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Bottom-up control on mobile invertebrate community in an eelgrass bed: Contribution of different functional groups of epibiotic organisms

Dissertation

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Chapter Ⅰ

Every single species has various functional traits (FTs; e.g., morphology, behavior, feeding habits, body size and development). Each functional trait of a species has an ecological and evolutionary linkage to the species identity. A study approach based on functional identity (FT based approach) beyond taxonomic identity is expected to lead to specific understanding of ecological and evolutionary issues. FT based approach has been developed with studies on terrestrial plants, and is now applied to various types of aquatic ecosystems including seagrass ecosystem. In recent community ecology on functional traits of organisms in biological interactions in seagrass systems, findings on functional responses of macroinvertebrates have been rapidly increasing, whereas those on functional roles of producers are still poor despite diverse producer species including various epibiotic organisms (epiphytic macroalagae and sessile invertebrates) occur in seagrass beds. Although functional traits of seagrass (e.g., morphology and shoot density) have been often focused in studies on seagrass beds, the functional roles of epibiotic organisms for macroinvertebrates have been rarely considered. In this thesis, I examined how mobile invertebrate community is structured in terms of bottom-up control focusing on functional traits of epibionts on eelgrass blades in an eelgrass bed of the Akkeshi-ko estuary and Akkeshi Bay. Additionally, I intended to extend

conventional paradigm for understanding of biological relationships in seagrass beds in this study, because epibiotic organisms have not been focused in the previous studies in seagrass beds despite the importance has been often reported.

Chapter Ⅱ

Eelgrass (*Zostera marina*) beds are known to have high ecological and economical values within coastal ecosystems of the temperate northern hemisphere although their biodiversity and functions varied greatly from sites to sites. The variation in the biomass, abundance and diversity of mobile invertebrates in eelgrass beds has been examined in relation to various abiotic and biotic factors, such as water temperature, salinity, eelgrass biomass and epiphytic microalgae presence. However, the importance of sessile epibionts, such as macroalgae and calcific spirorbid polychaetes attached to eelgrass blades, has not been the focus of previous studies. In the present study, I examined the effects of three different sessile epibionts, namely, branched red algae, filamentous green algae, and calcific spirorbid polychaetes, on the biomass and diversity of mobile invertebrates in the eelgrass beds of Akkeshi in northeastern Japan. The relationships between seven abiotic and biotic variables including three types of epibionts, and biomass of 11 dominant mobile invertebrate species as well as three community-level variables (the total biomass of mobile invertebrates, species richness and the Shannon-Weiner species diversity index) were analyzed using a linear mixed model. My results show that branched red algae are correlated with *Pontogeneia rostrata*, Lacuna spp., *Nereis* sp., *Syllis* sp. and the total biomass of mobile invertebrates, filamentous green algae with *Pontogeneia rostrata*, *Ansola angustata* and the species diversity of mobile invertebrates, and spirorbid polychaetes with *Ansola angustata*, Lacuna spp., *Siphonacmea oblongata*, *Syllis* sp., the species richness and diversity of mobile invertebrates. The effect size of the epibionts was similar or even higher than that of abiotic and eelgrass factors on the total biomass of mobile invertebrates, species richness, species diversity and most of dominant invertebrate populations across the taxonomic groups. Consequently, epibiotic macroalgae and spirorbid polychaetes can be good predictors of the variation in the total biomass, species richness and species diversity of mobile invertebrates and the biomass of major dominant species, especially for species that have a relatively high dependency on eelgrass blades. These results suggest that the different functional groups of sessile epibionts have significant roles in determining the biomass and diversity of mobile invertebrates in eelgrass beds.

Chapter Ⅲ

Biological communities in seagrass beds are composed of a diversity of organisms including plants, algae and animals. Seagrass-associated macroinvertebrates (epifauna: e.g., crustaceans, gastropods and polychate worms) especially play important roles as mediators between primary producers and higher consumers in seagrass beds. However, many aspects of the variation in epifaunal community in seagrass beds still remain to be investigated. In this study, I examined how epifaunal community structure varied seasonally and spatially with abiotic and biotic factors in an eelgrass bed of Akkeshi, northeastern Japan using redundancy analysis (RDA). I especially focused on the influence of epibiotic organisms (epiphytic macroalgae and spirorbid polychates) on eelgrass blades, that has been poorly investigated. I hypothesized that: (1) abiotic factors are more definitive for the variation in epifaunal community than biotic factors when the eelgrass bed is less productive in early spring and late autumn; (2) biotic factors including macro-epibionts (i.e., epiphytic macroalgae and sessile animals) are more influential when the eelgrass bed is more productive from late spring and early autumn. Consequently, the epifaunal community composition was temporally and spatially varied with different sets of abiotic/biotic factors. Also, epibiotic organisms contributed to explain some parts of the variation in addition to other abiotic/biotic factors especially in summer productive seasons, despite relatively low biomass compared to that of eelgrass and epiphytic microalgae. Inconsistent with my hypothesis, RDAs showed that abiotic factors were more definitive for the spatial patterns of the epifaunal communities from spring to summer when the productivity of eelgrass bed are increasing, whereas biotic factors are relating more important in autumn when the productivity declines. The results implicate that the effects of epibiotic factors on environmental condition and biological/functional interaction should be more taken into account in eelgrass community studies to comprehensively understand the variation in seagrass systems.

Chapter Ⅳ

Invertebrate consumers, such as insects and small crustaceans, are affected by multiple abiotic factors and plant traits that are essential bottom-up factors affecting their

variability. The complex networks of their interactions likely vary seasonally with changes in these factors. In this study, I assessed a seasonal shift in the network structure focusing on the variation of epifaunal community abundance (ECA) along the bottom-up cascade from abiotic factors (water temperature, salinity and dissolved inorganic nitrogen) via biotic factors (eelgrass and epibionts: epiphytic micro- and macro-algae and sessile spirorbid polychaetes) in eelgrass beds in Akkeshi (eastern Hokkaido, Japan). Structural equation models (SEMs) were constructed for the seven months from May to November. My SEMs based on the bottom-up network explained over 50% of the spatial variation in ECA in most months, and showed that great variations of the direct and indirect interactions among months. The network structure was generally more complex in spring to summer than in autumn. In the former, in addition to abiotic variables like water temperature and salinity, intermediate variables like eelgrass biomass, epiphytic micro/macroalgae and sessile polychaetes contributing more frequently to the variation in ECA. In autumn, however, only the water temperature and eelgrass biomass were selected as significant factors affecting ECA variation. The relative importance of selected variables and their direction and patterns of interactions also varied from months to months, partially reflecting the seasonal changes in the degree of spatial variability and life history traits of dominant species. My results demonstrated that the network analyses using SEM are effective in elucidating temporal shifts in the interacting effects of abiotic/biotic factors affecting abundance of consumers. My monthly observation also highlighted the influential roles of epibionts such as macroalgae and sessile animals on eelgrass blade affecting abundance of mobile epifauna, which aspect had been focused only in few studies of seagrass ecology.

Chapter Ⅴ

Although the effects of seagrass factors (e.g., biomass, shoot density, morphology and patch structure) and epiphytic microalgae on macroinvertebrate community have been main topics in the previous studies on producer-consumer interactions in seagrass beds, I demonstrated that mobile invertebrate community structure was influenced by epibiotic organisms (i.e., macroalgae and sessile invertebrates) through the variation in functional relationships among them in an eelgrass bed in the Akkeshi-ko estuary and Akkeshi Bay. My extension of the framework and change of perspective for biological network and relationships are expected to be effective for further understand of seagrass ecosystem. Additionally, my results suggest that functional traits of both reactor and influencer should simultaneously be considered to precisely understand functional interactions across terrestrial and aquatic systems.

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Chapter Ⅰ

General introduction

1.1. Functional trait approach in ecology and evolution

A variety of functional traits (e.g., morphology, behavior, feeding habits, body size and development) are packed in every single species. Each functional trait of a species has an ecological and evolutionary linkage to the species identity. Those linkages can be often found in trait matching in plant-animal interaction (e.g., predator-prey and plant-pollinator interaction). For example, morphological traits of birds are filtered by various traits of plants which offer fruits to the birds (Dehling *et al*., 2016), and flower traits, such as color and corolla length, affect the pollinator's behavior (Vázquez *et al*., 2009). Thus, a concept based on functional identity (functional traits: FTs) beyond taxonomic identity is expected to lead to specific understanding of ecological and evolutionary issues.

With an increased interest in prevention of ecosystem deterioration associated with species/biodiversity loss induced by various environmental changes (e.g., global warming, ocean acidification over global scale and eutrophication, overexploitation, pollution at regional scale), understanding how community dynamics and ecosystem functioning are related to biodiversity at various levels (e.g. genetic diversity, species diversity and landscape diversity) has been a main stream in ecology. Especially, it is generally believed that species diversity reduction with species loss would cause reductions in ecosystem functions (Worm *et al*. 2006; Stachowicz *et al*. 2007). Over various spatial scales, it has been demonstrated that the variation of species diversity can influence ecosystem functioning by interfering major environmental drivers of metabolism, organismal fitness, and ecosystem processes (Hooper *et al*., 2005; Cardinale *et al*., 2012; Duffy *et al*., 2015). However, an approach with species diversity could not fully contribute to understand how ecosystem functioning vary with biodiversity because the target community is composed of various organisms with multiple ecological functions.

Taxonomic classification (TC) based approach is useful to understand community properties, namely abundance, biomass and biodiversity, but not to understand environment-species and species-species interactions considering those detailed causalities (Villéger *et al*., 2010; Mouillot *et al*., 2013). While FT based approach is often effective to describe how functional traits are related with environmental gradients, and to identify causal links on ecosystem properties and functions (referred in Gravel *et al*., 2016), TC based approach shows only variation patterns but not fully explain processes causing variations. Phylogenetic approach, by which phylogenetic diversity (see Chao *et al*., 2014) are considered, is often as ineffective as TC approach because it is also difficult to consider the causalities if clades include functionally diverse species. Phylogenetic classification does not always match with FT based classification in most cases. For the points as mentioned above, FT based approach is considered to be more powerful than TC based approach and phylogenetic approach to investigate relationships between biodiversity and ecosystem function in biological communities. We can understand more realistic relationships by applying FT based approach to answer ecological and evolutionary questions on responses to environmental changes and the variation in biological interactions, because the concepts based on FTs enable us to link state of an individual or a relationship between individuals to pattern and processes at community and ecosystem level (Gravem & Morgan, 2016).

Over the two decades, approaches incorporating FTs and/or phylogenies of organisms have been increasingly spotlighted in addition to TC based approach. FT based approach refers to the value and range of species or organismal traits and the diversity based on the traits (i.e., functional diversity [FD]) and have been actively applied to ecology (e.g., Tilman *et al.*, 1997; Petchey & Gaston, 2002, 2006; Mouillot *et al.*, 2013). FT based approach is regarded as key to understanding community dynamics, ecosystem processes and individual species' responses to environmental stresses or disturbance (McGill *et al.*, 2006; Cadotte *et al.*, 2011; Mouillot *et al.*, 2013).

Commencing with terrestrial systems including forests and grasslands, FT based approach is now applied to various types of terrestrial and aquatic ecosystems. FT based approach has been already shown to be useful in studies on terrestrial plants. For example, some global-scale studies demonstrated that several plant traits are highly correlated to life cycle strategies and production (Wright *et al.*, 2004) and to environmental factors (Díaz *et al.*, 2016). Moreover, functional diversity or functional traits of dominant species within a community determine primary production (Petchey *et al.*, 2004; Mokany *et al.* 2008) and the spatial stability (Weigelt *et al.* 2008).

In aquatic systems, various types of phytoplankton and algae appear in addition to vascular plants. Thus, biodiversity of primary producers in aquatic systems are higher than terrestrial systems. In particular, productivity and biodiversity of primary producers in coastal marine ecosystems, such as seagrass beds where various plants and algae serve as the foundation, are higher than in terrestrial systems and among other aquatic systems including freshwater streams, lakes, rocky shore and pelagic plankton ecosystem (Duarte & Cebrian, 1996; Duarte & Chiscano, 1999). By manipulating experiment in which the effect of diversity of marine macroalgal species on the stability of higher trophic levels was tested, Ramus and Long (2015) demonstrated that higher producer diversity contributed to increasing in productivity and diversity of consumers' community and the maintenance of the stability of food webs, and that producer biomass and productivity did not affect consumer production or diversity. Their results suggest that producer diversity can affect the mechanisms of trophic relationships through some kind of functional interactions in biological community. Because aquatic producers serve not only as food resource but also as habitat provider or modifier (i.e., ecosystem engineering: Jones *et al.*, 1997; Crooks 2002), FT based approach is expected to be effective to understand variation patterns and processes in aquatic systems as studies in terrestrial ecosystems.

1.2. Seagrass beds

Seagrasses are marine angiosperms that occur globally from cold to tropical shallow coastal zones (den Hartog & Kuo, 2006). They form extensive meadows known as "seagrass beds". There are approximately 60 seagrass species across the world and 16 around Japanese coastal zones (Aioi and Nakaoka, 2003; Short *et al.*, 2007). Among them, *Zostera mariana* (eelgrass) has a vast distributional range in the northern hemisphere (Aioi and Nakaoka, 2003). In Japan, *Z. marina* is found from Hokkaido to Kyushu (Aioi and Nakaoka, 2003).

Seagrasses are important marine foundation species, providing not only high primary productivity (Duarte & Chiscano, 1999), but also critical habitat for various organisms, such as fish, invertebrates and algae (Larkum *et al*., 2006; Hughes *et al*., 2009). The habitat provision enhances biodiversity and productivity within the coastal ecosystems (Hemminga & Duarte, 2000; Duffy, 2006; Valentine & Duffy, 2006). Furthermore, seagrass beds can make highly valuable impacts on supporting, regulating and provisioning services including productive fisheries (Unsworth *et al*., 2010), water quality improvement (Burkholder *et al*., 2007), nutrient cycling (McGlathery *et al*., 2007) and coastline/bottom sediment stabilization (Barbier *et al*., 2008; Christianen *et al*., 2013). Recently, researches on the function as organic carbon storage (i.e., "blue carbon sink") have been paid attention in relation to mitigation ability of seagrass beds toward reduction of CO_2 to retard the speed of global warming and ocean acidification (Fourqurean *et al*., 2012; Marbà *et al*., 2015).

Seagrass beds are globally threatened by human-induced environmental changes such as reduction in water quality, overgrazing, and coastal development (Orth *et al*., 2006; Hughes *et al*., 2009; Waycott *et al*., 2009). As one of efforts against global seagrass loss, the variation of the capacity for resistance and resilience to environmental stress and perturbations have been recently investigated in relation to food web structure in seagrass beds (Unsworth *et al*., 2015; Maxwell *et al*., 2016; Östman *et al*., 2016). These efforts in seagrass beds are expected to contribute to improvement of management for coastal habitats including seagrass beds. However, most natural ecosystems are not only shaped by food webs based on trophic interactions (e.g., predator-prey and plant-herbivore interactions), but also by interaction webs incorporating non-trophic interactions (e.g., ecosystem engineering and host-parasite interaction) with food webs, which has been less studied compared to trophic interactions. Therefore, broadening the scope of studies investigating more diverse relationships in networks will lead to more realistic understanding of current status of seagrass beds threatened by multiple human-induced impacts (Kéfi *et al.*, 2012; Sanders *et al*. 2014).

1.3. Biological community of seagrass bed

Ecosystem of seagrass beds has a complex network structured by a variety of plants, algae and animals. Especially, seagrass-associated small mobile invertebrates (epifauna; e.g., crustaceans, molluscs and polychaete worms) are abundant in their biodiversity and biomass (Duffy, 2006; Valentine & Duffy, 2006), and play important roles in the network that links energy and materials from primary producers to higher consumers (Duffy & Hay, 2000; Duffy, *et al.*, 2005). Consequently, top-down and bottom-up controls focusing on functional roles (e.g., herbivory and prey for higher consumers) of epifaunal species or community have been actively investigated in seagrass beds (Whalen *et al.*, 2013; Amundrud *et al.*, 2015; Duffy *et al.*, 2015). In addition, epifaunal populations and communities are directly and indirectly susceptible to various abiotic and biotic factors. Understanding the relationships of epifaunal invertebrates with environmental conditions and other species, has been a central issue of community ecology on seagrass systems (e.g., Orth *et al.*, 1984; Boström & Bonsdorff, 1997; Boström *et al.*, 2006; Duffy *et al.*, 2015).

Biological communities of seagrass beds are structurally similar to that of terrestrial grasslands, because those are largely structured by plants as foundation species which exhibit high spatial and temporal variation in biomass and production in relation to their life cycles and abiotic and biotic interactions. Macroinvertebrate communities in seagrass beds are often composed largely by plant resource utilizers, such as herbivores and detritivores (Valentine & Heck, 1999; Heck *et al.*, 2000). Plant-animal interaction has been a major topic in studies on seagrass beds (e.g., van Montfrans 1984; Attrill *et al.*, 2000; Jaschinski *et al.*, 2009; Reynolds *et al.*, 2014). However, a great difference between terrestrial and aquatic systems including seagrass beds, i.e., more generalists on diet (feeding modes) and habitat (host specificity) are included in aquatic systems than terrestrial systems. Shurin *et al*. (2006) pointed out that aquatic food webs contain more generalists than terrestrial webs because aquatic macrophytes have limited structural defenses in comparison to terrestrial plants. Most epifaunal species in seagrass beds generally show low host-specificity (Jernakoff *et al.*, 1996; Nakaoka, 2005). Moreover, more diverse primary producers appear especially in seagrass beds than terrestrial ecosystems because primary producers in seagrass beds consist not only of seagrass species (vascular plants), but also of various algal species, such as unicellular diatoms, dinoflagellates and multicellular algae. Therefore, plant-animal interactions are expected to be temporally and spatially more variable with more diverse functional groups of plants that can interact differently with animals in seagrass beds.

1.4. Effectiveness of functional trait approach on

understanding community dynamics in seagrass beds

Functional traits of organisms are directly linked to ecosystem properties and offer an important insight to understand species coexistence mechanisms, and the relationship between taxonomic diversity (species diversity) and ecosystem functioning (Díaz & Cabido, 2001; Hillebrand & Matthiessen, 2009; Cadotte *et al*., 2011; Gagic *et al*., 2015). In recent community ecology on functional traits of organisms in seagrass systems, findings on functional traits of macroinvertebrates have been rapidly increasing (e.g., Jaschinski & Sommer, 2008; Best *et al*., 2012; Best & Stachowicz, 2012, 2013, 2014; Dolbeth *et al*., 2013). More concrete understanding of the variation in macroinvertebrate populations and community has been attempted considering causal relationships attributed to functional traits of macroinvertebrates because the dynamics of macroinvertebrate populations and community are key determinations of the dynamics of whole seagrass communities (see previous section). Examples of the relevant studies include studies on how the variation in macroinvertebrate populations and community is caused by ecological processes (i.e., environmental filtering, niche differentiation, food availability and predation) in connection with the relationships between environmental condition and functional traits, such as feeding habits, microhabitat use, mobility and body size (e.g., Best & Stachowicz, 2012, 2013, 2014; Dolbeth *et al*., 2013; Wong & Dowd, 2015).

Also, primary producers in seagrass beds, such as seagrass, unicellular epiphytes and macroalgae, are very diverse with various functional traits (see previous section). Additionally, sessile animals, such as spirorbids, bryozoans, hydrozoans and tunicates, often appear (Hamamoto *et al*., 1999; Kouchi *et al*., 2006). Because primary producers and sessile animals are linked by trophic/non-trophic interactions to different macroinvertebrates (e.g., food-consumer, habitat-resident and host-parasite: Attrill *et al*., 2000; Thomsen, 2010; Poore *et al*., 2012; Gartner *et al*., 2013; Lefcheck *et al*., 2014; Long & Grosholz, 2015), an approach to examine the variation in macroinvertebrate community focusing on functional traits/roles of primary producers and sessile animals is necessary to understand the bottom-up processes acting in seagrass beds more comprehensively. However, such studies are still less conducted than studies focusing on functional traits/roles of macroinvertebrates (Thomsen, 2010; Lefcheck *et al*., 2014; Long & Grosholz, 2015).

Currently, the functional roles of epibiotic organisms have been rarely considered as compared to those of eelgrass and epiphytic microalgae in the studies on macroinvertebrate community in seagrass beds (conventional paradigm: Fig. 1-2). Especially, epiphytic microalgae have been traditionally focused as major epibiotic factor serving as food resource for most macroinvertebrates in seagrass beds (e.g., van Montfrans 1984; Jernakoff *et al*., 1996; Jaschinski *et al*., 2009; Duffy *et al*., 2015). Additionally, approaches from the perspective of functional traits of animals have been still dominant in most studies on functional relationships between plants and animals (Poore *et al*., 2012; Best & Stachowicz, 2012, 2013; Wong & Dowd, 2015). Therefore, quantitative evaluation on the roles of various types of epibiotic organisms is necessary to comprehensively understand the variation in seagrass ecosystems.

1.5. Study area

The Akkeshi-ko estuary (local name: Akkeshi Lake) and Akkeshi Bay are located in northeastern Hokkaido, Japan (Fig. 1-1). A narrow channel (width: approximately 500 m, depth: approximately 10 m) connects the Akkeshi-ko estuary and Akkeshi Bay.

The Akkeshi-ko estuary is a brackish water of approximately 32.3 km^2. The depth range in most of the estuary is very shallow (0.8-1.7 m in mean tide level) with the maximum difference in tide levels of up to approximately ± 0.6 m), and the bottom is mostly muddy. The estuary is known for aquaculture of asari clams *Venerupis philippinarum* and pacific oysters *Crassostrea gigas*. Moreover, this estuary has been registered an important wetland under the Ramsar Convention since 1993 and offers habitat for various water birds, such as whooper swans *Cygnus cygnus*, brant geese *Branta bernicla*, red-crowned cranes *Grus japonensis*, various ducks and submerged aquatic plants. Here, eelgrass (*Zostera marina*) is the most dominant and covers a large part of the bottom area except for the clam aquaculture farms in the intertidal zone near the channel (Kashim & Mukai, 2006; Hasegawa *et al*., 2007; Yamada *et al*., 2007a, b). This extensive eelgrass bed offers suitable habitat or spawning ground to commercial important species, such as pacific herrings *Clupea pallasii pallasii* and Hokkai shrimp *Pandalus latirostris*. In addition to eelgrass, two other seagrass species (dwarf eelgrass *Zostera japonica* and ditch grass *Ruppia maritima*) and a diversity of algal species occur. Freshwater inflow from the Bekanbeushi River, which accounts for 98.8 % of all of the flow volume and 5.8 % of total volume of the estuary per day on average, and tidal seawater input from the Akkeshi Bay characterize physical and chemical environmental gradients with great seasonal fluctuation (Iizumi *et al*., 1996; Yamada *et al*., 2007a, b). Water temperature reaches the peak from July to September though it varies considerably from year to year (Iizumi *et al*., 1996; Yamada *et al*., 2007a; this study). The estuary is partly covered by ice from December to March (Iizumi *et al*., 1996).

Akkeshi Bay opens to the Pacific Ocean at the south end. This bay is also an important habitat for pacific oyster aquaculture and for fishing ground of various fishes, shellfishes and kelps. From the intertidal zone to the subtidal zone (5 m below mean low water), two seagrass species *Z. marina* and *Zostera asiatica* occur. *Zostera marina* occurs at depths shallower than 2 m and *Z. asiatica* dominates in deeper water (Watanabe *et al*., 2005). Also, kelps *Saccharina* spp. and surfgrass *Phyllospadix iwatensis* are often found in some seagrass meadows where hard bottoms and gravels are mixed with sedimentary bottoms.

1.6. Objectives of this study

The main aim of this thesis is to examine how mobile invertebrate community is structured in terms of bottom-up control focusing on functional traits of epibionts on eelgrass blades (macroalgae and sessile invertebrates) in an eelgrass bed of the Akkeshi-ko estuary and Akkeshi Bay. Because those epibionts have not been focused in the previous studies in seagrass beds despite the importance has been often reported as mentioned above, I extend conventional paradigm for understanding of biological relationships in seagrass beds in this study (Fig. 1-2). Additionally, I attempt to find general patterns and processes operating within biological communities by understanding the dynamics of macroinvertebrate (epifaunal) community to effectively comprehend complex systems of seagrass beds because macroinvertebrates are ubiquitous and important not only in seagrass ecosystems but also in other complex aquatic and terrestrial ecosystems, such as insects, crustaceans and snails. I conducted this study from a perspective of the bottom-up control, because the bottom-up control on mobile invertebrate community in eelgrass meadows was stronger than the top-down control in my study site (Momota, 2013; Duffy *et al.*, 2015).

In Chapter 2, I examined the effects of three different sessile epibionts, namely, branched red algae, filamentous green algae, and calcific spirorbid polychaetes, on the biomass and diversity of mobile invertebrates in the eelgrass beds. The relationships between multiple abiotic and biotic variables including three types of epibionts, and biomass of dominant mobile invertebrate species as well as community-level variables (e.g., the total biomass of mobile invertebrates, species richness and the Shannon-Wiener species diversity index) were analyzed using a linear mixed model.

In Chapter 3, I examined how mobile invertebrate community structure varied seasonally and spatially with abiotic and biotic factors in the eelgrass bed. I compared the relative importance of abiotic and biotic factors on mobile invertebrates using multivariate analyses, especially by focusing on epiphytic macroalgae and calcific spirorbid polychaetes on eelgrass blades.

In Chapter 4, I assessed a seasonal shift in the network structure describing interrelationships among abiotic factors, primary producers and mobile invertebrates (epifaunas). I specially tried to explain seasonal variation in epifaunal community abundance (ECA) by a combination of abiotic factors (water temperature, salinity and dissolved inorganic nitrogen) and biotic factors (eelgrass and epibionts: epiphytic micro- and macro-algae and sessile spirorbid polychaetes) in an eelgrass bed using structural equation modeling (SEM).

Finally in Chapter 5, I synthesized the findings obtained in the series of my studies in Chapters 2, 3 and 4. I discussed the effectiveness of functional trait approach on understanding the variation in mobile invertebrate community in seagrass beds and its implications and further use in community ecology.

I targeted most mobile invertebrates inhabiting eelgrass shoots (i.e., epifaunal species) but excluded some species (e.g., mysids and decapods) for the following reasons: (1) my sampling method was not unsuited to accurately collect them with remarkably high mobility. I used mesh bags to spatially measure the abundance and species composition of mobile invertebrates, which is a standard method for quantitative study of epifauna in eelgrass beds (Duffy *et al*. 2015), whereas Yamada *et al*. (2007b) used an epibenthic sledge which were suitable for catching highly mobile species; (2) mysids and most decapods (e.g., crabs, hermit crabs, crangonidae and ghost shrimps) show low dependency on eelgrass shoots although Hokkai shrimp *P. latirostris* inhabits eelgrass meadow in Akkeshi (see previous section); (3) most decapods are too large to be regarded as “epifauna” on eelgrass shoots. Small invertebrates (i.e., mesofauna and mesograzers) have been traditionally focused in most previous studies on mobile invertebrate community in eelgrass beds (Heck *et al*., 2000; Larkum *et al*., 2006; Whalen *et al*, 2013; Duffy *et al*. 2015); (4) some shrimps and crabs are often regared as mesopredator in seagrass beds (Amundrud *et al*., 2015; Gross *et al*., 2017).

Figures

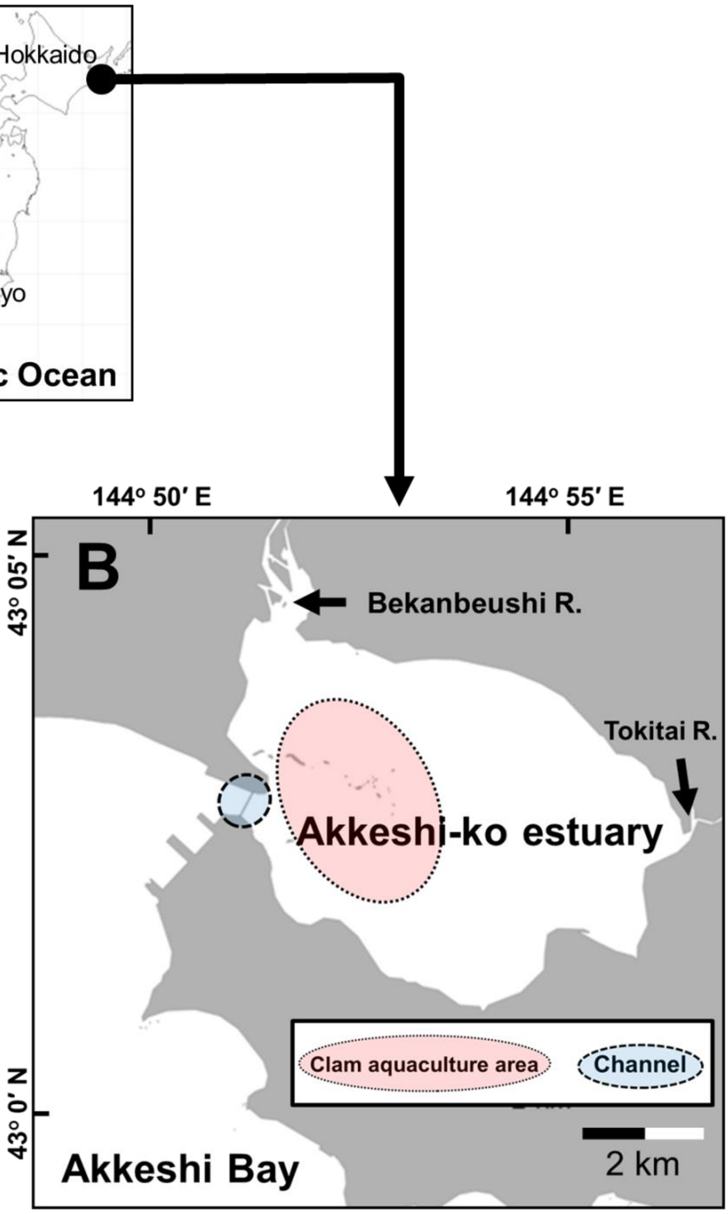

Fig. 1-1. Maps of (A) Japan and (B) the Akkeshi-ko estuary and Akkeshi Bay, northeastern Japan.

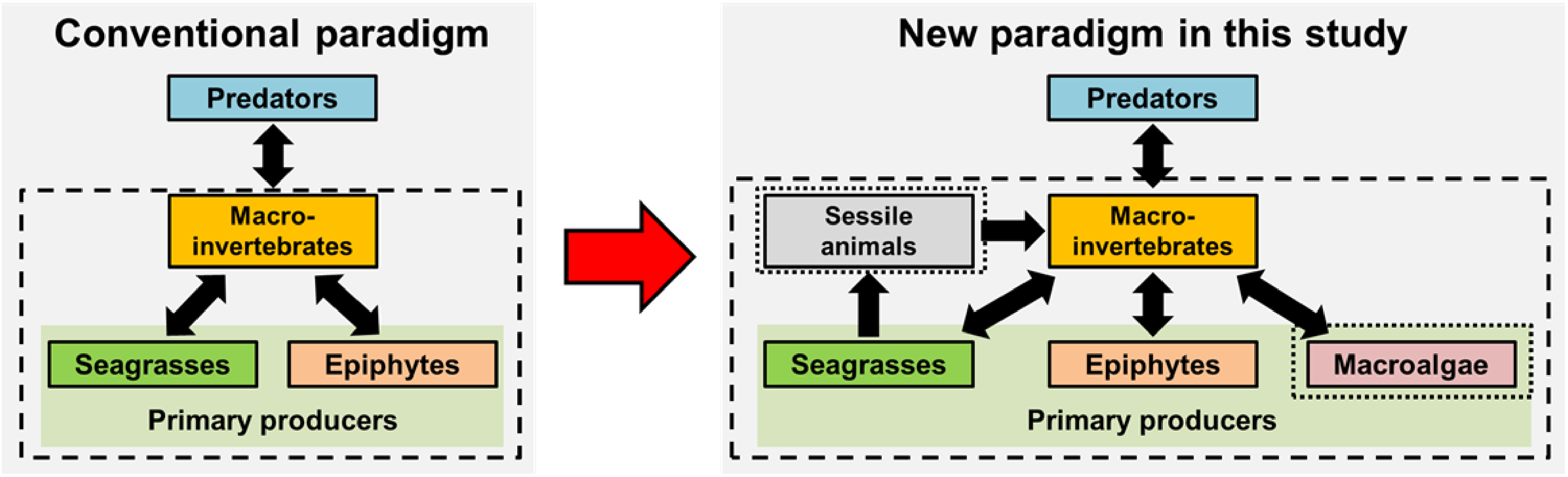

Fig. 1-2. Paradigm development of biological relationships in seagrass ecosystem in this study. Dashed boxes indicate scope of targeted relationships in this study. I integrated epibiotic factors, which have been rarely focused, to conventional paradigm of studies on producer-consumer relationships in seagrass beds.

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Chapter Ⅱ

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

2.1 Introduction

The abundance, biomass and species diversity of marine benthic invertebrate communities vary greatly with multiple abiotic/biotic factors. The effects of temperature and salinity as environmental filters have been known to be critical factors that regulate population/community patterns and processes in coastal habitats, especially in estuaries where strong environmental gradients are generated by tidal fluctuation and freshwater inflow (e.g. Ysebaert *et al*., 2003; Yamada *et al*., 2007b; Douglass *et al*., 2010; Blake & Duffy, 2010). Water temperature can either increase or decrease the abundance and diversity of component species (e.g. Somero, 2002; Harley *et al*., 2006;

Hoegh-Guldberg & Bruno, 2010; Meager *et al*. 2011), whereas a decrease in salinity generally leads to a lower species diversity and higher dominance by tolerant species (e.g. Ysebaert *et al*., 2003; Yamada *et al*., 2007b). Marine plants act as both a food resource because plant resource utilizers dominate in marine benthic invertebrate communities (e.g. Valentine & Heck, 1999; Harley, 2006; Poore *et al*., 2012) and as habitat-former (e.g. Attrill *et al*. 2000; Lee *et al*., 2001; Thomsen, 2010; Gartner *et al*. 2013).

Eelgrass (*Zostera marina*) is an important marine foundation species that is widely distributed along the coast of the northern hemisphere (Hughes et al., 2009). The complex physical structures created by eelgrass provide a habitat for many organisms (Jernakoff *et al*. 1996; Heck *et al*. 2003), which leads to an enhanced biodiversity and secondary production (Hemminga & Duarte, 2000; Duffy, 2006; Valentine & Duffy, 2006). A benthic invertebrate community in the above-ground parts of seagrass beds mainly consists of small crustaceans, gastropod mollusks and polychaetes, most of which are herbivores and detritivores (Valentine & Heck, 1999; Heck *et al*. 2000). These invertebrates play an important role in mediating the energy flow in the eelgrass bed ecosystem (Duffy & Hay, 2000; Duffy *et al*. 2005). To explore plant-animal interactions in eelgrass bed communities, many studies have investigated the relationship between animal abundance and various eelgrass traits, such as biomass, shoot density, leaf length, habitat patch structure, and epiphytic microalgal biomass of (e.g. diatoms) that serve as food resources (Attrill *et al*. 2000; Gartner *et al*. 2013; Whalen *et al*. 2013). However, large epibiotic organisms, such as macroalgae and sessile animals (e.g. spirorbid polychaetes, tunicates, bryozoans, hydrozoans), attached to eelgrass blades can also affect the mobile invertebrate community through resource provisioning and/or habitat modification. Despite some studies noting that the role of macroalgae on seagrass blades as a food resource or as a habitat provision can be one of the determinants of the abundance of mobile invertebrates (Valentine & Duffy, 2006; Gartner *et al*., 2013; Whalen *et al*. 2013), most studies have focused only on the importance of seagrass and/or microalgae (e.g. Jernakoff *et al*., 1996; Heck & Valentine, 2006). Whilst relevant studies are few, the sessile organisms such as invasive tunicates and juvenile bay scallops attaching on eelgrass blade can either affect mobile invertebrates by providing refuge from predation (Long & Grosholz, 2015) and by becoming a food resource (Lefcheck *et al*., 2014). Interpreting variations in the mobile invertebrate community in relation to various functional groups of epibiotic organisms that differ in size, morphology, habitat requirement and life history traits is thus necessary to deepen my understanding of the organization of animal assemblages in eelgrass beds and of the influences these organisms have on each other and eelgrass.

An extensive eelgrass meadow, consisting mostly of *Zostera marina* and partly of *Z. japonica* and *Ruppia maritima*, is located in the Akkeshi-ko estuary and the Akkeshi Bay in eastern Hokkaido, Japan (Hasegawa, Hori & Mukai, 2007). From early summer to late fall, a large variety of algae and sessile animals (epibiotic species), which attach to eelgrass blades, are observed, including microalgae, branched red algae, *Neosiphonia* sp., *Chondria dasyphylla*, filamentous green algae, *Cladophora* sp., calcareous algae, *Circeis spirillum*, and spirorbid polychaetes, such as *Neodexiospira brasiliensis*, bryozoans, hydrozoans, and tunicates. Among them, microalgae, the branched red algae and the spirorbid polychaetes are dominant in eelgrass beds for a long term, between early summer and late fall, with the peak of abundance between August and September (Hamamoto & Mukai, 1999; Kasim & Mukai, 2006; Hasegawa *et al*. 2007; Momota, unpublished data). Previous studies on benthic invertebrate assemblages in the Akkeshi-ko estuary and Akkeshi Bay have focused on their variability in relation to the salinity gradient (Yamada *et al*. 2007a; Yamada *et al*. 2007b). In addition to salinity, the spatial heterogeneity of other abiotic/biotic factors (e.g. water temperature, microalgal biomass and eelgrass biomass) is also high in estuarine systems, such as the Akkeshi-ko estuary (Iizumi *et al*. 1996; Kashim & Mukai, 2006; Hasegawa *et al*. 2007). Nevertheless, no previous study has investigated the mobile invertebrate community structure using an approach that simultaneously accounts for the details of sessile epibionts and environmental control by abiotic factors in the seagrass beds in Akkeshi.

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

2.2 Materials and methods

2.2.1 Study area

The Akkeshi-ko estuary (locally called Akkeshi Lake) and Akkeshi Bay are located in Northeastern Hokkaido, Japan (Fig. 2-1) and are connected to each other through a narrow channel (width: approximately 500 m, depth: approximately 10 m). The Akkeshi-ko is a brackish estuary, shallow water (depth range in most of the lake: 0.8-1.7 m with the maximum difference in tide levels of up to approximately ± 0.6 m), with an area of approximately 32 km^2. Most bottom areas of the Akkeshi-ko estuary are muddy and covered with eelgrass (*Z. marina*) except for the aquaculture farms of the clam *Venerupis philippinarum* in the intertidal zone near the channel (Kashim & Mukai, 2006; Hasegawa *et al*. 2007; Yamada *et al*. 2007a, Yamada *et al*. 2007b). Here, freshwater input from the Bekanbeushi River, which accounts for 98.8% of all of the flow volume (Iizumi *et al*. 1996), and tidal seawater input from the Akkeshi Bay cause steep physical and chemical environmental gradients (Iizumi *et al*. 1996; Yamada *et al*. 2007a).

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

In this study, I established stations in the Akkeshi-ko estuary, (BK: river mouth of the Bekanbeushi River, HN: Horonitai, TB: Toubai, SL: the southern lakeside, CL: the central lake and CK: Chikarakotan) and one station in Akkeshi Bay (SR: Shinryu) (Fig. 1). BK (mean sea level, MSL hereafter: 0.9 m) is located at the mouth of the

Bekanbeushi River and is strongly affected by freshwater inflow. The vegetation is dense with small-sized *Z. marina* (average shoot length of 1.0 m in August). HN (MSL: 1.1 m) is in a location with a high water temperature and medium salinity relative to the other stations. In addition to *Z. marina*, *Rupia maritima*, a seagrass species that is more tolerant to low-saline water, occurs at HN. The eelgrass beds at HN are mostly continuous but have some gaps, and the average shoot length in August is 1.3 m. TB (MSL: 1.1 m) and SL (MSL: 1.0 m) have a relatively low salinity compared to that of the other stations and are the furthest stations from the Akkeshi Bay. Although these two stations are in a similar environment, the water is often more turbid and the eelgrass bed is patchier at TB than SL. SL has a higher seagrass biomass and shoot density than TB. The average shoot length of eelgrass is approximately 1.3 m in August at both of these stations. CL (MSL: 1.4 m) and CK (MSL: 1.5 m) are deeper stations with a higher salinity and are dominated by longer eelgrass (shoot length: 1.5-3.5 m at the peak season). The eelgrass at SR (MSL: 1.5 m) in the Akkeshi Bay, has a similar shoot size to that in CL and CK. Here, the dominant seagrass species changes from *Z. marina* bed to *Z. asiatica* at a depth of approximately 2 m, as mentioned above.

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

2.2.2 Field sampling

I conducted a field survey in August 2012. Sample collection was performed when the tidal current was slow. I collected mobile invertebrates on eelgrass blades when the water level was deeper than the average sheath length of the eelgrass at each station (BK: 20 cm; HN, TB, SL: 30 cm; CL, CK, SR: 40 cm). Because the eelgrass at my study stations is tall (> 1 m) compared to the average water depth of each station, the canopy usually reaches the water surface (except for at extremely high tides). All sampling was performed under these conditions. I targeted mobile invertebrates and excluded some species with remarkably higher mobility and less dependency on eelgrass habitat, such as mysids and decapods (Yamada *et al*. 2007b), which were not quantitatively collected by my method (see below).

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

I collected three replicate samples (a total of 21 samples from all stations) of mobile invertebrates, spirorbid polychaetes and epiphytic macroalgae together with the entire above-ground parts of the eelgrass using a mesh bag (bore diameter: 20 cm, mesh size: 0.1 mm) based on the mouth area of the mesh bag (314 cm^2). Upon collection, we counted the number of eelgrass shoots to determine shoot density. For microalgae, five replicate samples were collected per station together with one eelgrass shoot using a plastic zip bag, because microalgae easily fell off from eelgrass blades when collected with the mesh bag.

2.2.3 Laboratory procedures

Immediately after being transported to the laboratory, the microalgae were scraped from the eelgrass blades using a glass slide; separated from other organisms such as macroalgae and spirorbid polychaetes; and then filtered using glass fiber filters (Whatman GF/F filter φ 47 mm, Whatman International Ltd., Maidstone, UK). If other organisms were present in the microalgal samples, I carefully removed them from the filters with forceps. Other epibiotic organisms collected using mesh bags were separated from the eelgrass by scraping them off with a glass slide; these organisms were classified as red algae, green algae, spirorbid polychaetes and mobile invertebrates. To obtain dry mass, eelgrass shoots, red algae, green algae, spirorbid polychaetes and filtered microalgae were dried at 60°C for 4 days in small aluminum foil bags, and then weighed. I counted and identified the mobile invertebrates after extraction with a sieve (500 μm) and fixation with 70% ethanol. Identification of mobile invertebrates was made to the lowest taxonomical unit possible (mostly to species) using detailed guides from the literature (Gammarid amphipod: Nishimura, 1995; Carlton, 2007; Ogawa, 2011; Caprella amphipod, Isopod, Copepod, Cumacea: Nishimura, 1995; Carlton, 2007; Gastropod: Okutani, 2000; Polychaeta: Nishimura, 1992; Imajima, 1996; Imajima, 2001; Turbellaria: Nishimura, 1992; Carlton, 2007; Hirunoidea: Nishimura, 1992) and the World Register of Marine Species online database (WoRMS: http://www.marinespecies.org).

2.2.4 Statistical analysis

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

To test which of the eight biotic/abiotic factors was a likely predictor of the variation in the mobile invertebrate community, I fitted linear mixed models (LMMs) with a Gaussian distribution (Bolker *et al.* 2009). The station was used as a random variable. As response variables, I used the biomass of 11 dominant species for the population-level analyses, and total invertebrate biomass, species richness and species diversity (Shannon-Wiener diversity index; calculated based on biomass data) for community-level analyses. The 11 most dominant species were selected by a threshold whereby the biomass proportion accounted for more than 1% of the total invertebrate biomass (see Table 2-S1). *Ostreobdella kakibir* (Hirudinoidea) was omitted from the analysis because it occurred only at one station (SR), even though they satisfied the requirement. R software (version 3.1.3) was used for all of the analyses (R Development Core Team, 2015).

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

I fitted the LMMs using the *lmer* function in the lme4 package (Bates *et al.* 2014). To obtain *P*-values of the LMMs, I used the *lmerTest* package (Kuznetsova et al., 2014). I selected the optimal model comparing the candidate models on all combinations of the predictors by the Akaike information criterion as corrected for the small sample size (AIC_c: Burnham & Anderson, 2002). I obtained AIC_c based on the maximum likelihood (ML) for comparisons among the LMMs because the restricted maximum likelihood (REML) is inappropriate in the case when the fixed structure is different between the candidate models (Zuur *et al.* 2009), but the parameters were estimated by REML. I used the *AIC_ctab* function in the *bbmle* library (Bolker & R Core Team, 2013) to compare the AIC_c. After setting the optimal models, I obtained the standardized coefficients as effect sizes by re-fitting using standardized variables that were scaled by the sample standard deviation and centered by sample mean values.

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

2.3. Results

2.3.1 Environmental factors

Water temperature was lower at the four stations (BK, CL, CK and SR) near the channel than at the other three stations in the inner parts of the estuary (HN, TB and SL) (Table 2-1). Salinity was lower at the lakeside stations (BK, HN, TB, SL and CK) that were influenced by freshwater inputs. For these stations, the inter-annual variation was also higher as shown by data collected by myself and other studies (Table 2-1).

Eelgrass biomass varied between 140 and 278 g DW m^{-2} among the stations. It was the lowest at TB, followed by HN and BK (Table 2-1). Eelgrass shoot density ranged between 85 and 234 shoot m^{-2}. It was highest at BK and second highest at SL. The mean densities were not largely different among other stations. Microalgal biomass varied by more than ten-fold between the lowest station (SL) and the highest station (CL). In the latter, the microalgal biomass exceeded the biomass of the eelgrass. Macroalgae were not present at TB and SR. Branched red algae were dominated by *Neosiphonia* sp. and *Chondria dasyphylla*, and filamentous green algae were dominated by *Cladophora* sp. The mean biomass of red algae was highest at HN and that of green algae was highest at CL, although their biomasses were less than 15 % that of eelgrass. Spirorbid polychaetes were not present at SL and CL. They were highly abundant at SR where their biomass was more than three-fold greater than the eelgrass biomass.

2.3.2 Mobile invertebrate community

A total of 32 mobile invertebrate species were collected in this study (Table 2-S1). At taxonomic levels, polychaete worms made up 32.2% of the total biomass, followed by gastropods (31.3%), gammarid amphipods (23.0%), and isopods (8.8%). At the species level, a polychaete *Nereis* sp. was most dominant (24.6%), followed by gastropods *Lacuna* spp. (23.4%) and the gammarid amphipod *Ampithoe lacertosa* (18.0%). For an additional eight species including two gammarid amphipods (*Monocorophium* spp. and *Pontogeneia rostrata*), two isopods (*Cymodoce japonica* and *Paranthura japonica*), two gastropods (*Ansola angustata* and *Siphonacmea oblongata*) and two polychaetes (*Exogone naidina* and *Syllis* sp.), their proportions were less than 5% at most.

The mean value of the total mobile invertebrate biomass was the highest at CK and much lower at stations along the coastline (HN, TB and SL). Species richness was the highest at CL, followed by CK and was approximately the same level at the other stations (Fig. 2-2). The mean value of species diversity was the highest at CL and the lowest at SR (Fig. 2-2).

2.3.3 Population level analyses

I found that each of the nine invertebrate populations belonging to gammaridae, gastropoda and polychaeta was predicted by a different combination of environmental factors in the optimal models (Table 2-2). The effect size of three epibionts on dominant invertebrate species was either similar or larger than abiotic and eelgrass factors (Fig. 2-3). For two isopods, no environmental factor correlated with their biomass.

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

For the two predictors relevant to the characteristics of the eelgrass bed, the above-ground biomass showed a significantly positive relationship only with *Syllis* sp., whereas shoot density was significantly correlated with *Monocorophium* spp. (positive), *P. rostrata* (negative) and *E. naidina* (negative) (Table 2-2).

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

Although epiphytic microalgae were removed from my analysis because of the collinearity with water temperature, no significant correlation was found for species that were correlated with water temperature (*A. lacertosa*: Pearson's r = 4.05, *P* = 0.25; *Lacuna* spp.: r = -3.42, *P* = 0.69; *E. naidina*: r = 0.31, *P* = 0.92; *Nereis* sp.: r = 10.87, *P* = 0.34; *Syllis* sp.: r = 0.21, *P* = 0.86).

2.3.4. Community level analyses

The total invertebrate biomass tended to decrease with water temperature, and significantly increased with increasing eelgrass biomass and red algal biomass (Table 2-2). Species richness showed a negative correlation with water temperature and spirorbid polychaetes. Species diversity was positively correlated with green algal biomass, but was negatively correlated with spirorbid polychaetes (Table 2-2). The effect size of red algal biomass on the total invertebrate biomass was similar to that of eelgrass biomass, and that of spirorbid polychaetes on species richness was also similar to that of water temperature (Fig. 2-3).

2.4. Discussion

The present study demonstrated that the biomass gradient of epibiotic organisms (e.g. macroalgae and spirorbid polychaetes) was a good predictor of the variation in some dominant mobile invertebrates in the eelgrass bed and the population biomass of the community parameters such as total biomass and diversity. Further, I found that the population biomasses and community components were not always influenced only by a single factor but also by multiple factors. The effect of the macroalgae is notable because these sessile epibionts have a much lower biomass compared to the biomass of eelgrass and epiphytic microalgae. However, the observed relationships between these functional groups and mobile invertebrate populations varied greatly among the species.

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

All three gastropods increased in correlation with spirorbid polychaetes, whereas the responses to the other factors were different (Table 2-2). Because the gastropods adhere to flat seagrass blades, the flat (simple) structure created by seagrass blades can be better than the rough structure of spirorbid polychaetes. Therefore, competition for space (negative effect) appears to be more expected than facilitation. Although I do not have a good answer for the positive relationships, one possibility for this unexpected result is that the rough structure acts as a shelter because small-sized individuals (< 3 mm) are frequent in gastropod populations during the summer season (*A. angustata*: Momota, personal observation; *Lacuna* spp.: Kanamori *et al*. 2004; *S. oblongata*: Toyohara, Nakaoka & Tsuchida, 2001).

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

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

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

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

Total biomass, species richness and species diversity were differentially correlated with abiotic/biotic factors, and varied in a complex manner although processes were unclear. The optimal model of the three community variables contains one or two variables of sessile epibionts. The positive correlation of red algae with total biomass reflects that with highly dominant species, such as *Lacuna* spp. and *Nereis* sp., which occupied more than 48 % of the total biomass. The negative interaction of spirorbid polychaetes with species richness and diversity suggest that spirorbid polychaetes can decrease the homogeneity of the biomasses of component species within a community by allowing some competitive species to dominate. The effect of green algae was found only on species evenness, but not on total biomass nor on species richness, suggesting that the green algae may increase species evenness by decreasing abundance of dominant species although the actual mechanisms remain to be cleared.

2.5. Conclusions

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

Recent studies demonstrated that the capacity for resistance and resilience to environmental stress and perturbations vary with food web structure in seagrass beds, which knowledge can contribute to improvement of coastal management (Unsworth *et* *al*., 2015; Maxwell *et al*., 2016; Östman *et al*., 2016). My study comparing population and community level responses of epifauna to different types of epibionts on eelgrass blades adds more knowledge on the complex trophic/non-trophic interactions of eelgrass communities, and will promote more understandings of the resilience and the feedback mechanisms of seagrass ecosystems which offer variable ecosystem services to human such as seafood provisioning and water quality controls (Cullen-Unsworth *et al*., 2013; Nordlund *et al*., 2016).

Figures

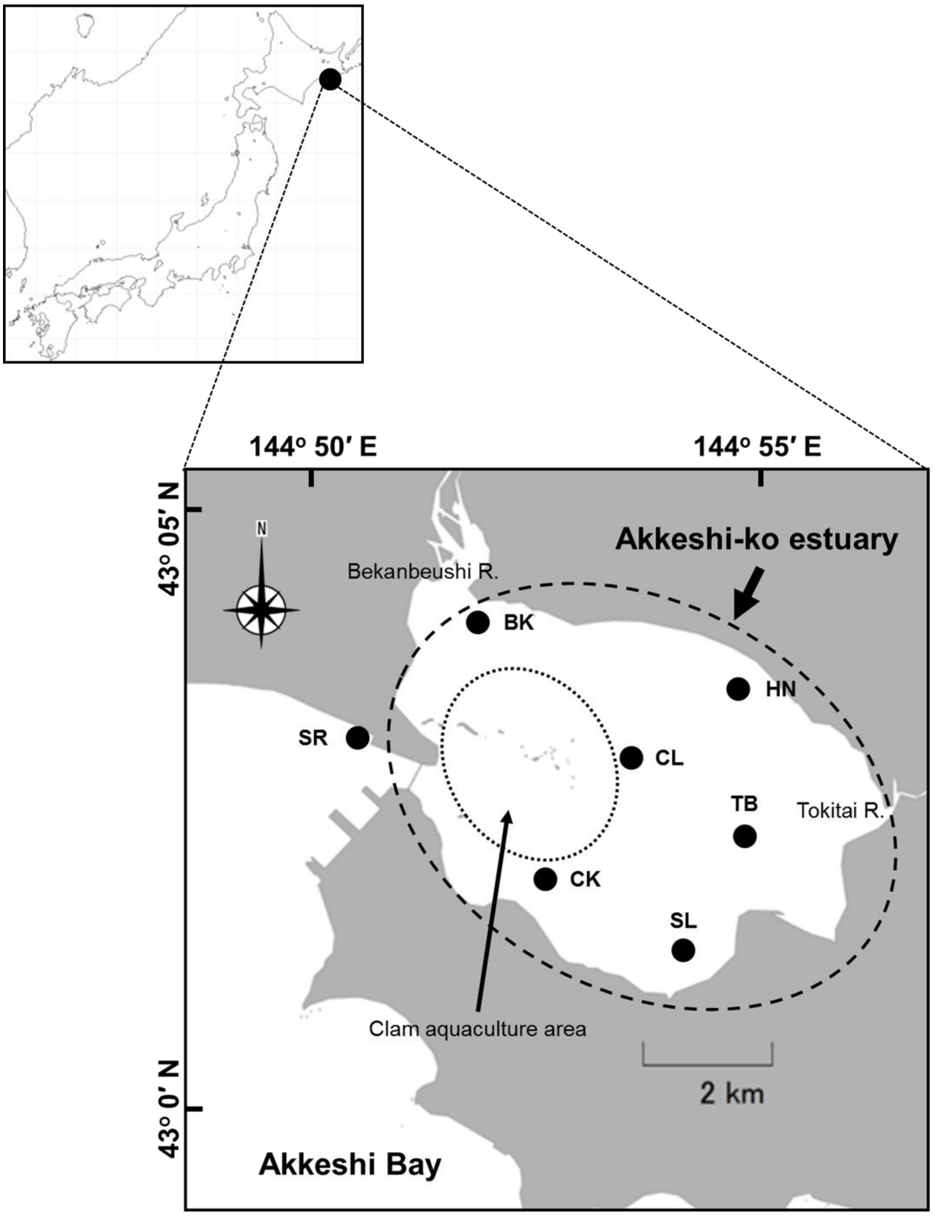


Fig. 2-1. Location of the study sites in the Akkeshi-ko estuary and the Akkeshi Bay in Northeastern Japan. The area enclosed by a dashed circle is the Akkeshi-ko estuary. Most of the clam aquaculture grounds are located in the western part of the estuary (indicated by a dotted circle).

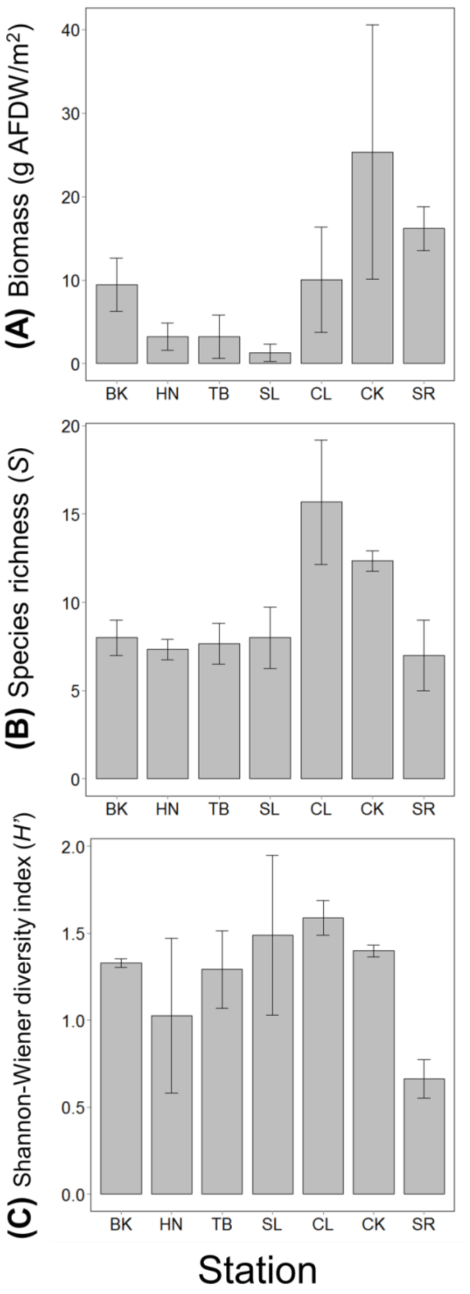
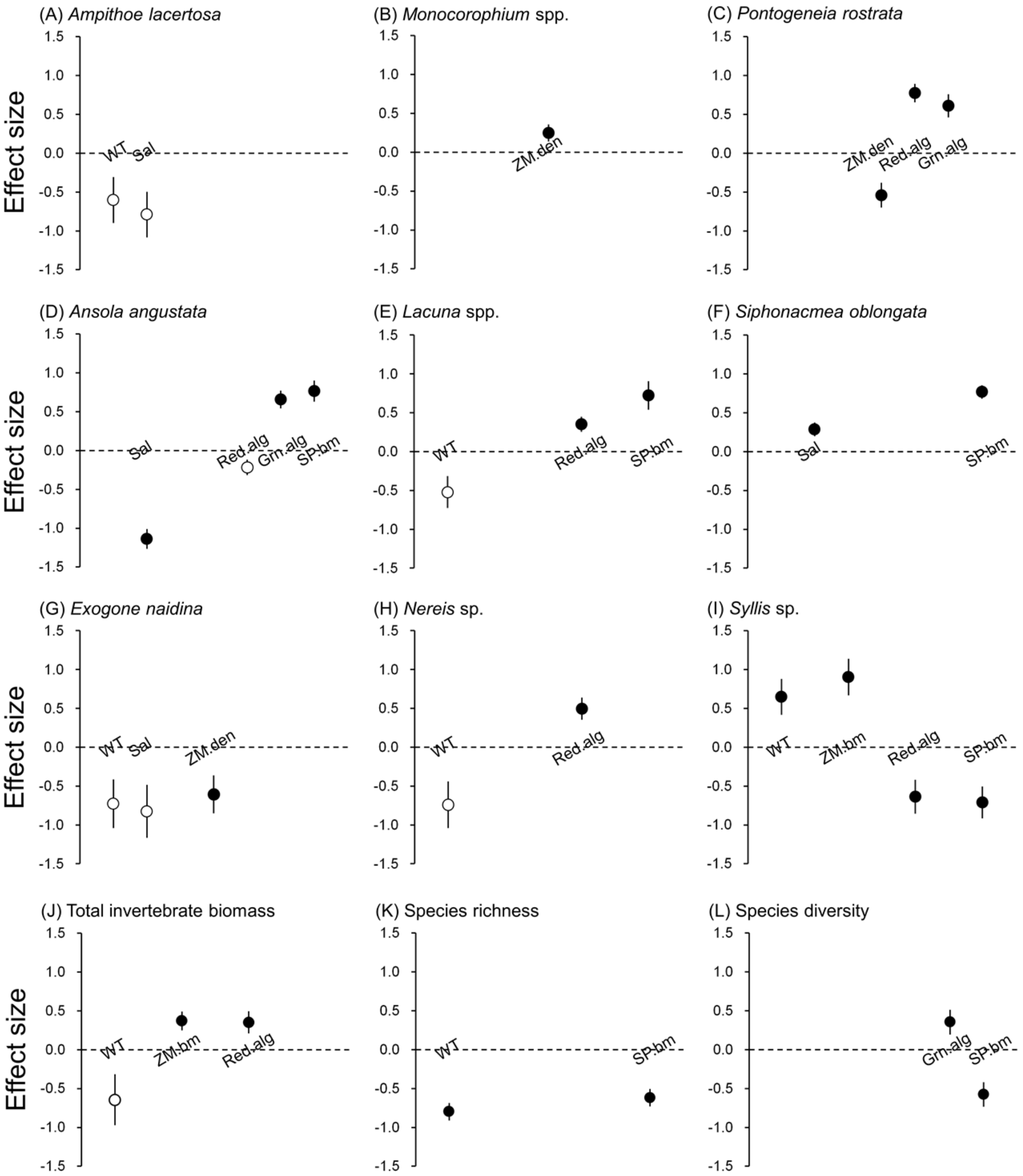


Fig. 2-2. (A) The total invertebrate biomass, (B) species richness and (C) Shannon-Wiener diversity index at the seven stations in the Akkeshi-ko estuary and Akkeshi Bay. The bars indicate the mean values with SDs. The order of the stations is lined up based on relative size of the impact of freshwater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


(A) *Ampithoe lacertosa*

(B) *Monocorophium* spp.

(C) *Pontogeneia rostrata*

(D) *Ansola angustata*

(E) *Lacuna* spp.

(F) *Siphonacmea oblongata*

(G) *Exogone naidina*

(H) *Nereis* sp.

(I) *Syllis* sp.

(J) Total invertebrate biomass

(K) Species richness

(L) Species diversity

Fig. 2-3. Effect size of abiotic and biotic factors on mobile invertebrate populations and community detected by linear mixed models.Water temperature (WT), salinity (Sal), eelgrass biomass (ZM.bm), eelgrass shoot density (ZM.den), branched red algae (Red.alg), filamentous green algae (Grn.alg)and spirorbid polychaetes (SP.bm). I only reported the results of predictors selected by the best models (Table 2). Open circles represent detected predictors without significance ($P > 0.05$) and filled circles represent detected predictors with significance ($P < 0.05$). Error bars indicate standard errors of effect sizes.

Tables

Table 2-1. Environmental conditions at seven stations in the Akkeshi-ko estuary and Akkeshi Bay. Abiotic factors in this study are indicated by **boldface**. For water temperature and salinity, I also presented data in August reported by the other studies.

Factors		**Stations**							**Ref.**
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	
Abiotic									
Water temperature (°C)		**23.8**	**26.1**	**25.9**	**25.5**	**21.0**	**22.6**	**22.5**	a
		21.4	22.4	22.9	—	22.5	20.0	18.8	b
		18.1	20.3	20.3	21.0	18.5	17.3	16.6	c
		21.7	24.1	23.8	23.9	21.9	22.7	18.8	d
Salinity		**25.0**	**26.4**	**27.0**	**27.1**	**29.2**	**26.3**	**29.9**	a
		16.8	28.1	28.4	—	29.6	32.0	28.6	b
		16.1	—	—	23.9	26.0	26.5	29.6	e
		26.7	25.0	13.6	22.4	27.4	28.4	29.9	c
		21.2	23.6	26.0	26.2	26.8	26.7	29.9	d
Biotic									
Eelgrass factor									
Dry mass (g m^{-2})	Mean	152.2	140.4	119.5	216.3	216.8	190.3	277.9	a
	SD	25.8	37.3	30.8	30.9	26.8	65.0	68.5	
Shoot density (m^{-2})	Mean	233.7	85.3	74.7	159.0	85.3	85.3	96.0	a
	SD	18.5	18.5	18.5	18.5	0.0	18.5	18.5	
Epibiont dry mass									
Microalgae (g m^{-2})	Mean	73.2	25.6	77.9	19.2	384.5	113.4	76.3	a
	SD	63.9	6.5	46.6	5.0	119.8	58.9	26.2	
Red algae (g m^{-2})	Mean	0.1	9.0	0.0	4.1	0.0	4.6	0.0	a
	SD	0.1	6.0	—	2.2	0.0	7.6	—	
Green algae (g m^{-2})	Mean	7.5	0.0	0.0	8.2	28.0	0.1	0.0	a
	SD	7.4	—	—	4.3	16.2	0.0	—	
Spirorbid shell (g m^{-2})	Mean	53.5	21.8	6.8	0.0	0.0	1.9	944.3	a
	SD	28.0	18.7	7.6	—	—	3.2	190.6	

[a] This study; [b] Iizumi et al., (1996); [c] Nakaoka et al., (unpubl.); [d] Momota, (unpubl.); [e] Kashim and Mukai, (2006)

Table 2-2. Results of LMMs for explaining responsible environmental factors on variation in mobile invertebrate populations and community components. AIC$_c$ scores and delta AIC$_c$ are also reported. Significant coefficients (*P*-values < 0.05 level) and the lowest AIC$_c$ scores are in **bold face**. Standardized coefficients (*Std.coef*) of the optimal models are reported right below each optimal model in *italic face*. Abbreviations as follows; WT: water temperature, Sal: salinity, ZM.bm: eelgrass biomass, ZM.den: eelgrass shoot density, Red.alg: red algal biomass, Grn.alg: green algal biomass, SP.bm: spirorbid polychaete biomass.

Response	**Model**	**Predictor**								**AIC$_c$**	**Δ AIC$_c$**
		(Intercept)	**WT**	**Sal**	**ZM.bm**	**ZM.den**	**Red.alg**	**Grn.alg**	**SP.bm**		
Population											
Gammarid amphipoda											
Ampithoe lacertosa	Null	**33.978**	—	—	—	—	—	—	—	193.9	1.0
	Full	673.282	-87.465	-48.498	-0.482	4.427	7.243	-0.761	-0.736	216.7	23.8
	Optimal	**1097.930**	-79.180	-129.700						**192.9**	—
Monocorophium spp.	Null	8.669	—	—	—	—	—	—	—	137.7	2.0
	Full	582.409	-42.288	-72.767	0.079	0.986	0.265	0.428	0.059	160.1	24.4
	Optimal	-6.824				**1.469**				**135.7**	—
Pontogeneia rostrata	Null	**10.068**	—	—	—	—	—	—	—	147.2	19.8
	Full	247.381	-11.576	-32.023	0.407	**-2.751**	**4.934**	**2.878**	0.111	139.5	12.1
	Optimal	**20.728**				**-1.842**	**5.023**	**2.523**		**127.4**	—

Table 2-2. (continued 1)

Response	**Model**	**Predictor**								**$\mathrm{AIC_c}$**	**$\Delta\ \mathrm{AIC_c}$**
		(Intercept)	**WT**	**Sal**	**ZM.bm**	**ZM.den**	**Red.alg**	**Grn.alg**	**SP.bm**		
Isopoda											
Cymodoce japonica	Null	**11.630**	—	—	—	—	—	—	—	**187.0**	0.0
	Full	605.245	-15.501	-100.306	4.532	-5.585	0.308	2.552	-0.113	211.1	24.1
	Optimal	**11.630**								**187.0**	—
Paranthura japonica	Null	**14.077**	—	—	—	—	—	—	—	**173.4**	0.0
	Full	132.132	8.691	-32.148	0.349	-0.924	2.623	4.411	0.463	200.0	26.6
	Optimal	**14.077**								**173.4**	—
Gastropoda											
Ansola angustata	Null	6.014	—	—	—	—	—	—	—	159.6	10.1
	Full	555.710	-12.507	**-96.596**	-0.524	1.180	-1.487	3.730	**0.923**	167.3	17.8
	Optimal	**600.167**		**-116.259**			-2.645	**5.102**	**1.149**	**149.5**	—
Lacuna spp.	Null	28.820	—	—	—	—	—	—	—	197.0	11.2
	Full	880.106	-129.988	-43.910	0.940	-2.094	**10.442**	-2.761	**2.607**	203.9	18.1
	Optimal	522.161	-106.591				**10.634**		**2.696**	**185.8**	—

Table 2-2. (continued 2)

Response	**Model**	**Predictor**								**AIC_c**	**ΔAIC_c**
		(Intercept)	**WT**	**Sal**	**ZM.bm**	**ZM.den**	**Red.alg**	**Grn.alg**	**SP.bm**		
Siphonacmea oblongata	Null	8.003	—	—	—	—	—	—	—	166.6	17.2
	Full	-350.288	-16.668	**82.450**	-1.654	2.567	1.664	-2.471	1.063	172.7	23.3
	Optimal	**-190.996**		**36.374**					**1.426**	**149.4**	—
Polychaeta											
Exogone naidina	Null	8.274	—	—	—	—	—	—	—	182.1	> 0.1
	Full	1003.724	-66.607	-119.185	1.236	-5.958	-1.510	0.675	-0.184	203.4	21.3
	Optimal	**988.365**	-75.725	-106.916		**-4.922**				**182.1**	—
Nereis sp.	Null	23.110	—	—	—	—	—	—	—	211.7	6.4
	Full	1994.677	-171.788	-216.463	5.651	-9.760	**13.928**	7.192	0.017	221.1	15.8
	Optimal	844.824	-171.482				**16.967**			**205.3**	—
Syllis sp.	Null	**6.678**	—	—	—	—	—	—	—	175.2	1.3
	Full	-342.880	**45.302**	14.699	**5.108**	-0.141	**-6.889**	-1.247	-1.140	191.5	17.6
	Optimal	**-269.866**	**45.615**		**4.886**		**-6.616**		**-0.908**	**173.9**	—

Table 2-2. (continued 3)

Response	**Model**	**Predictor**								**AIC$_c$**	**Δ AIC$_c$**
		(Intercept)	**WT**	**Sal**	**ZM.bm**	**ZM.den**	**Red.alg**	**Grn.alg**	**SP.bm**		
Community component											
Total invertebrate biomass	Null	**2.785**	—	—	—	—	—	—	—	72.0	10.3
	Full	60.096	-5.936	-5.985	**0.214**	-0.097	**0.456**	0.184	0.056	81.0	19.3
	Optimal	23.569	-4.937		**0.219**		**0.401**			**61.7**	—
Species richness	Null	**3.027**	—	—	—	—	—	—	—	28.0	14.7
	Full	**16.581**	**-1.485**	-1.211	0.080	-0.094	-0.036	0.080	-0.023	33.6	20.3
	Optimal	**13.909**	**-2.185**						**-0.031**	**13.3**	—
Species diversity	Null	**1.255**	—	—	—	—	—	—	—	19.9	8.6
	Full	2.056	-0.126	-0.049	0.010	0.012	-0.068	0.034	-0.026	36.7	25.4
	Optimal	**1.288**						**0.065**	**-0.020**	**11.3**	—

Supplemental Table

Table 2-S1. Biomass of 32 mobile invertebrates at seven stations in the Akkeshi-ko estuary and Akkeshi Bay. The biomass proportion and the rank are also shown.

Species	Mean AFDW ± SD (mg m^{-2}: n=3 per site)								Prop. (%)	Rank
	BK	HN	TB	SL	CL	CK	SR	Among sites		
Gammarid amphipoda										
Ampithoe lacertosa	2908 ± 745	1283 ± 1201	566 ± 510	71 ± 52	1733 ± 705	1183 ± 1105	31 ± 34	1107 ± 1156	17.97	3
Aoroides curvipes	15 ± 26	—	43 ± 22	—	2 ± 4	—	—	9 ± 19	0.14	21
Ischyroceridae sp.	—	—	—	—	—	—	24 ± 25	3 ± 12	0.06	26
Grandidierella japonica	—	—	—	—	54 ± 94	15 ± 26	—	10 ± 37	0.16	20
Hyalidae sp.	—	—	—	—	—	—	39 ± 68	6 ± 26	0.09	23
Monocorophium spp.	1131 ± 251	1 ± 2	3 ± 5	1 ± 1	49 ± 16	24 ± 23	2 ± 4	173 ± 409	2.81	8
Pleustes panoplus	—	—	—	—	6 ± 11	—	—	1 ± 4	0.01	28
Pontogeneia rostrata	21 ± 16	256 ± 139	6 ± 11	73 ± 25	237 ± 166	146 ± 161	1 ± 2	106 ± 134	1.72	12
Caprellid amphipoda										
Caprella acanthogaster	—	—	—	—	152 ± 152	—	—	22 ± 73	0.22	18
Caprella kroyeri	58 ± 100	99 ± 126	135 ± 155	10 ± 15	—	—	—	43 ± 88	0.61	16
Caprella laeviuscula	403 ± 349	—	—	—	132 ± 229	—	5 ± 8	77 ± 195	0.79	13
Caprella scaura	—	—	—	—	—	44 ± 76	—	6 ± 29	0.06	25
Caprella tsugarensis	—	—	—	30 ± 51	35 ± 40	286 ± 294	9 ± 16	51 ± 137	0.52	17

Table 2-S1. (continued 1)

Species	**Mean AFDW ± SD (mg m^{-2}: n=3 per site)**								**Prop. (%)**	**Rank**
	BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**Among sites**		
Isopoda										
Cymodoce japonica	16 ± 28	221 ± 324	79 ± 68	12 ± 19	754 ± 1300	870 ± 779	—	279 ± 605	4.47	5
Munna sp.	—	1 ± 1	—	23 ± 17	2 ± 1	6 ± 7	—	4 ± 10	0.07	24
Paranthura japonica	—	157 ± 147	604 ± 319	39 ± 68	306 ± 271	734 ± 469	—	263 ± 351	4.27	7
Copepoda										
Kushia zosteraphila	1 ± 1	—	54 ± 47	37 ± 19	2 ± 2	13 ± 14	18 ± 7	11 ± 16	0.18	19
Miraciidae sp.	—	1 ± 1	—	—	4 ± 5	—	—	1 ± 4	0.01	29
Thalestridae sp.	—	—	—	—	3 ± 2	—	—	1 ± 1	0.01	30
Cumacea										
Diastylis sp.	—	—	—	—	2 ± 4	—	—	0 ± 2	0.01	31

Table 2-S1. (continued 2)

Species	**Mean AFDW ± SD (mg m^{-2}: n=3 per site)**								**Prop. (%)**	**Rank**
	BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**Among sites**		
Gastropoda										
Ansola angustata	1187 ± 819	—	—	—	—	—	—	170 ± 498	2.78	9
Batillaria attramentaria	—	—	—	314 ± 544	—	—	—	45 ± 205	0.73	15
Lacuna smithi	1 ± 1	—	—	—	—	—	—	0 ± 0	0.00	32
Lacuna spp.	318 ± 205	3 ± 4	1 ± 1	—	162 ± 166	2342 ± 2267	7164 ± 2147	1427 ± 2717	23.42	2
Nassarius fraterculus	—	—	—	—	9 ± 15	—	—	1 ± 6	0.02	27
Siphonacmea oblongata	—	—	—	—	17 ± 30	—	1838 ± 953	265 ± 724	4.36	6
Polychaeta										
Exogone naidina	—	—	—	—	45 ± 31	1995 ± 2141	—	291 ± 983	4.57	4
Harmothoe imbricata	—	—	—	—	310 ± 248	35 ± 60	—	49 ± 136	0.77	14
Nereis sp.	—	—	—	—	2333 ± 2873	8741 ± 6504	—	1582 ± 3835	24.62	1
Syllis sp.	—	—	585 ± 1013	208 ± 208	181 ± 262	14 ± 25	—	141 ± 395	2.22	11
Platyhelminthes										
Rhabdocoela sp.	4 ± 5	10 ± 3	20 ± 19	17 ± 8	—	2 ± 3	—	8 ± 10	0.12	22
Hirudinoidea										
Ostreobdella kakibir	—	—	—	—	—	—	1097 ± 1552	157 ± 629	2.22	10

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Chapter Ⅲ

Seasonal change in eelgrass epifaunal community in response to gradients of abiotic and biotic factors

3.1 Introduction

Biological communities in seagrass beds are composed of a diversity of organisms including plants, algae and animals. Physical structure provided by seagrasses offers habitat as substrate, shelter and nursery to the organisms, and consequently enhances their diversity, biomass and production (Duffy, 2006). Seagrass-associated macroinvertebrates (e.g., crustaceans, gastropods and polychate worms) especially play important roles as mediators between primary producers and higher consumers (Duffy & Hay, 2000; Duffy *et al*., 2005). Because the macroinvertebrate assemblages in the above-ground part of eelgrass bed (epifaunal communities) are mainly composed of plant resource utilizers, such as grazers, herbivores and detritivores (Valentine & Heck, 1999; Heck *et al*., 2000), they are susceptible to direct/indirect effects of plant traits (e.g., Attrill *et al*., 2000; Jaschinski *et al*., 2009). Additionally, many studies reported that macroinvertebrate communities show various responses to physical and chemical conditions in coastal habitats including seagrass beds (e.g., Ysebaert *et al*., 2003;

Yamada *et al*., 2007b; Douglass *et al*., 2010; Barnes & Hendy, 2015). Thus, understanding the variation in patterns and processes of epifaunal community is a key to evaluate the condition and ecosystem functioning of seagrass beds (Duffy *et al*., 2001, 2003; Duffy, 2006; Gullström *et al*., 2012). However, many aspects of epifaunal community in seagrass beds still remain to be investigated. The relationship between epibiotic organisms (i.e., seagrass sessile/associated macroalgae and animals) and mobile epifaunal community in above-ground part of seagrasses is one of the less studied subjects (Momota & Nakaoka, 2017 [Chapter 2]). Although epiphytic microalgae on seagrass blades (e.g., diatoms) have mainly been focused as key factor in studies on the bottom-up effects in seagrass habitats, some recent studies have pointed out that the effects of larger epibiotic organisms (e.g., juvenile scallops, tunicates and spirorbid polychaetes) should also be taken into consideration (Lefcheck *et al*., 2014; Long & Grosholz, 2015; Momota & Nakaoka, 2017 [Chapter 2]). Epibiotic organisms in eelgrass beds are morphologically diverse and differently influence each epifaunal population and community properties via habitat modification and/or provisioning food resource (Momota & Nakaoka, 2017 [Chapter 2]). Marine invertebrate species including seagrass epifaunal species generally show low host plant dependency (Nakaoka, 2005; Best *et al*., 2014) and are more susceptible to the value of a shelter from predators or abiotic stresses rather than its food value (e.g., Duffy & Hay, 1991; reviewed in Best *et al*., 2014). Therefore, epifaunal community is expected to vary reflecting different response of each epifaunal population to microhabitat change caused by epibiotic organisms.

Given that sessile epibiotic organisms are influential as shown above, mobile epifaunal communities in seagrass habitats with abundant epibiotic organisms are expected to change in more complex ways than those in simper structured seagrass habitats with little epibiotic organisms. Best & Stachowicz (2014) found that dominant community assembly processes were different between marine amphipod communities in two habitat types: environmental filtering was the dominant process in macroalgae habitats whereas niche partitioning was the dominant process in seagrass habitats. Their results imply that epifaunal community composition in seagrass habitats with epibiotic organisms may be changed with the interference of multiple community assembly processes depending on habitat structure. Thomsen (2010) reported positive effects of invasive red macroalga *Gracilaria vermiculophylla* on native invertebrates via habitat-formation in a seagrass habitat. Understanding relationships of epifaunal species not only with seagrass and microalgae but also epibiotic organisms will thus advance our knowledge in the variation and maintenance mechanisms within seagrass systems in conjunction with traditional abiotic factors (e.g., water temperature and salinity) that also determine distributional patterns of epifaunal species. However, studies focusing on the effects of seasonal habitat change on epifaunal community have been still limited. Toward a comprehensive understanding of the variation in natural epifaunal community, it is necessary as a first step to examine how the relationships between multiple factors and epifaunal community vary seasonally. Relative importance of multiple factors can vary seasonally and that can influence the strength of community assembly mechanisms (Best & Stachowicz 2014).

In this study, I examined how epifaunal community structure varied seasonally and spatially with abiotic and biotic factors in an eelgrass bed of Akkeshi, northeastern Japan. I especially focused on the influence of epiphytic macroalgae and spirorbid polychates on eelgrass blades, which were found to influence population sizes of dominant epifaunal species in summer when the eelgrass bed was productive (Hasegawa *et al*., 2007; Yamada *et al*., 2007b). However, the temporal changes in the relationships and the importance have not been examined yet. Because a large variety of epibiotic algae and sessile animals seasonally appear one after another from early summer to late fall (Hamamoto *et al*., 1999; Hasegawa *et al*., 2007), these effects on mobile epifaunal community may change seasonally here. I expected that relative importance of multiple abiotic/biotic factors changes with season in relation to spatial variability of each factors.

3.2. Materials and methods

3.2.1. Study area

I established a total of eight study stations in eelgrass (*Zostera marina*) meadows in the Akkeshi-ko estuary and Akkeshi Bay, located in northeastern Hokkaido, Japan (Fig. 3-1): six stations were established in the Akkeshi-ko estuary (BK: river mouth of the Bekanbeushi River, HN: Horonitai, TB: Toubai, SL: the southern lakeside, CL: the central lake and CK: Chikarakotan) and two stations in Akkeshi Bay (SR: Shinryu and AK: Aininkappu).

The Akkeshi-ko estuary is linked by a narrow channel (width: approximately 500 m, depth: approximately 10 m) to Akkeshi Bay (Fig. 3-1). Freshwater inflow from Bekanbeushi River to Akkeshi-ko estuary accounts for 98.8% of the total inflow volume, and tidal seawater input from Akkeshi Bay generate spatially and temporally different environmental gradients across my study stations (e.g. water temperature, salinity, nitrogen/phosphate concentration and landscape of eelgrass medews: Iizumi *et* *al*., 1995, Yamada *et al*., 2007a, b; Momota & Nakaoka, 2017 [Chapter 2]).

The Akkeshi-ko estuary is a shallow (less than 2 m deep) and brackish area with a mud bottom. Eelgrass meadows in the Akkeshi-ko estuary extend over a large part of the subtidal zone except for clam (*Venerupis philippinarum*) farms in the intertidal zone near the channel (Momota & Nakaoka, 2017 [Chapter 2]).

Eelgrass meadows in Akkeshi Bay occur in the soft bottom from the intertidal zone to the subtidal zone of shallower than 2 m deep. The bottom sediment type is different between SR (sandy mud bottom) and AK (sand bottom) in Akkeshi Bay.

3.2.2. Field sampling and laboratory procedures

I carried out field surveys once each month from May to November in 2013. I did not carry out surveys between December and April because most of the study stations were covered with ice in the Akkeshi-ko estuary. The biomass and productivity of eelgrass and algae are low during the winter (Watanabe *et al*., 2005, Hasegawa *et al*., 2007). I conducted the field surveys at a time when tidal current was slow around mid-tide level in order to reduce biases in measurements associated with tidal cycles.

In this study, I collected data on two abiotic factors (water temperature [°C] and salinity) and on four biotic factors (eelgrass biomass [g dry weight per unit area; g DW m^{-2}], epiphytic microalgal biomass [micro-algae on eelgrass blades in this study, g DW m^{-2}], epiphytic macroalgal biomass [g DW m^{-2}] and spirorbid polychaete biomass [shell biomass g DW m^{-2}]). I defined microscopic algae (mostly unicellular diatoms and dinoflagellates) as microalgae whereas visible multicellular red, green and brown algae as macroalgae. For the response variables, I collected data on epifaunal community structures (epifaunal species composition and abundances per species [individuals per unit bottom area: inds. m^{-2}]).

I obtained the data on abiotic factors, biotic factors and epifaunal community structures (i.e. species abundances and composition) according to sampling and taxonomic identification methods in Chapter 2. Five replicate samples were collected at each station.

3.2.3. Statistical analyses

To examine which abiotic/biotic factors influence temporal and spatial variation in the similarity and composition of epifaunal communities, I performed multivariate analyses: a non-metric multi-dimensional scaling (NMDS: Clarke, 1993), a permutational multivariate analysis of variance (PERMANOVA: Anderson, 2001), redundancy analysis (RDA: ter Braak & Prentice, 1988) and analysis of similarities (ANOSIM: Clarke, 1993). All these multivariate analyses were performed using the *vegan* package (Oksanen *et al*., 2013) in R software (version 3.3.3: R Development Core Team, 2017). Epifaunal abundances were log-transformed (x+1) to reduce the influence of abundance bias before providing a Bray-Curtis dissimilarity matrix.

I performed NMDSs based on a Bray-Curtis dissimilarity matrix to illustrate the similarity of communities among stations and months using the *metaMDS* function. Stress value of the NMDS was checked to evaluate the accuracy of the two-dimensional ordinations, where the value <0.2 was acceptable (Clarke, 1993). Then, I conducted PERMANOVA using the *adonis* function in the vegan package to statistically test the temporal and spatial variation in epifaunal community composition.

PERMANOVA is a robust test for multivariate datasets without non-normality and can handle a non-Euclidian distance matrix (McArdle & Anderson 2001). I used 9999 permutations for NMDS ordination and PERMANOVA.

RDAs were applied to understand variation in response of community composition to the abiotic/biotic factors. I separately performed RDAs on data sets of each month to clearly illustrate the temporal changes in the responsible factors on epifaunal community. Prior to RDAs, all the abiotic/biotic factors were log-transformed (x+1) and epifaunal abundance data was transformed by the Hellinger transformation. RDA using the Hellinger transformation has relatively high statistical power (Fuzessy *et al*., 2015) and produces contrasting results (Dray *et al*., 2012), because this transformation does not give high weights to the rare species (Legendre & Gallagher, 2001). To explain the observed patterns of community changes, a forward selection model with Monte Carlo permutation (999 times) test was used to detect the relevant subset and significance ($p < 0.05$) of the factors. I also tested the contribution of each selected factor to the variation in epifaunal community composition with Monte Carlo permutation (999 times) test. Before the model fit, I tested multicollinearity among the predicting factors using the variance inflation factor (VIF) analysis with a cutoff of 10 (e.g., Dormann *et al*., 2013). However, strong multicollinearity (VIF > 10) was not detected.

ANOSIM based on a Bray-Curtis dissimilarity matrix was performed to follow RDAs by statistically test for the similarity of epifaunal community composition among stations for each month using *anosim* function. I used Monte Carlo permutation (999 times) test to obtain global R values and associated *p* values. When global R is negative or close to 0 (where R is scaled between 0 and 1), similarities within the same station and among stations are equivalent. Conversely, global R values approaching 1 indicate strong separation of epifaunal communities among stations.

3.3. Results

3.3.1. Variation in abiotic/biotic factors and epifaunal community

Water temperature increased from May to August and then decreased (Fig. 3-2A; Table 3-1). The spatial variation was great during June and August. Salinity was mostly stable throughout the sampling term except for September when the field sampling was conducted nine days after a heavy rain (Fig. 3-2B; Table 3-1).

Eelgrass shoot biomass increased from May to July and drastically decreased after August (Fig. 3-2C; Table 3-1). The spatial variation was large from June to August.

Epiphytic microalgal biomass rapidly increased from May to June and gradually decreased after August (Fig. 3-2D; Table 3-1). The spatial variation was especially large in June.

Epiphytic macroalgal biomass increased from May to August, drastically decreased from August to September, and showed a slight increase in October (Fig. 3-2E; Table 3-1).

Spirorbid shell biomass increased from June to August, and rapidly declined from August to September (Fig. 3-2F; Table 3-1). The spatial variation was large in July and August. *Neodexiospira brasiliensis* was predominant and *Circeis spirillum* was the second most abundant.

I collected a total of 113 epifaunal species in this study (Table 3-2). Among all the samples, gammarid amphipods *Monocorophium* spp. was most dominant (25.2%), followed by a gammarid amphipod *Ampithoe lacertosa* (10.9%), a polychaete *Exogone naidina* (7.4%), ostracods Cytheroidea spp. (6.6%), a copepod *Kushia zosteraphila* (6.5%), a gammarid amphipod *Pontogeneia rostrata* (6.19%), a gastropod *Ansola angustata* (4.8%), gastropod *Lacuna* spp. (4.7%) and copepods Miraciidae spp. (4.5%). Epifaunal community size was high in July and August, and showed rapid increase or decrease before or after this peak (Fig. 3-2G). The spatial variation was also large in July and August.

3.3.2. Temporal and spatial variation in epifaunal community

The variation in epifaunal community composition was explained for 33.7 % by stations (PERMANOVA, $F = 43.83$, $p < 0.0001$; Fig.4), 13.8 % by months ($F = 20.94$, $p < 0.0001$) and 28.0 % by the interactive effect of station and month variation ($F = 6.08$, $p < 0.0001$). The NMDS ordination plot showed that the similarity of epifaunal communities in BK was greatly different from the other seven stations (Fig. 3-3). This trend was also shown in RDAs in most months except for September and October (Fig. 3-4). The epifaunal community composition at the inner most part of the Akkeshi-ko estuary (HN, TB and SL) showed high similarity, and so between the two stations in the center of the estuary (CL and CK). For SR and AK in Akkeshi Bay, the epifaunal community composition was different. Except for BK, there was a linear trend in the shift in community composition from Akkeshi Bay to the inner part of the estuary along the first axis of NMDS (Fig. 3-3).

The spatial variation in epifaunal community composition was significant in all months (Table 3-3). The spatial variation was higher in summer.

3.3.3. Relationships between abiotic/biotic factors and epifaunal community composition

RDAs explained 63 to 76 % of spatial variation in epifaunal community composition by the first and second axes (Fig. 3-4). Totally, abiotic/biotic factors accounted for 52 to 68 % of the variation (Fig. 3-5). The variation was relatively better explained by the factors from May to August. The similarity of epifaunal community composition gradually became low among stations but high within stations from spring to summer, and then high among stations on and after September (Fig. 3-4). The similarity among stations was roughly separated into two groups (BK and the other stations) from May to August and in November. Moreover, the similarity was clear between lake-side stations (HN, SL and TB) and the other stations (CL, CK, SR and AK) in July and August. The spatial variation in the similarity among stations was less clear in September and October than from May to August and in November.

Water temperature and salinity were selected as significant factors affecting the epifaunal community composition throughout the seven months (Fig. 3-4). The effects of the two abiotic factors were stronger from May to August. Those effects explained 36 to 46 % of the variation in the epifaunal community composition in these months (Fig. 3-5). Although both of those effects were weaker from September to November, the effect of water temperature still explained >10 % of the variation in the epifaunal community composition.

The four biotic factors explained 19 to 35 % of the variation in the epifaunal community composition among months (Fig. 3-5). The relative importance of the biotic factors was higher than that of abiotic factors from September to November. The significant effect of eelgrass biomass was detected in most months except for July and November, and especially contributed to >10 % of the variation in the epifaunal community composition in May and October. The effects of epibiotic factors (i.e., epiphytic microalgae, epiphytic macroalgae and spirorbid polychaetes) were detected throughout the survey period (Fig. 3-4). The effects of epiphytic microalgae showed higher contribution from September to November. The effect of epiphytic macroalgae was detected in all months, and was relatively higher from June to October. The effect of spirorbid polychaetes was also detected in most months except for October, and was relatively higher from July to August.

3.4. Discussion

The present study showed that epifaunal community structure in the eelgrass bed of Akkeshi varied temporally and spatially. Overall, the spatial variation was much greater than the temporal variation. I expected that abiotic factors were more definitive for the variation in epifaunal community than biotic factors when the eelgrass bed was less productive in early spring and late autumn whereas biotic factors including macro-epibionts (i.e., epiphytic macroalgae and sessile animals) were more influential when the eelgrass bed was more productive from late spring and early autumn, because the effects of biotic factors was supposed to be detected when the spatial variations were high in productive seasons. Inconsistent with my expectation, however, RDAs showed that abiotic factors were more definitive for the spatial patterns of the epifaunal communities from spring to summer when the productivity of eelgrass bed are increasing (Hasegawa *et al*., 2007), whereas biotic factors are relating more important in autumn when the productivity declines (Fig. 3-5). The general pattern of the similarity of epifaunal communities in this study was quite similar to that of Yamada *et al*. (2007b) who studied more highly mobile epifaunal communities such as mysids and shrimps. This results common to these two studies indicated that macroinvertebrate species in eelgrass bed of Akkeshi is under the strong influence of abiotic factors.

The spatial pattern of the similarity of epifaunal communities could be roughly distinguished by grouping the eight stations into two areas (BK and the other stations) by water temperature and salinity gradients. Because BK is located at river mouth of the Bekanbeushi River where epifaunal community is exposed to severe environmental fluctuations, the similarity of epifaunal community composition is greatly different between BK and the other stations (Yamada *et al*., 2007b). The increasing in the similarity of epifaunal community composition between BK and the other stations on and after September was presumably caused by the spatial pattern of water temperature gradually varied from September (i.e., water temperature inversion) (Fig. 3-4). The similarity was clear between lake-side stations (HN, SL and TB) and the other stations (CL, CK, SR and AK) in July and August, because epifaunal community structure was largely determined by abiotic/biotic processes. Contrastingly, the spatial variation in the similarity of epifaunal community composition among stations was less clear in September and October than from May to August and in November (Fig. 3-4). This result suggests that the effects of abiotic factors (environmental control) are stronger than biotic factors to determine epifaunal community composition (Fig. 3-5).

Water temperature and salinity, the two abiotic factors were influential on spatial patterns of epifaunal communities from spring to summer, which was inconsistent with my expectation, that predict their prevalence in less productive season in early spring and late autumn. Similar results were reported in previous studies on the spatial variation in seagrass-associated species (Montague & Ley, 1993; Yamada *et al*., 2007b). Water temperature was also considered to contribute to increase or decrease the abundance and diversity of component species as reported in studies on marine benthic invertebrate communities (e.g., Somero, 2002; Harley *et al*., 2006; Hoegh-Guldberg & Bruno, 2010; Meager *et al*., 2011). Overall, water temperature and salinity can spatially differentiate epifaunal community composition through the abundance, diversity and distribution of component species, as evidenced by results of RDAs show that the similarity of epifaunal communities gradually diverged among stations and converged within stations from spring to summer (Fig. 3-4).

Although less important than abiotic factors, biotic factors contributed significantly to the variation in epifaunal community composition. Among them, two epibiotic factors (macroalgae and spirorbid polychaetes) contributed similar or higher than that of eelgrass and epiphytic microalgal biomass in most months (Fig. 3-5). This is especially notable that epiphytic (both sessile and drift/seagrass-associated) macroalgae and spirorbid polychaetes were much less abundant than eelgrass and epiphytic microalgae throughout the survey period (Fig. 3-2E, 3-2F). The seasonall variation in the contributing these biotic factors agreed with my expectation in that the relatively higher contributions were found from late spring and early autumn (epiphytic macroalgae: from June to October; spirorbid polychaetes: from July to

August). The contributions basically appeared to be high when the biomass and the spatial variation of epibionts were also high (except for macroalgae in September and October).

For the contribution of epiphytic macroalgae in September and October, the effect was likely to be different though the reason remained unknown exactly. In other eelgrass bed, the positive effect of drift macroalgae on gastropods was reported (Thomsen, 2010). For spirorbid polychaetes, abundant biomass of them beyond a certain level appears to become influential for epifaunal community through the variation in epifaunal species richness and diversity Momota and Nakaoka (2017) (Chapter2).

Epiphytic microalgal biomass was not always important for the variation in epifaunal community despite that epifaunal communities were mostly composed of plant resource utilizers, such as amphipods, copepods and gastropods (Table 3-2). Higher contribution of epiphytic microalgal biomass was found in autumn when their abundance was decreasing and their spatial variation was not great (Fig. 3-5). The causal mechanisms for the observed relationship in autumn remain to be investigated.

Surprisingly, eelgrass biomass was influential only in limited months such as May and October. Although the effect has been traditionally regarded as key factor determing epifaunal abundance and diversity in eelgrass beds (e.g., Heck *et al.*, 1995; Attrill *et al.*, 2000), it may contribute to the variation in epifaunal abundance but not to that in epifaunal community composition in Akkeshi. Furthermore, eelgrass biomass was less important than epiphytic macroalgae and spirorbid polychaetes in most months. Although it is difficult to independently deal with eelgrass and epibiotic organisms and to compare the relative importance among them, microhabitat variation caused by epibiotic organisms is likely more important for epifaunal community composition rather than eelgrass habitat space. The diversification of microhabitat by epibaiotic organisms seems to change the epifaunal community composition by promoting the microhabitat differentiation and coexistence of epifaunal species (Lürig *et al*., 2016). Momota and Nakaoka (2017) (Chapter2) found that biomass of the epibiotic organisms positively correlated with that of dominant epifaunal species whereas eelgrass biomass was not correlated with most dominant species. It remains unknown why the contribution of eelgrass biomass was higher in May and October.

3.5. Conclusioms

In conclusion, the present study demonstrated that the epifaunal community composition was temporally and spatially varied with different sets of abiotic/biotic factors. I also found that epibiotic factors (i.e., epiphytic macroalgae and spirorbid polychaetes) contributed to explain some parts of the variation in addition to other abiotic/biotic factors especially in summer productive seasons, despite relatively low biomass compared to that of eelgrass and epiphytic microalgae. The results implicate that the effects of epibiotic factors on environmental condition and biological/functional interaction should be more taken into account in eelgrass community studies to comprehensively understand the variation in seagrass systems.

Figures

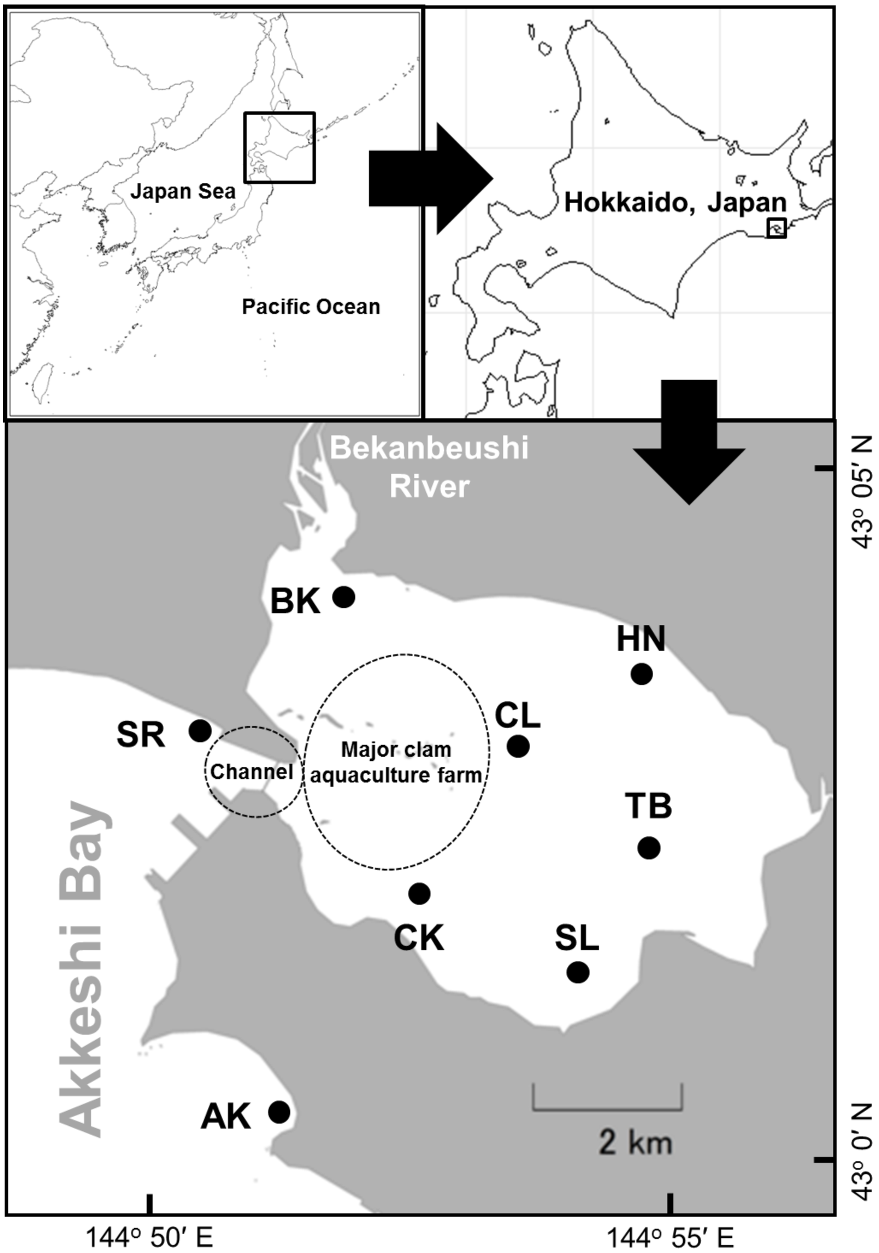
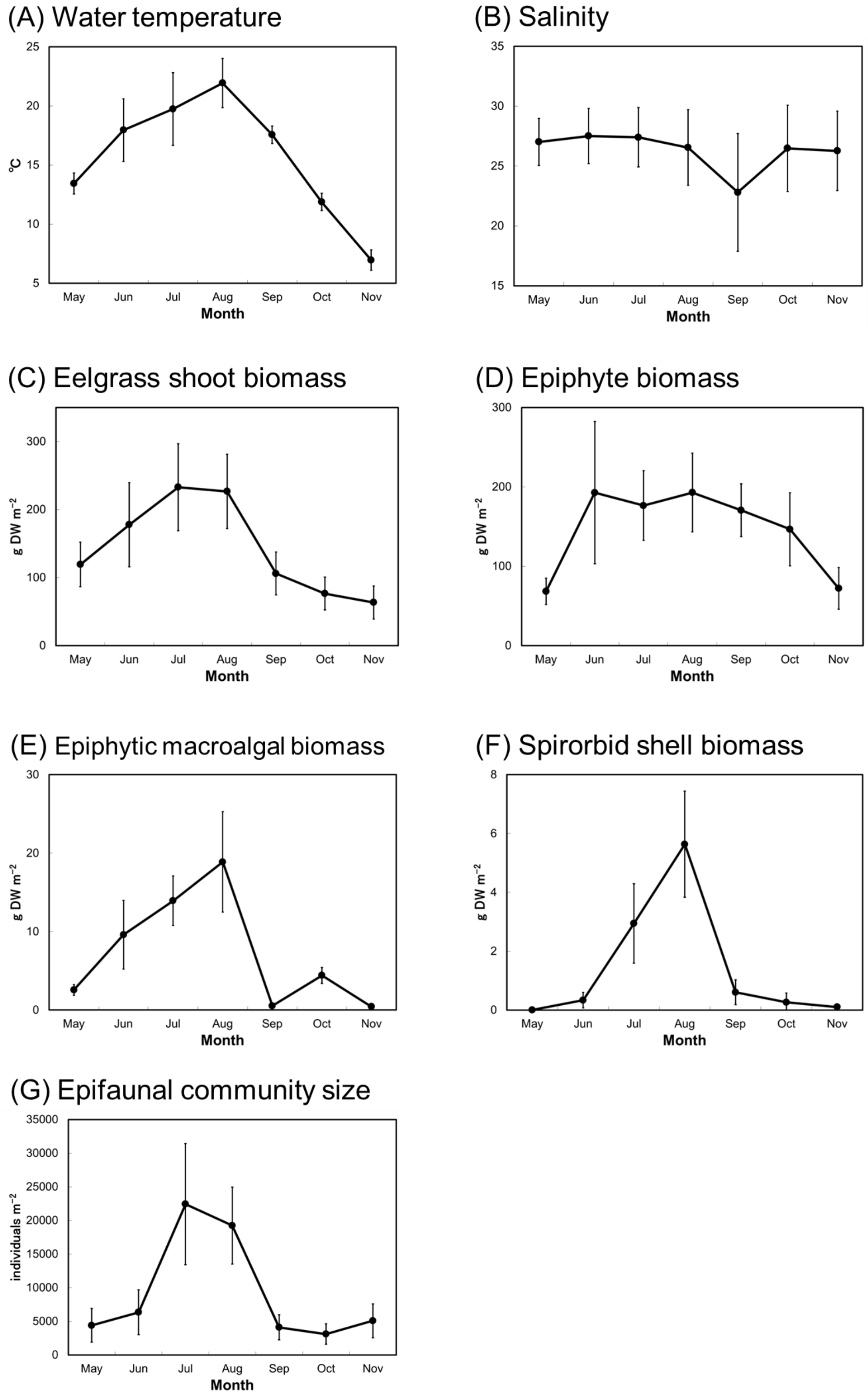


Fig. 3-1. Location of the study stations in Akkeshi-ko estuary and Akkeshi Bay, northeastern Japan.



(A) Water temperature

(B) Salinity

(C) Eelgrass shoot biomass

(D) Epiphyte biomass

(E) Epiphytic macroalgal biomass

(F) Spirorbid shell biomass

(G) Epifaunal community size

Fig. 3-2. Monthly change in (A) water temperature, (B) salinity, (C) eelgrass shoot biomass, (D) epiphyte biomass, (E) epiphytic macroalgal biomass, (F) spirorbid shell biomass and (G) epifaunal abundance. The points indicate mean values in each study month. The error bars indicate SDs.

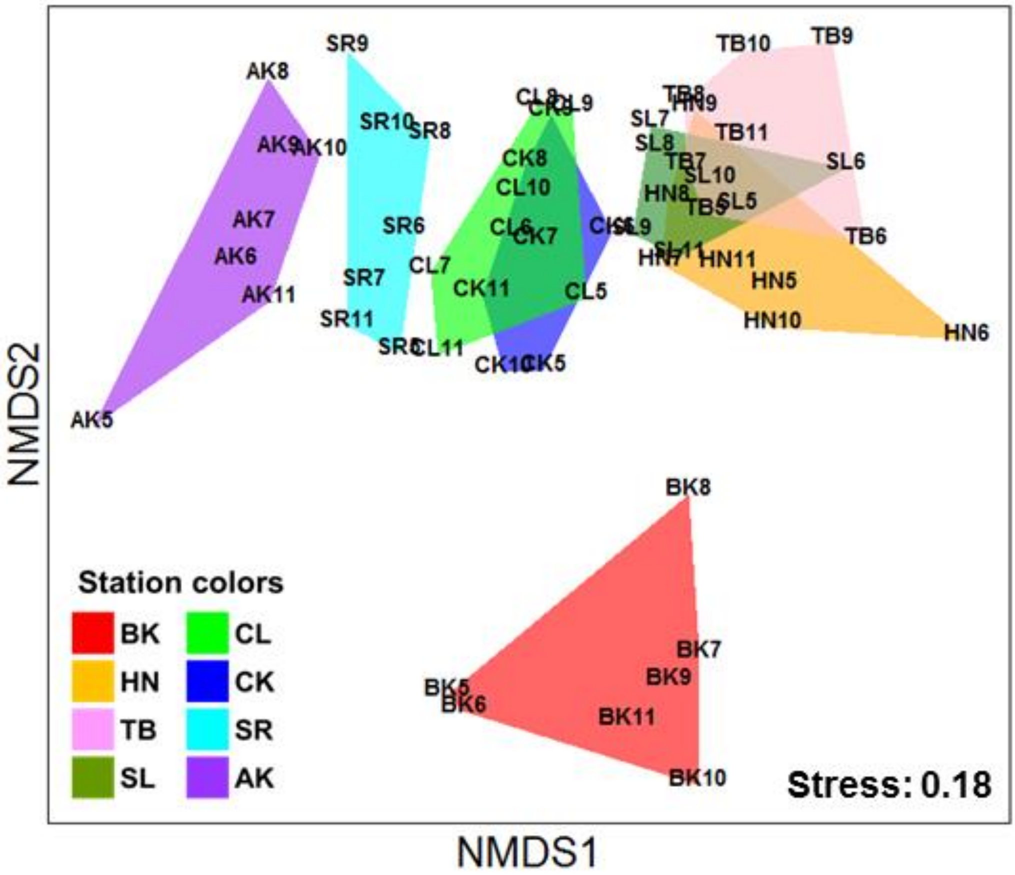
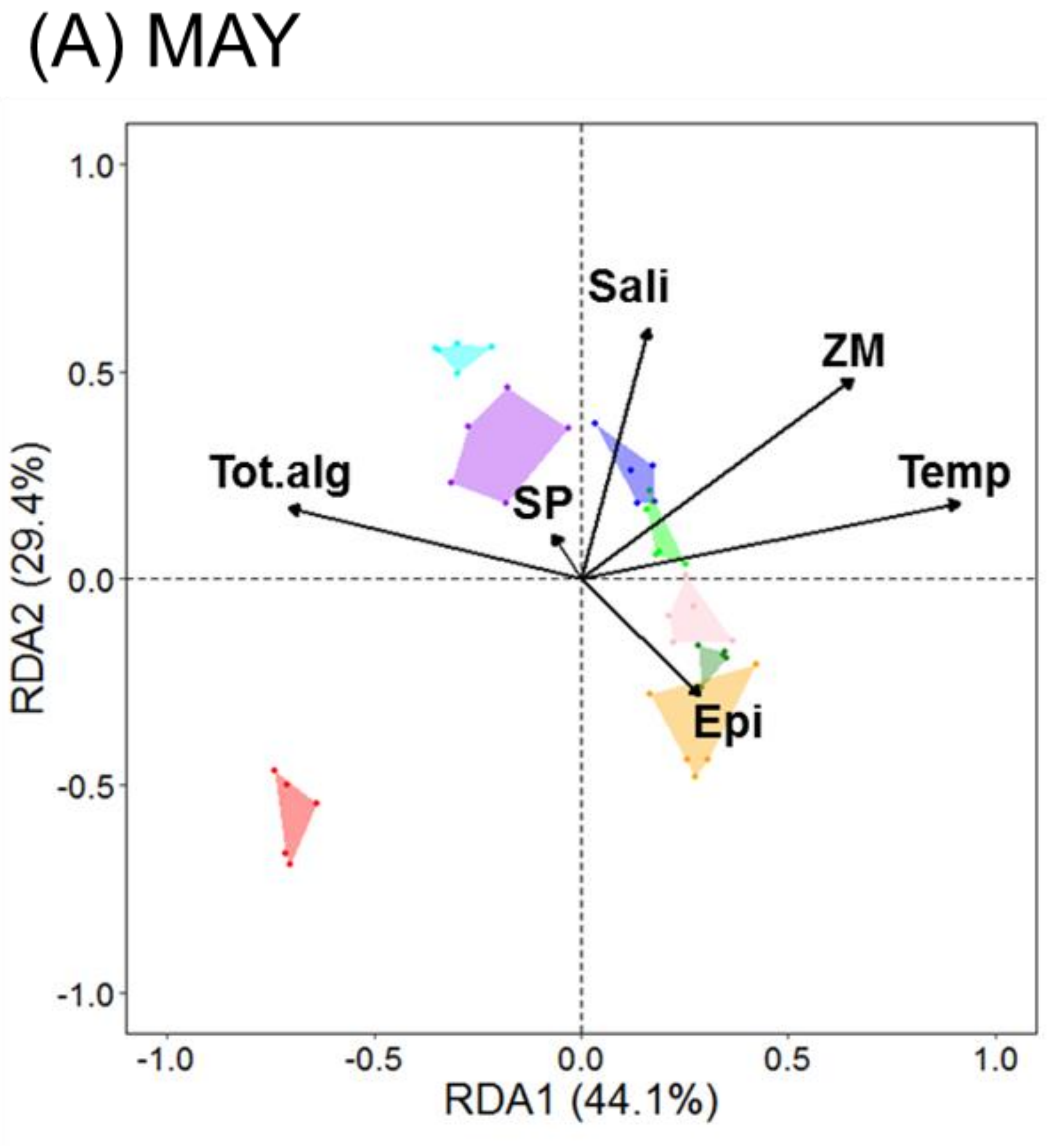
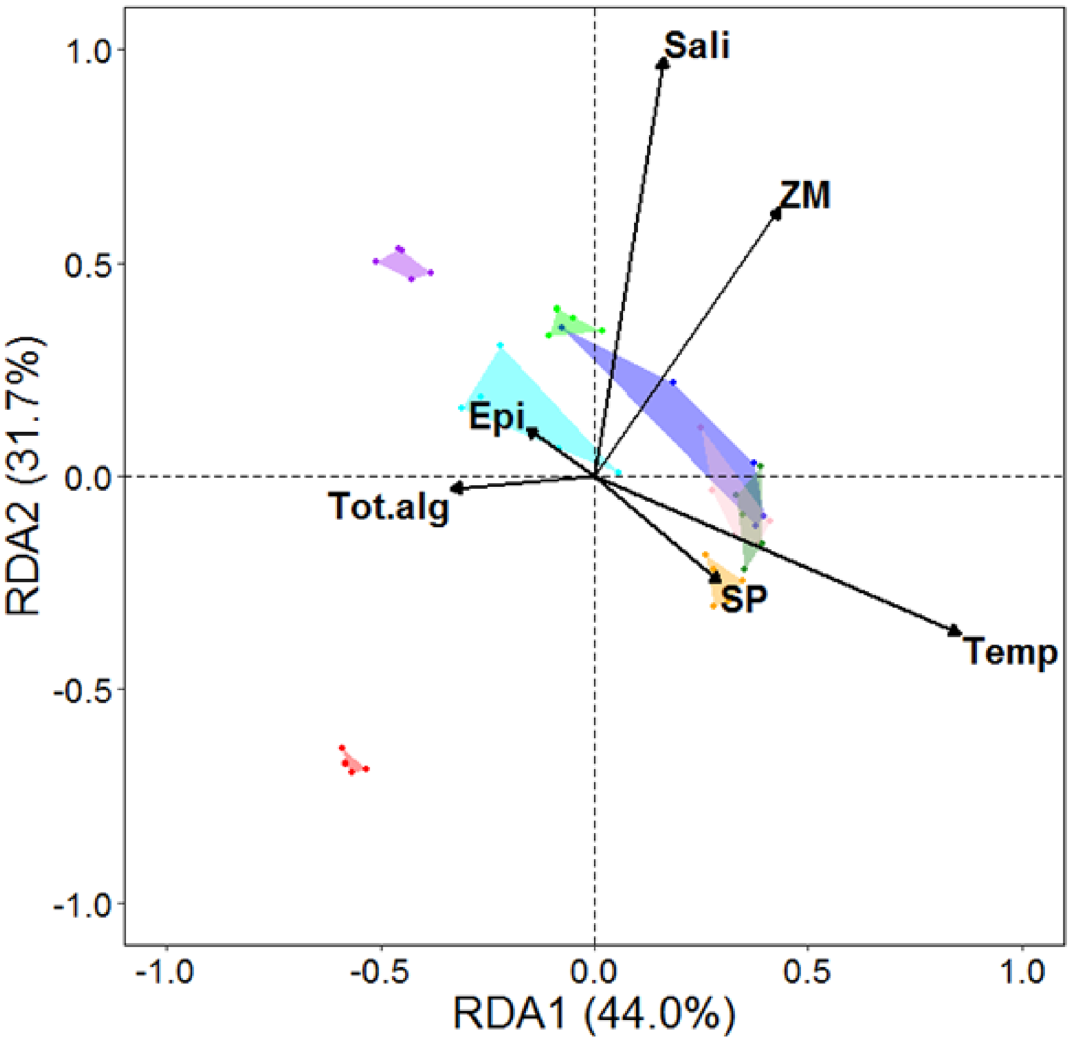


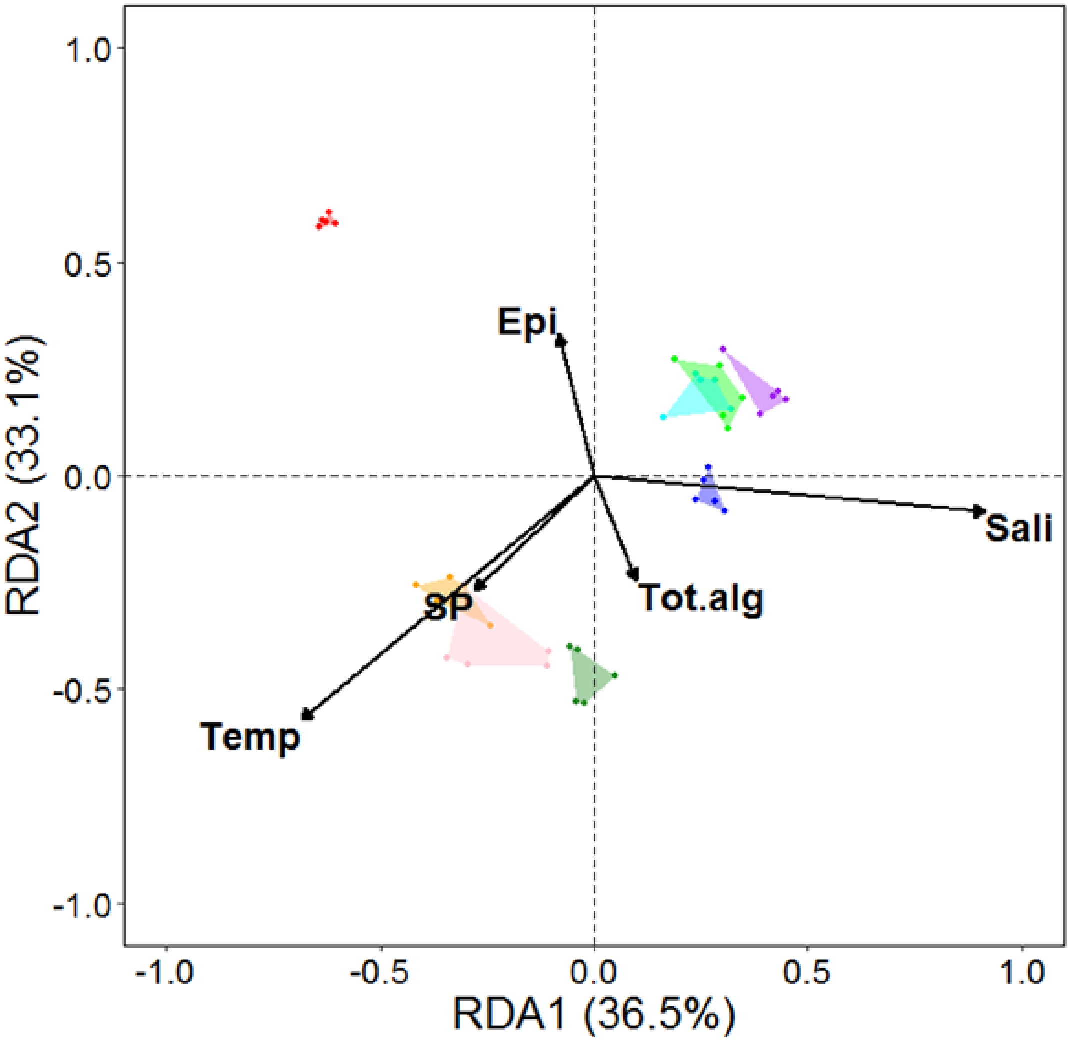
Fig. 3-3. NMDS ordination of epifaunal communities at the eight study stations across months. Each plot represents station name with sampling month from May to November. Species names are reported in Table 1. Convex hulls indicate the similarity of community composition among month across stations.


(A) MAY
Sali
ZM
Temp
Tot.alg
SP
Epi
RDA2 (29.4%)
RDA1 (44.1%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0

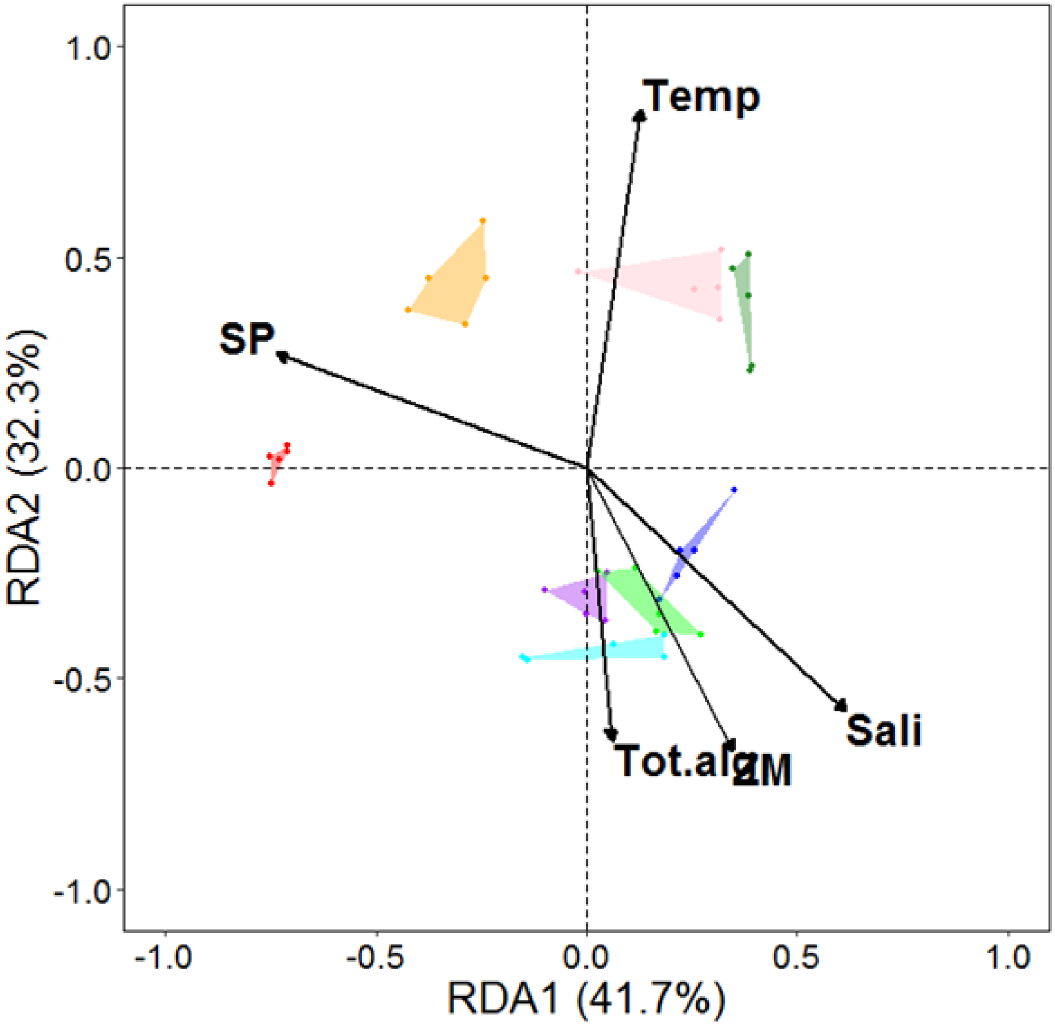
(B) JUNE

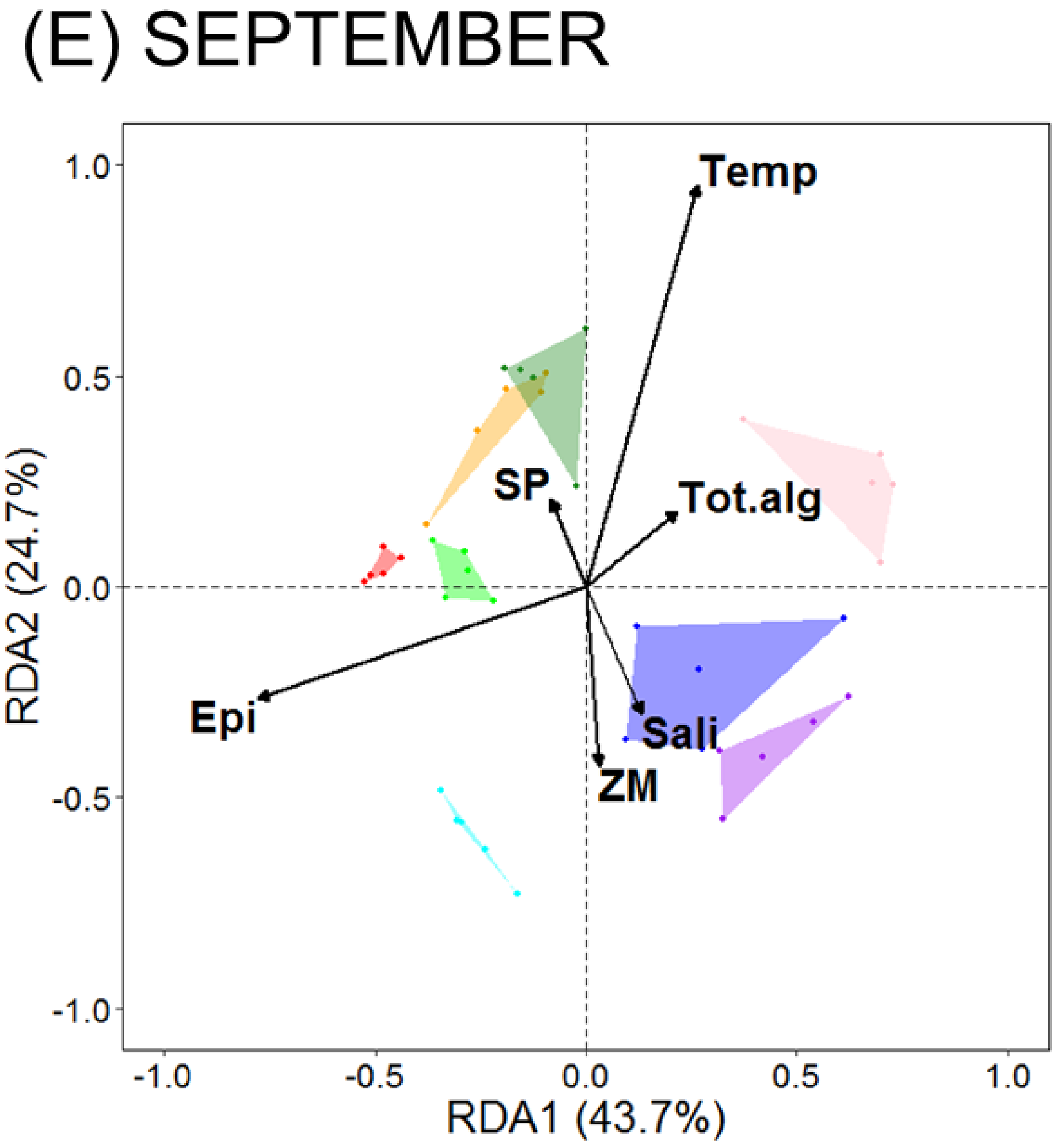

Sali
ZM
Epi
Tot.alg
SP
Temp
1.0
0.5
0.0
-0.5
-1.0
RDA2 (31.7%)
-1.0
-0.5
0.0
0.5
1.0
RDA1 (44.0%)

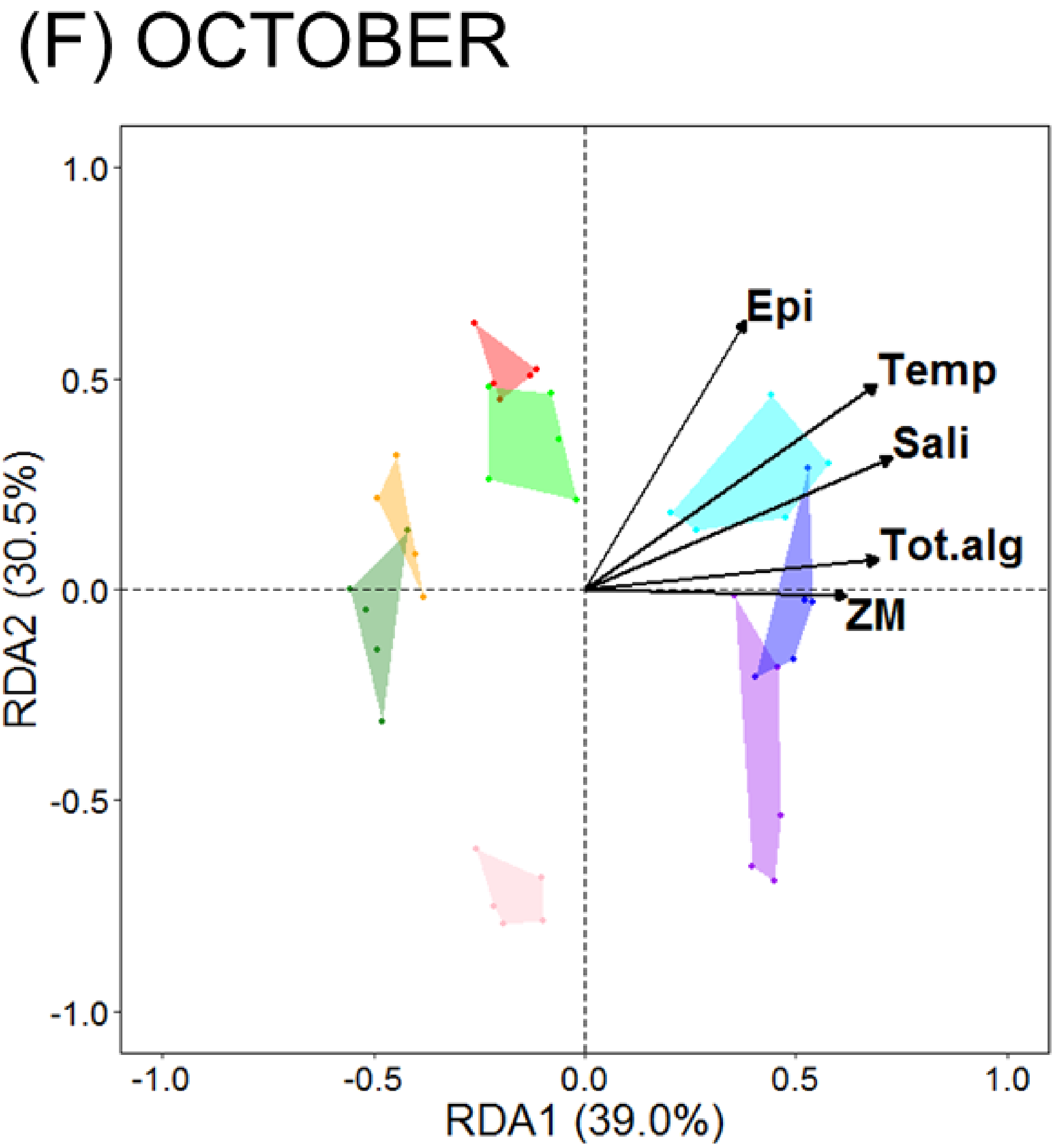
(C) JULY

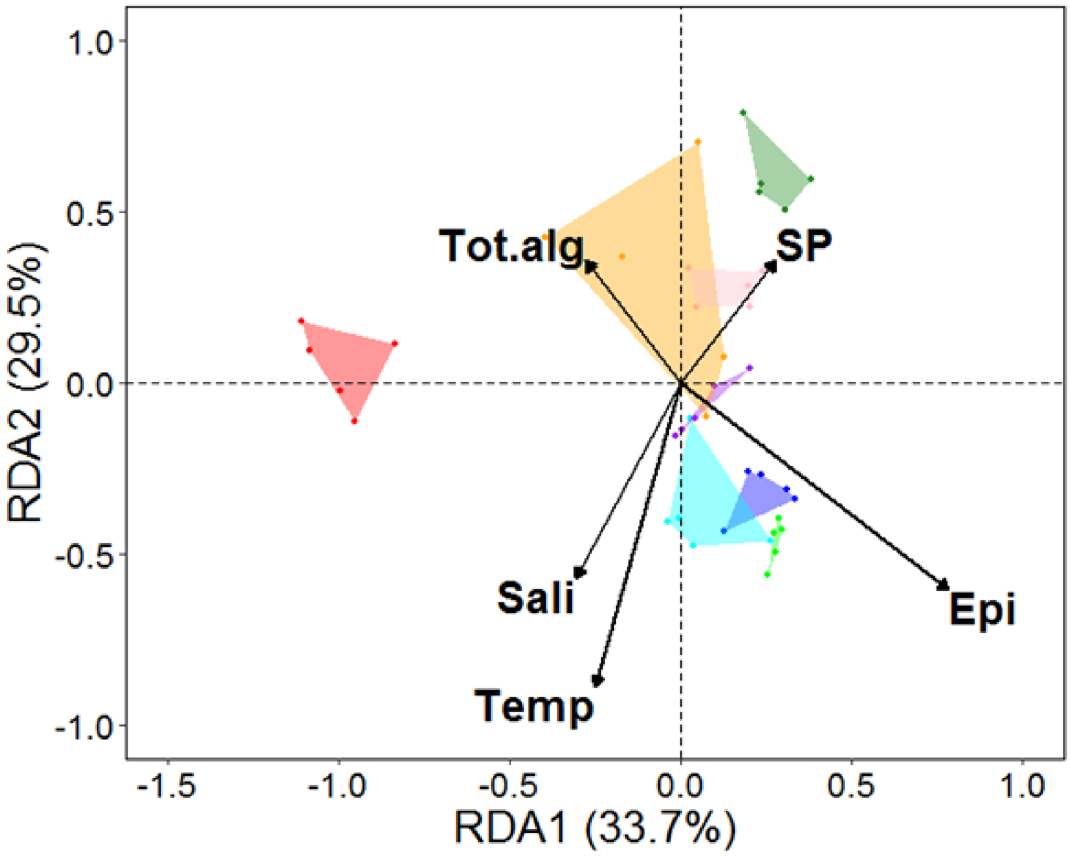

RDA2 (33.1%)
RDA1 (36.5%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Epi
Sali
Tot.alg
SP
Temp

(D) AUGUST


Temp
SP
Sali
Tot.alg
ZM
RDA1 (41.7%)
RDA2 (32.3%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0


(E) SEPTEMBER
Temp
SP
Tot.alg
Epi
Sali
ZM
RDA2 (24.7%)
RDA1 (43.7%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0


(F) OCTOBER
Epi
Temp
Sali
Tot.alg
ZM
RDA2 (30.5%)
1.0
0.5
0.0
-0.5
-1.0
RDA1 (39.0%)
-1.0
-0.5
0.0
0.5
1.0



(G) NOVEMBER

Fig. 3-4. RDA biplots of significant factors ($p < 0.05$) explaining the variation in epifaunal community composition for each month between May (A) and (G). Convex hulls in each biplot (A~G) indicate the community composition within each station. Percentages of the variation explained by first two axes of RDAs were given in each axis. See Fig. 3-3 for the color for each station.

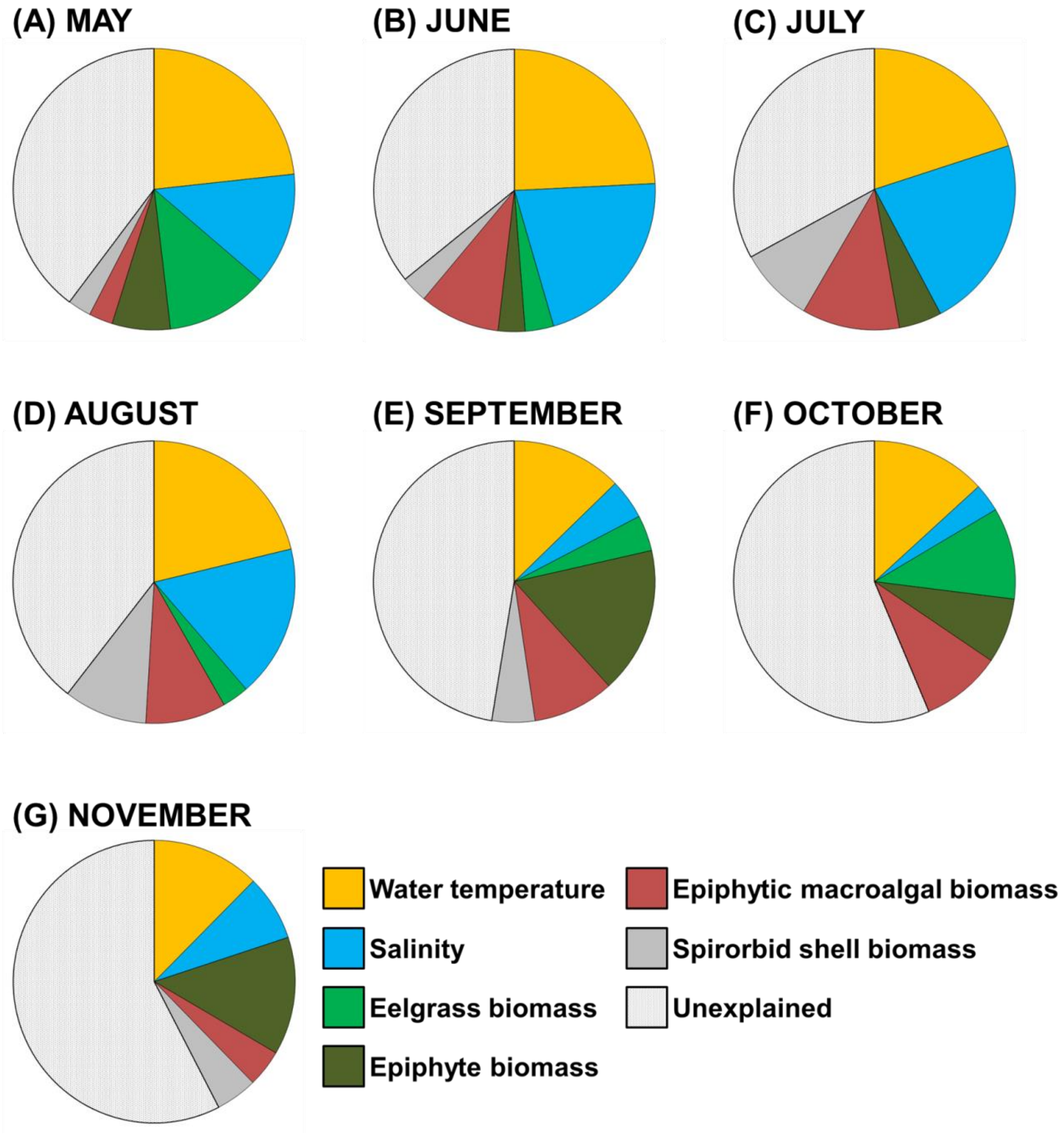


Fig. 3-5. The variation in the epifaunal compositions explained by the six abiotic/biotic factors in RDAs for each month from May (A) to November (G).

Tables

Table 3-1. Environmental conditions at eight stations in the Akkeshi-ko estuary and Akkeshi Bay from May to November.

Factors	**Month**	**Mean value (SD)**								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
Water temperature (°C)	MAY	11.71	13.61	13.78	14.16	14.53	13.75	12.35	13.59	13.43
	JUN	16.39	20.50	20.45	21.13	16.69	19.61	14.24	14.65	17.96
	JUL	20.45	23.01	22.57	22.70	17.91	20.06	17.70	13.58	19.75
	AUG	21.72	24.09	23.84	23.86	21.89	22.70	18.76	18.64	21.94
	SEP	17.43	18.28	18.56	18.31	17.33	17.48	16.29	16.84	17.57
	OCT	12.34	11.56	11.36	10.47	12.25	11.88	13.02	12.18	11.88
	NOV	7.53	5.92	5.78	6.01	7.60	7.28	8.16	7.37	6.96
Salinity	MAY	24.65	24.96	26.24	27.82	27.66	27.38	26.15	31.24	27.01
	JUN	22.59	26.42	26.90	27.33	29.42	28.27	28.42	30.73	27.51
	JUL	23.52	24.63	27.01	27.55	27.83	28.53	27.93	32.22	27.40
	AUG	21.25	23.59	26.00	26.23	26.81	26.71	29.85	31.95	26.55
	SEP	25.04	16.88	18.02	21.72	26.31	25.72	17.31	31.40	22.80
	OCT	26.70	25.17	21.87	21.29	28.76	26.28	29.03	32.78	26.49
	NOV	28.70	22.16	21.72	24.23	26.34	26.73	28.13	32.15	26.27

Table 3-1. (Continued 1)

Factors	Month	Mean value (SD)								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
Eelgrass biomass (g m^{-2})	MAY	22.71	118.13	130.32	125.43	144.06	186.85	186.62	41.29	119.43
	JUN	37.03	126.59	153.69	177.60	210.58	259.35	295.09	163.13	177.88
	JUL	224.31	221.17	197.94	172.22	247.62	316.13	372.59	112.49	233.06
	AUG	140.32	150.88	104.22	240.76	324.94	241.39	267.75	344.18	226.80
	SEP	83.83	57.92	34.36	80.88	118.31	108.38	67.30	297.65	106.08
	OCT	57.75	19.93	31.98	69.68	86.76	109.89	65.13	172.17	76.66
	NOV	27.31	12.20	35.31	54.96	58.30	94.92	86.04	139.16	63.52
Epiphyte biomass (g m^{-2})	MAY	49.96	103.67	83.69	111.90	31.64	50.05	100.76	15.21	68.36
	JUN	183.68	255.14	103.49	63.86	311.93	273.80	220.07	131.21	192.90
	JUL	236.87	84.73	288.26	57.05	114.77	333.64	169.59	128.18	176.64
	AUG	408.35	74.18	99.82	145.91	246.92	111.81	260.59	196.79	193.05
	SEP	170.86	88.23	29.82	259.83	301.12	123.34	282.11	110.49	170.72
	OCT	90.42	59.65	17.21	166.51	294.72	220.20	169.22	155.71	146.71
	NOV	12.83	23.93	41.14	39.58	244.38	55.02	92.28	68.94	72.26

Table 3-1. (Continued 2)

Factors	**Month**	**Mean value (SD)**								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
Macroalgal biomass (g m^{-2})	MAY	5.50	0.02	2.40	0.00	1.64	0.00	10.22	0.77	2.57
	JUN	9.44	1.39	0.54	0.00	14.49	5.37	45.50	0.00	9.59
	JUL	0.51	51.96	0.89	1.89	5.33	42.00	8.86	0.00	13.93
	AUG	0.43	13.34	0.46	0.00	49.65	9.18	8.14	69.82	18.88
	SEP	0.00	0.00	0.00	2.63	0.00	0.00	0.00	1.59	0.53
	OCT	0.00	2.73	0.00	0.00	0.00	26.87	4.62	1.05	4.41
	NOV	0.32	3.08	0.06	0.01	0.00	0.00	0.00	0.00	0.43
Spirorbid biomass (g m^{-2})	MAY	0.000	0.008	0.001	0.000	0.000	0.001	0.003	0.009	0.003
	JUN	0.000	2.619	0.000	0.000	0.003	0.020	0.032	0.019	0.337
	JUL	0.076	22.019	0.025	0.031	0.046	0.464	0.386	0.505	2.944
	AUG	8.149	29.575	0.087	0.619	1.044	0.209	0.553	4.851	5.636
	SEP	0.942	0.376	0.006	1.388	0.094	0.026	0.008	1.991	0.604
	OCT	0.016	0.029	0.000	1.403	0.025	0.030	0.026	0.572	0.263
	NOV	0.000	0.004	0.003	0.423	0.024	0.032	0.164	0.181	0.104

Table 3-2. Abundances of 113 epifaunal species in the Akkeshi-ko estuary and Akkeshi Bay. The abundance proportion and the rank in this table are based on the total abundance across all sampling stations and months.

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Gammarid amphipoda											
Ampithoe lacertosa	Amp.lac	35	315	2154	4778	993	427	155	**8857**	10.896	2
Aoroides curvipes	Aor.cur	31	288	147	486	46	85	151	**1234**	1.518	14
Atylus sp1.	Atylus1	—	—	—	—	—	—	3	**3**	0.004	80
Atylus sp2.	Atylus2	22	2	—	—	1	—	1	**26**	0.032	49
Byblis japonicus	Byb.jap	1	—	—	—	—	1	1	**3**	0.004	80
Carinonajna sp.	Carino	—	—	1	—	—	—	2	**3**	0.004	80
Cerapus nudus	Cer.nud	—	1	—	—	10	—	50	**61**	0.075	42
Dexaminidae sp.	Dexam	—	1	—	—	—	—	—	**1**	0.001	95
Dogielinotidae sp.	Dogie	—	1	—	—	—	—	—	**1**	0.001	95
Eogammarus spp.	Eogam	19	193	49	—	—	4	15	**280**	0.344	28
Grandidierella japonica	Gra.jap	—	—	—	13	—	1	—	**14**	0.017	62
Harpiniopsis sp.	Harpini	—	—	1	4	1	2	1	**9**	0.011	70
Haustorioides sp.	Haust	—	—	—	—	—	1	—	**1**	0.001	95

Table 3-2. (Continued 1)

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Hyalidae sp.	Hyali	1	—	—	—	—	—	—	**1**	0.001	95
Ischyroceridae sp.	Ischy	4	99	80	31	17	32	35	**298**	0.367	27
Jassa morinoi	Jas.mor	1	—	1	—	—	—	—	**2**	0.002	85
Jassa sp.	Jassa	3	36	18	1	1	13	24	**96**	0.118	36
Lysianassidae sp1.	Lysiana1	—	—	—	—	—	—	1	**1**	0.001	95
Lysianassidae sp2.	Lysiana2	—	—	1	—	2	—	6	**9**	0.011	70
Lysianassidae sp3.	Lysiana3	2	—	—	—	—	—	—	**2**	0.002	85
Lysianassidae sp4.	Lysiana4	1	—	—	—	—	—	—	**1**	0.001	95
Lysianassidae sp5.	Lysiana5	—	—	1	—	—	—	—	**1**	0.001	95
Monocorophium spp.	Mon.cor	140	664	8927	8033	1643	364	683	**20454**	25.162	1
Orchomene sp.	Orcho	7	6	—	7	4	—	6	**30**	0.037	46
Pyatakovestia pyatakovi	Pac.pya	—	—	1	—	—	—	—	**1**	0.001	95
Paradexamine sp.	Parad	—	—	—	5	1	—	—	**6**	0.007	73
Paragrubia sp.	Parag	—	1	—	—	—	—	—	**1**	0.001	95
Photis sp1.	Photis1	2	—	—	—	—	—	18	**20**	0.025	53
Photis sp2.	Photis2	1	—	—	1	5	—	24	**31**	0.038	45
Photis sp3.	Photis3	1	—	6	16	24	12	14	**73**	0.090	39
Pleustes sp.	Pleus	1	—	—	—	—	—	—	**1**	0.001	95

Table 3-2. (Continued 2)

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Polycheria sp.	Polyche	—	—	1	—	—	—	1	**2**	0.002	85
Pontogeneia rostrata	Pon.ros	383	1321	1119	1397	268	373	170	**5031**	6.189	6
Pontogeneia sp.	Ponto	1	—	—	—	—	—	—	**1**	0.001	95
Stenothoe sp.	Steno	93	169	4	—	1	12	26	**305**	0.375	26
Sunamphitoe sp.	Sunam	—	—	—	2	—	—	—	**2**	0.002	85
Urothoe sp1.	Urothoe1	—	1	—	—	—	—	—	**1**	0.001	95
Urothoe sp2.	Urothoe2	1	1	—	—	—	—	—	**2**	0.002	85
Caprellid amphipoda											
Caprella acanthogaster	Cap.aca	2	71	308	1242	5	—	—	**1628**	2.003	12
Caprella aino	Cap.ain	—	3	35	163	5	3	—	**209**	0.257	31
Caprella algaceus	Cap.alg	16	30	1516	502	1	16	3	**2084**	2.564	11
Caprella bispinosa	Cap.bis	—	—	3	62	3	3	—	**71**	0.087	40
Caprella danilevskii	Cap.dan	—	—	4	28	3	1	—	**36**	0.044	44
Caprella kyoyeri	Cap.kro	2	138	53	550	39	13	9	**804**	0.989	18
Caprella laeviuscula	Cap.lae	—	3	—	—	—	1	—	**4**	0.005	75
Caprella mutica	Cap.mut	1	—	1	4	3	—	1	**10**	0.012	68
Caprella obtusifrons	Cap.obt	—	—	—	1	—	—	—	**1**	0.001	95

Table 3-2. (Continued 3)

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Caprella polyacantha	Cap.pol	—	—	—	—	—	—	4	**4**	0.005	75
Caprella scaura	Cap.sca	11	156	276	188	94	119	78	**922**	1.134	16
Caprella septentrionalis	Cap.sep	—	—	—	7	1	—	—	**8**	0.010	72
Caprella tsugarensis	Cap.tsu	8	32	203	189	6	17	8	**463**	0.570	23
Caprella venusta	Cap.ven	18	57	21	29	62	27	—	**214**	0.263	30
Isopoda											
Cymodoce japonica	Cym.jap	2	—	12	41	19	12	3	**89**	0.109	37
Gnorimosphaeroma rayi	Gno.ray	3	3	69	2	19	8	12	**116**	0.143	35
Idotea ochotensis	Ido.och	9	3	3	6	13	16	15	**65**	0.080	41
Munna sp.	Munna	5	41	163	576	25	16	3	**829**	1.020	17
Paranthura japonica	Par.jap	8	9	30	28	21	11	19	**126**	0.155	34
Pleurogonium hispidum	Ple.his	1	1	1	—	3	—	11	**17**	0.021	56
Copepoda											
Calanoida sp.	Calano	46	4	42	26	6	3	2	**129**	0.159	33
Caligidae sp.	Caligia	1	—	—	—	1	—	—	**2**	0.002	85
Dactylopusia sp.	Dacty	69	6	—	2	—	34	233	**344**	0.423	24
Ectinosomatidae sp.	Ectino	4	7	—	4	2	—	6	**23**	0.028	50

Table 3-2. (Continued 4)

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Harpacticidae spp.	Harpac	248	140	110	15	1	3	20	**537**	0.661	21
Kushia zosteraphila	Kus.zos	571	576	451	1779	447	579	897	**5300**	6.520	5
Laophontidae sp.	Laopho	39	114	102	99	49	4	86	**493**	0.606	22
Metidae sp.	Metidae	1	—	—	—	—	—	—	**1**	0.001	95
Miraciidae spp.	Miracii	1530	940	220	473	68	53	362	**3646**	4.485	9
Thalestridae spp.	Thales	450	33	523	375	56	22	134	**1593**	1.960	13
Tanaidacea											
Sinelobus sp.	Sinelo	—	1	—	—	—	—	—	**1**	0.001	95
Cumacea											
Diastylis sp.	Diast	1	1	1	—	—	2	7	**12**	0.015	63
Leptostraca											
Nebalia sp.	Nebal	—	1	—	2	—	1	1	**5**	0.006	74
Ostracoda											
Cytheroidea spp.	Cythe	54	11	4528	—	2	—	726	**5321**	6.546	4
Vargula hilgendorfii	Var.hil	1	—	—	1	3	2	5	**12**	0.015	63
Acari											
Halacaridae sp.	Halacari	57	34	1850	63	62	2	75	**2143**	2.636	10

Table 3-2. (Continued 5)

Species	Abbr.	Total abundance (inds.)								Prop. (%)	Rank
		MAY	JUN	JUL	AUG	SEP	OCT	NOV	ALL		
Pycnogonida											
Pycnogonida sp.	Pycno	1	1	1	7	4	1	5	**20**	0.025	53
Gastropoda											
Alvania concinna	Alv.con	3	—	—	—	2	3	8	**16**	0.020	57
Ansola angustata	Ans.ang	29	26	1808	737	604	509	157	**3870**	4.761	7
Assiminea sp.	Assim	—	—	—	—	—	—	—	**1**	0.001	95
Batillaria attramentaria	Bat.att	—	1	6	2	—	—	—	**10**	0.012	68
Buccinum sp.	Bucci	—	1	—	—	—	—	—	**1**	0.001	95
Ercolania boodleae	Erc.boo	4	—	—	—	—	—	—	**4**	0.005	75
Lacuna spp.	Lacuna	62	105	1468	1003	214	666	336	**3854**	4.741	8
Lacuna uchidai	Lac.uch	—	5	11	6	—	—	—	**22**	0.027	51
Lirularia iridescens	Lir.iri	—	—	—	1	—	—	—	**1**	0.001	95
Lucidestea sp.	Lucid	—	—	1	—	—	—	1	**2**	0.002	85
Margarites pilsbryi	Mar.pil	1	—	—	1	—	—	—	**2**	0.002	85
Mitrella sp.	Mitrel	2	—	2	2	2	4	4	**16**	0.020	57
Nassarius fraterculus	Nas.fra	4	3	4	3	—	4	—	**18**	0.022	55
Retusa sp.	Retusa	—	5	—	—	2	3	1	**11**	0.014	66
Siphonacmea oblongata	Sip.obl	25	15	61	96	13	14	7	**231**	0.284	29

Table 3-2. (Continued 6)

Species	Abbr.	Total abundance (inds.)								Prop. (%)	Rank
		MAY	JUN	JUL	AUG	SEP	OCT	NOV	ALL		
Bivalvia											
Arcuatula senhousia	Arc.sen	7	4	—	1	—	2	—	**15**	0.018	60
Myidae sp.	Myidae	1	3	1	—	—	1	5	**11**	0.014	66
Mytilus sp.	Mytilus	3	8	1	—	5	5	22	**44**	0.054	43
Veneridae sp1.	Veneri1	1	4	3	—	—	2	12	**22**	0.027	51
Veneridae sp2.	Veneri2	—	—	—	—	—	2	2	**4**	0.005	75
Veneridae sp3.	Veneri3	1	1	5	1	2	4	2	**16**	0.020	57
Veneridae sp4.	Veneri4	2	—	1	—	—	—	—	**3**	0.004	80
Veneridae sp5.	Veneri5	—	3	—	—	—	—	—	**3**	0.004	80
Veneridae sp6.	Veneri6	—	—	—	—	2	—	—	**2**	0.002	85
Polychaeta											
Echiurus sp.	Echiur	7	—	—	—	4	—	1	**12**	0.015	63
Exogone naidina	Exo.nai	854	2093	1073	422	84	128	1357	**6011**	7.395	3
Harmothoe imbricata	Har.imb	—	10	2	7	—	4	5	**28**	0.034	47
Lumbrineris japonica	Lum.jap	7	4	1	2	—	—	14	**28**	0.034	47
Nereis sp.	Nereis	18	16	76	103	46	49	34	**342**	0.421	25
Sphaerodoridae sp.	Sphaero	1	—	—	—	—	1	2	**4**	0.005	75
Syllis sp.	Syllis	117	55	59	124	60	67	97	**579**	0.712	19

Table 3-2. (Continued 7)

Species	**Abbr.**	**Total abundance (inds.)**								**Prop. (%)**	**Rank**
		MAY	**JUN**	**JUL**	**AUG**	**SEP**	**OCT**	**NOV**	**ALL**		
Platyhelminthes											
Polycladida sp.	Polycla	2	—	2	—	11	—	—	**15**	0.018	60
Rhabdocoela sp1.	Rhabd1	144	8	497	390	46	29	33	**1147**	1.411	15
Rhabdocoela sp2.	Rhabd2	128	24	1	—	—	—	9	**162**	0.199	32
Rhabdocoela sp3.	Rhabd3	1	—	—	—	—	—	—	**1**	0.001	95
Nemertea											
Nemertea spp.	Nemer	200	64	46	29	24	18	169	**550**	0.677	20
Hirudinea											
Ostreobdella kakibir	Ost.kak	—	20	17	12	11	16	5	**81**	0.100	38
Incecta											
Chironomidae sp. (larva)	Chirno	2	—	—	—	—	—	—	**2**	0.002	85

Table 3-3. Results of ANOSIM to test for spatial variation in epifaunal community composition among eight stations in the Akkeshi-ko estuary and Akkeshi Bay from May to November.

Month	**Global R**	**P**
MAY	0.799	0.001
JUN	0.865	0.001
JUL	0.970	0.001
AUG	0.931	0.001
SEP	0.921	0.001
OCT	0.870	0.001
NOV	0.713	0.001

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Chapter Ⅳ

Seasonal variation in the direct and indirect linkages between environmental factors, biotic factors and epifaunal abundance in eelgrass beds

4.1 Introduction

Understanding the spatial and temporal variation in complex networks of biological communities in natural ecosystems is a great challenge of ecology. To do so, the evaluation of relationships inherent in the networks is imperative (Paine, 1992). Spatial and temporal shift in the interactions is ubiquitous in most natural ecosystems (Abrams, 2001), and arise from changes in environmental conditions and community component species (e.g. density, functional traits, behavior), resulting in the variation in the intensity and direction of the interactions at various spatial and temporal scales (Menge *et al*., 1994; Abrams *et al*., 1996; Berlow *et al*., 1999; Navarrete & Berlow, 2006). Finding out the variation pattern and processes on multiple spatial and temporal scales, therefore, can provide a better understanding of natural community dynamics.

Trophic/non-trophic interactions within communities have been investigated by experimental and observational approaches. One of the mainstream methods in the observational approach has been that exploring the causal relationships by testing correlation between multiple abiotic and biotic variables with response variables such as consumer abundance, biomass and diversity by multivariate statistics (e.g. Ter Braak, 1986; Ter Braak & Prentice, 1988; Palmer, 1993; Legendre & Gallagher, 2001; Cottenie, 2005; Peres-Neto *et al*., 2006; Hansen *et al*., 2008; Gullström *et al*., 2012; Barnes *et al*., 2016). For population/community of consumers, the effects of abiotic factors and plant traits are essential bottom-up factors affecting their variability (e.g. Hunter & Price, 1992; Power, 1992; Polis & Strong, 1996; Siemann, 1998; Loreau *et al*., 2001; Werner & Peacor, 2003; Wardle *et al*., 2004; Brönmark & Hansson 2005; Shurin *et al*., 2006; Bukovinszky *et al*., 2008; Stam *et al*., 2014). However, the indirect effects of abiotic factors via plant traits cannot be estimated by methods in which plant traits are treated together with abiotic factors as independent variables of the same level (such as in multi-regression analyses). To this problem, structural equation modeling (SEM), that is a path analyzing method, provides the solution by assessing indirect or cascading effects, and is recently increasing in the study of ecology and evolution (e.g. Grace, 2006; Grace *et al*., 2007; Lefcheck, 2015). Especially, piecewise SEM, where the component models (a set of linear equations) are solved individually (*local estimation*), allows to handle various distributions and sampling designs even in the case with small sample size (Lefcheck, 2015). Therefore, the path analysis is expected to promote a more detailed understanding of the process of community dynamics and formation based on observational data collected in empirical biological communities.

Small invertebrates (e.g. arthropods such as insects and crustaceans, mollusks, annelids), that mediate bottom-up and top-down cascade of biological communities, are indispensable componets in a wide variety of terrestrial and aquatic ecosystems

(Shurin *et al*., 2002; Werner & Peacor, 2003; Duffy *et al*., 2007, 2015). The effect of primary producers on production and consumption of those invertebrates via herbivory is higher in aquatic than terrestrial systems (Cyr & Pace, 1993; Polis, 1999; Shurin *et al*., 2002). Marine benthic communities are characterized by the presence of multiple primary producers, such as phytoplankton, micro- and macro-algae, seagrass, which vary greatly in their size and spatial and temporal variability (Cebrian, 1999; Shurin *et al*., 2002). Temporal shift in the interaction between plants and epiphytic animals can be expected to be great in such marine habitats because different types of plants exhibit different patterns of seasonal abundance (Kentula & DeWitt, 2003; Hasegawa *et al*., 2007; Mabrouk *et al*., 2015).

Here, I assessed a seasonal shift in the network structure of the interactions among mobile and sessile invertebrate assemblages, various types of primary producers (eelgrass, epiphytic micro- and macro-algae) and abiotic factors (water temperature, salinity and dissolved inorganic nitrogen) in eelgrass beds in Akkeshi (eastern Hokkaido, Japan) using exploratory path analysis based on an integrative evaluation approach (piecewise SEM). In Akkeshi, eelgrass beds develop along steep gradients of various physico-chemical factors (Iizumi *et al*., 1995; Yamada *et al*., 2007a, b). Eelgrass associated organisms (e.g. epiphytic algae, sessile invertebrates and mobile invertebrates) also show great spatial and seasonal variation in their community structure (Hamamoto & Mukai, 1999; Kasim & Mukai, 2006; Hasegawa *et al*., 2007; Yamada *et al*., 2007a, b). By comparing the selected network structures among successive months, I discuss how direct and indirect interactions among biotic/abiotic factors changes to explain variability in the epifaunal community abundance (hereafter ECA). I focused on the bottom-up control of abiotic factors on ECA via primary producers and sessile animals, because the bottom-up control is dominant process in eelgrass bed of Akkeshi (Momota, 2013; Duffy *et al*., 2015).

4.2. Materials and methods

4.2.1 Study area

I carried out monthly field survey from May to November, 2013 (a total of seven occasions) in eelgrass meadows at eight study stations in Akkeshi-ko estuary and Akkeshi Bay, located in northeastern Hokkaido, Japan (Fig. 4-1). Six stations were established in Akkeshi-ko estuary (BK: river mouth of the Bekanbeushi River, HN: Horonitai, TB: Toubai, SL: the southern lakeside, CL: the central lake and CK: Chikarakotan) and two stations in Akkeshi Bay (SR: Shinryu and AK: Aininkappu). I did not carry out surveys between December and April because most of these stations were covered with ice. The biomass and productivity of eelgrass and other plants is known to be low during winter (Watanabe *et al*., 2005; Hasegawa *et al*., 2007).

Akkeshi-ko estuary and Akkeshi Bay are connected to each other via a narrow channel (width: approximately 500 m, depth: approximately 10 m; Fig. 4-1). Freshwater inflow from Bekanbeushi River to Akkeshi-ko estuary, accounting for 98.8% of the total inflow volume, together with tidal seawater input from Akkeshi Bay generate spatio-temporally different environmental gradients (e.g. water temperature, salinity, nitrogen and phosphate) across our study stations (Iizumi *et al*., 1995; Yamada *et al*., 2007a, b).

Akkeshi-ko estuary is a shallow (less than 2 m deep) and semi-closed brackish water with a muddy bottom. Two eelgrass species (*Zostera marina* and *Z. japonica*) occur almost all the part of the estuary except for aquaculture farms of the clam *Venerupis philippinarum* in the intertidal sand flats near the channel (Kashim & Mukai, 2006; Hasegawa *et al*., 2007; Yamada *et al*., 2007a, b). *Zostera marina* is dominant over the estuary and covers a large part of the subtidal area, whereas *Z. japonica* appears in the intertidal zone. Another vascular plant *Rupia maritima*, which prefers low-salinity area, is often found at the stations near Bekanbeushi River, such as BK and HN (Nakaoka *et al*., 2013).

In Akkeshi Bay (open to the Pacific Ocean at the south end), *Z. marina* is present from the intertidal zone to the subtidal zone at depths shallower than 2 m. In the deeper water (~ 5 m), another eelgrass species *Zostera asiatica* replaces *Z. marina* (Watanabe *et al*., 2005). SR is adjacent to a muddy beach, whereas AK to a sandy beach and a rocky shore. In addition to two *Zostera* species, surfgrass *Phyllospadix iwatensis* also occurs with kelps and sargassums at AK where rocky bottom are mixed with sandy bottom.

4.2.2. Field and laboratory procedures

In this study, I collected data on three abiotic factors (water temperature [°C], salinity and total dissolved inorganic nitrogen content [hereafter TDN; μM]) and on four biotic factors (eelgrass biomass [dry weight g m^{-2}; DW g m^{-2}], epiphyte biomass [microalgae with sediments in this study, DW g m^{-2}], epiphytic macroalgal biomass [DW g m^{-2}], spirorbid polychaete biomass [shell biomass DW g m^{-2}] and abundance of epifaunal mobile invertebrates (epifaunal community abundance, or ECA, [number of individuals m^{-2}]). I defined the combined total content of nitrate, nitrite and ammonium as TDN. Here, microalgae are defined as microscopic algae (mostly unicellular diatoms and dinoflagellates) whereas macroalgae are multicellular green, red and brown algae which size is more than a few millimeters. I conducted the field surveys at a time when tidal current was slow around mid-tide level in order to reduce biases in measurements associated with tidal cycles.

I measured water temperature and salinity using a memory sensor (AAQ-1183; JFE Advantech Co. Ltd., Japan) at each station before the sample collection. To accurately reflect the environment inside the eelgrass beds, we placed the sensor approximately 50 cm above the bottom within eelgrass meadow. For TDN, we collected water with plastic bottles approximately 50 cm above the bottom within eelgrass meadow three times at each station. After the sampling, the water samples were kept in a cool box with ice, and filtered through 0.45 μm Millex syringe filters (Merck Millipore, Germany) in the laboratory. The filtered water samples were stored in a freezer at -30 °C until the analysis using an auto-analyzer (TRAACS 2000 system, Bran and Luebbe, Norderstedt, Germany).

I collected epifaunal mobile invertebrates and spirorbid polychaetes with above-ground eelgrass shoots using a 0.1 mm mesh bag within a range of the mouth area of the mesh bag (314 cm^2). Five replicate samples were collected at each station. After collection, the number of eelgrass shoot was counted to determine the shoot density per unit area. Later in the laboratory, epifaunal mobile invertebrates were separated from the eelgrass and other epibiotic organisms by scraping with a glass slide and counted after extraction using a 500 μm sieve and fixation with 70% ethanol. Eelgrass and spirorbid worms were dried at 60 °C for 4 days and then weighed.

Five replicate samples of epiphytic algae (i.e., micro- and macro-algae) were taken together with one above-ground eelgrass shoot using a plastic zip bag. After transporting to the laboratory in a cool box, these algae were immediately scraped from the eelgrass blades using a glass slide and separated from other organisms. To obtain homogeneous subsamples for epiphyte (microalgal) biomass from each sample, epiphytic microalgae were simultaneously filtered twice per sample using glass fiber filters (Whatman GF/F filter φ 47 mm; Whatman International Ltd., Maidstone, UK). I separated macroalgae into four types (branched red algae, filamentous green algae, membranous green algae and membranous brown algae). Filters for epiphyte biomass and separated macroalgae were dried and weighed as with eelgrass and spirorbid worms collected using mesh bag.

4.2.3. Statistical analyses

To describe the direct and indirect effects of water temperature, salinity, total dissolved inorganic nitrogen, eelgrass biomass, epibiont biomasses (epiphyte [microalge], epiphytic macroalgae and spirorbid polychaetes) on ECA for each month, I constructed a piecewise structural equation model (piecewise SEM), which combines information from multiple separate linear models (component models) into a single causal network (Shipley, 2009; Lefcheck, 2015). I assumed causal relationships for each variable pair in SEM (Fig. 4-2). To keep the number of explainable variables minimum, I only use TDN as an indicator of nutrient for eelgrass and algae because phosphate concentration in the water column is less variable than nitrate and ammonium within the Akkeshi-ko estuary (Iizumi *et al.*, 1995). Because our component models (linear mixed models: LMM) incorporated the variation of station as a random effect and because sample size was small, I fitted a piecewise SEM which is more powerful and flexible than traditional variance-covariance based SEM (Shipley, 2013). Each component model was fit using restricted maximum likelihood with a Gaussian distribution. I selected the best fitting SEMs by the corrected Akaike information criterion for small sample size (AIC_c) with Shipley's D-separation (D-sep) test, that yields Chi-squared distributed Fisher's C statistic (Shipley, 2009, 2013). Eventually, the best SEM with the lowest AIC_c value was selected from candidate models that passed D-sep test of adequate fit ($P > 0.05$) for each month.

Because our primary interest was the influence of abiotic/biotic factors on ECA, I found the best SEM meeting the three conditions as follows: (1) the variation in ECA was explained as far as possible, (2) passing D-sep test and (3) showing the lowest AIC_c value under the condition that previous two conditions already had been met. Before fitting SEMs, I selected influential factors from the candidate factors, which have a direct causality with ECA (except for TDN) by fitting LMM (i.e. component model of ECA). Then, I also fit LMMs for selected mediators (i.e. eelgrass and epibionts) to find the important paths from abiotic factors including TDN and to optimize SEM fit. I basically fit SEMs keeping the explanatory power for the variation in ECA by preferentially leaving the influential environmental factors.

Once the best SEMs were selected, I obtained coefficients standardized to the relevant ranges of the component variables to compare the relative importance of its predictor variables according to recommendation in Grace (2006). Moreover, I calculated the marginal R^2 value for endogenous variables using an approach described by Nakagawa and Schielzeth (2013).

To facilitate model fitting, all variables were $\log_{10}$ transformed. Because ECA across stations varied up to three orders of magnitude, I transformed them to converge on a solution of component model through likelihood algorithms, although the fitting criterion is scale-free (Bollen, 1989; see also Byrnes *et al*., 2011). Also, I tested multicollinearity among abiotic/biotic predictors in LMMs using the variance inflation factor (VIF) analysis based on the *vif.mer* function (Frank, https://raw.githubusercontent.com/aufrank/R-hacks/master/mer-utils.R). If VIF > 5, the appropriate variable was cut off before fitting models. Moreover, I calculated Pearson's correlation coefficients for exogenous abiotic variables which are independent each other in SEM, and cut off the appropriate variable given that the absolute value of the coefficient (r) was greater than 0.7 (Dormann *et al*., 2013). As a result from multicollinearity tests based on VIF and Pearson's correlation coefficients, I cut off TDN in May (correlated with water temperature; Pearson's r = -0.90, P < 0.01), water temperature in July (with salinity; r = -0.73, P < 0.01), and salinity in August (with water temperature; r = -0.66, P < 0.01 but cut off by VIF), October (with water temperature; r = 0.81, P < 0.01) and November (with water temperature; r = 0.83, P < 0.01). I used the *piecewiseSEM* package for piecewise SEM fit (Lefcheck, 2015), and the *lme* function in the *nlme* package for fitting of component models. In selecting the best component models for endogenous variables, I obtained AIC_c based on the maximum likelihood to compare LMMs because the restricted maximum likelihood (REML) is inappropriate in the case when the fixed structure is different between the candidate models (Zuur *et al*., 2009). I compared the AIC_c using the *AICctab* function in the *bbmle* package (Bolker & R Core Team, 2013). All analyses were performed using R software version 3.1.3 (www.r-project.org).

4.3. Results

4.3.1. Temporal variation in abiotic/biotic factors and epifaunal community

Water temper increased from May to August and decreased after August (Fig. 4-3A; Table 4-1). The gradient (i.e. spatial variation among stations) was steep from June to August, whereas small in the other months. Salinity did not show clear seasonal variation and mean salinity was most stable except in September when low salinity was observed in most stations (Fig. 4-3B; Table 4-1). Total dissolved inorganic nitrogen (TDN) increased steadily from July to November with a large among-site variation in August and September (Fig. 4-3C; Table 4-1).

Above-ground eelgrass biomass increased from spring to early summer and was the highest in July and August (Fig. 4-3D; Table 4-1). It decreased from August to November. Among-site variation was large in all the months except in October and November.

Epiphyte biomass increased from May to June, and then gradually decreased (Fig. 4-3E; Table 4-1). The variation was the lowest in May, but remained high from June to November.

Epiphytic macroalgal biomass increased from May to August, and suddenly dropped in September and remained low in October and November (Fig. 4-3F; Table 4-1). The spatial variation was relatively high from June to August (Fig. 4-3F).

Spirorbid polychaete biomass and their spatial variation were higher in July and August than other months (Fig. 4-3G).

ECA tended to increase from May to July, reach the maximum during July and August, and then start decreasing after August (Fig. 4-3H).

The composition of epiphytic macroalgae changed greatly among months. Various types of epiphytic macroalgae occurred in May and June, and then branched red algae dominated in and after July (Fig. 4-4). Branched red algae consisted of *Campylaephora* sp., *Chondria dasyphylla* and *Neosiphonia* sp., filamentous green algae of *Chaetomorpha crassa*, *Cladophora* spp., *Ectocarpus* sp. and *Spongomorpha* sp., and membranous brown algae of *Punctaria flaccida*. The species composition of membranous green algae showed seasonal turnover that *Kornmannia leptoderma* developed from May to June and then was replaced with *Ulva* spp. (e.g. *Ulva intestinalis* and *Ulva australis*) on and after August.

The contribution of component epifaunal groups was also different among months with the diversity tended to high in July, October and November. Gammarids dominated from early summer to fall. Copepods and polychaete worms were abundant in May, June, October and November. Gastropods gradually increased from July and reached a peak in October.

4.3.2. Structural equation models

The best SEM of each month explained more than 50% of the variation in epifaunal community abundance (ECA) except for August (45%) and September (47%) (Fig. 4-6). In contract, the SEM did not explain much of the spatial variation in the intermediate biotic variables (eelgrass, macroalgae, epiphytes and spirorbid polychages) based on the explainable variables, with marginal R^2 values were less than

50% (except for macroalgae in May and September) (Fig. 4-6).

A great monthly variation was found in the selected pathways of the best SEMs (Fig. 4-6). Three to five abiotic/biotic factors were selected as significant variables affecting ECA from May to September, whereas only two of them (water temperature and eelgrass biomass) were selected in October and November, making the network structure simpler during autumn when most biotic variables were decreasing in biomass/abundance.

For the direct effect of physical factors to ECA, water temperature was influential in May, June, October and November, whereas salinity was influential in July and September. The effect of water temperature on ECA was negative in May and June, but positive in October and November. The effect of salinity was negative in July, but positive in September (Fig. 4-6).

For the indirect effects of those physical factors on ECA, the driver-mediator pathways drastically changed among months (Fig. 4-6). Eelgrass biomass was selected as a significant intermediate variable with water temperature or TDN in May, August, October and November. Its effect on ECA was positive except in August.

The effects of epibionts (epiphyte, macroalgae and spirorbid polychaetes) were found in all the months except in October and November. Microalgae were affected by water temperature, eelgrass biomass and TND in May, August and September, respectively, and they influenced ECA positively in the former two months and negatively in September. Epiphyte biomass was affected by eelgrass biomass in August and by TDN in September, but not related to abiotic and/or eelgrass in June and July. In all the four months, it positively affected ECA. Spirorbid polychaetes were related to salinity in July and September, but in different directions. It was positively correlated to ECA in May, September, but negatively in June and July.

4.4. Discussion

Our results using a path analyzing approach demonstrated that over 50% of the variation in epifaunal community abundance (ECA), consisting of various invertebrate species in seagrass bed, could be explained for the most months by combinations of abiotic and biotic factors which affects eelgrass bed communities as bottom-up forces. I also found that the networks of direct and indirect effects on ECA greatly varied among months. More complex networks were observed in spring to summer when eelgrass communities are developing (with the increase in primary productivity), and in mature stages (when the primary productivity reaches maximum; Hasegawa *et al.*, 2007) than in autumn when the biomass and productivity of eelgrass are decreasing. The path analyzing approach was effective to identify how and when the abiotic and biotic factors were influential and to understand the relative importance of influence pathways.

The direct and indirect effects of abiotic factors were temporally different in terms of the direction and the importance (Fig. 4-6). The direct effects of water temperature or salinity on ECA were found in most months as confirmed in previous studies on invertebrate community of seagrass beds in estuaries (e.g. Montague & Ley, 1993; Edgar & Barrett, 2002; Yamada *et al.*, 2007b; Douglass *et al.*, 2010). However, our time-series observation also suggested that the effects of the two factors were influential on ECA through different pathways of interactions at different timings. Water temperature is a well-known driver of species distributions and interactions in aquatic systems beyond seagrass systems (Clarke & Gaston, 2006; Tittensor *et al*., 2010). In the present study, the direct effect of water temperature on ECA was negative in spring (May and June) but positive in autumn (October and November), indicating that the processes were different between the two phases. The negative effect in spring is likely related to the mass occurrence of cold-tolerant species in sites with lower temperature, such as copepods, such as Miraciidae spp. *Kushia zosteraphila* Thalestridae spp. and Harpacticoida spp. In contract, the positive correlation in autumn is considered to be observed because the range of water temperature dropped to the level which limits the activities of epifaunal species on eelgrass blades in some sites.

The effect of salinity was detected only in July and September. The less contribution to ECA in comparison with that of temperature is against our first expectation because many previous studies pointed out that salinity is a major driven factor causing variation in benthic abundance in estuarine systems, leading to lower species diversity and higher dominance by tolerant species (e.g. Remane & Schlieper, 1971; Montagna and Kalke, 1992; Mannino and Montagna, 1997; Ysebaert *et al*., 1998; 2003; Yamada *et al*., 2007b). The positive relationship between salinity and ECA in September may be ascribed to abnormal weather condition the estuarine experienced a few days before our sample collection. Precipitation of 201 mm rain was recorded over a day in a nearest weather station (Ohta, Akkeshi) nine days before our sampling whereas average rainfall of September is 184 mm; Japan Meteorological Agency; http:// http://www.jma.go.jp/jp/amedas/). This was reflected by the low average salinity compared to other months (Fig. 4-3B). The positive correlation of salinity with ECA may indicate that mobile epifauna may negatively affected by the disturbance associated with freshwater runoff as shown by very low ECA values at most stations near rivers (e.g. HN, TB, SL and SR).

Nutrient condition in water, represented by TDN was assumed to affect ECA indirectly through the changes in plant abundance/biomass. Here, its effect appeared to be poorly important for the variation in ECA although it was detected in association with plant factors only in August and September. In these months, among-site variation in TDN is great which is partially due to local eutrophication caused by flux from sediment and oyster aquaculture in some parts of the estuary (Hasegwa *et al.*, 2008; Abe, 2016). The negative correlation of TDN with eelgrass, macroalgae and microalgae suggests that the eutrophication limits the growth of these plants as demonstrated in other studies (McGlathery *et al.* 2007, Schmidt *et al.* 2012). Its effect indirectly affect the abundance of epifauna which dynamics are tightly associated with those of these plants.

Our results showed that eelgrass and epiphyte biomass, which had been considered as main factors responsible for the variation in epifaunal community in seagrass bed (e.g. Orth 1977; van Montfrans *et al.*, 1984; Irlandi & Peterson, 1991; Jernakoff *et al.*, 1996; Heck & Valentine, 2006), were not always influential throughout the study period (Fig. 4-6). The strong effect of eelgrass biomass was detected in May, August, October and November, but the effect was unexpectedly negative in August. The result of the positive effect can be interpreted by temporal limitation of habitat capacity due to seasonal prevalence of eelgrass. Because eelgrass biomass was low throughout our sampling sites in the spring and the fall (Fig. 4-3D), the importance appeared to increase. For the negative effect in August, presumably, excessive eelgrass indirectly influenced ECA by interfering with multiple physical and biological processes, such as water current and flux, detritus and drifting algae trapping, recruitment and predation intensity (Robbins & Bell, 1994; Boström & Bonsdorff, 2000; Lee *et al*., 2001; Hovel *et al*., 2002).

The positive effect of epiphyte biomass on ECA was detected between June and September, but not in May, October and November (Fig 4-6). This indicates the possibility that food availability can determine ECA during the productive term of the eelgrass beds (Fig. 3E). The mobile epifaunal invertebrates during the season largely comprised of gammarid and caprellid amphipods (Fig 4-5, 4-6) that mostly consume microalgae and/or detritus (e.g. Duffy, 1990, Cruz-Rivera & Hay, 2000; Guerra-García & Tierno de Figueroa, 2009; Best & Stachowicz, 2012, 2013; Martínez-Laiz & Guerra-García, 2015). The importance of epiphyte biomass also changes with temporal shift in compositions of epifaunal community. Gammarids and caprellids could be more strongly related to epiphyte biomass than the other microalgal grazers and detritivores, such as copepods and gastropods which were abundant in May, October or November.

Epibionts on eelgrass blades, such as macroalgae and sessile animals (such as spiorobid polychates and bryozoan) have been overlooked in most of the past studies on eelgrass community although some recent studies pointed out their importance (Duffy & Harvilicz, 2001; Duffy *et al.*, 2003; Lefcheck *et al.*, 2014; Long and Grosholz, 2015). In my study, both the epiphytic macroalgae and spirorbid polychaetes on eelgrass blades had significant contribution to explain the variability in ECA, especially from May to September (Fig. 4-6). Positive correlation between macroalgae and ECA in May and August is probably due to provision of additional habitats as major epifaunal animals collected here (such as small crustacean species including gammarids and copepods) were observed to dwell among the space provided by the macroalgae. Negative correlation in September is difficult to explain at the present stage although the biomass of macroalgae was very low in this sampling occasion probably due to massive runoff by the heavy rain as mentioned above. Any apparent correlation could occur with unexplored factors.

The effect of spirorbid polychaetes was significant from spring to summer (except for August), but the direction and intensity varied even among month. The indicates that the types of interactions between sessile polychates and mobile invertebrates may change among seasons with different abundance and life history stages of both functional groups. In May, for example, abundance of both sessile and mobile invertebrates were small and thus their spatial variation may be caused by the same process (such as temperature dependent colonization rate), which may have caused apparent positive correlation. The relationship then became negative as their abundance increased. One of the predominant spirorbid polychaetes *Neodexiospira brasiliensis* in Akkeshi recruits on seagrasses from June to July (Hamamoto *et al.*, 1996; Hamamoto & Mukai, 1999). They intensely compete for space on eelgrass blades with mobile epifaunal species during their recruitment period, which likely led to the negative effect.

4.5. Conclusions

In conclusion, this study showed that the importance of the direct and indirect factors on seagrass epifaunal abundance varies seasonally depending on temporal changes in the components. Overall, the network for the variation in ECA was dominantly driven by direct abiotic factors which are especially important early and late seasons of seagrass phenology, whereas the biotic intermediate factors become more predominant in spring to summer when the eelgrass ecosystem become productive. The variation in functional response and interactions determines the important pathways in the network.

Figures

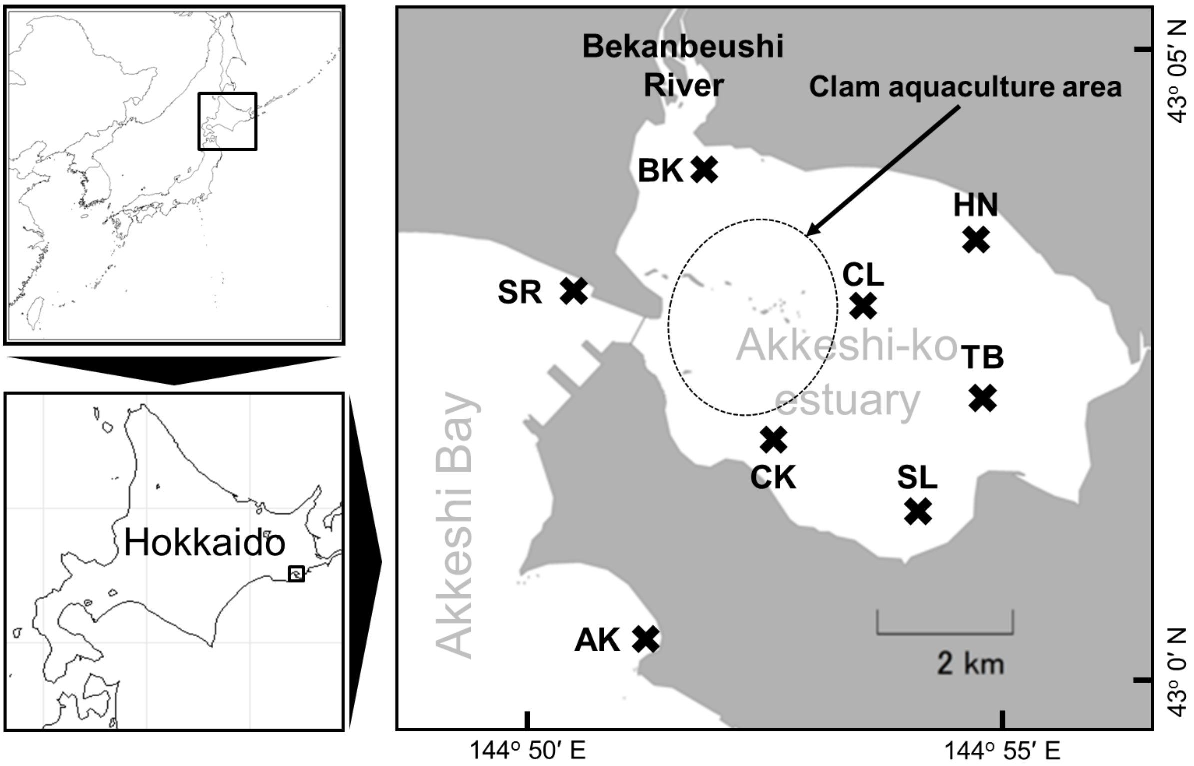

Fig. 4-1. Location of the study stations in the Akkeshi-ko estuary and Akkeshi Bay, northeastern Japan.

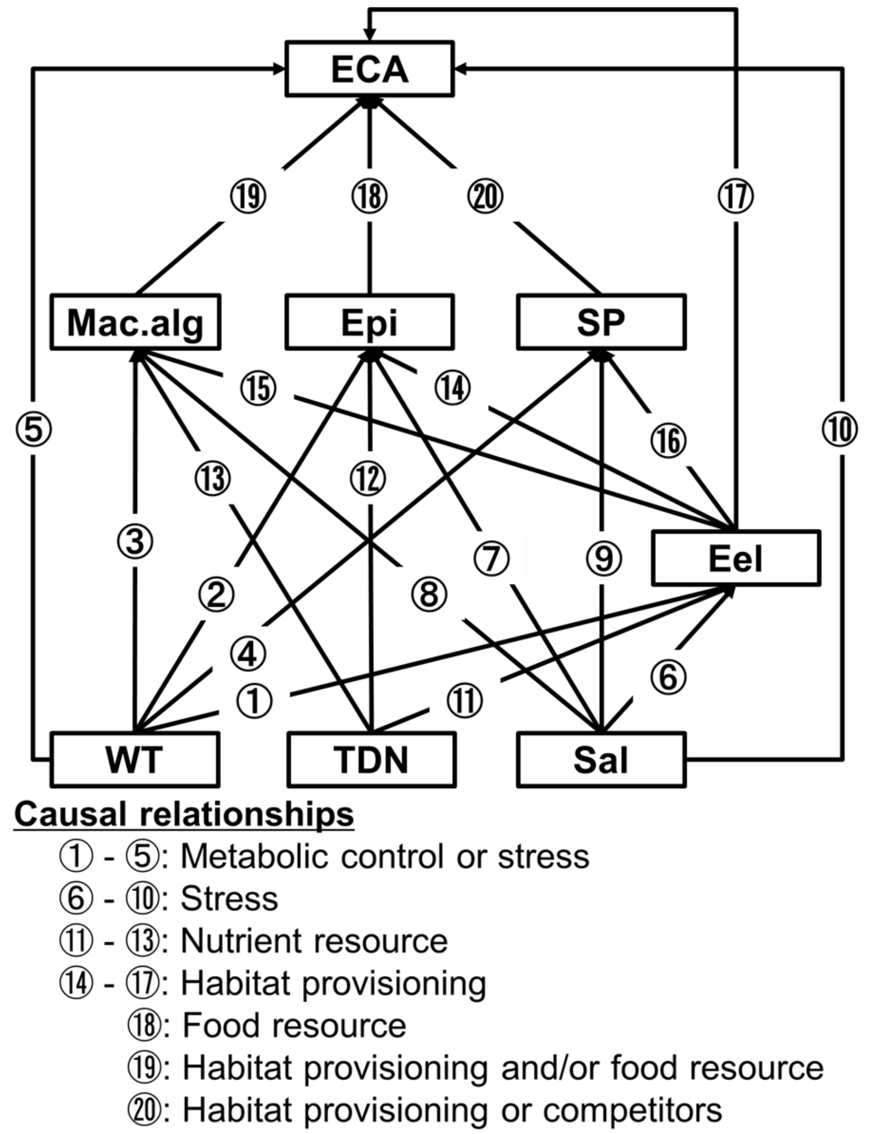

Fig. 4-2. The full modell of SEM with causalities of numbered arrows. Abbreviated words in each box indicate as follow: water temperature (WT), salinity (Sal), total dissolved inorganic nitrogen (TDN), above-ground eelgrass biomass (Eel), epiphyte biomass (Epi), epiphytic macroalgal biomass (Mac.alg), spirorbid shell biomass (SP) and epifaunal community abundance (ECA).

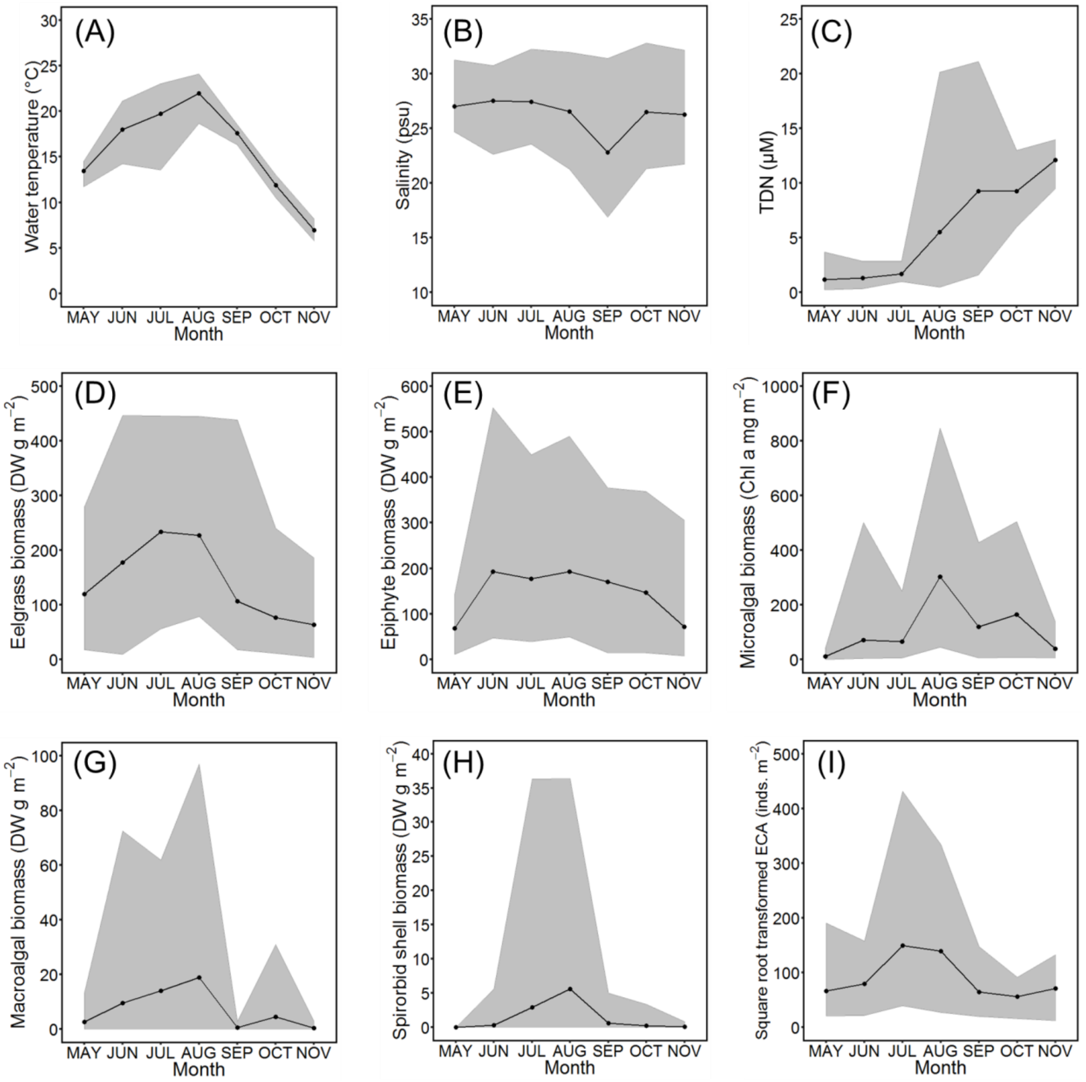

Fig. 4-3. Monthly variation in (A) water temperature, (B) salinity, (C) total dissolved inorganic nitrogen content (TDN), (D) above-ground eelgrass biomass, (E) epiphytic microalgal biomass, (F) epiphytic macroalgal biomass, (G) spirorbid shell biomass and (H) log transformed abundace of epifaunal communities. The points indicate mean values of the eight study stations. The shaded area shows the range of variation among stations.

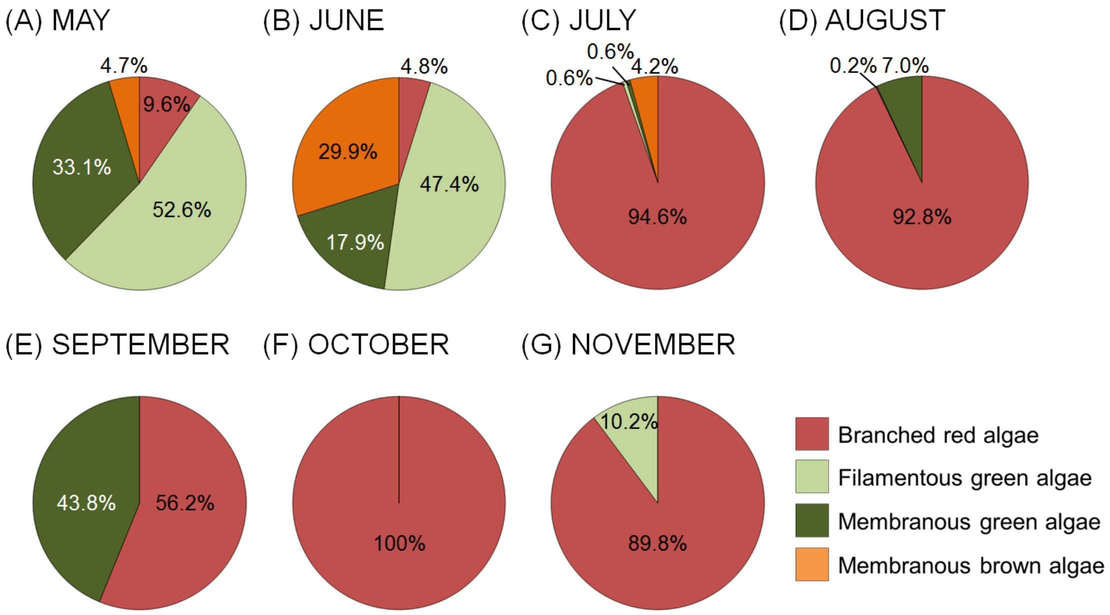

Fig. 4-4. Monthly variation in different types of epiphytic macroalgale.

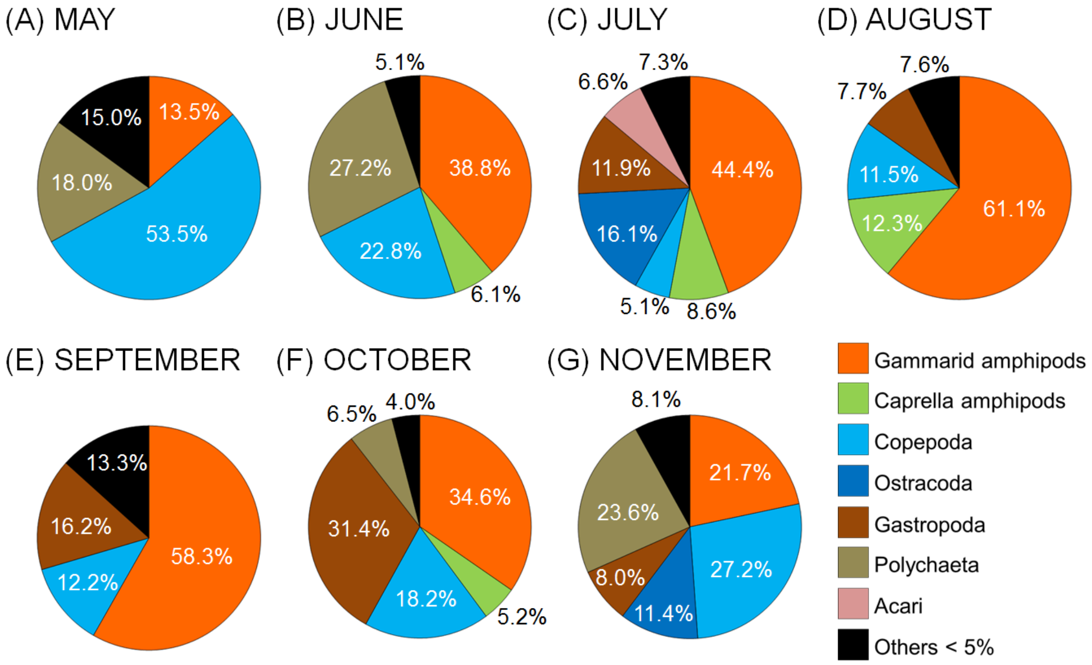

Fig. 4-5. Monthly variation in epifaunal community composition. All the dominant epifaunal group over 5% of total epifaunal abundance was presented. The blank area represents pooled minor groups, each of which was less than 5% of total epifaunal abundance.

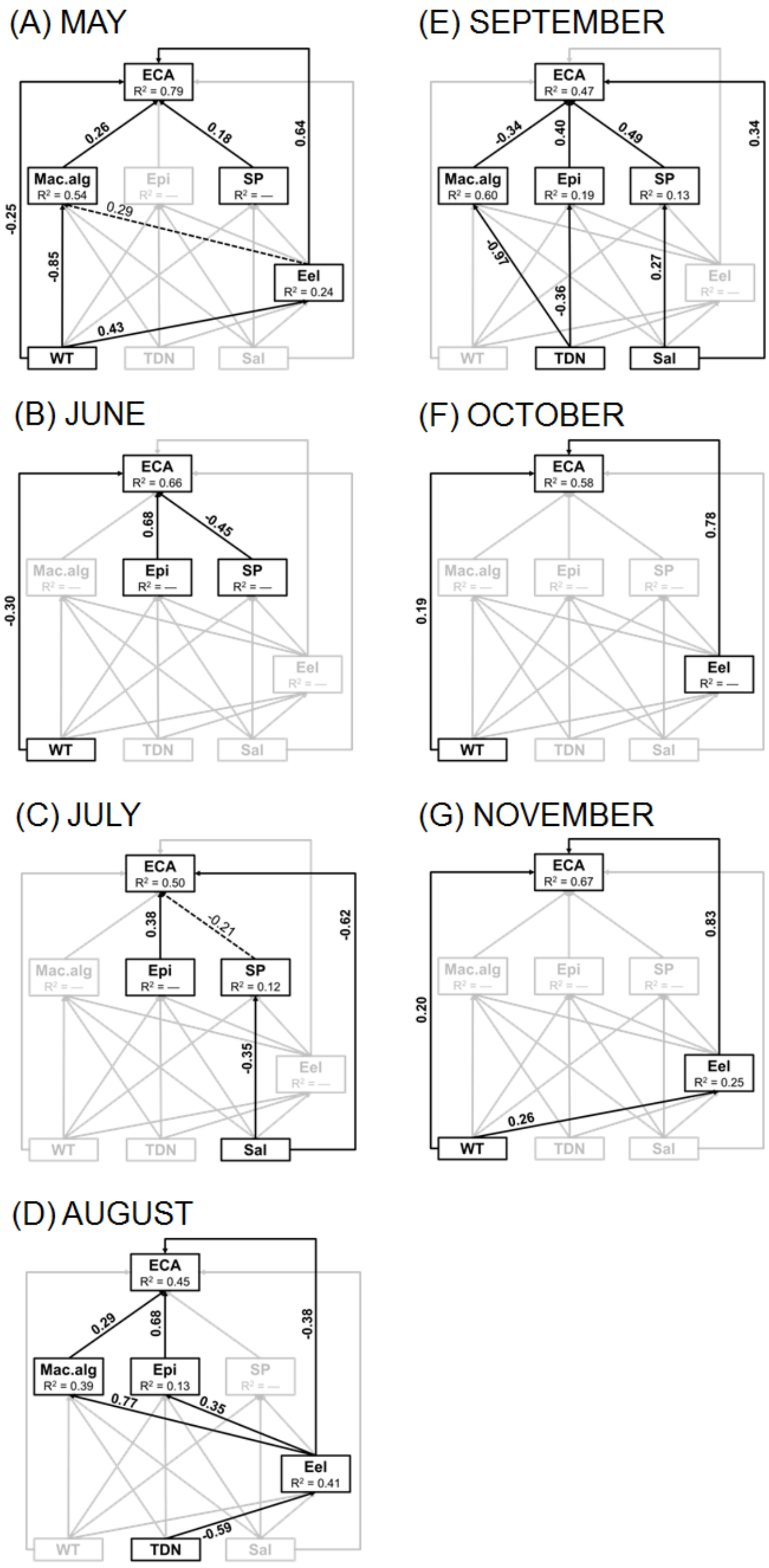

Fig. 4-6. The best SEMs for each month. Black boxes are selected variables. Abbreviated words in each box indicate as follow: water temperature (WT), salinity

(Sal), total dissolved inorganic nitrogen (TDN), above-ground eelgrass biomass (Eel), epiphyte biomass (Epi), epiphytic macroalgal biomass (Mac.alg), spirorbid shell biomass (SP) and epifaunal community abundance (ECA). Black arrows indicate selected causal relationships (solid arrows: significant paths [$P < 0.05$], dashed arrows: non-significant paths). Values on arrows represent range-standardised path coefficients. Marginal R^2 values are shown for endogenous variables in boxes.

Tables

Table 4-1. Environmental conditions at eight stations in the Akkeshi-ko estuary and Akkeshi Bay from May to November.

Factors	**Month**	**Mean value (SD)**								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
Water temperature (°C)	MAY	11.71	13.61	13.78	14.16	14.53	13.75	12.35	13.59	13.43
	JUN	16.39	20.50	20.45	21.13	16.69	19.61	14.24	14.65	17.96
	JUL	20.45	23.01	22.57	22.70	17.91	20.06	17.70	13.58	19.75
	AUG	21.72	24.09	23.84	23.86	21.89	22.70	18.76	18.64	21.94
	SEP	17.43	18.28	18.56	18.31	17.33	17.48	16.29	16.84	17.57
	OCT	12.34	11.56	11.36	10.47	12.25	11.88	13.02	12.18	11.88
	NOV	7.53	5.92	5.78	6.01	7.60	7.28	8.16	7.37	6.96
Salinity	MAY	24.65	24.96	26.24	27.82	27.66	27.38	26.15	31.24	27.01
	JUN	22.59	26.42	26.90	27.33	29.42	28.27	28.42	30.73	27.51
	JUL	23.52	24.63	27.01	27.55	27.83	28.53	27.93	32.22	27.40
	AUG	21.25	23.59	26.00	26.23	26.81	26.71	29.85	31.95	26.55
	SEP	25.04	16.88	18.02	21.72	26.31	25.72	17.31	31.40	22.80
	OCT	26.70	25.17	21.87	21.29	28.76	26.28	29.03	32.78	26.49
	NOV	28.70	22.16	21.72	24.23	26.34	26.73	28.13	32.15	26.27

Table 4-1. (Continued 1)

Factors	**Month**	**Mean value (SD)**								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
TDN (μM)	MAY	3.68	1.07	0.26	0.23	0.68	0.94	2.00	0.62	1.18
	JUN	1.03	0.94	0.54	2.86	1.42	1.52	1.64	0.33	1.29
	JUL	2.87	1.03	1.50	0.98	2.01	2.56	1.51	1.08	1.69
	AUG	5.03	3.66	20.12	1.23	3.07	8.65	2.01	0.46	5.53
	SEP	9.99	9.97	21.12	3.57	11.19	11.58	5.09	1.60	9.26
	OCT	6.65	6.37	12.72	12.03	9.53	12.97	7.63	5.96	9.23
	NOV	11.99	10.16	13.92	11.10	13.98	13.01	13.29	9.47	12.11
Eelgrass biomass (g m^{-2})	MAY	22.71	118.13	130.32	125.43	144.06	186.85	186.62	41.29	119.43
	JUN	37.03	126.59	153.69	177.60	210.58	259.35	295.09	163.13	177.88
	JUL	224.31	221.17	197.94	172.22	247.62	316.13	372.59	112.49	233.06
	AUG	140.32	150.88	104.22	240.76	324.94	241.39	267.75	344.18	226.80
	SEP	83.83	57.92	34.36	80.88	118.31	108.38	67.30	297.65	106.08
	OCT	57.75	19.93	31.98	69.68	86.76	109.89	65.13	172.17	76.66
	NOV	27.31	12.20	35.31	54.96	58.30	94.92	86.04	139.16	63.52

Table 4-1. (Continued 2)

Factors	**Month**	**Mean value (SD)**								
		BK	**HN**	**TB**	**SL**	**CL**	**CK**	**SR**	**AK**	**Among sites**
Epiphyte biomass (g m^{-2})	MAY	49.96	103.67	83.69	111.90	31.64	50.05	100.76	15.21	68.36
	JUN	183.68	255.14	103.49	63.86	311.93	273.80	220.07	131.21	192.90
	JUL	236.87	84.73	288.26	57.05	114.77	333.64	169.59	128.18	176.64
	AUG	408.35	74.18	99.82	145.91	246.92	111.81	260.59	196.79	193.05
	SEP	170.86	88.23	29.82	259.83	301.12	123.34	282.11	110.49	170.72
	OCT	90.42	59.65	17.21	166.51	294.72	220.20	169.22	155.71	146.71
	NOV	12.83	23.93	41.14	39.58	244.38	55.02	92.28	68.94	72.26
Macroalgal biomass (g m^{-2})	MAY	5.50	0.02	2.40	0.00	1.64	0.00	10.22	0.77	2.57
	JUN	9.44	1.39	0.54	0.00	14.49	5.37	45.50	0.00	9.59
	JUL	0.51	51.96	0.89	1.89	5.33	42.00	8.86	0.00	13.93
	AUG	0.43	13.34	0.46	0.00	49.65	9.18	8.14	69.82	18.88
	SEP	0.00	0.00	0.00	2.63	0.00	0.00	0.00	1.59	0.53
	OCT	0.00	2.73	0.00	0.00	0.00	26.87	4.62	1.05	4.41
	NOV	0.32	3.08	0.06	0.01	0.00	0.00	0.00	0.00	0.43

Table 4-1. (Continued 3)

Factors	Month	Mean value (SD)								
		BK	HN	TB	SL	CL	CK	SR	AK	Among sites
Spirorbid biomass (g m^{-2})	MAY	0.000	0.008	0.001	0.000	0.000	0.001	0.003	0.009	0.003
	JUN	0.000	2.619	0.000	0.000	0.003	0.020	0.032	0.019	0.337
	JUL	0.076	22.019	0.025	0.031	0.046	0.464	0.386	0.505	2.944
	AUG	8.149	29.575	0.087	0.619	1.044	0.209	0.553	4.851	5.636
	SEP	0.942	0.376	0.006	1.388	0.094	0.026	0.008	1.991	0.604
	OCT	0.016	0.029	0.000	1.403	0.025	0.030	0.026	0.572	0.263
	NOV	0.000	0.004	0.003	0.423	0.024	0.032	0.164	0.181	0.104

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Chapter Ⅴ

General discussion

This thesis mainly aimed to examine how mobile invertebrate community varies in terms of bottom-up cascade focusing on functional traits of macroalgae and sessile invertebrates in an eelgrass bed in Akkeshi.

In Chapter 2, I examined the effects of three different sessile epibionts (branched red algae, filamentous green algae, and calcific spirorbid polychaetes) on the biomass and diversity of mobile invertebrates in the eelgrass beds using a linear mixed model. Epibionts can be good predictors of the variation in the total biomass, species richness and species diversity of mobile invertebrates and the biomass of major dominant species. Each response variable was differently correlated with different set of epibionts.

In Chapter 3, I examined how mobile invertebrate community structure varied seasonally and spatially with abiotic and biotic factors in the eelgrass bed, especially focusing on macroalgae and calcific spirorbid polychaetes. Mobile invertebrate community composition varied with different sets of abiotic/biotic factors explaining observed spatial variation among different months. I found that the contribution of macroalgae and calcific spirorbid polychaetes to the variation in mobile invertebrate community was comparable to traditional factors in seagrass studies (i.e., water temperature, salinity, eelgrass traits and epiphyte biomass).

In Chapter 4, I assessed a seasonal shift in the network structure describing interrelationships among abiotic factors, primary producers and abundance of mobile invertebrates (epifaunas). I tried to explain seasonal variation in epifaunal community abundance (ECA) by a combination of abiotic factors (water temperature, salinity and dissolved inorganic nitrogen) and biotic factors (eelgrass and epibionts: epiphytic micro- and macro-algae and sessile spirorbid polychaetes) in an eelgrass bed using structural equation modeling (SEM). The SEMs are effective in elucidating temporal shifts in the interacting effects of abiotic/biotic factors affecting epifaunal abundance. This study highlighted the effects of epibionts on epifaunal abundance, that aspect had been focused only in few studies of seagrass ecology.

5.1. Importance of epibiotic factors on macroinvertebrate communities in seagrass beds

Although the effects of seagrass factors (e.g., biomass, shoot density, morphology and patch structure) and epiphytic microalgae on macroinvertebrate community have been main topics in the previous studies on producer-consumer interactions in seagrass beds (e.g., van Montfrans 1984; Jernakoff *et al*., 1996; Attrill *et al*., 2000; Jaschinski *et al*., 2009), those of epibiotic organisms including macroalgae and sessile organisms have been rarely focused despite they are found in many seagrass beds and are potentially important as shown in some studies (Thomsen, 2010; Lefcheck *et al.*, 2014; Long & Grosholz, 2015). This study focusing on the effects of epibiotic organisms pointed out the insufficiency state of conventional studies to understand the variation in seagrass ecosystems accurately.

I found the effects of epibiotic organisms (red and green algae, spirorbid polychaetes) on macroinvertebrate populations and community structure in Chapter 2 and the seasonal and spatial variations in the effects in Chapter 3 and 4. Notably, the results in Chapter 2 and 3 showed that the effects of epibiotic organisms are never ignorable relative to traditional factors in seagrass beds. These results suggest the importance of consideration of the effects of epibiotic organisms with traditional factors as described in Fig. 1-2. Moreover, an approach on multiple epibiotic organisms in this study has high novelty. More studies with this approach are expected to be accumulated in the future.

5.2. Effectiveness of application of functional trait approach to understanding ecosystem in seagrass beds

In addition to functional traits of epibiotic organisms based on morphology in this study, more evaluation of each functional relationship (functional match) between epibiotic organisms and macroinvertebrates is required to understand causalities of biological processes operating in seagrass beds. Apparently, studies focusing on functional traits of macroinvertebrates (e.g., feeding habits, habitat preference and mobility) are now dominant in seagrass beds whereas studies focusing on the functional roles of epibiotic organisms (epiphytic macroalgae and sessile animals) are still very few (Thomsen, 2010; Lefcheck *et al*., 2014; Long & Grosholz, 2015). Especially, grazing of macroinvertebrates has drawn a lot of interest in studies on trophic interactions in seagrass beds (e.g., van Montfrans 1984; Jaschinski et al., 2009; Duffy *et al*., 2015). In most of the relevant studies, the relationship between epiphytic microalgae and macroinvertebrates have been main topic but not between epiphytic macroalgae and macroinvertebrates, although some studies included some macroalgae (Best & Stachowicz, 2012; Gartner *et al*., 2013; Whalen *et al*., 2013). The current conventional studies which focus only on specific functional relationships are insufficient to understand the full scope of seagrass ecosystems is difficult.

To quote an example of study on functional traits of macroinvertebrates in seagrass beds, Best & Stachowicz (2014) showed that species coexisting in seagrass in the field differed not in their feeding niche but in traits related to microhabitat use. In Chapter 2 in this study, I found various relationships between epibiotic organisms and populations of macroinvertebrates with different functional traits cross diverse taxonomic groups. To reduce conjectural parts of understanding of biological processes in seagrass beds, these findings suggest that the demand of covering functional traits of more diverse macroinvertebrates, an approach with integration of trophic interactions (i.e., food web) and non-trophic interactions, understanding of biological interactions from perspectives of both species sides.

However, in association with the state where studies on specific functional relationships (i.e., grazer-epiphyte relationship) have been accumulated as mentioned above, studies based on trophic interactions have been still dominant. In the case of studies dealing with epibiotic organisms, their functional roles should include not only food resource but also habitat provision and/or alteration. For example, the importance of the effects of macroalgae and sessile animals (e.g., mussels) on macroinvertebrate communities through habitat provision and/or alteration have been reported in studies in rocky shores where epibiotic organisms are especially dominant among coastal marine ecosystems (Maggi *et al.*, 2009; Koivisto & Westerbom, 2010). Macroinvertebrate communities in seagrass beds are often composed largely by grazers and detritivores (Valentine & Heck, 1999; Heck *et al.*, 2000). Thus, macroalgae and sessile animals should also be important for macroinvertebrates through habitat provision and/or alteration in seagrass beds. Because several studies suggest that many marine consumers respond more to the value of a habitat rather than its food value (Duffy & Hay 1991; Best & Stachowicz, 2014), an approach considering functional relationships from a perspective of non-trophic relationships as shown in Chapter 4 in this study is necessary for studies on seagrass ecosystems. Therefore, much more information on functional roles of various epibiotic organisms should be obtained in future studies.

5.3. Future application and prospects of this study

This study revealed that the populations and community structure of mobile invertebrates, which were important in the ecosystem of seagrass beds, varied with multiple factors spatially and temporally. Here, I refer to how the results would contributie to future studies on seagrass beds and future conservation and management of seagrass beds.

As a highlight of this study, the roles of epibiotic organisms are found as important as other abiotic/biotic factors for determining community structure of eelgrass beds. This result shows that focus on individual functional relationships based on the new framework of this study and an integrated perspective taking into account both trophic and non-trophic relationships is necessary for biodiversity consevation and maintenance/improvement of ecosystem function (especially productivity) in seagrass beds (Chapter 2 and 4).

Furthermore, several future tasks for biodiversity consevation and maintenance/improvement of ecosystem function in seagrass beds was clarified by dealing with different (but related) response variables in each chapter in this study (Chapter 2: species and community structure; Chapter 3: species composition; Chapter 4: community size and causal links). Over the results of chapters 2 to 4, contents related to species/biodiversity and contents related to production (abundance and biomass) are thought to be varied through different processes. While expanding the research framework presented in this study is important, it is also necessary to reevaluate the influence of traditional factors on biological communities and ecosystem function/properties as seen in the difference in the seasonal importance of traditional factors (e.g., eelgrass biomass) between different objective variables (e.g., similarity in Chapter 3 and abundance in Chapter 4).

Although I comprehensively evaluated the variation in mobile invertebrate community by treating all mobile invertebrates with various functional traits together in the approach of this research, it is necessary to compare approaches based on trophic relationships that have been traditionally dealt with in ecology and on other function groups with my approach for more accurate evaluation in future. In addition, accumulation and organization of information on individual species is also necessary.

5.4. Future prospects of application of functional trait approach to ecology and evolution

As shown in this study on a seagrass ecosystem, application of functional trait approach can provide more biological and ecological insights into specific understanding of the variations in species interactions and macroinvertebrate community. Especially, this study strongly suggests the importance of considering functional traits not only of one species side (reactor or influencer) but both species sides to understand biological interactions in ecology and evolution.

Currently, studies based on functional traits show the effectiveness because the approach using functional traits can clearly link the relationship between environment and species, species and species by functional response or match in ecology and evolution. Functional responses of individual species to various abiotic/biotic factors can change the composition of functional traits within biological populations and communities through ecological (e.g., environmental filtering, niche differentiation) or evolutionary (e.g., evolutionary diversification and selection) processes, and furthermore the processes operating the ecosystems on various temporal and spatial scales (Reiss *et al.*, 2009; Stegen *et al.*, 2009). If in the case that I consider only biological processes but not the feedback, it is essential to focus on responses or functional match of both species for a species interaction although it is sufficient to focus on biological/functional response of a species for a relationship between environment and species.

Up to now, studies on functional responses or interactions have been developed differently between terrestrial and aquatic systems. While studies on functional traits of plants have been dominant in terrestrial systems, studies on functional traits of animals have been dominant in aquatic systems including seagrass beds targeted in this study. Regardless of systems, such bias in the approach may cause delay in understanding of producer-consumer interactions (e.g., plant-animal interactions). Therefore, extension of the framework and change of perspective tried in this study are promising to solve such bias.

5.5. Concluding remarks

In this study, I demonstrated that mobile invertebrate community structure was influenced by the bottom-up control by epibiotic organisms (i.e., macroalgae and sessile invertebrates), in addition to traditional abiotic/biotic factors (i.e., water temperature, salinity, eelgrass traits and epiphyte biomass), through the variation in functional relationships among them in an eelgrass bed in the Akkeshi-ko estuary and Akkeshi Bay. I found that the effects of epibiotic organisms varied temporally and spatially, partially contributed to the variation in mobile macroinvertebrate community as in traditional abiotic/biotic factors. It is also found that the effects of epibiotic organisms were different depending on mobile invertebrate species and even on community structure. I expect that taking into account the effects of epibiotic organisms leads to understand more realistic relationships in seagrass beds. Additionally, this study advocated the importance of application of synthesizing approach considering both trophic and non-trophic interactions beyond food web concept in community ecology.

Regarding the relationships that have been investigated so far, the results obtained using functional trait based approach seems to be more valid than the results based only on quantitative information (abundance and biomass). However, studies based on topics that focus on taxonomic classification approach are still dominant in discipline in biological interactions and/or communities. One of the reasons is that it takes a huge amount of effort to evaluate the information on the functional traits of individual species. Therefore, it will be necessary to systematically organize the data on physiology, morphology, and ecology of individual species in order to further develop functional trait based approach.

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