0.4	18.8 ± 1.1	2.0 ± 0.8	447.1 ± 206.7	97.3 ± 26.8	362.7 ± 155.4	66.2 ± 18.3
CV	1.3	6.6	38.1	46.2	27.6	42.9	27.6

Table 3.2. Alpha, beta, and gamma diversities based on species richness and Simpson's diversity index calculated for epifauna and infauna communities in Akkeshi, Notoro, and Saroma.

	Species richness			Simpson's diversity index		
	Alpha	Beta	Gamma	Alpha	Beta	Gamma
Epifauna						
Akkeshi	28	44	72	0.71	0.20	0.90
Notoro	30	24	54	0.56	-0.02	0.54
Saroma	22	22	44	0.60	0.15	0.75
Infauna						
Akkeshi	8	13	21	0.53	0.30	0.83
Notoro	7	14	21	0.70	0.15	0.85
Saroma	6	8	14	0.55	0.18	0.73

Table 3.3. The contribution of turnover and nestedness based on Jaccard and Bray-Curtis dissimilarity matrix for epifauna and infauna in Akkeshi, Notoro, and Saroma.

	Jaccard			Bray-Curtis		
	Turnover	Nestedness	Overall	Turnover	Nestedness	Overall
Epifauna						
Akkeshi	0.77	0.06	0.84	0.80	0.08	0.88
Notoro	0.60	0.10	0.70	0.32	0.31	0.63
Saroma	0.69	0.05	0.75	0.65	0.07	0.72
Infauna						
Akkeshi	0.85	0.03	0.88	0.80	0.06	0.86
Notoro	0.77	0.05	0.82	0.72	0.08	0.80
Saroma	0.50	0.21	0.71	0.50	0.28	0.78

Table 3.4. The results of RDA for the presence-absence data followed by forward step wise tests for epifauna and infauna from Akkeshi, Notoro, and Saroma. F values and statistically significant values ($P < 0.05$) included in the best models chosen by the forward step wise tests were bolded. n.d. indicates no result. Above and Below indicate above-ground and below-ground eelgrass biomass, respectively, and SOM indicate sediment organic matter.

	RDA loading (RDA1)	RDA loading (RDA2)	F	*P*
Epifauna				
Akkeshi				
Above	**0.67**	**-0.57**	**1.66**	**0.04**
SOM	**0.75**	**0.26**	**1.75**	**0.04**
Below	-0.80	-0.31	1.28	0.22
Canopy height	0.16	0.17	0.98	0.49
Shoot density	-0.18	-0.77	1.04	0.52
Salinity	-0.74	0.11	0.57	0.88
Notoro				
Shoot density	0.85	0.29	1.27	0.11
Above	0.81	0.14	1.20	0.16
Below	-0.24	0.68	1.03	0.52
Canopy height	0.62	-0.00	1.00	0.58
Saroma				
Below	-0.76	-0.21	1.14	0,34
Shoot density	-0.38	-0.08	0.80	0.78
Canopy height	0.45	0.23	0.77	0.87
Above	0.10	0.14	0,63	0.99
Infauna				
Akkeshi				
Below	**-0.84**	**-0.46**	**2.06**	**0.03**
Canopy height	0.12	0.87	1.48	0.11
Above	0.58	0.06	1.08	0.39
SOM	0.80	0.01	1.06	0.48
Salinity	-0.72	0.16	1.03	0.49
Shoot density	-0.20	-0.63	0.75	0.73

Notoro				
Shoot density	-0.68	0.24	1.22	0.23
Above	-0.70	0.16	1.23	0.26
Canopy height	-0.62	0.36	1.15	0.30
Below	0.45	0.26	0.90	0.48
Saroma				
Above	0.70	0.17	1.27	0.23
Canopy height	0.52	0.13	0.91	0.53
Shoot density	0.46	0.15	0.84	0.58
Below	-0.02	0.21	0.57	0.85

Table 3.5. The results of RDA for the abundance data followed by forward step wise tests for epifauna and infauna from Akkeshi, Notoro, and Saroma. F values and statistically significant values ($P < 0.05$) included in the best models chosen by the forward step wise tests were bolded. Abbreviations used in this Table are explained in the legend of Table 3.4.

	RDA loading (RDA1)	RDA loading (RDA2)	F	*P*
Epifauna				
Akkeshi				
Salinity	**-0.85**	**0.26**	**1.91**	**0.005**
Canopy height	-0.16	0.79	1.51	0.14
Above	0.73	-0.36	1.46	0.22
Below	-0.53	0.69	1.35	0.24
Shoot density	0.28	0.55	1.26	0.38
SOM	0.64	-0.12	1.06	0.52
Notoro				
Above	**-0.97**	**-0.19**	**2.83**	**0.01**
Below	0.25	0.84	1.70	0.29
Shoot density	-0.95	-0.02	1.33	0.35
Canopy height	-0.77	-0.31	0.30	0.93
Saroma				
Shoot density	**0.93**	**-0.17**	**2.19**	**0.01**
Below	0.85	0.37	3.68	0.08
Canopy height	0.23	-0.77	2.09	0.22
Above	0.72	-0.67	1.28	0.32
Infauna				
Akkeshi				
SOM	**0.25**	**0.81**	**3.08**	**>0.01**
Salinity	**-0.80**	**-0.11**	**3.36**	**0.02**
Shoot density	0.09	-0.53	1.27	0.36
Below	-0.38	-0.55	0.94	0.53
Canopy height	0.08	-0.11	0.87	0.55
Above	0.68	-0.16	0.89	0.57

Notoro				
Shoot density	0.63	-0.34	1.16	0.31
Above	0.59	-0.48	1.06	0.37
Canopy height	0.41	-0.88	1.09	0.47
Below	-0.22	0.64	0.91	0.57
Saroma				
Above	0.82	-0.42	3.56	0.09
Canopy height	0.61	-0.37	1.46	0.24
Shoot density	0.57	-0.32	1.22	0.29
Below	0.10	-0.16	0.19	0.97

CHAPTER 4

THE RELATIVE IMPORTANCE OF TEMPORAL, ENVIRONMENTAL, SPATIAL, AND PLANT BIOMASS FACTORS IN VARIATIONS OF COMSUMER PRODUCTIONS IN EELGRASS ECOSYSTEMS

4.1. Introduction

Understanding spatio-temporal variations of abundance, species richness, and biomass of organisms has been one of the main goals of ecological studies (Buonaccorsi et al. 2001; Cavanaugh et al. 2013). Evaluating the variation in biomass of small invertebrates has been especially important as a proxy of secondary production and for quantifying seasonal and spatial changes in productions of a given system in the context of analyzing ecosystem functions and services such as local fisheries activities (Duffy et al. 2003). Here, we estimate the production by using biomass of consumers, the heterotrophic organisms that consume primary producers or other heterotrophs, as a proxy (Stites 1999). In coastal marine ecosystems including seagrass and macroalgal beds, factors such as habitat complexity, geographical distance, and environmental variabilities are responsible for the spatial variations of invertebrate species in the scales of several to hundreds of km (Namba et al. 2018; Leopardas et al. 2018; Whippo et al. 2018). Moreover, temporal variations in production are observed in daily, monthly, and yearly basis (Hasegawa et al. 2007; Ciavatta et al. 2008; Douglass et al. 2010). In some cases, synchrony, the term explaining similar change or fluctuations in metrics such as biomass of multiple populations or groups of organisms, affects productions in various spatial and temporal scales (Bjørnstad et al. 1999; Cavanaugh et al., 2013).

Spatial and temporal variations in invertebrate biomass of coastal marine ecosystems have been connected to simultaneous effects of multiple abiotic and biotic factors, and many past studies identified dominant factors that are responsible for the variations in different temporal and spatial scales. Examples of long-term time series analysis come from Douglass et al. (2010), where yearly and monthly changes in seawater temperature and salinity anomalies are responsible for fluctuation of the biomass of consumers in seagrass beds over longer temporal scales. At large spatial scales extended to more than hundreds of km in coastlines, geographical distances can be dominant factors influencing differences in productions through dispersal processes (Cavanaugh et al. 2013). At estuarine and lagoonal scales extended to several to tenths of km, geographical distances among sites are often correlated to the distance of environmental stress gradients (Weilhoefer et al. 2020). In these scales, the effects of environmental gradients are often the dominant factors of the variations. For example, Momota and Nakaoka (2017) identified the effects of spatial salinity differences on the biomass of multiple crustacean and gastropod species in eelgrass (*Zostera marina*) ecosystem in a lagoonal system with a salinity gradient extended to approximately 10 km. Moreover, both non-trophic and trophic plant-animal interactions such as habitat complexity and food availability affect the variation in biomass in lagoonal/estuarine to patch scales (Momota and Nakaoka 2017; Namba and Nakaoka 2018; Ruesink et al. 2019). Synchronous patterns of temporal and spatial variations in the biomass of primary producers and associated grazing invertebrates have been observed in multi-specific macrophyte beds (Kanamori et al. 2004) and kelp forests (Morton et al. 2016), indicating that plant-animal interactions related to functional traits of animals such as feeding modes are important determinants of the variations.

Although previous studies identified that environmental, temporal, spatial, and trophic/non-trophic plant-animal interactions affect spatial and temporal variations of biomass and the synchronous relationship between the secondary production of multiple functional groups, there are a number of unsolved issues regarding to the evaluation process of the variations. First, the knowledge on the relative importance of multiple factors that simultaneously affect the variations is limited. This could be solved by applying techniques such as redundancy analysis and multiple regressions on distance matrices (MRM) with variation partitioning that were used in previous studies examining the relative importance of simultaneously operating factors on community compositions (Lichstein 2007; Yamada et al. 2014). Second, many studies have been more focused on incorporating various spatial scales for analyzing the variations of secondary productions, yet studies examining the variations at different temporal scales, especially at seasonal and monthly scales, are still scarce compared to studies based on discreet or one-time sampling (e.g. Momota and Nakaoka 2017; Namba and Nakaoka 2018). It is necessary to incorporate seasonal scale data as many coastal marine ecosystems are structured by submerged aquatic vegetations with distinct phenology (Douglass et al. 2010; Canavaugh et al. 2013). Lastly, the importance of comparing systems (e.g. lagoons, etc.) with the same ecosystems (e.g. eelgrass ecosystems) but different environmental properties (e.g.

steepness of stress gradients) should be recognized as it gives insights into how the relative importance of the factors is influenced by the ambient environments. This would be especially important from management perspectives, where accurate evaluation of ecosystem functions and services is crucial. The comparison can be effectively done in coastal marine ecosystems that are widely distributed and found in systems with diverse environmental properties and that are important providers of ecosystem functions, such as eelgrass beds (Namba et al. 2020).

In this study, I evaluate the spatio-temporal variations of the biomass of invertebrate functional groups inhabiting eelgrass beds and the relative importance of temporal, environmental, spatial, and plant biomass factors responsible for the variations in production. Eelgrass beds are formed by eelgrass, a species of angiosperm seagrass, in shallow coastal areas, and they are one of the most ubiquitous and best-studied coastal marine ecosystems (Duffy et al. 2015). Eelgrass ecosystems provide habitats for diverse invertebrate species as well as primary producers such as epiphytic microalgae attached to the aboveground parts of eelgrass, and the biodiversity support primary and secondary productions (Nordlund et al. 2017; Momota and Nakaoka 2017). The ability of eelgrass to hold sediments by rhizomes and roots enables microphytobenthos such as diatoms to settle on and within the seafloor of eelgrass beds (Moncreiff et al. 1992; Reidenbach and Timmerman 2019), and reduced currents within the beds helps water column phytoplankton to be gathered (Lemmens et al. 1996; Reidenbach and Timmerman 2019). These primary producers are utilized by invertebrate consumers with different functional traits. Environmental properties such as salinity gradients affect productivity and distribution of invertebrates, operating as a strong filtering factor for these organisms (Josefson and Göke, 2013; Namba et al. 2020).

I hypothesize that (1) the spatio-temporal variations in the productions of major functional groups of invertebrates are affected by the combinations of environmental, temporal, and plant biomass factors, and the relative importance of these factors in the variations vary among the functional groups and that (2) the patterns vary between invertebrates from eelgrass ecosystems with and without strong salinity gradients. Finding from this research adds new perspectives on how each factor affect the variations of secondary production of each functional group and the ecosystem functions they provide.

4.2. Material and Method

4.2.1. Study sites and field sampling

This study was conducted in two lagoons, Akkeshi and Notoro in eastern Hokkaido, Japan (Figure 4.1). Here, eelgrass beds occurred in shallow subtidal zones with the depth of 0.2 to 1.6 m. Akkeshi had a steep salinity gradient created by freshwater input from a river, while Notoro had a marginal salinity gradient due to lack of a large amount of river inflow (Namba and Nakaoka 2018; Namba et al. 2020). Field sampling of eelgrass bed communities was done every month from April to November 2018, and sampling sites were chosen to evenly space each site along salinity gradients in Akkeshi and Notoro (Figure 4.1). Five replicate samples of aboveground part of eelgrass and phytal invertebrates (hereafter epifauna) was collected at each site by mesh bags with 20 cm diameter and 0.1 mm mesh size for measuring eelgrass and epifauna biomass. Additionally, five shoots of eelgrass were separately collected for measuring the biomass of epiphytic microalgae, and five replicates of sediment samples for microphytobenthos were collected by inserting a syringe core with 5 cm diameter into the seafloor sediment to 5 cm depth. One water sample per site was taken for measuring the amount of water column phytoplankton. All samples were stored in a darkened cooler box and brought back to the laboratory.

4.2.2. Laboratory procedures

The biomass of epiphytic microalgae, microphytophytobenthos, and water column phytoplankton were measured as Chl-*a* concentrations of the samples using non-acidification method of Welschmeyer (1994). Epiphytic microalgae were scraped off from each eelgrass shoot by a slide glass and filtered through a GF/F glass-fiber filter (Whatman International Ltd., UK). Similarly, the water samples containing water column phytoplankton were filtered through GF/F filters. The filters and 1.5 g of sediment samples for microphytobenthos were then extracted in 6 mm N,N-dimethylformamide for 24 hr and stored at –20 °C in the dark until analysis. Chl-*a* concentration of the extracted solutions were then measured by a fluorometer (10-AU-005-CE Fluorometer, Turner Designs, USA).

Eelgrass samples were taken out from the mesh bags, and the attached epifauna were scraped off from eelgrass and filtered through a 0.5 mm sieve. Eelgrass dry weight were measured after drying the samples at 60 °C for 48 hr. Epifauna retained on the sieve were fixed with 70 % ethanol. Only the samples with the sizes between 0.5 mm and 8 mm were counted and identified to the lowest possible taxon. In order to conduct functional trait analysis, feeding type of each epifaunal species was identified as this information indicates the resource use pattern and association of epifauna to each functional group of the primary producers (Ebeling et al. 2018). As same species or same genus of epifauna can be found in both northeastern and northwestern Pacific Ocean, we used a list provided by MacDonald et al. (2010) for identifying traits, assuming functional traits did not vary spatially and temporally. Epifauna were categorized into 10 groups based on their feeding types (MacDonald et al. 2010), and ash-free dry weight of each groups were calculated by formula provided by Edgar (1990). Because most of the groups were rarely observed (> 0.23 g m^{-2} averaged per lagoon for each month), biomass data of the three most dominant groups (grazers, predators, and suspension feeders) were used for statistical analyses.

4.2.3. Statistical analysis

I compared the spatio-temporal differences in biomass of primary producers and epifauna for each functional group by linear models with sites and seasons as fixed factors. Because water column phytoplankton was only taken one replicate per site in every month, the data was not analyzed by linear model. The models were created for Akkeshi and Notoro separately, and data were log(y+1) transformed prior to the analysis. To visualize the among-site and month variation in epifaunal species composition, we used Euclidean distance based on square-root transformed species-specific biomass data of each functional group for non-metric multidimensional scaling (NMDS) analysis. Data for Akkeshi and Notoro were analyzed separately, and the analyses were done by PRIMER (version 7).

To examine the factor which best explained the variations in biomass of each trait group of epifauna, I used multiple regressions on distance matrices (MRM). MRM is an extension of simple Mantel tests and incorporates distance matrices for the analyses (Tuomisto and Ruokolainen 2006; Lichstein 2007). Unlike simple Mantel tests which are used for examining correlations between two matrices (e.g. Legendre 2015), MRM uses multiple regression to calculate partial matrix correlation between one response matrix and one or more explanatory matrices, which enables regression analyses for multivariate datasets (Lichstein 2007). Significance tests are done by first permutating the response matrix for a given number of times while explanatory matrices are kept fixed to obtain a distribution of correlation and then determine whether the observed correlation is greater than expected by chance from pseudo-t tests (Legendre et al. 1994; Lichstein 2007). I applied MRM to my data in order to prevent loss of information when collapsing the data to vectors as done in RDA and other multivariate tests (Legendre et al. 1994). Moreover, my data were composed of pairwise multivariate environmental, temporal, spatial, and biomass data that were readily made into distance-based explanatory and response matrices, making MRM as a suitable test for the dataset (Nunes et al. 2020; Murphy et al. 2021).

The MRM test was done for each trait and lagoon separately, creating six independent tests with a unique matrix containing 8 columns (the number of sampling months) and 4 rows (for Akkeshi) or 3 rows (for Notoro). Spatio-temporal variations in biomass of each of the 4 functional group of primary producer and temporal, spatial, and environmental (salinity and water temperature) distances were the 8 explanatory matrices used for the full models, and each response and explanatory variable was transformed to Euclidean distance matrices (Lichstein 2007; Nunes et al. 2020). Temporal distance (hereafter temporal factor) was based on how many days each date of monthly sampling was separated. Spatial distances (hereafter spatial factor) was based on distance of each site from the major river mouth of each lagoon and used as an indicator of relative location fo each site along salinity gradients. Environmental distances (hereafter environmental factor) were calculated based on salinity and water temperature of each site at each sampling month. Salinity and water temperature were used because these factors are known to affect the variation in epifaunal biomass in previous studies (e.g. Douglass et al. 2010). Significance tests were applied to explanatory matrices in the full models, and the significant matrices were included in the reduced models. To calculate the relative importance of sole and shared effects of temporal, spatial, environmental, and plant biomass factors, we used variation partitioning equations for each reduced model (Borcard et al. 1992). Variation partitioning measures the amount of variation explained by a single response matrix and by multiple matrices (Nunes et al. 2020). Linear modeling and MRM were done by R (version 4.0.0, R Core Team 2020) using the 'ecodist' (Goslee and Urban 2020) packages.

4.3. Results

Linear models for each group of the primary producers showed the biomass varied among months and sites for both lagoons with significant interaction of month and site except for microphytobenthos in Notoro (Table 4.1). Eelgrass biomass increased from spring to summer and declined in autumn in Akkeshi, while it increased in autumn in Notoro (Figure 4.2). Biomass of epiphytic microalgae increased from spring to summer, peaked in September, and declined towards November in AL1 and 2 of Akkeshi (Figure 4.3). However, it continuously increased towards November in AL3, 4, and all sites in Notoro. Biomass of microphytobenthos stayed relatively consistent throughout the months for all sites (Figure 4). Biomass of water column phytoplankton increased in July to September and declined afterwards in Akkeshi sites and NO2, while it peaked in October at NO1 and NO3 of Notoro (Figure 4.5).

In total, 78 and 59 species of epifauna were found in Akkeshi and Notoro, respectively (Table B1 and B2). We recorded 13 and 13 species of grazers, 20 and 16 species of predators, and 19 and 15 species of suspension feeders in Akkeshi and Notoro sites, respectively (Table B3 and B4). Biomass of grazers, predators, and suspension feeders varied among months and sites for both lagoons (Table 4.1). Grazer biomass increased from spring to autumn in Akkeshi, while the biomass was relatively low in all sites of Notoro throughout months (Figure 4.6). Predator biomass was higher in the spring to summer and decreased towards autumn in both Akkeshi and Notoro sites (Figure 4.7). Suspension feeder biomass had spatio-temporal variation patterns similar to grazer, and increase in the biomass from September to November was notable in AL1 of Akkeshi (Figure 4.8). The NMDS analysis showed separation in biomass-based community composition for each functional group between sites in Akkeshi, with some overwraps (Figure B1). By contrast, no consistent spatial patterns in community composition was observed from the NMDS analysis based on Notoro data (Figure B2).

The results of MRM showed that spatial factor, time, and eelgrass biomass were important in explaining the variation in grazer biomass of Akkeshi (Table 4.2). Variation partitioning indicated that the shared effect of spatial, temporal, and eelgrass biomass factors to spatio-temporal dynamics of grazer biomass was 14.3 % of the variation, and combinations of two factors ranged from 4 to 12 % (Table 4.3). The sole effect of spatial factor was largest, followed by time and eelgrass biomass. The sole effects of each factor were lower than the shared effects, and the unexplained variation was more than 45 %. Water temperature explained the predator biomass of Notoro, and approximately 5 % of the variation was explained by this factor. By contrast, no explanatory matrix was chosen for Notoro grazer biomass and Akkeshi predator biomass. Spatial factor, biomass of microphytobenthos, and water temperature were important for explaining suspension feeder biomass of Akkeshi, and the shared effects of the 3 factors were approximately 4.5 % of the variation. The combinations of two factors ranged from 3 to 1.5 %, and the sole effects of each factor ranged from 1.3 to 0.3 %. Unknown variation remained approximately 87 %. Biomass of eelgrass was the only important factor for suspension feeder biomass of Notoro with 18 % variation explained by this factor.

4.4. Discussion

The results of my study indicated that the spatio-temporal variations of secondary production in eelgrass ecosystems were simultaneously affected by factors related to temporal and spatial factors, environmental heterogeneity, and the biomass of primary producers. The relative importance of each factor varied among functional groups of epifauna from different lagoons. I will first explore the effects of the factors on the variations of each epifaunal groups and relate the results to traits such as life history and utilization of resources from macrophyte beds. Then I will discuss how the relationship between the spatio-temporal variations of epifaunal invertebrate groups and other factors affects functioning of eelgrass ecosystems with small-scale environmental gradients.

The effects of spatial distance, time, and eelgrass biomass on variation of grazers from Akkeshi suggest that these factors simultaneously operate and affect the spatio-temporal variations of grazer biomass. Partitioning of variation indicated that distance from the river mouth was the main factor affecting the variation of the biomass, followed by temporal distance and eelgrass biomass, in the ecosystem with a steep environmental gradient. By contrast, absence of strong association between the grazer variations and any of the response factors in Notoro indicates that the spatio-temporal variations of grazers are not associated with any of these examined factors. Variation in the effect of environmental filtering causes higher beta diversity in communities along the axis of environmental gradients, while lower beta diversity in relation to homogeneity in environmental conditions is observed in communities from ecosystems without steep gradients (Namba et al. 2020). Similarity in the community composition between spatially close sites along a gradient axis may contribute to the synchronous pattern of the grazer biomass and distance of the sites from river mouth. Moreover, the presence of similar species may contribute to the effect of temporal factor, as communities with similar species component tend to exhibit temporal synchrony in production rates (Crump and Hobbie 2005). Grazers in marine ecosystems feed on macrophytes and epiphytic microalgae and utilize macrophytes as habitats (Valentine and Heck 1999; Duffy et al. 2015). The synchronous pattern in spatio-temporal variations of grazer and eelgrass biomass suggested interaction between foundation species and the associated fauna, as observed in terrestrial grassland (Bruckerhoff et al. 2020) and marine ecosystems (Kanamori et al. 2004; Morton et al. 2016). Absence of clear pattern in temporal variation nor association between primary producers and grazers in Notoro could be explained by the relatively stable temporal changes of grazer and primary producer biomass compared to Akkeshi.

The spatio-temporal variations of predator biomass from Akkeshi were not affected by any factors, but those from Notoro were affected by the variation in water temperature. Although the communities shared similar species component when the sites were spatially close along the salinity gradient in Akkeshi (Momota and Nakaoka 2018), I found that the spatial variation in predator biomass was not strongly associated with the gradient. The predator species identified in this study were mobile meso-predators (size between 0.5 and 8 mm) mainly feeding on zooplankton (MacDonald et al. 2010), and their ability to move between habitats results in weak association with environmental gradients compared to other functional groups (Yamada et al. 2007). Association between predator biomass and water temperature in Notoro would be explained by seasonal change in biomass in response to increase in water temperature from spring to summer. Temporal stability of predator biomass could be related to the presence of individuals with relatively similar body size and biomass throughout the season. Biomass of predators is often associated with the spatio-temporal variations of biomass of their prey populations (Bauer et al. 2014). Absence of clear synchronous patterns between predator and primary producer biomass was expected as predators frequently move between macrophyte beds and other habitats (Orth et al. 1984). The result showed high value of residuals, and this suggested that most of the variation may be associated with the factors that were not investigated in this study. Further study is needed for the role of species interaction between predators and their preys such as zooplankton by incorporating the spatio-temporal variations of the biomass of these groups into the analysis (e.g. Durant et al. 2019).

Significance in the association of the variations of suspension feeders with environmental factors and microphytobenthos biomass in Akkeshi indicates that spatio-temporal variations of suspension feeders were more likely to exhibit synchronous patterns when the organisms inhabit similar environments along a gradient axis. Although smaller proportion in explaining the variation, the relationship between microphytobenthos and suspension feeder biomass suggested the availability of food sources was an important component of the variations of this functional group, as microphytobenthos including diatoms were important food for suspension feeders in macrophyte beds (Lebreton et al. 2011). Moreover, seasonal change in water temperature was associated with the biomass of suspension feeders, indicating that the biomass increases in most sites as the water temperature increases towards summer except AL1 where the biomass continuously increased towards winter. Without a steep salinity gradient, the variations of suspension feeder biomass in Notoro were not explained by the environmental factor. Interestingly, the linkage between the variation of eelgrass biomass, instead of microphytobenthos, and suspension feeders was suggested. The dominant species of suspension feeders in Notoro included bivalve *Arcuatula senhousia* and amphipod *Erichtonius pugnax* that physically attach themselves to eelgrass aboveground parts for foraging (M. Namba, *personal observation*). Eelgrass provides habitats for these suspension feeders, and the dependency of the organisms to the standing stock of the plants is partially explaining the observed pattern between eelgrass and suspension feeders. However, the large value of residuals for both Akkeshi and Notoro suspension feeders suggest that the variation in biomass was not fully explained by the tested factors only. Additional investigations should incorporate factors that were not used in this study, such as sediment grain size of the eelgrass beds and eelgrass shoot density, for identifying the condition in which suspension feeders is influenced (e.g. Reise 2002).

Variation partitioning of the effects of environmental, temporal, spatial, and species interaction factors on the variations of the functional groups indicates that understanding the relative importance of each factor for organisms with different functional traits is important for evaluation of ecosystem functions such as productions. The spatio-temporal variations of biomass indicate how the amount of production in the local communities fluctuate (Douglass et al. 2010; Hessing-Lewis and Hacker 2013). Disentangling the relationship between the biomass of various populations and each factor reveals in what conditions the production is increased or decreased and how the change is anticipated (Jänes et al. 2017). When multiple factors operate simultaneously, variation partitioning is effective in identifying and quantitatively assess the effect of dominant and minor factors alike (Peres-Neto et al. 2006; Namba and Nakaoka 2018). Moreover, my results showed that both grazers and suspension feeders were affected by the spatial factor but that the strength of the effect varied between the groups, suggesting the importance of evaluating the effect of each factor separately for functional groups.

Differences in the relative importance of each factor on the same functional groups of epifauna between the lagoons with and without steep salinity gradients suggest that the spatio-temporal variations of secondary production can be variable among systems. Responses of organisms to environmental, temporal, spatial, and plant biomass factors are thought to be more congruent within functional groups or guilds than among groups (Matias et al. 2017). However, my results suggested that there were between-system differences in the spatio-temporal variations of the same functional group and the affecting factors. When the steepness of environmental gradients between two systems is different, there are variations in the factors shaping community assembly processes (Namba et al. 2020) as well as stress responses of populations (Salo et al. 2014; Namba and Nakaoka *in prep.*). Therefore, I suggest that system-specific understanding of the spatio-temporal variations of secondary productions is required for further investigation in functioning of ecosystems and management practices.

Ecosystems such as terrestrial grasslands and forests as well as marine macrophyte beds provide important functions by supporting primary and secondary productions (Duffy et al. 2015; Defriez and Reuman 2017). In addition to investigations based on single-time sampling, the needs and importance of quantifying the productions by the analysis of synchrony among spatio-temporal variations of multiple functional groups from time-series data had been suggested (Douglass et al. 2010; Canavaugh et al. 2013; Namba and Nakaoka 2018). My results from the seasonal sampling covering sites from two lagoons revealed the presence of various synchronous patterns in the eelgrass ecosystem and the importance of each of temporal, spatial, environmental, and plant biomass factors varied among functional groups of primary producers and epifauna, suggesting that evaluating the synchronous patterns of spatio-temporal dynamics is effective in quantifying production in coastal marine ecosystems shaped by macrophytes. As the loss of macrophyte foundation species followed by decline in associated fauna and flora due to increase in human activities and climate change is concerned (Waycott et al. 2009; Duffy et al. 2019), quantitative assessment of productions has become ever more important in both terrestrial and marine ecosystems. Research on how each factor shapes the spatio-temporal variations of production and the synchrony among multiple populations and groups and how this varies among the same ecosystems but different environmental gradients may be essential in the era of rapid change and loss in ecosystem functions, and additional studies to cover longer and larger temporal and spatial scales of the variations are required.

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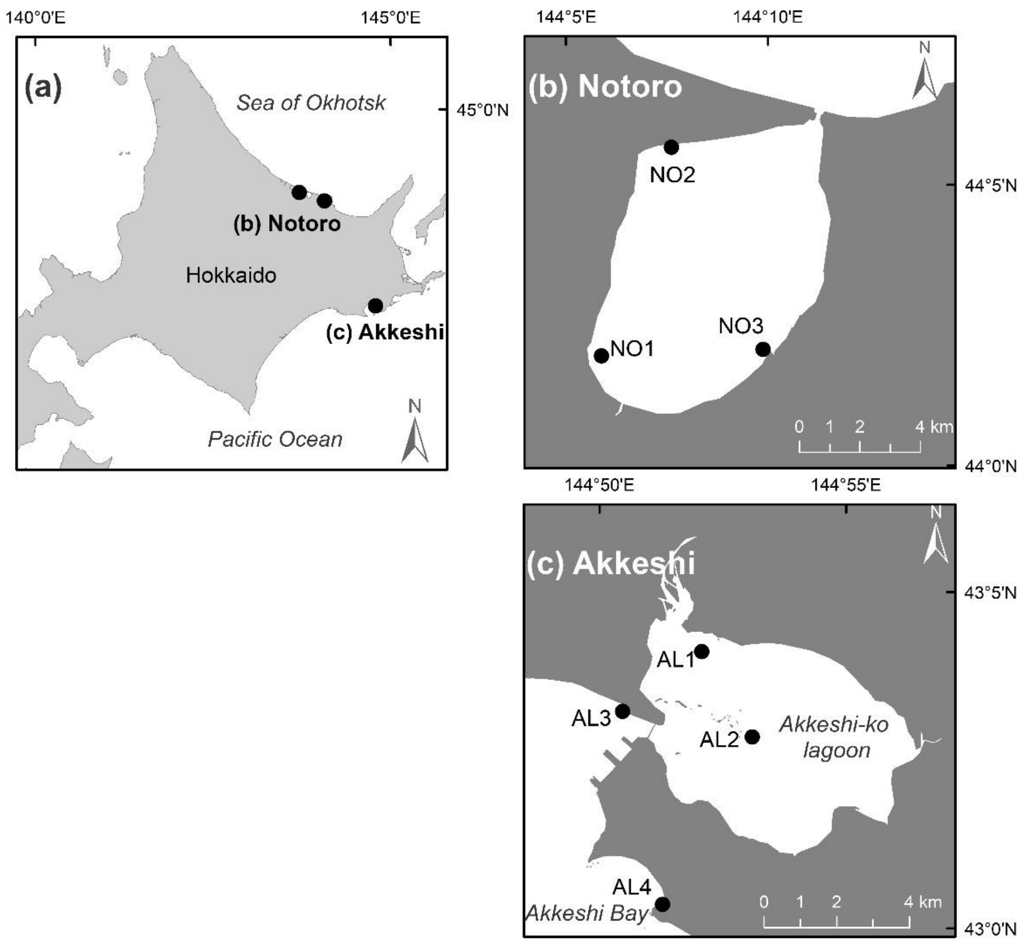

Figure 4.1. Study sites in (a) Eastern Hokkaido, Japan; (b) Notoro and (c) Akkeshi.

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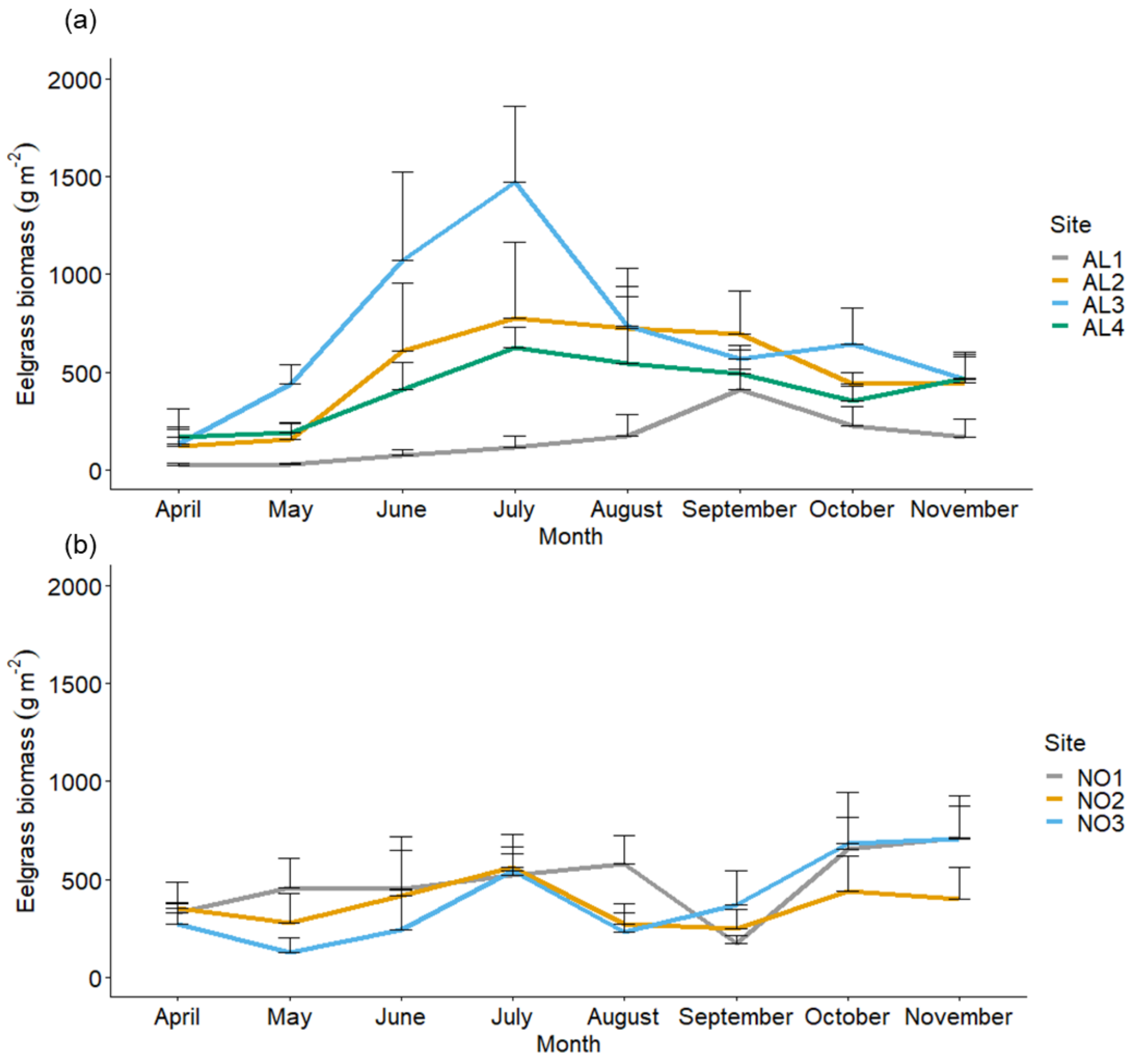

Figure 4.2. Spatio-temporal changes in eelgrass biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation

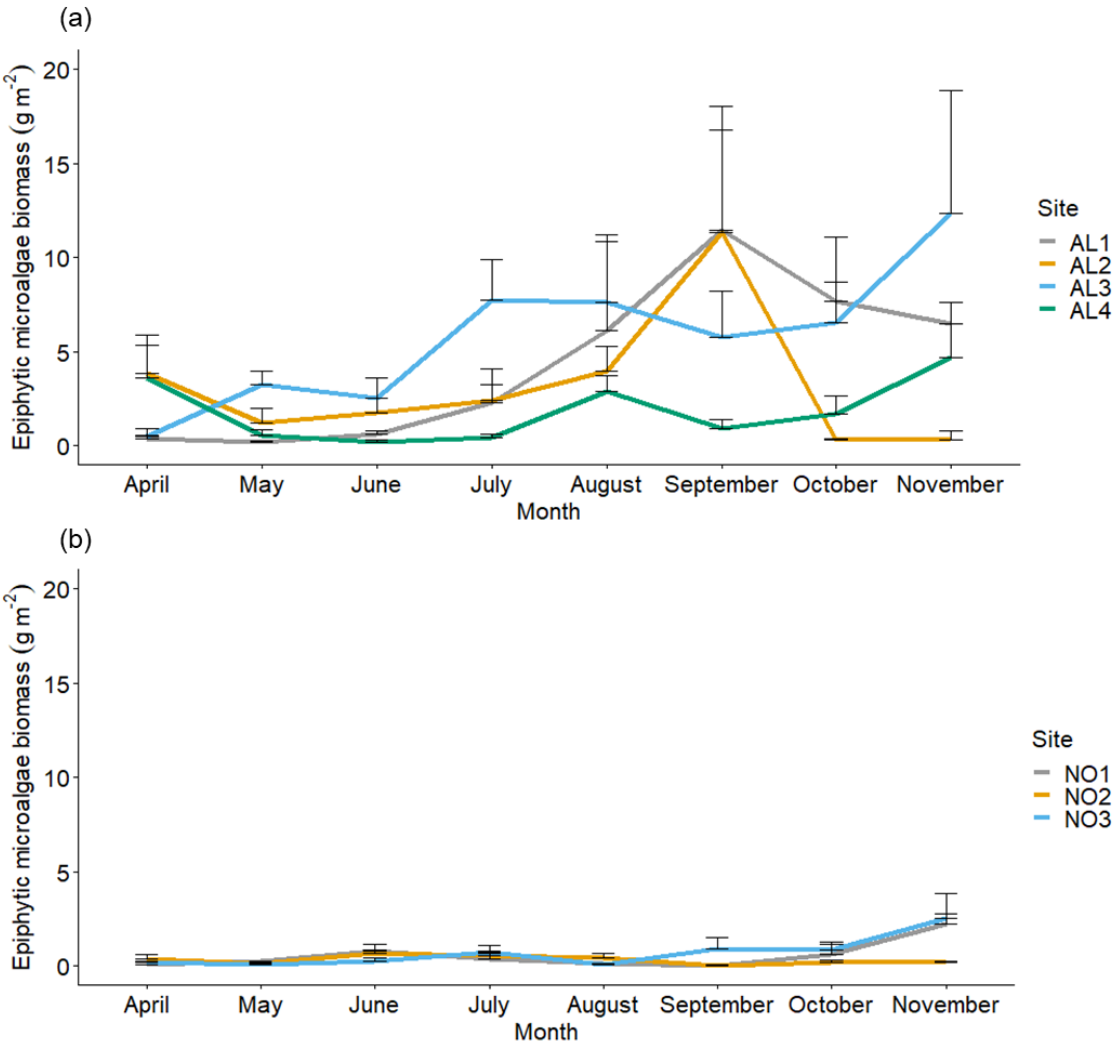

Figure 4.3. Spatio-temporal changes in epiphytic microalgae biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation.

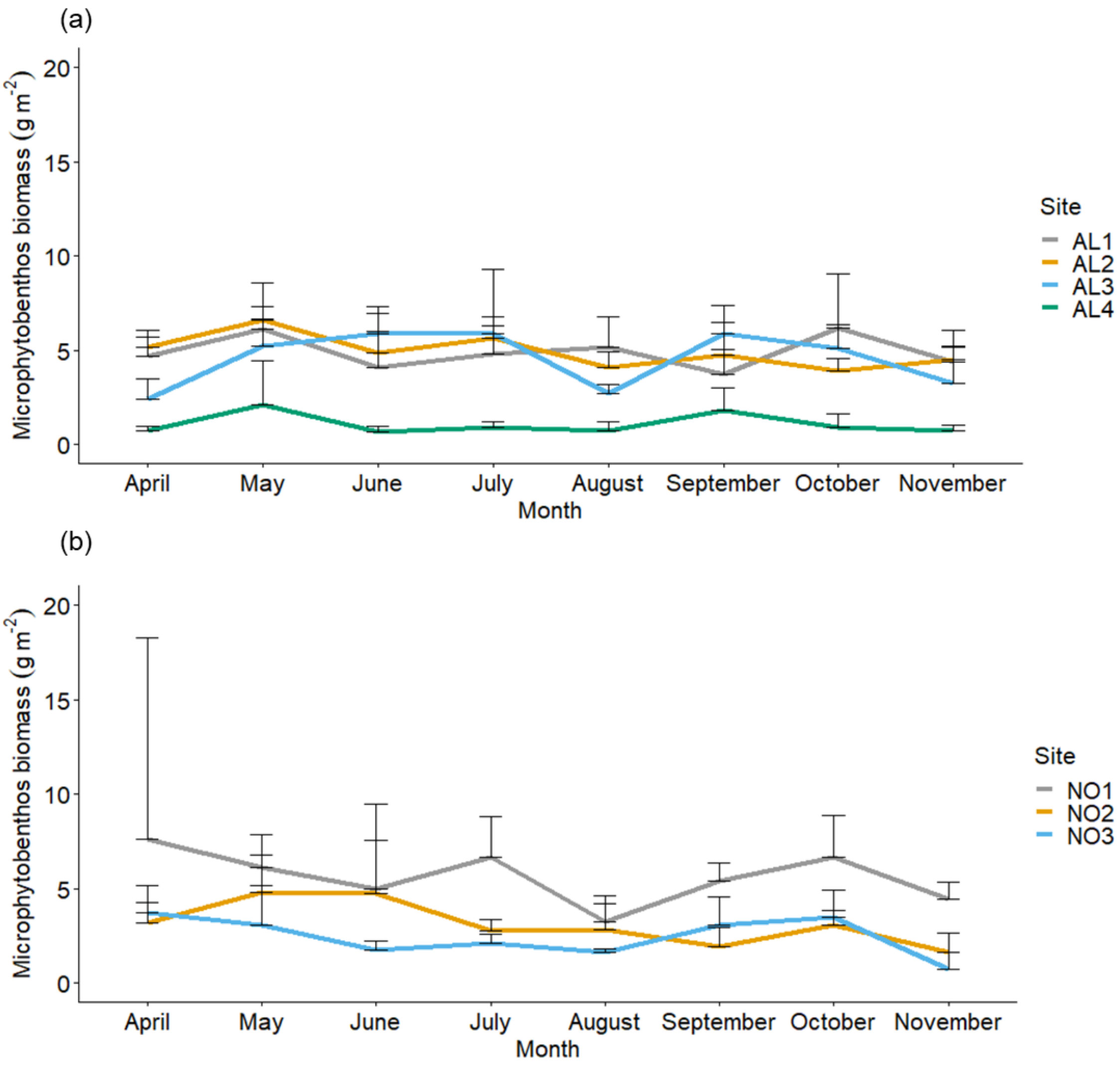

Figure 4.4. Spatio-temporal changes in microphytobenthos biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation.

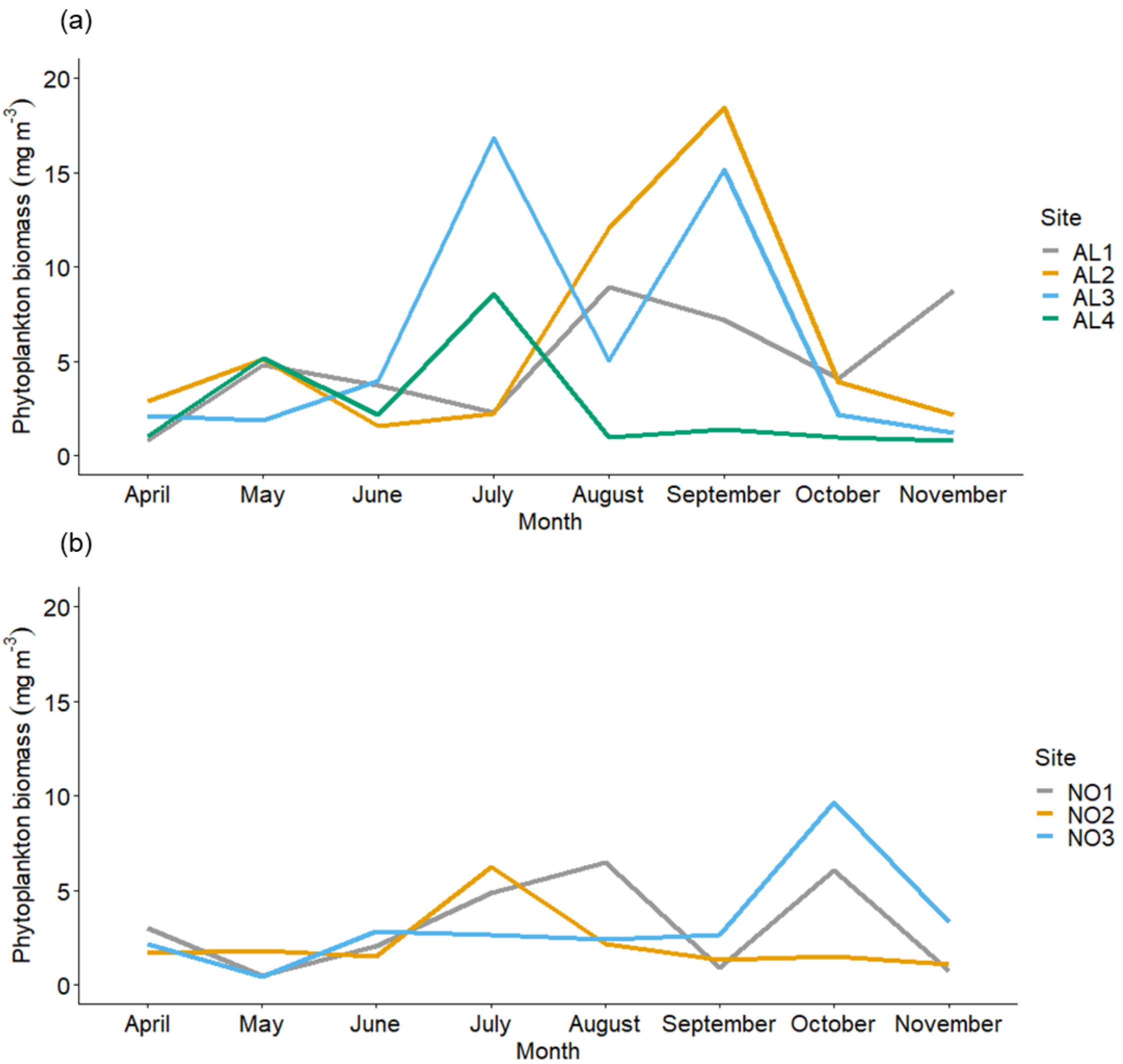

Figure 4.5. Spatio-temporal changes in water column phytoplankton biomass from each site in (a) Akkeshi and (b) Notoro.

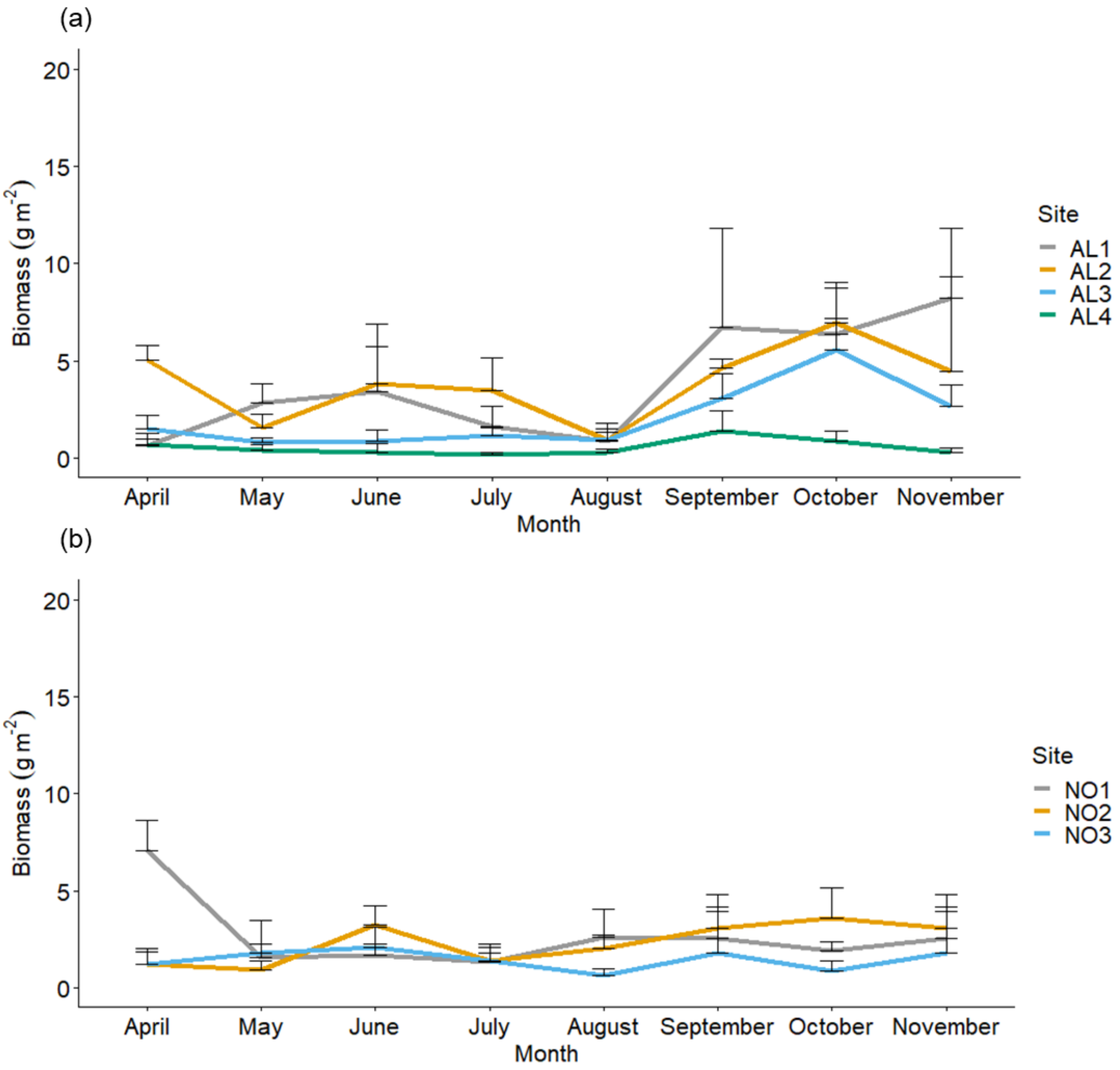

Figure 4.6. Spatio-temporal changes in grazer biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation.

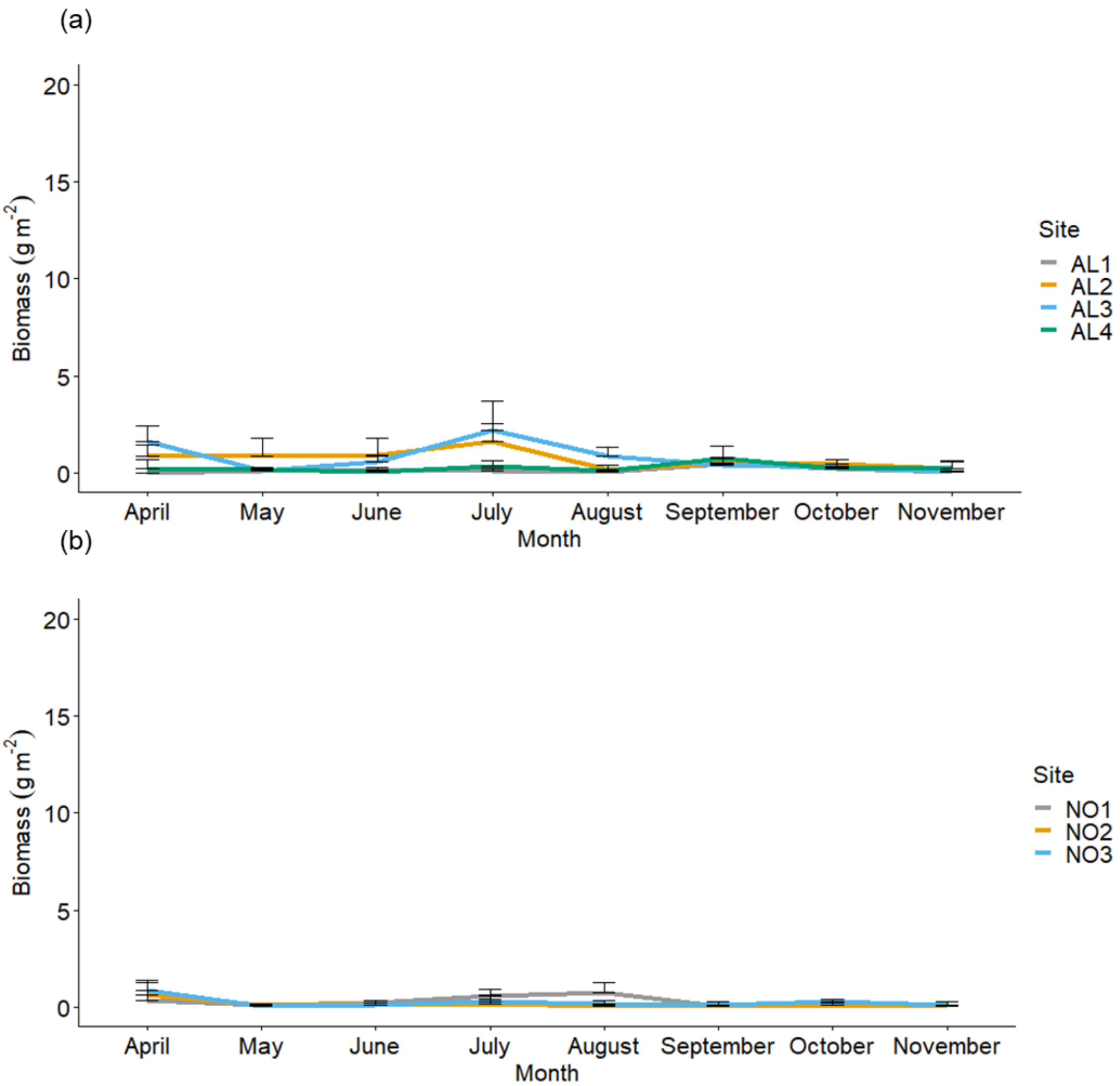

Figure 4.7. Spatio-temporal changes in predator biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation.

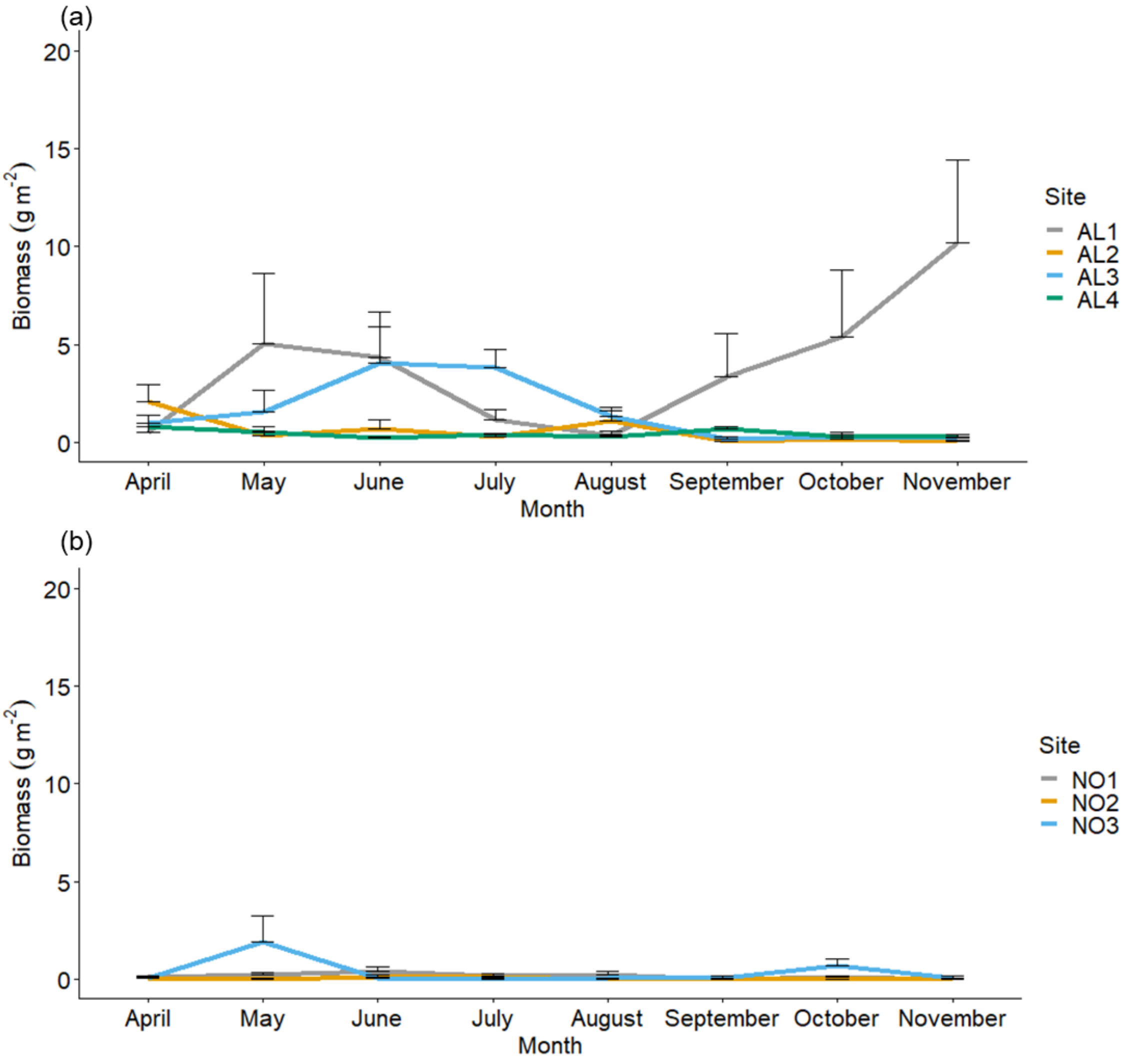

Figure 4.8. Spatio-temporal changes in suspension feeder biomass from each site in (a) Akkeshi and (b) Notoro. The error bars indicate standard deviation.

Table 4.1. Significance test based on linear models for each functional group of primary producers and epifauna.

	Akkeshi			Notoro		
	df	F value	P value	df	F value	P value
Eelgrass						
Site	3	109.628	< 0.001	2	6.156	0.003
Month	7	45.881	< 0.001	7	9.513	< 0.001
Site*Month	21	3.849	< 0.001	14	3.361	< 0.001
Residuals	127			96		
Epiphytic microalgae						
Site	3	47.071	< 0.001	2	11.529	< 0.001
Month	7	38.401	< 0.001	7	30.667	< 0.001
Site*Month	21	19.806	< 0.001	14	14.513	< 0.001
Residuals	127			96		
Microphytobenthos						
Site	3	124.268	< 0.001	2	32.636	< 0.001
Month	7	4.015	< 0.001	7	4.940	< 0.001
Site*Month	21	1.948	0.013	14	1.765	0.055
Residuals	127			96		
Grazer						
Site	3	78.020	< 0.001	2	13.290	< 0.001
Month	7	24.291	< 0.001	7	2.528	0.02
Site*Month	21	4.872	< 0.001	14	4.139	< 0.001
Residuals	127			96		
Predator						
Site	3	24.894	< 0.001	2	3.124	0.048
Month	7	9.446	< 0.001	7	9.217	< 0.001
Site*Month	21	4.612	< 0.001	14	2.333	0.008
Residuals	127			96		
Suspension feeder						
Site	3	88.027	< 0.001	2	15.344	< 0.001
Month	7	5.331	< 0.001	7	10.687	< 0.001
Site*Month	21	18.428	< 0.001	14	10.801	< 0.001
Residuals	127			96		

Table 4.2. MRM full model results for grazer, predator, and suspension feeders from Akkeshi and Notoro. Statistically significant values ($P < 0.05$) were bolded. Location indicates distance from river mouth, ZosteraBM indicates eelgrass biomass, EpiphyteBM indicates epiphytic microalgae biomass, PhytobenthosBM indicates microphytobenthos biomass, WaterBM indicates water column phytoplankton biomass, Time indicates temporal distance from the sampling date in April, Salinity indicates the averagesalinity value at the time of sampling, and WT indicates average water temperature measured at the time of sampling.

	Akkeshi		**Notoro**	
	Regression coefficient	P	Regression coefficient	P
Grazer				
Intercept	0.530	0.970	0.366	0.629
Location	0.044	**0.006**	0.004	0.704
ZosteraBM	-0.078	**0.049**	-0.084	0.369
EpiphyteBM	0.040	0.305	-0.075	0.552
PhytobenthosBM	0.004	0.974	0.067	0.544
WaterBM	-0.012	0.820	-0.007	0.921
Time	0.002	**0.005**	0.001	0.211
Salinity				
WT				
Predator				
Intercept	0.324	0.493	0.127	0.887
Location	-0.012	0.210	< -0.001	0.999
ZosteraBM	0.055	0.094	-0.019	0.711
EpiphyteBM	-0.022	0.408	-0.039	0.618
PhytobenthosBM	0.042	0.617	-0.042	0.423
WaterBM	0.031	0.447	0.061	0.138
Time	< -0.001	0.554	< 0.001	0.522
Salinity	-0.004	0.610	0.005	0.769
WT	-0.002	0.776	0.007	**0.041**
Suspension feeder				
Intercept	0.626	0.681	0.112	0.74
Location	0.040	**0.041**	-0.007	0.381

ZosteraBM	0.102	0.112	0.291	**0.026**
EpiphyteBM	0.005	0.945	-0.170	0.127
PhytobenthosBM	-0.322	**0.052**	-0.075	0.471
WaterBM	0.077	0.304	0.119	0.065
Time	0.001	0.150	< 0.001	0.661
Salinity	-0.008	0.644	-0.014	0.573
WT	-0.025	**0.043**	-0.005	0.379

Table 4.3. Variation partitioning of temporal, spatial, environmental, and species interaction factor (i.e. primary producer biomass) based on the significant variables chosen from the MRM full models.

	R squared	P	% variance partitioning
Akkeshi			
Grazer			
Location + ZosteraBM + Time	0.143	0.001	14.346
Location + ZosteraBM	0.110	0.002	10.983
Location + Time	0.121	0.002	12.070
ZosteraBM + Time	0.041	0.008	4.081
Location	0.095	0.002	9.488
ZosteraBM	0.013	0.073	1.310
Time	0.021	0.017	2.118
Residuals			45.603
Predator			
No significant explanatory variables	NA		NA
Suspension feeder			
Location + PhytobenthosBM+WT	0.045	0.093	4.541
Location + PhytobenthosBM	0.034	0.072	3.350
Location + WT	0.015	0.288	1.533
PhytobenthosBM + WT	0.017	0.237	1.718
Location	0.003	0.480	0.280
PhytobenthosBM	0.004	0.435	0.414
WT	0.013	0.158	1.286
Residuals			86.877
Notoro			
Grazer			
No significant explanatory variable	NA		NA
Predator			
WT	0.049		4.890
Residuals			95.110

Suspension feeder		
ZosteraBM	0.182	18.200

CHAPTER 5

INCREASED SALINITY STRESS CHANGES PLANT PRODUCTIVITY AND ABUNDANCE BY ALTERING THE TOP-DOWN CONTROLS IN EELGRASS BEDS

The work presented in Chapter 5 is in the preparation process for the submission as:
Namba, M., and M. Nakaoka. Increased salinity stress changes plant productivity and abundance by altering the top-down controls in eelgrass beds. *Ecosphere*.

5.1. Introduction

Natural and anthropogenic environmental stress affects various organisms at the individual, population, and community levels and their roles in providing ecosystem functions (Gough and Grace 1998; Winder and Schindler 2004). The degree of environmental stress varies among and within ecosystems (Barnes and Ellwood 2012; Cheng and Grosholz 2016; Namba et al. 2020), while spatial variations create stress gradients. Individual responses, such as changes in respiration, feeding, growth, and reproductive rates (Salo et al. 2014; McDonald et al. 2016) and population responses, such as changes in abundance and biomass (Rodríguez-Gallego et al. 2015) can vary along stress gradients. Individual and/or population responses can influence community responses directly or indirectly through species interactions, including bottom-up (i.e., limited resources; Whalen et al. 2013) and top-down controls (i.e., change in predation pressure; Dunn and Hovel 2019). Species interactions, such as predation (Cheng and Grosholz 2016), herbivory (Rand 2002; Daleo and Iribarne 2009), and competition (Maestre et al. 2009) also vary along the stress gradients in terms of their differences in direction and strength (Bertness and Callaway 1994; Schöb et al. 2013). Thus, evaluating the impact of stress gradients on individuals, populations, and species interactions can uncover community dynamics and ecosystem functions in fluctuating environments.

Stress responses of organisms and their effects on species interactions have been studied in various stress gradients, such as altitudinal gradients (Bertness and Callaway 1994; Mori et al. 2013; Schöb et al. 2013), distances from the shoreline (Alberti et al. 2010), and salinity gradients in estuaries (Barnes and Ellwood 2012; Namba and Nakaoka 2018; Namba et al. 2020). In previous studies, stress gradients and the responses of organisms were mainly assumed to be temporally and spatially fixed. The aspect of evaluating the possible alteration of stress gradients and stress levels by natural and anthropogenic disturbances was often missing as well as how these changes affect the responses of organisms and their interactions remain largely unsolved. Another aspect to consider when evaluating the stress response of organisms along stress gradients is the relationship between the susceptibility of the organisms to stress and the ambient stress level of their habitat. Previous studies have shown that organisms from more stressful sites along a stress gradient are less affected by stressful conditions. For example, marine macrophyte eelgrass (*Zostera marina*) from a brackish environment were more tolerant to low salinity than those from the marine environment (Salo et al. 2014) and ectotherms from warmer environments were less stressed by heat than those from colder environments (Bahrndorff et al. 2009). The stress effects that are caused by disturbances may differ among populations in environments with different stress levels, where higher ambient stress would be related to higher resistance to fluctuating stress levels. As stress levels and alterations in stress gradients increase in many ecosystems due to climate change (Boudouresque et al. 2009), understanding the variation in stress responses of organisms and their effects on species interactions is becoming more important for evaluating the robustness and resilience of ecosystems, which refers to the capacity of a system to absorb the effects of disturbances and retain its functions (Folke et al. 2004; Mori et al. 2013).

In coastal ecosystems, stress gradients created by salinity differences are commonly observed in semi-enclosed coastal systems, where sites near river mouths experience low and fluctuating salinity, thus, are stressful environments for marine organisms (Palmer et al. 2011; Van Diggelen and Montagna 2016). Freshwater input to coastal ecosystems due to the increasing amount of precipitation can cause higher frequency and longer fluctuation of salinity (Van Diggelen and Montagna 2016). This is considered a major disturbance event and is becoming more frequent as more concentrated precipitation occurs in many parts of the world due to ongoing climate change (Short and Neckles 1999; Duarte 2002; IPCC 2014). Changes in salinity affect the functioning and productivity of coastal ecosystems, including macroalgal (Rodríguez-Gallego et al. 2015) and seagrass beds (Fernández-Torquemada and Sánchez-Lizaso 2005; Salo et al. 2014; Namba and Nakaoka 2018). Organisms from naturally stressful sites along the gradient may be less stressed by salinity changes compared to those from naturally stable sites in terms of mortality (Rosenberg and Rosenberg 1972), physiological responses such as osmoregulation and nutrient content (Nejrup and Pedersen 2008; Salo et al. 2014), and changes in growth rate and productivity (Garrote-Moreno et al. 2014). Less is known about the community-level responses to salinity changes through species interactions or how the responses vary among communities from sites with different ambient stress levels. Moreover, higher frequency and longer duration of stress is known to negatively affect organisms (Hanley et al. 2017), and the effects of pulse and press disturbances have been found to affect organisms differently (e.g. Salo et al. 2018). Yet the relative effects of frequency and duration of stress on organisms' responses such as growth rate and interactions remain unsolved.

Here, I experimentally examined the effect of the pulse and press disturbances of salinity decline and fluctuation on primary producers and animals as well as their species interactions by using marine organisms from sites with different ambient stress levels. I focused on the changes in amount, growth rate, and survival of different functional groups of primary producers and animals in eelgrass beds (i.e., eelgrass, epiphytic microalgae, and a grazer gastropod, *Lacuna decorata*) and their interactions based on grazing activities and thus the top-down controls, while examining how they were affected by different frequency and duration of salinity changes. I hypothesized that 1) higher frequency and longer duration of salinity changes would negatively affect the amount, growth rate, and survival of primary producers and animal as well as the top-down controls, and that 2) populations of primary producers and animals from naturally more stressful and unstable environments along salinity gradients would have less changes in the amount and growth rate as well as top-down controls during experiencing both press and pulse disturbances than those from stable environments. I also expected that the second hypothesis is more evident in a system with a stronger salinity gradient. Hence, my study sheds new light on the connections between the disturbance effects on species interactions as well as how the robustness and resilience of ecosystems and their functions against disturbances are related to the condition of ambient environments.

5.2. Material and Method

5.2.1. The studied system

Eelgrass (*Zostera marina*) is a species of seagrass that occurs in the intertidal to subtidal areas of coasts, estuaries, and lagoons in the northern hemisphere and forms a dense meadow, called the eelgrass bed, in sandy or muddy sea bottoms (Duffy et al. 2015). Eelgrass beds support the biodiversity in the coastal environment by hosting a wide variety of plants and animal species, and both plant-plant and plant-animal interactions are important in determining community structures (Duffy et al. 2015). Aside from eelgrass, various macroalgae and microalgae attach to the aboveground part of eelgrass (Momota and Nakaoka 2017). These epiphytic algae and eelgrass often compete for light resources, where the negative effect of overgrown epiphytic algae on the photosynthetic activities of eelgrass has been documented (Hauxwell et al. 2003). Grazing herbivores (hereafter grazers), such as sea urchins and waterfowls, feed on eelgrass directly, while grazers, such as amphipods and gastropods, feed on epiphytic microalgae or both eelgrass and microalgae (Duffy et al. 2015). Direct feeding of eelgrass can have a negative impact on eelgrass growth and biomass, while feeding of epiphytic microalgae would have an indirect positive effect on eelgrass (Duffy et al. 2013; Whalen et al. 2013).

5.2.2. Studied sites and organisms

I collected organisms for the experiment from eelgrass beds at two sites (AK and BK: 43°00'14.9"N 144°51'29.5"E and 43°04'20.0"N 144°51'35.5"E, respectively) in Akkeshi, and at two sites (NTR and UBR; 44°05'29.9"N 144°07'42.6"E and 44°01'44.3"N 144°06'09.6"E, respectively) in Notoro of eastern Hokkaido, Japan (see Namba and

Nakaoka 2018 and Namba et al. 2020 for detailed site information). Akkeshi has an estuarine system with a strong salinity gradient that is created by a large freshwater input from the river inflow (Momota and Nakaoka 2017; Namba and Nakaoka 2018; Namba et al. 2020). Moreover, tidal forces create diurnal variations in the site salinity within the lagoons (Figure C1 A and B). AK is located in the outer part of the estuarine system, where salinity remains stable at 32 throughout the day. BK is located close to the river mouth and is approximately 4 km away from AK, where the diurnal variation of salinity ranges between 0 to 32. Hence, it has an unstable saline environment. In contrast, Notoro is also an estuary, but it lacks a strong salinity gradient due to the small amount of freshwater input from the surrounding watersheds and it has a relatively uniform saline environment (Namba and Nakaoka 2018; Namba et al. 2020) (Figure C1 C and D). The effect of the tidal forces is minimal in Notoro, and thus no diurnal variation in salinity is observed. UBR is close to the river mouth, yet the salinity is constant at 32. NTR is approximately 4 km away from UBR and has a stable saline environment similar to UBR. Eelgrass, epiphytic microalgae attached to the aboveground eelgrass, and gastropod *L. decorata* collected at AK, BK, NTR, and UBR were used for the experiment. This gastropod is one of the most abundant and widely distributed species in eelgrass beds in both Akkeshi and Notoro sites and is found in both unstable and stable saline environments (Namba et al. 2020). Living on the aboveground parts of eelgrass, they feed on various food sources, including microalgae and eelgrass leaves (Kanamori et al. 2004; Kajihara et al. 2010).

5.2.3. The salinity fluctuation experiment

I tested the effects of the different frequency and duration of salinity decline and fluctuation on each functional group (eelgrass, epiphytic microalgae, and the grazer gastropod) as well as their interactions using a flow-through indoor mesocosm at the Akkeshi Marine Station (AMS), Hokkaido University, Japan (43°01'14.9"N 144°50'12.7"E). Owing to the limitations in the facility, the experiment was conducted in two blocks. The first block consisted of organisms from Notoro and the experiment ran for 28 days (17 July – 13 August 2019). The second block consisted of organisms from Akkeshi and the experiment ran for 18 days (7 September – 26 September 2019). The experimental duration of the second block was shorter than originally planned (28 days) because the survival of the grazer started to decrease earlier than expected. I collected eelgrass and grazers from each site one week prior to the start of each experiment. For each block, the eelgrass shoots with four leaves (the average ± standard deviation values of the length of the longest shoots were 94.60 ± 44.03 cm and 106.08 ± 24.68 cm for Akkeshi and Notoro, respectively) and the belowground tissues (rhizomes and roots) that were not damaged by grazing were used. The grazers of similar shell length (height of the shell, average ± standard deviation values were 4.69 ± 0.87 mm and 5.14 ± 0.03 mm for Akkeshi and Notoro, respectively) and wet weight (0.020 ± 0.010 g and 0.025 ± 0.007 g for Akkeshi and Notoro, respectively) were used. The eelgrass shoots and the grazers were kept in separate aquaria with running seawater and the grazers were fed fresh eelgrass leaves for one week until the beginning of the experiments to acclimatize them to the laboratory environment.

The experiment in each block was conducted using a three-way orthogonal design consisting of four salinity fluctuation levels, two grazer levels (with and without the grazer), and two source site levels (AK and BK in the Akkeshi block as well as UBR and NTR in the Notoro block). Salinity fluctuation levels included the (1) control (C hereafter: ambient saline seawater from the ocean next to AMS, ranged between 30-32), (2) the low frequency and short duration treatment thus simulating single occurrence of pulse disturbance (T1 hereafter: salinity reduced from the ambient level to 10 for 2 days at the beginning of the experiment and returned to the ambient level afterwards until the end of the experiment), (3) the high frequency and short duration treatment thus simulating frequent pulse disturbance (T2 hereafter: repeating the 1-week cycle of 2-day salinity reduction to 10 and following the 5-day ambient salinity treatment throughout the experiment), and (4) the low frequency and long duration treatment thus simulating press disturbance (T3 hereafter: salinity reduced to 10 throughout the experiment). A total of 10 was the lowest salinity level recorded in a previous survey of eelgrass beds around eastern Hokkaido after heavy rainfall events (Namba and Nakaoka 2018). The T1 and T2 simulated the occurrences of one and multiple heavy rain events, respectively, while T3 simulated the situation where freshwater from heavy rainfall is retained in a part of an estuarine as previously observed when multiple typhoons hit Akkeshi in 2016 (Namba, personal observation), and thus, was the most severe disturbance. A salinity reduction within the aquaria was created by the addition of dechlorinated tap water into the seawater reservoirs. The grazer levels were manipulated with 0 or 5 individuals per eelgrass shoot and the density was determined by field observation of *L. decorata* at the studied sites (Namba et al. *unpublished data*). The shell length of the grazers was measured using a caliper and the wet weight of the grazers was measured before the experiment by draining them on a piece of paper for 5 s. In each salt treatment and source site, I prepared 10 replicates with and without-grazer treatments, respectively, resulting in a total of 160 replicates. Each replicate plot (an experimental bottle; 11 cm in diameter and 60 cm tall, which had 12 holes with a 3 cm diameter that was covered by a 1 mm mesh for water exchange) with one eelgrass shoot and either 0 or 5 grazers. Eelgrass shoots were planted in 0.1 mm glass bead substrates with the addition of 0.14 g of NeXCOTE (HYPONeX Japan Corp., Ltd., Japan), a slow diffusing fertilizer, to standardize the effect of the soil nutrients. The bottles were randomly placed in the aquaria and assigned to different salinity treatments.

The same aquarium tanks with a flow-through system were used in both blocks of the experiment. The room temperature, seawater salinity, and temperature of the tanks were continuously measured by the loggers (HOBO conductivity logger U24-002-C and HOBO water level logger U20L-02, Onset, USA). The temperature changes across all tanks were kept constant using thermostat regulators (Figure C2 (A to H) and Figure C3 (A to H)). Mixing of freshwater and seawater to reach the target salinity level was performed in the reservoir tanks of the system. Water entering the system was filtered through the cotton aquarium filter. In addition, the system had a circulating water pump and the water discharge rate was maintained so that the water in the aquarium tanks was completely replaced every 12 h. Effectively, each step of the salinity reduction to 10 and the following recovery to the ambient salinity level took 12 h. The light condition in the system was equalized by completely covering the tanks with black sheets. Furthermore, I used a standardized light source (light emitting diode GLRS1122/FUV) (Volx Japan, Japan) with a 12 hour dark-light cycle and kept the light intensity of the tanks at 700-800 $\mu mol\ m^{-2}\ s^{-1}$ on the water surface, which is close to the field conditions in the eelgrass beds (Dennison and Alberte 1982). To standardize the dissolved oxygen level to be 7.5 to 8.5 $mg\ L^{-1}$, the water in the tanks was aerated. In addition, bottles were moved in the aquarium tanks to randomize the effect of light, while both tanks and bottles were cleaned every 3 days to remove epiphytes that could potentially hinder the experiment.

5.2.4. Measurements

To measure the eelgrass leaf growth rate, two holes were punched on each leaf by a needle at the beginning of the experiment that were 0.5 mm apart and 1 cm above the oldest leaf of each eelgrass shoot, which was adapted from Short and Duarte (2001). The needle was inserted so that it penetrated all the leaves of the eelgrass. During the experiment, the salinity and water temperature levels were measured daily using the portable salinity and temperature instrument EcoSense EC300A (YSI Inc., Xylem Inc., USA). Dissolved oxygen (DO) levels of the aquarium tanks were measured using the DO meter LAQUAct OM-71-2 (HORIBA, Ltd., Japan) every other day, while the nutrient levels of NO_2, NO_3, NH_4, and PO_4 in the aquarium tanks were checked on a weekly basis using the water quality testing kits of Pack Test (Kyoritsu Chemical-Check Lab., Corp., Japan) to confirm that all the tanks had equal levels of DO and nutrients. Grazer survival was monitored daily by counting the dead and living individuals during the experiment. Afterwards, eelgrass shoots were removed from the experimental units and the epiphytic microalgae were gently removed from the aboveground part of the eelgrass using a glass slide and were filtered through GF/F filters (Whatman International Ltd., UK). To estimate the amount of epiphytic microalgae, chlorophyl-*a* (Chl-*a*) concentrations measured by the non-acidification method of Welschmeyer (1994) were used. The filters were extracted in 6 mL of N,N-dimethylformamide for more than 24 h and stored at −20 °C until further analysis. The Chl-*a* concentration within the extract was measured using the 10-AU-005-CE fluorometer (Turner Designs Inc., USA).

After scraping off the epiphytes, each eelgrass shoot was separately placed between a plastic lamination film and scanned using the photo scanner RICOH MP C3004

JPN (Ricoh Company, Ltd., Japan). Photo files created from this process were used to measure the leaf area and the grazing scar area by the grazers using the image analysis software of Fiji with a Python extension (Schindelin et al. 2012). I used the holes punched on the oldest leaf of each shoot as a reference mark to measure the specific growth rate of eelgrass, as the oldest leaves ceased their growth, and the mark remained in the same position throughout the experiment. The biomass of the newly grown eelgrass leaves was defined as the segment between the two holes on the leaf surface and the relative location of the reference mark (Figure 8-3 in Short and Duarte 2001). The newly grown part of each leaf was separated per shoot from the other parts and placed in a pre-weighed aluminum bag. The newly emerged leaves without hole marks were also collected per shoot and placed together with the newly grown part in a separate aluminum foil bag. The bags containing eelgrass were then dried at 60 °C for 48 h and weighed to measure the biomass. Next, the specific growth rate of each eelgrass shoot was calculated by first dividing the proportion of the dry biomass of the newly grown part by the total dry biomass of the whole eelgrass shoot, and then by the duration of the experiment, which was modified from Aioi and Pollard (1993). Grazers were collected from each bottle, in which the shell length and wet weight were measured. The specific growth rate and wet weight changes were determined using the formula and method of Salo et al. (2018). The effects of top-down controls by the grazer on eelgrass and epiphyitic microalgae were measured separately. To examine the amount of grass that was grazed, the area of the grazing scars on the eelgrass shoots was calculated from the scanned images. Because some grazers died during the experiment, the area was then standardized by multiplying the days of survival and the number of surviving individuals. Moreover, the amount of epiphytic microalgae that was consumed by the grazers was estimated by subtracting Chl-

a of each eelgrass shoot of the grazer treatment from the average Chl-*a* amount of the non-grazer treatment per salinity treatment. Finally, the amount of consumption was then standardized using the same method used for the grazing scar area.

5.2.5. Statistical analysis

All statistical analyses were performed using the R software v3.5.1 (R Core Team 2018). I tested the effects of the grazer, the source site, and the salinity treatments on the eelgrass specific growth rate and the Chl-*a* amount of epiphytic microalgae by three-way analysis of variance (ANOVA) in each block of the experiment. The first hypothesis regarding that the higher frequency and longer duration of salinity changes would negatively affect the responses of plants and animals as well as their interactions was tested by the presence/absence of significant salinity effects and the significant interactions between the salinity and grazer treatments. The second hypothesis regarding that the populations of primary producers and animals from naturally more stressful and unstable environments along salinity gradients would have higher tolerance to the disturbances than those from stable environments was tested by the presence/absence of significant interactions between the salinity and site treatments. I also tested the effects of site and salinity treatments on the grazer-specific growth rate, wet weight changes, grazing scar area, and the amount of microalgae consumption using a factorial two-way ANOVA. The response variables were log-transformed to meet the assumption of both normality and homoscedasticity. In addition, the effect size of each response variable was calculated based on Hedges' *d* with a 95 % confidence interval using the R package 'effsize'. Furthermore, the variation in the survival of grazers across salinity treatments was tested by log-rank test followed by a pairwise comparison using the Bonferroni correction as the adjusting method for P-value using the R package ‘survival’. The tests were performed separately for each of the four sites.

5.3. Results

The eelgrass specific growth rate was generally higher under the grazer treatment than under the non-grazer treatment across all salinity treatments and sites (Figure 5.1a). Salinity treatments significantly affected the growth rate and the presence/absence of the grazer only had a marginal effect in Akkeshi (Table 5.1). However, no significant interactions among the site, salinity, and grazer treatments were detected. The results also showed that the effects of salinity were the highest when the disturbance level was intermediate and the presence of grazers enhanced the growth of eelgrass. Lack of significant interactions indicated that the top-down control of grazers was not affected by salinity and that the origin of eelgrass was not related to how the eelgrass growth rate was affected by salinity. Effect size analysis also showed that the difference in eelgrass growth rate between AK and BK was not large for most of the treatments, except C of the grazer treatment, which was congruent with the ANOVA results (Figure C4 A and B). In contrast, the single effect of grazers and the interactions of the site and salinity treatments were detected in Notoro. This indicated that the grazer enhanced the eelgrass growth regardless of the salinity and site treatments. Moreover, the effect of salinity differed between the two sites, where the growth rate was lowest in T3 of NTR and the lowest in T2 of UBR. The effect size analysis of Notoro showed a large difference between the sites across salinity treatments with small differences across grazer treatments.

Furthermore, the amount of epiphytic microalgae was generally lower in the grazer treatment than in the non-grazer treatment in both Akkeshi and Notoro, showing the effect of grazing pressure on the microalgae abundance and productivity (Figure 5.1b). A significant interaction was found between the grazer and salinity treatments as well as between the site and salinity treatments in both Akkeshi and Notoro (Table 5.1). Moreover, the interaction of the grazer and the salinity treatment factors showed that the presence or absence of the grazer affected the variation in the amount of epiphytic microalgae across salinity treatments, in which the difference between the grazer/non-grazer treatments was generally higher at the intermediate level of salinity stress (T1 and T2). In addition, the interaction of salinity and site factors indicated that the patterns of change in the amount of epiphytic microalgae across salinity treatments differed between the sites, where the amount of epiphytes was greater in lower stress treatments in BK than in AK of Akkeshi and in NTR than in UBR of Notoro. The effect size analysis of epiphytic algae supported these results, as shown in the similar patterns of the measured effect sizes between the grazer treatments (Figure C4 C and D). Furthermore, the survival of grazers varied across the sites and treatments in both Akkeshi and Notoro (Figure 5.2). At Akkeshi, all grazers in AK survived the control treatment, but similar patterns of acute declines in survival were observed in T1, T2, and T3 (Figure 5.2a). The survival of grazers from BK was higher than 70% across all salinity treatments and the patterns of change in survival between the control and other salinity treatments did not vary (Figure 5.2b). Interestingly, T3 had significantly higher survival rates than T1 and T2. All individuals of NTR in Notoro survived the control treatment, but the survival severely declined in T3 at the beginning of the experiment (Figure 5.2c). Individuals in T1 and T2 had similar patterns of survival across salinity treatments, where the survival of the grazers in UBR in T1 was as high as in the control, while in T2 and T3 it was similar, but lower than in the control and T1 (Figure 5.2d).

The specific shell growth rate and wet weight changes of the grazers were generally the greatest in the control treatment and decreased with the salinity stress intensity and frequency in both AK and BK of Akkeshi and in NTR and UBR of Notoro (Figure 5.3a). In Akkeshi, the shell growth was significantly different between the salinity treatments and the sites, but the interaction was not significant (Table 5.2). Grazers in BK grew better than those in AK. In Notoro, significant interactions between the site and the salinity treatment was detected, showing a greater decline in shell growth rate with more severe salinity in NTR than in UBR. Wet weight changes of grazers at Akkeshi were only affected by salinity, but a significant interaction between the salinity and the sites was also detected in Notoro, similarly to the shell growth rate (Figure 5.3b, Table 5.2). The effect magnitude of the specific growth rate as well as the wet weight changes of grazers were overall small in both the Akkeshi and Notoro sites across salinity treatments, except for the control treatment in Notoro, showing small site differences in these variables (Figure C4 E and F). The grazing scar area of eelgrass, which is a proxy for the consumption of eelgrass by the grazers, was highest in T1 and lowest in T3 in AK and BK and decreased with the intensity of the salinity in Notoro sites (Figure 5.3c). Site differences were not significant in Akkeshi and were only marginal in Notoro (Table 5.2) with a small effect size (Figure C4 G). Furthermore, the amount of consumption of epiphytic microalgae increased between the control treatment to the medium levels of salinity (T1 and T2), and again decreased as the salinity disturbance became more severe (T3) across all the sites (Figure 5.3d). Variations between sites was not significant in both Akkeshi and Notoro (Table 5.2), which also had small effect sizes (Figure C4 H).

5.4. Discussion

I hypothesized that the prolonged decline (i.e. longer press disturbance) and the more frequent salinity changes (i.e. more pulse disturbance) due to freshwater input from intensified rainfalls can negatively affect the amount, survival, and growth rate of primary producers and animals as well as their interactions, using eelgrass bed ecosystem as an example. I also expected that the organisms from naturally unstable, and hence, stressful environments along a stress gradient would be less affected by the change in stress levels than those from more stable environments. My results mostly supported these hypotheses, showing various patterns of changes in growth rate, biomass, survival, and species interactions as salinity changes became more severe. Differences in the patterns between sites were mainly observed in the lagoon with a salinity gradient (Akkeshi), but some were also observed in the lagoon without a gradient (Notoro). To understand the mechanisms causing the observed variations, I first examined the effects of salinity decline and fluctuation on the responses of primary producers and animals as well as the change in top-down controls. Then, I explored the differences in these responses among the populations from different sites on the ecosystem functions and compared the robustness and resilience of the systems with and without the stress gradient.

5.4.1. Effect of salinity stress on plants, animals, and their interactions

My results suggested that individual and population responses to salinity fluctuation and decline differed among the functional groups of the primary producers (eelgrass and epiphytic microalgae), in which their responses varied between the presence and absence of grazers. The more frequent and longer salinity decline was related to the decline in growth rate of eelgrass regardless of the presence or absence of grazers.

Eelgrass growth rate was mostly higher when grazers were present in both Akkeshi and Notoro. This suggested that press and pulse disturbances caused by salinity fluctuation and decline negatively affected eelgrass, but having a grazer ensured a higher growth rate under stressful conditions. In contrast to the specific eelgrass growth rate, the amount of epiphytic microalgae increased with the increased duration and frequency of the salinity changes across all sites. The epiphytic microalgae on the above ground tissues of eelgrass consisted of various species of diatoms and dinoflagellates (Hasegawa et al. 2007). Moreover, it is possible that the decline in salinity provided a favorable condition for the growth and proliferation of these species. Additionally, a lower amount of epiphytic microalgae with grazers indicated the presence of a top-down control, in which the grazers consumed epiphytic microalgae from the above ground tissues of eelgrass (Duffy et al. 2015). The top-down control of the epiphytic microalgae by the grazers may indirectly improve eelgrass growth rate because light attenuation by fouling of the overgrowing epiphytes on eelgrass can negatively affect the photosynthetic activity of the plant and cause a decline in its growth rate (Short and Neckles 1999). Overall, the results suggested that a higher amount of epiphytic microalgae associated with the absence of grazers had negative effects on eelgrass growth, in which the effects increased with longer and more frequent salinity changes. In addition, the top-down control of grazers positively affected eelgrass by removing microalgae, especially under most disturbed conditions. However, whether press or pulse disturbance affected more than another remained unclear from the results. Such roles of grazers were also observed in other conditions, such as eutrophication, in which grazers effectively removed the microalgae that was growing on the eelgrass (Whalen et al. 2013).

As mentioned earlier, the specific growth rate of eelgrass and the amount of epiphytic microalgae was affected by the presence of grazers. Top-down control of grazers on the primary producers varied with their abundance and condition, where higher grazer abundance would intensify herbivory in the ecosystem (Wernberg et al. 2008). However, factors such as salinity decline cause higher mortality and thus lower survival (Irlandi et al. 1997; Zhang et al. 2016). The results of my experiment showed a decline in survival when salinity decline was longer and more frequent in most cases. Decline in grazer abundance results in weakened herbivory and eventually declines in top-down controls in the ecosystem, as observed previously where a decline in the abundance of herbivorous fish and invertebrates lowered feeding pressure on epiphytes, hindering the growth of marine macrophytes (Heck and Valentine 2006; Myers and Heck 2013). The condition of gastropod grazers can be measured by their physical parameters, such as specific growth rate and wet weight change, and are used as indicators of their performances (Salo et al. 2018). In my experiment, a decline in specific growth rate and wet weight change was observed when salinity stress increased. When exposed to stressors such as low salinity, growth of small marine invertebrates is decreased due to stress-induced changes in physiological and osmoregulatory responses (Chaparro et al. 2008; Zhang et al. 2016), such as a decline in metabolic rates (Paganini et al. 2010; Sokolova 2013). Growth decline is further linked to a decline in feeding rates in environmental conditions outside of the natural range of stress levels, as observed for gastropods (Irlandi et al. 1997; Zhang et al. 2016) and bivalves (Chaparro et al. 2008).

The stress responses of grazers are closely tied to species interactions, such as herbivory (Rand 2002; Daleo and Iribarne 2009). In my study, grazing scars on eelgrass and consumption of epiphytic microalgae were measured as indicators of herbivory. In both naturally unstable and stable sites in Akkeshi and Notoro, the area of grazing scars on eelgrass declined with press disturbances as well as more frequent pulse disturbances. This implies that the grazers were less feeding on eelgrass, and reduction of direct grazing may explain the increase in eelgrass growth rate in the treatments with more severe salinity change as the damage to eelgrass by such grazing activities inhibits eelgrass growth (Zimmerman et al. 2001). Changes in the consumption of microalgae with different salinity treatments had a different pattern from the change in the area of grazing scars on eelgrass. For all sites, consumption increased from the control to the treatments with the middle level of stress intensity and declined in the treatment with the most severe stress. The grazer *L. decorata* feeds on both microalgae and eelgrass (Kajihara et al. 2010), and the grazers seem to feed on epiphytic microalgae first and directly feed on eelgrass in the absence of dense algal mat on eelgrass above ground tissues (M. Namba, *personal observation*). The peak consumption at a moderate level of salinity stress can be associated with an increase in algal food sources, but a decline in the consumption at the longer and most frequent salinity decline may be related to decreased vital rates of grazers, as shown by the lowest survival and growth rates observed for this treatment (T3). Decline in the consumption of epiphytic microalgae by the grazers also suggests that it indirectly helps increase in epiphytic microalgae. Decline in the consumption of microalgae by grazers means weakened top-down control, and eelgrass may be negatively affected by the increase in epiphytic microalgae caused by reduced herbivory.

5.4.2. Variation in the stress responses of organisms from habitats with different environmental stabilities

Site differences in the growth rate of seagrass species along salinity gradients have been observed both experimentally and in the fields where populations from sites with higher stress levels grew better when exposed to stressors (Salo et al. 2014; McDonald et al. 2016). In contrast, my results showed that the eelgrass growth rate declined with more intense stress in both groups from the less stressful site and highly stressful site in Akkeshi. Although site differences in the response of the growth rate to salinity stress were observed in Notoro, a decline in growth with increased stress levels indicated that eelgrass was affected by salinity stress regardless of source population. My results also showed that the amount of epiphytic algae increased with intensified stress for all populations and that only the site differences were found when epiphytic algae were exposed to longer decline and more frequent fluctuation of salinity. The response of epiphytic algae to stressors was similar regardless of source population. This may be because environmental filtering by salinity stress affected all the treatments in similar ways and resulted in the selection of epiphyte communities that grew well in highly stressful environments. Epiphyte composition and abundance varied along the salinity gradients, in which an increase in the abundance of some diatom species relative to the decline in salinity was previously observed (Underwood et al. 1998; Potapova 2011). Hence, species and taxa level studies on the changes in epiphyte communities with salinity stress are required for further explanation.

My results of survival, growth rate, and wet weight change of the grazers showed variations in the stress responses among the populations from different ambient stress levels, suggesting that the top-down control may or may not be maintained at some sites along the stress gradients when a disturbance event occurs. For the grazers from naturally stable environments, a higher frequency and a longer duration of salinity changes caused a drastic decline in the survival and reduced the abundance, as shown in AK of Akkeshi. In contrast, survival and abundance were maintained for the grazers from a naturally stressful environment, as shown in the BK of Akkeshi. However, my prediction that grazers from stable UBR and NTR of Notoro would have a survival level as low as those from AK was not supported, where the reason for this is unclear. However, relatively small differences in survival among salinity treatments in BK were observed, while clear differences between the less (control and T1) and more severe stress treatments (T2 and T3) were observed among grazers from UBR and NTR. Furthermore, the decrease in shell growth rate observed in all sites suggested that the grazers were overall affected by the salinity changes regardless of the stress levels of their natural habitats. Interestingly, the results of the changes in wet weight and shell lengths showed that grazers from AK and BK of Akkeshi had similar responses to the salinity changes. This suggested that the surviving grazers from the two sites have similar responses to salinity decline and fluctuation.

Moreover, the degree and direction of the effects of salinity change varied among the organisms from sites with different natural stress levels along the stress gradients, as shown by the significant treatment-site interactions in eelgrass growth, epiphyte abundance, grazer growth, and feeding rates. In systems with variable ambient stress levels, such as Akkeshi, ecosystem functions were likely partially lost at first, but recovered later when disturbance events occured. The site with naturally stable salinity (AK) in Akkeshi experienced a decline in the survival of grazers and herbivory and an increase in eelgrass growth rate as well as epiphytic microalgae abundance with increased stress levels. In contrast, the sites with naturally unstable and highly fluctuating salinity environments (BK) did not lose most of the grazers even when the level of stress was maximized. Despite the decline in herbivory, the growth rates of eelgrass and microalgae biomass were maintained at this site. Therefore, AK had a decline in the top-down control, while BK did not, when a salinity decline occurred in the ecosystem. However, both NTR and UBR of Notoro were sites with a stable natural salinity environment (Namba and Nakaoka 2018; Namba et al. 2020). The survival of the grazers from NTR decreased with decline and fluctuation of salinity levels. The decline in the survival in UBR was not as drastic as NTR, yet it was affected by the higher frequency and longer duration of salinity decline more than in the case of BK of Akkeshi. Despite the increase in herbivory, it would be difficult for the grazers to control the amount of overgrowing microalgae. A uniform loss of grazers from sites in Notoro would occur when salinity decline occurs more frequently or in the long term, and this would affect the top-down control operated by the grazers. When the system undergoes such a regime shift, decreased ecosystem functions can be expected in both sites.

5.4.3. Conclusions and outlook

Using the eelgrass bed ecosystem and its salinity gradients as an example, I demonstrated that the individual and population responses of plants and grazers are intertwined by complex species interactions and are affected by different patterns of change in stress levels. In addition, the variation in the stress responses could be related to the differences in the ambient stress levels of the organisms' habitats. Top-down controls by grazers can play an important role in maintaining ecosystem functions and a decline in the productivity of macrophytes was expected with increasing stress levels following higher mortality of grazers. The effects of pulse and press disturbances caused by salinity decline and fluctuation varied among organisms and populations, thus which type of disturbance is more influential than another is unclear from this research. Because the mortality of grazers is low in the population from a naturally stressful site, such as in

BK of Akkeshi, I expected to observe that the ecosystem functions would be better maintained at sites with naturally stressful environments than in stable environments with more severe stresses/disturbances.

Various ecosystems are prone to disturbances at local and global scales that are caused by ongoing climate changes (Short and Neckles 1999; Duarte 2002) and increase in human disturbances (Peterson et al. 2014), hence, it is crucial to simulate different frequency and duration of disturbance events as well as to incorporate habitat ambient stress levels when evaluating the potential effects of disturbance events on organisms at various organizational levels in further research. As demonstrated in coastal eelgrass ecosystems that are vulnerable to disturbances, comprehensive research on connectivity of different ecosystems and trophic levels as well as long-term monitoring of population dynamics affected by disturbances are necessary (Peterson et al. 1994; Peterson et al. 2008). In the present study, I evaluated the effects of extreme rainfall events and the subsequent salinity decline and fluctuation on eelgrass bed ecosystems that would occur with ongoing climate change. With this disturbance event, I observed a loss of grazers and weakened top-down controls in the ecosystem, followed by an increase in epiphytic algae and a decrease in eelgrass growth. This cascading process would eventually trigger regime shifts and a decline in ecosystem functions of eelgrass beds. Loss of grazers has also resulted in regime shifts in many coastal ecosystems, where fast-growing algae dominate plant-plant competition and outgrow the foundation species in coastal ecosystems, resulting in a decline in ecosystem functions (e.g. Svensson et al. 2012). Weakened top-down controls within the food web eventually cause microalgae to overgrow eelgrass, causing the ecosystem to be less productive, while algal-dominated ecosystems would be affected by other disturbances, such as eutrophication (Hauxwell et al. 2003) and heat waves (Li et al. 2017). In addition to the salinity decline, further investigations on the effects of multiple stressors on the individual and population levels as well as the species interactions in various ecosystems should be conducted. In addition, understanding the causes and consequences of regime shifts that are triggered by such disturbances are needed in the era of climate change as well as the increase in anthropogenic stress.

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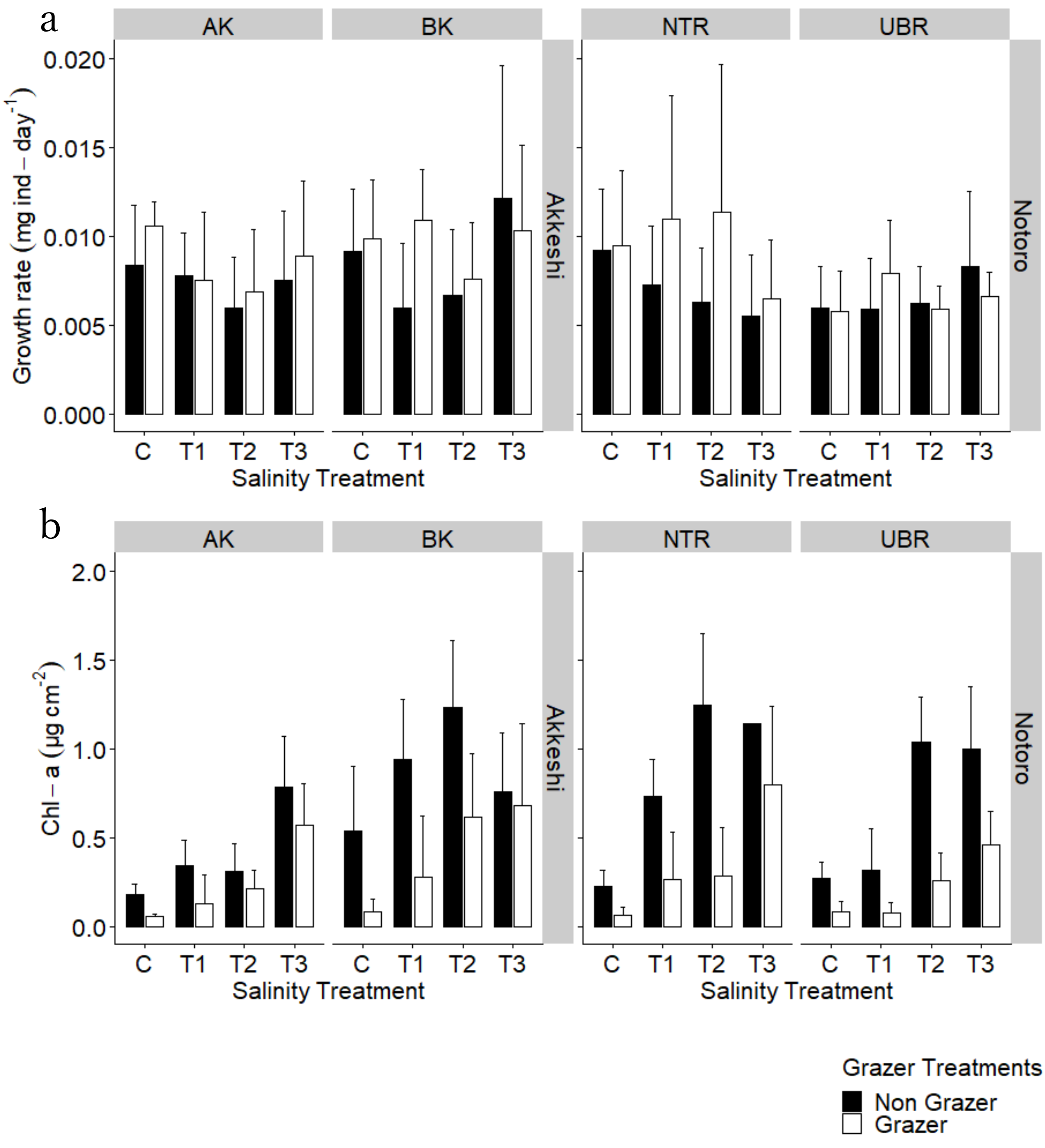

Figure 5.1. Variation among different salinity and grazer treatments in (a) specific growth rate of eelgrass and (b) Chl-a amount of epiphytes from AK and BK of Akkeshi and NTR and UBR of Notoro. The error bars indicate standard deviation of the data.

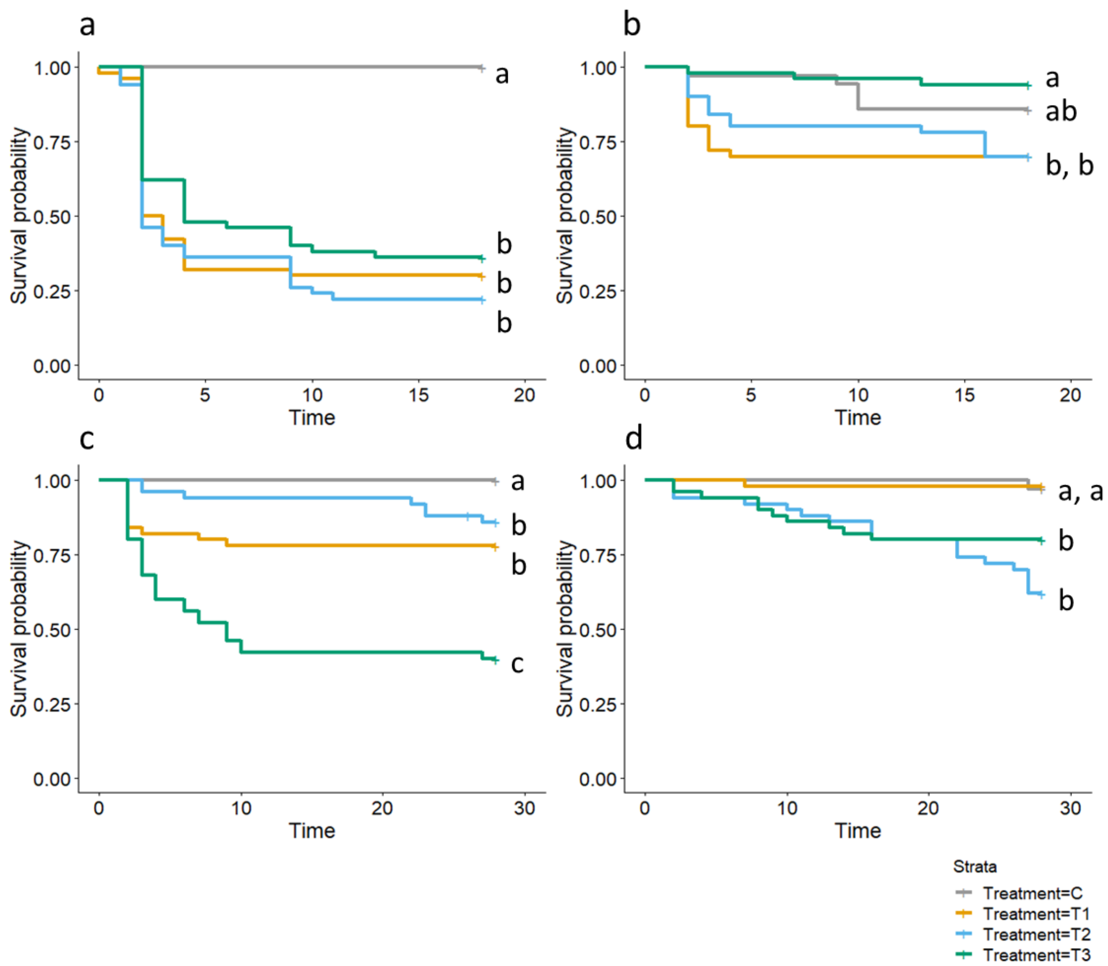

Figure 5.2. Survival of grazers from (a) AK, (b) BK, (c) NTR, (d) UBR. The lower-case letters indicate significant differences among salinity treatments detected by the pair-wise post hoc test followed by log-rank test ($P < 0.05$). Bonferroni correction was used as adjusting method for P-value.

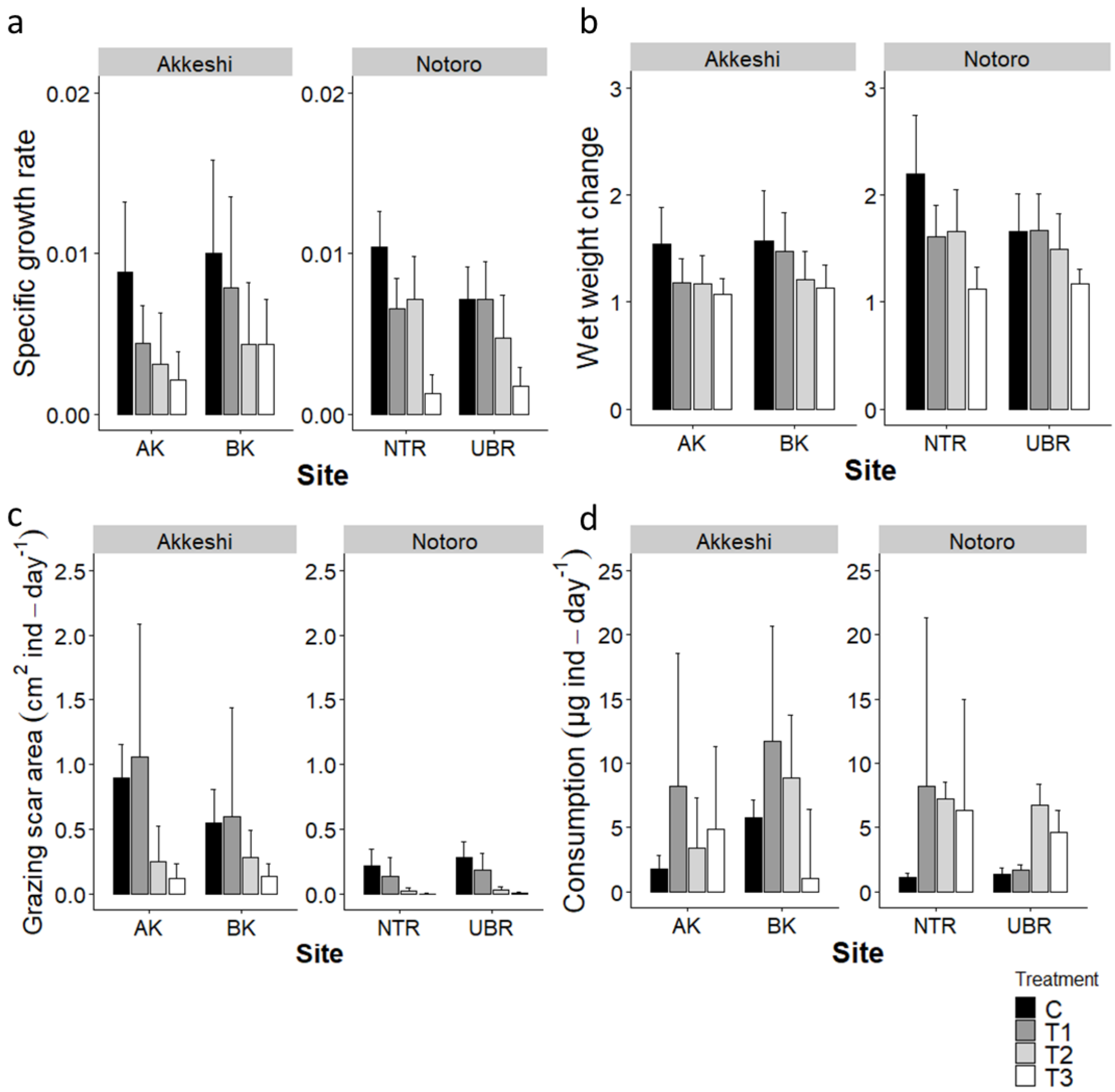

Figure 5.3. Variation among different salinity treatments (C, T1, T2, and T3) in (a) specific growth rate of grazer shell lengths, (b) wet weight change of grazers, (c) grazing scar area, and (d) consumption of epiphytes by grazers from AK and BK of Akkeshi and NTR and UBR of Notoro. The values are standardized by individuals per day. The error bars indicate standard deviation of the data.

Table 5.1. Three-way ANOVA testing variation in eelgrass specific growth rate and Chl-*a* amount of epiphytes among salinity treatments (Treatment: Control, T1, T2, and T3), grazer treatments (Grazer: non-grazer and grazer), and between sites (Site). P values < 0.05 are shown in boldface.

	Eelgrass specific growth rate						*Chl-a amount*					
	Akkeshi			Notoro			Akkeshi			Notoro		
Variable	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Treatment	3	4.43	**<0.01**	3	1.35	0.26	3	39.72	**<0.01**	3	56.81	**<0.01**
Grazer	1	3.68	0.06	1	4.18	**0.04**	1	84.78	**<0.01**	1	143.58	**<0.01**
Site	1	2.77	0.10	1	1.17	0.28	1	40.21	**<0.01**	1	7.69	**<0.01**
Treatment × Grazer	3	0.61	0.61	3	0.71	0.55	3	9.37	**<0.01**	3	6.91	**<0.01**
Treatment × Site	3	0.77	0.51	3	3.99	**0.01**	3	7.75	**<0.01**	3	5.96	**<0.01**
Grazer × Site	1	0.17	0.68	1	2.48	0.12	1	3.15	0.08	1	0.08	0.77
Treatment × Grazer × Site	3	2.29	0.08	3	0.62	0.60	3	1.13	0.34	3	0.90	0.44
Error	132			132			132			131		

Table 5.2. Two-way ANOVA testing variation in (A) grazer shell length specific growth rate, (B) grazer wet weight change, (C) area of grazing scar, and (D) consumption of epiphytic microalgae among salinity treatments (Treatment: Control, T1, T2, and T3) and between sites (Site). *P* values < 0.05 are shown in boldface.

	Akkeshi			Notoro		
Variable	df	*F*	*P*	df	*F*	*P*
(A)						
Treatment	3	25.21	**<0.01**	3	157.1	**<0.01**
Site	1	10.47	**<0.01**	1	16.55	**<0.01**
Treatment × Site	3	0.92	0.43	3	13.67	**<0.01**
Error	212			280		
(B)						
Treatment	3	20.78	**<0.01**	3	68.82	**<0.01**
Site	1	2.79	0.10	1	9.48	**<0.01**
Treatment × Site	3	1.79	0.15	3	9.72	**<0.01**
Error	212			280		
(C)						
Treatment	3	12.03	**<0.01**	3	42.78	**<0.01**
Site	1	2.07	0.16	1	3.92	**0.05**
Treatment × Site	3	1.84	0.15	3	0.28	0.84
Error	66			66		
(D)						
Treatment	3	3.88	**0.01**	3	3.64	**0.02**
Site	1	1.85	0.18	1	1.39	0.24
Treatment × Site	3	2.25	0.10	3	1.07	0.37
Error	66			66		

CHAPTER 6

GENERAL DISCUSSION

Understanding how biological communities respond to multiple stressors has become a major interest of ecologists and managers alike with growing pressures of anthropogenic impacts on marine ecosystems (Duffy et al. 2019). Stress gradients are present in various forms, and they are suitable test grounds for examining how biodiversity and functions of communities are affected by different stress levels. Identifying the factors that shape the community assembly processes as well as ecosystem functions such as primary and secondary productions is an important step in understanding the conditions and changes in ecosystems in which the communities are most vulnerable to, and this can be effectively acheived by comparing responses of organisms from the same ecosystem but with differences in the degree of ambient stress gradients. Salinity gradients in eelgrass beds are one of the best examples to study this topic among marine ecosystems that are ubiquitous around the globe and provide important ecosystem functions to the use of the society (Nordlund et al. 2017). Based on the two hypotheses in the Chapter 1, this thesis showed that (1) there was a variation in underlying processes determining biodiversity and production of different functional groups and communities among sites with variable stress levels and between systems with and without a steep stress gradient and that (2) the variation was related to the responses of inhabiting organisms to alteration of stress gradients by pulse and press disturbances in eelgrass bed ecosystems (Figure 6.1).

6.1. Variation in biodiversity and functions of eelgrass bed communities shaped by stress gradients

My study on identifying the effects of various factors on the biomass of eelgrass, epiphytic microalgae, and epifauna from the three lagoons with distinct environmental properties (Chapter 2) indicated that primary producer and consumer biomass of eelgrass ecosystem was influenced by different factors that vary at different spatial scales, and that how the stress gradients shape ecosystem functions varied among lagoons. Large body of past research is limited to investigate the extent of the effect of stress gradients in a single ecosystem rather than targeting on multiple ecosystems with the gradients created by same stressors (e.g. Salo et al. 2014; Cheng and Grosholz 2016). By comparing multiple ecosystems with variable gradient properties, I was able to consider the spatial scales in which the effect of the gradients was most notable. I identified that salinity gradients were important for determining eelgrass and epifauna biomass within lagoons, especially those with strong influences of freshwater discharges from major rivers, whereas variability in biomass of epiphytic microalgae was more attributed to environmental variations in larger spatial scales such as water temperature (Chapter 2).

Further investigation of the effects of salinity gradients on invertebrate communities of eelgrass beds from the lagoon with a steep gradient and the lagoon with a marginal gradient added insights into the variations in responses of the organisms with different functional traits (Chapter 3, 4). Although salinity gradients appeared to affect biodiversity and functions provided by epifaunal communities from the ecosystem with a steep gradient (Chapter 2, 3, 4), the effect of salinity gradient was not as prominent in infaunal communities of the same ecosystem, suggesting that the ambient stress levels of habitats influence how organisms are affected by stressors (Chapter 3). Moreover, salinity gradients more affected spatio-temporal variations of biomass of epifaunal grazers and suspension feeders than predators, indicating that feeding mode of epifauna was another factor to determine the stress response of organisms (Chapter 4). Benefits of incorporating functional trait analysis into evaluating the effects of stressors on marine organisms have been recognized in the fields such as assessment of wastewater impacts (Krumhansl et al. 2015) and eutrophication (Baden et al. 2010). My thesis demonstrated that analyzing production and biodiversity of organisms based on functional trait classification can be used to evaluate the responses of marine communities to stressors and identify potential vulnerability of the communities experiencing different levels of ambient stress.

Variations in plant-animal interactions were important components in determining biodiversity and functions of the ecosystem along stress gradients (Chapters 2, 3, 4 and 5). Positive relationships between overall epifaunal biomass and eelgrass standing stock indicated the use of eelgrass by multiple faunal species through non-trophic interaction which has been known as important species interactions in eelgrass bed communities (Chapter 2; Wong 2017). Community compositions of epifauna based on abundance data were affected by eelgrass biomass and shoot density in the lagoons without a steep salinity gradient, whereas the strong influence of environmental filtering masked the effect of the foundation species when a steep salinity gradient is present (Chapter 3). Besides eelgrass, I showed the roles of epiphytic microalgae and microphytobenthos on determining spatio-temporal patterns of grazers and suspension feeders biomass, respectively, in the lagoon with a steep salinity gradient, suggesting that the trophic linkage between animals and plants should be considered together with the effect of variable stress levels when evaluating variation in biodiversity and ecosystem functions along stress gradients.

6.2. Response of marine ecosystems to increasing stress levels and potential regime shifts

When steep stress gradients were present, variation in community compositions and production was observed with variation in stress levels among sites (Chapters 2, 3 and 4). I experimentally showed that stress responses among populations of epifaunal grazers varied when a steep stress gradient was present within an ecosystem, where populations from sites with high ambient stress levels were more resistant to increase in stress than those from sites with low stress levels (Chapter 5). Having more diverse populations in terms of stress tolerance within an ecosystem enables diverse responses to stressors as some communities within the system are exposed to higher stress levels at ambient conditions than the others are. My thesis pointed out that this heterogeneity in ambient stress levels along gradient axes was related to the heterogeneous stress responses of organisms and that it was important to maintain ecosystem functions and biodiversity when the ecosystem underwent increase in stress levels. Because my study in Chapter 5 only focused on one gastropod species, further investigations are needed to target wider ranges of species present in eelgrass beds to understand community-wide responses to stressors for better understanding of this topic.

I documented that increase in stress levels triggered changes in primary producer-epifaunal grazer interactions and that the change was less severe when the organisms were from habitats with high ambient stress levels (Chapter 5). Spatio-temporal variations of grazer was closely linked to epiphytic microalgae biomass

(Chapter 4), and this trophic interaction acted as a top-down control on epiphytic microalgae to overgrow eelgrass (Whalen et al. 2013). Weakened top-down control by the loss of consumers caused abrupt and persistent changes in marine ecosystems such as seagrass beds to alternative states, usually by outbreak of competitors of eelgrass such as fast-growing algae (Moksnes et al. 2008; Whalen et al. 2013; Tomas et al. 2015). These regime shifts often result in further cascading effects on trophic interactions within the ecosystem (Vergés et al. 2014; Tomas et al. 2015). Change in species interaction that was observed in my study is a warning sign that alteration in stress gradients triggers further loss of ecosystem functions in eelgrass beds, yet stress resistance of the communities from habitats with high ambient stress levels showed that there were possibilities that the function may be maintained in some parts of the ecosystem.

Defining the stability of ecosystems is important in understanding the mechanisms of regime shifts and their consequences, yet it is often challenging because detailed knowledge on biodiversity and function of ecosystems as well as long-term data covering a large spatial scale are not accumulated yet. My thesis mainly covered spatio-temporal extent of stress gradients ranging < 50 km and within one year, simulating sudden shifts of the ecosystem to an alternative stable state by increase in salinity stress at local scales. Similar abrupt changes in ecosystems are occurring worldwide, mainly due to changes in population size of keystone and/or foundation species with increases in stress levels (Rocha et al. 2018). Persistent changes in ecosystems at broader spatial scales are not covered in this thesis, and these are the topics that need to be addressed in further research as many of regime shifts in marine ecosystems are attributed to long-term influences of stressors such as rising seawater temperature and overexploitation across geographical regions (Vergés et al. 2019; Duffy et al. 2019).

6.3. Application of the results

The results of this thesis give insights into how the eelgrass bed ecosystems are shaped by various environmental factors and how human societies by the coasts may coexist with these ecosystems. The importance of eelgrass beds and other macrophyte ecosystems in terms of blue carbon and sources of food and other resources to human beings has been examined by the scientific society (Kuwae and Hori 2017; Nordlund et al. 2017) and is gradually recognized by local stakeholders such as municipal governments and fishery sectors. As the coastal communities rely heavily on the functions and services provided by ecosystems like eelgrass beds, understanding factors affecting the biodiversity and functions is fundamental for effective management and conservation for sustainable use of the ecosystems. For example, salinity is one factor determining the biodiversity in Akkeshi, whereas eelgrass biomass is the most influential factor in Notoro (Chapter 3). This suggests that the focus of management practices for consumers should differ in the two lagoons, such as constant monitoring of salinity especially at the sites with stable saline environments as an important component of the practice in Akkeshi and incorporating the evaluation of eelgrass biomass and distribution in Notoro. Moreover, knowing which ecosystems are more susceptible to environmental changes is important for prioritizing the activities based on ecosystems' vulnerability within limited time and budgets. My study revealed the vulnerability of eelgrass ecosystems to salinity fluctuation and decline in naturally stable environments (Chapter 5), and this results suggested that plans for mediating the impacts of increase in freshwater input by heavy rainfalls, such as creating terrestrial buffer zones to absorb excess freshwater, are crucial for maintaining functioning of ecosystems in lagoons such as Notoro and Saroma. Understanding the threshold of salinity decline and fluctuation is important for assessing the condition in which regime shifts of eelgrass beds may occur. As my research suggests that the salinity decline to 10 causes mortality of the grazer gastropod and decline of top-down controls (Chapter 5), it is suggested that salinity at 10 is already past the threshold. Continuous monitoring of salinity changes as well as the amount of precipitation is important for detecting the early warning signs of such regime shifts in eelgrass beds. The results from this thesis provided such fundamental information and contribute to the local best practices by showing that eelgrass beds were not affected by same factors uniformly, that salinity decline could be one reason for the loss of eelgrass beds, and the responses of the organisms to the salinity may vary among functional groups.

In addition, the results of this thesis are applicable to local fisheries and aquaculture activities. Understanding how biodiversity and secondary productions are affected by the presence of salinity gradients gives insights into how the stocks of consumers, such as amphipods and polychaetes that are important food for commercially important crustaceans and fish (Wong 2017; Unsworth et al. 2019), are maintained in different sites within lagoonal and estuarine systems. My studies showed that the species pool of consumers and the range of stress tolerance of species varied among lagoons and that some eelgrass beds were more vulnerable to decline in salinity than the others are (Chapter 3, 5). This would have impacts on local fisheries in the vulnerable systems as decline in consumers and eelgrass beds indicate loss of food sources, spawning grounds, and habitats for commercially harvested shrimps, crabs, and fish such as herrings. For example, the presence of salinity gradient causes the variation in community structures in eelgrass beds of Akkeshi, and the fishery practices for sustainable use of resources such as fish and crustaceans should recognize the site differences and consider establishing site-specific plans for the use. By contrast, eelgrass ecosystems in Notoro and Saroma are less spatially variable. This means that fishery plans for the resource use of these two lagoons should be different from Akkeshi and that more uniform approaches may be applied. Therefore, site-specific monitoring on changes in primary and secondary productions as well as shifts of biodiversity in eelgrass beds is important to understand the negative impacts on fisheries activities and possibly suggest mitigation plans for preparing the local communities to the forthcoming changes.

6.4. Conclusion

Based on the findings from this thesis, I conclude that salinity gradients affect variability in biodiversity and functions of eelgrass bed ecosystems through mechanisms operated at individual, population, and community levels, and that resistance and vulnerability of the ecosystems are associated with the presence of steep salinity gradients with various ambient stress levels. Increase in stress levels and alteration of stress gradients are already occurring globally and locally (Silliman and He 2018). Shifts of marine ecosystems, including eelgrass beds, to alternative stable states that are less productive and lower in biodiversity have been documented and some of them are often irreversible (Rocha et al. 2018; Filbee-Dexter and Wernberg 2018). As a result, understanding the mechanisms of abrupt changes in communities and their potentials to resist such changes has become ever more important in the recent decades for evaluating the causes and consequences of regime shifts, and long-term monitoring of ecosystems and manipulated field and laboratory experiments is necessary for this purpose.

Although we should not be too optimistic about the future of marine ecosystems, it is also necessary to look into silver lining and brighter sides of the issues. For example, we need to know in more detail about resilience of marine ecosystems against abrupt and persistent increase in stress levels and alteration of stress gradients. If some organisms within the ecosystem are more resistant to increasing stress levels than others (as shown in Chapter 5), there are some hopes that the original function and biodiversity of the ecosystem may be at least partially maintained. Theoretical and empirical studies show that high biodiversity enables the ecosystem to maintain its functions even after impacted by disturbances (e.g. Yachi and Loreau 1999; Blake and Duffy 2010; Gonzalez et al. 2020). I suggest that diversity of individuals, populations, and communities in terms of stress tolerance and resistance is as well important for ecosystem resilience against regime shifts and that further research is needed to reveal the underlying processes. Moreover, directional selection triggered by increase in stress induces shifts of the populations to be more tolerant against the stressors (Coleman and Wernberg 2020). The roles of stressors and stress gradients on shaping communities and the mechanisms leading to community changes should be investigated from various perspectives by incorporating innovative tools and diverse knowledge. For example, I suggest uses of a combination of various remote sensing techniques that are most suitable for given environments, such as underwater video surveys followed by flame analyses with machine learning techniques for deep subtidal zones (e.g. Filbee-Dexter and Scheibling 2016), acoustic monitoring surveys for subtidal ecosystems with low visibility (e.g. Sonoki et al. 2016), and drone or satellite image analysis for shallower zones with high visibility (Wilson et al. 2020). Other useful methods would be long-term field experiments for documenting and elucidating the mechanisms of regime shifts of macrophytes occurring at large spatial scales and longer temporal scales (e.g. Menge et al. 2017). Incorporating numerical models such as ecosystem models and population dynamics models may be helpful for forecasting the changes (e.g. Krumhansl et al. 2014; Abe 2016; Kumagai et al. 2018). Use of historical and archaeological data such as invertebrate remains from middens may help identifying the baseline shifts in coastal marine ecosystems (e.g. Ainis et al. 2014). I hope that this thesis serves as a step for better and deeper understanding in biodiversity and functions of coastal marine ecosystems and shifts that they may undergo with increasing anthropogenic disturbances and ongoing climate change.

6.5. References

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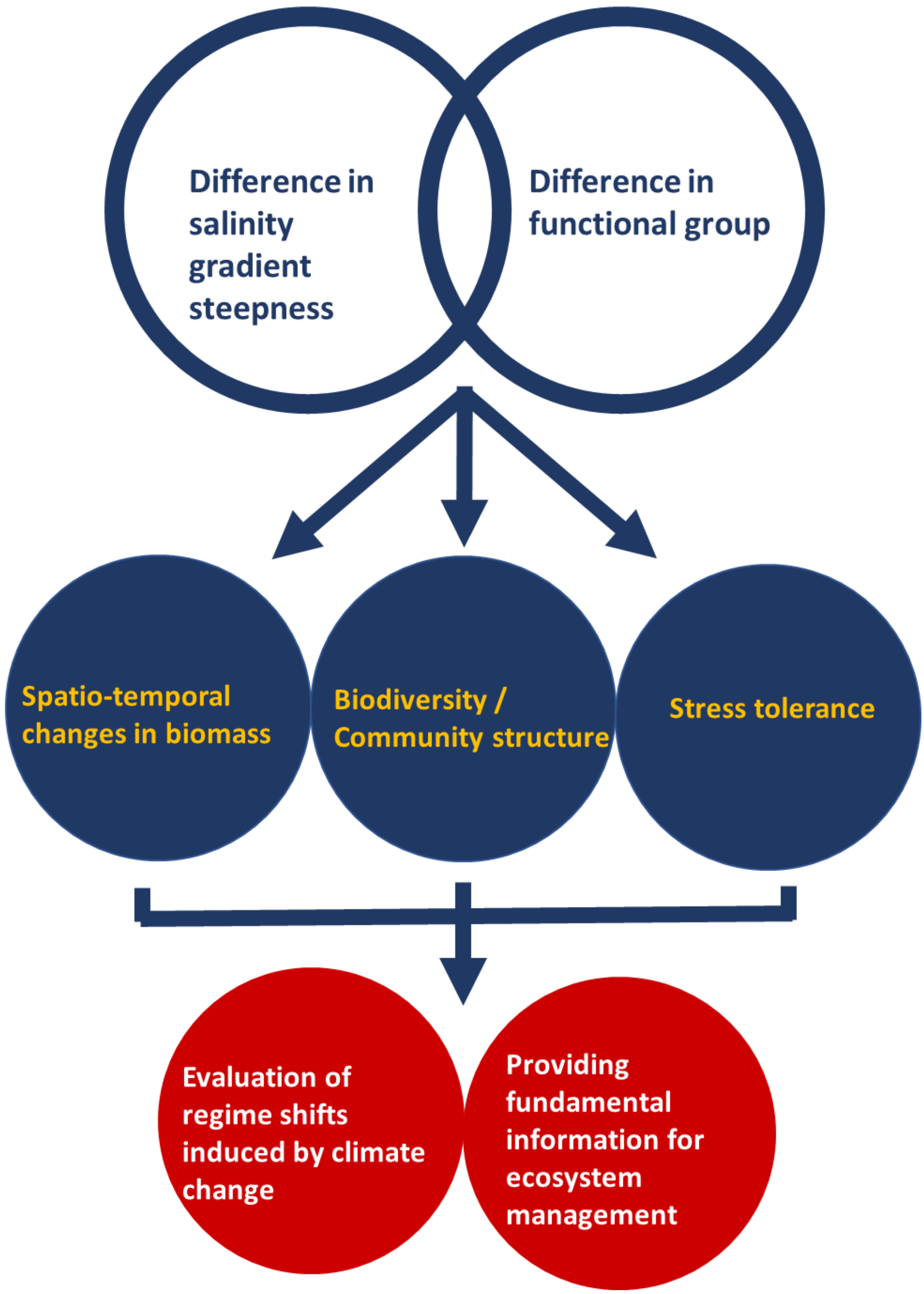

Figure 6.1. Schematic diagram for summarizing the findings of this thesis. The white circles indicate the two main hypotheses of the thesis, the blue circles indicate three aspects for testing the hypotheses, and the red circles indicate the possible applications of the results of this thesis.

Appendix A. Additional information on environmental conditions and epifauna biomass for Chapter 2.

Table A1. Water column nutrients (μM) and water Chl-a concentrations (μg/l) in the summer and fall 2016 at the study sites in Akkeshi, Saroma, and Notoro. N.d. indicates no data.

Site	PO_4		NO_2		NO_3		NH_4		Water Chl-*a*	
	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall	Summer	Fall
Akkeshi										
AK	0.4	0.3	0.1	0.4	0.04	0.3	1.0	0.6	24.3	15.1
BK	0.3	3.2	0.3	0.9	4.1	3.6	0.7	6.3	1.0	11.5
CK	0.6	4.3	0.4	0.6	0.2	0.7	0.4	3.2	4.8	6.8
CL	0.3	5.0	0.4	0.7	0.5	0.5	0.5	4.9	1.9	10.2
HN	1.6	4.6	0.4	0.9	0.2	0.6	0.6	5.9	9.3	38.0
SL	0.9	7.2	0.1	0.6	0.002	0.3	0.5	2.3	4.6	7.2
SR	0.2	1.7	0.1	1.3	0.1	6.4	0.4	5.6	23.9	6.3
TB	1.3	6.7	0.5	1.0	0.2	1.9	0.5	9.3	6.4	21.2
Mean±SD	0.7 ± 0.5	4.1 ± 2.4	0.3 ± 0.2	0.8 ± 0.3	0.7 ± 1.4	1.8 ± 2.2	0.6 ± 0.2	4.8 ± 2.7	9.5 ± 9.7	14.5 ± 10.7
Saroma										
SA1	0.1	1.4	0.04	0.4	0.02	1.4	0.2	2.4	3.0	4.9
SA2	n.d.	1.4	n.d.	0.4	n.d.	1.6	n.d.	2.3	n.d.	8.1
SA3	0.2	0.9	0.1	0.2	0.2	1.1	1.1	1.1	1.2	1.5
SA5	0.08	n.d.	0.04	n.d.	0.01	n.d.	0.4	n.d.	2.8	n.d.
SA6	0.08	0.7	0.02	0.2	0.005	2.2	0.06	4.1	2.2	3.3
SA7	0.1	n.d.	0.03	n.d.	0.03	n.d.	0.2	n.d.	2.4	n.d.
SA8	0.2	0.7	0.05	0.2	0.03	0.6	0.05	2.1	1.7	3.8
Mean±SD	0.1 ± 0.04	1.0 ± 0.4	0.05 ± 0.03	0.3 ± 0.1	0.05 ± 0.08	1.4 ± 0.6	0.3 ± 0.4	2.4 ± 1.1	2.2 ± 0.7	4.3 ± 2.5
Notoro										
NO1	0.3	0.9	0.02	0.1	0.03	0.4	0.4	0.2	2.6	9.0

NO2	1.0	0.8	0.04	0.2	0.2	0.8	0.4	2.6	0.7	5.4
NO3	0.1	0.4	0.04	0.2	0.07	0.5	0.8	0.9	1.0	2.3
NO4	0.2	0.3	0.03	0.1	0.03	0.3	0.5	0.4	0.9	4.1
NO5	0.4	0.8	0.1	0.09	0.4	0.4	0.66	0.2	0.6	2.6
Mean±SD	0.4 ± 0.3	0.6 ± 0.3	0.05 ± 0.03	0.1 ± 0.05	0.1 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.8 ± 1.1	1.2 ± 0.8	4.7 ± 2.7

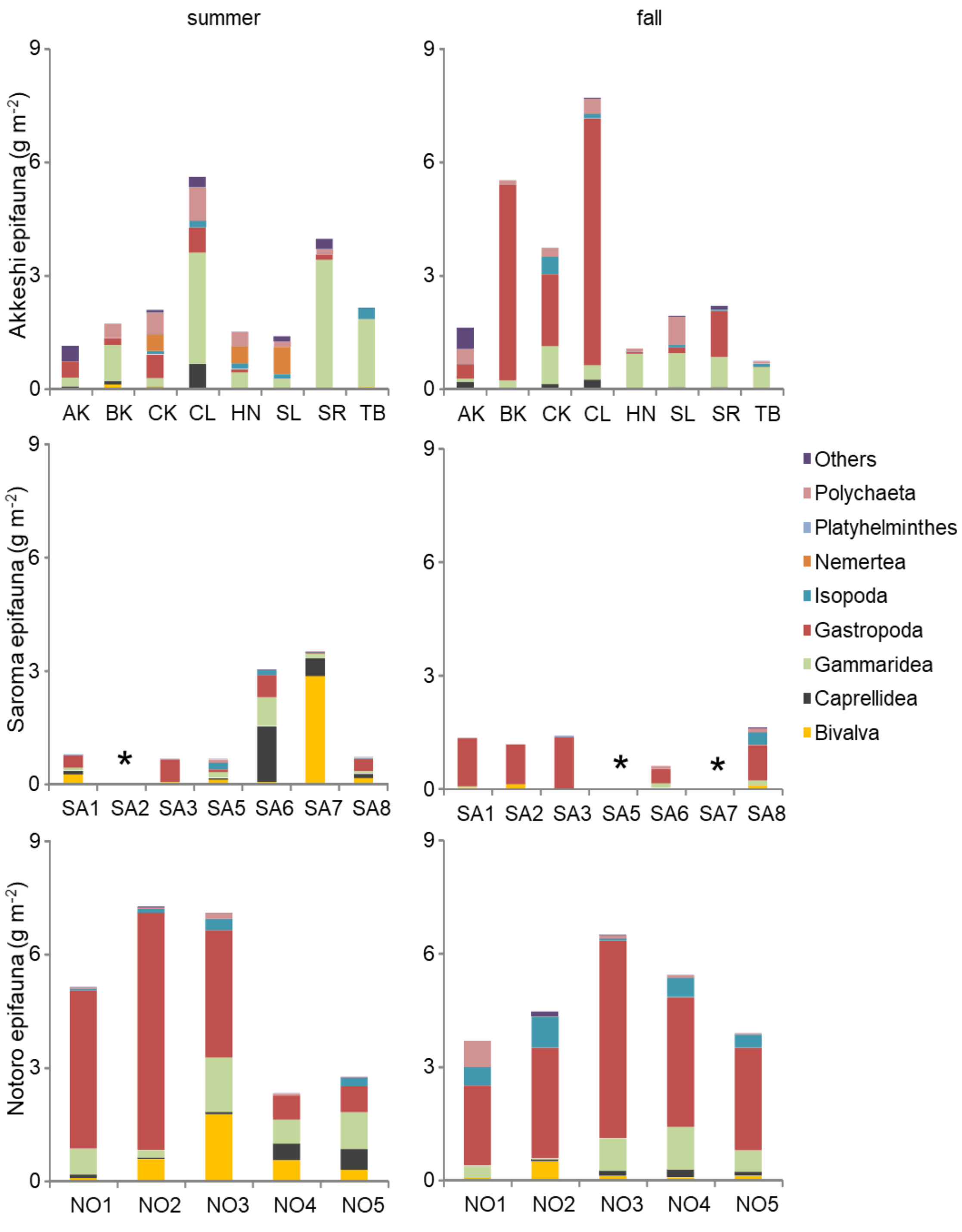

Figure A1. Mean biomass of epifauna (g m^{-2}; n = 5). The biomass is expressed by 9 major taxa for each site in Akkeshi, Saroma, and Notoro in the summer and fall. Minor taxonomical groups were grouped to one category (shown as Others). The symbols (*) on the graphs indicate no data.

Appendix B. Species lists and NMDS results for Chapter 4.

Table B1. Species list for Akkeshi epifauna. Species observed in all sites and months were combined.

Akkeshi		
Acari sp.	*Idotea ochotensis*	*Phoxocephalidae* spp.
Alvania concinna	*Ischyroceridae* spp.	*Phyllodocidae* spp.
Ampithoe spp.	*Jassa* spp.	*Platynereis bicanaliculata*
Ansola angustata	*Kushia zosteraphila*	*Pleustes* spp.
Aoroides curvipes	*Lacuna decorata*	*Hoploplana* spp.
Atylus spp.	*Lacuna* spp.	*Pontogeneia rostrata*
Balanus glandula	*Lacuna uchidai*	*Pontogeneia* spp.
Capitellidae spp.	*Lumbricillus nipponicus*	*Pycnogonida* sp.
Caprella spp.	*Lysianassidae* spp.	*Ruditapes philippinarum*
Carinonajna sp.	*Macoma calcarea*	*Siphonacmea oblongata*
Cerapus tubularis	*Maldanidae* spp.	*Sipuncula* spp.
Cirriformia spp.	*Melita* spp.	*Sphaerodoridae* sp.
Copepoda spp.	*Mizuhopecten yessoensis*	*Stenothoe* spp.
Cryptomya busoensis	*Monocorophium* spp.	*Syllis* spp.
Cymodoce japonica	*Munna* spp.	*Veneroida* sp.*1*
Daylithos parmatus	*Mysis* spp.	*Veneroida* sp.*2*
Decapoda spp.	*Mytilidae* spp.	
Diastylidae spp.	*Nassa hypolia*	
Dimorphostylis spp.	*Nassarius multigranosus*	
Echiura spp.	*Nemertellina yamaokai*	
Eogammarus sp.	*Nemerthea* spp.	
Euphilomedes spp.	*Neomysis* spp.	
Exogone naidina	*Nereididae* spp.	
Gnorimosphaeroma rayi	*Nereis vexillosa*	
Harmothoe imbricata	*Orchomene* sp.	
Harmothoe spp.	*Ostracoda* spp.	
Hediste diadroma	*Ostreobdella kakibir*	
Heptacarpus flexus	*Pachydrilus nipponicus*	
Heptacarpus sp.	*Pagurus* sp.	
Hirudinea sp.	*Paranthura japonica*	
Hyallelidae spp.	*Photis* spp.	

Table B2. Species list for Notoro epifauna. Species observed in all sites and months were combined.

Notoro	
Alvania concinna	*Lycastopsis augeneri*
Ampithoe spp.	*Macoma calcarea*
Ansola angustata	*Maldanidae* spp.
Arcuatula senhousia	*Margarites* spp.
Atylus spp.	*Mizuhopecten yessoensis*
Capitellidae spp.	*Monocorophium* spp.
Caprella spp.	*Mya arenaria*
Cnidaria spp.	*Mytilidae* spp.
Copepoda spp.	*Nassarius multigranosus*
Cryptomya busoensis	*Nemerthea* spp.
Cymodoce japonica	*Neomysis* spp.
Diastylidae spp.	*Nereididae* spp.
Eogammarus sp.	*Nereis vexillosa*
Ericthonius pugnax	*Oliva* spp.
Euphilomedes spp.	*Ophiurida* spp.
Exogone naidina	*Orchomene* sp.
Glycinde spp.	*Pagurus middendorffii*
Gnorimosphaeroma rayi	*Paranthura japonica*
Harmothoe imbricata	*Photis* sp.
Hippolytidae sp.	*Phyllodocidae* spp.
Homalopoma amussitatum	*Platyhelminthes* spp.
Imajimapholoe sp.	*Platynereis bicanaliculata*
Ischyroceridae spp.	*Pleustes* spp.
Kushia zosteraphila	*Hoploplana* spp.
Lacuna decorata	*Pontogeneia rostrata*
Lirularia iridescens	*Pycnogonida* spp.
Lirularia pygmaea	*Rissoina* spp.
Littorina squalida	*Siphonacmea oblongata*
Lumbricillus nipponicus	*Syllis* spp.
Lumbrineridae spp.	

Table B3. Species list for grazer, predator, and suspension feeders of Akkeshi. Species observed in all sites and months are combined.

Akkeshi		
Grazer	Predator	Suspension Feeder
Alvania concinna	*Decapoda* spp.	*Aoroides curvipes*
Ampithoe spp.	*Eogammarus* sp.	*Balanus glandula*
Ansola angustata	*Harmothoe imbricata*	*Cerapus tubularis*
Caprella spp.	*Harmothoe* spp.	*Copepoda* spp.
Carinonajna sp.	*Heptacarpus flexus*	*Cryptomya busoensis*
Diastylidae spp.	*Heptacarpus* sp.	*Hediste diadroma*
Dimorphostylis spp.	*Hoploplana* spp.	*Ischyroceridae* spp.
Exogone naidina	*Nemertellina yamaokai*	*Jassa* spp.
Hyallelidae spp.	*Nemerthea* spp.	*Kushia zosteraphila*
Lacuna decorate	*Nereididae* spp.	*Macoma calcarea*
Lacuna spp.	*Paranthura japonica*	*Maldanidae* spp.
Lacuna uchidai	*Phoxocephalidae* spp.	*Mizuhopecten yessoensis*
Siphonacmea oblongata	*Phyllodocidae* spp.	*Monocorophium* spp.
	Platynereis bicanaliculata	*Mytilidae* spp.
	Pleustes spp.	*Neomysis* spp.
	Pontogeneia rostrata	*Photis* spp.
	Pontogeneia spp.	*Ruditapes philippinarum*
	Pycnogonida sp.	*Veneroida* sp.1
	Stenothoe spp.	*Veneroida* sp.2
	Syllis spp.	

Table B4. Species list for grazer, predator, and suspension feeders of Notoro. Species observed in all sites and months are combined.

Notoro		
Grazer	Predator	Suspension Feeder
Alvania concinna	*Cnidaria* spp.	*Arcuatula senhousia*
Ampithoe spp.	*Eogammarus* sp.	*Copepoda* spp.
Ansola angustata	*Glycinde* spp.	*Cryptomya busoensis*
Caprella spp.	*Harmothoe imbricate*	*Ericthonius pugnax*
Diastylidae spp.	*Hippolytidae* sp.	*Ischyroceridae* spp.
Homalopoma amussitatum	*Hoploplana* spp.	*Jassa* spp.
Lacuna decorate	*Imajimapholoe* sp.	*Kushia zosteraphila*
Lirularia iridescens	*Lumbricillus nipponicus*	*Macoma calcarea*
Lirularia pygmaea	*Lumbrineridae* spp.	*Mizuhopecten yessoensis*
Littorina squalida	*Nemerthea* spp.	*Monocorophium* spp.
Margarites spp.	*Nereididae* spp.	*Mya arenaria*
Rissoina spp.	*Paranthura japonica*	*Mytilidae* spp.
Siphonacmea oblongata	*Phyllodocidae* spp.	*Oliva* spp.
	Pleustes spp.	*Ophiurida* spp.
	Pontogeneia rostrate	*Photis* sp.
	Syllis spp.	

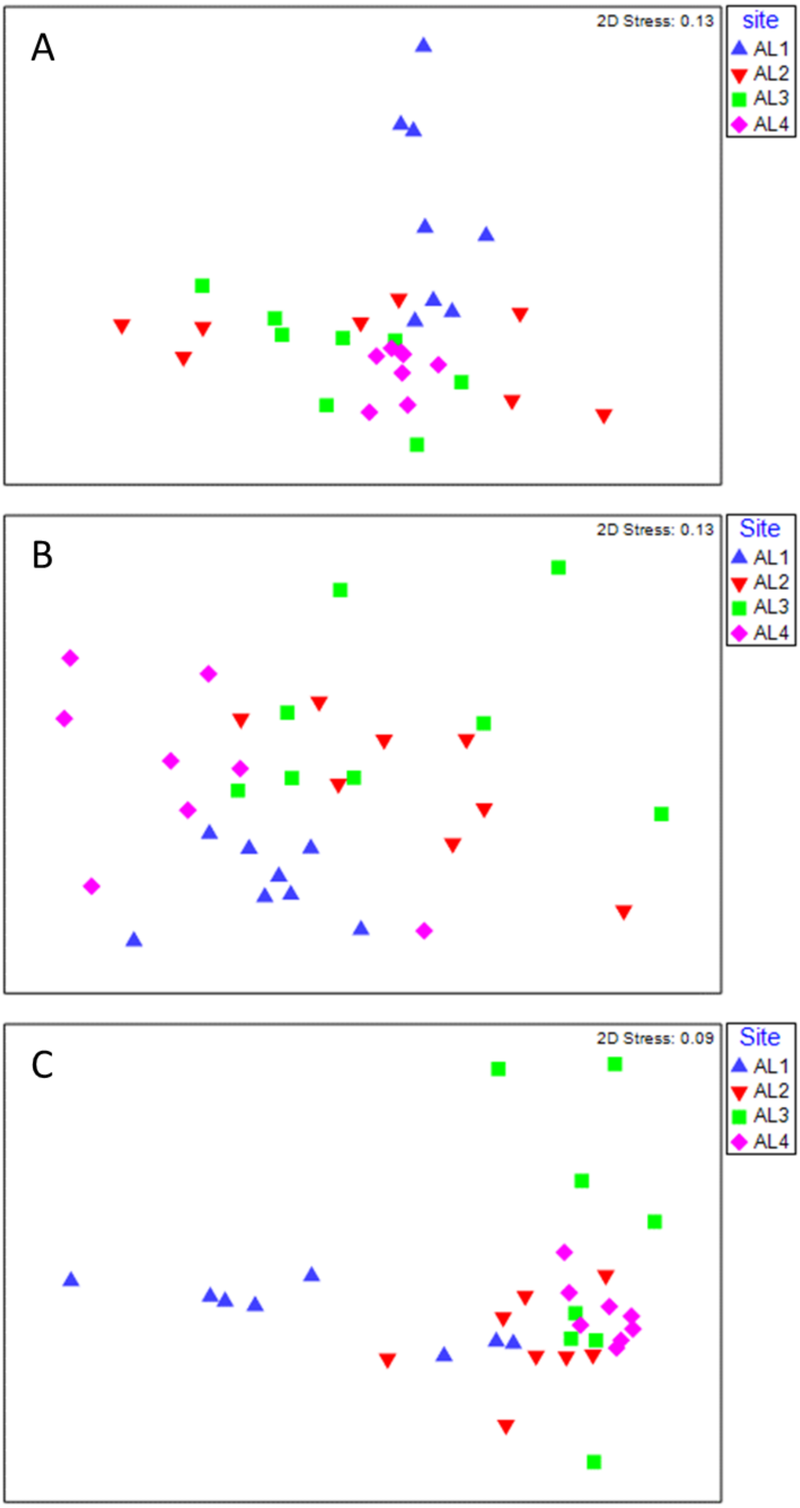

Figure B1. NMDS results based on Euclidean distance of square-root transformed biomass data for (A) grazers, (B) predators, and (C) suspension feeders from Akkeshi.

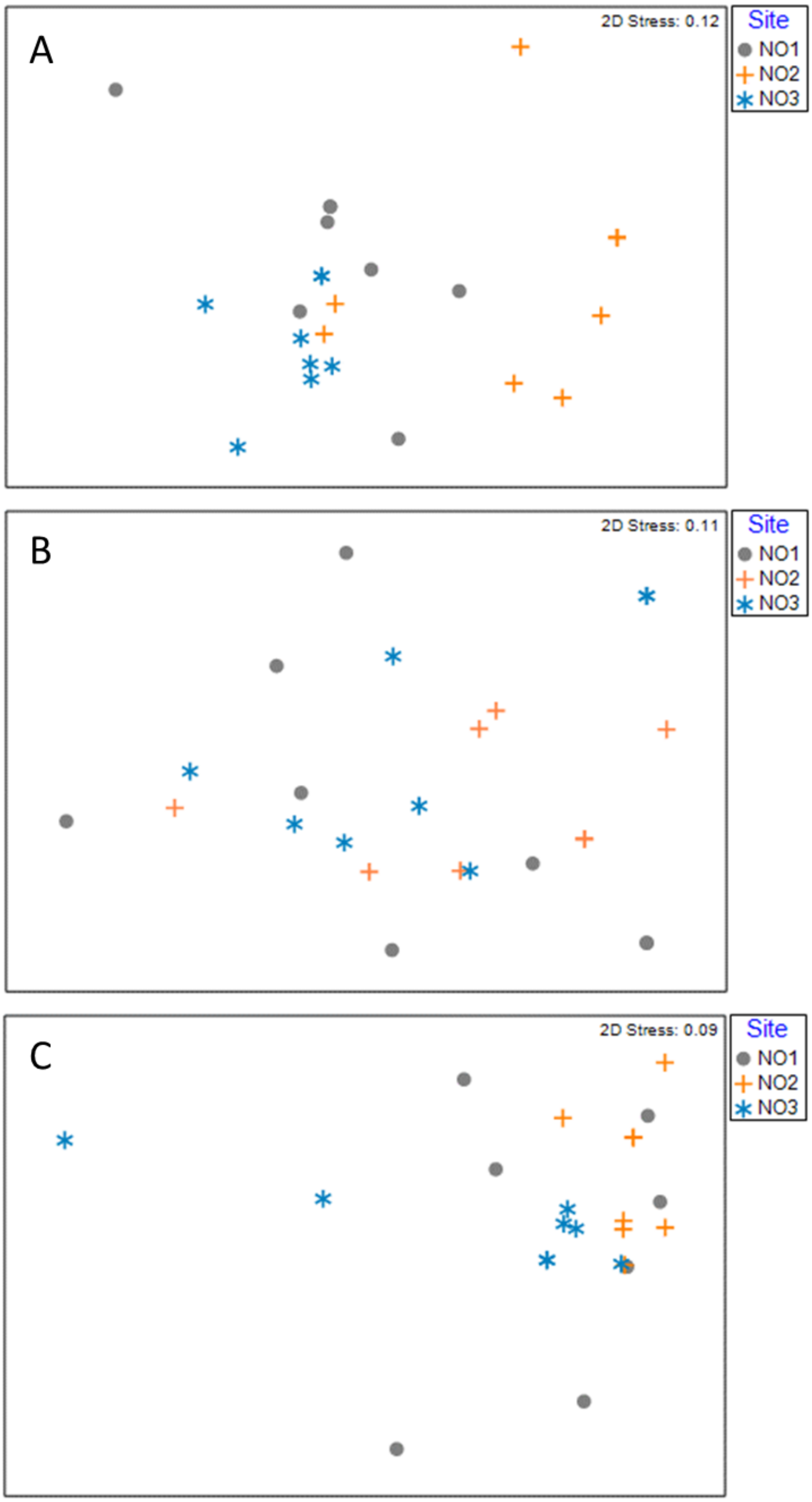

Figure B2. NMDS results based on Euclidean distance of square-root transformed biomass data for (A) grazers, (B) predators, and (C) suspension feeders from Notoro.

Appendix C. Continuous measurements of salinity and sea water temperature and the results for effect size analysis for Chapter 5.

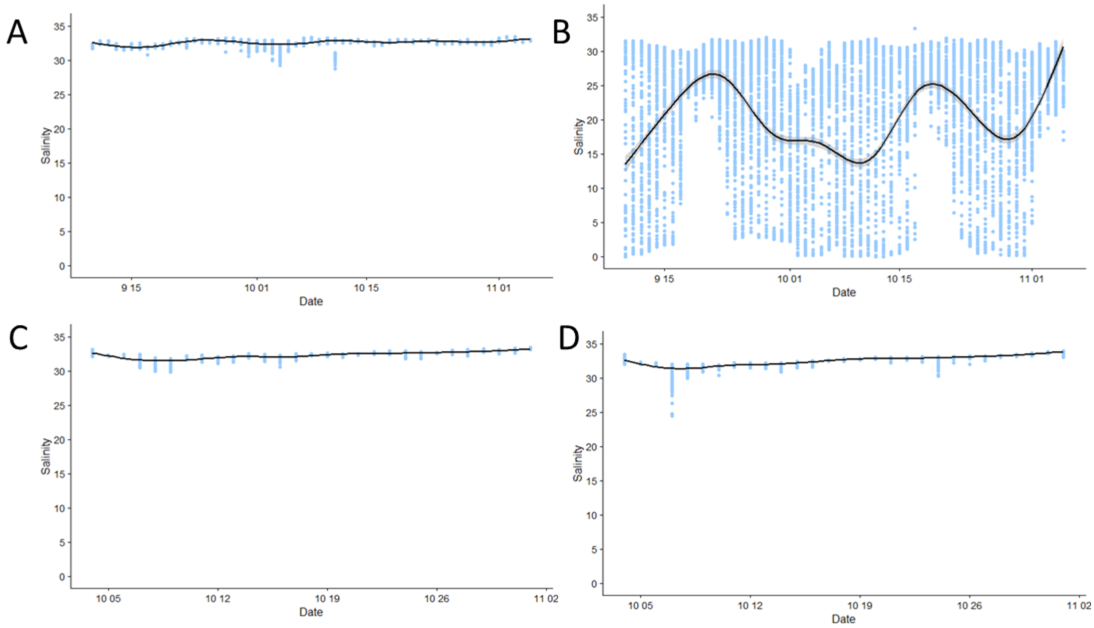

Figure C1. Time-series field data of change in salinity at (A) AK, (B) BK, (C) NTR, and (D) UBR measured in September to November 2018. The blue dots indicate data points measured every 10 minutes, and black sold curve indicates smoothed conditional means of the data. Measurements were done by conductivity loggers (for (A), (C), and (D) HOBO U24-002-C, Onset, USA and for (B) Infinity-CT A7CT-USB, JFE Advantech, Japan) tethered to rope and fixed at 50 cm above the sea floor.

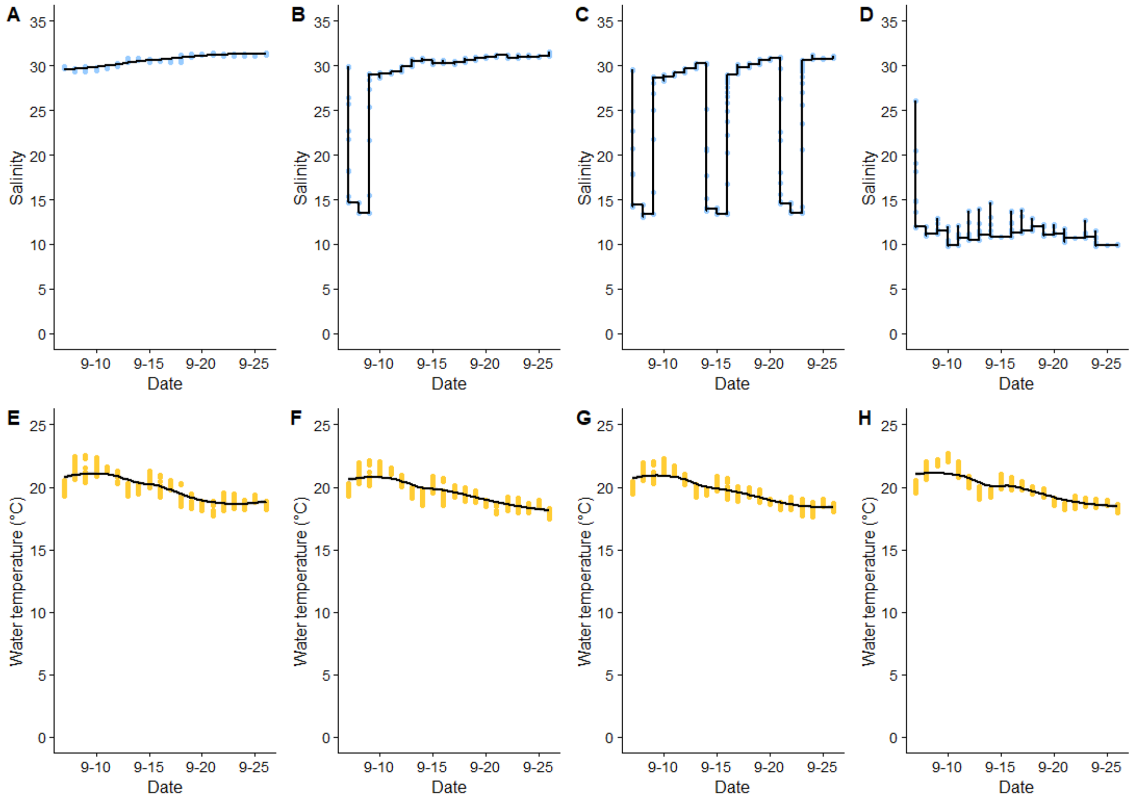

Figure C2. Time-series data of change in salinity and temperature at each salinity treatment aquarium for Akkeshi organisms. (A), (B), (C), and (D) are measured salinity in the aquaria for C, T1, T2, and T3 treatments, respectively, and (E), (F), (G), and (H) are measured temperature in the aquaria for C, T1, T2, and T3 treatments, respectively. Date is shown as month – day on the x axis.

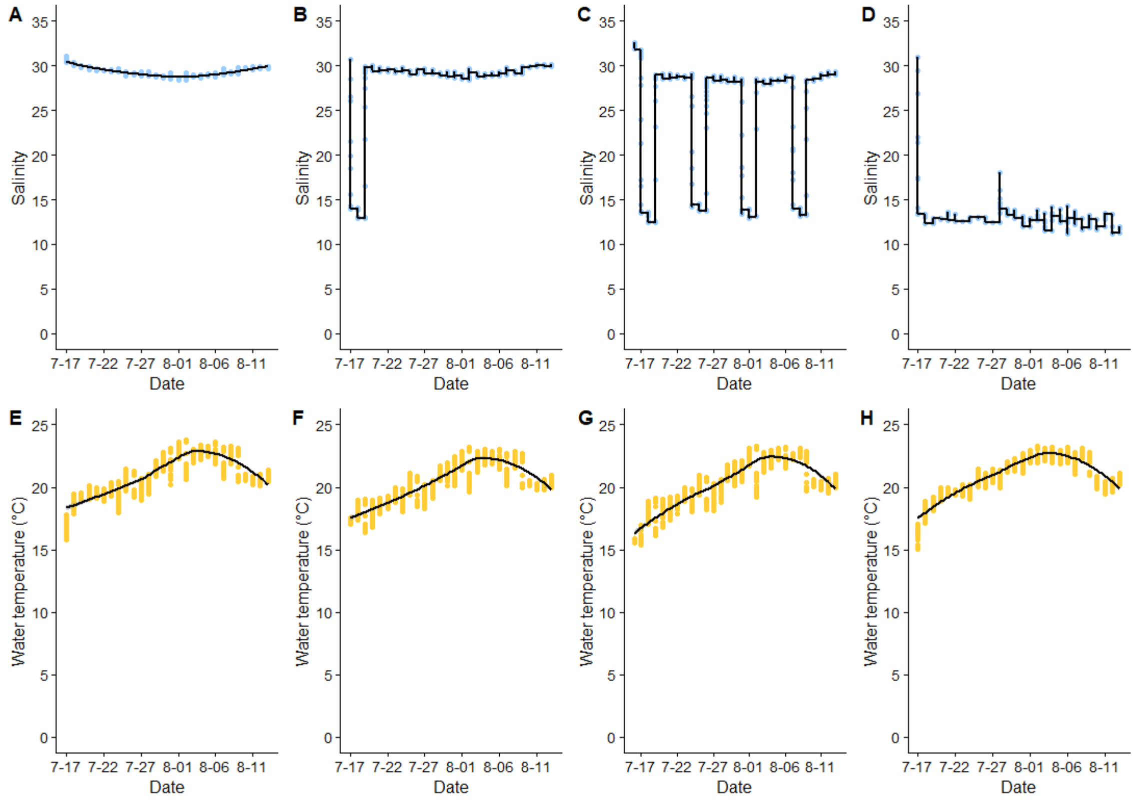

Figure C3. Time-series data of change in salinity and temperature at each salinity treatment aquarium for Notoro organisms. (A), (B), (C), and (D) are measured salinity in the aquaria for C, T1, T2, and T3 treatments, respectively, and (E), (F), (G), and (H) are measured temperature in the aquaria for C, T1, T2, and T3 treatments, respectively. Date is shown as month – day on the x axis.

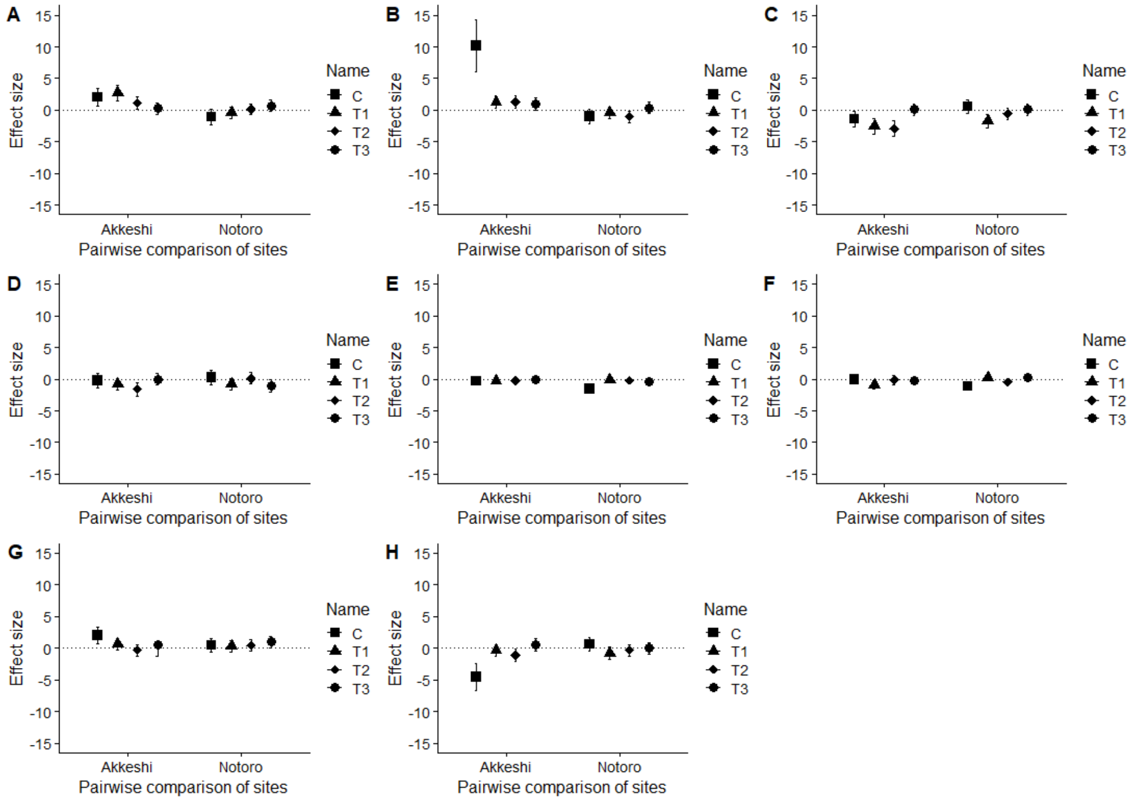

Figure C4. Site-comparison of effect sizes (Hedge's *d*) for eelgrass specific growth rate (A) without and (B) with grazer, Chl-*a* biomass (C) without and (D) with grazer, (E) grazer shell specific growth rate, (F) grazer wet weight change, (G) grazing scar area and (H) consumption of microalgae. Different symbols indicate different salinity treatments, and error bars indicate 95% confidence interval.