Variation in phenology, biological traits, and associated epifaunal community between native and non-native populations of the seagrass *Zostera japonica*

（海草コアマモの在来集団と移入集団の間における季節性、形質、葉上動物群集の変異）

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ABSTRACT

Biological invasions, or human-mediated translocations of species to regions outside their native range, are increasing worldwide, including in seagrass ecosystems. Understanding of how seagrass and seagrass ecosystems respond to invasions are important not only for management and conservation of the ecosystem, but also provide opportunity to deepen our understandings on seagrass ecology in general.

Zostera japonica is an intertidal seagrass native in Asia, with wide distribution extending from tropical to temperate regions. In addition to its native distribution, *Z. japonica* also occurs in North America, where it was introduced in the 1950s and has established its populations since then. Because of its established non-native populations in North America, the impacts of *Z. japonica* introduction had been occasionally studied by comparing with its native populations in Asia, or by comparing with a native congener *Z. marina* in its introduced region, which is native in both Asia and North America. However, previous studies comparing native and non-native populations cannot discriminate if observed difference arose from the introduction effects or from regional differences, nor studies comparing with native congener in the introduced region alone cannot discriminate if observed difference arose from introduction effects or from species differences.

In this thesis, I proposed a use of combined methods of native and non-native regional comparison with *Z. japonica* and *Z. marina* species comparison for robust investigation of *Z. japonica* introduction effects. The proposed combination method is promising to separate the introduction effects from those caused by regional or species differences. The main objective of this thesis is to investigate introduction effects **ON** and

OF *Z. japonica*, with special focuses on three aspects of seagrass ecosystems, (1) seagrass phenology, (2) seagrass biological traits and (3) seagrass-associated animal communities, which were examined in three independent chapters (Chapters 2 to 4).

In Chapter 2, I investigated the influence of abiotic factors on seagrass biomass and reproductive phenology of *Z. japonica*. By conducting a large-scale analysis covering tropical to temperate region and including both native and non-native populations, I found that phenological traits varied greatly among regions affected by abiotic factors, such as latitude and temperatures, and that traits varied among populations, but results also varied among analyzed traits. While maximum biomass and peak reproductive timings were significantly affected by temperature for *Z. japonica* populations in general, growth durations and reproductive ratios were affected by latitude and only for non-native populations. These observed difference in responses between native and non-native populations indicate the presence of the introduction effects on seagrass phenology.

In Chapter 3, I conducted multi-site comparisons of seagrass biological traits of *Z. japonica* between native (Japan) and non-native (Canada) regions, along with those of *Z. marina* that is native in both regions. Results suggested that non-native *Z. japonica* showed constant shoot size, lower shoot density, leaf area index and biomass, and higher reproductive ratio compared to native populations, whereas *Z. marina* showed no regional difference in any of analyzed traits. Regional trait differences observed only for *Z. japonica* support that these differences are in fact induced by introduction and not by regional environmental difference which would affect both *Zostera* species. These results indicated that significant trait change occurred during the introduction of *Z. japonica* to North America, potentially through rapid evolution.

In Chapter 4, I compared seagrass-associated epifaunal invertebrate community between *Z. japonica* and *Z. marina* from multiple sites in Japan and Canada, where both seagrass species are native and where *Z. japonica* is non-native, respectively. I found that epifaunal abundance were similar between native and non-native *Z. japonica*, whereas epifaunal diversity was lower for *Z. japonica* compared to *Z. marina*, only in Canada, and was equally diverse for both species in Japan. Additionally, while epifaunal community on native *Z. japonica* were distinct from that of *Z. marina* in Japan, species associated with non-native *Z. japonica* were mainly a subset of that on *Z. marina* in Canada, suggesting that non-native *Z. japonica* has not established a species-specific community in its introduced region. These results demonstrated that observed diversity reduction for non-native *Z. japonica* is caused by its non-native status and not by seagrass species identity.

From three independent studies shown above, I constantly found that non-native *Z. japonica* populations differ from native populations highlighting the significant introduction effects **ON** and **OF** *Z. japonica*. My study indicated that the non-native seagrass differs in their phenological and biological traits from native populations and hosts less diverse epifaunal community compared to the native congeneric species in the introduced region. Significant introduction effects suggested from this study pointed out the importance of accounting non-native seagrass differently from native seagrass for seagrass ecosystem management, which had been overlooked in seagrass ecology. Findings of this thesis contribute to deepen our understanding on invasion ecology and seagrass ecology in general, in addition to giving valuable implications on marine biodiversity conservation and ecosystem management.

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Chapter 1

General Introduction

1.1 Biological Invasions

Biological invasions, or human-mediated translocations of species to regions outside their native range, are increasing worldwide at an unprecedented rate (Ricciardi 2007; van Kleunen et al. 2015a; Diagne et al. 2021). Biological invasions increase with and are intensified by globalization and climate change (Ricciardi 2007; Seebens et al. 2015; Essl et al. 2020), and can occur both intentionally and unintentionally. The intentional pathway involves active translocations of species to a region for specific purposes, such as uses as agricultural crops, ornamental plants, and animals for pets and biocontrol agents (Hulme et al. 2008; Watari et al. 2008; Davis and Landis 2011). In contrast, the unintentional pathway happens when species are translocated by accidental transport to a region. Global trade and human travel increase the likelihood of accidental translocation of organisms, such as plant seeds attached to travelers' clothes, soil/sediment contaminants, and insects and pathogens transported with agricultural crops (Hulme et al. 2008; Davis and Landis 2011; Chown et al. 2012; Seebens et al. 2015). Biological invasions are not limited to terrestrial environments but are also found in marine ecosystems (Wonham and Carlton 2005; Williams 2007; Rilov and Crooks 2009).

Introductions through aquaculture, aquarium trade, and shipping ballast water are common pathways for marine biological invasions (Hewitt et al. 2009; Minchin et al. 2009; Katsanevakis et al. 2013; Grosholz et al. 2015; Mach et al. 2017).

Although there are numerous terms expressing species translocated to novel habitat (i.e., alien, exotic, introduced, invasive, naturalized, non-indigenous), a non-native species does not necessarily mean that the species is invasive to a region (Richardson et al. 2000, Colautti and MacIsaac 2004). The biological invasion processes consist of multiple stages that can occur in this order: transport, establish, spread, and impact (Blackburn et al. 2011). Among the numerous species that are transported, established, and spread, only those species with significant negative impacts on their novel environments are considered invasive (McNeely et al. 2001; Beck et al. 2008). The question on what makes a species invasive and others not, had led scientists to pursue common traits for invasive species known as "invasive traits" (Kolar and Lodge 2001; Pyšek and Richardson 2007; Hayes and Barry 2008; van Kleunen et al. 2010a; van Kleunen et al. 2015b). Rapid growth, fast reproduction, high environmental tolerance, and high dispersal ability (Pyšek and Richardson 2007; Mason et al. 2008; van Kleunen et al. 2015b; Cardeccia et al. 2018) are examples of invasive traits. Throughout this thesis, I used term "native" and "non-native" exclusively to describe the species or population status in respect to their original habitat or to their introduced habitat, whereas the term "invasive" is restrictedly used to describe harmful status of non-native species or in respect to the traits involved.

Various impacts of biological invasions are reported worldwide. Biological invasions negatively affect human welfare by causing agricultural damage and spreading disease, and burden high cost for non-native species management (Ogden et al. 2019;

Diagne et al. 2021; Watari et al. 2021). Ecosystems and local biodiversity are also negatively affected by invasions, as non-native species can reduce native species abundances and alter ecosystem services and functions by shifting community composition, which can lead to the extinction of native species (Vilà et al. 2011; Brantley et al. 2015; Bellard et al. 2016; Vivó-Pons et al. 2020). However, biological invasions of species with the ability to modify environments or facilitate biodiversity, such as ecosystem engineers or foundation species (*sensu* Jones et al. 1994 and Ellison 2019; Crooks 2002; Crooks 2009; Ehrenfeld 2010), may cause positive effects on introduced communities and ecosystems. Habitat introduced by non-native foundation species can sometimes benefit from biological invasions, as foundation species increase local nutrient and carbon storage, and facilitate higher biodiversity and abundances of associated biological community (Rodriguez 2006; Ehrenfeld 2010; Gribben et al. 2013; Davidson et al. 2018), and thus non-native foundation species have high potential values for biodiversity conservation (Schlaepfer et al. 2011).

Studying biological invasions is not only important for conservation of biodiversity and ecosystem management but also provides an opportunity to deepen our understanding of basic ecology, evolution, population biology, and biogeography (Sakai et al. 2001; Briggs 2007; Sax et al. 2007; Keller and Taylor 2008). For example, rapid adaptive evolution during invasion is suggested to play important role for determining invasion success of non-native species (Sakai et al. 2001; Novak 2007). There is building evidence on how such rapid evolution occurs and benefits non-native species during invasion. Rapid evolution in plant phenology enables plants to adapt to environmental conditions in introduced regions and facilitate range expansion of invasive plants (Colautti and Barrett 2013; Novy et al. 2013), and genetic analyses confirm that such

phenotypic evolution occurred during invasion and range expansion of red seaweed, *Gracilaria vermiculophylla* (Sotka et al. 2018). Interestingly, biological invasions not only cause rapid evolution of introduced species but also trigger evolution of native species in their introduced range (Gillis and Walsh 2017).

1.2 Seagrass Ecosystems

As mentioned earlier, biological invasions also happen in marine environments, including seagrass beds (Williams 2007). Seagrasses are marine angiosperms that mainly inhabit shallow, protected areas in tropical to subarctic coasts around the world (Green and Short 2003; Short et al. 2007). Seagrasses form extensive meadows known as seagrass beds, which provide various ecosystem services to humans and are economically valuable coastal ecosystems (Costanza et al. 1997, 2014; Nordlund et al. 2016; United Nations Environment Programme 2020).

Despite the importance of seagrasses, there are multiple threats to seagrass ecosystems, such as eutrophication, disease, extreme climatic events, coastal development, dredging, and biological invasions (Orth et al. 2006; Grech et al. 2012; Roca et al. 2016; Unsworth et al. 2019; Turschwell et al. 2021). Due to both natural and anthropogenic stressors, the areal extent of seagrass beds is rapidly declining worldwide, especially in Southeast Asia (Waycott et al. 2009; Dunic et al. 2021; Sudo et al. 2021). Nevertheless, seagrass beds in some areas are increasing or had been successfully restored (Leschen et al. 2010; Tomasko et al. 2018; Orth et al. 2020; Guerrero-Meseguer et al. 2021; Dunic et al. 2021; Turschwell et al. 2021). However, seagrass ecosystems are expected to experience further change due to climate change in future (Duarte 2002; Hyndes et al. 2016; Nguyen et al. 2021). For effective ecosystem management and

conservation, it would be extremely important to understand how seagrass and seagrass ecosystems respond to any threat or change in environmental factors.

Seagrass ecosystems are threatened by the introduction of non-native species, and the numbers of non-native species in coastal marine communities are increasing (Ruiz et al. 2000; Williams 2007). Reports highlight non-native seaweeds and invertebrates are common in seagrass beds (Williams 2007). Although ecological effects of non-native species on seagrass beds are largely unassessed, most of the assessed species show negative impacts on seagrasses. For example, non-native fouling species, including tunicates, sponges, and bryozoan, reduce seagrass growth and survival rates, and can lead to a decrease in seagrass areal extent (Williams 2007; Wong and Vercaemer 2012). Experimental and observation studies show there is direct space competition between non-native seaweeds and seagrasses, and that seagrasses can be outcompeted by non-native seaweeds under disturbance (den Hartog 1997; Garbary et al. 2004). However, the cascading effects of seagrass loss and transformation to seaweed bed to associated community or ecosystems are largely context dependent, depending on whether non-native seaweeds are morphologically or functionally similar to native seagrasses (Williams and Heck 2001; Williams 2007). Functionally similar seaweeds can support similar abundances of fish but not species (Eklöf et al. 2005). Seaweed species that are very different from seagrass (i.e., morphologically, palatability, and toxicity), such as *Caulerpa* spp., significantly reduces the abundance and diversity of invertebrates and fishes, which leads to altered food web structures (York et al. 2006; Gab-Alla 2007; Deudero et al. 2011).

While there are numerous studies on the impacts of non-native seaweed and/or invertebrate to seagrass or seagrass ecosystems, non-native seagrasses are mostly not well

investigated. As of December 2021, there are five seagrass species known to be non-native in different areas of the world: *Halophila decipiens* from the Indian, the Atlantic and the Pacific Ocean, introduced to Hawai'i; *Halophila stipulacea* from the Indian Ocean to the Mediterranean and the Caribbean Sea; *Halophila ovalis* from Indo-Pacific region to southeast coast of Florida, USA, which was known as *Halophila johnsonii*; *Zostera tasmanica* from Australia and New Zealand to Chile; and *Zostera japonica* from the northwest Pacific Ocean to the northeast Pacific Ocean (Williams 2007 and references therein; Waycott et al. 2021). Among them, *Z. japonica* and *H. stipulacea* are relatively well-studied for their ecology and impacts on non-native environments (Fisher et al. 2011; Mach et al. 2014; Shafer et al. 2014; Viana et al. 2019; Winters et al. 2020).

Because of valuable ecosystem services and functions provided by the seagrass, it would be of extreme importance to clarify how seagrass differ or similar between native and non-native regions. When there is non-native seagrass introduced, the most important things to clarify is to decide whether the species is invasive or not. This is commonly conducted by evaluating its impact to introduced habitat or by evaluating its traits and see if they are invasive traits or not. On the other hand, accounting their high primary production and use of seagrass beds as blue carbon sinks (Nordlund et al. 2016; United Nations Environment Programme 2020), production and reproductive ecology of seagrasses are important points to consider if they are different between native and non-native populations. For example, if native and non-native populations differ in response to abiotic factors, it would not be suitable to apply the knowledge of native populations to non-native and vice versa, which makes it difficult for coastal managers to decide their management and conservation planning. Furthermore, seagrasses provide habitat and nursery to various organisms which is one of their important ecosystem services and

functions (Nordlund et al. 2016; Lefcheck et al. 2019). Thus, if non-native seagrasses differ in their habitat provisioning function, the impacts would not be limited to seagrass and associated animals but further cascade to wider ranges of organisms through food web.

1.3 Study species: *Zostera japonica* and *Z. marina*

Eelgrass, *Zostera marina* L. is the most widespread and dominant species in temperate regions throughout the Northern Hemisphere (Green and Short 2003). *Z. marina* inhabits subtidal and lower intertidal areas and is the primary foundation species of seagrass beds, especially in subtidal areas (Nakaoka and Aioi 2001; Short et al. 2007; Ruesink et al. 2018). *Z. marina* is the most well-studied species of all seagrasses and current research includes various aspects of biology like genetics, physiology, life history, and ecology. Research in these fields often involve laboratory experiments and field observations, as well developing applications for eelgrass restoration and conservation (i.e., Hughes and Stachowicz 2009; Duffy et al. 2015; Olsen et al. 2016; van Katwijk et al. 2016; Reynolds et al. 2018; Ruesink et al. 2018; Orth et al. 2020).

Dwarf eelgrass, *Zostera japonica* Aschers et Graeben, is a smaller-sized seagrass compared to *Z. marina* and mostly inhabits intertidal areas (Nakaoka and Aioi 2001; Shafer et al. 2014). *Z. japonica* is native to Asia (Green and Short 2003) and was likely introduced to North America in the 1950s, via accidental transport with oyster (*Crassostera gigas*) imports, and it has established populations since then (Hitchcock 1969; Harrison and Bigley 1982). Genetic study suggested that non-native *Z. japonica* in North America originated from Miyagi Prefecture, Japan (Tanaka 2012), which is a place with a history of oyster exports to the world, including North America. In its non-native

region, *Z. japonica* is currently found from California to southern British Columbia (Shafer et al. 2014). However, it is possible that the species is still expanding its distribution since the species has only colonized a small portion of its suitable habitat in North America (Harrison and Bigley 1982; Shafer et al. 2011; Shafer et al. 2014). In its native region, *Z. japonica* is distributed along a wide latitudinal range covering tropical to temperate zones (Green and Short 2003; Short et al. 2007), whereas non-native *Z. japonica* has not established in tropical regions. While *Z. japonica* is increasing in North America, it is facing significant reduction and is threatened in some areas in its native habitat (Lee 1997). In this thesis, any *Z. japonica* populations from Asia is treated as native populations, regardless of temperate and tropical distribution, and any *Z. japonica* populations from North America is treated as non-native populations, regardless of introduction timings or establishment status.

In both Asia, where *Z. japonica* and *Z. marina* are native, and North America, where *Z. japonica* is non-native, these two seagrass species are found adjacent or mixing with each other in coastal habitats (Nakaoka and Aioi 2001; Ruesink et al. 2010; Shafer et al. 2014). Different zonation patterns suggest that interaction strengths between *Z. japonica* and *Z. marina* varies among sites (Shafer et al. 2014), in both regions. Before *Z. japonica* colonization, upper intertidal areas of the west coast of North America were mainly empty without any species that provided habitat structure, and *Z. japonica*'s introduction to the region transformed unvegetated mud- and sandflat to a vegetated habitat (Mach et al. 2014; Shafer et al. 2014).

The biology of *Z. japonica*, especially in relation to *Z. marina*, has been studied more often in North America than in Asia, most likely to investigate the effects of its introduction. In North America, there are multiple studies comparing physiology and

ecology of seagrasses, and nutrient fluxes and associated animal communities between the two *Zostera* species (i.e., Harrison 1982; Thom et al. 1995; Larned 2003; Lamberson et al. 2011; Ruesink et al. 2010; Shafer and Kaldy 2014; Knight et al. 2015; Boardman and Ruesink 2022). However similar comparative studies are rarely conducted in Asia. Studies comparing biomass and reproductive dynamics (Lee et al. 2005, 2006), zonation patterns (Kim et al. 2020), and epiphytic diatom communities (Chung and Lee 2008) are examples of the few comparative studies in Asia. The different research objectives between North America and Asia make it difficult to evaluate if and how *Z. japonica* is affected by its introduction.

1.4 How to investigate introduction effects

There are two common methods for investigating the introduction effects on and of non-native species: (1) interspecific comparisons with other species in an introduced range (Mack 1996; van Kleunen et al. 2010a) and (2) intraspecific comparisons of non-native species from their introduced and native ranges (Bossdorf et al. 2005; Hierro et al. 2005; Parker et al. 2013). The first method, interspecific comparison, is more commonly used in invasion ecology, especially in attempts to examine invasive traits (Hamilton et al. 2005; van Kleunen et al. 2010a; Leffler et al. 2014; Mathakutha et al. 2019). The second method, intraspecific comparison, is more widely used in evolutionary biology and biogeography contexts in addition to invasion ecology, where biological invasions can provide clues for solving fundamental questions in research, such as rapid evolution process as mentioned above (Maron et al. 2004; Bossdorf et al. 2005; Hierro et al. 2005; Briggs 2007).

While each comparative method has strengths for investigating introduction effects, they also have limitations. Interspecific comparisons cannot discriminate if observed differences between native and non-native species are really based on its native/non-native status or only caused by difference in species identity. On the other hand, intraspecific comparisons cannot distinguish if observed differences between native and non-native populations arose from introduction effects or are solely dependent on latent environmental differences in compared regions. Due to strengths and weaknesses in both methods alone, the combined use of these two methods is strongly suggested for robust investigations (van Kleunen et al. 2010b; Fig. 1.1). However, such combined use has been rarely conducted because of limitations of appropriate pairs of species from native and non-native regions that enables such combination.

1.5 Objectives and chapter outline

The main objective of this thesis is to investigate introduction effects **ON** and **OF** *Z. japonica*. To investigate this, I focused on 3 different aspects of seagrass ecosystems: (1) seagrass phenology, (2) seagrass biological traits, and (3) seagrass-associated animal communities (Fig. 1.2). I hypothesize that introduction affects all 3 aspects of seagrass ecosystems, and that native and non-native *Z. japonica* will exhibit significant variation in its phenology, biological traits, and its association with animal communities. I conducted three independent studies to address this objective and to test the hypothesis on each aspect of seagrass ecosystems separately, with additional objectives and hypothesis specific to each analyzed aspect, which will be introduced in each chapter.

This thesis consists of three main chapters on original research in addition to the General Introduction and General Discussion. After the general introduction in this chapter (Ch. 1), I evaluated the impacts of introduction on *Z. japonica*, in terms of phenology (Ch. 2) and biological traits (Ch. 3), and then evaluated the impact of the *Z. japonica* introduction on its associated animal communities (Ch. 4), ending with a general discussion to conclude the thesis (Ch. 5).

In Chapter 2, I conducted a large-scale comparison of biomass and reproductive phenology of *Z. japonica*. Seasonal data on *Z. japonica* biomass and reproduction traits are synthesized from my original field observations and by collating available information from the literature. I investigated how traits vary among regions and evaluated the influence of abiotic factors, such as latitude and temperature, on traits variations. Abiotic influences were further compared between native and non-native populations to examine the potential introduction effects on *Z. japonica* phenology. Results on *Z. japonica* phenology were also discussed by comparing them with previous findings on *Z. marina* phenology. This chapter emphasizes the importance of abiotic factors on seagrass phenology and how it may be altered by introduction.

In Chapter 3, I compared biological traits of *Z. japonica* collected from multiple sites in Hokkaido, Japan, where *Z. japonica* is native, and multiple sites in British Columbia, Canada, where *Z. japonica* is non-native, to explore if non-native *Z. japonica* experiences trait shift during introduction. To clarify if observed variations are independent of regional difference, trait variations were further compared with that of *Z. marina*, which is native in both regions. This chapter specifically examines if biological traits of non-native *Z. japonica* exhibit signs of population bottlenecks and/or rapid evolution, both of which are common phenomena in biological invasions.

In Chapter 4, I examined if non-native seagrass differs with native seagrass in terms of seagrass-associated invertebrate (epifauna) communities. Epifaunal abundance and species richness between the two seagrass species, *Z. japonica* and *Z. marina*, were compared at multiple sites in Canada and Japan. Effect of seagrass species on epifaunal communities were further compared among region to discriminate if the seagrass effect differs between *Z. japonica*'s native and non-native status. This chapter highlights the potential cascading effects of *Z. japonica*'s introduction to native epifaunal invertebrate communities.

This thesis is concluded with a general discussion (Ch. 5) where novel findings and future implications of these studies are discussed with current limitations and future directions.

1.6 References

Beck KG, Zimmerman K, Schardt JD, et al (2008) Invasive species defined in a policy context: Recommendations from the Federal Invasive Species Advisory Committee. Invasive Plant Sci Manag 1:414–421. https://doi.org/10.1614/IPSM-08-089.1

Bellard C, Cassey P, Blackburn TM (2016) Alien species as a driver of recent extinctions. Biol Lett 12:20150623. https://doi.org/10.1098/rsbl.2015.0623

Blackburn TM, Pyšek P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. https://doi.org/10.1016/j.tree.2011.03.023

Boardman FC, Ruesink JL (2022) Competition and coexistence in a rare Northeastern Pacific multispecies seagrass bed. Aquat Bot 176:103450. https://doi.org/10.1016/j.aquabot.2021.103450

Bossdorf O, Auge H, Lafuma L, et al (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11. https://doi.org/10.1007/s00442-005-0070-z

Brantley ST, Miniat CF, Elliott KJ, et al (2015) Changes to southern Appalachian water yield and stormflow after loss of a foundation species. Ecohydrology 8:518–528. https://doi.org/10.1002/eco.1521

Briggs JC (2007) Marine biogeography and ecology: invasions and introductions. J Biogeogr 34:193–198. https://doi.org/10.1111/j.1365-2699.2006.01632.x

Cardeccia A, Marchini A, Occhipinti-Ambrogi A, et al (2018) Assessing biological invasions in European Seas: Biological traits of the most widespread non-indigenous species. Estuar Coast Shelf Sci 201:17–28. https://doi.org/10.1016/j.ecss.2016.02.014

Chown SL, Huiskes AHL, Gremmen NJM, et al (2012) Continent-wide risk assessment for the establishment of nonindigenous species in Antarctica. Proc Natl Acad Sci 109:4938–4943. https://doi.org/10.1073/pnas.1119787109

Chung M-H, Lee K-S (2008) Species composition of the epiphytic diatoms on the leaf tissues of three *Zostera* species distributed on the southern coast of Korea. Algae 23:75–81. https://doi.org/10.4490/algae.2008.23.1.075

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366. https://doi.org/10.1126/science.1242121

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define 'invasive' species. Divers Distrib 10:135–141. https://doi.org/10.1111/j.1366-9516.2004.00061.x

Costanza R, d'Arge R, de Groot R, et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260. https://doi.org/10.1038/387253a0

Costanza R, Groot R De, Sutton P, et al (2014) Changes in the global value of ecosystem services. Glob Environ Chang 26:152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002

Crooks JA (2009) The Role of Exotic Marine Ecosystem Engineers. In: Rilov G, Crooks JA (eds) Biological Invasions in Marine Ecosystems. Springer, Berlin, Heidelberg, pp 287–304

Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153–166. https://doi.org/10.1034/j.1600-0706.2002.970201.x

Davidson IC, Cott GM, Devaney JL, Simkanin C (2018) Differential effects of biological invasions on coastal blue carbon: A global review and meta-analysis. Glob Chang Biol 24:5218–5230. https://doi.org/10.1111/gcb.14426

Davis A, Landis D (2011) Invasive species in agriculture. In: Rejmanek M, Simberloff D (eds) Encyclopedia of Introduced Invasive Species. University of California Press, Berkeley, CA, pp 7–11

den Hartog C (1997) Is *Sargassum muticum* a threat to eelgrass beds? Aquat Bot 58:37–41

Deudero S, Box A, Alós J, et al (2011) Functional changes due to invasive species: Food web shifts at shallow *Posidonia oceanica* seagrass beds colonized by the alien macroalga *Caulerpa racemosa*. Estuar Coast Shelf Sci 93:106–116. https://doi.org/10.1016/j.ecss.2011.03.017

Diagne C, Leroy B, Vaissière A-C, et al (2021) High and rising economic costs of biological invasions worldwide. Nature 592:571–576. https://doi.org/10.1038/s41586-021-03405-6

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

Duffy JE, Reynolds PL, Boström C, et al (2015) Biodiversity mediates top-down control in eelgrass ecosystems: A global comparative-experimental approach. Ecol Lett 18:696–705. https://doi.org/10.1111/ele.12448

Dunic JC, Brown CJ, Connolly RM, et al (2021) Long-term declines and recovery of meadow area across the world's seagrass bioregions. Glob Chang Biol 27:4096–4109. https://doi.org/10.1111/gcb.15684

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80. https://doi.org/10.1146/annurev-ecolsys-102209-144650

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

Ellison AM (2019) Foundation species, non-trophic interactions, and the value of being common. iScience 13:254–268. https://doi.org/10.1016/j.isci.2019.02.020

Essl F, Lenzner B, Bacher S, et al (2020) Drivers of future alien species impacts: An expert-based assessment. Glob Chang Biol 26:4880–4893. https://doi.org/10.1111/gcb.15199

Fisher JP, Bradley T, Patten K (2011) Invasion of Japanese Eelgrass, *Zostera japonica* in the Pacific Northwest: A preliminary Analysis of Recognized Impacts, Ecological Functions, and Risks. Ocean Park, WA

Gab-Alla AA-FA (2007) Ecological study on community of exotic invasive seaweed *Caulerpa prolifera* in Suez Canal and its associated macro invertebrates. J Appl Sci 7:679–686. https://doi.org/10.3923/jas.2007.679.686

Garbary DJ, Fraser SJ, Hubbard C, Kim KY (2004) *Codium fragile*: rhizomatous growth in the *Zostera* thief of eastern Canada. Helgol Mar Res 58:141–146. https://doi.org/10.1007/s10152-004-0173-7

Gillis MK, Walsh MR (2017) Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. Proc R Soc B Biol Sci 284:20170814. https://doi.org/10.1098/rspb.2017.0814

Grech A, Chartrand-Miller K, Erftemeijer P, et al (2012) A comparison of threats, vulnerabilities and management approaches in global seagrass bioregions. Environ Res Lett 7:024006. https://doi.org/10.1088/1748-9326/7/2/024006

Green EP, Short FT (2003) World Atlas of Seagrasses. University of California Press, Berkeley, CA

Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. Oikos 122:816–824. https://doi.org/10.1111/j.1600-0706.2012.20868.x

Grosholz ED, Crafton RE, Fontana RE, et al (2015) Aquaculture as a vector for marine invasions in California. Biol Invasions 17:1471–1484. https://doi.org/10.1007/s10530-014-0808-9

Guerrero-Meseguer L, Veiga P, Sampaio L, Rubal M (2021) Resurgence of *Zostera marina* in the Ria de Aveiro lagoon, Portugal. Aquat Bot 169:103338. https://doi.org/10.1016/j.aquabot.2020.103338

Hamilton MA, Murray BR, Cadotte MW, et al (2005) Life-history correlates of plant invasiveness at regional and continental scales. Ecol Lett 8:1066–1074. https://doi.org/10.1111/j.1461-0248.2005.00809.x

Harrison PG (1982) Comparative growth of *Zostera japonica* Aschers. & Graebn. and *Z. marina* L. under simulated intertidal and subtidal conditions. Aquat Bot 14:373–379

Harrison PG, Bigley RE (1982) The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can J Fish Aquat Sci 39:1642–1648. https://doi.org/10.1139/f82-221

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biol Invasions 10:483–506. https://doi.org/10.1007/s10530-007-9146-5

Hewitt CL, Gollasch S, Minchin D (2009) The Vessel as a Vector – Biofouling, Ballast Water and Sediments. In: Rilov G, Crooks JA (eds) Biological Invasions in Marine Ecosystems. Springer, Berlin, Heidelberg, pp 117–131

Hierro JL, Maron JL, Callaway RM (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. J Ecol 93:5–15. https://doi.org/10.1111/j.1365-2745.2004.00953.x

Hitchcock CL, Cronquist A, Ownbey M, Thompson JW (1969) Vascular Plants of the Pacific Northwest Volume 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. Univ. Washington Press, Seattle, WA

Hughes AR, Stachowicz JJ (2009) Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 90:1412–1419. https://doi.org/10.1890/07-2030.1

Hulme PE, Bacher S, Kenis M, et al (2008) Grasping at the routes of biological invasions: a framework for integrating pathways into policy. J Appl Ecol 45:403–414. https://doi.org/10.1111/j.1365-2664.2007.01442.x

Hyndes GA, Heck KL, Vergés A, et al (2016) Accelerating tropicalization and the transformation of temperate seagrass meadows. Bioscience 66:938–948. https://doi.org/10.1093/biosci/biw111

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386. https://doi.org/10.2307/3545850

Katsanevakis S, Zenetos A, Belchior C, Cardoso AC (2013) Invading European Seas: Assessing pathways of introduction of marine aliens. Ocean Coast Manag 76:64–74. https://doi.org/10.1016/j.ocecoaman.2013.02.024

Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol Lett 11:852–866. https://doi.org/10.1111/j.1461-0248.2008.01188.x

Kim SH, Kim JW, Kim YK, et al (2020) Factors controlling the vertical zonation of the intertidal seagrass, *Zostera japonica* in its native range in the northwestern Pacific. Mar Environ Res 157:104959. https://doi.org/10.1016/j.marenvres.2020.104959

Knight NS, Prentice C, Tseng M, O'Connor MI (2015) A comparison of epifaunal invertebrate communities in native eelgrass *Zostera marina* and non-native *Zostera japonica* at Tsawwassen, BC. Mar Biol Res 11:564–571. https://doi.org/10.1080/17451000.2014.985230

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204. https://doi.org/10.1016/S0169-5347(01)02101-2

Lamberson JO, Frazier MR, Nelson WG, Clinton PJ (2011) Utilization Patterns of Intertidal Habitats by Birds in Yaquina Estuary, Oregon. U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division, Newport OR

Larned ST (2003) Effects of the invasive, nonindigenous seagrass *Zostera japonica* on nutrient fluxes between the water column and benthos in a NE Pacific estuary. Mar Ecol Prog Ser 254:69–80. https://doi.org/10.3354/meps254069

Lee SY (1997) Annual cycle of biomass of a threatened population of the intertidal seagrass *Zostera japonica* in Hong Kong. Mar Biol 129:183–193. https://doi.org/10.1007/s002270050159

Lee SY, Kim JB, Lee SM (2006) Temporal dynamics of subtidal *Zostera marina* and intertidal *Zostera japonica* on the southern coast of Korea. Mar Ecol 27:133–144. https://doi.org/10.1111/j.1439-0485.2006.00089.x

Lee SM, Lee SY, Choi C Il (2005) Reproductive phenology of four Korean seagrasses, *Zostera caespitosa*, *Z. caulescens*, *Z. japonica* and *Z. marina*. Ocean Polar Res 27:125–133 (in Korean with English abstract)

Lefcheck JS, Hughes BB, Johnson AJ, et al (2019) Are coastal habitats important nurseries? A meta-analysis. Conserv Lett 12:e12645. https://doi.org/10.1111/conl.12645

Leffler AJ, James JJ, Monaco TA, Sheley RL (2014) A new perspective on trait differences between native and invasive exotic plants. Ecology 95:298–305

Leschen AS, Ford KH, Evans NT (2010) Successful eelgrass (*Zostera marina*) restoration in a formerly eutrophic estuary (Boston Harbor) supports the use of

a multifaceted watershed approach to mitigate eelgrass loss. Estuaries and Coasts 33:1340–1354. https://doi.org/10.1007/s12237-010-9272-7

Mach ME, Levings CD, Chan KMA (2017) Nonnative species in British Columbia eelgrass beds spread via shellfish aquaculture and stay for the mild climate. Estuaries and Coasts 40:187–199. https://doi.org/10.1007/s12237-016-0124-y

Mach ME, Wyllie-Echeverria S, Chan KMA (2014) Ecological effect of a nonnative seagrass spreading in the Northeast Pacific: A review of *Zostera japonica*. Ocean Coast Manag 102:375–382. https://doi.org/10.1016/j.ocecoaman.2014.10.002

Mack RN (1996) Predicting the identity and fate of plant invaders: Emergent and emerging approaches. Biol Conserv 78:107–121. https://doi.org/10.1016/0006-3207(96)00021-3

Maron JL, Vilà M, Bommarco R, et al (2004) Rapid evolution of an invasive plant. Ecol Monogr 74:261–280. https://doi.org/10.1890/03-4027

Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Glob Ecol Biogeogr 17:633–640. https://doi.org/10.1111/j.1466-8238.2008.00402.x

Mathakutha R, Steyn C, le Roux PC, et al (2019) Invasive species differ in key functional traits from native and non-invasive alien plant species. J Veg Sci 30:994–1006. https://doi.org/10.1111/jvs.12772

McNeely JA, Mooney HA, Neville LE, et al (eds) (2001) Global Strategy on Invasive Alien Species. IUCN, Gland, Switzerland, and Cambridge, UK

Minchin D, Gollasch S, Cohen AN, et al (2009) Characterizing Vectors of Marine Invasion. In: Rilov G, Crooks JA (eds) Biological Invasions in Marine Ecosystems. Springer, Berlin, Heidelberg, pp 109–116

Nakaoka M, Aioi K (2001) Ecology of seagrasses *Zostera* spp. (Zosteraceae) in Japanese waters: A review. Otsuchi Mar Sci 26:7–22

Nguyen HM, Ralph PJ, Marín-Guirao L, et al (2021) Seagrasses in an era of ocean warming: a review. Biol Rev 7:. https://doi.org/10.1111/brv.12736

Nordlund LM, Koch EW, Barbier EB, Creed JC (2016) Seagrass ecosystem services and their variability across genera and geographical regions. PLoS One 11:e0163091. https://doi.org/10.1371/journal.pone.0163091

Novak SJ (2007) The role of evolution in the invasion process. Proc Natl Acad Sci 104:3671–3672. https://doi.org/10.1073/pnas.0700224104

Novy A, Flory SL, Hartman JM (2013) Evidence for rapid evolution of phenology in an invasive grass. J Evol Biol 26:443–450. https://doi.org/10.1111/jeb.12047

Ogden NH, Wilson JRU, Richardson DM, et al (2019) Emerging infectious diseases and biological invasions: a call for a One Health collaboration in science and management. R Soc Open Sci 6:181577. https://doi.org/10.1098/rsos.181577

Olsen JL, Rouzé P, Verhelst B, et al (2016) The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. Nature 530:331–335. https://doi.org/10.1038/nature16548

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

Orth RJ, Lefcheck JS, McGlathery KS, et al (2020) Restoration of seagrass habitat leads to rapid recovery of coastal ecosystem services. Sci Adv 6:1–10. https://doi.org/10.1126/sciadv.abc6434

Parker JD, Torchin ME, Hufbauer RA, et al (2013) Do invasive species perform better in their new ranges? Ecology 94:985–994. https://doi.org/10.1890/12-1810.1

Pyšek P, Richardson DM (2007) Traits Associated with Invasiveness in Alien Plants: Where Do we Stand? In: Nentwig W (ed) Biological Invasions. Springer-Verlag, Berlin, Heidelberg, pp 97–125

Reynolds PL, Stachowicz JJ, Hovel K, et al (2018) Latitude, temperature, and habitat complexity predict predation pressure in eelgrass beds across the Northern Hemisphere. Ecology 99:29–35. https://doi.org/10.1002/ecy.2064

Ricciardi A (2007) Are modern biological invasions an unprecedented form of global change? Conserv Biol 21:329–336. https://doi.org/10.1111/j.1523-1739.2006.00615.x

Richardson DM, Pysek P, Rejmanek M, et al (2000) Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib 6:93–107. https://doi.org/10.1046/j.1472-4642.2000.00083.x

Rilov G, Crooks JA (2009) Biological Invasions in Marine Ecosystems. Springer, Berlin, Heidelberg

Roca G, Alcoverro T, Krause-Jensen D, et al (2016) Response of seagrass indicators to shifts in environmental stressors: A global review and management synthesis. Ecol Indic 63:310–323. https://doi.org/10.1016/j.ecolind.2015.12.007

Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biol Invasions 8:927–939. https://doi.org/10.1007/s10530-005-5103-3

Ruesink JL, Hong J-S, Wisehart L, et al (2010) Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Biol Invasions 12:1773–1789. https://doi.org/10.1007/s10530-009-9588-z

Ruesink JL, Stachowicz JJ, Reynolds PL, et al (2018) Form-function relationships in a marine foundation species depend on scale: a shoot to global perspective from

a distributed ecological experiment. Oikos 127:364–374. https://doi.org/10.1111/oik.04270

Ruiz GM, Fofonoff PW, Carlton JT, et al (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu Rev Ecol Syst 31:481–531. https://doi.org/10.1146/annurev.ecolsys.31.1.481

Sakai AK, Allendorf FW, Holt JS, et al (2001) The Population Biology of Invasive Species. Annu Rev Ecol Syst 32:305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sax DF, Stachowicz JJ, Brown JH, et al (2007) Ecological and evolutionary insights from species invasions. Trends Ecol Evol 22:465–471. https://doi.org/10.1016/j.tree.2007.06.009

Schlaepfer MA, Sax DF, Olden JD (2011) The potential conservation value of non-native species. Conserv Biol 25:428–437. https://doi.org/10.1111/j.1523-1739.2010.01646.x

Seebens H, Essl F, Dawson W, et al (2015) Global trade will accelerate plant invasions in emerging economies under climate change. Glob Chang Biol 21:4128–4140. https://doi.org/10.1111/gcb.13021

Shafer DJ, Kaldy JE (2014) Comparison of photosynthetic characteristics of the seagrass congeners *Zostera marina* L. And *Zostera japonica* Ascher. & Graeb. Aquat Bot 112:91–97. https://doi.org/10.1016/j.aquabot.2013.09.002

Shafer DJ, Kaldy JE, Gaeckle JL (2014) Science and management of the introduced seagrass *Zostera japonica* in North America. Environ Manage 53:147–162. https://doi.org/10.1007/s00267-013-0172-z

Shafer DJ, Kaldy JE, Sherman TD, Marko KM (2011) Effects of salinity on photosynthesis and respiration of the seagrass *Zostera japonica*: A comparison of two established populations in North America. Aquat Bot 95:214–220. https://doi.org/10.1016/j.aquabot.2011.06.003

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

Sotka EE, Baumgardner AW, Bippus PM, et al (2018) Combining niche shift and population genetic analyses predicts rapid phenotypic evolution during invasion. Evol Appl 11:781–793. https://doi.org/10.1111/eva.12592

Sudo K, Quiros TEAL, Prathep A, et al (2021) Distribution, temporal change, and conservation status of tropical seagrass beds in Southeast Asia: 2000-2020. Front Mar Sci 8:637722. https://doi.org/10.3389/fmars.2021.637722

Tanaka N (2012) Itan no Shokubutsu “Mizukusa” wo Kagaku suru (Scientific evaluation of outlier in plants “Aquatic Plants”). Beret, Tokyo, Japan

Thom R, Miller B, Kennedy M (1995) Temporal patterns of grazers and vegetation in a temperate seagrass system. Aquat Bot 50:201–205

Tomasko D, Alderson M, Burnes R, et al (2018) Widespread recovery of seagrass coverage in Southwest Florida (USA): Temporal and spatial trends and management actions responsible for success. Mar Pollut Bull 135:1128–1137. https://doi.org/10.1016/j.marpolbul.2018.08.049

Turschwell MP, Connolly RM, Dunic JC, et al (2021) Anthropogenic pressures and life history predict trajectories of seagrass meadow extent at a global scale. Proc Natl Acad 118:e2110802118. https://doi.org/10.1073/pnas.2110802118/-/DCSupplemental.Published

United Nations Environment Programme (2020) OUT OF THE BLUE THE VALUE OF SEAGRASSES. UNEP, Nairobi

Unsworth RKF, McKenzie LJ, Collier CJ, et al (2019) Global challenges for seagrass conservation. Ambio 48:801–815. https://doi.org/10.1007/s13280-018-1115-y

van Katwijk MM, Thorhaug A, Marbà N, et al (2016) Global analysis of seagrass restoration: The importance of large-scale planting. J Appl Ecol 53:567–578. https://doi.org/10.1111/1365-2664.12562

van Kleunen M, Dawson W, Essl F, et al (2015a) Global exchange and accumulation of non-native plants. Nature 525:100–103. https://doi.org/10.1038/nature14910

van Kleunen M, Dawson W, Maurel N (2015b) Characteristics of successful alien plants. Mol Ecol 24:1954–1968. https://doi.org/10.1111/mec.13013

van Kleunen M, Weber E, Fischer M (2010a) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecol Lett 13:235–245. https://doi.org/10.1111/j.1461-0248.2009.01418.x

van Kleunen M, Dawson W, Schlaepfer D, et al (2010b) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958. https://doi.org/10.1111/j.1461-0248.2010.01503.x

Viana IG, Siriwardane-de Zoysa R, Willette DA, Gillis LG (2019) Exploring how non-native seagrass species could provide essential ecosystems services: a perspective on the highly invasive seagrass *Halophila stipulacea* in the Caribbean Sea. Biol Invasions 21:1461–1472. https://doi.org/10.1007/s10530-019-01924-y

Vilà M, Espinar JL, Hejda M, et al (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. Ecol Lett 14:702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x

Vivó-Pons A, Alós J, Tomas F (2020) Invasion by an ecosystem engineer shifts the abundance and distribution of fish but does not decrease diversity. Mar Pollut Bull 160:111586. https://doi.org/10.1016/j.marpolbul.2020.111586

Watari Y, Takatsuki S, Miyashita T (2008) Effects of exotic mongoose (*Herpestes javanicus*) on the native fauna of Amami-Oshima Island, southern Japan,

estimated by distribution patterns along the historical gradient of mongoose invasion. Biol Invasions 10:7. https://doi.org/10.1007/s10530-007-9100-6

Watari Y, Komine H, Angulo E, et al (2021) First synthesis of the economic costs of biological invasions in Japan. NeoBiota 67:79–101. https://doi.org/10.3897/neobiota.67.59186

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

Waycott M, van Dijk KJ, Calladine A, et al (2021) Genomics-based phylogenetic and population genetic analysis of global samples confirms *Halophila johnsonii* Eiseman as *Halophila ovalis* (R.Br.) Hook.f. Front Mar Sci 8:1–13. https://doi.org/10.3389/fmars.2021.740958

Williams SL, Heck Jr. KL (2001) Seagrass comunity ecology. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Associates, Inc., Sunderland, MA, pp 317–337

Williams SL (2007) Introduced species in seagrass ecosystems: Status and concerns. J Exp Mar Bio Ecol 350:89–110. https://doi.org/10.1016/j.jembe.2007.05.032

Winters G, Beer S, Willette DA, et al (2020) The tropical seagrass *Halophila stipulacea*: Reviewing what we know from its native and invasive habitats, alongside identifying knowledge gaps. Front Mar Sci 7:300. https://doi.org/10.3389/fmars.2020.00300

Wong M, Vercaemer B (2012) Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (*Zostera marina*). Aquat Invasions 7:315–326. https://doi.org/10.3391/ai.2012.7.3.003

Wonham MJ, Carlton JT (2005) Trends in marine biological invasions at local and regional scales: the Northeast Pacific Ocean as a model system. Biol Invasions 7:369–392. https://doi.org/10.1007/s10530-004-2581-7

York PH, Booth DJ, Glasby TM, Pease BC (2006) Fish assemblages in habitats dominated by *Caulerpa taxifolia* and native seagrasses in south-eastern Australia. Mar Ecol Prog Ser 312:223–234. https://doi.org/10.3354/meps312223

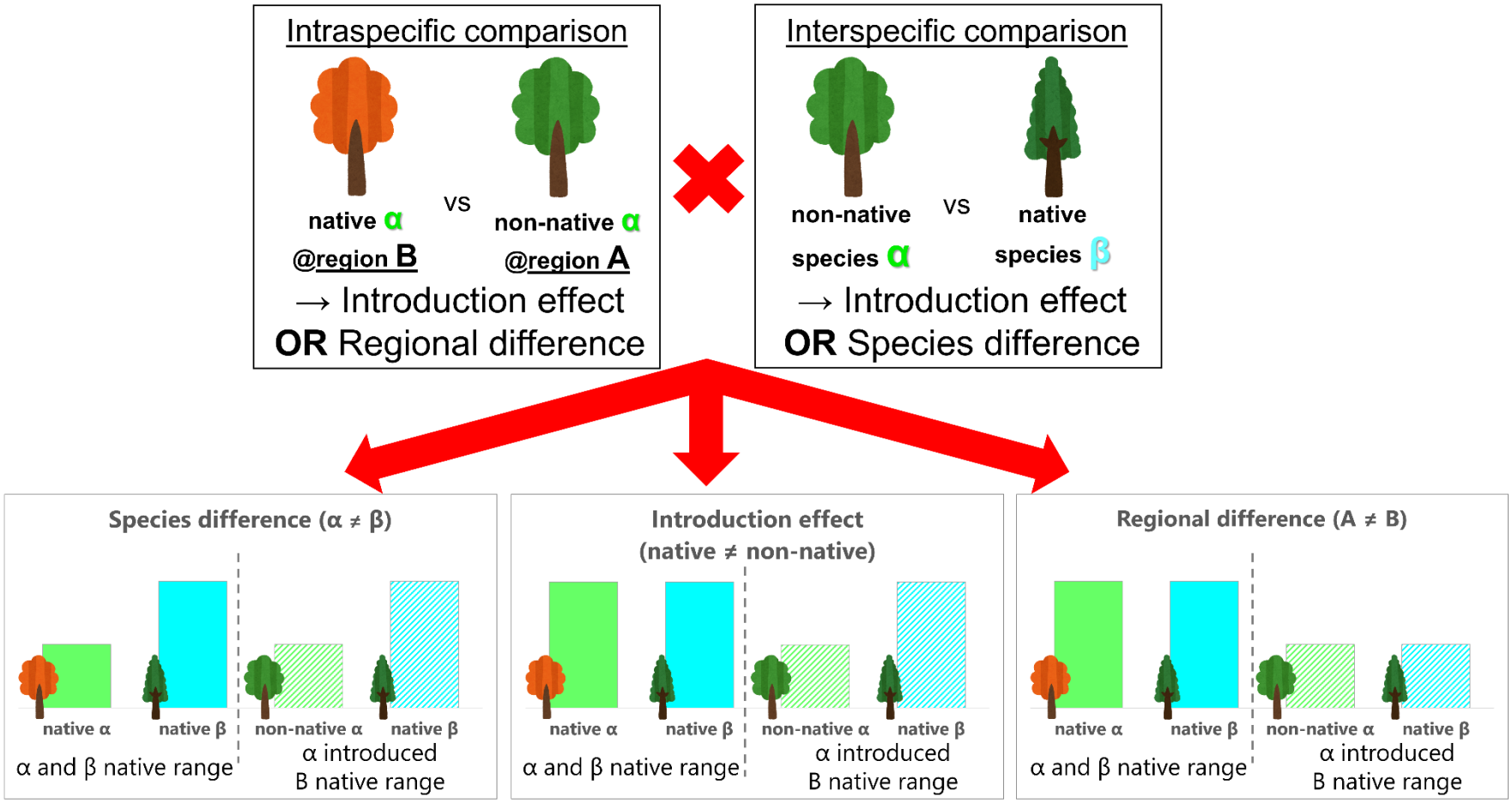

Figure 1.1. Schematic graphics showing limitations of comparison methods used alone and how proposed combined methods approach can improve them.

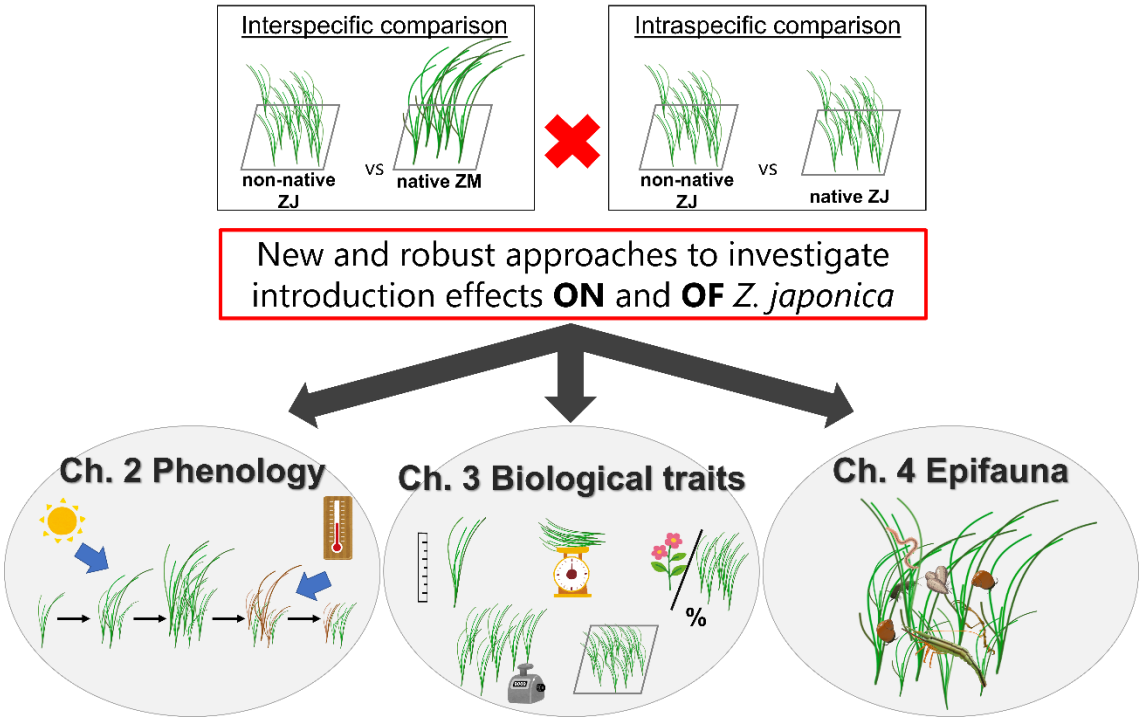

Figure 1.2. Schematic graphics showing combined approaches proposed in this thesis and three seagrass aspects focused on each chapter.

Chapter 2

Large-scale comparison of biomass and reproductive phenology among native and non-native populations of the seagrass *Zostera japonica*

The work presented in Chapter 2 appears in:

Ito MA, Lin H-J, O'Connor MI, Nakaoka M (2021) Large-scale comparison of biomass and reproductive phenology among native and non-native populations of the seagrass *Zostera japonica*. *Marine Ecology Progress Series* **675:** 1–21. https://doi.org/10.3354/meps13884

Abstract: Large-scale analysis along latitude or temperature gradients can be an effective method for exploring the potential roles of light and temperature in controlling seagrass phenology. In this study, I investigated effects of latitude and temperature on seagrass biomass and reproductive seasonality. *Zostera japonica* is an intertidal seagrass with a wide latitudinal distribution expanding from tropical to temperate zones in its native range in Asia, with an additional non-native distribution in North America. I collated available data on phenological traits (timings of peak biomass or reproduction, durations of biomass growth and reproductive season, and maximum biomass or reproductive ratio) from publications and my own observations. Traits were compared among geographic groups: Asia-tropical, Asia-temperate, and North America-temperate. I further examined relationships between traits and latitude and temperature for 3 population groups: Asian, North American, and all populations. The analysis revealed significant variation among geographic groups in maximum biomass, peak reproductive timing, and maximum reproductive ratio, but not in other traits. Maximum biomass and peak reproductive timing for Asian and all populations were significantly correlated with latitude and temperature. Maximum biomass was highest at mid-latitudes or intermediate temperatures and decreased toward distribution range limits, and peak reproductive timing occurred later in the year at higher latitudes or cooler sites. North American populations showed shorter growth durations and greater reproductive ratios at higher latitude. Different responses observed for North American populations may reflect effects of introduction. This study demonstrates potential variation among geographic regions and between native and non-native populations.

2.1 Introduction

Seagrasses are marine angiosperms that form extensive meadows in shallow coastal environments, which are known as seagrass beds (Hemminga and Duarte 2000). Seagrass meadows provide multiple important ecosystem services and are recognized as one of the most valuable habitats in the world (Costanza et al. 1997). Despite their importance, seagrasses are rapidly declining worldwide due to various types of anthropogenic stressors and disturbances that act at different scales (Orth et al. 2006; Waycott et al. 2009). While most stressors and disturbances generally affect seagrasses at local scales, ongoing climate change is considered to substantially impact seagrasses globally. Increased temperatures are expected to shift seagrass distribution to higher latitudes, alter growth rates and other physical functions, and change sexual reproduction patterns (Short and Neckles 1999; Duarte 2002; Hyndes et al. 2016). To predict future changes in seagrass meadows and to promote effective conservation, it is important to understand how life history traits of each seagrass species vary with environmental conditions.

Seagrasses maintain their populations through both vegetative growth and sexual reproduction (Hemminga and Duarte 2000; Kendrick et al. 2012). Abiotic factors such as temperature, light, and nutrients play key roles in regulating both vegetative growth and flowering in seagrasses (Lee et al. 2007). The relative importance of these factors varies among species and location. For example, the seagrass *Posidonia oceanica* exhibits growth patterns that are primarily controlled by large-scale variation in the solar cycle and are less affected by local environmental variability (Alcoverro et al. 1995; Marbà et al. 1996). Pergent-Martini et al. (2005) found that the effects of nutrients on *Zostera noltii* growth and biomass vary among locations. Other studies found that seagrass growth

dynamics are more strongly affected by temperature than irradiance (Lee et al. 2005; Hosokawa et al. 2009). Variation among the findings of studies conducted at local scales makes it difficult to generalize effects of abiotic factors on seagrass phenology (the timing of recurring life history events).

Large-scale analyses covering broad environmental gradients are effective tools for understanding how abiotic factors affect the phenology of terrestrial species (e.g., Zhang et al. 2004; Primack et al. 2009; Gill et al. 2015), and such approaches are increasingly used to study marine species (Racault et al. 2012; Tala and Chow 2014; James et al. 2015). Furthermore, such large-scale analyses can be useful for predicting species' responses to ongoing or historical climate change through space-for-time substitution (Fukami and Wardle 2005; Buyantuyev et al. 2012). The amount of light available on a certain day of the year depends on latitude; temperature also shows a high correlation with latitude but varies more with local influences (Lalli and Parsons 1997). Therefore, studies that encompass wide latitudinal ranges could lead to insights on both light and temperature effects.

To date, several studies covering wide latitudinal ranges have been conducted for seagrass biomass, a proxy for growth condition, and reproduction. Duarte (1989) found that temperate seagrasses commonly showed unimodal patterns of biomass throughout the year, with peak biomass occurring in summer, with the timing of peak biomass influenced by its latitudinal distribution. Comparisons of multiple *Zostera* species revealed latitudinal influences on flowering duration and peak timing (Phillips et al. 1983; Ramage and Schiel 1998; Nakaoka and Aioi 2001; Dos Santos and Matheson 2017). Recent studies of *Z. marina* revealed that biomass and flowering patterns are strongly influenced by latitude and temperature; biomass and flowering peaks occurred

earlier for low-latitude/warmer populations, while growth and flowering durations are constant across latitude (Clausen et al. 2014; Blok et al. 2018). However, tropical seagrasses show different characteristics in their seasonal pattern which are occasionally independent from light and temperature and are instead affected by factors such as riverine influence, tidal exposure, or water motion (Erftemeijer and Herman 1994; Huong et al. 2003). Furthermore, intertidal seagrass species are suggested to be more susceptible to change in temperature and/or light than subtidal species (Marbà et al. 1996). In fact, manipulative experiments testing light and temperature effects on intertidal species suggested that seagrass growth rate is a function of duration of exposure to warm temperature, and light is not the dominant factor controlling growth (Shafer and Kaldy 2014; Kaldy et al. 2015b). Although there is an increasing understanding of general phenological patterns observed in seagrasses, species-specific responses to global factors are not well investigated for intertidal and/or tropical seagrasses.

Among species belonging to the genus *Zostera*, whose distribution is mostly restricted to temperate ocean regions, *Z. japonica* has an exceptionally wide native distribution range extending from the tropical coast of Vietnam (10° N) to Kamchatka, Russia (55° N) on the western coast of the Pacific Ocean (Green and Short 2003). In addition to its native habitat, *Z. japonica* also extends its distribution to the eastern coast of the Pacific Ocean in North America, where it is believed to have been introduced to that region with the Pacific oyster (Harrison and Bigley 1982). Non-native populations often show different traits (i.e., higher growth rate, extended flowering duration, higher reproductive output) than their native populations, suggesting trait changes during introduction processes (Mason et al. 2008; Leffler et al. 2014). However, introduction effects on phenology are context dependent; some non-native species maintain

phenological traits from their native habitats even after being introduced to new region (Godoy et al. 2009), whereas other non-native species show higher phenological plasticity, enabling them to adapt to environmental change (Zettlemoyer et al. 2019). Observations of both native and non-native populations could therefore provide insights for how *Z. japonica* phenological traits are affected by introduction. Due to its wide latitudinal distribution in its native region, additional habitat in its non-native region, and habitat preference for fluctuating intertidal environment, *Z. japonica* is considered an ideal organism for large-scale analyses on latitudinal and temperature effects on its phenological traits.

The aim of this study was to examine broad-scale variation in phenology of the intertidal seagrass *Z. japonica* throughout its distributional range. I systematically reviewed and synthesized data from published literature on *Z. japonica* phenological traits (biomass and reproductive traits). Synthesized data were further analyzed to test for variation among different geographic groups, namely Asia-tropical, Asia-temperate, and North America-temperate, and evaluated for the effects of latitude and temperature. Through the analyses, I specifically tried to answer the following 3 questions: (1) How do *Z. japonica* phenological traits vary among geographic groups? (2) How are the traits influenced by latitude or temperatures? and (3) Is there a difference between native and non-native populations, suggesting an introduction effect?

2.2 Materials and methods

2.2.1 Data collection and synthesis

I systematically reviewed research articles to build a dataset for assessing seasonal changes in seagrass biomass and/or reproduction. I used ISI Web of Science to

conduct the literature search by using seagrass species names as key words. Considering past changes in taxonomic identity, I also included currently unaccepted species names as search terms. Final key search terms were: 'Zostera japonica' OR 'Zostera nana' OR 'Zostera americana'. To include articles written in Japanese, I used J-STAGE (www.jstage.jst.go.jp) to collect additional articles. For searches on J-STAGE, 'Zostera japonica' and the Japanese name 'koamamo' were used. Both literature searches on the Web of Science and on J-STAGE were conducted on 26 January 2020. Searches resulted in 137 articles from the Web of Science and 220 from J-STAGE. Cross-referencing articles revealed additional papers, including a study written in Korean (Ok and Lee 2014). In addition to data collected from research articles, I used seasonal data collected by my research group to minimize gaps in the data coverage for the analysis (Table S2.1). To the best knowledge, no seasonal data were available for populations in Russia, which is the northern range limit of *Zostera japonica*.

For each research article, I first skimmed through the abstract for relevant information pertaining to this study. With the relevant articles, I then collated data on seagrass biomass and reproductive seasonality. To ensure a standard quality of data, I further filtered the articles based on the following criteria: (1) articles must contain one or more variables on phenological traits and where and how they were collected (see Section 2.2.2 for details), (2) only data collected in situ are used and data from laboratory experiments are excluded to assure a strong influence from environmental conditions, and (3) if an article is based on a manipulative experiment, only data from the control or untreated samples are used. If an article contained more than 1 year of data, the data were either merged or separated into multiple datasets, depending on whether the number of data points available per year exceeded 5 or not (see Section 2.2.2 for descriptions). If an

article contained data from more than a single site, data from each site were analyzed separately to increase spatial heterogeneity in the dataset. Therefore, some articles contributed more than 1 dataset in the compiled data (Table 2.1).

After systematic review and quality control, I collated 24 articles and 7 datasets from my research group on seagrass phenological traits, covering a wide range in latitude and temperature, and from both native and non-native populations (Table 2.1, Fig. 2.1; Table S2.1). Compiled datasets were divided into 3 groups based on the geographic distribution of *Z. japonica*: the Asia-tropical group consists of native populations at latitudes <30° N in Asia, the Asia-temperate group includes native populations at latitudes >30° N in Asia, and the North America-temperate group includes non-native populations in North America. Spatial and temporal variations in the datasets differed greatly among geographic groups; the Asia-tropical and North America-temperate groups showed a narrower range of latitudinal and temperature variation compared to the Asia-temperate group, while the North America-temperate group showed the largest variation in temporal scales spanning from 1978 to 2018 (Tables 2.1 & 2.2, Fig. 2.1). The North American group only included temperate populations, as there are no reports on non-native *Z. japonica* distributed in tropical areas. In southern Japan, seagrass species composition drastically changes from temperate to tropical around 30° N (Kawano et al. 2012) and thus 30° N was selected as the cut-off point between tropical and temperate regions.

2.2.2 Biomass and reproductive trait acquisition

Differences in seagrass biomass and reproduction seasonality among geographic groups or populations were evaluated in the form of timings of peak biomass/reproduction, durations of biomass growth/reproductive season, and maximum biomass/reproductive

ratio observed. Biomass was used as a proxy reflecting seagrass vegetative growth. From each article, I extracted data from tables or the main text whenever possible. If data were only available in a figure and not provided in any numerical form, a web-based graphical digitizer was used to extract data (WebPlotDigitizer version 4.2; Rohatgi 2019). After assigning fixed values, acquired from the original figure and calibrating the digitizer axes, I used manual methods to extract each data point from the figure by directly clicking on the graph.

For the expected unimodal annual biomass pattern, one can analyze biomass seasonality by fitting quadratic equations to seasonal data, based on the method described by Clausen et al. (2014). The peak biomass timing was determined using the x-coordinate of a parabola vertex and the growth duration using 2 parabola roots (Clausen et al. 2014). Start and end of biomass growth season corresponded with 2 parabola roots, and therefore the number of days between the 2 roots was considered the growth duration. To minimize uncertainty in estimated relationships, I used a minimum of 5 datapoints per dataset. Although a quadratic fit can be applied to any variable that shows a unimodal pattern, I only used data on dry weight of above-ground biomass (AGB) whenever available to maintain consistent biological measurements in the data. However, these strict limitations would result in reduced analytical power and hence I also used other variables such as total biomass, ash-free dry weight, and density, but only if AGB was not provided in the article. The quadratic fit performed well with my dataset, with a mean and median r-squared value (r^2) of 0.79 and 0.81, respectively. However, I excluded data with a criterion of $r^2 = 0.65$, as suggested in studies of unimodal curve fitting (Frisén 1986; Murtaugh 2003). I retained approximately 85% (62 out of 73) of all datasets, and there was no obvious bias in regional groups or latitude/temperature that showed a poor quadratic fit.

The method used here to acquire biomass peak and growth duration are in consistent with the method used in a previous study on *Z. marina* by Clausen et al. (2014).

For the comparison of maximum biomass, I used values obtained from the articles rather than using values estimated from the quadratic equation. For maximum biomass, I used AGB if available; if not, AGB was calculated from data on total biomass and either of above- to below-ground biomass ratios or below-ground biomass, whichever was available. Most data on AGB were presented in g m^{-2}, and if not, units were converted to g m^{-2} to make direct comparisons possible. For the sites with multiple years of observations, only the maximum biomass observed per site was used in the analysis.

Seagrass biomass was reported quantitatively in most of the articles, making it possible to conduct analysis using the quadratic fit. However, seagrass sexual reproduction was usually only reported when reproductive shoots were present, and the absence of reproductive shoots was rarely stated, making it impossible to apply the quadratic fit to annual datasets. There are a variety of ways to report seagrass reproduction: reproductive shoot density, biomass, ratio (both density- or biomass-based) or verbally (presence/absence). For a verbal statement like 'Sexual reproduction is also limited to a brief period in the year, between March and May' (Lee 1997, p. 190), I was unable to extract any information other than timing and duration of the reproductive season since there was no statement about the reproductive peak or maximum reproductive ratio. Nonetheless, I included information to increase sample size and analytical strength. I did not include different reproductive stages (i.e., spathe emergence, flowering, or fruiting) due to limited information available in most articles.

For the duration of the reproductive season, I used the number of days between the start (i.e., when a reproductive shoot was first observed) to the end (i.e., when a

reproductive shoot was last observed) of the year. When data were available in verbal form, I used mid-month (15th day of the month) as the date, unless the article stated an early or late period in a certain month, in which case the 5th and 25th day of the month were used, respectively.

I extracted or calculated reproductive ratio from the articles. There are 2 common methods to calculate reproductive ratio: density-based or biomass-based. I used both ratio types for analysis of reproductive ratio. If both types were available from the same study, I used only density-based data. Maximum reproductive ratio observed per site was used in the analysis.

2.2.3 Latitude and temperature data

Geographical coordinates, or latitude and longitude, of each site were directly obtained from the original publications. When authors of the original publications did not provide the coordinates, coordinates were georeferenced from the maps or text in the publications. Based on the obtained site coordinates, I extracted site-specific air temperatures from WorldClim Version 2 (www.worldclim.org; Fick and Hijmans 2017), as a monthly mean temperature over a 30 yr period (1970–2000). Although the analyzed temperature data spanned 30 yr, I confirmed that there was no consistent trend in temperature during this period or between old and recent temperature from the same study sites with large temporal variation (Roberts Bank and Boundary Bay, Canada; data not shown). Similarly, water temperature data were derived from MODIS-Aqua 11 μm daytime sea surface temperature (NASA 2014), as monthly mean temperatures from 4 July 2002 to 25 January 2020. Both WorldClim and MODIS-Aqua were accessed on 26 January 2020.

Intertidal seagrasses are influenced by both air and water temperatures (Lee et al. 2005). However, only air temperature was used for analysis in this study, due to its high correlation with water temperature measured in situ and scientific support from previous research. Among the articles I examined, 6 publications reported water temperatures measured in situ (Lee et al. 2005, 2006; Park et al. 2011; Zhang et al. 2015; Kim et al. 2016; Suonan et al. 2017). When on-site water measurements were compared with extracted air and water temperatures, half of the sites showed higher correlations with extracted air temperatures while the other half showed higher correlations with extracted water temperatures (data not shown). Across studies with on-site temperatures, average coefficient of determination was also high between on-site temperatures and extracted air temperature ($n = 6$, $r^2 = 0.80$). Additionally, Clausen et al. (2014) showed that air temperature has a significant effect on the seasonality of subtidal populations of the seagrass *Z. marina*. Therefore, I can justify using air temperature data to test for a correlation between temperature and intertidal seagrass seasonality.

From monthly mean temperatures, I calculated the annual mean (average from January to December), summer mean (June to August), and winter mean (December to February) for each site. These values were used in the rest of the analyses. I conducted a separate analysis for each mean temperature variable, to evaluate and distinguish the effects of temperatures in different seasons.

Prior to the analysis, the relationships between latitude and each temperature variable were investigated (Figs. S2.1 & S2.2). As expected, I found a high negative correlation between latitude and temperature (Pearson's correlation analysis), where increases in latitude were associated with decreases in temperature. While the amount of light available and photoperiod are determined exclusively by latitude, substantial

variation in temperature is attributable to other local factors (Clausen et al. 2014), and thus comparison of latitude with temperature allowed me to differentiate the influence of light vs. temperature. Additionally, relationships between variables differed significantly among seagrass geographic groups. The North America-temperate group showed a reversed relationship that summer temperature increases as latitude increases, since it is driven by coastal upwelling events along the western coast of North America (Bograd et al. 2009). Among-group variation in latitude and temperature relationship would enable me to distinguish the primary driver of variation in phenological traits.

I initially used photosynthetically available radiation (PAR) data from NASA MODIS-Aqua for a measure of light availability in terms of total radiation received in a day. However, analysis using PAR is not presented because it was mostly consistent with the latitude analysis (data not shown) and did not yield new findings. Therefore, I only used latitude as a proxy for light availability in terms of daylength (total hours of sunlight).

2.2.4 Statistical analysis and inference

All analyses were performed in R version 3.6.2 (R Core Team 2019). I expected significant among-group variation for traits affected by latitude/temperature or altered during introduction. To assess whether phenological traits differ among the 3 geographic groups, I conducted a non-parametric Kruskal-Wallis rank sum test for each trait. The non-parametric test was chosen because all trait variables violated assumptions for parametric tests; data were not normally distributed and/or showed non-homogeneous variances among groups. To evaluate the effect size of each among-group variation, eta-squared based on the *H*-statistic was also calculated for a Kruskal test using the 'kruskal_effsize' function in the R package 'rstatix' (Kassambara 2020). Eta-squared is

calculated as sums of squares for the effect of interest divided by the total sums of squares (Cohen 1973), and magnitudes of effect are interpreted as follows: 0.01 to <0.06 (small effect), 0.06 to <0.14 (medium), and ≥0.14 (large) (Maher et al. 2013; Kassambara 2020). When a significant ($p < 0.05$) effect of geographic group was found, I performed Dunn's multiple comparison test to examine which group or groups showed the significant difference, using the 'dunnTest' function in the R package 'FSA' (Ogle et al. 2020), with the p-value adjusted using the Benjamini-Hochberg method.

I expected significant relationships between latitude or temperature and traits influenced by light and temperature, and expected that the trends would differ between native and non-native populations, if the traits are altered during introduction. To test whether there is a difference between native and non-native groups, I analyzed Asian and North American populations separately. Due to limitations in data from the tropical region, I could not conduct a separate analysis for the tropical populations. Prior to the main analysis, I compared trends between the Asian populations with and without the tropical group; combining of the tropical group would significantly decrease explanatory power (R^2) if trends differ between the tropical and temperate groups, whereas combining would strengthen explanatory power if trends are consistent. Combining the tropical and temperate Asian groups did not significantly reduce explanatory strength, but rather increased strength in most cases, indicating a similar trend among the Asian populations. Furthermore, I investigated latitude and temperature effects on all populations by combining Asian and North American populations together, to evaluate if *Z. japonica* as a species shows consistent trends across its entire distributional range. Hence, I focused on comparison among native and non-native populations (Asian vs. North American populations), and all populations for evaluation of latitude/temperature effects. To

evaluate the effects of latitude and annual, summer, and winter mean temperature parameters on seagrass biomass and reproduction, and to examine how trends differ among population groups, I performed linear and quadratic regression analyses.

To assess latitude and temperature effects on phenological traits, I first examined if the regression model with latitude or temperature was better than the null model without the explanatory variable. The best regression model was selected based on Akaike's information criterion corrected for small sample size (AICc) using the 'dredge' function in the R package 'MuMIn' (Barton 2019). If the models with predictors were better than the null models (ΔAICc > 2), I then compared AICc for linear and quadratic models and selected the best models; quadratic models were only selected if their ΔAICc was lower than the linear models. For each trait, the best predictor among latitude and temperature was determined based on adjusted r-squared (R^2) values; the parameter with the highest R^2 was selected as the most influential variable for variation in the phenological traits. Significance of fit based on the p-value and goodness of fit using an adjusted r-squared value (R^2) were used for the examination. Cohen (1988) suggested that R^2 values are interpreted as follows: 0.26 (substantial), 0.13 (moderate), and 0.02 (weak). In this sense, results with $R^2 > 0.13$ were considered significantly addressing a good fit. When the quadratic model was selected as the best model, the quadratic equation was solved to calculate the latitude/temperature value where the quadratic maximum/minimum occurs.

2.3 Results

Compiled datasets covered a wide latitudinal range, from 20.9 to 49.1°N. Annual mean air temperature ranged from 6.2 to 22.9°C, summer mean air temperature ranged from 14.7 to 28.4°C, and winter mean air temperature ranged from –4.9 to 16.9°C (Tables

2.1 & 2.2). Latitude and temperature were significantly different among seagrass geographic groups except for the winter temperature between the North America-temperate and Asia-temperate groups (Table 2.2; Fig. S2.1).

2.3.1 Comparisons of biomass and reproductive traits among geographic groups

Mean ± SD peak biomass timing for Asia-tropical, Asia-temperate, and North America-temperate groups were day of year (DOY) 174.9 ± 90.3, 207.4 ± 38.3, and 209.7 ± 28.5, respectively, and the differences among the groups were not significant (Kruskal-Wallis test: chi-squared [χ^2] = 2.67, df = 2, p = 0.26, eta-squared [η^2] = 0.01; Fig. 2.2A). Average growth duration for Asia-tropical, Asia-temperate, and North America-temperate groups were 192.6 ± 54.6, 207.2 ± 68.1, and 199.6 ± 62.8 d, respectively. Variations in growth duration were not significantly different among the groups (χ^2 = 0.14, df = 2, p = 0.93, η^2 = –0.03; Fig. 2.2B). Maximum biomass showed significant differences among the groups (χ^2 = 23.38, df = 2, p < 0.001, η^2 = 0.44; Fig. 2.2C). Average maximum biomass was significantly higher in Asia-temperate (154.0 ± 75.1 g m^{-2}) than North America-temperate (60.0 ± 27.6 g m^{-2}; Dunn's test p < 0.001) and Asia-tropical groups (42.6 ± 20.5 g m^{-2}; p < 0.001). The difference between Asia-tropical and North America-temperate groups was not significant (p = 0.33).

Peak reproductive timing was significantly different among geographic groups (χ^2 = 23.76, df = 2, p < 0.001, η^2 = 0.44; Fig. 2.2D). The peak reproductive timing was earliest for the Asia-tropical group (DOY 119.9 ± 22.1), followed by the Asia-temperate (DOY 183.5 ± 57.1) and lastly the North America-temperate group (DOY 231.3 ± 31.2). All differences between each pair of groups were significant (Asia-tropical vs. Asia-temperate: p < 0.05; Asia-tropical vs. North America-temperate: p < 0.001; Asia-

temperate vs. North America-temperate: $p < 0.01$). Average reproductive duration for Asia-tropical, Asia-temperate, and North America-temperate groups was 125.6 ± 86.0, 135.7 ± 51.0, and 106.5 ± 45.6 d, respectively. Among-group variation in reproductive duration was not significant ($\chi^2 = 2.39$, df = 2, $p = 0.30$, $\eta^2 = 0.01$; Fig. 2.2E). For reproductive ratios, we performed analyses with and without data from Park et al. (2011) for Koje Bay, where 63% of shoots flowered in the first year after clamming activity (Fig. 2.2F; Fig. S2.3). Original data including this outlier showed marginally significant variation of reproductive ratio among geographic groups ($\chi^2 = 5.07$, df = 2, $p = 0.08$, $\eta^2 = 0.07$; Fig. S2.3), however, when I removed the outlier, the trend became significant ($\chi^2 = 7.13$, df = 2, $p < 0.05$, $\eta^2 = 0.12$; Fig. 2.2F). Mean reproductive ratios for each group were 17.3 ± 10.8% for Asia-tropical and 23.6 ± 12.9% for North America-temperate groups, whereas the Asia-temperate group had values of 15.9 ± 15.1 and 12.7 ± 8.7% with and without the outlier, respectively. Analysis without the outlier revealed a significant difference between the Asia-temperate and North America-temperate groups ($p < 0.05$), but not between the other combinations (Asia-tropical vs. Asia-temperate: $p = 0.35$, Asia-tropical vs. North America-temperate: $p = 0.44$; Fig. 2.2F).

2.3.2 Effects of latitude and temperature on biomass and reproductive traits

Variation in biomass and reproductive traits were compared along latitude and temperature gradients, and relationships between traits and latitude or temperature were examined through regression analysis and model selection (see Figs. 2.3 & 2.4). The best models were selected based on AICc for each pair of biomass or reproductive traits and latitude or temperature, which are summarized in Table 2.3. Additional information on linear and quadratic regression models is provided in Table S2.2.

Peak biomass timing was not affected by latitude or temperature in any geographical group ($R^2 < 0.13$ for all combinations; Fig. 2.3A–D, Table 2.3; Table S2.2). For peak biomass timing, the model fits were statistically significant or marginally significant ($p < 0.05$) for Asian and all populations in linear regression models, but the linear models were not selected as the best model (Table S2.2). Growth durations for the North American populations were negatively correlated with latitude and summer mean temperatures (latitude: $F_{1,23} = 14.99$, $p < 0.001$, $R^2 = 0.37$; summer mean: $F_{1,23} = 5.71$, $p < 0.05$, $R^2 = 0.16$; Fig. 2.3E,G; Table S2.2) and positively with annual and winter mean temperatures (annual mean: $F_{1,23} = 4.87$, $p < 0.05$, $R^2 = 0.14$; winter mean: $F_{1,23} = 6.36$, $p < 0.05$, $R^2 = 0.18$; Fig. 2.3F,H; Table S2.2). Among parameters affecting variation in growth duration in the North American populations, latitude is the strongest predictor; growth duration decreases by 22.45 d per 1° of latitude north (Fig. 2.3E, Table 2.3). Growth durations of the Asian and all populations were not affected by latitude or temperature ($p > 0.05$, $R^2 < 0.13$; Fig. 2.3E–H, Table 2.3; Table S2.2).

For maximum AGB, significant relationships between latitudes/temperatures and biomass were observed in the quadratic regression for Asian and all populations, but not for the North American populations (Fig. 2.3I–L, Table 2.3; Table S2.2). For the Asian populations, the biomass peaked at latitude 34.4° N ($F_{2,26} = 12.37$, $p < 0.001$, $R^2 = 0.45$) and at 13.5, 21.9, and 4.3°C for annual, summer, and winter mean temperatures, respectively (annual mean: $F_{2,26} = 13.73$, $p < 0.001$, $R^2 = 0.48$; summer mean: $F_{2,26} = 14.09$, $p < 0.001$, $R^2 = 0.48$; winter mean: $F_{2,26} = 11.92$, $p < 0.001$, $R^2 = 0.44$). For all populations, the biomass peaked at latitude 36.9° N ($F_{2,48} = 18.85$, $p < 0.001$, $R^2 = 0.42$), at 15.7°C for annual mean temperature ($F_{2,48} = 12.27$, $p < 0.001$, $R^2 = 0.31$), and at 21.7°C for summer mean temperature ($F_{2,48} = 20.80$, $p < 0.001$, $R^2 = 0.44$). Winter mean

temperature did not explain variation in AGB for all populations due to an R^2 value below the threshold of 0.13 ($F_{2,48} = 3.93$, $p < 0.05$, $R^2 = 0.10$). Among latitude and temperatures, summer mean temperature was the strongest predictor for maximum biomass in both Asian and all populations (Table 2.3).

Peak reproductive timing was significantly affected by latitude in all geographic groups, and in contrast, temperature effects were only observed for Asian and all populations (Fig. 2.4A–D, Table 2.3; Table S2.2). An increase in latitude delayed the reproductive peak timing, with 4.8 and 4.3 d per 1° latitude for the Asian and all populations, respectively (Asia: $F_{1,27} = 10.90$, $p < 0.01$, $R^2 = 0.26$; all: $F_{1,50} = 45.89$, $p < 0.001$, $R^2 = 0.47$; Fig. 2.4A). For the North American populations, reproductive peak timing showed a unimodal relationship with latitude ($F_{2,20} = 7.49$, $p < 0.005$, $R^2 = 0.37$); however, an unrealistic U-shaped curve within a small latitudinal range with large residuals, represented in Fig. 2.4A, suggests that the model was statistically overfitted without ecological significance. Temperatures were correlated with reproductive peak timing for both Asian and all populations, yet the relationships were varied. Annual mean temperatures showed significant negative relationships with reproductive peak timing in the Asian and all populations, where I found that a 1°C increase in temperature advanced timing by 6.7 and 8.8 d for the Asian and all populations, respectively (Asia: $F_{1,27} = 12.17$, $p < 0.01$, $R^2 = 0.29$; all: $F_{1,50} = 43.07$, $p < 0.001$, $R^2 = 0.45$; Fig. 2.4B). Summer mean temperatures showed a quadratic relation with reproductive peak timings, where the quadratic maximum occurred at 20.1 and 16.5°C for the Asian and all populations, respectively (Asia: $F_{2,26} = 7.57$, $p < 0.005$, $R^2 = 0.32$; all: $F_{2,49} = 24.30$, $p < 0.001$, $R^2 = 0.48$; Fig. 2.4C). Finally, winter mean temperatures showed a negative linear relationship with the Asian populations ($F_{1,27} = 15.93$, $p < 0.001$, $R^2 = 0.35$). A 1°C increase in

temperature caused an earlier reproductive peak by 5.3 d, whereas the relationship was quadratic for all populations with the quadratic maxima at 0°C ($F_{1,50} = 32.65$, $p < 0.001$, $R^2 = 0.37$; Fig. 2.4D). The strongest predictor for reproductive peak timing varied among groups: latitude for the North American populations, summer mean temperature for all populations, and winter mean temperature for the Asian populations (Table 2.3).

Reproductive durations were not affected by latitude nor temperatures in any geographic groups ($p > 0.05$, $R^2 < 0.13$ in all combinations; Fig. 2.4E–H, Table 2.3; Table S2.2). For reproductive ratios, only the North American populations showed a significant positive effect of latitude ($F_{1,21} = 4.57$, $p < 0.05$, $R^2 = 0.14$; Table 2.3, Fig. 2.4I), while all other combinations of geographic groups with latitude or temperature were not significant ($p > 0.05$, $R^2 < 0.13$; Fig. 2.4I–L, Table 2.3; Table S2.2). In North American populations, an increase of 1° latitude caused a 3.55% increase in reproductive ratio.

2.4 Discussion

2.4.1 Seagrass biomass and growth

Generally, seagrass biomass peaks during summer and remains low during winter, while the date of peak biomass timing shows latitudinal variation (Duarte 1989; Hemminga and Duarte 2000). Studies on kelp production and reproduction suggested that environmental conditions during winter and summer temperatures are both important to their seasonality (Krause-Jensen et al. 2012; James et al. 2015). In this study, the intertidal seagrass *Zostera japonica* showed no geographical variation in peak biomass timing and growth duration, nor was it affected by latitude or temperature, except for the North American populations where latitude and temperature had significant effects on their growth duration. For the predominantly subtidal congener *Z. marina*, Clausen et al.

(2014) found significant latitude and temperature effects on seagrass peak biomass timing, but the effects were not significant on growth duration. Average growth duration for *Z. japonica* was 201.6 ± 63.1 d, which is comparable to the growth duration reported for *Z. marina*, 192.6 d (Clausen et al. 2014). In their study, Clausen et al. (2014) suggested that the earlier start and earlier end of the growth season in southern populations, and the later start and later end in northern populations, resulted in an overall constant growth duration across latitudes. An analysis of the start or end of the growth season was outside the scope of this study, yet the lack of significant latitude/temperature effects on peak biomass timing in this study implies that *Z. japonica* biomass is controlled differently from *Z. marina*.

High spatial and temporal variation, especially in peak biomass timing, may have masked potential effects of global factors, such as latitude and temperature. The study results suggest that the northern distributed group tended to show delayed biomass peak timing (Figs. 2.2A & 2.3A–D; Table S2.2). However, high spatial and temporal variation within the group were observed. Peak biomass timing from the same site but from a different tidal zonation could differ as much as 87 to 95 d (Kim et al. 2016; Suonan et al. 2017). Even at the same location, observations from 2 consecutive years resulted in peak timing differences of 57 d in an undisturbed condition (Kaldy 2006) and up to 192 d when disturbed; biomass peaked in March–April in an undisturbed year and in late October in the year disturbed by clamming activity (Park et al. 2011). This high variation suggests relatively stronger local effects than factors showing effects at larger scales, such as latitude or temperature, due to the fluctuating intertidal environment, compared to the stable subtidal environment experienced by *Z. marina*. Local environmental conditions, such as disturbance, tidal exposure, and ocean currents, may certainly affect the observed

variation in seasonality traits; however, with limited case studies investigating the effect of each condition and no evidence of latitudinal trends in disturbance, the analysis of local conditions remained outside of scope in this study.

Differences between temperate and tropical populations may have contributed to the observed variation in peak biomass timing and growth duration. In the tropics, stress from heat or desiccation is especially strong for intertidal seagrasses (Lin and Shao 1998; Björk et al. 1999; Lan et al. 2005). High within-group variation for the tropical region might be caused by an autumn–winter peak, instead of a spring–summer peak for the temperate region, which occurred after heat and desiccation stress during late spring to summer. To this extent, an autumn–winter peak may not have fit well with this study's analysis, which assumed a spring–summer peak. Furthermore, the tropical population growth is largely affected by the rainy season, salinity, turbidity, riverine influence, tidal exposure, or water motion (Erftemeijer and Herman 1994; Lee 1997; Fong et al. 1998; Huong et al. 2003), suggesting that seasonal fluctuations in temperature or light may not be the most important factors controlling the growth of intertidal seagrass in the tropics. For example, biomass and growth of the tropical seagrass *Thalassia hemprichii* increased during the monsoon season, in which heavy rain brings more terrestrial nutrients into the beds (Lin and Shao 1998; Lin et al. 2018). Future investigation on seagrass seasonality in tropical regions will help resolve this uncertainty.

In the analysis, *Z. japonica* showed significant variation among geographical groups and significant latitude and temperature effects on maximum biomass, where the maximum biomass was the highest at mid-distribution, mid-latitude, or intermediate temperature, and decreased towards the edge of its range distribution (Fig. 2.3I–L). To the best knowledge, this relationship between biomass and latitude or temperature was

not found in the well-studied congener *Z. marina*, or in any other seagrass species (Olesen and Sand-Jensen 1994; Nakaoka and Aioi 2001; Olesen et al. 2015), although the size of *Z. marina* shoots peaks at intermediate latitudes (Ruesink et al. 2018). In native *Z. japonica*, variation in maximum biomass was described best by a quadratic relationship between latitude and temperature, and peaked around 34.4°N latitude or 21.9°C in summer mean temperature; summer mean temperature was the most influential predictor for variation in biomass (Fig. 2.3I,K). The quadratic relationship indicates a substantial reduction in maximum biomass at both the northern/colder range limit as well as the southern/warmer range limit. A significant reduction in biomass caused by storms, waterfowl grazing, ice scouring, and freezing in winter was observed in northern *Z. japonica* populations (Harrison 1982a, b; Sato et al. 2020; M. A. Ito pers. obs.), whereas similar biomass reduction caused by heat and desiccation in summer was observed in southern populations (Lee 1997; Fong et al. 1998). Such stressors at both the northern/colder and southern/warmer range limits may have caused biomass reduction or change in growth patterns. However, such stressors have also been reported in other seagrass species, especially in the well-studied *Z. marina* (freezing or ice scour: Keddy and Patriquin 1978; Robertson and Mann 1984; summer heat or desiccation: Phillips et al. 1983; Orth and Moore 1986), although that species does not show significant biomass variation among latitude or temperature. The new findings from this study on latitude/temperature–biomass relationships address the potential risk of applying insight from one species to another.

Optimal and upper critical temperatures for growth rate, photosynthesis, and distribution of *Z. japonica* from both native and non-native regions have been reported in past studies. An investigation of temperature effects on *Z. japonica* growth rates revealed

maximum growth around 18–23°C in native (Lee et al. 2005) and around 20°C in non-native populations (Shafer et al. 2008; Kaldy et al. 2015b). Similarly, Uede (2007) found that seagrass standing biomass increases significantly up to 20°C, and then drastically decreases at 25–28°C in its native region. Two experimental studies on upper critical temperatures for photosynthetic activity for this species reported an identical value of 29°C (Abe et al. 2009; Morita et al. 2010), which corresponds to the upper critical temperature for its native geographic range (Abe et al. 2009 and references therein). The optimal and upper critical temperatures demonstrated from this study, 22 and 28°C, respectively, agreed well with other studies. Additionally, Kaldy et al. (2015b) suggested that *Z. japonica* growth rate is a function of the duration of exposure to warm temperature (20°C) and implies that temperature predominantly drives the intertidal zonation pattern. Although an analysis of the temperature experienced during the seagrass growth season was outside of the research scope, Kaldy et al. (2015b) clearly highlights the importance of temperature effects on maximum biomass achieved by each population. Based on the results (Fig. 2.3K), most of the *Z. japonica* populations are suggested to be experiencing higher than optimum temperatures at least some of the time within the summer. Further arguments considering ongoing climate change and its effect on seagrass biomass are discussed in Section 2.4.4.

2.4.2 Seagrass sexual reproduction

Seagrass sexual reproduction shows a seasonal pattern with a later peak at higher latitude and lower temperature sites (Phillips et al. 1983; Nakaoka and Aioi 2001). This study demonstrated strong latitude/temperature effects on peak reproductive timing of *Z. japonica*, but not on reproductive duration nor on maximum reproductive ratio. Average

peak reproductive timing was significantly different among the geographic groups; the reproductive peak occurred in late spring for the Asia-tropical group, in early summer for the Asia-temperate group, and late summer for the North America-temperate group (Fig. 2.2D). Regression analysis using summer mean temperatures showed that a decrease in temperature delayed the peak timing of the Asia-tropical group, which plateaued around DOY 230 (Fig. 2.4C). This plateau corresponded with the late-summer peak for the North America-temperate group, suggesting that reproduction cannot happen later than this timepoint. Interestingly, this timing coincides with the timeframe during which coastal upwelling starts to relax in North America (Bograd et al. 2009). For successful sexual reproduction, seagrass must flower, produce fruit, and carry out seed maturation (Alexandre et al. 2006). The number of days needed for *Z. japonica* to complete its reproductive stages have not been documented, although one study on the closely related congener *Z. noltii* reported that 47 d were required for the whole flowering to fruiting process (Alexandre et al. 2006). If this period is applicable to *Z. japonica*, limits on peak reproductive timing may arise from necessity to complete its reproductive process before seasonal die-off in winter. Most of the studies screened here did not report reproductive stages, which prevented me from conducting detailed analyses on different stages.

Average reproductive durations for *Z. japonica* and *Z. marina* appear similar, i.e., 121 and 128 d, respectively (*Z. japonica*: this study, *Z. marina*: Blok et al. 2018). *Z. japonica* showed constant reproductive duration among groups without latitude/temperature effects. For *Z. marina*, reproductive duration showed significant latitude/temperature effects as an earlier start of flowering for lower latitude/warmer populations, while end of flowering was constant along latitude/temperature (Blok et al.

2018). Although start or end flowering dates were not analyzed in the present study, contrasting results suggest that such events may have not occurred in *Z. japonica*.

Non-significant latitude and temperature effects on Asian or all population groups confirmed that the reproductive ratio in *Z. japonica* might be independent of large-scale factors, such as light or temperature, but may rather depend on local environmental conditions. When under stress, seagrass allocates more energy to sexual reproduction and thus reproductive effort can be used as an ecological indicator of environmental stress (Cabaço and Santos 2012; Kendrick et al. 2012). In this study, *Z. japonica* demonstrated relatively low (<20%) reproductive ratios for native populations, whereas non-native populations showed higher reproductive ratios, although the difference was only significant between the Asia-temperate and North America-temperate groups (Fig. 2.2F). An exceptionally high reproductive effort (63%), treated as an outlier in the analysis, was observed at a site with high anthropogenic disturbance by clam-harvesting activity (Park et al. 2011). In fact, experimental disturbance resulted in a negative correlation between flowering and vegetative biomass of non-native *Z. japonica* (Henderson and Hacker 2015), yet there was no observed negative relationship between reproductive effort and vegetative biomass in this study (Fig. S2.4C).

Compared to the vegetative growth (biomass results, Section 2.4.1), seagrass sexual reproduction was more strongly correlated with variation in latitude and temperature. This was also suggested by Blok et al. (2018, p. 88), who stated that 'sexual reproduction is more responsive to latitudinal climatic variation than seasonal biomass development' in *Z. marina*. Investigation of the direct relationship between biomass and reproduction remains outside of the study scope; however, none of comparisons between peak biomass and reproductive timing, duration, or maximum output showed significant

correlations in any geographic group (Fig. S2.4). These results highlight that drivers for biomass and reproduction might be different or have different mechanisms in *Z. japonica*.

2.4.3 Effects of introduction

Non-native *Z. japonica* populations in North America enabled me not only to extend latitude or temperature coverage, but also to investigate the potential effects of introduction on seagrass phenology. Among-group geographic comparisons resulted in significant differences between native and non-native groups for maximum biomass, peak reproductive timing, and reproductive ratio. Nonetheless, observed results must be cautiously interpreted because native and non-native groups differed in their latitudinal distribution. In this study, the non-native *Z. japonica* (North America-temperate) group covered only small latitudinal and temperature ranges compared to the native (Asia-tropical and Asia-temperate) groups (Table 2.2), reflecting their current and limited distribution in North America (Shafer et al. 2014). Furthermore, the eastern coast of the Pacific Ocean, which is inhabited by non-native *Z. japonica* populations, is strongly affected by seasonal coastal upwelling in summer (Bograd et al. 2009), and an inverse relationship between summer temperature and latitude was observed relative to what is observed in the western Pacific (Fig. S2.1). Differences in latitudinal distribution and latitude–temperature relationships in non-native populations may confound the observed results.

I found that non-native populations exhibited higher reproduction and shorter growth duration at higher latitudes, while other populations did not. Kaldy (2006) reported that sexual reproduction in non-native *Z. japonica* differed between southern populations in Oregon, where the timing and intensity of sexual reproduction was more

variable than among populations in British Columbia. Experiments on non-native *Z. japonica* demonstrated higher germination rates in warmer temperatures (15 and 20°C) compared to lower temperatures (10°C) (Kaldy et al. 2015a). In *Z. marina*, higher flowering ratios and longer reproductive durations were reported for lower temperature sites (Qin et al. 2020). Populations at northern cooler sites experiencing unfavorable germination but good flowering conditions may experience higher sexual reproduction as observed in this study, although effects of temperature were not significant. These interpretations, however, do not explain why this relationship is absent in native populations. The magnitude of environmental change effects on seagrass generally increases at higher latitude (Hemminga and Duarte 2000). The fact that non-native *Z. japonica* occurs at the upper end of latitudes where data were not available for native populations may explain why there are stronger abiotic effects at higher latitudes compared to introduction effects. Furthermore, coastal upwelling that only affects North American populations may moderate the latitudinal effect on growth duration. Kaldy et al. (2015b) suggested that non-native *Z. japonica* growth rate is highest at 20°C and decreased in lower temperature. Such low water temperature conditions caused by coastal upwelling may limit seagrass growth during summer. Further investigation will be possible once seasonality data on the northern-most native populations from Russia become available.

High reproductive ratios observed in non-native *Z. japonica* could have occurred during the introduction process, as it is one of the common characteristics for successful establishment and population growth of non-native species (Ruesink et al. 2010). *Z. japonica* has been established in North America since the 1970s; however, an invasion lag-phase (interval between non-native species becoming established and becoming fully

invasive) is 20–30 yr on average, and some species show a lag-phase >40 yr (Sakai et al. 2001; Aikio et al. 2010), suggesting the possibility that *Z. japonica* is still in an invasion lag-phase. As stated earlier, reproductive ratios can be used as an ecological indicator of stress (Cabaço and Santos 2012). Kaldy and Shafer (2013) pointed out that a non-native population in Padilla Bay, WA, USA, is susceptible to the combination of heat stress and low salinity. Higher sexual reproduction observed in non-native groups indicates that non-native *Z. japonica* may be under stress while adapting to local environmental conditions in the introduced regions. Likewise, enhanced sexual reproduction was demonstrated in *Z. noltii* during its colonization stage (Cabaço et al. 2012), which suggests that non-native *Z. japonica* could be rapidly reproducing as they colonize new habitats.

Interestingly, latitude was a stronger predictor than temperature in North American *Z. japonica* populations, while temperature was always the strongest predictor for the Asian and all populations. It is known that *Z. marina* has lost its photo-recognition gene (Olsen et al. 2016); however, the present results suggest that *Z. japonica* may retain some mechanism to recognize photoperiod. On the other hand, significant latitudinal correlation found in North American populations could be confounded with other environmental factors, such as coastal upwelling. Two of the northern-most study sites included in the analysis, Boundary Bay and Roberts Bank, Canada, are located in inner estuaries compared to other sites in North America that are located on the outer coast (Fig. 2.1). Upwelling intensity is generally weaker at northern sites (Bograd et al. 2009). Pawlowicz et al. (2019) showed that the Salish Sea, where the 2 sites are located, have a long water residence time, especially during the summer, and this long residence time could lead to warmer water temperatures being optimal for *Z. japonica* to grow. However, limited latitudinal and temperature coverage in the non-native region and limited

knowledge on upwelling effects on seagrass will restrict further investigation. More data on seasonality from both northern and southern ranges will help clarify this uncertainty in the future.

2.4.4 Conclusions and future implications

The present study demonstrates that the effects of latitude and temperature on seagrass biomass and reproduction vary among analyzed traits and among geographic groups. Phenological traits with significant among-group geographical variation coincided with traits with significant latitude or temperature effects, highlighting the strong influences of globally controlled factors on maximum biomass and peak reproductive timing. In contrast, some traits showed significant latitude effects but only for the non-native group, suggesting an alteration in traits during the introduction process. Many of the results obtained from this study for *Z. japonica* differed from the previous findings of other seagrass species. These new findings revealed the complexity of seagrass phenology under fluctuating intertidal environments, and highlight the importance of including data from tropical and non-native regions, which cannot be addressed in other seagrass species aside from *Z. japonica*. Thus, I have presented the limitations of applying these findings to other species and the necessity of understanding species-specific phenological responses to changing abiotic factors.

This study provides considerable insight in predicting how an intertidal seagrass species may respond to climate change. Large-scale analyses covering wide temperature ranges enabled me to use a space-for-time substitution approach to predict plant response to future climate change (Fukami and Wardle 2005; Buyantuyev et al. 2012). In this study, I found significant temperature effects on *Z. japonica* maximum biomass and peak

reproductive timing. More than half of the studied populations are already experiencing higher temperatures than the growth optimum, and these populations are highly likely to experience substantial decreases in maximum biomass observed in the growth season. Furthermore, increases in temperature will push all tropical and some temperate populations beyond their upper critical temperatures. This study suggests that climate change can cause detrimental reductions in biomass and may alter the range of *Z. japonica* in the future.

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2.6 References

Abe M, Yokota K, Kurashima A, Maegawa M (2009) High water temperature tolerance in photosynthetic activity of *Zostera japonica* Ascherson & Graebner seedlings from Ago Bay, Mie Prefecture, central Japan. Fish Sci 75:1117–1123. https://doi.org/10.1007/s12562-009-0141-x

Abe M, Yokota K, Kurashima A, et al (2012) Structures and seasonal changes of *Zostera japonica* population in Tategami-ura, Ago Bay, Mie Prefecture. Aquac Sci 60:215–225 (in Japanese with English abstract)

Aikio S, Duncan RP, Hulme PE (2010) Lag-phases in alien plant invasions: Separating the facts from the artefacts. Oikos 119:370–378. https://doi.org/10.1111/j.1600-0706.2009.17963.x

Alcoverro T, Duarte CM, Romero J (1995) Annual growth dynamics of *Posidonia oceanica*: contribution of large-scale versus local factors to seasonality. Mar Ecol Prog Ser 120:203–210. https://doi.org/10.3354/meps120203

Alexandre A, Cabaço S, Santos R, Serrão EA (2006) Timing and success of reproductive stages in the seagrass *Zostera noltii*. Aquat Bot 85:219–223. https://doi.org/10.1016/j.aquabot.2006.05.002

Barton K (2019) MuMIn: Multi-Model Inference. R package version 1.43.15. https://cran.r-project.org/src/contrib/Archive/MuMIn/

Bigley RE, Harrison PG (1986) Shoot demography and morphology of *Zostera japonica* and *Ruppia maritima* from British Columbia, Canada. Aquat Bot 24:69–82. https://doi.org/10.1016/0304-3770(86)90118-X

Björk M, Uku J, Weil A, Beer S (1999) Photosynthetic tolerances to desiccation of tropical intertidal seagrasses. Mar Ecol Prog Ser 191:121–126. https://doi.org/10.3354/meps191121

Blok SE, Olesen B, Krause-Jensen D (2018) Life history events of eelgrass *Zostera marina* L. populations across gradients of latitude and temperature. Mar Ecol Prog Ser 590:79–93. https://doi.org/10.3354/meps12479

Bograd SJ, Schroeder I, Sarkar N, et al (2009) Phenology of coastal upwelling in the California Current. Geophys Res Lett 36:L01602. https://doi.org/10.1029/2008GL035933

Buyantuyev A, Xu P, Wu J, et al (2012) A space-for-time (SFT) substitution approach to studying historical phenological changes in urban environment. PLoS One 7:e51260. https://doi.org/10.1371/journal.pone.0051260

Cabaço S, Santos R (2012) Seagrass reproductive effort as an ecological indicator of disturbance. Ecol Indic 23:116–122. https://doi.org/10.1016/j.ecolind.2012.03.022

Cabaço S, Santos R, Sprung M (2012) Population dynamics and production of the seagrass *Zostera noltii* in colonizing versus established meadows. Mar Ecol 33:280–289. https://doi.org/10.1111/j.1439-0485.2011.00494.x

Clausen K, Krause-Jensen D, Olesen B, Marbà N (2014) Seasonality of eelgrass biomass across gradients in temperature and latitude. Mar Ecol Prog Ser 506:71–85. https://doi.org/10.3354/meps10800

Cohen J (1973) Eta-squared and partial eta-squared in fixed factor anova designs. Educ Psychol Meas 33:107–112. https://doi.org/10.1177/001316447303300111

Cohen J (1988) Statistical Power Analysis for the Behavioral Sciences, 2nd edn. Lawrence Erlbaum Associates Publishers, Hillsdale, NJ

Costanza R, d'Arge R, de Groot R, et al (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260. https://doi.org/10.1038/387253a0

Dos Santos VM, Matheson FE (2017) Higher seagrass cover and biomass increases sexual reproductive effort: A rare case study of *Zostera muelleri* in New Zealand. Aquat Bot 138:29–36. https://doi.org/10.1016/j.aquabot.2016.12.003

Duarte CM (1989) Temporal biomass variability and production/biomass relationships of seagrass communities. Mar Ecol Prog Ser 51:269–276. https://doi.org/10.1080/14786419.2015.1027893

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

Erftemeijer PLA, Herman PMJ (1994) Seasonal changes in environmental variables, biomass, production and nutrient contents in two contrasting tropical intertidal seagrass beds in South Sulawesi, Indonesia. Oecologia 99:45–59. https://doi.org/10.1007/BF00317082

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Climatol 37:4302–4315. https://doi.org/10.1002/joc.5086

Fong TCW, Lee SY, Wu RSS (1998) Seasonal cycle of growth and reproduction of the seagrass *Zostera japonica* in Hong Kong. In: Morton B (ed) The Marine Biology of the South China Sea. Proceedings of the Third International Conference on the Biology of the South China Sea. pp 247–260

Frisén M (1986) Unimodal regression. Stat 35:479–485. https://doi.org/10.2307/2987804

Fukami T, Wardle DA (2005) Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. Proc R Soc B 272:2105–2115. https://doi.org/10.1098/rspb.2005.3277

Gill AL, Gallinat AS, Sanders-DeMott R, et al (2015) Changes in autumn senescence in northern hemisphere deciduous trees: a meta-analysis of autumn phenology studies. Ann Bot 116:875–888. https://doi.org/10.1093/aob/mcv055

Godoy O, Castro-Díez P, Valladares F, Costa-Tenorio M (2009) Different flowering phenology of alien invasive species in Spain: evidence for the use of an empty temporal niche? Plant Biol 11:803–811. https://doi.org/10.1111/j.1438-8677.2008.00185.x

Green EP, Short FT (2003) World Atlas of Seagrasses. University of California Press, Berkeley, CA

Harrison PG (1982a) Spatial and temporal patterns in abundance of two intertidal seagrasses, *Zostera americana* den hartog and *Zostera marina* L. Aquat Bot 12:305–320. https://doi.org/10.1016/0304-3770(82)90024-9

Harrison PG (1982b) Seasonal and year-to-year variations in mixed intertidal populations of *Zostera japonica* Aschers. & Graebn. and *Ruppia maritima* L. S.L. Aquat Bot 14:357–371. https://doi.org/10.1016/0304-3770(82)90109-7

Harrison PG, Bigley RE (1982) The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can J Fish Aquat Sci 39:1642–1648. https://doi.org/10.1139/f82-221

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

Henderson J, Hacker SD (2015) Buried alive: an invasive seagrass (*Zostera japonica*) changes its reproductive allocation in response to sediment disturbance. Mar Ecol Prog Ser 532:123–136. https://doi.org/10.3354/meps11335

Hosokawa S, Nakamura Y, Kuwae T (2009) Increasing temperature induces shorter leaf life span in an aquatic plant. Oikos 118:1158–1163. https://doi.org/10.1111/j.1600-0706.2009.17288.x

Huong TTL, Vermaat JE, Terrados J, et al (2003) Seasonality and depth zonation of intertidal *Halophila ovalis* and *Zostera japonica* in Ha Long Bay (northern Vietnam). Aquat Bot 75:147–157. https://doi.org/10.1016/S0304-3770(02)00172-9

Hyndes GA, Heck KL, Vergés A, et al (2016) Accelerating tropicalization and the transformation of temperate seagrass meadows. Bioscience 66:938–948. https://doi.org/10.1093/biosci/biw111

Inoue C, Tanaka J, Nagumo T (2006) Seasonal Change of Biomass in *Zostera japonica* Ascherson et Graebner at Tateyama Bay, Chiba Prefecture. Bull Nippon Dent Univ 35:65–67 (in Japanese with English abstract)

James K, Kibele J, Shears NT (2015) Using satellite-derived sea surface temperature to predict the potential global range and phenology of the invasive kelp *Undaria pinnatifida*. Biol Invasions 17:3393–3408. https://doi.org/10.1007/s10530-015-0965-5

Kaldy JE (2006) Production ecology of the non-indigenous seagrass, dwarf eelgrass (*Zostera japonica* Ascher. & Graeb.), in a Pacific Northwest Estuary, USA. Hydrobiologia 553:201–217. https://doi.org/10.1007/s10750-005-5764-z

Kaldy JE, Shafer DJ (2013) Effects of salinity on survival of the exotic seagrass *Zostera japonica* subjected to extreme high temperature stress. Bot Mar 56:75–82. https://doi.org/10.1515/bot-2012-0144

Kaldy JE, Shafer DJ, Ailstock MS, Magoun AD (2015a) Effects of temperature, salinity and seed age on induction of *Zostera japonica* germination in North America, USA. Aquat Bot 126:73–79. https://doi.org/10.1016/j.aquabot.2015.06.006

Kaldy JE, Shafer DJ, Magoun AD (2015b) Duration of temperature exposure controls growth of *Zostera japonica*: Implications for zonation and colonization. J Exp Mar Bio Ecol 464:68–74. https://doi.org/10.1016/j.jembe.2014.12.015

Kamohara S, Yamada S, Sone R, Waku M (2015) Relationship between characteristics of *Zostera japonica* population and the conditions of their bottom sediment at Rokujo tidal flats in Mikawa Bay. Aichi Suishi Kenpou 20:10–18 (in Japanese with English abstract)

Kassambara A (2020) rstatix: Pipe-Friendly Framework for Basic Statistical Test. R package version 0.5.0 https://cran.r-project.org/src/contrib/Archive/rstatix/

Kawano T, Igari T, Imayoshi Y, et al (2012) Distribution of temperate/tropical seagrass in Satsunan Islands and adjacent waters, Kagoshima Prefecture, Japan. Aquac Sci 60:359–369 (in Japanese with English abstract)

Keddy CJ, Patriquin DG (1978) An annual form of eelgrass in Nova Scotia. Aquat Bot 5:163–170. https://doi.org/10.1016/0304-3770(78)90059-1

Kendrick GA, Waycott M, Carruthers TJB, et al (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience 62:56–65. https://doi.org/10.1525/bio.2012.62.1.10

Kim JH, Kim SH, Kim YK, et al (2016) Growth dynamics of the seagrass *Zostera japonica* at its upper and lower distributional limits in the intertidal zone. Estuar Coast Shelf Sci 175:1–9. https://doi.org/10.1016/j.ecss.2016.03.023

Koshikawa Y, Nakamura H, Tanaka M (2009) Formative and decline mechanism of eelgrass community *Zostera japonica* in wetland subjected to influence of hinterland environmental condition. J Japan Soc Civ Eng Ser B2 (Coastal Eng 65:1076–1080. https://doi.org/10.2208/kaigan.65.1076 (in Japanese with English abstract)

Krause-Jensen D, Marbà N, Olesen B, et al (2012) Seasonal sea ice cover as principal driver of spatial and temporal variation in depth extension and annual production of kelp in Greenland. Glob Chang Biol 18:2981–2994. https://doi.org/10.1111/j.1365-2486.2012.02765.x

Lalli CM, Parsons TR (1997) Biological Oceanography: an introduction, 2nd edn. Elsevier Butterworth-Heinemann, Oxford

Lan C-Y, Kao W-Y, Lin H-J, Shao K-T (2005) Measurement of chlorophyll fluorescence reveals mechanisms for habitat niche separation of the intertidal

seagrasses *Thalassia hemprichii* and *Halodule uninervis*. Mar Biol 148:25–34. https://doi.org/10.1007/s00227-005-0053-y

Larned ST (2003) Effects of the invasive, nonindigenous seagrass *Zostera japonica* on nutrient fluxes between the water column and benthos in a NE Pacific estuary. Mar Ecol Prog Ser 254:69–80. https://doi.org/10.3354/meps254069

Lee K-S, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. J Exp Mar Bio Ecol 350:144–175. https://doi.org/10.1016/j.jembe.2007.06.016

Lee SY (1997) Annual cycle of biomass of a threatened population of the intertidal seagrass *Zostera japonica* in Hong Kong. Mar Biol 129:183–193. https://doi.org/10.1007/s002270050159

Lee SY, Kim JB, Lee SM (2006) Temporal dynamics of subtidal *Zostera marina* and intertidal *Zostera japonica* on the southern coast of Korea. Mar Ecol 27:133–144. https://doi.org/10.1111/j.1439-0485.2006.00089.x

Lee SY, Oh JH, Choi C Il, et al (2005) Leaf growth and population dynamics of intertidal *Zostera japonica* on the western coast of Korea. Aquat Bot 83:263–280. https://doi.org/10.1016/j.aquabot.2005.07.004

Leffler AJ, James JJ, Monaco TA, Sheley RL (2014) A new perspective on trait differences between native and invasive exotic plants. Ecology 95:298–305. https://doi.org/10.1890/13-0102.1

Lin H-J, Shao K-T (1998) Temporal changes in the abundance and growth of intertidal *Thalassia hemprichii* seagrass beds in southern Taiwan. Bot Bull Acad Sin 39:191–198

Lin H-J, Lee C-L, Peng S-E, et al (2018) The effects of El Niño-Southern Oscillation events on intertidal seagrass beds over a long-term timescale. Glob Chang Biol 24:4566–4580. https://doi.org/10.1111/gcb.14404

Maher JM, Markey JC, Ebert-May D (2013) The other half of the story: Effect size analysis in quantitative research. CBE Life Sci Educ 12:345–351. https://doi.org/10.1187/cbe.13-04-0082

Marbà N, Cebrián J, Enríquez S, Duarte C (1996) Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Mar Ecol Prog Ser 133:203–215. https://doi.org/10.3354/meps133203

Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Glob Ecol Biogeogr 17:633–640. https://doi.org/10.1111/j.1466-8238.2008.00402.x

Morita T, Kokubu H, Miyamatsu A, et al (2010) The effects of water temperature in the sediment and the desiccation period on the growth and survival of transplant seagrass *Zostera japonica* shoots in mesocosm tank culture. Aquac Sci 58:261–267

Murtaugh PA (2003) On detecting hump-shaped relationships in ecology: a bootstrap test for monotonicity. Environmetrics 14:611–616. https://doi.org/10.1002/env.607

NASA (2014) NASA Goddard Space Flight Center, Ocean Ecology Laboratory, Ocean Biology Processing Group. Moderate-resolution Imaging Spectroradiometer (MODIS) Aqua 11μm Day Sea Surface Temperature Data; 2014 Reprocessing.NASA OB.DAAC, Greenbelt, MD, USA. data/10.5067/AQUA/MODIS/L3M/SST/2014. Accessed 17 Jun 2020

Nakaoka M, Aioi K (2001) Ecology of seagrasses *Zostera* spp. (Zosteraceae) in Japanese waters: A review. Otsuchi Mar Sci 26:7–22

Nomme KM, Harrison PG (1991) A multivariate comparison of the seagrasses *Zostera marina* and *Zostera japonica* in monospecific versus mixed populations. Can J Bot 69:1984–1990. https://doi.org/10.1139/b91-249

Ogle DH, Wheeler P, Dinno A (2020) FSA: Fisheries Stock Analysis. R package version 0.8.31 https://github.com/droglenc/FSA

Ok J-S, Lee S-Y (2014) The autecology of *Zostera marina* and *Z. japonica* at Sagumi Bay in the southwestern coast of Korea. J Environ Sci Int 23:1563–1572 (in Korean with English abstract)

Olesen B, Krause-Jensen D, Marbà N, Christensen PB (2015) Eelgrass *Zostera marina* in subarctic Greenland: dense meadows with slow biomass turnover in cold waters. Mar Ecol Prog Ser 518:107–121. https://doi.org/10.3354/meps11087

Olesen B, Sand-Jensen K (1994) Biomass-density patterns in the temperate seagrass *Zostera marina*. Mar Ecol Prog Ser 109:283–291. https://doi.org/10.3354/meps109283

Olsen JL, Rouzé P, Verhelst B, et al (2016) The genome of the seagrass *Zostera marina* reveals angiosperm adaptation to the sea. Nature 530:331–335. https://doi.org/10.1038/nature16548

Orth RJ, Moore KA (1986) Seasonal and year-to-year variations in the growth of *Zostera marina* L. (eelgrass) in the lower Chesapeake Bay. Aquat Bot 24:335–341. https://doi.org/10.1016/0304-3770(86)90100-2

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

Park SR, Kim YK, Kim J-H, et al (2011) Rapid recovery of the intertidal seagrass *Zostera japonica* following intense Manila clam (*Ruditapes philippinarum*) harvesting activity in Korea. J Exp Mar Bio Ecol 407:275–283. https://doi.org/10.1016/j.jembe.2011.06.023

Pawlowicz R, Hannah C, Rosenberger A (2019) Lagrangian observations of estuarine residence times, dispersion, and trapping in the Salish Sea. Estuar Coast Shelf Sci 225:106246. https://doi.org/10.1016/j.ecss.2019.106246

Pergent-Martini C, Pasqualini V, Ferrat L, et al (2005) Seasonal dynamics of *Zostera noltii* Hornem. in two Mediterranean lagoons. Hydrobiologia 543:233–243. https://doi.org/10.1007/s10750-004-7454-7

Phillips RC, McMillan C, Bridges KW (1983) Phenology of eelgrass, *Zostera marina* L., along latitudinal gradients in North America. Aquat Bot 15:145–156. https://doi.org/10.1016/0304-3770(83)90025-6

Primack RB, Ibáñez I, Higuchi H, et al (2009) Spatial and interspecific variability in phenological responses to warming temperatures. Biol Conserv 142:2569–2577. https://doi.org/10.1016/j.biocon.2009.06.003

Qin L-Z, Kim SH, Song H-J, et al (2020) Influence of regional water temperature variability on the flowering phenology and sexual reproduction of the seagrass *Zostera marina* in Korean coastal waters. Estuaries and Coasts 43:449–462. https://doi.org/10.1007/s12237-019-00569-3

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at www.R-project.org

Racault M-F, Le Quéré C, Buitenhuis E, et al (2012) Phytoplankton phenology in the global ocean. Ecol Indic 14:152–163. https://doi.org/10.1016/j.ecolind.2011.07.010

Ramage DL, Schiel DR (1998) Reproduction in the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. Mar Biol 130:479–489. https://doi.org/10.1007/s002270050268

Robertson AI, Mann KH (1984) Disturbance by ice and life-history adaptations of the seagrass *Zostera marina*. Mar Biol 80:131–141. https://doi.org/10.1007/BF02180180

Rohatgi A (2019) WebPlotDigitizer: Web based tool to extract data from plots, images, and maps. Version 4.2. https://automeris.io/WebPlotDigitizer. Accessed 19 Mar 2021

Ruesink JL, Hong J-S, Wisehart L, et al (2010) Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Biol Invasions 12:1773–1789. https://doi.org/10.1007/s10530-009-9588-z

Ruesink JL, Stachowicz JJ, Reynolds PL, et al (2018) Form-function relationships in a marine foundation species depend on scale: a shoot to global perspective from a distributed ecological experiment. Oikos 127:364–374. https://doi.org/10.1111/oik.04270

Sakai AK, Allendorf FW, Holt JS, et al (2001) The Population Biology of Invasive Species. Annu Rev Ecol Syst 32:305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sato F, Tanaka S, Kirihara S, Tanaka Y (2020) The influence of migratory birds on the distribution of the seagrass *Zostera japonica*. Bot Mar 63:521–525. https://doi.org/10.1515/bot-2020-0045

Shafer DJ, Kaldy JE (2014) Comparison of photosynthetic characteristics of the seagrass congeners *Zostera marina* L. and *Zostera japonica* Ascher. & Graeb. Aquat Bot 112:91–97. https://doi.org/10.1016/j.aquabot.2013.09.002

Shafer DJ, Wyllie-Echeverria S, Sherman TD (2008) The potential role of climate in the distribution and zonation of the introduced seagrass *Zostera japonica* in North America. Aquat Bot 89:297–302. https://doi.org/10.1016/j.aquabot.2008.03.005

Shafer DJ, Kaldy JE, Gaeckle JL (2014) Science and management of the introduced seagrass *Zostera japonica* in North America. Environ Manage 53:147–162. https://doi.org/10.1007/s00267-013-0172-z

Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. Aquat Bot 63:169–196. https://doi.org/10.1016/S0304-3770(98)00117-X

Suonan Z, Kim SH, Qin L-Z, Lee KS (2017) Reproductive strategy of the intertidal seagrass *Zostera japonica* under different levels of disturbance and tidal inundation. Estuar Coast Shelf Sci 197:185–193. https://doi.org/10.1016/j.ecss.2017.08.031

Tala F, Chow F (2014) Phenology and photosynthetic performance of *Porphyra* spp. (Bangiophyceae, Rhodophyta): Seasonal and latitudinal variation in Chile. Aquat Bot 113:107–116. https://doi.org/10.1016/j.aquabot.2013.11.005

Thom R, Miller B, Kennedy M (1995) Temporal patterns of grazers and vegetation in a temperate seagrass system. Aquat Bot 50:201–205. https://doi.org/10.1016/0304-3770(95)00449-A

Uede T (2007) Seasonal changes of *Zostera japonica* at intertidal zone in Takinai and Uchinoura, Tanabe Bay, Wakayama Prefecture, Japan. Nippon Suisan Gakkaishi 73:478–486 (in Japanese with English abstract)

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

Wheatcroft RA, Sanders RD, Law BA (2013) Seasonal variation in physical and biological factors that influence sediment porosity on a temperate mudflat: Willapa Bay, Washington, USA. Cont Shelf Res 60S:S173–S184. https://doi.org/10.1016/j.csr.2012.07.022

Zettlemoyer MA, Schultheis EH, Lau JA (2019) Phenology in a warming world: differences between native and non-native plant species. Ecol Lett 22:1253–1263. https://doi.org/10.1111/ele.13290

Zhang X, Friedl MA, Schaaf CB, Strahler AH (2004) Climate controls on vegetation phenological patterns in northern mid- and high latitudes inferred from

MODIS data. Glob Chang Biol 10:1133–1145. https://doi.org/10.1111/j.1529-8817.2003.00784.x

Zhang X, Zhou Y, Liu P, et al (2015) Temporal pattern in biometrics and nutrient stoichiometry of the intertidal seagrass *Zostera japonica* and its adaptation to air exposure in a temperate marine lagoon (China): Implications for restoration and management. Mar Pollut Bull 94:103–113. https://doi.org/10.1016/j.marpolbul.2015.03.004

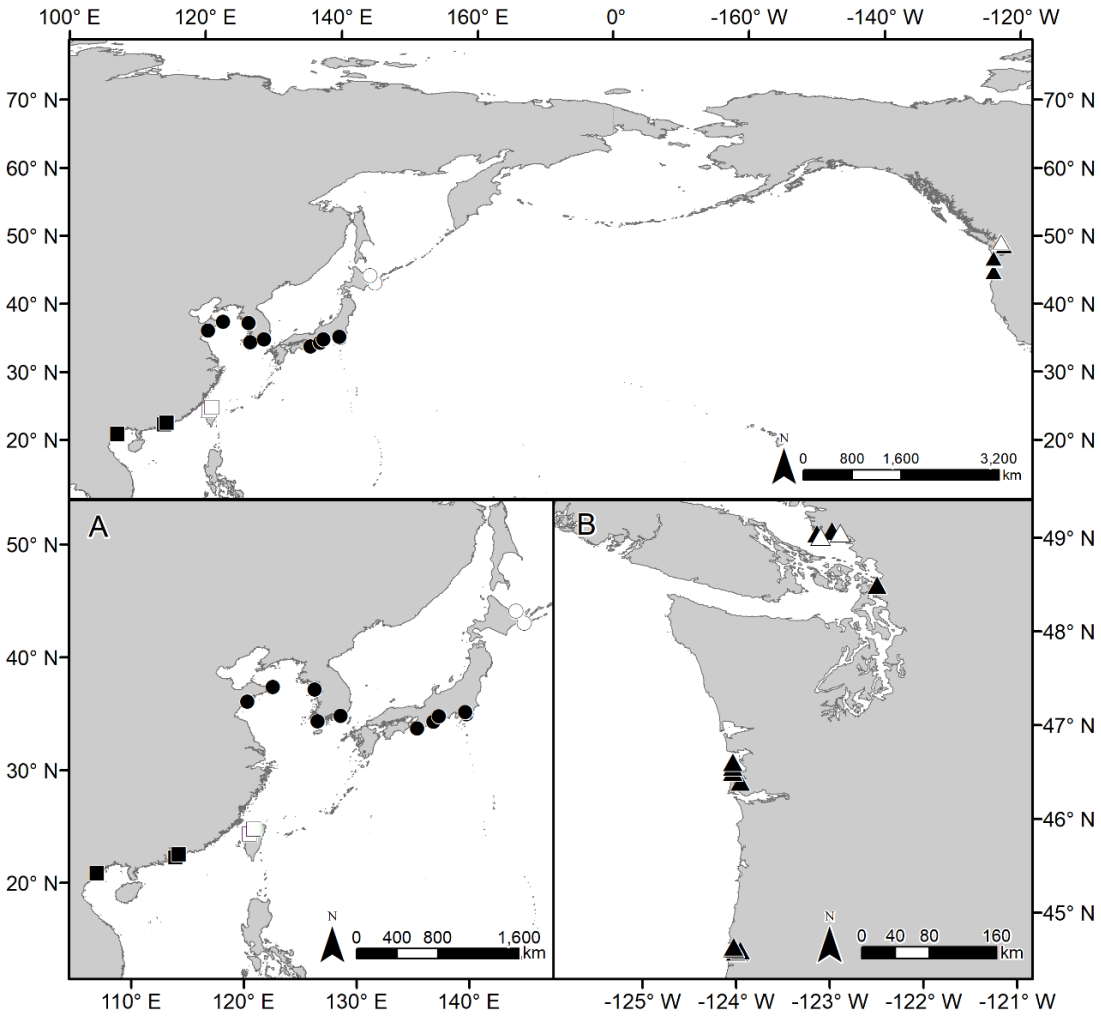

Figure 2.1. Study locations, enlarged for (A) native *Zostera japonica* populations from Asia and (B) non-native populations from North America. Different symbols represent different geographic groups; Asia-tropical (square), Asia-temperate (circle) and North America-temperate (triangle), where filled symbols represent data from the literature review and open symbols from my unpublished data (available in Supplementary Table S2.1).

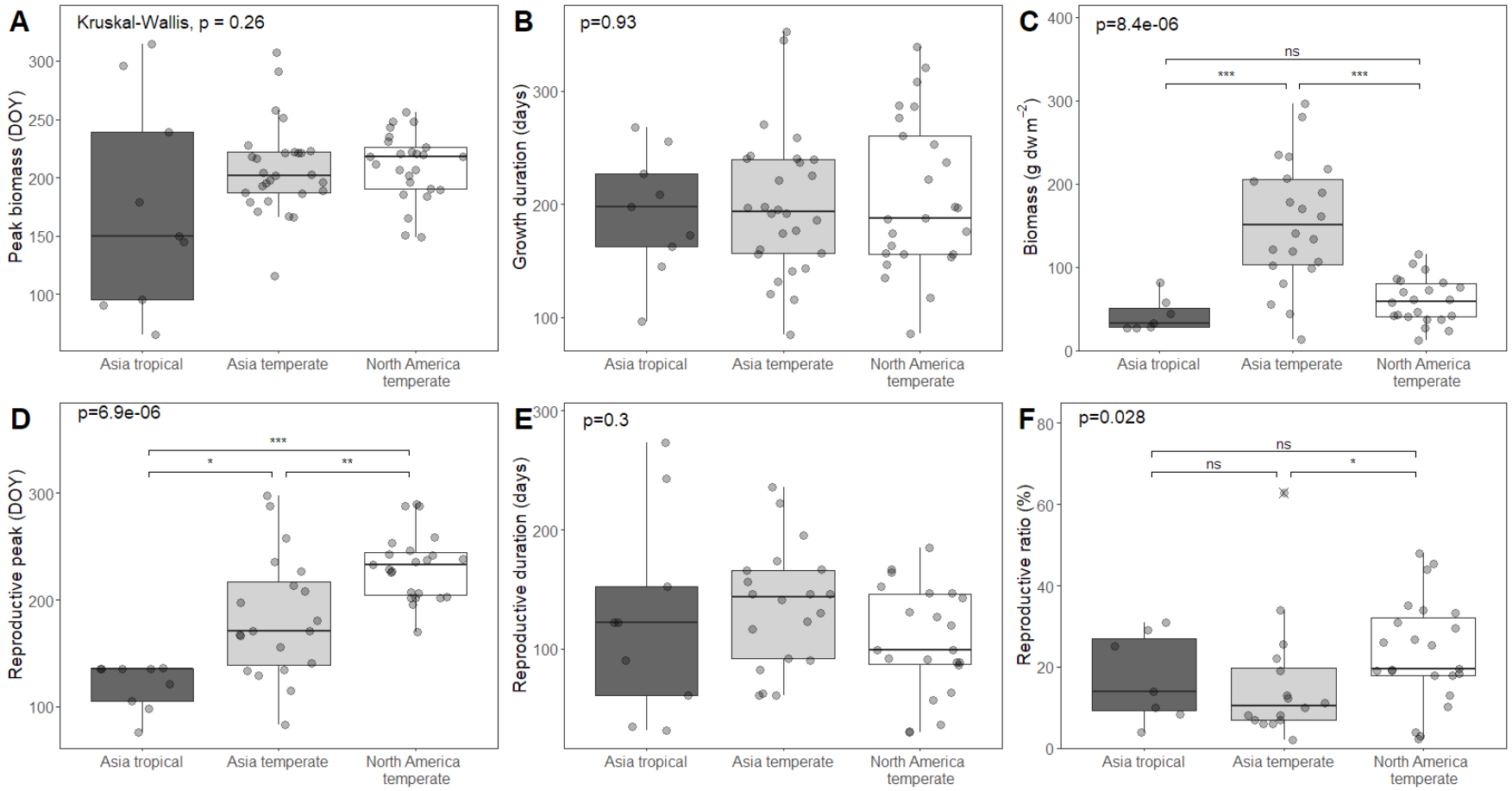

Figure 2.2. Comparison of biomass and reproductive traits among *Zostera japonica* geographic groups for: (A) peak biomass timing by day of year (DOY), (B) growth duration, (C) maximum biomass, (D) peak reproductive timing, (E) reproductive duration, and (F) maximum reproductive ratio. A crossed circle in (F) represents an outlier removed from the analysis. Boxes delimit upper and lower

quartiles, the horizontal line within the box is the median, and the whiskers above and below the box indicate the interquartile range. Dots represent actual values observed for each group. Results from the Kruskal-Wallis rank sum test are presented in the top-left corner of each plot, and significance of the post hoc Dunn's test results is indicated as: ns: not significant; $p > 0.05$, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

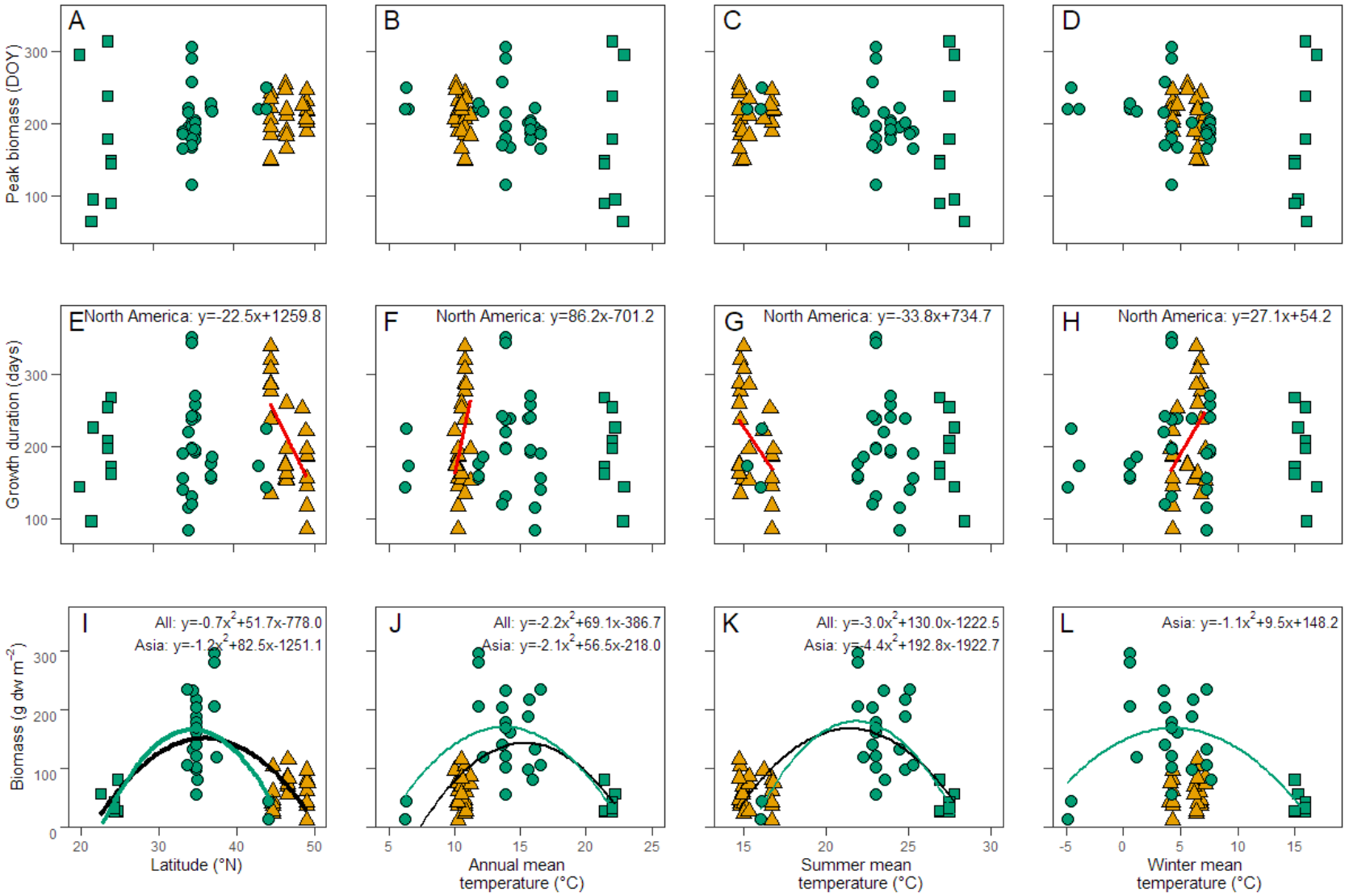
A
B
C
D
Peak biomass (DOY)
E North America: y=-22.5x+1259.8
F North America: y=86.2x-701.2
G North America: y=-33.8x+734.7
H North America: y=27.1x+54.2
Growth duration (days)
I All: y=-0.7x²+51.7x-778.0
Asia: y=-1.2x²+82.5x-1251.1
J All: y=-2.2x²+69.1x-386.7
Asia: y=-2.1x²+56.5x-218.0
K All: y=-3.0x²+130.0x-1222.5
Asia: y=-4.4x²+192.8x-1922.7
L Asia: y=-1.1x²+9.5x+148.2
Biomass (g dw m⁻²)
Latitude (°N)
Annual mean temperature (°C)
Summer mean temperature (°C)
Winter mean temperature (°C)

Figure 2.3. Biomass variables in relation to latitudes and temperature. (A-D) Peak biomass timing by day of year (DOY), (E-H) growth durations, and (I-L) maximum biomass are compared to latitude (A, E, I), annual mean (B, F, J), summer mean (C, G, K), and winter mean (D, H, L) temperatures. Shapes represent different geographic groups; Asia-tropical (square), Asia-temperate (circle) and North-America temperate (triangle). Significant regression lines in panels (I-L) are green for the Asian populations, red for the North American populations, and black for all populations, with the equation at the top of the panel.

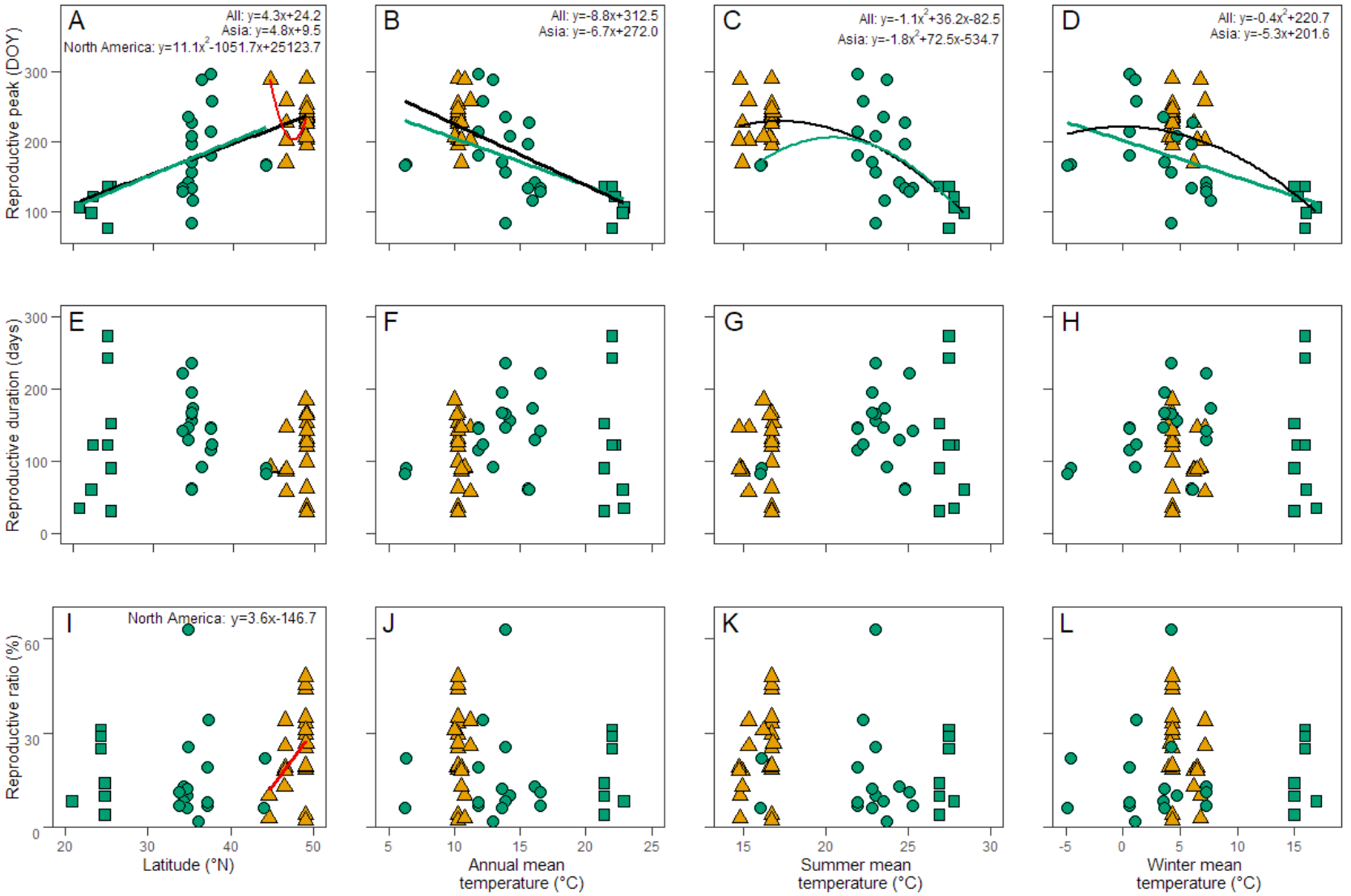
A
All: y=4.3x+24.2
Asia: y=4.8x+9.5
North America: y=11.1x²-1051.7x+25123.7
B
All: y=-8.8x+312.5
Asia: y=-6.7x+272.0
C
All: y=-1.1x²+36.2x-82.5
Asia: y=-1.8x²+72.5x-534.7
D
All: y=-0.4x²+220.7
Asia: y=-5.3x+201.6
Reproductive peak (DOY)
E
F
G
H
Reproductive duration (days)
I
North America: y=3.6x-146.7
J
K
L
Reproductive ratio (%)
Latitude (°N)
Annual mean temperature (°C)
Summer mean temperature (°C)
Winter mean temperature (°C)

Figure 2.4. Reproductive variables in relation to latitude and temperatures. (A-D) Peak reproductive timing by day of year (DOY), (E-H) reproductive durations, and (I-L) maximum reproductive ratio are compared to latitude (A, E, I), annual mean (B, F, J), summer mean (C, G, K), and winter mean (D, H, L) temperatures. Other details as in Fig. 2.3.

Table 2.1. List of studies included in the analysis for seagrass biomass and reproduction. Numbers of data for seagrass biomass and/or reproduction that are noted as a single study may contain data from several locations or several years. Dashes mean no data from the study were used in the analysis. For biomass, data for [a] density, [b] AFDW, [c] total biomass are used instead of above-ground biomass.

Geographic group Study site	Latitude (°N)	Annual temp (°C)	Sampling years	No. of data for Biomass	No. of data for Reproduction	Reference
North America temperate						
Boundary Bay, BC	49.1	10.3	1978	1	1	Harrison (1982a)
Boundary Bay, BC	49.1	10.2	2017-2018	1	1	This study
Roberts Bank, BC	49.0	10.3	1980-1981	3[a]	3	Bigley & Harrison (1986)
Roberts Bank, BC	49.0	10.3	1979-1981	4	8	Harrison (1982b)
Roberts Bank, BC	49.0	10.3	1987	1	1	Nomme & Harrison (1991)
Roberts Bank, BC	49.0	10.0	2017-2018	1	1	This study
Padilla Bay, WA	48.5	10.5	1989-1990	1[a]	-	Thom et al. (1995)
Willapa Bay, WA	46.6	10.5	2004	6	6	Ruesink et al. (2010)
Willapa Bay, WA	46.4	10.1	2009-2010	2[a]	-	Wheatcroft et al. (2013)
Yaquina Bay, OR	44.6	10.8	2001-2003	2	2	Kaldy (2006)
Yaquina Bay, OR	44.6	10.8	1999-2000	7	-	Larned (2003)
Asia temperate						
Notoro-ko, Japan	44.1	6.3	2018	1	1	This study
Notoro-ko, Japan	44.0	6.2	2018	1	1	This study
Akkeshi-ko, Japan	43.0	6.5	2018	1	-	This study
Swan Lake, China	37.4	12.2	2011-2013	1	2	Zhang et al. (2015)
Sungbondo Isl., Korea	37.2	11.8	2001-2002	3	3	Lee et al. (2005)
Ena Bay, Japan	35.1	15.8	2007-2009	5[a]	-	Koshikawa et al. (2009)
Tateyama Bay, Japan	35.0	15.9	2004-2005	1	1	Inoue et al. (2006)
Koje Bay, Korea	34.8	13.9	2004-2006	3	-	Kim et al. (2016)
Koje Bay, Korea	34.8	13.9	2003-2005	3	1	Park et al. (2011)

Koje Bay, Korea	34.8	13.9	2015-2016	5	3	Suonan et al. (2017)
Mikawa Bay, Japan	34.8	15.6	2011-2012	3	3	Kamohara et al. (2015)
Dadae Bay, Korea	34.7	14.2	2001-2002	1	1	Lee et al. (2006)
Sagumi Bay, Korea	34.3	13.9	2008-2011	2	1	Ok & Lee (2014)
Ago Bay, Japan	34.3	16.1	2003-2004	2	1	Abe et al. (2012)
Tanabe Bay, Japan	33.7	16.5	2004-2005	3	2	Uede (2007)
Asia tropical						
Hsiangshan, Taiwan	24.8	21.4	2007-2008	3	3	This study
Kaomei, Taiwan	24.3	22.0	2007-2008	3	3	This study
Lai Chi Wo, Hong Kong	22.5	22.2	1995-1996	1	1	Fong et al. (1998)
San Tau, Hong Kong	22.3	22.8	1993-1994	1[b]	1	Lee (1997)
Ha Long Bay, Vietnam	20.9	22.9	1999-2001	1[c]	1	Huong et al. (2003)

Table 2.2. Range of latitude and temperature of 3 geographic groups of *Zostera japonica*. For each variable, values for each group are summarized as minimum−maximum (mean ± SD). Kruskal-Wallis test and post-hoc Dunn test examined the variation in these variables among groups. Data are also available as a graphical form in Figure S2.1.

Variable	North America-temperate (NAtemp)	Asia-temperate (Atemp)	Asia-tropical (Atrop)	Kruskal-Wallis test p	Post hoc Dunn test
Latitude (°N)	44.6−49.1 (47.2±1.9)	33.7−44.1 (35.7±2.6)	20.9−24.8 (23.7±1.4)	< 0.001	NAtemp > Atemp > Atrop
Annual air temp. (°C)	10.0−11.2 (10.5±0.3)	6.2−16.5 (13.8±2.7)	21.4−22.9 (22.0±0.6)	< 0.001	NAtemp < Atemp < Atrop
Summer air temp. (°C)	14.7−16.8 (15.8±0.9)	15.2−25.3 (22.9±2.4)	26.9−28.4 (27.5±0.5)	< 0.001	NAtemp < Atemp < Atrop
Winter air temp. (°C)	4.0−7.2 (5.4±1.2)	-4.9−7.7 (4.1±3.4)	15.0−16.9 (15.7±0.6)	< 0.001	NAtemp = Atemp < Atrop

Table 2.3. Best regression models for biomass and reproductive traits selected based on Akaike's information criterion corrected for small sample size (AICc). Estimate ± SE are noted for quadratic and/or linear coefficients and the intercept of selected models. Null indicates neither linear nor quadratic models are selected. Dashes indicate that the estimate was not applicable to the selected model. Within each trait, the environmental variable with the highest explanatory strength (R^2) is **bolded**.

Phenological trait	Asian populations					North American populations					All populations				
Environmental variable	model	quadratic (SE)	linear (SE)	intercept (SE)	R^2	model	quadratic (SE)	linear (SE)	intercept (SE)	R^2	model	quadratic (SE)	linear (SE)	intercept (SE)	R^2
Biomass peak															
Latitude	Null	———	—	———	—	Null	———	—	———	—	Null	———	—	———	—
Annual mean	Null	———	—	———	—	Null	———	—	———	—	Null	———	—	———	—
Summer mean	Null	———	—	———	—	Null	———	—	———	—	Null	———	—	———	—
Winter mean	Null	———	—	———	—	Null	———	—	———	—	Null	———	—	———	—
Growth duration															
Latitude	Null	———	—	———	—	Linear	———	**-22.5 (5.8)**	**1259.8 (272.1)**	**0.37**	Null	———	—	———	—
Annual mean	Null	———	—	———	—	Linear	———	86.2 (39.0)	-701.2 (411.7)	0.14	Null	———	—	———	—
Summer mean	Null	———	—	———	—	Linear	———	-33.8 (14.2)	734.7 (221.1)	0.16	Null	———	—	———	—
Winter mean	Null	———	—	———	—	Linear	———	27.1 (10.7)	54.2 (61.9)	0.18	Null	———	—	———	—
Maximum biomass															
Latitude	Quadratic	-1.2 (0.3)	82.5 (18.9)	-1251.1 (298.6)	0.45	Null	———	—	———	—	Quadratic	-0.7 (0.1)	51.7 (9.3)	-778.0 (167.4)	0.42
Annual mean	Quadratic	-2.1 (0.5)	56.5 (14.3)	-218.0 (108.5)	0.48	Null	———	—	———	—	Quadratic	-2.3 (0.5)	69.1 (14.0)	-386.7 (100.7)	0.31
Summer mean	Quadratic	**-4.4 (0.9)**	**192.8 (38.7)**	**-1922.7 (432.1)**	**0.48**	Null	———	—	———	—	Quadratic	**-3.0 (0.5)**	**130.0 (22.3)**	**-1222.5 (219.4)**	**0.44**
Winter mean	Quadratic	-1.1 (0.3)	9.5 (4.6)	148.2 (17.4)	0.44	Null	———	—	———	—	Null	———	—	———	—
Reproductive peak															
Latitude	Linear	———	4.8 (1.5)	9.5 (47.6)	0.26	Quadratic	**11.1 (3.0)**	**-1051.7 (281.1)**	**25123.7 (6631.2)**	**0.37**	Linear	———	4.3 (0.6)	24.2 (25.7)	0.47

Annual mean	Linear	———	-6.7 (1.9)	272.0 (32.3)	0.29	Null	———	———	———	—	Linear	———	-8.8 (1.3)	312.5 (19.1)	0.45
Summer mean	Quadratic	-1.8 (0.7)	72.5 (29.5)	-534.7 (333.4)	0.32	Null	———	———	———	—	Quadratic	**-1.1 (0.4)**	**36.2 (17.3)**	**-82.5 (174.5)**	**0.48**
Winter mean	Linear	———	**-5.3 (1.3)**	**201.6 (12.8)**	**0.35**	Null	———	———	———	—	Quadratic	-0.4 (0.1)	———	220.7 (7.9)	0.38
Reproductive duration															
Latitude	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Annual mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Summer mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Winter mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Reproductive ratio															
Latitude	Null	———	———	———	—	Linear	———	**3.5 (1.7)**	**-146.7 (79.7)**	**0.14**	Null	———	———	———	—
Annual mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Summer mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—
Winter mean	Null	———	———	———	—	Null	———	———	———	—	Null	———	———	———	—

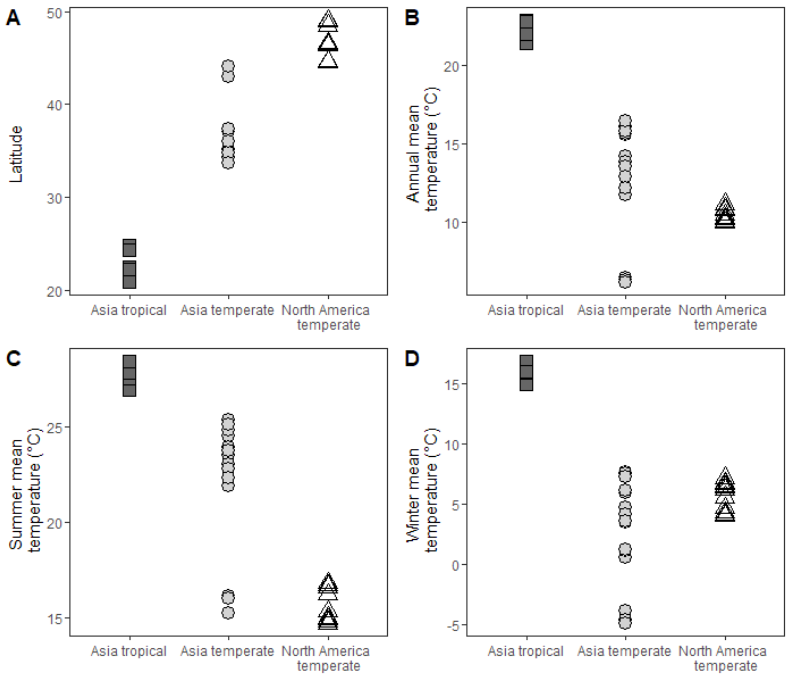

Figure S2.1. Among geographic group comparisons of latitude and annual, summer and winter mean temperatures.

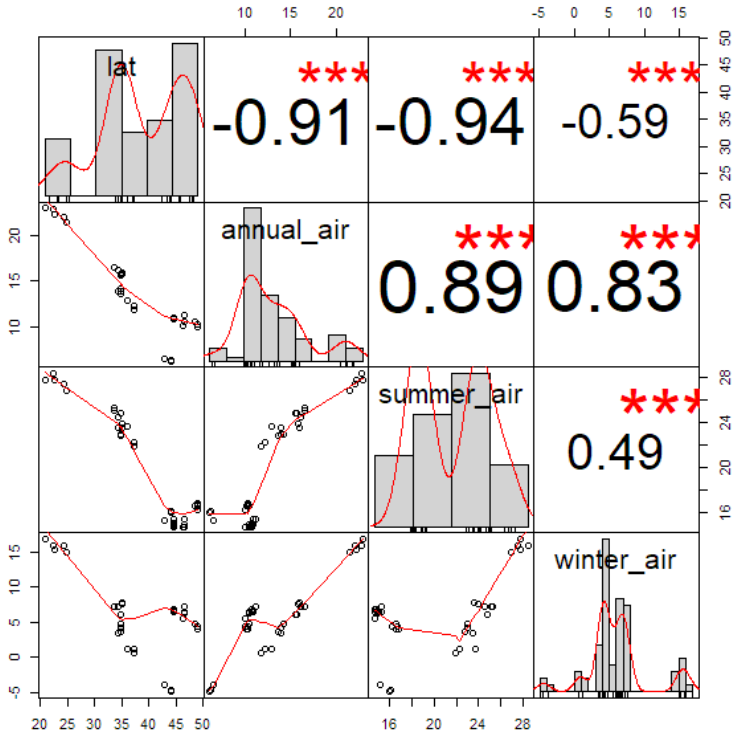

Figure S2.2. Correlation chart among environmental variables (latitude and temperature).

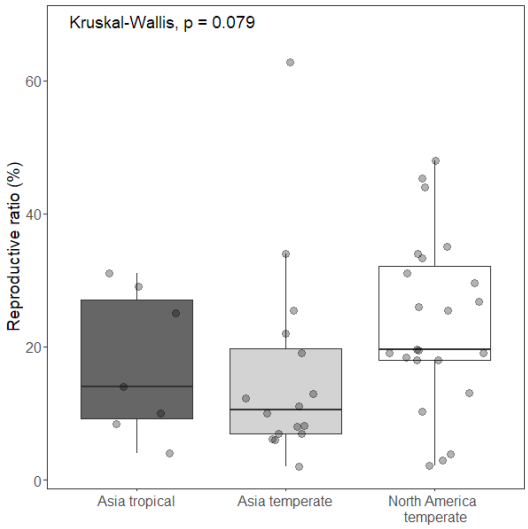

Figure S2.3. Among geographic group comparisons for reproductive ratios without removing an outlier.

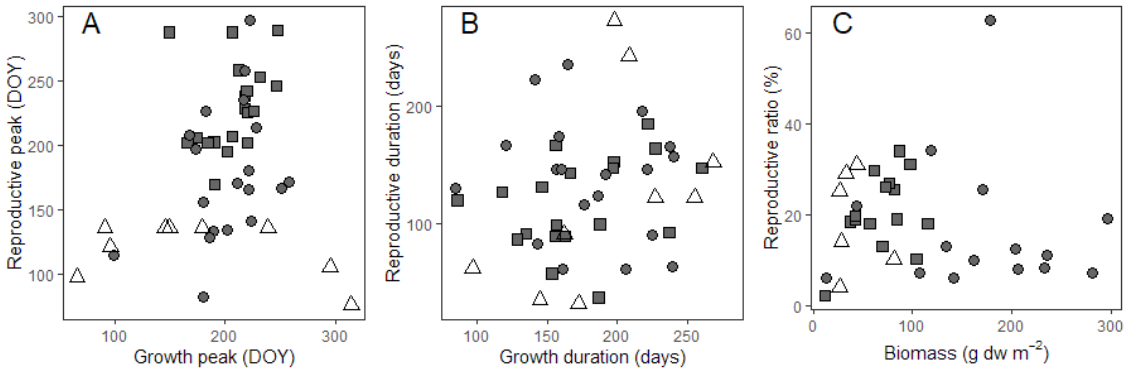

Figure S2.4. Comparisons between biomass and reproductive traits. (A) peak timings, (B) durations and (C) maximum outputs for growth and reproductive seasons are compared. Shapes represent different regional groups; Asia tropical (square), Asia temperate (circle) and North America temperate (triangle). Shapes are filled for the Asian populations.

Table S2.1. Biomass, density and reproductive ratio data used in the analysis. Data listed here are limited to author's unpublished data that are not publicly-accessible in other format upon the publication. For each data, name of the collector, the site and locality of collection, date and day of year (DOY) collected are noted. Ratio type specifies the type of reproductive ratio used. NU represents the data Not Used in the study.

Collector	Site	Locality	Date	DOY	Biomass	Density	Rep ratio (%)	Ratio type
Ito	Boundary Bay, BC	Crescent Beach	Apr 2017	117	16.99	NU	0.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	May 2017	135	18.19	NU	0.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Jun 2017	160	42.68	NU	7.2	density-based
Ito	Boundary Bay, BC	Crescent Beach	Jul 2017	206	23.62	NU	18.6	density-based
Ito	Boundary Bay, BC	Crescent Beach	Aug 2017	230	22.07	NU	13.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Sep 2017	259	21.51	NU	15.1	density-based
Ito	Boundary Bay, BC	Crescent Beach	Oct 2017	294	11.25	NU	5.6	density-based
Ito	Boundary Bay, BC	Crescent Beach	Nov 2017	324	5.67	NU	2.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Dec 2017	349	0.86	NU	0.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Jan 2018	16	1.44	NU	0.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Feb 2018	45	4.79	NU	0.0	density-based
Ito	Boundary Bay, BC	Crescent Beach	Mar 2018	61	3.28	NU	0.0	density-based
Ito	Roberts Bank, BC	Tsawwassen	Apr 2017	104	20.05	NU	1.0	density-based
Ito	Roberts Bank, BC	Tsawwassen	May 2017	132	43.63	NU	2.0	density-based
Ito	Roberts Bank, BC	Tsawwassen	Jun 2017	163	83.69	NU	12.4	density-based
Ito	Roberts Bank, BC	Tsawwassen	Jul 2017	207	97.66	NU	30.6	density-based
Ito	Roberts Bank, BC	Tsawwassen	Aug 2017	229	79.75	NU	29.6	density-based

Ito	Roberts Bank, BC	Tsawwassen	Sep 2017	258	64.89	NU			11.2	density-based
Ito	Roberts Bank, BC	Tsawwassen	Oct 2017	281	57.76	NU			11.1	density-based
Ito	Roberts Bank, BC	Tsawwassen	Nov 2017	321	14.38	NU			6.9	density-based
Ito	Roberts Bank, BC	Tsawwassen	Dec 2017	348	5.62	NU			9.2	density-based
Ito	Roberts Bank, BC	Tsawwassen	Jan 2018	15	10.37	NU			0.0	density-based
Ito	Roberts Bank, BC	Tsawwassen	Feb 2018	44	12.34	NU			0.0	density-based
Ito	Roberts Bank, BC	Tsawwassen	Mar 2018	60	13.04	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO2	May 2018	121	5.6	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO2	Jun 2018	167	22	NU			21.5	density-based
Ito	Notoro-ko, Japan	NO2	Jul 2018	194	32.5	NU			21.3	density-based
Ito	Notoro-ko, Japan	NO2	Aug 2018	220	34	NU			17.8	density-based
Ito	Notoro-ko, Japan	NO2	Sep 2018	257	43.9	NU			0.8	density-based
Ito	Notoro-ko, Japan	NO2	Oct 2018	277	40.8	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO2	Nov 2018	305	31.3	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO3	May 2018	122	0.2	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO3	Jun 2018	166	5.7	NU			5.9	density-based
Ito	Notoro-ko, Japan	NO3	Jul 2018	193	11.2	NU			3.5	density-based
Ito	Notoro-ko, Japan	NO3	Aug 2018	220	13.1	NU			2.7	density-based
Ito	Notoro-ko, Japan	NO3	Sep 2018	248	12	NU			0.3	density-based
Ito	Notoro-ko, Japan	NO3	Oct 2018	278	5	NU			0.0	density-based
Ito	Notoro-ko, Japan	NO3	Nov 2018	306	0.5	NU			0.0	density-based
Ito	Akkeshi-ko, Japan	CK	May 2018	136	NU		2091	NU		NU
Ito	Akkeshi-ko, Japan	CK	Jun 2018	169	NU		5177	NU		NU

Ito	Akkeshi-ko, Japan	CK	Jul 2018	197	NU	7312	NU		NU
Ito	Akkeshi-ko, Japan	CK	Oct 2018	282	NU	3689	NU		NU
Ito	Akkeshi-ko, Japan	CK	Nov 2018	308	NU	2741	NU		NU
Lin & Hsiao	Hsiangshan, Taiwan	H1	Mar 2003	75	28.46	NU		1.5	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Apr 2003	106	30.7	NU		8.5	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	May 2003	136	50.93	NU		9.7	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Jun 2003	167	45.8	NU		3.4	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Jul 2003	197	35.17	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Aug 2003	228	18.02	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Sep 2003	258	23.89	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Oct 2003	288	33.87	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Nov 2003	319	33.31	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Dec 2003	349	61.28	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Jan 2004	15	65.85	NU		5.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Feb 2004	45	48.51	NU		1.5	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H1	Mar 2004	76	81.52	NU		8.5	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Mar 2003	75	12.61	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Apr 2003	106	13.26	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	May 2003	136	27.06	NU		4.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Jun 2003	167	25.66	NU		3.1	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Jul 2003	197	19.98	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Aug 2003	228	4.97	NU		0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Sep 2003	258	4.22	NU		0.0	density-based

Lin & Hsiao	Hsiangshan, Taiwan	H2	Oct 2003	288	5.34	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Nov 2003	319	2.54	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Dec 2003	349	3.01	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Jan 2004	15	4.03	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Feb 2004	45	5.06	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H2	Mar 2004	76	7.86	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Mar 2003	75	9.16	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Apr 2003	106	18.48	NU	2.8	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	May 2003	136	17.93	NU	14.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Jun 2003	167	28.37	NU	4.8	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Jul 2003	197	10.37	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Aug 2003	228	3.75	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Sep 2003	258	8.88	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Oct 2003	288	10	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Nov 2003	319	7.58	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Dec 2003	349	3.66	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Jan 2004	15	7.95	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Feb 2004	45	5.52	NU	0.0	density-based
Lin & Hsiao	Hsiangshan, Taiwan	H3	Mar 2004	76	8.97	NU	2.6	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Mar 2003	75	9.42	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Apr 2003	106	17.57	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	May 2003	136	22.85	NU	25.2	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Jun 2003	167	18.99	NU	17.3	density-based

Lin & Hsiao	Kaomei, Taiwan	K1	Jul 2003	197	23.88	NU	13.3	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Aug 2003	228	26.18	NU	14.4	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Sep 2003	258	19.06	NU	4.2	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Oct 2003	289	26.73	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Nov 2003	319	20.19	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Dec 2003	350	5.59	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Jan 2004	15	6.63	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Feb 2004	46	2.69	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K1	Mar 2004	76	3.93	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Mar 2003	75	9.13	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Apr 2003	106	14.6	NU	1.7	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	May 2003	136	35.04	NU	31.3	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Jun 2003	167	44.06	NU	27.8	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Jul 2003	197	22.35	NU	2.1	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Aug 2003	228	32.71	NU	7.9	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Sep 2003	258	19.64	NU	4.9	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Oct 2003	289	33.07	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Nov 2003	319	15.87	NU	3.4	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Dec 2003	350	7.12	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Jan 2004	15	9.52	NU	6.3	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Feb 2004	46	7.2	NU	5.9	density-based
Lin & Hsiao	Kaomei, Taiwan	K2	Mar 2004	76	6.81	NU	9.4	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Mar 2003	75	19.6	NU	0.0	density-based

Lin & Hsiao	Kaomei, Taiwan	K3	Apr 2003	106	5.66	NU	1.3	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	May 2003	136	14.59	NU	10.8	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Jun 2003	167	20.24	NU	13.1	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Jul 2003	197	12.65	NU	7.4	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Aug 2003	228	14.94	NU	6.8	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Sep 2003	258	15.12	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Oct 2003	289	27.79	NU	0.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Nov 2003	319	32.97	NU	1.1	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Dec 2003	350	28.25	NU	8.1	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Jan 2004	15	17.01	NU	10.6	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Feb 2004	46	15.27	NU	18.0	density-based
Lin & Hsiao	Kaomei, Taiwan	K3	Mar 2004	76	22.46	NU	29.4	density-based

Table S2.2a. Details for linear and quadratic regression models for biomass and reproductive traits. Best models selected based on AICc are bolded, for each trait and variable combination for (a) Asian, (b) North American, and (c) all populations. Environmental variables with the highest explanatory strength (R^2) for each trait is noted with an asterisk.

	Asian populations										
Phenological trait	Linear						Quadratic				
Environmental variable	F	df	*p*	R^2	AICc		F	df	*p*	R^2	AICc	
Biomass peak												
Latitude	3.20	1,35	0.08	0.06	405.1		1.66	2,34	0.21	0.04	407.4	
Annual mean	3.77	1,35	0.06	0.07	404.6		1.84	2,34	0.18	0.04	407.1	
Summer mean	3.55	1,35	0.07	0.07	404.8		1.96	2,34	0.16	0.05	406.8	
Winter mean	3.52	1,35	0.07	0.07	404.8		1.84	2,34	0.17	0.04	407.1	
Growth duration												
Latitude	0.03	1,35	0.86	0.00	414.0		0.52	2,34	0.60	0.00	415.4	
Annual mean	0.01	1,35	0.91	0.00	414.0		0.46	2,34	0.64	0.00	415.5	
Summer mean	0.01	1,35	0.93	0.00	414.0		0.60	2,34	0.56	0.00	415.2	
Winter mean	0.00	1,35	0.98	0.00	414.0		0.44	2,34	0.65	0.00	415.6	
Maximum biomass												
Latitude	5.08	1,27	<0.05	0.13	338.6		12.37	2,26	**<0.001**	**0.45**	**326.9**	
Annual mean	4.17	1,27	0.05	0.10	339.4		13.73	2,26	**<0.001**	**0.48**	**325.4**	
Summer mean	0.89	1,27	0.35	0.00	342.7		14.09	2,26	**<0.001**	**0.48**	**325.0**	*
Winter mean	6.58	1,27	<0.05	0.17	337.3		11.92	2,26	**<0.001**	**0.44**	**327.4**	
Reproductive peak												
Latitude	10.90	1,27	**<0.01**	**0.26**	**312.9**		6.09	2,26	<0.001	0.27	314.3	
Annual mean	12.17	1,27	**<0.01**	**0.29**	**312.0**		7.72	2,26	<0.005	0.32	312.0	
Summer mean	6.16	1,27	<0.05	0.16	316.8		7.57	2,26	**<0.005**	**0.32**	**312.2**	
Winter mean	15.93	1,27	**<0.001**	**0.35**	**309.3**	*	8.13	2,26	<0.005	0.34	311.4	
Reproductive duration												
Latitude	0.00	1,27	0.97	0.00	328.0		1.69	2,26	0.20	0.05	327.2	
Annual mean	0.00	1,27	0.94	0.00	328.0		0.87	2,26	0.43	0.00	328.9	
Summer mean	0.02	1,27	0.89	0.00	328.0		0.95	2,26	0.40	0.00	328.7	
Winter mean	0.01	1,27	0.92	0.00	328.0		0.78	2,26	0.47	0.00	329.0	
Reproductive ratio												
Latitude	0.04	1,21	0.84	0.00	191.9		0.04	2,20	0.96	0.00	194.8	
Annual mean	0.05	1,21	0.82	0.00	191.9		0.03	2,20	0.97	0.00	194.8	
Summer mean	0.02	1,21	0.89	0.00	191.9		0.01	2,20	0.99	0.00	194.8	
Winter mean	0.09	1,21	0.76	0.00	191.8		0.06	2,20	0.95	0.00	194.7	

Table S2.2b. Details for linear and quadratic regression models for biomass and reproductive traits. Best models selected based on AICc are bolded, for each trait and variable combination for (a) Asian, (b) North American, and (c) all populations. Environmental variables with the highest explanatory strength (R^2) for each trait is noted with an asterisk.

	North American populations										
Phenological trait	Linear						Quadratic				
Environmental variable	F	df	*p*	R^2	AICc		F	df	*p*	R^2	AICc	
Biomass peak												
Latitude	1.85	1,23	0.19	0.03	242.6		0.95	2,22	0.40	0.00	245.4	
Annual mean	3.96	1,23	0.06	0.11	240.6		2.47	2,22	0.11	0.11	242.4	
Summer mean	1.21	1,23	0.28	0.01	243.3		0.97	2,22	0.39	0.00	245.3	
Winter mean	3.17	1,23	0.09	0.08	241.3		2.23	2,22	0.13	0.09	242.8	
Growth duration												
Latitude	14.99	1,23	**<0.001**	**0.37**	**275.6**	*	9.52	2,22	<0.005	0.42	275.4	
Annual mean	4.87	1,23	**<0.05**	**0.14**	**283.3**		3.41	2,22	0.05	0.17	284.2	
Summer mean	5.71	1,23	**<0.05**	**0.16**	**282.6**		3.45	2,22	<0.05	0.17	284.2	
Winter mean	6.36	1,23	**<0.05**	**0.18**	**282.0**		3.73	2,22	<0.05	0.19	283.7	
Maximum biomass												
Latitude	0.26	1,20	0.61	0.00	214.4		3.32	2,19	0.06	0.18	211.1	
Annual mean	0.05	1,20	0.83	0.00	214.7		0.70	2,19	0.51	0.00	216.2	
Summer mean	0.55	1,20	0.47	0.00	214.1		0.28	2,19	0.76	0.00	217.1	
Winter mean	0.35	1,20	0.56	0.00	214.3		0.42	2,19	0.66	0.00	216.8	
Reproductive peak												
Latitude	0.67	1,21	0.42	0.00	229.1		7.49	2,20	**<0.005**	**0.37**	**219.9**	*
Annual mean	0.82	1,21	0.37	0.00	228.9		0.59	2,20	0.57	0.00	231.4	
Summer mean	0.09	1,21	0.76	0.00	229.7		0.22	2,20	0.80	0.00	232.2	
Winter mean	0.02	1,21	0.88	0.00	229.8		0.67	2,20	0.52	0.00	231.3	
Reproductive duration												
Latitude	0.35	1,20	0.56	0.00	236.4		0.17	2,19	0.84	0.00	239.4	
Annual mean	0.76	1,20	0.39	0.00	236.0		1.58	2,19	0.23	0.05	236.4	
Summer mean	0.08	1,20	0.78	0.00	236.7		0.30	2,19	0.74	0.00	239.1	
Winter mean	0.34	1,20	0.57	0.00	236.4		0.40	2,19	0.67	0.00	238.9	
Reproductive ratio												
Latitude	4.57	1,21	**<0.05**	**0.14**	**184.6**	*	2.62	2,20	0.10	0.13	186.7	
Annual mean	0.47	1,21	0.50	0.00	188.6		1.85	2,20	0.18	0.07	188.1	
Summer mean	3.51	1,21	0.07	0.10	185.5		2.45	2,20	0.11	0.12	187.0	
Winter mean	1.99	1,21	0.17	0.04	187.0		1.54	2,20	0.24	0.05	188.7	

Table S2.2c. Details for linear and quadratic regression models for biomass and reproductive traits. Best models selected based on AICc are bolded, for each trait and variable combination for (a) Asian, (b) North American, and (c) all populations. Environmental variables with the highest explanatory strength (R^2) for each trait is noted with an asterisk.

	All populations									
Phenological trait	Linear					Quadratic				
Environmental variable	F	df	*p*	R^2	AICc	F	df	*p*	R^2	AICc
Biomass peak										
Latitude	3.99	1,60	0.05	0.05	654.1	2.15	2,59	0.13	0.04	656.0
Annual mean	5.98	1,60	<0.05	0.07	652.2	2.95	2,59	0.06	0.06	654.4
Summer mean	3.11	1,60	0.08	0.03	654.9	3.10	2,59	0.05	0.06	654.1
Winter mean	6.17	1,60	<0.05	0.08	652.0	3.09	2,59	0.05	0.06	654.2
Growth duration										
Latitude	0.01	1,60	0.93	0.00	695.4	1.12	2,59	0.33	0.00	695.4
Annual mean	0.10	1,60	0.75	0.00	695.3	0.65	2,59	0.53	0.00	696.3
Summer mean	0.65	1,60	0.42	0.00	694.7	0.33	2,59	0.72	0.00	697.0
Winter mean	0.08	1,60	0.78	0.00	695.3	0.81	2,59	0.45	0.00	696.0
Maximum biomass										
Latitude	2.28	1,49	0.14	0.03	584.1	18.85	2,48	**<0.001**	**0.42**	**559.2**
Annual mean	0.05	1,49	0.82	0.00	586.4	12.27	2,48	**<0.001**	**0.31**	**567.8**
Summer mean	6.26	1,49	<0.05	0.10	580.3	20.80	2,48	**<0.001**	**0.44**	**557.0** *
Winter mean	6.04	1,49	<0.05	0.09	580.5	3.93	2,48	<0.05	0.10	581.1
Reproductive peak										
Latitude	45.89	1,50	**<0.001**	**0.47**	**541.3**	23.87	2,49	<0.001	0.47	542.1
Annual mean	43.07	1,50	**<0.001**	**0.45**	**542.8**	23.39	2,49	<0.001	0.47	542.7
Summer mean	37.80	1,50	<0.001	0.42	545.9	24.30	2,49	**<0.001**	**0.48**	**541.7** *
Winter mean	21.30	1,50	<0.001	0.28	556.7	32.65	1,50	**<0.001**	**0.38**	**549.0**
Reproductive duration										
Latitude	1.72	1,49	0.20	0.01	560.5	1.94	2,48	0.15	0.04	560.6
Annual mean	1.09	1,49	0.30	0.00	561.1	1.76	2,48	0.18	0.03	561.0
Summer mean	2.34	1,49	0.13	0.03	559.9	2.34	2,48	0.11	0.05	559.8
Winter mean	0.13	1,49	0.72	0.00	562.1	0.14	2,48	0.88	0.00	564.3
Reproductive ratio										
Latitude	2.88	1,44	0.10	0.04	373.6	2.41	2,43	0.10	0.06	374.1
Annual mean	0.86	1,44	0.36	0.00	375.7	0.43	2,43	0.66	0.00	378.1
Summer mean	1.59	1,44	0.21	0.01	374.9	0.85	2,43	0.43	0.00	377.2
Winter mean	0.03	1,44	0.87	0.00	376.5	0.53	2,43	0.59	0.00	377.9

Chapter 3

Regional comparison revealed reduced shoot density and enhanced sexual reproduction in non-native seagrass populations

Abstract: Study of non-native species traits is important not only for management purposes but also provides beneficial opportunities to examine potential trait shifts during biological invasions. In this study, I examined biological traits for native (Japan) and non-native (Canada) populations of the seagrass *Zostera japonica*, along with its congener species, *Z. marina* which is native both in Japan and Canada. By using a combination of intraspecific and interspecific comparisons, I was able to distinguish introduction effects from regional environmental differences. Non-native *Z. japonica* showed significantly lower shoot density, leaf area index (LAI) and biomass, and significantly higher reproductive ratio than native populations, whereas shoot length was similar between regions. In contrast, all traits were not significantly different between regions for *Z. marina*. Likewise, multivariate analyses revealed that traits differed greatly between regions only for *Z. japonica*. Due to constant shoot size, reduced LAI and biomass were considered as resulting from reduced shoot density. While enhanced reproduction observed for non-native *Z. japonica* may be a cause of successful introduction or a result of adaptation to environmental stresses, concurrent reduced shoot density suggests a trade-off between vegetative and sexual production. In conclusion, these results suggest that non-native *Z. japonica* experienced potential trait shifts during introduction and settlement in North America.

3.1 Introduction

A fundamental research question in invasion biology is why some non-native species establish, spread, and impact novel ecosystems while others do not. It is extremely important for scientists and managers to find key biological traits of a non-native species that are related to invasiveness to mitigate their effects on native populations. Numerous researchers have collated information on non-native species and their biological traits, documenting specific traits affecting species' invasiveness (Kolar and Lodge 2001; Pyšek and Richardson 2007; Hayes and Barry 2008; van Kleunen et al. 2010a; van Kleunen et al. 2015; but see Thompson and Davis 2011). Rapid growth, large body size and biomass, high reproductive output, high dispersal ability, and high environmental tolerance, are examples of traits common to successful invaders (Pyšek and Richardson 2007; Mason et al. 2008; van Kleunen et al. 2015; Cardeccia et al. 2018).

Studying non-native species traits is important not only for management purposes but also provides beneficial opportunities for facilitating a deeper understanding of invasion events in the contexts of ecology, evolutionary and population biology, and biogeography (Sakai et al. 2001; Sax et al. 2005; Sax et al. 2007; Keller and Taylor 2008). The biological invasion processes consist of multiple stages that occur in order: transport, introduction, establishment, and spread (Blackburn et al. 2011). During these processes, a species undergoes filtering at each stage which often leads the species to experience a population bottleneck (Sakai et al. 2001; Blackburn et al. 2011), and severe population bottleneck occasionally resulting in reduced trait variability (Prôa and Nanova 2020). Study on plant functional traits revealed that traits involved in individual-level processes and functions could be scaled up to evaluate demographic and population-level processes

(Drenovsky et al. 2012). Thus, reduced trait variability under severe bottleneck would affect traits in individual and higher organization levels.

Different traits play important roles in each stage of invasion processes. For example, high dispersal abilities could enhance rapid colonization in disturbed habitats and increase potential for further spread (Viard et al. 2006; Mason et al. 2008; Ros et al. 2020). Tolerance to environmental stressors can help species survive during dispersal, as well as out-competing native species during establishment and spread (Rudnick et al. 2003; van Kleunen et al. 2011; Tingley et al. 2014). For example, studies on invasive amphipods, ascidian, macroalgae, and seagrass showed that the invasive species can tolerate wide ranges of abiotic factors, such as salinity, temperature, and light (Jofré Madariaga et al. 2014; Oscar et al. 2018; Casties et al. 2019). These adaptive traits are not necessarily inherent to species in their native range but can evolve rapidly through the process of introduction (Keller and Taylor 2008; Elst et al. 2016). Studies on invasive plants suggest that species altered their traits through local adaptation to their introduced environment (Maron et al. 2004; Whitney and Gabler 2008; Colautti and Barrett 2013; Novy et al. 2013). Furthermore, invasive species frequently increase their dispersal ability after their establishment to non-native habitats, leading to further range expansion of the species (Whitney and Gabler 2008; Perkins et al. 2013; Ochocki and Miller 2017). As shown, invasive traits may be the causes of successful introduction or may be obtained through adaptive evolution during the introduction processes.

Key biological traits for invasiveness have been tested in the following two methods: (1) interspecific comparison with native/non-invasive species in an introduced regions (Mack 1996; van Kleunen et al. 2010a) and (2) intraspecific comparison between populations of alien species in their introduced and native ranges (Bossdorf et al. 2005;

Hierro et al. 2005; Parker et al. 2013). The first method, interspecific comparison, has been widely used in invasion biology, especially in attempts to identify traits involved with invasiveness (Hamilton et al. 2005; van Kleunen et al. 2010a). However, there are difficulties in selecting appropriate spatial scales of study and the native comparators (van Kleunen et al. 2010b, Hulme and Bernard-Verdier 2018). For example, relationships between non-native and native congeners can be interpreted differently at different spatial scales: continental, regional, and local scales among habitats or within-habitats (Hamilton et al. 2005; Diez et al. 2008). Also, trait comparisons between invasive species and native species that are invasive elsewhere, resulted in a non-significant difference (van Kleunen et al. 2010a).

The second method, intraspecific comparison, became more popular as researchers found the value of biogeographical comparisons in invasion studies (Jakobs et al. 2004; Hierro et al. 2005). This method is especially useful for understanding genetic and/or ecological changes during invasion (Huey et al. 2005, Sax et al. 2007) and testing specific hypotheses related for how traits influence invasion success, such as the enemy-release hypothesis (Torchin et al. 2001) and the evolution-of-increased-competitive-ability hypothesis (Blossey and Nötzold 1995). However, it is difficult to interpret intraspecific biogeographical comparisons when the target species shows high phenotypic plasticity and when studied traits show strong environmental effects (Hulme and Bernard-Verdier 2018; Milanović et al. 2020). Combined use of these two methods is promising because both methods have strengths and limitations (van Kleunen et al. 2010b), but has rarely been conducted in empirical studies due to limitations in selecting appropriate pairs of species that occur both native and non-native regions.

Here, I propose that a pair of seagrass species, *Zostera japonica* and *Z. marina*, would provide an ideal opportunity to study the changes in biological traits of invading species based on the combined method proposed above. *Zostera japonica* is a seagrass native to Asia and was introduced to North America in the 1950s, most likely along with oysters (*Crassostrea gigas*) imported from Japan (Hitchcock et al. 1969; Harrison and Bigley 1982). Since its introduction, it has established and spread its distribution along the western coast of North America, with recent distribution from northern California to British Columbia (Shafer et al. 2014). Due to its rapid and extensive spread around North America, this species is considered "invasive" and removal is being attempted in Washington and California, whereas neither protection or removal action has been taken in British Columbia (Williams 2007; Mach et al. 2014; Shafer et al. 2014). To date, management status varies among locations and between stakeholder groups because there is a limited understanding of how *Z. japonica* impacts coastal ecosystems (Shafer et al. 2014). In both native and non-native regions of Asia and North America, *Z. japonica* often forms mixed meadows or adjacently inhabits its congeneric species, *Zostera marina* (Nomme and Harrison 1991; Nakaoka and Aioi 2001; Shafer et al. 2014). *Z. marina* is widely distributed throughout the Northern Hemisphere and is native in both Asia and North America (Green and Short 2003). Thus, comparisons between different sides of the North Pacific Ocean, i.e., between East Asia where both species are native, and the west coast of North America where only *Z. marina* is native will contribute to a significant understanding and robust evaluation of potential introduction effects of non-native *Z. japonica*.

In this study, I specifically tried to test these three hypotheses: (1) Population bottleneck will cause significant reduction in trait variation among sites for non-native *Z.*

japonica, but not for native *Z. japonica* nor *Z. marina*, and (2) trait altered by rapid evolution will exhibit significant regional difference between native and non-native *Z. japonica*, whereas (3) trait altered by regional environmental difference will exhibit significant regional difference for both *Z. japonica* and *Z. marina*. I examined and compared biological traits of the seagrass *Z. japonica* from multiple sites within its native region, Japan, and multiple sites within its non-native region, Canada. Traits were further compared with the native congener *Z. marina* from both regions. Seagrass biological traits were analyzed for among-site variation within each region and between the two regions, for each species separately, and the trends were compared between the two seagrass species. With these comparisons, I aimed to clarify the introduction effect on non-native *Z. japonica* and provide fundamental knowledge necessary for ecological management of the non-native species.

3.2 Materials and methods

3.2.1 Study sites

I selected sites inhabited by both *Zostera japonica* and *Z. marina* in eastern Hokkaido, Japan and in southern British Columbia, Canada. Aside from the co-occurrence of the two seagrass species, sites were selected without any specification of environmental characteristics. In total, there were nine sites in the native region (Japan) and six sites in the introduced region (Canada), covering wide ranges of salinity and temperature in each region (Table 3.1, Fig. 3.1). Seagrasses in the genus *Zostera* show seasonal fluctuation in biomass and sexual reproduction, where timing of biomass/reproductive peaks are influenced by temperature (Phillips et al. 1983; Clausen et al. 2014; Blok et al. 2018; Ito et al. 2021). For this reason, I selected areas with similar

summer temperatures for comparison. To minimize seasonal differences, seagrass samplings were conducted in the same season (between July 20, 2017 and August 11, 2017 in Canada, and between July 25, 2018 and August 17, 2018 in Japan), when seagrasses are expected to show maximum biomass (Duarte 1989; Clausen et al. 2014).

Due to haphazard site selection in both native and introduced regions, other potential environmental factors affecting seagrass biological traits likely varied among sites, such as sediment type, nutrient concentrations, and zonation patterns. Both *Z. japonica* and *Z. marina* are known to inhabit sandy and muddy sediments and are significantly affected by sediment characteristics and nutrient availability (Short 1983; Moore and Short 2006; Zhang et al. 2021). Zhang et al. (2021) reported longer shoot length, heavier biomass and greater shoot density in *Z. japonica* inhabiting finer, nutrient-rich sediments. Observations during field collections revealed various sediment types in both Japan and Canada (Table 3.1), which affirms unbiased collection on sediment perspective. Shafer et al. (2014) suggested that distribution of *Z. japonica* and *Z. marina* can be described as three vertical zonation patterns (disjunct, mosaic overlapping and overlapping patterns; refer to Shafer et al. 2014), depending on bathymetric slope and microtopography of the site. Differences in zonation patterns may affect competition between the two seagrass species (Ruesink et al. 2010; Hannam and Wyllie-Echeverria 2015). At seagrass collection sites, all three zonation patterns were observed in both regions (Table 3.1). The overlapping patterns were most frequently observed in both Japan and Canada, followed by disjunct patterns, and mosaic overlapping patterns were rare and found only in single site in each region.

3.2.2 Field collection and trait measurements

At each site, I collected seagrass samples and abiotic data. Above-ground part of seagrass shoots were carefully harvested with scissor or by hand, by cutting the shoots off at sediment surface. All seagrass shoots within 20-cm diameter circle (sampled area: 0.0314 m^2) or 25-cm quadrat (0.0625 m^2) were collected. Circle and quadrat methods were used depending on water depth and tool availability during field collection. All *Z. marina* samples from Canada and all *Z. japonica* samples were collected using the quadrat method, whereas *Z. marina* samples from Japan were collected using both the mesh bag and quadrat: quadrat for TFT and CKR, and meshbag for the rest. I haphazardly collected nine samples per seagrass species at each site, separating at least several meters apart from each other, with a maximum of 15 m apart for large and flat seagrass meadows. Seagrass samples were collected from monospecific stands of each species, however, up to several shoots of the other species were occasionally found in the collected samples.

In addition to collecting seagrass, I collected abiotic data including water temperature and salinity with a handheld multiparameter probe (YSI Pro2030; YSI/Xylem Inc., Yellow Springs, OH, USA) in Canada and with handheld conductivity meter (YSI EcoSense EC300A; YSI/Xylem Inc.) in Japan, except at FTM and NTR where conductivity temperature depth sensors (RINKO Profiler ASTD102-ALC-R02; JFE Advantech Co., Ltd., Japan) were used. These abiotic data were collected at one or two points within each site with adequate depth and water-flow for measurement, which were mostly within *Z. marina* meadows at each site, and one or two measurements were taken at each point. Multiple measurements taken from each site were averaged to describe abiotic conditions during the field collection at each site (Table 3.1).

Based on collected seagrass samples, I quantified five biological traits for each sample; shoot length, shoot density, leaf area index (LAI), biomass, and reproductive ratio. Randomly selected five to ten vegetative shoots from each sample were measured for the length from the first rhizome node to the longest leaf tip of each shoot; values from multiple shoots were then averaged and used for further analysis. Seagrass shoots were separated into vegetative and reproductive shoots and the shoot numbers of each type were counted. LAI was calculated as the average shoot leaf area (shoot length multiplied by shoot width) multiplied by shoot density per sampled area. Seagrass samples were dried at 60°C until constant weight to gain dry weight, and we used the dry weight to estimate seagrass above-ground biomass. Reproductive ratio was calculated as the ratio of reproductive shoots to total shoot density. To standardize sampling methods across sites, the total number of shoots and seagrass biomass per sample were transformed into a 1 m^2 value and analyzed.

Biological traits used for comparative studies were selected among common invasive traits in plants; It is common for invasive plants to display, larger shoot size, higher shoot density, greater LAI, greater biomass, and higher reproductive ratio than native congener or native populations (Pyšek and Richardson 2007; Mason et al. 2008; van Kleunen et al. 2015). Larger shoot size and greater LAI are beneficial for seagrass for effective photosynthesis and increase competitive ability among other macrophytes. Both higher shoot density and greater biomass, often induced by high clonal production and by high vegetative growth, respectively, increase competitive ability and increase the potential to replace the native occupants. Whereas high reproductive ratio is advantageous with high dispersal ability and increasing likelihood of establishment.

3.2.3 Statistical analysis

All statistical analyses were performed in R (version 3.6.2; R Core Team 2019). Prior to trait comparisons, I checked if variation in environmental factors for each region were statistically different or not. I performed two sample Welch's *t*-test with unequal variance to compare regional differences in salinity and water temperature. I also used Pearson's correlation tests to check if salinity or temperature were highly correlated with analyzed biological traits. Biological trait data were averaged per site and were evaluated separately for each seagrass species and for each region, by correlation matrix between biological traits and abiotic factors.

Among-site variation in biological traits for each seagrass species were compared separately. I performed Generalized Linear Models (GLMs) for each region separately. To evaluate if traits differ among sites in each region, GLMs with Site as an explanatory variable were performed. For each biological trait, different distribution was assumed for response variables: Gamma distribution for shoot length, LAI, and biomass; Poisson distribution for shoot density; and binomial distribution for reproductive ratio.

Analysis on regional effects on the biological traits were performed through Generalized Linear Mixed-effects Models (GLMMs) using the *lme4* package (Bates et al. 2015). For GLMMs, we set Region (2 levels; Japan and Canada) as a fixed factor and Site (9 and 6 levels for Japan and Canada, respectively) as random factors nested under each Region. Distributions assumed for each biological trait are the same as with GLMs. Due to model convergence problems during random effects integration, I changed the number of adaptive Gauss-Hermite quadrature points (nAGQ) to zero (nAGQ=0) from the default setting of one (nAGQ=1). This change resulted a faster but less exact form of parameter estimation for GLMMs by optimizing the random effects (Bates et al. 2015). Post-hoc

power analysis was conducted using *powerSim* function in the *simr* package (Green and MacLeod 2016). Due to small sample sizes in the analysis, especially for GLMMs, power was generally low (<80%) under the common significance level (p = 0.05). To increase the power to sufficiently (>80%) detect the effect of Region with large effect size (odds ratio (OR) = 9 or 0.11), I set significance level used to p = 0.1for analysis with GLMMs. With this significance level, I could detect small effect size (OR = 1.48 or 0.67) with statistical power of 19.40% (lower and upper 95% confidence interval: 16.02% and 23.14%), medium effect (OR = 3.45 or 0.29) with 64.00% (59.62% and 68.21%), and large effect with 97.40% (95.59% and 98.61%).

To explore how biological traits vary regionally in multivariate space, I further conducted Principal Component Analysis (PCA) for each seagrass species separately using *vegan* package (Oksanen et al. 2019) in R. For the PCA, data points were not averaged per site and original replicate data were used. Before conducting the PCA, I scaled trait variables to standardize each variable (mean = 0; SD =1). I conducted the PCA using the *rda* function and plotted the results with the biplot function. After plotting the PCA as biplot, I overlayed polygons for each region, using the *ordihull* function, to visually check the overlap between data points from the two regions. Finally, I performed ANODIS (analysis of distance; Skalski et al. 2018) to evaluate the statistical significance in regional effect on the PCA.

3.3 Results

3.3.1 Abiotic conditions

Abiotic conditions varied greatly among sites within each region. Among sites in Japan, average salinity was 26.5 ± 6.0 (mean ± SD), and 25.4 ± 1.5 for sites in Canada.

Similarly, average water temperature was 19.7 ± 2.2 ℃ for sites in Japan, and 18.3 ± 3.3 ℃ for sites in Canada. Variation in salinity and temperature characteristic of the sites I sampled in did not differ significantly between the two regions (Welch's *t*-test: salinity $t = 0.52$, df = 9.44, $p = 0.61$; water temperature $t = 0.94$, df = 7.98, $p = 0.37$), indicating that regional comparisons were not confounded with measured differences in abiotic factors. For both *Z. japonica* and *Z. marina*, I did not find a significant correlation between abiotic environmental factors and analyzed biological traits (Table S3.1).

3.3.2 Among-site and between-region variations in plant trait

Among-site variation was significant for all biological traits in both Japan and Canada, and for both seagrass species, *Z. japonica* and *Z. marina* ($p < 0.001$ for all combinations; Fig. 3.2, Table 3.2).

Between the two regions, shoot length was not significantly different for both *Z. japonica* and *Z. marina* (Figs. 3.2a, b, Table 3.2). For shoot density, LAI and biomass, significant inter-regional variation was found for *Z. japonica*; average values in Japan were higher than in Canada (Figs. 3.2c, e, g, Table 3.2). However, for *Z. marina*, no significant regional variation was found for these variables (Figs. 3.2d, f, h, Table 3.2). Reproductive ratio was significantly higher in Canada than in Japan for *Z. japonica* (Fig. 3.2i, Table 3.2), whereas it was not significantly different between the two regions for *Z. marina* (Fig. 3.2j, Table 3.2).

PCA using all five biological traits revealed that the first axis of the PCA (PC1) represented 62.0% and the second axis (PC2) represented 21.8% of variation for *Z. japonica* across sties and regions, whereas PC1 represented 44.5% and PC2 represented 30.2% for *Z. marina* (Fig. 3.3, Table 3.3). The traits that most contributed to the PC1 were

LAI and biomass for both *Z. japonica* and *Z. marina*, whereas the traits contributed to the PC2 were shoot density and reproductive ratio for *Z. japonica* and shoot length and density for *Z. marina* (Table 3.3). Visual examinations of the PCA showed that the data points from Canada and Japan highly overlapped for *Z. marina* (Fig. 3.3b), whereas only partially overlapped for *Z. japonica* (Fig. 3.3a). ANODIS revealed a significant regional effect for *Z. japonica* ($F_{2,266} = 18.26$, $p < 0.001$) but a non-significant effect for *Z. marina* ($F_{2,266} = 0.53$, $p = 0.591$).

3.4 Discussion

The introduction of *Z. japonica* to North America provided an opportunity to examine potential trait shifts during biological invasions. Furthermore, combined use of intraspecific and interspecific comparisons enabled us to distinguish introduction effects from regional differences. The results demonstrated that significant between-region variation in biological traits were only found in *Z. japonica*, which is non-native in Canada, but not for *Z. marina*, which is native in both Canada and Japan. Likewise, multivariate analyses revealed that traits differed greatly between regions for only *Z. japonica*. If there were any environmental differences between the region, including factors not observed or not analyzed in this study, both *Z. japonica* and *Z. marina* would be equally affected by the difference and both species would result in significant regional differences. However, significant regional differences were only observed for *Z. japonica*, affirming that these differences were independent of regional difference in environmental conditions. These findings suggest that non-native *Z. japonica* experienced potential trait shifts during introduction and settlement phases since its introduction in North America.

3.4.1 Among-site variation and population bottleneck

Population bottlenecks are common during biological invasion when a limited number of individuals are introduced to the non-native habitat (Sakai et al. 2001; Dlugosch and Parker 2008; Blackburn et al. 2011) and are often associated with reduction or alteration in genetic diversity (Lande 1988; Huey et al. 2005; Novak 2007; Dlugosch and Parker 2008; but see Novak and Mack 2005; Vicente et al. 2021). I thus hypothesized that non-native populations would show reduced phenotypic variation among sites when the species undergoes a severe genetic bottleneck. In this study, however, I did not observe significant reduction in phenotypic variation among sites in non-native *Z. japonica* populations in Canada and instead found significant among-site variation for all analyzed biological traits. These results suggest that *Z. japonica* did not experience a genetic bottleneck during its introduction to North America. For example, multiple introduction events are known to mediate the bottleneck effects during introduction (Sakai et al. 2001; Novak 2007). It is also possible that *Z. japonica* exhibits high phenotypic plasticity which could be masking its genetic bottleneck. This occurrence has been demonstrated in different plant species (Li et al. 2019; Villellas et al. 2021), and high phenotypic plasticity is a common characteristic for seagrass (Collier et al. 2008; Cabaço et al. 2009; Maxwell et al. 2014; McDonald et al. 2016). Thus, it is likely that *Z. japonica* did not reveal reduced phenotypic variation even it underwent genetic bottleneck. Further analysis may be possible once genetic data on native and non-native *Z. japonica* become available.

3.4.2 Reduced shoot density, LAI, and biomass for non-native *Z. japonica*

Common invasive traits in plants include higher abundance and morphometrics (e.g., shoot/individual density, biomass, and LAI) in invasive species than native (van

Kleunen et al. 2010a; Jelbert et al. 2015). In this study, I found a significant regional difference on some analyzed biological traits for *Z. japonica* but not for *Z. marina*. Nonetheless, introduced *Z. japonica* showed significantly lower shoot density, lower biomass, and lower LAI than native populations, which contradicts our expectations for invasive species. Studies comparing invasive and non-invasive non-native, and native species found that traits are similar between non-invasive and native species, whereas invasive species differ in traits from non-invasive or native (van Kleunen et al. 2010a; Mathakutha et al. 2019). In terms of abundance and morphometrics, significantly lower performance observed for non-native *Z. japonica* may suggest its non-invasiveness. In addition, Zenni and Nuñez (2013) claimed that many studies on "non-native" species are biased toward studying invasive or highly invasive species, and studies on non-invasive species are rare. Such biased case studies in invasion biology suggest that reduced abundances for non-native populations maybe common yet underrepresented in the literature.

Seagrasses, including species in genus *Zostera*, are known to show high seasonal variation in their size, density, biomass, and reproduction (Duarte et al. 1989; Nakaoka and Aioi 2001; Clausen et al. 2014; Blok et al. 2018; Ito et al. 2021). In this study, I tried to minimize the seasonal effect by conducting seagrass collection in mid-summer when seagrasses are expected to be at their maximum biomass. However, study on biomass and reproductive phenology of *Z. japonica* and *Z. marina* revealed that these species differ in their phenological responses to latitude and temperature, and that native and non-native *Z. japonica* populations also differ in their responses (Clausen et al. 2014; Blok et al. 2018; Ito et al. 2021, Chapter 2 of this thesis). Although this study found significant trait variations during summer, it would worth further investigating if such trait variations are

observed in different seasons, especially during winter when seagrasses are not in their growing season.

Among the three biological traits that showed significant reduction in Canada, biomass and LAI are functions of shoot density under constant shoot size (Enríquez and Pantoja-Reyes 2005). Thus, reduced biomass and LAI could be considered as a result from reduced shoot density for non-native *Z. japonica*. Density reduction can happen as a response to various factors: i.e., light reduction, eutrophication, and wasting disease (Olesen et al. 2002; Orth et al. 2006; Collier et al. 2007; Ralph et al. 2007; Schmidt et al. 2012; Graham et al. 2021). However, similar reductions in density were not observed for *Z. marina*, suggesting that observed difference in *Z. japonica* is not affected by such factors. Observed reduced shoot density for non-native *Z. japonica* can be explained considering trade-offs between sexual and asexual production. Seagrasses reproduce sexually, via flowering and seed production, as well as asexually through clonal production (Hemminga and Duarte 2000, Kendrick et al. 2012). An experimental study on non-native *Z. japonica* showed that there is a tradeoff between vegetative biomass and flowering under modest disturbance of sediment deposition (Henderson and Hacker 2015). The present results indicate that non-native *Z. japonica* exhibited enhanced sexual reproduction by showing higher reproductive ratio compared to native populations (further discussed in section 3.4.3). This enhanced reproduction may have caused reduced clonal production and reduced shoot density for the non-native *Z. japonica* populations.

3.4.3 Enhanced reproduction for non-native *Z. japonica*

Another common trait of invasive species is high per capita reproduction rates (Hayes and Barry 2008; Mason et al. 2008; Jelbert et al. 2015). Invasive species often

exhibit higher reproductive allocation and produce more seeds than their native congener or population from their native habitat (Mason et al. 2008; Jelbert et al. 2015). I found that reproductive ratios are significantly higher in Canada for *Z. japonica*, but the ratios are similar in Japan and Canada for *Z. marina*. Because regional difference in reproductive ratio was observed only for *Z. japonica* but not *Z. marina*, this confirms that the observed difference is independent from regional environmental differences that I did not measure. The higher reproductive ratio observed for non-native *Z. japonica* is consistent with findings from other studies (Ito et al. 2021).

There are two processes that may lead to enhanced reproduction during the introduction: pre-adapted/selection before or during species introduction, or rapid evolution/adaptation after the species was introduced (Keller and Taylor 2008; Elst et al. 2016). High reproduction is beneficial during introduction and establishment, and increases the likelihood of successful invasion (Sakai et al. 2001). Under the pre-adapted scenario, enhanced reproduction is the cause of successful invasion for *Z. japonica*. On the other hand, seagrasses are known to show enhanced reproduction during their colonization to new habitats or when under stress (Cabaço and Santos 2012; Cabaço et al. 2012; Kendrick et al. 2012). It is probable that *Z. japonica* exhibits enhanced reproduction as a response to colonization or as an adaptation to handle the stressful environment of non-native habitat. Furthermore, Yue et al. (2020) revealed that *Z. japonica* reproductive strategy, dependency on asexual and sexual reproduction, is affected by geographic locations and environmental factors, such as overwintering conditions. Unfortunately, the observational data from this study are limited to summer. Future investigations in winter, including temperature and other abiotic conditions, will help elucidating the potential mechanisms that induced the observed differences in reproduction.

To differentiate the pre-adapted scenario from the rapid evolution scenario, genetic investigation on both native and non-native *Z. japonica* would be effective. Currently, there are only limited genetic information available for *Z. japonica*, especially for non-native populations, however, genetic origin of non-native *Z. japonica* populations is suggested as Miyagi Prefecture, Japan (Tanaka 2012). Biological trait comparisons between genetic origin and non-native populations will help understanding if observed traits difference in this study is influenced by traits of genetic origin population or if traits had been altered through introduction processes. Furthermore, an experiment coupled with genomic and transcriptomic analysis would be beneficial for understanding how biological processes, such as sexual reproduction, are controlled and to compare if they are different between native and non-native populations. Potential effects of regional or between-site environmental variation can be negated by conducting a common garden experiment, which makes it possible to compare native and non-native populations under the same environmental conditions. To date, genetic mechanisms related to seagrass flowering or reproduction had not been identified. By using non-native seagrass with enhanced reproduction, it may be possible to detect genes and metabolites that are responsible for sexual reproduction in seagrass. Once genes and/or metabolites are detected, it would be possible to compare if such genes and/or metabolites are consistent between native and non-native populations and to evaluate if those had been undergone influence of evolutionary processes or not.

Among various potential invasive traits, I only observed enhanced reproduction for non-native *Z. japonica*. High sexual reproduction is considered as invasive trait due to its high potential for increasing abundance and for long dispersal (Mason et al. 2008; Jelbert et al. 2015). In seagrasses, areal expansion is limited by clonal growth, whereas

sexual reproduction has higher potential for farther dispersal and distant colonization (Olesen et al. 2004; Kendrick et al. 2012). Dispersal distance for seagrass sexual propagules has been estimated to be tens to hundreds of kilometers based on microsatellite connectivity data (Reusch 2002; Tanaka et al. 2011) and may reach up to 130-150 km estimated from model-based approaches (Erftemeijer et al. 2008; Källström et al. 2008). Thus, higher sexual reproduction increases the likelihood of secondary introduction within its non-native region. In British Columbia, Canada, strong habitat connectivity among seagrass beds were revealed using biophysical model and network analysis of invertebrate community dispersal (Cristiani et al. 2021). Considering increased dispersal ability with enhanced reproduction and strong seagrass habitat connectivity in the region, it is highly likely that *Z. japonica* further spread among coastal areas in North America. In fact, our paper reports the first observation of *Z. japonica* presence in Sarita (see Table 3.1 and Fig. 3.1), indicating ongoing spread of *Z. japonica*.

3.4.4 Conclusion

The present study illustrated the strengths of using a species pair for evaluating whether and how biological traits may be influenced by introduction processes. I successfully demonstrated that non-native *Z. japonica* showed enhanced reproduction and reduced shoot density compared to native populations, and that the differences in traits are independent from the regional difference between Japan and Canada. Even recently, it is still under discussion whether *Z. japonica* is considered invasive or not (Shafer et al. 2014). Based on these study results, non-native *Z. japonica* only exhibited enhanced reproduction among potential invasive traits analyzed.

However, we must note that these traits may further change as non-native *Z. japonica* experiences longer establishment. It is common for non-native species to undergo an invasion lag-phase (interval between non-native species becoming established and becoming fully invasive) and durations of invasion lag-phase may be more than 40 years (Sakai et al. 2001; Aikio et al. 2010). Observational data from this study can provide biological trait reference for future.

These results may provide fundamental knowledge necessary for seagrass management and conservation decision-making. Genetic studies are currently still limited for *Z. japonica* and will facilitate further understanding of evolutionary processes involved during the introduction. Ongoing and future spread of *Z. japonica* is concerning especially considering their enhanced reproductive traits and potential trait change after invasion lag-phase, and thus, continued monitoring of the species' distribution and traits is highly advised.

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3.6 References

Aikio S, Duncan RP, Hulme PE (2010) Lag-phases in alien plant invasions: Separating the facts from the artefacts. Oikos 119:370–378. https://doi.org/10.1111/j.1600-0706.2009.17963.x

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Blackburn TM, Pyšek P, Bacher S, et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339. https://doi.org/10.1016/j.tree.2011.03.023

Blok SE, Olesen B, Krause-Jensen D (2018) Life history events of eelgrass *Zostera marina* L. populations across gradients of latitude and temperature. Mar Ecol Prog Ser 590:79–93. https://doi.org/10.3354/meps12479

Blossey B, Nötzold R (1995) Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. J Ecol 83:887. https://doi.org/10.2307/2261425

Bossdorf O, Auge H, Lafuma L, et al (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11. https://doi.org/10.1007/s00442-005-0070-z

Cabaço S, Machás R, Santos R (2009) Individual and population plasticity of the seagrass *Zostera noltii* along a vertical intertidal gradient. Estuar Coast Shelf Sci 82:301–308. https://doi.org/10.1016/j.ecss.2009.01.020

Cabaço S, Santos R (2012) Seagrass reproductive effort as an ecological indicator of disturbance. Ecol Indic 23:116–122. https://doi.org/10.1016/j.ecolind.2012.03.022

Cabaço S, Santos R, Sprung M (2012) Population dynamics and production of the seagrass *Zostera noltii* in colonizing versus established meadows. Mar Ecol 33:280–289. https://doi.org/10.1111/j.1439-0485.2011.00494.x

Cardeccia A, Marchini A, Occhipinti-Ambrogi A, et al (2018) Assessing biological invasions in European Seas: Biological traits of the most widespread non-indigenous species. Estuar Coast Shelf Sci 201:17–28. https://doi.org/10.1016/j.ecss.2016.02.014

Casties I, Clemmesen C, Briski E (2019) Environmental tolerance of three gammarid species with and without invasion record under current and future global warming scenarios. Divers Distrib 25:603–612. https://doi.org/10.1111/ddi.12856

Clausen K, Krause-Jensen D, Olesen B, Marbà N (2014) Seasonality of eelgrass biomass across gradients in temperature and latitude. Mar Ecol Prog Ser 506:71–85. https://doi.org/10.3354/meps10800

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366. https://doi.org/10.1126/science.1242121

Collier C, Lavery P, Masini R, Ralph P (2007) Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. Mar Ecol Prog Ser 337:103–115. https://doi.org/10.3354/meps337103

Collier C, Lavery P, Ralph P, Masini R (2008) Physiological characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. Mar Ecol Prog Ser 353:65–79. https://doi.org/10.3354/meps07171

Cristiani J, Rubidge E, Forbes C, et al (2021) A biophysical model and network analysis of invertebrate community dispersal reveals regional patterns of

seagrass habitat connectivity. Front Mar Sci 8:717469. https://doi.org/10.3389/fmars.2021.717469

Diez JM, Sullivan JJ, Hulme PE, et al (2008) Darwin's naturalization conundrum: dissecting taxonomic patterns of species invasions. Ecol Lett 11:674–681. https://doi.org/10.1111/j.1461-0248.2008.01178.x

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Drenovsky RE, Grewell BJ, D'Antonio CM, et al (2012) A functional trait perspective on plant invasion. Ann Bot 110:141–153. https://doi.org/10.1093/aob/mcs100

Duarte CM (1989) Temporal biomass variability and production/biomass relationships of seagrass communities. Mar Ecol Prog Ser 51:269–276. https://doi.org/10.1080/14786419.2015.1027893

Elst EM, Acharya KP, Dar PA, et al (2016) Pre-adaptation or genetic shift after introduction in the invasive species *Impatiens glandulifera*? Acta Oecologica 70:60–66. https://doi.org/10.1016/j.actao.2015.12.002

Enríquez S, Pantoja-Reyes NI (2005) Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*. Oecologia 145:234–242. https://doi.org/10.1007/s00442-005-0111-7

Erftemeijer PLA, van Beek JKL, Ochieng CA, et al (2008) Eelgrass seed dispersal via floating generative shoots in the Dutch Wadden Sea: a model approach. Mar Ecol Prog Ser 358:115–124. https://doi.org/10.3354/meps07304

Graham OJ, Aoki LR, Stephens T, et al (2021) Effects of seagrass wasting disease on eelgrass growth and belowground sugar in natural meadows. Front Mar Sci 8:768668. https://doi.org/10.3389/fmars.2021.768668

Green EP, Short FT (2003) World Atlas of Seagrasses. University of California Press, Berkeley, CA

Green P, MacLeod CJ (2016) SIMR: an R package for power analysis of generalized linear mixed models by simulation. Methods Ecol Evol 7:493–498. https://doi.org/10.1111/2041-210X.12504

Hamilton MA, Murray BR, Cadotte MW, et al (2005) Life-history correlates of plant invasiveness at regional and continental scales. Ecol Lett 8:1066–1074. https://doi.org/10.1111/j.1461-0248.2005.00809.x

Hannam MP, Wyllie-Echeverria S (2015) Microtopography promotes coexistence of an invasive seagrass and its native congener. Biol Invasions 17:381–395. https://doi.org/10.1007/s10530-014-0736-8

Harrison PG, Bigley RE (1982) The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can J Fish Aquat Sci 39:1642–1648. https://doi.org/10.1139/f82-221

Hayes KR, Barry SC (2008) Are there any consistent predictors of invasion success? Biol Invasions 10:483–506. https://doi.org/10.1007/s10530-007-9146-5

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

Henderson J, Hacker SD (2015) Buried alive: an invasive seagrass (*Zostera japonica*) changes its reproductive allocation in response to sediment disturbance. Mar Ecol Prog Ser 532:123–136. https://doi.org/10.3354/meps11335

Hierro JL, Maron JL, Callaway RM (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. J Ecol 93:5–15. https://doi.org/10.1111/j.1365-2745.2004.00953.x

Hitchcock CL, Cronquist A, Ownbey M, Thompson JW (1969) Vascular Plants of the Pacific Northwest Volume 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. Univ. Washington Press, Seattle, WA

Huey RB, Gilchrist GW, Hendry AP (2005) Using invasive species to study evolution: case studies with Drosophila and Salmon. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species Invasions: Insights into Ecology, Evolution and Biogeography. Sinauer, Sunderland, MA, pp 139–164

Hulme PE, Bernard-Verdier M (2018) Comparing traits of native and alien plants: Can we do better? Funct Ecol 32:117–125. https://doi.org/10.1111/1365-2435.12982

Ito MA, Lin H-J, O'Connor MI, Nakaoka M (2021) Large-scale comparison of biomass and reproductive phenology among native and non-native populations of the seagrass *Zostera japonica*. Mar Ecol Prog Ser 675:1–21. https://doi.org/10.3354/meps13884

Jakobs G, Weber E, Edwards PJ (2004) Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. Divers Distrib 10:11–19. https://doi.org/10.1111/j.1472-4642.2004.00052.x

Jelbert K, Stott I, McDonald RA, Hodgson D (2015) Invasiveness of plants is predicted by size and fecundity in the native range. Ecol Evol 5:1933–1943. https://doi.org/10.1002/ece3.1432

Jofré Madariaga D, Rivadeneira MM, Tala F, Thiel M (2014) Environmental tolerance of the two invasive species *Ciona intestinalis* and *Codium fragile*: their invasion potential along a temperate coast. Biol Invasions 16:2507–2527. https://doi.org/10.1007/s10530-014-0680-7

Källström B, Nyqvist A, Åberg P, et al (2008) Seed rafting as a dispersal strategy for eelgrass (*Zostera marina*). Aquat Bot 88:148–153. https://doi.org/10.1016/j.aquabot.2007.09.005

Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol Lett 11:852–866. https://doi.org/10.1111/j.1461-0248.2008.01188.x

Kendrick GA, Waycott M, Carruthers TJB, et al (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience 62:56–65. https://doi.org/10.1525/bio.2012.62.1.10

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204. https://doi.org/10.1016/S0169-5347(01)02101-2

Lande R (1988) Genetics and demography in biological conservation. Science 241:1455–1460. https://doi.org/10.1126/science.3420403

Li F-F, Gong L, Li J-S, et al (2019) Low genetic differentiation yet high phenotypic variation in the invasive populations of *Spartina alterniflora* in Guangxi, China. PLoS One 14:e0222646. https://doi.org/10.1371/journal.pone.0222646

Mach ME, Wyllie-Echeverria S, Chan KMA (2014) Ecological effect of a nonnative seagrass spreading in the Northeast Pacific: A review of *Zostera japonica*. Ocean Coast Manag 102:375–382. https://doi.org/10.1016/j.ocecoaman.2014.10.002

Mack RN (1996) Predicting the identity and fate of plant invaders: Emergent and emerging approaches. Biol Conserv 78:107–121. https://doi.org/10.1016/0006-3207(96)00021-3

Maron JL, Vilà M, Bommarco R, et al (2004) Rapid evolution of an invasive plant. Ecol Monogr 74:261–280. https://doi.org/10.1890/03-4027

Mason RAB, Cooke J, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Glob Ecol Biogeogr 17:633–640. https://doi.org/10.1111/j.1466-8238.2008.00402.x

Mathakutha R, Steyn C, le Roux PC, et al (2019) Invasive species differ in key functional traits from native and non-invasive alien plant species. J Veg Sci 30:994–1006. https://doi.org/10.1111/jvs.12772

Maxwell PS, Pitt KA, Burfeind DD, et al (2014) Phenotypic plasticity promotes persistence following severe events: physiological and morphological responses of seagrass to flooding. J Ecol 102:54–64. https://doi.org/10.1111/1365-2745.12167

McDonald AM, Prado P, Heck KL, et al (2016) Seagrass growth, reproductive, and morphological plasticity across environmental gradients over a large spatial scale. Aquat Bot 134:87–96. https://doi.org/10.1016/j.aquabot.2016.07.007

Milanović M, Knapp S, Pyšek P, Kühn I (2020) Trait-environment relationships of plant species at different stages of the introduction process. NeoBiota 58:55–74. https://doi.org/10.3897/neobiota.58.51655

Moore KA, Short FT (2006) Zostera: Biology, Ecology, and Management. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands, pp 361–386

Nakaoka M, Aioi K (2001) Ecology of seagrasses *Zostera* spp. (Zosteraceae) in Japanese waters: A review. Otsuchi Mar Sci 26:7–22

Nomme KM, Harrison PG (1991) A multivariate comparison of the seagrasses *Zostera marina* and *Zostera japonica* in monospecific versus mixed populations. Can J Bot 69:1984–1990. https://doi.org/10.1139/b91-249

Novak SJ (2007) The role of evolution in the invasion process. Proc Natl Acad Sci 104:3671–3672. https://doi.org/10.1073/pnas.0700224104

Novak SJ, Mack RN (2005) Genetic Bottlenecks in Alien Plant Species: Influence of mating systems and introduction dynamics. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species Invasions: Insights into Ecology, Evolution and Biogeography. Sinauer, Sunderland, MA, pp 201–228

Novy A, Flory SL, Hartman JM (2013) Evidence for rapid evolution of phenology in an invasive grass. J Evol Biol 26:443–450. https://doi.org/10.1111/jeb.12047

Ochocki BM, Miller TEX (2017) Rapid evolution of dispersal ability makes biological invasions faster and more variable. Nat Commun 8:14315. https://doi.org/10.1038/ncomms14315

Oksanen AJ, Blanchet FG, Friendly M, et al (2019) Vegan: Community Ecology Package

Olesen B, Enríquez S, Duarte C, Sand-Jensen K (2002) Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. Mar Ecol Prog Ser 236:89–97. https://doi.org/10.3354/meps236089

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

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

Oscar MA, Barak S, Winters G (2018) The tropical invasive seagrass, *Halophila stipulacea*, has a superior ability to tolerate dynamic changes in salinity levels compared to its freshwater relative, *Vallisneria americana*. Front Plant Sci 9:1–19. https://doi.org/10.3389/fpls.2018.00950

Parker JD, Torchin ME, Hufbauer RA, et al (2013) Do invasive species perform better in their new ranges? Ecology 94:985–994. https://doi.org/10.1890/12-1810.1

Perkins TA, Phillips BL, Baskett ML, Hastings A (2013) Evolution of dispersal and life history interact to drive accelerating spread of an invasive species. Ecol Lett 16:1079–1087. https://doi.org/10.1111/ele.12136

Phillips RC, McMillan C, Bridges KW (1983) Phenology of eelgrass, *Zostera marina* L., along latitudinal gradients in North America. Aquat Bot 15:145–156. https://doi.org/10.1016/0304-3770(83)90025-6

Prôa M, Nanova O (2020) Severe population bottleneck and cranial morphology change in the Mednyi Island subspecies of Arctic fox *Vulpes lagopus* (Carnivora: Canidae). Mammalia 84:162–170. https://doi.org/10.1515/mammalia-2018-0165

Pyšek P, Richardson DM (2007) Traits Associated with Invasiveness in Alien Plants: Where Do we Stand? In: Nentwig W (ed) Biological Invasions. Springer-Verlag, Berlin, Heidelberg, pp 97–125

R Core Team (2019) R: A language and environment for statistical computing

Ralph PJ, Durako MJ, Enríquez S, et al (2007) Impact of light limitation on seagrasses. J Exp Mar Bio Ecol 350:176–193. https://doi.org/10.1016/j.jembe.2007.06.017

Reusch TBH (2002) Microsatellites reveal high population connectivity in eelgrass (*Zostera marina*) in two contrasting coastal areas. Limnol Oceanogr 47:78–85. https://doi.org/10.4319/lo.2002.47.1.0078

Ros M, Navarro-Barranco C, González-Sánchez M, et al (2020) Starting the stowaway pathway: the role of dispersal behavior in the invasion success of low-mobile marine species. Biol Invasions 22:2797–2812. https://doi.org/10.1007/s10530-020-02285-7

Rudnick DA, Hieb K, Grimmer KF, Resh VH (2003) Patterns and processes of biological invasion: The Chinese mitten crab in San Francisco Bay. Basic Appl Ecol 4:249–262. https://doi.org/10.1078/1439-1791-00152

Ruesink JL, Hong J-S, Wisehart L, et al (2010) Congener comparison of native (*Zostera marina*) and introduced (*Z. japonica*) eelgrass at multiple scales within a Pacific Northwest estuary. Biol Invasions 12:1773–1789. https://doi.org/10.1007/s10530-009-9588-z

Sakai AK, Allendorf FW, Holt JS, et al (2001) The Population Biology of Invasive Species. Annu Rev Ecol Syst 32:305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sax DF, Stachowicz JJ, Gaines SD (eds) (2005) Species Invasions: Insights into Ecology, Evolution, and Biogeography. Sinauer, Sunderland, MA

Sax DF, Stachowicz JJ, Brown JH, et al (2007) Ecological and evolutionary insights from species invasions. Trends Ecol Evol 22:465–471. https://doi.org/10.1016/j.tree.2007.06.009

Schmidt AL, Wysmyk JKC, Craig SE, Lotze HK (2012) Regional-scale effects of eutrophication on ecosystem structure and services of seagrass beds. Limnol Oceanogr 57:1389–1402. https://doi.org/10.4319/lo.2012.57.5.1389

Shafer DJ, Kaldy JE, Gaeckle JL (2014) Science and management of the introduced seagrass *Zostera japonica* in North America. Environ Manage 53:147–162. https://doi.org/10.1007/s00267-013-0172-z

Short FT (1983) The seagrass, *Zostera marina* L.: Plant morphology and bed structure in relation to sediment ammonium in Izembek Lagoon, Alaska. Aquat Bot 16:149–161. https://doi.org/10.1016/0304-3770(83)90090-6

Skalski JR, Richins SM, Townsend RL (2018) A statistical test and sample size recommendations for comparing community composition following PCA. PLoS One 13:e0206033. https://doi.org/10.1371/journal.pone.0206033

Tanaka N (2012) Itan no Shokubutsu “Mizukusa” wo Kagaku suru (Scientific evaluation of outlier in plants “Aquatic Plants”). Beret, Tokyo, Japan

Tanaka N, Demise T, Ishii M, et al (2011) Genetic structure and gene flow of eelgrass *Zostera marina* populations in Tokyo Bay, Japan: implications for their restoration. Mar Biol 158:871–882. https://doi.org/10.1007/s00227-010-1614-2

Thompson K, Davis MA (2011) Why research on traits of invasive plants tells us very little. Trends Ecol Evol 26:155–156. https://doi.org/10.1016/j.tree.2011.01.007

Tingley R, Vallinoto M, Sequeira F, Kearney MR (2014) Realized niche shift during a global biological invasion. Proc Natl Acad Sci 111:10233–10238. https://doi.org/10.1073/pnas.1405766111

Torchin ME, Lafferty KD, Kuris AM (2001) Release from parasites as natural enemies: increased performance of a globally introduced marine crab. Biol Invasions 3:333–345. https://doi.org/10.1023/A:1015855019360

van Kleunen M, Weber E, Fischer M (2010a) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecol Lett 13:235–245. https://doi.org/10.1111/j.1461-0248.2009.01418.x

van Kleunen M, Dawson W, Schlaepfer D, et al (2010b) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958. https://doi.org/10.1111/j.1461-0248.2010.01503.x

van Kleunen M, Schlaepfer DR, Glaettli M, Fischer M (2011) Preadapted for invasiveness: Do species traits or their plastic response to shading differ between invasive and non-invasive plant species in their native range? J Biogeogr 38:1294–1304. https://doi.org/10.1111/j.1365-2699.2011.02495.x

van Kleunen M, Dawson W, Maurel N (2015) Characteristics of successful alien plants. Mol Ecol 24:1954–1968. https://doi.org/10.1111/mec.13013

Viard F, Ellien C, Dupont L (2006) Dispersal ability and invasion success of *Crepidula fornicata* in a single gulf: insights from genetic markers and larval-dispersal model. Helgol Mar Res 60:144–152. https://doi.org/10.1007/s10152-006-0033-8

Vicente S, Máguas C, Richardson DM, et al (2021) Highly diverse and highly successful: invasive Australian acacias have not experienced genetic bottlenecks globally. Ann Bot 128:149–157. https://doi.org/10.1093/aob/mcab053

Villellas J, Ehrlén J, Crone EE, et al (2021) Phenotypic plasticity masks range-wide genetic differentiation for vegetative but not reproductive traits in a short-lived plant. Ecol Lett ele.13858. https://doi.org/10.1111/ele.13858

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: Challenges for predicting invasive potential. Divers Distrib 14:569–580. https://doi.org/10.1111/j.1472-4642.2008.00473.x

Williams SL (2001) Reduced genetic diversity in eelgrass transplantations affects both population growth and individual fitness. Ecol Appl 11:1472–1488. https://doi.org/10.1890/1051-0761(2001)011[1472:RGDIET]2.0.CO;2

Williams SL (2007) Introduced species in seagrass ecosystems: Status and concerns. J Exp Mar Bio Ecol 350:89–110. https://doi.org/10.1016/j.jembe.2007.05.032

Yue S, Zhang X, Xu S, et al (2020) Reproductive strategies of the seagrass *Zostera japonica* under different geographic conditions in Northern China. Front Mar Sci 7:1–14. https://doi.org/10.3389/fmars.2020.574790

Zenni RD, Nuñez MA (2013) The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122:801–815. https://doi.org/10.1111/j.1600-0706.2012.00254.x

Zhang J, Wang K, Yi Q, et al (2021) Growth of *Zostera japonica* in different sediment habitats of the Yellow River estuary in China. Environ Sci Pollut Res 28:31151–31162. https://doi.org/10.1007/s11356-021-12925-7

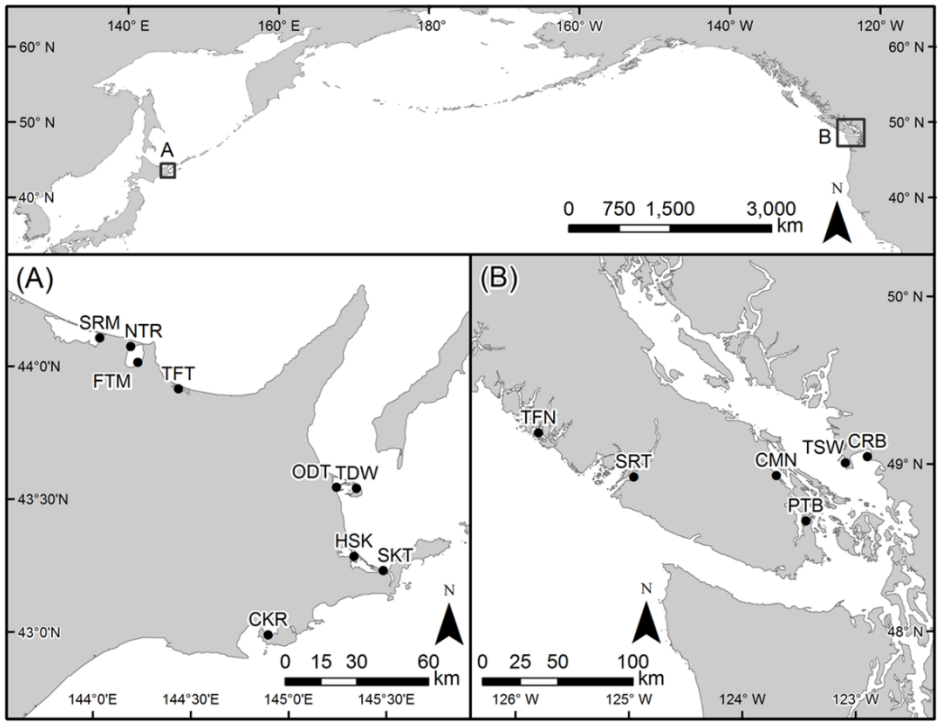

Figure 3.1. Map of sampling sites in Japan (A) where both *Zostera japonica* and *Z. marina* are native, and in Canada (B), where *Z. japonica* is introduced.

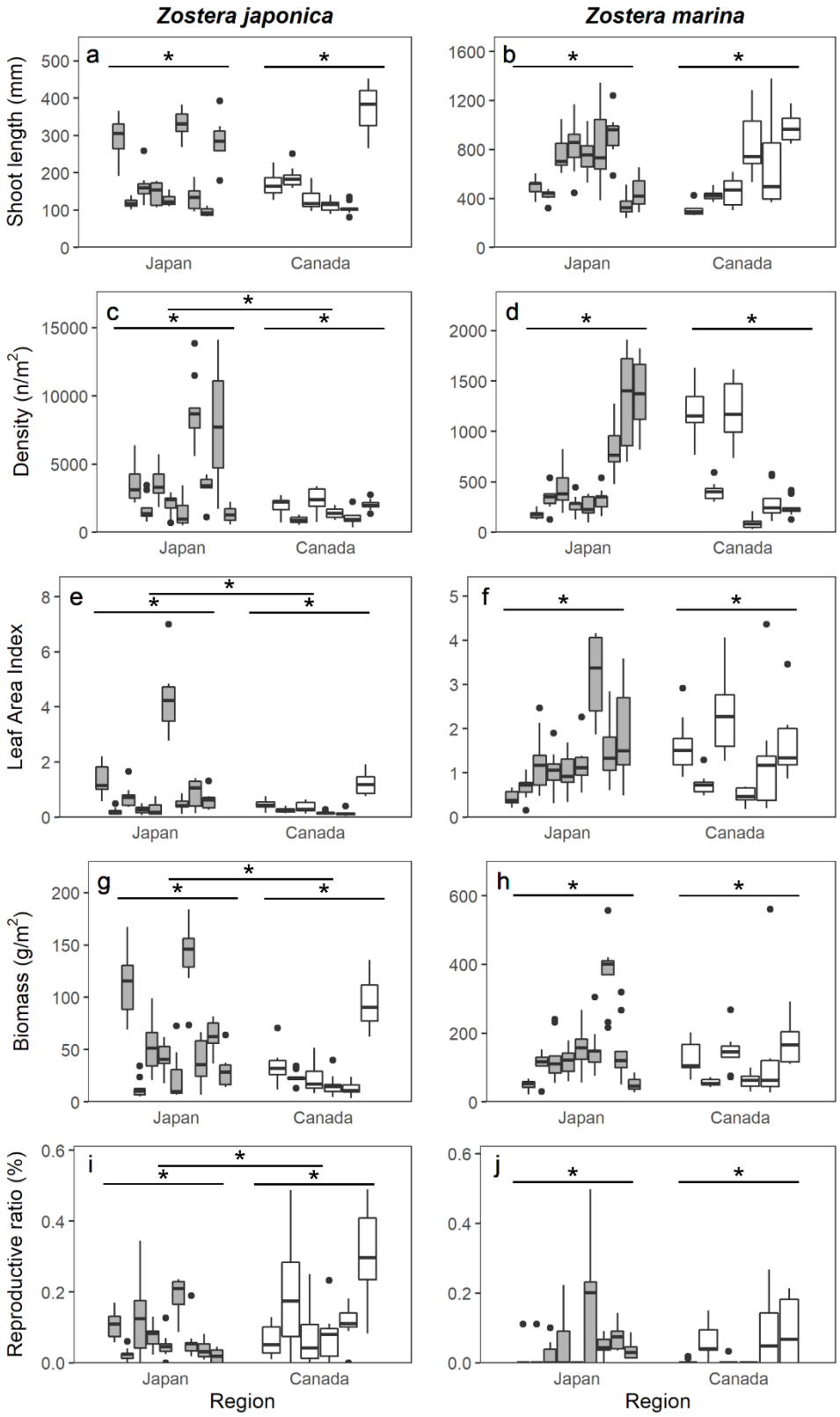

Figure 3.2. Among-site and between-region variation in biological traits of *Zostera japonica* (left panels) and *Z. marina* (right): **a, b** shoot length, **c, d** density, **e, f** leaf area

index (LAI), **g, h** biomass, and **i, j** reproductive ratio. Significant among-site variation is represented by bar and asterisk above each region, and significant between-region difference is represented by the same symbol on top middle crossing the two regions.

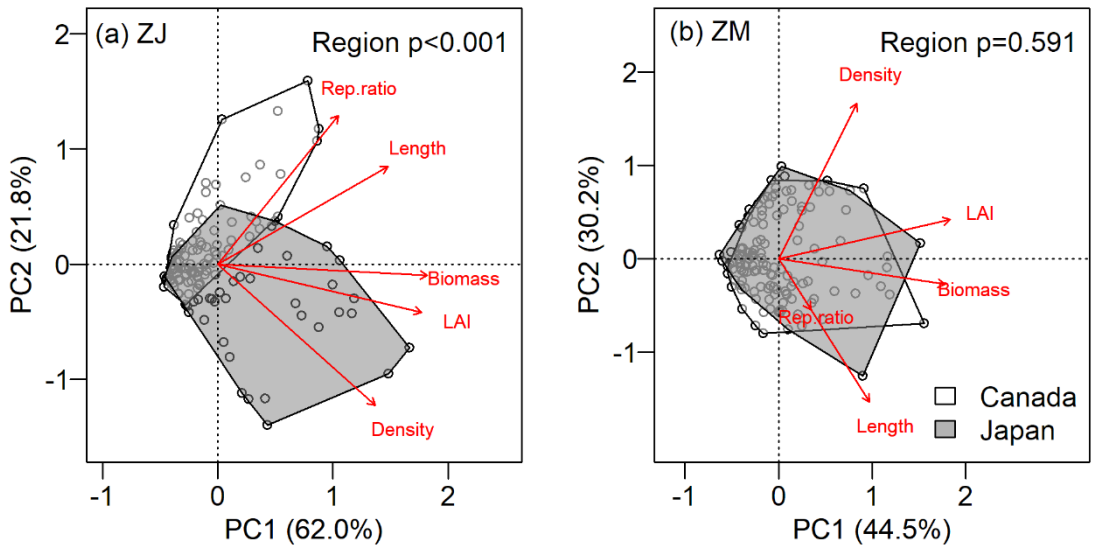

Figure 3.3. Regional variation in biological traits in **a** *Zostera japonica* (ZJ) and **b** *Zostera marina* (ZM) defined by first two axes of PCA. Each point represents sample replicate with a convex hull enclosing the points from each region. Arrows indicating magnitude and direction of loadings of traits.

Table 3.1. Geographical coordinates and environmental factors of sampling sites in Canada and Japan. Each site is assigned a specific code and the codes are used throughout the figure and text and figures. Water temperature (Water Temp) and salinity data were measured on-site during field collections. Sediment types are based on visual observations at each site. Zonation is classified into three patterns: disjunct, overlap, and mosaic overlap (sensu Shafer et al. 2014).

Region	Site	Code	Latitude	Longitude	Date	Water Temp (°C)	Salinity	Sediment Type	Zonation Pattern
Canada	Tofino	TFN	49.151 N	125.893 W	2017/7/23	14.3	25.3	sandy mud	disjunct
	Sarita	SRT	48.907 N	125.011 W	2017/8/11	17.3	27.0	mud	disjunct
	Chemainus	CMN	48.930 N	123.719 W	2017/7/21	17.9	23.0	sand	overlap
	Patricia Bay	PTB	48.659 N	123.450 W	2017/7/20	17.5	25.1	muddy sand	overlap
	Tsawwassen	TSW	49.007 N	123.094 W	2017/7/25, 26	18.4	25.0	mud	overlap
	Crescent Beach	CRB	49.044 N	122.894 W	2017/7/25	24.3	27.1	muddy sand	mosaic
Japan	Akkeshi	CKR	43.022 N	144.884 E	2018/8/12	18.0	24.3	mud	mosaic
	Shunkunitai	SKT	43.273 N	145.470 E	2018/7/29	22.5	28.6	sand	overlap
	Hashirikotan	HSK	43.325 N	145.318 E	2018/7/28	21.1	20.2	sand	overlap
	Odaitou	ODT	43.584 N	145.220 E	2018/7/26	18.7	19.0	muddy sand	disjunct
	Todowara	TDW	43.581 N	145.324 E	2018/7/25	18.9	28.7	sandy mud	overlap
	Tofutsu	TFT	43.938 N	144.384 E	2018/8/7	22.5	19.0	mud	overlap
	Futamigaoka	FTM	44.033 N	144.169 E	2018/8/8	17.9	33.4	muddy sand	disjunct
	Notori	NTR	44.092 N	144.128 E	2018/8/8	21.4	33.3	muddy sand	overlap
	Saroma	SRM	44.119 N	143.964 E	2018/8/17	16.5	32.2	sand	overlap

Table 3.2. Results of generalized linear models testing among-site variation in biological traits of *Zostera japonica* and *Z. marina*, and those of generalized mixed models testing between-region variation.

		among-site						between-region		
		Japan			Canada					
	Traits	LR χ^2	df	*p*	LR χ^2	df	*p*	χ^2	df	*p*
Zostera japonica	Length	466.79	8	<0.001	367.51	5	<0.001	0.18	1	0.673
	Density	137001.00	8	<0.001	9630.80	5	<0.001	6.83	1	0.009
	LAI	246.79	8	<0.001	135.61	5	<0.001	5.14	1	0.023
	Biomass	135.92	8	<0.001	117.52	5	<0.001	4.37	1	0.037
	Rep.ratio	10679	8	<0.001	4065.40	5	<0.001	3.61	1	0.058
Zostera marina	Length	148.87	8	<0.001	98.87	5	<0.001	0.29	1	0.591
	Density	24080.00	8	<0.001	18899.00	5	<0.001	0.08	1	0.778
	LAI	118.90	8	<0.001	46.42	5	<0.001	<0.01	1	0.958
	Biomass	165.48	8	<0.001	22.37	5	<0.001	0.21	1	0.646
	Rep.ratio	1148.00	8	<0.001	1213.00	5	<0.001	0.40	1	0.528

Table 3.3. Loadings of biological traits and proportion of variance explained by the first two axes of PCA for *Zostera japonica* and *Z. marina*. Traits with the two highest loading in each component are shown in bold.

	Zostera japonica		*Zostera marina*	
Traits	PC1	PC2	PC1	PC2
Length	1.74	1.00	1.14	**-1.80**
Density	1.61	**-1.44**	0.99	**1.96**
Leaf Area Index	**2.08**	-0.49	**2.16**	0.49
Biomass	**2.14**	-0.12	**2.10**	-0.32
Reproductive ratio	1.23	**1.52**	0.40	-0.64
% Variance explained	62.0%	21.8%	44.5%	30.2%
(*n*)	135		135	

Table S3.1. Bivariate correlation between environmental variable and biological traits. Pearson's correlation factor (*r*) and statistical significance (*p*) for each variable-trait combination are shown.

Region	Species	Variable	Length		Density		LAI		Biomass		Rep ratio	
			p	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*
Japan	*Z. japonica*	Water Temp	0.143	0.529	0.715	0.142	0.239	0.437	0.541	0.236	0.300	0.390
		Salinity	0.415	-0.311	0.587	0.210	0.914	0.042	0.960	0.020	0.979	-0.010
	Z. marina	Water Temp	0.868	0.065	0.818	0.090	0.621	-0.192	0.179	-0.491	0.204	0.468
		Salinity	0.796	0.101	0.755	-0.121	0.729	0.135	0.278	0.406	0.434	0.300
Canada	*Z. japonica*	Water Temp	0.575	0.291	0.618	-0.261	0.884	0.077	0.837	0.109	0.475	0.366
		Salinity	0.767	-0.156	0.202	-0.606	0.516	-0.335	0.597	-0.276	0.755	0.165
	Z. marina	Water Temp	0.593	-0.279	0.957	-0.029	0.556	-0.306	0.362	-0.457	0.995	-0.003
		Salinity	0.504	0.344	0.142	-0.674	0.121	-0.700	0.147	-0.668	0.821	0.120

Chapter 4

Regional comparison revealed less diverse epifaunal community associated with non-native seagrass compared to its native habitat

Abstract: Foundation species is a dominant and disproportionately important species that defines the ecosystem, determines local and regional diversity, and community dynamics. Seagrasses are amongst the most dominant foundation species in shallow coastal areas and provide habitat for various types of organisms, making them known as one of the richest coastal ecosystems for marine biodiversity. Seagrass-associated epifaunal invertebrate communities are a major component of seagrass ecosystems and play a key role in food web dynamics. The addition of non-native foundation species through biological invasion can alter the epifaunal communities in seagrass beds. Biological invasions are increasing worldwide, including the seagrass *Zostera japonica*, which is native from Asia and introduced to North America. In their introduced region, *Z. japonica* epifaunal communities are occasionally compared with the native congener *Z. marina*. However, these results have not been compared with epifaunal communities in their native region, making it difficult to distinguish between species differences and invasion impact. In this study, I compared epifaunal communities associated with *Z. japonica* and *Z. marina* from multiple sites in Japan where both species are native, and multiple sites in Canada where *Z. japonica* is introduced. I analyzed and compared variation in abundance, diversity, and composition of species specifically associated with each seagrass species, and then compared seagrass species effect between the regions. Abundance and diversity comparisons showed that epifaunal abundance was similar between the two seagrass species without regional difference, but epifaunal communities for *Z. japonica* were less diverse than *Z. marina* only in Canada. Species composition result showed that distinct epifaunal community associated to each seagrass species were observed in Japan, whereas non-native *Z. japonica* community was mainly a subset of *Z. marina* in Canada. These findings revealed that *Z. japonica* in its introduced region can

provide additional habitat for epifaunal communities that extend to shallower intertidal areas. However, non-native *Z. japonica* has not yet established a species-specific community as in its native region, and epifaunal species associated with non-native *Z. japonica* is still limited, even after decades of introduction.

4.1 Introduction

Foundation species, defined as a key species that determines local and regional diversity and controls community dynamics (Ellison 2019) is disproportionately important in the ecosystem. Examples of foundation species include corals in coral reefs and trees in forests. The various ecosystem functions provided by the foundation species includes nutrient control, environmental stabilization, and habitat provisioning (Ellison et al. 2005; Ellison 2019). Foundation species provide habitats for other species and thus increases community diversity (Stachowicz and Byrnes 2006; Scott and terHorst 2020; Ellison 2019). What is unique about foundation species compared to other important species in ecosystems is that ecological facilitation by foundation species happens via non-trophic interactions (Borst et al. 2018; Ellison 2019). Due to its diverse and ecosystem-wide contributions compared to other species, it is extremely important to understand status of foundation species and how the change in foundation species cascade to the ecosystem. Abundance and diversity of foundation species are rapidly changing with various types of anthropogenic and natural disturbances (Ellison et al. 2005; Peters and Yao 2012; Dalgleish et al. 2016; Castorani et al. 2018), causing reduction in abundance (Allen et al. 2010; Sorte et al. 2017; Lindbladh et al. 2019), and in some cases, complete loss of the foundation species (Anderson et al. 2010; Brantley et al. 2015; Thomson et al. 2015). On the other hand, new foundation species are introduced through biological introductions and range expansion with climate changes (Prevéy et al. 2010; Ramus et al. 2017; Dudley et al. 2020; Scott and terHorst 2020). Effects of a change in abundance, loss and addition of foundation species may not be limited to the foundation species themselves but lead to greater impacts through their associated community and ecosystem functions.

Because foundation species are major determinants of abundance and diversity of associated biological community, it is important to know how associated community responds to the changes in foundation species. Evolutionary history of species interactions can induce strong association between foundation species and associated community (Strauss and Irwin 2004; Vázquez et al. 2009), although such association may be interrupted upon the change in foundation species (Fenner and Lee 2001; Zuefle et al. 2008). However, responses of associated community to the changes in foundation species are largely context dependent, especially in terms of responses to non-native foundation species. When non-native foundation species function similarly to native foundation species, it can lead to an increase in biodiversity through functional redundancy (Ramus et al. 2017; Valdez et al. 2021). Whereas, if non-native species function differently, they can cause drastic changes in community (Lanta et al. 2013; Sotka and Byers 2019; Lindbladh et al. 2019; Navarro-Barranco et al. 2019). However, comparisons of associated community between native and non-native foundation species are difficult to interpret because we cannot generally discriminate the following two cases; (1) the results arose from native vs non-native difference, or (2) the results arose from host A vs host B difference, when community comparisons were only conducted in the introduced region. Regional comparison of species comparison, with the same species pair in both regions, can solve this problem and enable us to discriminate native/non-native difference to host species identity effect. Although important, such study has not been conducted, to my best knowledge, potentially due to difficulty in the selection of appropriate species pair that will make us possible to test in this proposed design.

Seagrasses are one of the most dominant foundation species in coastal environment (Crooks 2002; Borst et al. 2018; Ruesink et al. 2018). Seagrass beds provide

habitat for various types of organisms, which is the best-known ecosystem services they provide (Nordlund et al. 2016; Lefcheck et al. 2019; United Nations Environment Programme 2020). Seagrass-associated epifaunal invertebrate communities are a major component of seagrass ecosystems and play a key role in food web dynamics (Orth et al. 1984; Valentine and Duffy 2006; Best and Stachowicz 2014; Reynolds et al. 2014). Among approximately 60 species of seagrasses (Green and Short 2003; den Hartog and Kuo 2006), only four are known to become non-native (Williams 2007). *Zostera japonica* is one of those non-native seagrass species that is native in Asia and introduced in North America (Green and Short 2003). Multiple studies have been conducted to study impacts of *Z. japonica* introduction to North America (Mach et al. 2014 and references therein; Shafer et al. 2014). In both native Asia and introduced North America, *Z. japonica* forms mixed or adjacently habits with congeneric seagrass, *Z. marina*, that are native in both regions. This setup allows me to examine how introduction of foundation species affect associated biological communities and their functions using the above-mentioned combined approach, regional comparison of species comparison.

In this study, I compared epifaunal communities associated with *Z. japonica* and *Z. marina* in Japan, where both species are native, and Canada, where *Z. japonica* is introduced. To reduce site-specific effects, I collected seagrass and epifauna from multiple sites in both regions. I analyzed and compared abundance and diversity measures and community composition that occur specifically to each seagrass species. I hypothesized that long evolutionary history made *Z. japonica* and *Z. marina* communities distinct from each other in native regions, whereas non-native *Z. japonica* community would be mainly a subset of *Z. marina* community in non-native regions. Thus, epifaunal abundance and diversity are expected to be significantly lower for non-native *Z. japonica*

than *Z. marina* in North America, whereas they are similar between the two species in Japan. With these comparisons, I specifically tried to investigate (1) how abundance and diversity of epifaunal community differ between *Z. japonica* and *Z. marina*, reflecting species identity, and (2) how epifaunal species composition that specifically associated to each seagrass species differ between Japan and Canada, reflecting the difference in native and non-native status for *Z. japonica*.

4.2 Materials and methods

4.2.1 Study sites and field collection

I selected a total of 12 study sites where both *Zostera japonica* and *Z. marina* present; seven sites from eastern Hokkaido, Japan, where both species are native, and five sites from southern British Columbia, Canada, where *Z. japonica* is non-native (Fig. 4.1). In both Canada and Japan, study sites were chosen without any preference on environmental characteristics. Nevertheless, environmental variables measured during each field collection showed that study sites covered wide ranges of salinity and temperature in each region (Table 4.1; see also section 3.2.2).

At each site, I collected seagrass and epifauna samples for each one of the two seagrass species. Four to nine haphazard samples per seagrass species were collected from monospecific stands of *Z. japonica* and *Z. marina*, although few shoots of another species were occasionally found in the collected samples. All above-ground part of seagrass and associated epifaunal community within 20 cm diameter circle (314 cm^2) or within 25 cm by 25 cm quadrat (625 cm^2) were sampled. For the 20-cm circle, I carefully placed 20-cm diameter meshbag (mesh size: 100 μm) on top of seagrass within the target area and harvested seagrass at the sediment surface. For the 25cm quadrat, I carefully harvested

all seagrass shoots within the target area and put them into the ziplock bag, with best care not to lose associated epifauna. Either one of the two sampling methods, meshbag or quadrat, was selected based on water depth and tool availability and was used during the collection for each seagrass species at each site. All *Z. marina* samples from Canada and all *Z. japonica* samples were collected with the quadrat method. For *Z. marina* samples from Japan, the quadrat was used for TFT and CKR, and the meshbag for the rest. During the collection, each sample was collected at least two meters apart from each other, and maximum of 15 m apart for large and flat seagrass meadows.

Collected seagrass and epifauna samples were brought back to the laboratory for further processing. Harvested seagrass were thoroughly washed with tap water in the plastic tab to detach any epifauna intact. Once all seagrasses were washed, water in the tab was drained through 500-µm sieve to retain epifauna. Collected epifauna were then fixed with ethanol (70% for Japan; 99% for Canada) and preserved until further processing. For collected seagrass, I measured and calculated various biological traits of seagrass (see Chapter 3, section 3.2.2 for details), however, only seagrass biomass was used in this study.

4.2.2 Epifaunal community and biodiversity measure

Epifaunal samples were counted and identified to the lowest possible taxa. However, resolution of species identification differed between Japan and Canada due to difference in available literature and knowledge on local community. To make a direct comparison between regions possible, I used higher taxonomic levels than identified for biodiversity analysis to avoid the effect of species identification resolution. Due to extremely high epifaunal abundance, some samples were occasionally divided into four

subsamples and only one of the four subsamples were processed. The subsampling was conducted for *Z. marina* samples from CMN, PTB, TFN and TSW, Canada. Prior to analysis, I confirmed that subsampling does not affect the analysis.

I measured or calculated five abundance and diversity measures to characterize the epifaunal community: Abundance per area, abundance per seagrass biomass, species richness, and Shannon's and Simpson's biodiversity indices. Number of epifauna individuals in each sample were converted to meter-squared area, based on bottom surface area sampled. For subsampled samples, counted number of individuals were multiplied by four before calculation of abundance per area. Once abundance per area (m^2) was calculated, we divided the calculated abundance by seagrass biomass (g/m^2) and obtained abundance/seagrass biomass (n/g). Number of taxa found in each sample were used as species richness. Shannon's and Simpson's biodiversity indices were calculated for log(x+1)-transformed number of individuals per sample.

4.2.3 Statistical Analysis

All statistical analyses were performed in R (version 3.6.2; R Core Team 2019) with assistances from certain packages for specific analyses. I performed Generalized Linear Models (GLMs) for evaluating the effect of seagrass species identity on abundance and diversity measures. GLMs were separately conducted for each site to evaluate site-level seagrass species effect. To evaluate if abundance and diversity measures differ between seagrass species, GLMs were performed with Seagrass (two levels: *Z. japonica* and *Z. marina*) as an explanatory variable. For each biodiversity measure, I assumed different distribution for response variables: Poisson distribution for abundance/area and species richness; Gamma distribution for abundance/seagrass and Shannon's *H*; and beta

distribution for Simpson's *D*. For each GLM result, I performed Chi-squared based ANOVA-like analysis using *Anova* function in *car* package. For Simpson's *D* with beta distribution, beta regression was performed using the *betareg* function in R package *betareg* (Cribari-Neto and Zeileis 2010).

After GLMs, I calculated effect size for seagrass. Log-transformed odds ratio (Log(OR)) were used as effect size measure. In this study, significant positive values (Log(OR) > 0) means that *Z. japonica* is superior to *Z. marina*, significant negative values (Log(OR) < 0) means that *Z. marina* is superior to *Z. japonica*, and values crossing zero means that seagrass species effects are negligible. The greater the value, either positive or negative, suggests stronger effect. Once effect sizes for each site were obtained, I calculated regional effect sizes by taking mean and standard deviation for site-level effect sizes within each region.

Species association to seagrass species were depicted as Venn's diagram using *venn.diagram* function from *VennDiagram* package (Chen and Boutros 2011). In the Venn's diagram, number of species associated to *Z. japonica* alone, *Z. marina* alone, and associated to both seagrass species are shown. To evaluate if ratio of number of *Z. japonica*-associated species to *Z. marina*-associated species is statistically different among region, I performed GLMs. GLMs with Region (two levels: Canada and Japan) as an explanatory variable were conducted. Binomial distribution was assumed for the ratio of *Z. japonica*-associated and *Z. marina*-associated species.

4.3 Results

4.3.1 Site-level comparisons

Comparisons between *Z. japonica* and *Z. marina* communities at site-level were conducted through GLMs (Table 4.2). All sites from both Japan and Canada showed significant differences ($p < 0.001$) in epifaunal abundance per area between *Z. japonica* and *Z. marina*, and the abundance was always lower for *Z. japonica*, except in TFT, Japan (Fig. 4.2A, B). For abundance per seagrass biomass, the pattern greatly varied among sites where three sites in Canada and one site in Japan show greater values for *Z. marina,* and one in Canada and two in Japan show greater values for *Z. japonica*, and other with non-significant results (Fig. 4.2C, D). For species richness, results also greatly varied among sites, however, with regional trend (Fig. 4.2E, F). Average species richness was always greater for *Z. marina* in sites in Canada and the differences were mostly significant except SRT ($p < 0.001$; Fig. 4.2E). In contrast, average species richness was not significantly different among seagrass species for all the sites in Japan except FTM (Fig. 4.2F). Among-site variation were also high for Shannon's *H* and Simpson's *D* indices (Fig. 4.2G-J). For both indices, *Z. marina* were always greater than *Z. japonica* for sites in Canada and difference was mostly significant except SRT ($p < 0.001$; Fig. 4.2G, I). In Japan, between-seagrass species difference was not significant in four sites in Japan (4 sites for Shannon's and 3 for Simpson's), higher for *Z. marina* in 2 sites for both diversity indices, and higher for *Z. japonica* in one site for Shannon and two sites for Simpson (Fig. 4.2H, K).

4.3.2 Effect sizes and regional comparison

Effect sizes for each site-level analysis were calculated and then tested for regional difference by obtaining regional averages of site-level effect sizes. For epifaunal abundance per area, we found that effect sizes were constantly negative and mostly

significant in Japan and Canada (Fig. 4.3A, B). Regional average effect sizes were significantly negative for both Japan and Canada, meaning that epifaunal abundance per area is lower for *Z. japonica* than *Z. marina*. For abundance per seagrass biomass, both positive and negative effect sizes were found at each site, and this resulted in overall non-significant effect in both region (Fig. 4.3.C, D), indicating that *Z. japonica* and *Z. marina* does not differ in epifaunal abundance per seagrass biomass. For species richness, and Shannon's *H* and Simpson's *D* diversity indices, site-level effect sizes were mainly negative for sites in Canada except SRT (Fig. 4.3E, G, I), whereas the difference were mostly negligible for sites in Japan (Log(OR) = 0; Fig. 4.3F, H, J). As expected, regional average effect size was significantly negative for Canada and did not deviate from zero for Japan, meaning that species richness, Shannon's, and Simpson's diversity indices were significantly lower in *Z. japonica* than *Z. marina* only in Canada.

4.3.3 Species association comparison

List of species found at each site and for each seagrass are summarized in Table S4.1 for Canada and in Table S4.2 for Japan. Venn diagram shows that number of species associated with *Z. japonica* is smaller than that associated with *Z. marina* for sites in Canada, whereas the species numbers associated to each seagrass species are similar for sites in Japan (Fig. 4.4). This trend was further confirmed with statistical analysis using GLM, which showed that ratio for *Z. japonica*-associated to *Z. marina*-associated species were significantly affected by the Region ($\chi^2 = 29.2$, df = 1, $p < 0.001$), and that the ratio is lower for Canada.

4.4 Discussion

Effects of non-native foundation species has been widely studied in both marine and terrestrial ecosystems to understand their impacts on associated community (i.e., Rodriguez 2006; Ehrenfeld 2010; Gribben et al. 2013; Scott and terHorst 2020). However, previous studies could not distinguish the effects of difference in host traits from the native vs non-native effect because comparative studies have been conducted only in introduced region. Regional comparison of species comparisons with the same species pair in both regions can solve this, however to my best knowledge, such suitable setup for comparisons were not found both in terrestrial and marine studies. Introduction of *Z. japonica* from Asia to North America, and its coexistence with *Z. marina* that are native in both regions provided me the best opportunity to discriminate the introduction effect from the difference in foundation species identity. Thus, my findings are promising in deepen understandings on how the effects of non-native foundation species on associated communities and ecosystem functioning vary from its native region and from congeneric native species.

Present analysis on seagrass epifaunal community revealed that epifaunal abundance did not differ between seagrass species when standardized by seagrass biomass. However, in terms of species richness and biodiversity indices, *Z. japonica* had a less diverse community than *Z. marina* only in Canada. Furthermore, the number of epifaunal species associated with *Z. japonica* was similar to those associated with *Z. marina* in Japan, with distinct species composition between two seagrass species. In contrast, the number is significantly lower for *Z. japonica* in Canada and species composition was mainly a subset of *Z. marina*. These findings highlighted that non-native

Z. japonica provide habitat for epifaunal community but only for limited number of species that originally associated with *Z. marina*.

4.4.1 Epifaunal abundance

Multiple studies have investigated the effect of habitat availability to epifaunal community (see Hemminga and Duarte 2000, and Valentine and Duffy 2006 for reviews). Some studies suggested that abundance of epifaunal community is mainly determined by the amount of structural component provided by foundation species; which is measured by variables like seagrass above-ground biomass, shoot density, and leaf surface area, for seagrass ecosystems (Stoner 1980; Attrill et al. 2000; Zwerschke et al. 2016; Namba and Nakaoka 2018). Aside from studies that compared morphologically or functionally different foundation species (i.e., Heiman et al. 2008; Sellheim et al. 2010; Thomsen et al. 2013), most studies do not find great variation in epifaunal abundance among different foundation species. This is especially true for seagrass species inhabiting the same environments, possibly reflecting similarity in morphological, physical, and chemical properties of seagrass leaves (Nienhuis et al. 1989; Edgar 1990; Nakaoka et al. 2001; Leopardas et al. 2014).

In this study, I found that epifaunal abundance per area were significantly greater for *Z. marina* than that of *Z. japonica* but epifaunal abundance per seagrass biomass were not significantly different between the two seagrass species, regardless of native or non-native status of *Z. japonica*. These results contradicted with my hypothesis, which predicted that epifaunal abundance will be lower for non-native *Z. japonica*, but not for the native vegetation. Significantly lower epifaunal abundance per area observed for *Z. japonica* is explained by its lower above-ground biomass, especially for non-native *Z.*

japonica in Canada where the biomass was significantly lower in BC than those in Hokkaido (see Ch. 3). To date, only a few studies previously compared epifaunal community between *Z. japonica* and *Z. marina*, and such comparisons have only been conducted in *Z. japonica* non-native region. Thom et al. (1995) found that grazer densities increased with shoot density and that non-native *Z. japonica* showed similar grazer densities to that of *Z. marina*. Another study found that non-native *Z. japonica* supports greater epifaunal abundance than *Z. marina* (Knight et al. 2015). Findings from these two previous studies in line with the present results from this study and suggest that non-native *Z. japonica* can in fact support similar epifaunal abundance with native congener, *Z. marina*. Thus, introduction effect of non-native species is not likely occurred in terms of epifaunal abundance, and non-native *Z. japonica* can provide additional habitats for epifaunal community.

4.4.2 Epifaunal diversity

The present results on biodiversity measures (species richness, Shannon's and Simpson's diversity indices) were contrasting to those on abundance. Significant seagrass effect was only found in Canada, where all three variables were significantly lower for *Z. japonica*, but not in Japan where they showed inconsistent patterns. The results indicate that *Z. japonica* communities are less diverse than *Z. marina* only in the non-native region. Because native *Z. japonica – Z. marina* comparisons result in non-significant differences, the less epifaunal diversity for the non-native *Z. japonica* is not induced by seagrass species difference but by introduction effects, supporting my hypothesis. Interestingly, while average epifaunal abundance per area or per seagrass biomass were similar between *Z. japonica* and *Z. marina* in both regions (Fig 4.2A-D), average species richness for *Z.*

marina in Canada were much higher than that of the other three groups (Fig. 4.2E, F), and richness comparisons between native and non-native *Z. japonica* communities did not yield to significant difference between the populations (data not shown). These highlight that if species richness were only compared between native and non-native *Z. japonica*, the significant diversity reduction in non-native *Z. japonica* in relation to *Z. marina* could have been overlooked.

Previous studies on non-native seagrasses provided various results. The only study compared epifaunal diversity between non-native *Z. japonica* and *Z. marina*, which took place in Tsawwassen, BC, Canada, one of the study sites included also in this study, found that all species except one (Caridea sp.) were found in both *Z. japonica* and *Z. marina*, suggesting that epifaunal diversity is similar between the two (Knight et al. 2015). Note that my observations at the same site found significantly lower species richness and diversity indices in *Z. japonica* than in *Z. marina* (TSW in Fig. 4.2E, G, I, 4.3E, G, I). However, studies on another non-native seagrass, *Halophila stipulacea*, found that invertebrate diversity (and abundance) can be higher for non-native seagrass compared to native seagrasses (Willette and Ambrose 2012; Valdez et al. 2021), although those results are not specific to epifaunal community.

To my best knowledge, there are only three studies that examined epifaunal community on *Z. japonica* in native Asia (Lee et al. 2001; Abe et al. 2004; Uede and Takahashi 2008), and none of them compared the results with *Z. marina*. Insufficient comparison data available from Asia, where both *Z. japonica* and *Z. marina* are native, limit me from further exploration on observed results for epifaunal diversity between the two seagrass species. However, this study results can thus benefit researchers in future as

references for epifaunal community data from both *Z. japonica* native and non-native regions.

4.4.3 Epifaunal species composition

Analysis of epifaunal species composition on the two seagrass species by Venn diagram shows that epifaunal species associated with *Z. japonica* is limited in Canada, but as many as that in *Z. marina* in Japan (Fig. 4.4). This result again supported my hypothesis that non-native seagrass cannot host as high biodiversity as that in native species. Declined diversity of associated communities with introduction of non-native foundation species is found in diverse occasions; arthropod diversity decreases with exotic plants introduction, invasive tree decreasing diversity of forest understories, fish diversity in non-native seagrass *H. stipulacea* is lower compared to native seagrass *Thalassia testudiunum*, and green alga *Caulerpa* spp. introduced to seagrass bed decreasing diversities in fish, invertebrate, and epiphytic algal communities (York et al. 2006; Gab-Alla 2007; Deudero et al. 2011; Becking et al. 2014; Cook-Patton et al. 2014; Winters et al. 2020; Dyderski and Jagodziński 2021).

There are multiple potential mechanisms explaining such declining pattern for non-native foundation species. Non-native toxic plants significantly reduce associated invertebrate and fish community for seagrass bed introduced by *Caulerpa* spp. (Gab-Alla 2007; Deudero et al. 2011). Nevertheless, temperate seagrasses, including *Z. japonica* and *Z. marina*, are not main food sources for associated epifaunal community (Heck and Valentine 2006; Valentine and Duffy 2006), and thus such toxicity or unpalatability of non-native seagrass are not likely to influence epifaunal association observed in this study. For temperate seagrasses, differences in trophic pathway may occur from seagrass

epiphytes, the main food source for many epifauna (Heck and Valentine 2006; Valentine and Duffy 2006). Chung and Lee (2008) found that epiphytic diatom abundance and diversity are significantly different between *Z. japonica* and *Z. marina* in Korea, where both species are native. Study on non-native macroalga suggests that shared epiphytic community between native and non-native macroalgae lead to higher similarity in epifaunal communities (Armitage and Sjøtun 2016). Regrettably, epiphytes are not investigated in this study. Future study incorporating epiphyte load would help elucidating if the epifaunal species difference between the two seagrasses occur from the difference in epiphytes. However, differences in toxicity nor epiphyte do not explain why there were lower numbers of species associated for non-native *Z. japonica*, and not for native *Z. japonica*.

Ultimately, potential mechanisms explaining this pattern is too short time scale for introduced species to establish species-specific interactions with native communities. Long coexistence and shared evolutionary history of species induce strong association between the foundation species and associated community (Strauss and Irwin 2004; Vázquez et al. 2009). Long evolutionary history between *Z. marina* and its associated community may not allow most associated species to switch their host seagrass species to newly introduced *Z. japonica*. *Z. japonica* introduced to North America in 1950s and has established populations since then (Hitchcock et al. 1969; Harrison and Bigley 1982), yet ~70 years of coexistence with *Z. marina* may be too short for evolutionary processes considering that these two species have evolved for 33 million years in its native region (Kato et al. 2003). Although past information on *Z. japonica* associated community is sparse in North America to compare the results with, future study conducted in the same or similar designs to this study will provide implications on this aspect.

More importantly, it would worth looking into epifaunal species that were associated only with non-native *Z. japonica* to investigate some potential characteristics that enabled those species to associate with non-native seagrass. Based on the epifaunal species list, there were 11 species that were only found in *Z. japonica*, at least in one of the study sites in Canada (Table S4.1). Among those 11 species, I focused on three species, namely Chironominae (Diptera), Nannastacidae (Cumacean) and Haminoeidae (Gastropod) to inspect their characteristics. First species, Chironominae is a species or taxonomic group in Diptera known to spend their larval stages depending on seagrass and seaweed (Cheng and Collins 1980). They are exclusively found at more estuarine site or shore with freshwater influences, and they are abundantly found at the site with the lower rate of water renewal (Drake and Arias 1995; Carlton 2008). Because *Z. japonica* mostly inhabit upper intertidal area compared to *Z. marina* (Shafer et al. 2014), the difference in habitat zonation between *Z. japonica* and *Z. marina* would be suggested for a reason why Chironominae is only found in *Z. japonica*. Second species, Nannastacidae is a taxonomic group under Cumacean; Cumaceans are known to mainly inhabit in soft bottom (Carlton 2008). Both *Z. japonica* and *Z. marina* inhabit in soft bottom, such as sand or mud flat (Green and Short 2003), however, *Z. japonica* bed usually locates in adjacent to mud/sand flat, where it was bare habitat before its colonization in North America (Shafer et al. 2014). This suggests that Nannastacidae found in non-native *Z. japonica* may be mainly inhabit in adjacent mudflat while some individuals spilled into or translocated to *Z. japonica* bed.

While species association of the former two species to non-native *Z. japonica* are potentially explained by difference in habitat preference between the two seagrass species, the third species Haimoeidae is different. This species or taxon group consists of two *Haminoea* species, *Haminoea vesicula*, a native species in North America, and *H.*

japonica, a non-native species which is suggested to be introduced with oyster aquaculture import from Japan (Carlton 2008; Hanson et al. 2013). Although those two species were analyzed together under taxon group Haminoeidae for consistent analysis between regions, only *H. japonica* was found in non-native *Z. japonica*. It is well acknowledged that non-native species may facilitate other non-native species (Heiman et al. 2008; O'Loughlin and Green 2015; Freeman et al. 2016), and thus there may be more non-native species associated with the non-native seagrass. In this study system, Wonham et al. (2005) suggested that non-native mudsnail *Batillaria attramentaria* increased percentage cover of *Z. japonica* and increased abundance of another non-native snail. Although such facilitation or strong association between *H. japonica* and *Z. japonica* are not known, it may be possible that *H. japonica* could easily colonize *Z. japonica* compared to *Z. marina*. Such facilitation or strong association among non-native species may lead to non-native seagrass community distinct from that of native species in future. Thus, I can suggest that epifaunal species association to the non-native *Z. japonica* could be explained by a match between habitat preference of epifaunal species and *Z. japonica* bed characteristics, such as intertidal or adjacent with mudflat, and possible association between non-native animals and non-native seagrass introduced from the same region.

4.4.4 Future implications and conclusion

The present study showed similar epifaunal abundance, but reduced epifaunal diversity with different species composition between non-native *Z. japonica* and *Z. marina* in North America. Even recently, management of *Z. japonica* varied greatly among states in North America; eradication and removal are attempted in Washington and California, whereas neither protection nor removal are attempted in British Columbia

(Mach et al. 2014; Shafer et al. 2014). Epifaunal abundance is constantly higher in *Z. japonica* than adjacent unvegetated habitat in any study conducted in native and non-native regions (Lee et al. 2001; Uede and Takahashi 2008; Mach et al. 2014 and references therein) and is similar with that of *Z. marina* (Thom et al. 1995; Knight et al. 2015; this study). Thus, we can conclude that *Z. japonica* introduction can extend additional habitats for epifaunal community to shallower coastal area in North America.

However, results suggested that non-native *Z. japonica* has not yet established a species-specific community as in its native, and epifaunal species associated with non-native *Z. japonica* is still limited, even after decades of introduction. Significantly less diverse epifaunal community associated with non-native *Z. japonica* may arose from differences in epiphyte load, short evolutionary history, or from any other uninvestigated factors. I have found that studies on epifaunal community on *Z. japonica* are sparse, especially in native Asia, and those were not compared with the results for *Z. marina*. Results from this study can benefit researcher as reference data for epifaunal community associated with native and non-native *Z. japonica*, as well can provide managers fundamental information on epifaunal community associated with native and non-native seagrass, independent from seagrass species identity.

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4.6 References

Abe F, Edagawa D, Kikuchi S, et al (2004) Eelgrass-shaped substrate for the growth of algae and its ability to attract marine life. Suisanzoshoku 52:109–120

Allen CD, Macalady AK, Chenchouni H, et al (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manage 259:660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Anderson P, Brundrett M, Grierson P, Robinson R (2010) Impact of severe forest dieback caused by *Phytophthora cinnamomi* on macrofungal diversity in the northern jarrah forest of Western Australia. For Ecol Manage 259:1033–1040. https://doi.org/10.1016/j.foreco.2009.12.015

Armitage CS, Sjøtun K (2016) Epiphytic macroalgae mediate the impact of a non-native alga on associated fauna. Hydrobiologia 776:35–49. https://doi.org/10.1007/s10750-016-2707-9

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

Becking LE, van Bussel T, Engel MS, et al (2014) Proximate response of fish, conch, and sea turtles to the presence of the invasive seagrass *Halophila stipulacea* in Bonaire

Best RJ, Stachowicz JJ (2014) Phenotypic and phylogenetic evidence for the role of food and habitat in the assembly of communities of marine amphipods. Ecology 95:775–786. https://doi.org/10.1890/13-0163.1

Borst ACW, Verberk WCEP, Angelini C, et al (2018) Foundation species enhance food web complexity through non-trophic facilitation. PLoS One 13:1–15. https://doi.org/10.1371/journal.pone.0199152

Brantley ST, Miniat CF, Elliott KJ, et al (2015) Changes to southern Appalachian water yield and stormflow after loss of a foundation species. Ecohydrology 8:518–528. https://doi.org/10.1002/eco.1521

Castorani MCN, Reed DC, Miller RJ (2018) Loss of foundation species: disturbance frequency outweighs severity in structuring kelp forest communities. Ecology 99:2442–2454. https://doi.org/10.1002/ecy.2485

Carlton JT (2008) The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon. University of California Press, Berkeley, CA.

Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 12:35. https://doi.org/10.1186/1471-2105-12-35

Cheng L, Collins JD (1980) Observations on behavior, emergence and reproduction of the marine midges *Pontomyia* (Diptera: Chironomidae). Mar Biol 58:1–5. https://doi.org/10.1007/BF00386872

Chung M-H, Lee K-S (2008) Species composition of the epiphytic diatoms on the leaf tissues of three *Zostera* species distributed on the southern coast of Korea. Algae 23:75–81. https://doi.org/10.4490/algae.2008.23.1.075

Cook-Patton SC, Agrawal AA (2014) Exotic plants contribute positively to biodiversity functions but reduce native seed production and arthropod richness. Ecology 95:1642–1650. https://doi.org/10.1890/13-0782.1

Cribari-Neto F, Zeileis A (2010) Beta Regression in R. J Stat Softw 34:1–24. https://doi.org/10.18637/jss.v034.i02

Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153–166. https://doi.org/10.1034/j.1600-0706.2002.970201.x

Dalgleish HJ, Nelson CD, Scrivani JA, Jacobs DF (2016) Consequences of shifts in abundance and distribution of American chestnut for restoration of a foundation forest tree. Forests 7:4. https://doi.org/10.3390/f7010004

den Hartog C, Kuo J (2006) Taxonomy and Biogeography of Seagrasses. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, THe Netherlands

Deudero S, Box A, Alós J, et al (2011) Functional changes due to invasive species: Food web shifts at shallow *Posidonia oceanica* seagrass beds colonized by the alien macroalga *Caulerpa racemosa*. Estuar Coast Shelf Sci 93:106–116. https://doi.org/10.1016/j.ecss.2011.03.017

Drake P, Arias AM (1995) Distribution and production of *Chironomus salinarius* (Diptera: Chironomidae) in a shallow coastal lagoon in the Bay of Cádiz. Hydrobiologia 299:195–206. https://doi.org/10.1007/BF00767326

Dudley MP, Freeman M, Wenger S, et al (2020) Rethinking foundation species in a changing world: The case for *Rhododendron maximum* as an emerging foundation species in shifting ecosystems of the southern Appalachians. For Ecol Manage 472:118240. https://doi.org/10.1016/j.foreco.2020.118240

Dyderski MK, Jagodziński AM (2021) Impacts of invasive trees on alpha and beta diversity of temperate forest understories. Biol Invasions 23:235–252. https://doi.org/10.1007/s10530-020-02367-6

Edgar GJ (1990) The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with Western Australian seagrass beds. J Exp Mar Bio Ecol 137:215–240. https://doi.org/10.1016/0022-0981(90)90186-G

Ehrenfeld JG (2010) Ecosystem consequences of biological invasions. Annu Rev Ecol Evol Syst 41:59–80. https://doi.org/10.1146/annurev-ecolsys-102209-144650

Ellison AM (2019) Foundation species, non-trophic interactions, and the value of being common. iScience 13:254–268. https://doi.org/10.1016/j.isci.2019.02.020

Ellison AM, Bank MS, Clinton BD, et al (2005) Loss of foundation species: Consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486. https://doi.org/10.1890/1540-9295(2005)003[0479:LOFSCF]2.0.CO;2

Fenner M, Lee WG (2001) Lack of pre-dispersal seed predators in introduced asteraceae in New Zealand. N Z J Ecol 25:95–99

Freeman AS, Frischeisen A, Blakeslee AMH (2016) Estuarine fouling communities are dominated by nonindigenous species in the presence of an invasive crab. Biol Invasions 18:1653–1665. https://doi.org/10.1007/s10530-016-1108-3

Gab-Alla AA-FA (2007) Ecological study on community of exotic invasive seaweed *Caulerpa prolifera* in Suez Canal and its associated macro invertebrates. J Appl Sci 7:679–686. https://doi.org/10.3923/jas.2007.679.686

Green EP, Short FT (2003) World Atlas of Seagrasses. University of California Press, Berkeley, CA

Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. Oikos 122:816–824. https://doi.org/10.1111/j.1600-0706.2012.20868.x

Hanson D, Hirano Y, Valdés Á (2013) Population genetics of *Haminoea* (*Haloa*) *japonica* Pilsbry, 1895, a widespread non-indigenous sea slug (Mollusca: Opisthobranchia) in North America and Europe. Biol Invasions 15:395–406. https://doi.org/10.1007/s10530-012-0294-x

Harrison PG, Bigley RE (1982) The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can J Fish Aquat Sci 39:1642–1648. https://doi.org/10.1139/f82-221

Heck KLJ, Valentine JF (2006) Plant-herbivore interactions in seagrass meadows. J Exp Mar Bio Ecol 330:420–436. https://doi.org/10.1016/j.jembe.2005.12.044

Heiman K, Vidargas N, Micheli F (2008) Non-native habitat as home for non-native species: comparison of communities associated with invasive tubeworm and native oyster reefs. Aquat Biol 2:47–56. https://doi.org/10.3354/ab00034

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

Hitchcock CL, Cronquist A, Ownbey M, Thompson JW (1969) Vascular Plants of the Pacific Northwest Volume 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. Univ. Washington Press, Seattle, WA

Kato Y, Aioi K, Omori Y, et al (2003) Phylogenetic analyses of *Zostera* species based on *rbcL* and *matK* nucleotide sequences: Implications for the origin and diversification of seagrasses in Japanese waters. Genes Genet Syst 78:329–342. https://doi.org/10.1266/ggs.78.329

Knight NS, Prentice C, Tseng M, O'Connor MI (2015) A comparison of epifaunal invertebrate communities in native eelgrass *Zostera marina* and non-native *Zostera japonica* at Tsawwassen, BC. Mar Biol Res 11:564–571. https://doi.org/10.1080/17451000.2014.985230

Lanta V, Hyvönen T, Norrdahl K (2013) Non-native and native shrubs have differing impacts on species diversity and composition of associated plant communities. Plant Ecol 214:1517–1528. https://doi.org/10.1007/s11258-013-0272-0

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

Lefcheck JS, Hughes BB, Johnson AJ, et al (2019) Are coastal habitats important nurseries? A meta-analysis. Conserv Lett 12:e12645. https://doi.org/10.1111/conl.12645

Leopardas V, Uy W, Nakaoka M (2014) Benthic macrofaunal assemblages in multispecific seagrass meadows of the southern Philippines: Variation among vegetation dominated by different seagrass species. J Exp Mar Bio Ecol 457:71–80. https://doi.org/10.1016/j.jembe.2014.04.006

Lindbladh M, Petersson L, Hedwall P-O, et al (2019) Consequences for bird diversity from a decrease in a foundation species—replacing Scots pine stands with Norway spruce in southern Sweden. Reg Environ Chang 19:1429–1440. https://doi.org/10.1007/s10113-019-01480-0

Mach ME, Wyllie-Echeverria S, Chan KMA (2014) Ecological effect of a nonnative seagrass spreading in the Northeast Pacific: A review of *Zostera japonica*. Ocean Coast Manag 102:375–382. https://doi.org/10.1016/j.ocecoaman.2014.10.002

Nakaoka M, Toyohara T, Matsumasa M (2001) Seasonal and between-substrate variation in mobile epifaunal community in a multispecific seagrass bed of Otsuchi Bay, Japan. Mar Ecol 22:379–395

Namba M, Nakaoka M (2018) Spatial patterns and predictor variables vary among different types of primary producers and consumers in eelgrass (*Zostera marina*) beds. PLoS One 13:e0201791. https://doi.org/10.1371/journal.pone.0201791

Navarro-Barranco C, Muñoz-Gómez B, Saiz D, et al (2019) Can invasive habitat-forming species play the same role as native ones? The case of the exotic marine macroalga *Rugulopteryx okamurae* in the Strait of Gibraltar. Biol Invasions 21:3319–3334. https://doi.org/10.1007/s10530-019-02049-y

Nienhuis PH, Coosen J, Kiswara W (1989) Community structure and biomass distribution of seagrasses and macrofauna in the flores sea, Indonesia.

Netherlands J Sea Res 23:197–214. https://doi.org/10.1016/0077-7579(89)90014-8

Nordlund LM, Koch EW, Barbier EB, Creed JC (2016) Seagrass ecosystem services and their variability across genera and geographical regions. PLoS One 11:e0163091. https://doi.org/10.1371/journal.pone.0163091

O'Loughlin LS, Green PT (2015) Invader–invader mutualism influences land snail community composition and alters invasion success of alien species in tropical rainforest. Biol Invasions 17:2659–2674. https://doi.org/10.1007/s10530-015-0903-6

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

Peters DPC, Yao J (2012) Long-term experimental loss of foundation species: consequences for dynamics at ecotones across heterogeneous landscapes. Ecosphere 3:27. https://doi.org/10.1890/ES11-00273.1

Prevéy JS, Germino MJ, Huntly NJ, Inouye RS (2010) Exotic plants increase and native plants decrease with loss of foundation species in sagebrush steppe. Plant Ecol 207:39–51. https://doi.org/10.1007/s11258-009-9652-x

R Core Team (2019) R: A language and environment for statistical computing

Ramus AP, Silliman BR, Thomsen MS, Long ZT (2017) An invasive foundation species enhances multifunctionality in a coastal ecosystem. Proc Natl Acad Sci 114:8580–8585. https://doi.org/10.1073/pnas.1700353114

Reynolds PL, Richardson JP, Duffy JE (2014) Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. Limnol Oceanogr 59:1053–1064. https://doi.org/10.4319/lo.2014.59.3.1053

Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biol Invasions 8:927–939. https://doi.org/10.1007/s10530-005-5103-3

Ruesink JL, Stachowicz JJ, Reynolds PL, et al (2018) Form-function relationships in a marine foundation species depend on scale: a shoot to global perspective from a distributed ecological experiment. Oikos 127:364–374. https://doi.org/10.1111/oik.04270

Scott ZR, terHorst CP (2020) The effect of an invasive foundation species on diversity is due to increased habitat availability. J Exp Mar Bio Ecol 528:151384. https://doi.org/10.1016/j.jembe.2020.151384

Sellheim K, Stachowicz JJ, Coates RC (2010) Effects of a nonnative habitat-forming species on mobile and sessile epifaunal communities. Mar Ecol Prog Ser 398:69–80. https://doi.org/10.3354/meps08341

Shafer DJ, Kaldy JE, Gaeckle JL (2014) Science and management of the introduced seagrass *Zostera japonica* in North America. Environ Manage 53:147–162. https://doi.org/10.1007/s00267-013-0172-z

Sorte CJB, Davidson VE, Franklin MC, et al (2017) Long-term declines in an intertidal foundation species parallel shifts in community composition. Glob Chang Biol 23:341–352. https://doi.org/10.1111/gcb.13425

Sotka EE, Byers JE (2019) Not so fast: promoting invasive species to enhance multifunctionality in a native ecosystem requires strong(er) scrutiny. Biol Invasions 21:19–25. https://doi.org/10.1007/s10530-018-1822-0

Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: Disentangling the influence of resource competition, facilitation, and extrinsic factors. Mar Ecol Prog Ser 311:251–262. https://doi.org/10.3354/meps311251

Stoner A (1980) Perception and choice of substratum by epifaunal amphipods associated with seagrasses. Mar Ecol Prog Ser 3:105–111. https://doi.org/10.3354/meps003105

Strauss SY, Irwin RE (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. Annu Rev Ecol Evol Syst 35:435–466. https://doi.org/10.1146/annurev.ecolsys.35.112202.130215

Thom R, Miller B, Kennedy M (1995) Temporal patterns of grazers and vegetation in a temperate seagrass system. Aquat Bot 50:201–205. https://doi.org/10.1016/0304-3770(95)00449-A

Thomsen M, Stæhr P, Nejrup L, Schiel D (2013) Effects of the invasive macroalgae *Gracilaria vermiculophylla* on two co-occurring foundation species and associated invertebrates. Aquat Invasions 8:133–145. https://doi.org/10.3391/ai.2013.8.2.02

Thomson JA, Burkholder DA, Heithaus MR, et al (2015) Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. Glob Chang Biol 21:1463–1474. https://doi.org/10.1111/gcb.12694

Uede T, Takahashi Y (2008) Benthos communities of the *Zostera japonica* meadows in the intertidal zone of Takinai and Uchinoura, Tanabe Bay, Wakayama Prefecture, Japan. Japanese J Benthol 63:42–55 (in Japanese with English abstract)

United Nations Environment Programme (2020) OUT OF THE BLUE THE VALUE OF SEAGRASSES. UNEP, Nairobi

Valdez SR, Shaver EC, Keller DA, et al (2021) A survey of benthic invertebrate communities in native and non-native seagrass beds in St. John, USVI. Aquat Bot 175:103448. https://doi.org/10.1016/j.aquabot.2021.103448

Valentine JF, Duffy JE (2006) The Central Role of Grazing in Seagrass Ecology. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, THe Netherlands

Vázquez DP, Blüthgen N, Cagnolo L, Chacoff NP (2009) Uniting pattern and process in plant–animal mutualistic networks: a review. Ann Bot 103:1445–1457. https://doi.org/10.1093/aob/mcp057

Willette DA, Ambrose RF (2012) Effects of the invasive seagrass *Halophila stipulacea* on the native seagrass, *Syringodium filiforme*, and associated fish and epibiota communities in the Eastern Caribbean. Aquat Bot 103:74–82. https://doi.org/10.1016/j.aquabot.2012.06.007

Williams SL (2007) Introduced species in seagrass ecosystems: Status and concerns. J Exp Mar Bio Ecol 350:89–110. https://doi.org/10.1016/j.jembe.2007.05.032

Winters G, Beer S, Willette DA, et al (2020) The tropical seagrass *Halophila stipulacea*: Reviewing what we know from its native and invasive habitats, alongside identifying knowledge gaps. Front Mar Sci 7:300. https://doi.org/10.3389/fmars.2020.00300

Wonham MJ, O'Connor M, Harley CDG (2005) Positive effects of a dominant invader on introduced and native mudflat species. Mar Ecol Prog Ser 289:109–116. https://doi.org/10.3354/meps289109

York PH, Booth DJ, Glasby TM, Pease BC (2006) Fish assemblages in habitats dominated by *Caulerpa taxifolia* and native seagrasses in south-eastern Australia. Mar Ecol Prog Ser 312:223–234. https://doi.org/10.3354/meps312223

Zuefle ME, Brown WP, Tallamy DW (2008) Effects of non-native plants on the native insect community of Delaware. Biol Invasions 10:1159–1169. https://doi.org/10.1007/s10530-007-9193-y

Zwerschke N, Emmerson MC, Roberts D, O'Connor NE (2016) Benthic assemblages associated with native and non-native oysters are similar. Mar Pollut Bull 111:305–310. https://doi.org/10.1016/j.marpolbul.2016.06.094

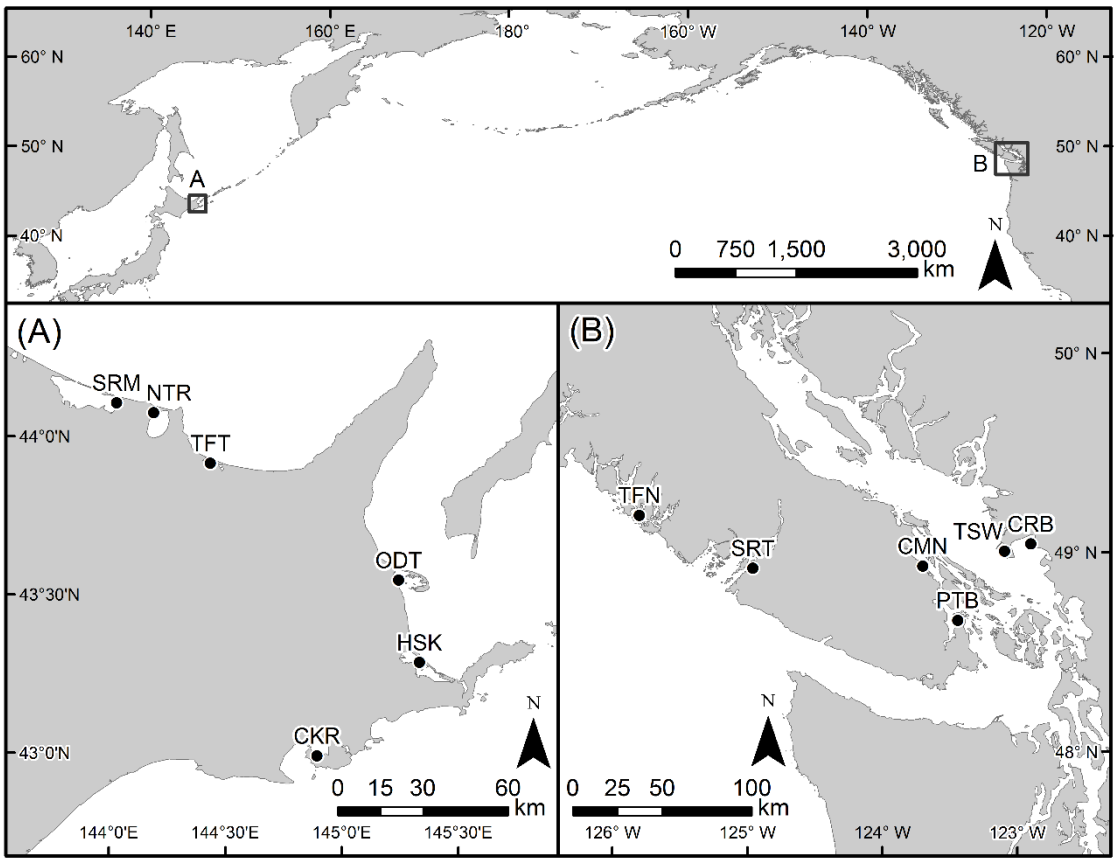

Figure 4.1. Map of study sites. See Table 4.1 for the code of site name.

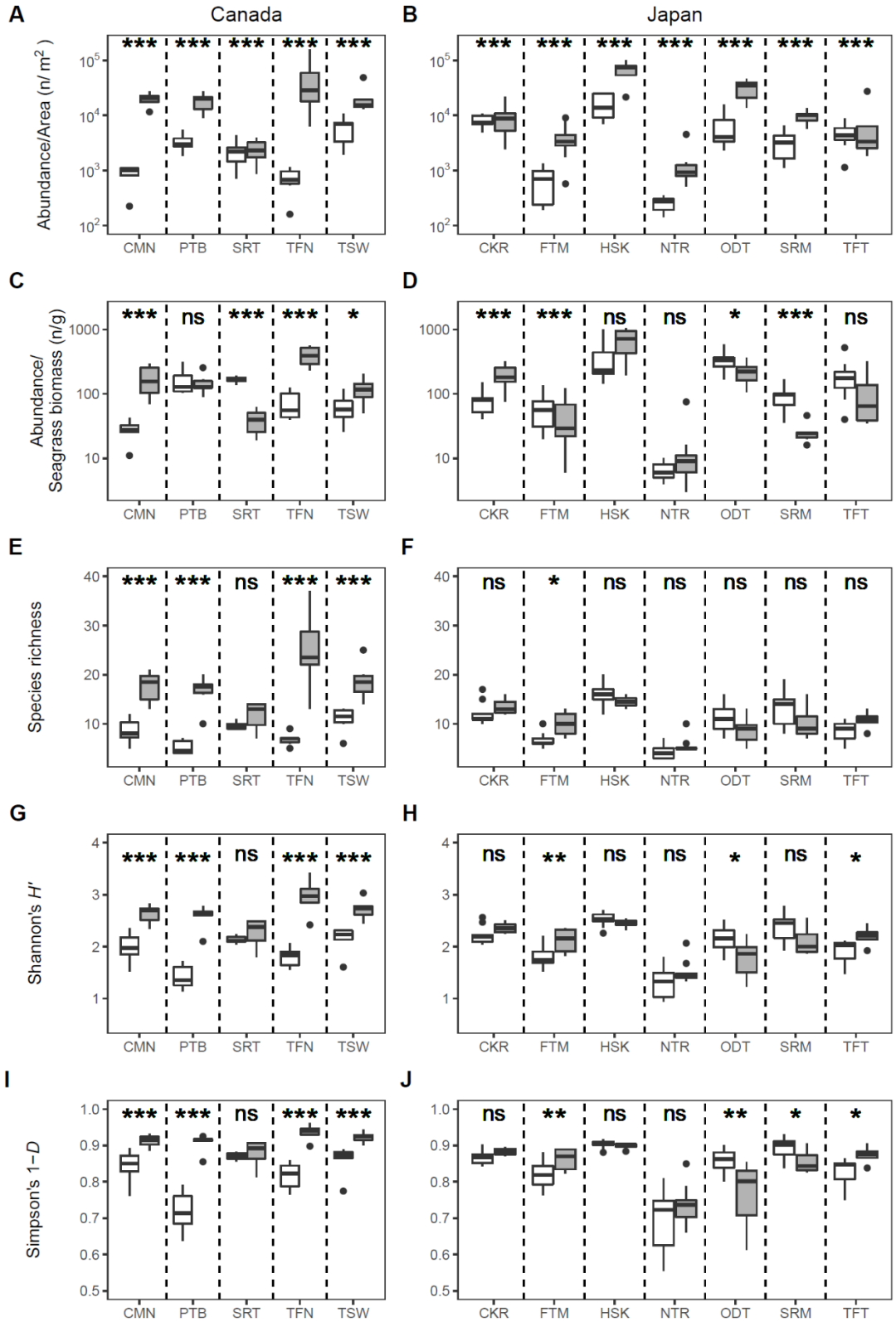

Figure 4.2. Boxplot charts showing the variation in abundance and biodiversity measures of epifauna between *Z. japonica* (white box) and *Z. marina* (grey box) at each site, for

epifaunal abundance per area (A, B), epifaunal abundance per seagrass (C, D), species richness (E, F), Shannon's *H* (G, H), and Simpson's *D* diversity indices (I, J). Letters and asterisks above the boxplots show the results of statistical comparisons between each seagrass species: "ns" not significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

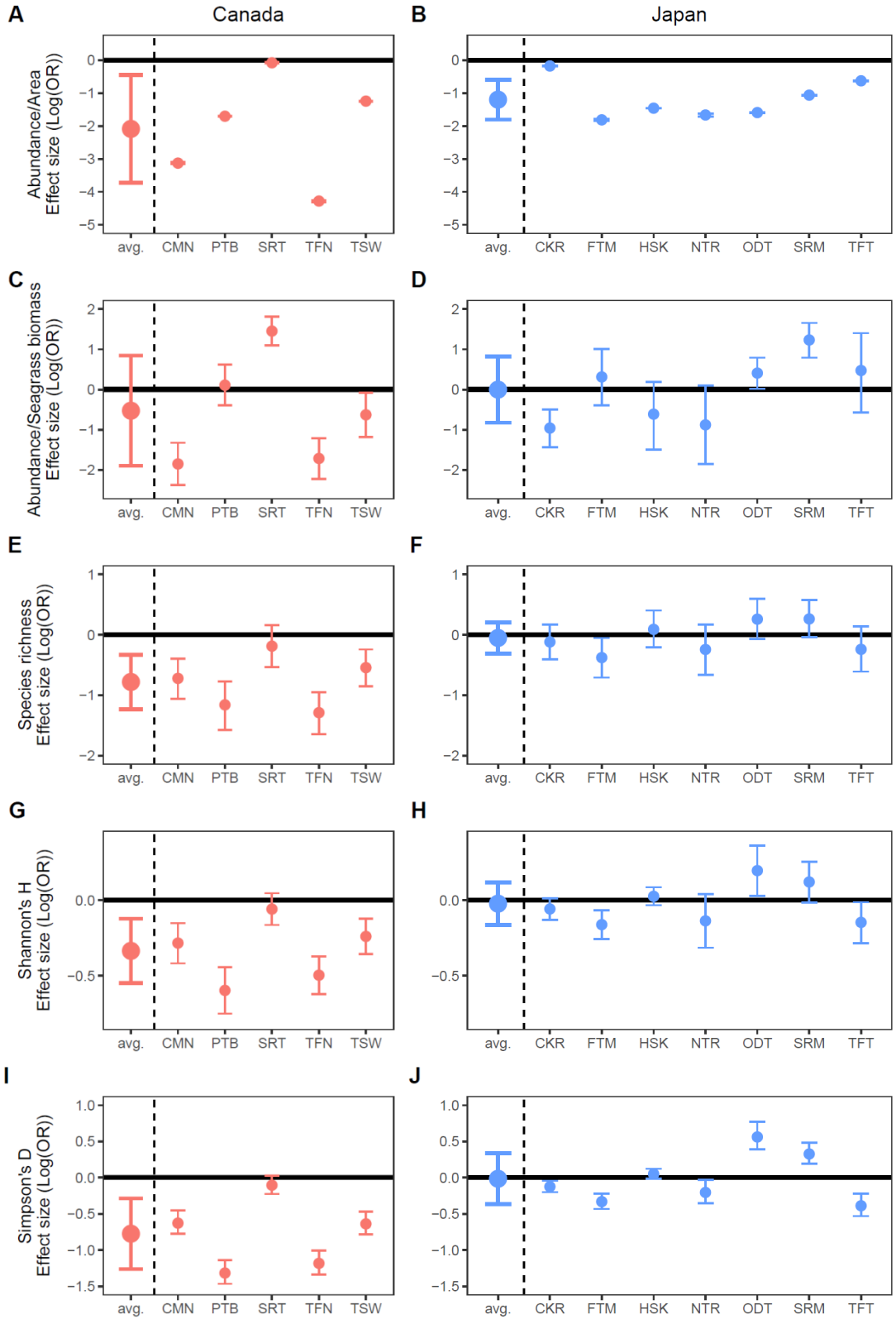

Figure 4.3. Results of effect size analysis, based on log-transformed odds ratio (Log(OR)), for epifaunal abundance per area (A, B), abundance per seagrass biomass (C, D), species

richness (E, F), Shannon's *H* (G, H), and Simpson's *D* (I, J) diversity indices. Effect sizes for species effect (*Z. japonica* vs *Z. marina*) are shown for each site in each region, and regional average calculated based on site-level effects size. Site-level effect sizes are shown as Log(OR) with upper and lower 95% confidence intervals, and regional average values are shown as mean ± standard deviation. Positive values indicate *Z. japonica* superior to *Z. marina* (Log(OR) >0), negative values indicate *Z. marina* superior to *Z. japonica* (Log(OR) <0), and values crossing zero indicates negligible effect.

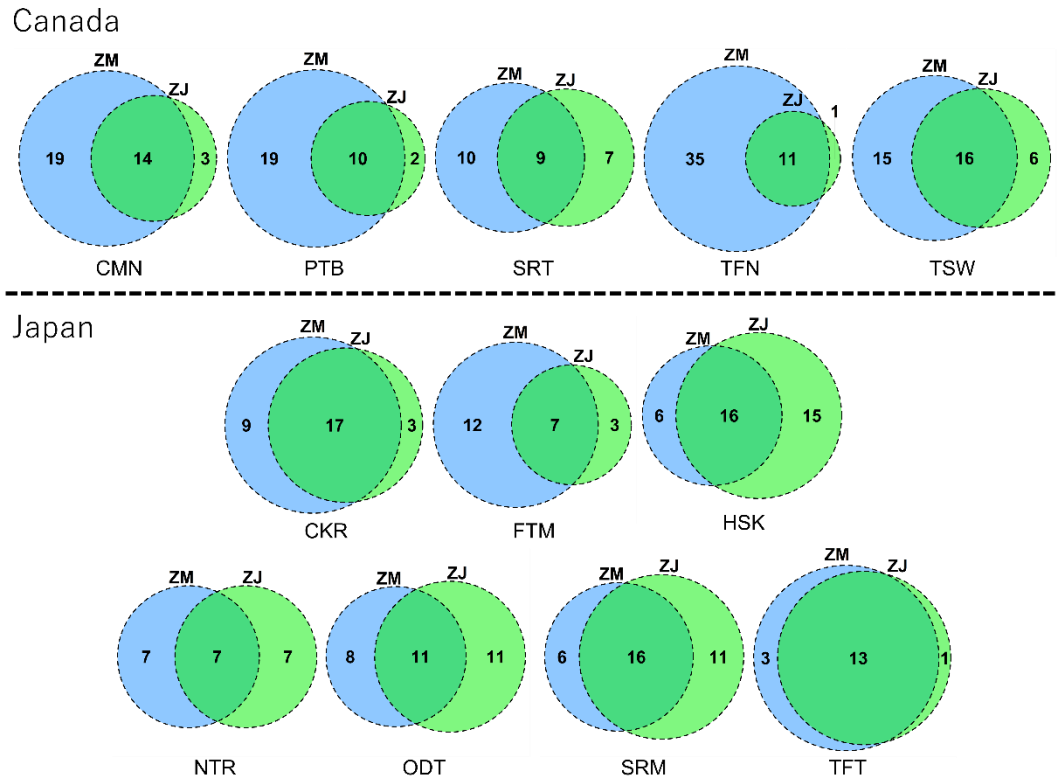

Figure 4.4. Venn diagrams showing number of epifaunal species associated with each seagrass species. The numbers of species associated with *Z. japonica* is shown in the right within green circle, the numbers of species associated with *Z. marina* in the left within blue circle, and the numbers of species associated to both seagrass species in the middle.

Table 4.1. Summary of information on study sites. Water temperature and salinity values shown here are based on measurements taken during field collection (see section 4.2.1, and section 3.2.2 in Ch. 3 for details).

Region	Site	Code	Latitude	Longitude	Collection Date	Water Temp (°C)	Salinity
Canada	Tofino	TFN	49.151 N	125.893 W	2017/7/23	14.3	25.3
	Sarita	SRT	48.907 N	125.011 W	2017/8/11	17.3	27.0
	Chemainus	CMN	48.930 N	123.719 W	2017/7/21	17.9	23.0
	Patricia Bay	PTB	48.659 N	123.450 W	2017/7/20	17.5	25.1
	Tsawwassen	TSW	49.007 N	123.094 W	2017/7/25,26	18.4	25.0
Japan	Akkeshi	CKR	43.022 N	144.884 E	2018/8/12	18.0	24.3
	Hashirikotan	HSK	43.325 N	145.318 E	2018/7/28	21.1	20.2
	Odaitou	ODT	43.584 N	145.220 E	2018/7/26	18.7	19.0
	Tofutsu	TFT	43.938 N	144.384 E	2018/8/7	22.5	19.0
	Futamigaoka	FTM	44.033 N	144.169 E	2018/8/8	17.9	33.4
	Notori	NTR	44.092 N	144.128 E	2018/8/8	21.4	33.3
	Saroma	SRM	44.119 N	143.964 E	2018/8/17	16.5	32.2

Table 4.2. Results of Generalized Linear Models showing variation in abundance and biodiversity measures between *Z. japonica* and *Z. marina* at each site. Assumed distribution of a variable are shown in parentheses after each variable.

		Abundance / Area (poisson)			Abundance / Seagrass biomass (Gamma)			Species richness (poisson)			Shannon's H (Gamma)			Simpson's D (betareg)		
Region	Site	X^2	df	*p*	X^2	df	*p*	X^2	df	*p*	X^2	df	*p*	X^2	df	*p*
Japan	CKR	1021.1	1,14	<0.001	3.392	1,14	<0.001	0.638	1,14	0.424	0.012	1,14	0.107	2.540	1,14	0.111
	FTM	24628.0	1,17	<0.001	0.440	1,17	0.376	5.258	1,17	0.022	0.119	1,17	0.001	9.736	1,17	0.002
	HSK	211643.0	1,12	<0.001	1.107	1,12	0.134	0.350	1,12	0.554	0.002	1,12	0.392	0.587	1,12	0.444
	NTR	7313.1	1,17	<0.001	3.365	1,17	0.076	1.333	1,17	0.248	0.085	1,17	0.130	1.680	1,17	0.195
	ODT	137102.0	1,14	<0.001	0.579	1,14	0.042	2.351	1,14	0.125	0.137	1,14	0.021	8.936	1,14	0.003
	SRM	23841.0	1,14	<0.001	4.771	1,14	<0.001	2.858	1,14	0.091	0.052	1,14	0.079	5.013	1,14	0.025
	TFT	7490.7	1,12	<0.001	0.582	1,12	0.353	1.562	1,12	0.211	0.061	1,12	0.032	6.402	1,12	0.011
Canada	CMN	129976.0	1,11	<0.001	9.060	1,11	<0.001	19.085	1,11	<0.001	0.242	1,11	<0.001	15.600	1,11	<0.001
	PTB	68771.0	1,11	<0.001	0.035	1,11	0.673	37.400	1,11	<0.001	1.058	1,11	<0.001	67.090	1,11	<0.001
	SRT	46.1	1,11	<0.001	5.843	1,11	<0.001	1.127	1,11	0.289	0.010	1,11	0.272	0.682	1,11	0.409
	TFN	388101.0	1,11	<0.001	7.909	1,11	<0.001	65.216	1,11	<0.001	0.733	1,11	<0.001	52.501	1,11	<0.001
	TSW	53031.0	1,11	<0.001	1.162	1,11	0.026	12.631	1,11	<0.001	0.173	1,11	<0.001	17.138	1,11	<0.001

Table S4.1. List of epifaunal species found at each site and for each seagrass species, *Zostera japonica* (ZJ) and *Z. marina* (ZM) in Canada. See Table 4.1 for the code of site name. Each cell with check (✔) represents species presence and blank cell represents species absence.

			CMN		PTB		SRT		TFN		TSW	
	taxon group	final_taxa	ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM
Mollusc	Bivalve	Bivalvia						✔				✔
		Cardiidae								✔		
		Hiatellidae		✔						✔		✔
		Myidae	✔									
		Mytilidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Tellinidae		✔	✔	✔	✔			✔	✔	✔
		Veneridae						✔				
	Gastropod	Aplysiidae		✔								✔
		Buccinidae		✔		✔				✔		
		Calyptraeidae								✔		✔
		Columbellidae		✔		✔		✔		✔	✔	✔
		Gastropoda								✔		
		Haminoeidae	✔	✔							✔	
		Littorinidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Lottiidae		✔		✔				✔	✔	✔
		Margaritidae		✔		✔	✔	✔		✔		✔
		Naticidae									✔	

		Nudibranchia								✔			
Mollusc		Rissoidae		✔	✔	✔		✔	✔	✔	✔	✔	
Crustacean	Caprellid	Caprellidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
	Copepod	Cyclopoida								✔			
		Harpacticoida	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
		Porcellidiidae	✔	✔	✔	✔		✔		✔		✔	
	Cumacean	Cumacea		✔		✔				✔		✔	
		Nannastacidae			✔		✔		✔		✔		
	Decapod	Brachyura		✔						✔			
		Caridea		✔									
		Epialtidae					✔						
		Oregoniidae								✔			
		Paguroidea	✔	✔						✔	✔		
		Varunidae		✔		✔							
	Gammarid	Amphipoda		✔		✔				✔		✔	
		Ampithoidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
		Anisogammaridae								✔		✔	
		Aoridae		✔		✔				✔		✔	
		Atylidae								✔			
		Corophiidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
		Hyalidae					✔				✔		
		Ischyroceridae					✔		✔	✔		✔	

		Photidae		✔		✔		✔		✔			✔
Crustacean		Podoceridae								✔			
		Pontogeneiidae						✔		✔			
		Stenothoidae								✔			
		Talitridae								✔			
	Isopod	Idoteidae	✔	✔		✔		✔		✔	✔	✔	
		Munnidae		✔		✔				✔			
	Tanaid	Leptocheliidae	✔	✔		✔	✔	✔		✔	✔	✔	
Annelida	Polychaete	Aphroditidae								✔			
		Glyceridae		✔		✔				✔			
		Goniadidae			✔								
		Lumbrineridae				✔				✔		✔	
		Maldanidae				✔							
		Nereididae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	
		Opheliidae						✔					
		Oweniidae										✔	
		Phyllodocidae		✔		✔				✔			
		Polychaeta								✔		✔	
		Syllidae		✔		✔				✔			
Other	Insect	Chironominae	✔				✔		✔	✔	✔		
		Insecta	✔										
	Halacaridae	Halacaridae	✔	✔		✔	✔			✔	✔	✔	

	Nemertea	Nemertea	✔	✔		✔			✔	✔	✔	✔
Other	Pycnogonid	Phoxichilidiidae						✔			✔	✔

Table S4.2. List of epifaunal species found at each site and for each seagrass species, *Zostera japonica* (ZJ) and *Z. marina* (ZM) in Japan. See Table 4.1 for the code of site name. Each cell with check (✔) represents species presence and blank cell represents species absence.

			CKR		FTM		HSK		NTR		ODT		SRM		TFT	
			ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM	ZJ	ZM
Mollusc	Bivalve	Myidae				✔										
		Mytilidae			✔	✔	✔	✔	✔	✔			✔	✔	✔	✔
		Pectinidae				✔			✔							
		Tellinidae	✔	✔					✔		✔	✔		✔	✔	✔
		Veneridae					✔									
	Gastropod	Assimineidae					✔								✔	✔
		Barleeiidae		✔			✔	✔			✔	✔	✔	✔		✔
		Batillariidae	✔	✔	✔		✔		✔		✔		✔		✔	✔
		Columbellidae	✔	✔												
		Gastropoda					✔		✔		✔		✔			
		Littorinidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Muricidae												✔		
		Nassariidae					✔	✔	✔		✔		✔	✔		
		Naticidae		✔			✔	✔			✔		✔			
		Olividae				✔	✔				✔		✔			
		Rissoidae				✔		✔		✔	✔	✔	✔	✔		
		Rissoinidae						✔		✔			✔	✔		

		Siphonariidae	✔													
Mollusc		Trochidae				✔	✔		✔					✔		✔
Crustacean	Caprellid	Caprellidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔		
	Copepod	Caligidae		✔												
		Harpacticoida	✔	✔		✔	✔	✔						✔		
		Porcellidiidae	✔	✔		✔					✔					
	Cumacean	Diastylidae					✔				✔	✔	✔			
		Leuconidae										✔				✔
		Nannastacidae		✔												
	Decapod	Caridea					✔									
		Thoridae								✔						
	Gammarid	Ampithoidae	✔	✔	✔	✔	✔	✔	✔	✔	✔		✔	✔	✔	✔
		Anisogammaridae	✔	✔			✔			✔			✔		✔	✔
		Aoridae	✔	✔	✔		✔	✔				✔	✔	✔	✔	✔
		Corophiidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Dexaminidae					✔	✔								
		Ischyroceridae	✔			✔										
		Lysianassoidea					✔				✔	✔	✔			
		Melitidae		✔												
		Pleustidae		✔				✔								
		Pontogeneiidae	✔	✔		✔		✔		✔						
		Stenothoidae	✔	✔								✔				

		Tryphosidae					✔	✔			✔		✔				
Crustacean		Urothoidae										✔					
	Isopod	Idoteidae	✔	✔			✔	✔			✔		✔				
		Isopoda		✔													
		Munnidae		✔													
		Paranthuridae		✔		✔		✔		✔		✔		✔			
		Sphaeromatidae	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
	Mysid	Mysidae	✔														
	Tanaid	Leptocheliidae					✔										
Annelid	Oligochaete	Enchytraeidae					✔				✔						
	Polychaete	Goniadidae			✔				✔				✔				
		Nereididae	✔	✔		✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
		Pholoidae											✔	✔			
		Phyllodocidae					✔					✔			✔		
		Polynoidae				✔	✔	✔		✔		✔	✔	✔			
		Sphaerodoridae									✔	✔					
		Syllidae			✔	✔											
Other	Halacaridae	Halacaridae												✔			
	Insect	Chironominae						✔					✔				
	Nemertea	Nemertea					✔								✔	✔	
	Platyhelminthes	Polycladida	✔	✔			✔			✔	✔	✔	✔	✔			
		Tricladida													✔	✔	

Pycnogonid	Phoxichilidiidae	✔

Chapter 5

General Discussion

5.1 Thesis overview

Studies on biological invasions are mandatory for management of any ecosystems affected by non-native species, but they are also useful for study understanding various aspects of biology and ecology in general (Sakai et al. 2001; Briggs 2007; Sax et al. 2005; Sax et al. 2007). Common methods used to study invasion were intraspecific and interspecific comparisons which both have limitations when used alone. Combining these two methods will enable us robust investigation of introduction effects (van Kleunen et al. 2010), however, was rarely conducted in practice. In this thesis, I proposed a use of a seagrass species pair, *Zostera japonica*, native in Asia and introduced to North America, and *Z. marina*, native in both regions, for this proposed approach of combined comparisons. This species pair provided opportunity to examine introduction effects independent from regional or species effect. Through three independent studies investigating introduction effects **ON** and **OF** *Z. japonica*, I found that non-native *Z. japonica* significantly differed from native populations in its phenology, biological traits, and in organization of seagrass associated epifaunal communities.

In Chapter 2, a large-scale analysis on seagrass phenological traits indicated that the phenological traits varied greatly among regions, affected by abiotic factors, such as latitude and temperatures, yet results also varied among analyzed traits. While significant latitude/temperature effects were observed for maximum biomass and peak reproductive timings for *Z. japonica* populations in general, non-native *Z. japonica* populations showed shorter growth durations and greater reproductive ratios at higher latitude, which were not observed for native populations. This observed differences in responses between native and non-native populations are suggested to reflect introduction effects.

In Chapter 3, multi-site comparisons of seagrass biological traits among *Z. japonica* native and non-native regions showed that constant shoot size, lower shoot density, leaf area index and biomass, and higher reproductive ratio for non-native *Z. japonica* compared to native populations. On the other hand, *Z. marina*, a native congener from both regions showed no regional differences in traits, affirming that observed regional differences for *Z. japonica* are in fact induced by introduction. These results emphasize strong introduction effects, potentially through rapid evolution, on some seagrass biological traits.

In Chapter 4, *Z. japonica – Z. marina* comparisons among regions showed that epifaunal communities of non-native *Z. japonica* are equally abundant but significantly less diverse than native populations. While native *Z. japonica* associated species-specific epifaunal community distinct from that of *Z. marina*, species associated with non-native *Z. japonica* were mainly a subset of *Z. marina* associated community, suggesting that non-native *Z. japonica* has not established its species-specific community in its introduced region. Significantly less diverse epifaunal community observed only for non-

native *Z. japonica* highlighted that observed diversity reduction is caused by non-native status and not by seagrass species identity.

Because other seagrass species do not have such extensive distribution, large-scale analyses covering both temperate and tropical regions and native vs non-native comparison were only possible for *Z. japonica* (Ch. 2). Furthermore, a combination of interspecific and intraspecific comparisons could not have been performed without appropriate congeneric pair species, *Z. marina* (Ch. 3, 4). Based on my studies, I will further discuss introduction effects on seagrass ecosystems in broader context (5.2), general findings of seagrass biology and ecosystems through a study on biological invasion (5.3), and current limitations of this study and further directions (5.4), then end the chapter with future implications (5.5). Findings of this thesis contribute to deepen our understanding on invasion ecology in general, in addition to giving valuable implications of planning marine biodiversity conservation and ecosystem management (Fig. 5.1).

5.2 Introduction effects on seagrass ecosystems

5.2.1 Introduction effects on seagrass phenology

Seagrasses maintain its populations through sexual reproduction (flowering and seeding) and through vegetative growth (clonal branching and shoot growth) (Hemminga and Duarte 2000; Kendrick et al. 2012). In general, seagrasses demonstrate seasonal dynamics in its growth, biomass, and reproduction (Duarte 1989). Studies suggested a strong correlation between their seasonal dynamics and latitude (Phillips et al. 1983; Duarte 1989; Ramage and Schiel 1998; Nakaoka and Aioi 2001). Because many of seagrass ecosystem functions and services depend on amount of seagrass, i.e., biomass, density and areal extent (Duffy et al. 2006; Lavery et al. 2013; Nordlund et al. 2016), it

is important to understand how seasonal dynamics in seagrasses are affected by environmental factors, not limited to latitude. Effects of various environmental factors, such as light, temperature and nutrients, on seagrass dynamics were investigated in previous studies (Marbà et al. 1996; Pergent-Martini et al. 2005; Lee et al. 2007; Hosokawa et al. 2009). However, such investigations were mostly conducted at local scales and rarely analyzed at large-scale covering wide ranges of species distribution (Clausen et al. 2014; Blok et al. 2018). Neither were they undertaken for intertidal species which is suggested to be more susceptible to change in environmental factors (Marbà et al. 1996). In Chapter 2, I had successfully demonstrated that seasonal dynamics of seagrass biomass and reproduction are significantly affected by global factors, such as latitude and temperatures although they varied greatly among analyzed traits. Results showed that maximum biomass and peak reproductive timings correlated with latitude and temperature, which maximum biomass is highest at mid-latitudes or intermediate temperature, and peak reproductive timing occurred later in the year at higher latitudes or cooler sites. While delay in peak reproductive timing observed in *Z. japonica* was in consistent with study results for *Z. marina* (Blok et al. 2018), observed correlation between maximum biomass and temperature was not reported in any other seagrass species, including well-studied *Z. marina* (Oelsen and Sand-Jensen 1994; Nakaoka and Aioi 2001; Clausen et al. 2014; Olesen et al. 2015; Ruesink et al. 2018). Thus, my result significantly contributed to understanding of abiotic influence on seagrass phenology and highlighted influences on phenology is species-specific. This finding will be important not only for seagrass ecology, but also for the management and conservation planning of ecosystems.

Seagrass ecosystems are rapidly changing with multiple threats to the ecosystem (Orth et al. 2006; Waycott et al. 2009; Roca et al. 2016; Unsworth et al. 2019) and expected to change further as global climate change intensifies (Short and Neckles 1999; Duarte 2002; Hyndes et al. 2016). Under such circumstances, large-scale analysis covering wide ranges of abiotic factors can provide useful information for predicting species responses to ongoing and future changes in environmental factors, such as temperature (Fukami and Wardle 2005; Buyantuyev et al. 2012). The results of Chapter 2 suggest that many of the analyzed *Z. japonica* populations are already experiencing higher temperature than its optimal temperature for biomass production, and that increase in temperature will decrease the biomass in most of the populations. My results also suggested that some *Z. japonica* populations are already in the edge of its critical temperature and further increase in temperature would highly threaten their persistence.

Biological invasions may also change plant phenology to adapt to environmental conditions in novel habitat (Colautti and Barrett 2013; Novy et al. 2013). Altered phenology through introduction may affect association with other species and potentially cause so called phenological mismatch (Edwards and Richardson 2004; Renner and Zohner 2018; Damien and Tougeron 2019). Because there are various organisms utilize seagrass ecosystems, such as fish for nursery, epifaunal community as habitat, and birds for foraging ground, the alteration in seagrass phenology may affect those organisms (Svensson et al. 2012; Sydeman et al. 2015; Unsworth 2021). Comparisons between native and non-native *Z. japonica* phenology showed that significant introduction effects on non-native *Z. japonica* (Ch. 2). Non-native populations showed shorter period of growth and higher reproductive ratio at higher latitude, which were not observed for native populations. To date, phenological mismatch had not been widely investigated in

seagrass ecosystems, especially not for biological invasions. This study indicated the importance of incorporating introduction effects in the study of seagrass phenology.

5.2.2 Introduction effects on seagrass biological traits

Seagrass biological traits have been well investigated focusing on how they vary among seasons, sites, regions and species (i.e., den Hartog and Kuo 2006; McDonald et al. 2016; Leopardas et al. 2018; Namba and Nakaoka 2018; Ruesink et al. 2018; Murphy et al. 2021). Past studies had shown interaction between seagrass biological traits and environmental factors, such as nutrient, light, depth and sediment types (Hemminga and Duarte 2000; Lee et. al. 2007; Zhang 2015; Vieira et al. 2018; Zhang et al. 2021). However, not many studies had been conducted to understand if those traits would change with species introduction to novel habitat, possibly due to limited occurrence of non-native seagrass species (Williams 2007). Biological invasions can alter species trait, through population bottleneck during invasion processes, and/or rapid adaptation to environmental conditions in introduced region (Sakai et al. 2001; Huey et al. 2005; Novak 2007; Sotka et al. 2018). It is also acknowledged that invasive species exhibit common traits known as “invasive traits” (Kolar and Lodge 2001; Pyšek and Richardson 2007; Mason et al. 2008; van Kleunen et al. 2015), and managers should be extremely cautious to see if non-native species exhibit such traits or not. Thus, study on non-native seagrass biological traits would be very important for both management and for deepening our understanding of seagrass responses to introduction.

To date, there are only four known seagrass species that had become non-native elsewhere (Williams 2007). *Halophila stipulacea* and *Z. japonica* are among those four species that had been investigated for their introduction effects. Studies on *H. sitpulacea*

suggested that non-native populations exhibit different flowering sex ratios and higher growth rate and production under heat stress, compared to native populations (see reviews by Winters 2020; Nguyen et al. 2018; Nguyen et al. 2020a, b). However, when compared only within species, one could not distinguish regional environmental difference to introduction effects. In Chapter 3, I overcome this difficulty by using species pair of *Z. japonica* and *Z. marina* to discriminate between regional difference which would affect both species equally, and the introduction effect which would affect only *Z. japonica*. I have found that strong inferences of the rapid evolution from significant reduction in shoot density and enhanced sexual reproduction for non-native *Z. japonica*, whereas reduction in phenotypic variation was not observed from this study, suggesting that population bottleneck did not occur or was masked by phenotypic plasticity in *Z. japonica*. Evidence of trait shifts suggested from the present study successfully demonstrated that *Z. japonica*'s introduction to North America would provide rare opportunity to investigate its evolutionary processes. In addition to evolutionary aspect, my results provided significant insights for management.

5.2.3 Introduction effects on seagrass associated community

Foundation species, including seagrasses, are known to facilitate biodiversity and are major determinants of abundance and diversity of associated biological communities (Ellison et al. 2005; Stachowicz and Byrnes 2006; Ellison 2019; Scott and terHorst 2020). Among various organisms associated with seagrass, small invertebrate community known as epifauna is especially important, because they connect primary producer, such as epiphytic algae, to higher trophic levels through predation by predatory fish and crustaceans and thus play a key role in seagrass food web dynamics (Orth et al.

1984; Valentine and Duffy 2006; Best and Stachowicz 2014). Introductions of new foundation species to a habitat occasionally cause drastic changes on associated community (Lanta et al. 2013; Lindbladh et al. 2019; Navarro-Barranco et al. 2019), whereas changes are limited when non-native species are functionally or morphologically similar with native foundation species (Ramus et al. 2017; Valdez et al. 2021).

Some studies had examined the impacts of non-native seagrass *Z. japonica* introduced to North America and compared associated epifaunal community between non-native *Z. japonica* with native congener *Z. marina* (Thom et al. 1995; Knight et al. 2015). However, these results in the *Z. japonica* introduced region had not been compared with the results from its native region, Asia, restricting us from discriminating seagrass species effects with introduction effects. In Chapter 4, I conducted the study to overcome this point by comparing seagrass species effect on epifaunal community between *Z. japonica* native and non-native regions. From this study, I have shown that epifaunal abundances are not affected by seagrass species identity in either region and are mainly controlled by seagrass biomass, or habitat availability, thus non-native *Z. japonica* can support similar epifaunal abundance with its native congener and with its native populations. On the other hand, I found that epifaunal community associated with non-native *Z. japonica* was less diverse than native *Z. marina* and that observed result was independent of species difference, because epifaunal communities associated with native *Z. japonica* and *Z. marina* are equally diverse. This result contradicted with previous findings that found equally diverse epifaunal community between the two species (Thom et al. 1995; Knight et al. 2015). Furthermore, species associated with non-native *Z. japonica* are mainly a subset of native *Z. marina* community, suggesting that only limited number of epifaunal species had successfully utilize non-native seagrass. Findings from

my study provide new insights on epifaunal association with non-native seagrass and provide fundamental knowledge on non-native species impacts which are important for management and conservation planning.

5.3 General findings of seagrass biology and ecosystems through a study on biological invasion

5.3.1 Utilizing biological invasions as study tools for ecology in general

Based on these results written above, I have successfully demonstrated that introduction of *Z. japonica* to North America provided us opportunity to deepen our understandings of basic ecology, biogeography, and evolutionary biology of seagrass, and thus biological invasions can be strong study tools not limited to applied field of invasion ecology. Findings from seagrass phenology (Ch. 2) and biological traits (Ch. 3) pointed out some clues on how seagrass biological processes, such as growth and reproduction are influenced by environmental factors and how they are reflected to traits. I found that biomass and reproductive phenology were differently affected by environmental factors, between native and non-native *Z. japonica* populations (Ch. 2), as well found that biomass and reproductive traits varied among native and non-native *Z. japonica* populations and that differences were independent of regional environmental difference, as regional difference was not found in *Z. marina* (Ch. 3). Nevertheless, my findings on altered phenological responses to environmental factors through introduction suggest that such altered responses may not be limited to phenological processes but other processes. Study in Chapter 3 assumed that biological traits responses to environmental conditions are consistent between native and non-native populations. However, accounting that

relationship between abiotic factors and biological processes may change through introduction, the assumption used may not have been appropriate. Thus, I suggest for future study involving non-native species, one must consider 1) if compared populations/sites/regions are experiencing similar environmental conditions, but also 2) if native and non-native populations respond to environmental conditions are similar. These suggestions are not limited to non-native seagrass species but apply broadly in invasion study. On the other hand, I revealed that while some traits were altered by introduction effects, some other traits were not affected by introduction and remained unchanged. For example, biomass or reproductive peak timings were not affected by introduction (Ch. 2) and that seagrass shoot size were similar between native and non-native *Z. japonica* populations (Ch. 3). These variation among analyzed traits suggest that introduction effects on seagrass traits are much more complex than what we had been expected. By finding out how seagrass traits differ between native and non-native populations and collating knowledge on which traits are more or less affected by introduction, we would be able to understand on which traits or biological processes involving such traits are more stable under evolutionary processes.

Furthermore, combination of seagrass biological traits study and seagrass-epifauna association study in the context of non-native seagrass provided broader insight about the influence of seagrass effect on epifaunal community. Based on Chapters 3 and 4, I found that epifaunal abundance varied among amount of seagrass biomass and that biomass was lower for non-native *Z. japonica.* However, epifauna abundance per seagrass biomass was similar among the native and non-native *Z. japonica* and *Z. marina*, confirming that abundance is not influenced by host species identity or regional difference. In contrast, epifaunal species richness showed significant regional difference and was less

diverse for the non-native *Z. japonica* (Ch. 4). Interestingly, when the same seagrass species were compared among region, species richness was similar for *Z. japonica* (Ch. 4) while biological traits were much different between native and non-native populations (Ch. 3). Similarly, although *Z. marina* did not show regional difference in traits between Japan and Canada, species richness was much higher for *Z. marina* in Canada. These findings suggest that epifaunal species richness is not influenced by seagrass biological traits, such as structural traits (shoot size, shoot density and biomass), highlighting that other factor which are independent from seagrass are responsible for species richness. Although other factors were not investigated in this study, my findings would help navigating the direction of future studies in seagrass-epifauna association as well on study on other foundation species and associated communities.

5.3.2 Promising approaches combing intraspecific and interspecific comparison

Use of biological invasions as the study tools could be even fortified by using combined methods of intraspecific and interspecific comparisons (van Kleunen et al. 2010). Throughout the thesis, I used the *Z. japonica – Z. marina* species pair both in native and non-native regions of *Z. japonica*. Without this combined use, I could not convince that observed shoot density reduction and enhanced sexual reproduction in non-native *Z. japonica* is caused by introduction effects and not by regional environmental difference (Ch. 3). Similarly, one could have argued that significantly less diverse community associated with non-native *Z. japonica* compared to *Z. marina* may result from seagrass species difference and not by introduction effects, without additional species comparison in Japan (Ch. 4). Based on these results, I clearly demonstrated the strengths of combined

methods for studying introduction effects and hope to convince researchers to use such study designs in future studies.

Although I had been proposing that *Z. japonica* and *Z. marina* species pair as a special opportunity for biological invasion studies, it will worth consider what makes them special, to explore potential characteristics of species or species pair that would enable the suggested comparative study designs. First, characteristics of seagrass, such as taxonomy, biogeography, and evolutionary history may play important role in making species comparable between each other. There are approximately 60 species of seagrass worldwide (den Hartog and Kuo 2006) and many species, including *Z. marina* and *Z. japonica*, have extensive geographical distribution (Green and Short 2003; Short et al. 2007). Small number of species and wide geographical distribution in their "native" region, would increase likelihood of one species to introduced to another's native range. Such small species diversity but wide distribution of each species may be caused by short evolutionary history of seagrass; marine families of angiosperm are suggested to have originated in the Cretaceous period and have multiple occasions to return into the sea around 70 to 100 million years ago (Les et al. 1997; Kato et al. 2003; Waycott et al. 2006). Nevertheless, wide distributions covering the multiple regions are more or less important than short evolutionary history for candidate species.

Another important factor will be trackable invasion history for non-native species. Introduction of *Z. japonica* had been documented from early phases of its introduction (Hitchcock et al. 1969; Harrison and Bigley 1982), and sightings at new habitat are constantly reported (Shafer et al. 2014). Background knowledge on invasion history will facilitate our understandings, especially for rapid evolution. Although not applicable to this study, if an origin of non-native populations is known, comparisons

between non-native populations and native populations from the origin would further improve the evolutionary studies. Use of species pair in invasion ecology has just started from this thesis, however, investigation on appropriate species pair, potentially one widely distributed or cosmopolitan species and another with invasion history background, would facilitate further understanding and broader implications on biological invasions.

5.4 Limitations of this study and further directions

Unfortunately, there are certain aspects that could not be fully covered in this study which are, if incorporated, would have significantly improved the understandings of seagrass ecology. They are spatial coverage, temporal variation, and genetic information.

Firstly, data used in this study did not cover the whole spatial distribution range of the target species. *Zostera japonica* exhibits wide latitudinal distribution covering both temperate and tropical regions (Green and Short 2003). Chapter 2 attempted to cover the whole distribution range by thorough literature survey, whereas comparative studies were only conducted in very small areas in eastern Hokkaido and southern British Columbia in Chapters 3 and 4. Even for large-scale comparison in Chapter 2, seasonal data from northernmost native (Russia), and southern non-native (California) were not available. Inclusion of studies and data from wider regions will strengthen the generality of my findings.

The second limitation occurs on the lack of data on temporally replicated data in Chapters 3 and 4. As discussed in detail in Chapter 2, seagrasses demonstrate seasonal dynamics in its biomass and reproduction. However, comparative studies (Ch. 3 and 4) were only conducted during summer, where seagrass biomass was at its maximum.

Seagrass biological traits would surely vary seasonally, but potentially differently among analyzed traits. Seasonal comparisons of seagrass biological traits between native and non-native seagrass may provide insights on seasonal trait variation which were partly discussed in Chapter 2. In addition to seagrass that varies among season, its associated community shows seasonal fluctuation in its abundance, diversity, and in the patterns of association with seagrass (Momota and Nakaoka 2018; Hinode et al. 2021). Because epifaunal communities may respond differently to seagrass host species at different time of year, it would be important to consider temporal variation in both seagrass and community into account. As altered phenology for non-native seagrass populations is suggested from the result of Chapter 2, this aspect would be extremely important for invasion study.

Furthermore, sparse information on *Z. japonica* genetics limited me to discuss introduction effects in detail, especially in terms of evolutionary context. To date, there are a few studies that had investigated population genetic structure of *Z. japonica* in its native regions (Hodoki et al. 2013; Lee et al. 2004; Zhang et al. 2016; Jiang et al. 2018). However, very few studies were available in its non-native regions where a study suggested that genetic origin of non-native *Z. japonica* populations is derived from Miyagi Pref., Japan (Tanaka 2012). Genetic information for non-native *Z. japonica* can provide more insights on evolutionary aspect of *Z. japonica* introduction. Trait shifts for non-native *Z. japonica* is suggested from this study (Ch. 2, 3), however, I could only discuss its evolutionary process in a very limited way due to lack of genetic data to either support or negate my speculation. Future studies incorporating genetic data would facilitate our understanding of introduction effects on *Z. japonica*, specifically with evolutionary processes.

5.5 Future implications

Significant effects of introduction **ON** and **OF** *Z. japonica* are already observed in North America. However, these may further change in future. Since its introduction in the 1950s, *Z. japonica* established in the west coast of North America over the 70 years (Hitchcock et al. 1969; Harrison and Bigley 1982). However, this duration may be still too short to establish stable species interactions and associations. Non-native species may still have been undergoing invasion lag-phase (interval between non-native species becoming established and becoming fully invasive; Sakai et al. 2001) after the 70 years (Ch. 2, 3), and this may be short for epifaunal community to specify into the new host seagrass (Ch. 4). Considering the invasion lag-phase, longer the establishment of *Z. japonica* in North America may induce species traits to become more "invasive", such as higher shoot density and larger shoot size, which will increase non-native *Z. japonica*'s competitive ability over *Z. marina*. Furthermore, epifaunal community would also change in time, by longer evolutionary history shared together and introduction of more non-native animal species.

Ongoing and future climate change would affect seagrass ecosystems. Global climate change may also affect *Z. japonica*, both in native and non-native regions. From this study, I find that seagrass biomass of *Z. japonica* is highly influenced by temperature and that increase in temperature will decrease the biomass in many of its distribution (Ch. 2). Other aspects may also change with temperature-induced change in seagrass biomass. Epifaunal community is strongly dependent on seagrass biomass and thus it may change with decline in seagrass biomass, especially in terms of abundance (Ch. 4). Critical upper temperature and optimal temperature for *Z. marina* are lower than that of *Z. japonica* (Lee

et al. 2005; Abe et al. 2009; Shafer et al. 2008; Morita et al. 2010; Shafer et al. 2014; Kaldy et al. 2015). This difference in heat tolerances in the two species suggest that temperature increase will more severely affect *Z. marina* than *Z. japonica*, leading to the prediction that the seagrass beds will probably be more dominated by *Z. japonica* in future. Results from Chapter 4 illustrated that epifaunal community is significantly less diverse for non-native *Z. japonica* than native *Z. marina*, suggesting that only limited number of epifaunal species has successfully switched its host to *Z. japonica*. These results imply that significant reduction in epifaunal community, especially in terms of diversity, is expected when *Z. japonica* outcompeted *Z. marina* under temperature stress.

Most importantly, *Z. japonica* traits and associated epifaunal community from its native habitats in Hokkaido, Japan, and most study sties from *Z. japonica* non-native regions, British Columbia, Canada, were described in detail for the first time by this study (Ch. 3, 4). Likely, this study provided the first comparative study between *Z. japonica* and *Z. marina* in its native region (Ch. 4). Thus, the results from this thesis could provide important references for both native and non-native *Z. japonica* at this timepoint. As discussed earlier, significant changes in seagrass and its associated community are expected in future. Under such circumstances, results from this thesis can be remarkably important. Results obtained from this study can be useful for management and conservation planning, such as to inspect habitat at risk on non-native *Z. japonica* expansion, and to predict future of *Z. japonica* distribution and abundance with climate changes.

5.6 References

Abe M, Yokota K, Kurashima A, Maegawa M (2009) High water temperature tolerance in photosynthetic activity of *Zostera japonica* Ascherson & Graebner seedlings from Ago Bay, Mie Prefecture, central Japan. Fish Sci 75:1117–1123. https://doi.org/10.1007/s12562-009-0141-x

Best RJ, Stachowicz JJ (2014) Phenotypic and phylogenetic evidence for the role of food and habitat in the assembly of communities of marine amphipods. Ecology 95:775–786. https://doi.org/10.1890/13-0163.1

Blok SE, Olesen B, Krause-Jensen D (2018) Life history events of eelgrass *Zostera marina* L. populations across gradients of latitude and temperature. Mar Ecol Prog Ser 590:79–93. https://doi.org/10.3354/meps12479

Briggs JC (2007) Marine biogeography and ecology: invasions and introductions. J Biogeogr 34:193–198. https://doi.org/10.1111/j.1365-2699.2006.01632.x

Buyantuyev A, Xu P, Wu J, et al (2012) A space-for-time (SFT) substitution approach to studying historical phenological changes in urban environment. PLoS One 7:e51260. https://doi.org/10.1371/journal.pone.0051260

Clausen K, Krause-Jensen D, Olesen B, Marbà N (2014) Seasonality of eelgrass biomass across gradients in temperature and latitude. Mar Ecol Prog Ser 506:71–85. https://doi.org/10.3354/meps10800

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366. https://doi.org/10.1126/science.1242121

Damien M, Tougeron K (2019) Prey–predator phenological mismatch under climate change. Curr Opin Insect Sci 35:60–68. https://doi.org/10.1016/j.cois.2019.07.002

den Hartog C, Kuo J (2006) Taxonomy and Biogeography of Seagrasses. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, THe Netherlands

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

Duarte CM (1989) Temporal biomass variability and production/biomass relationships of seagrass communities. Mar Ecol Prog Ser 51:269–276. https://doi.org/10.1080/14786419.2015.1027893

Duffy JE (2006) Biodiversity and the functioning of seagrass ecosystems. Mar Ecol Prog Ser 311:233–250. https://doi.org/10.1021/ja00173a016

Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430:881–884

Ellison AM (2019) Foundation species, non-trophic interactions, and the value of being common. iScience 13:254–268. https://doi.org/10.1016/j.isci.2019.02.020

Ellison AM, Bank MS, Clinton BD, et al (2005) Loss of foundation species: Consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486. https://doi.org/10.1890/1540-9295(2005)003[0479:LOFSCF]2.0.CO;2

Freeman AS, Frischeisen A, Blakeslee AMH (2016) Estuarine fouling communities are dominated by nonindigenous species in the presence of an invasive crab. Biol Invasions 18:1653–1665. https://doi.org/10.1007/s10530-016-1108-3

Fukami T, Wardle DA (2005) Long-term ecological dynamics: reciprocal insights from natural and anthropogenic gradients. Proc R Soc B 272:2105–2115. https://doi.org/10.1098/rspb.2005.3277

Green EP, Short FT (2003) World Atlas of Seagrasses. University of California Press, Berkeley, CA

Harrison PG, Bigley RE (1982) The recent introduction of the seagrass *Zostera japonica* Aschers. and Graebn. to the Pacific coast of North America. Can J Fish Aquat Sci 39:1642–1648. https://doi.org/10.1139/f82-221

Heiman K, Vidargas N, Micheli F (2008) Non-native habitat as home for non-native species: comparison of communities associated with invasive tubeworm and native oyster reefs. Aquat Biol 2:47–56. https://doi.org/10.3354/ab00034

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

Hinode K, Kamisaki H, Nishihara GN, Terada R (2021) The phenology of epiphytic diatoms and epifauna observed on *Zostera marina* of Arikawa Bay, Nagasaki Prefecture, Japan. Plankt Benthos Res 16:1–12. https://doi.org/10.3800/pbr.16.

Hitchcock CL, Cronquist A, Ownbey M, Thompson JW (1969) Vascular Plants of the Pacific Northwest Volume 1: Vascular Cryptogams, Gymnosperms, and Monocotyledons. Univ. Washington Press, Seattle, WA

Hodoki Y, Ohbayashi K, Tanaka N, Kunii H (2013) Evaluation of genetic diversity in *Zostera japonica* (Aschers. et Graebn.) for seagrass conservation in brackish lower reaches of the Hii River System, Japan. Estuaries and Coasts 36:127–134. https://doi.org/10.1007/s12237-012-9564-1

Hosokawa S, Nakamura Y, Kuwae T (2009) Increasing temperature induces shorter leaf life span in an aquatic plant. Oikos 118:1158–1163. https://doi.org/10.1111/j.1600-0706.2009.17288.x

Huey RB, Gilchrist GW, Hendry AP (2005) Using invasive species to study evolution: case studies with Drosophila and Salmon. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species Invasions: Insights into Ecology, Evolution and Biogeography. Sinauer, Sunderland, MA, pp 139–164

Hyndes GA, Heck KL, Vergés A, et al (2016) Accelerating tropicalization and the transformation of temperate seagrass meadows. Bioscience 66:938–948. https://doi.org/10.1093/biosci/biw111

Jiang K, Tsang P-KE, Xu N-N, Chen X-Y (2018) High genetic diversity and strong differentiation in dramatically fluctuating populations of *Zostera japonica* (Zosteraceae): implication for conservation. J Plant Ecol 11:789–797. https://doi.org/10.1093/jpe/rtx053

Kaldy JE, Shafer DJ, Magoun AD (2015) Duration of temperature exposure controls growth of *Zostera japonica*: Implications for zonation and colonization. J Exp Mar Bio Ecol 464:68–74. https://doi.org/10.1016/j.jembe.2014.12.015

Kato Y, Aioi K, Omori Y, et al (2003) Phylogenetic analyses of *Zostera* species based on rbcL and matK nucleotide sequences: Implications for the origin and diversification of seagrasses in Japanese waters. Genes Genet Syst 78:329–342. https://doi.org/10.1266/ggs.78.329

Kendrick GA, Waycott M, Carruthers TJB, et al (2012) The central role of dispersal in the maintenance and persistence of seagrass populations. Bioscience 62:56–65. https://doi.org/10.1525/bio.2012.62.1.10

Knight NS, Prentice C, Tseng M, O'Connor MI (2015) A comparison of epifaunal invertebrate communities in native eelgrass *Zostera marina* and non-native *Zostera japonica* at Tsawwassen, BC. Mar Biol Res 11:564–571. https://doi.org/10.1080/17451000.2014.985230

Lanta V, Hyvönen T, Norrdahl K (2013) Non-native and native shrubs have differing impacts on species diversity and composition of associated plant communities. Plant Ecol 214:1517–1528. https://doi.org/10.1007/s11258-013-0272-0

Lavery PS, Mateo MÁ, Serrano O, Rozaimi M (2013) Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon

ecosystem service. PLoS One 8:e73748. https://doi.org/10.1371/journal.pone.0073748

Lee K-S, Park SR, Kim YK (2007) Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. J Exp Mar Bio Ecol 350:144–175. https://doi.org/10.1016/j.jembe.2007.06.016

Lee SY, Oh JH, Choi C Il, et al (2005) Leaf growth and population dynamics of intertidal *Zostera japonica* on the western coast of Korea. Aquat Bot 83:263–280. https://doi.org/10.1016/j.aquabot.2005.07.004

Lee S, Ma S, Lim Y, et al (2004) Genetic diversity and its implications in the conservation of endangered *Zostera japonica* in Korea. J Plant Biol 47:275–281. https://doi.org/10.1007/BF03030519

Leopardas V, Hori M, Mukai H, et al (2018) Broad scale variation in seagrass benthic macrofaunal assemblages along the coast of Japan. Ecol Res 33:105–117. https://doi.org/10.1007/s11284-017-1517-5

Les DH, Cleland MA, Waycott M (1997) Phylogenetic studies in Alismatidae, II: Evolution of marine angiosperms (Seagrasses) and hydrophily. Syst Bot 22:443–463. https://doi.org/10.2307/2419820

Lindbladh M, Petersson L, Hedwall P-O, et al (2019) Consequences for bird diversity from a decrease in a foundation species—replacing Scots pine stands with Norway spruce in southern Sweden. Reg Environ Chang 19:1429–1440. https://doi.org/10.1007/s10113-019-01480-0

Marbà N, Cebrián J, Enríquez S, Duarte C (1996) Growth patterns of Western Mediterranean seagrasses: species-specific responses to seasonal forcing. Mar Ecol Prog Ser 133:203–215. https://doi.org/10.3354/meps133203

McDonald AM, Prado P, Heck KL, et al (2016) Seagrass growth, reproductive, and morphological plasticity across environmental gradients over a large spatial scale. Aquat Bot 134:87–96. https://doi.org/10.1016/j.aquabot.2016.07.007

Momota K, Nakaoka M (2018) Seasonal change in spatial variability of eelgrass epifaunal community in relation to gradients of abiotic and biotic factors. Mar Ecol 1–11. https://doi.org/10.1111/maec.12522

Morita T, Kokubu H, Miyamatsu A, et al (2010) The effects of water temperature in the sediment and the desiccation period on the growth and survival of transplant seagrass *Zostera japonica* shoots in mesocosm tank culture. Aquac Sci 58:261–267 (in Japanese with English abstract)

Murphy CE, Orth RJ, Lefcheck JS (2021) Habitat primarily structures seagrass epifaunal communities: A regional-scale assessment in the Chesapeake Bay. Estuaries and Coasts 44:442–452. https://doi.org/10.1007/s12237-020-00864-4

Nakaoka M, Aioi K (2001) Ecology of seagrasses *Zostera* spp. (Zosteraceae) in Japanese waters: A review. Otsuchi Mar Sci 26:7–22

Namba M, Nakaoka M (2018) Spatial patterns and predictor variables vary among different types of primary producers and consumers in eelgrass (*Zostera marina*) beds. PLoS One 13:e0201791. https://doi.org/10.1371/journal.pone.0201791

Navarro-Barranco C, Muñoz-Gómez B, Saiz D, et al (2019) Can invasive habitat-forming species play the same role as native ones? The case of the exotic marine macroalga *Rugulopteryx okamurae* in the Strait of Gibraltar. Biol Invasions 21:3319–3334. https://doi.org/10.1007/s10530-019-02049-y

Nguyen HM, Kleitou P, Kletou D, et al (2018) Differences in flowering sex ratios between native and invasive populations of the seagrass *Halophila stipulacea*. Bot Mar 61:337–342. https://doi.org/10.1515/bot-2018-0015

Nguyen HM, Yadav NS, Barak S, et al (2020a) Responses of invasive and native populations of the seagrass *Halophila stipulacea* to simulated climate change. Front Mar Sci 6:1–16. https://doi.org/10.3389/fmars.2019.00812

Nguyen HM, Savva I, Kleitou P, et al (2020b) Seasonal dynamics of native and invasive *Halophila stipulacea* populations—A case study from the northern Gulf of Aqaba and the eastern Mediterranean Sea. Aquat Bot 162:103205. https://doi.org/10.1016/j.aquabot.2020.103205

Nordlund LM, Koch EW, Barbier EB, Creed JC (2016) Seagrass ecosystem services and their variability across genera and geographical regions. PLoS One 11:e0163091. https://doi.org/10.1371/journal.pone.0163091

Novak SJ (2007) The role of evolution in the invasion process. Proc Natl Acad Sci 104:3671–3672. https://doi.org/10.1073/pnas.0700224104

Novy A, Flory SL, Hartman JM (2013) Evidence for rapid evolution of phenology in an invasive grass. J Evol Biol 26:443–450. https://doi.org/10.1111/jeb.12047

O'Loughlin LS, Green PT (2015) Invader–invader mutualism influences land snail community composition and alters invasion success of alien species in tropical rainforest. Biol Invasions 17:2659–2674. https://doi.org/10.1007/s10530-015-0903-6

Olesen B, Krause-Jensen D, Marbà N, Christensen PB (2015) Eelgrass *Zostera marina* in subarctic Greenland: dense meadows with slow biomass turnover in cold waters. Mar Ecol Prog Ser 518:107–121. https://doi.org/10.3354/meps11087

Olesen B, Sand-Jensen K (1994) Biomass-density patterns in the temperate seagrass *Zostera marina*. Mar Ecol Prog Ser 109:283–291. https://doi.org/10.3354/meps109283

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

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

Pergent-Martini C, Pasqualini V, Ferrat L, et al (2005) Seasonal dynamics of *Zostera noltii* Hornem. in two Mediterranean lagoons. Hydrobiologia 543:233–243. https://doi.org/10.1007/s10750-004-7454-7

Phillips RC, McMillan C, Bridges KW (1983) Phenology of eelgrass, *Zostera marina* L., along latitudinal gradients in North America. Aquat Bot 15:145–156. https://doi.org/10.1016/0304-3770(83)90025-6

Ramage DL, Schiel DR (1998) Reproduction in the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. Mar Biol 130:479–489. https://doi.org/10.1007/s002270050268

Ramus AP, Silliman BR, Thomsen MS, Long ZT (2017) An invasive foundation species enhances multifunctionality in a coastal ecosystem. Proc Natl Acad Sci 114:8580–8585. https://doi.org/10.1073/pnas.1700353114

Renner SS, Zohner CM (2018) Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. Annu Rev Ecol Evol Syst 49:165–182. https://doi.org/10.1146/annurev-ecolsys-110617-062535

Roca G, Alcoverro T, Krause-Jensen D, et al (2016) Response of seagrass indicators to shifts in environmental stressors: A global review and management synthesis. Ecol Indic 63:310–323. https://doi.org/10.1016/j.ecolind.2015.12.007

Ruesink JL, Stachowicz JJ, Reynolds PL, et al (2018) Form-function relationships in a marine foundation species depend on scale: a shoot to global perspective

from a distributed ecological experiment. Oikos 127:364–374. https://doi.org/10.1111/oik.04270

Sakai AK, Allendorf FW, Holt JS, et al (2001) The Population Biology of Invasive Species. Annu Rev Ecol Syst 32:305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037

Sax DF, Stachowicz JJ, Brown JH, et al (2007) Ecological and evolutionary insights from species invasions. Trends Ecol Evol 22:465–471. https://doi.org/10.1016/j.tree.2007.06.009

Sax DF, Stachowicz JJ, Gaines SD (eds) (2005) Species Invasions: Insights into Ecology, Evolution, and Biogeography. Sinauer, Sunderland, MA

Scott ZR, terHorst CP (2020) The effect of an invasive foundation species on diversity is due to increased habitat availability. J Exp Mar Bio Ecol 528:151384. https://doi.org/10.1016/j.jembe.2020.151384

Shafer DJ, Kaldy JE, Gaeckle JL (2014) Science and management of the introduced seagrass *Zostera japonica* in North America. Environ Manage 53:147–162. https://doi.org/10.1007/s00267-013-0172-z

Shafer DJ, Wyllie-Echeverria S, Sherman TD (2008) The potential role of climate in the distribution and zonation of the introduced seagrass *Zostera japonica* in North America. Aquat Bot 89:297–302. https://doi.org/10.1016/j.aquabot.2008.03.005

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

Short FT, Neckles HA (1999) The effects of global climate change on seagrasses. Aquat Bot 63:169–196. https://doi.org/10.1016/S0304-3770(98)00117-X

Sotka EE, Baumgardner AW, Bippus PM, et al (2018) Combining niche shift and population genetic analyses predicts rapid phenotypic evolution during invasion. Evol Appl 11:781–793. https://doi.org/10.1111/eva.12592

Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: Disentangling the influence of resource competition, facilitation, and extrinsic factors. Mar Ecol Prog Ser 311:251–262. https://doi.org/10.3354/meps311251

Svensson CJ, Baden S, Moksnes PO, Åberg P (2012) Temporal mismatches in predator-herbivore abundance control algal blooms in nutrient-enriched seagrass ecosystems. Mar Ecol Prog Ser 471:61–71. https://doi.org/10.3354/meps10014

Sydeman WJ, Poloczanska E, Reed TE, Thompson SA (2015) Climate change and marine vertebrates. Science 350:772–777. https://doi.org/10.1126/science.aac9874

Tanaka N (2012) Itan no Shokubutsu “Mizukusa” wo Kagaku suru (Scientific evaluation of outlier in plants “Aquatic Plants”). Beret, Tokyo, Japan

Thom R, Miller B, Kennedy M (1995) Temporal patterns of grazers and vegetation in a temperate seagrass system. Aquat Bot 50:201–205. https://doi.org/10.1016/0304-3770(95)00449-A

Unsworth RKF, Butterworth EG (2021) Seagrass meadows provide a significant resource in support of avifauna. Diversity 13:363. https://doi.org/10.3390/d13080363

Unsworth RKF, McKenzie LJ, Collier CJ, et al (2019) Global challenges for seagrass conservation. Ambio 48:801–815. https://doi.org/10.1007/s13280-018-1115-y

Valdez SR, Shaver EC, Keller DA, et al (2021) A survey of benthic invertebrate communities in native and non-native seagrass beds in St. John, USVI. Aquat Bot 175:103448. https://doi.org/10.1016/j.aquabot.2021.103448

Valentine JF, Duffy JE (2006) The Central Role of Grazing in Seagrass Ecology. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, THe Netherlands

van Kleunen M, Dawson W, Schlaepfer D, et al (2010) Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol Lett 13:947–958. https://doi.org/10.1111/j.1461-0248.2010.01503.x

Vieira VMNCS, Lopes IE, Creed JC (2018) The biomass – density relationship in seagrasses and its use as an ecological indicator. BMC Ecol 1–16. https://doi.org/10.1186/s12898-018-0200-1

Waycott M, Procaccini G, Les DH, Reusch TBH (2006) Seagrass Evolution, Ecology and Conservation: A Genetic Perspective. In: Larkum AWD, Orth RJ, Duarte CM (eds) Seagrasses: Biology, Ecology and Conservation. Springer, Dordrecht, The Netherlands, pp 25–50

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

Williams SL (2007) Introduced species in seagrass ecosystems: Status and concerns. J Exp Mar Bio Ecol 350:89–110. https://doi.org/10.1016/j.jembe.2007.05.032

Winters G, Beer S, Willette DA, et al (2020) The tropical seagrass *Halophila stipulacea*: Reviewing what we know from its native and invasive habitats, alongside identifying knowledge gaps. Front Mar Sci 7:300. https://doi.org/10.3389/fmars.2020.00300

Wonham MJ, O'Connor M, Harley CDG (2005) Positive effects of a dominant invader on introduced and native mudflat species. Mar Ecol Prog Ser 289:109–116. https://doi.org/10.3354/meps289109

Zhang J, Wang K, Yi Q, et al (2021) Growth of *Zostera japonica* in different sediment habitats of the Yellow River estuary in China. Environ Sci Pollut Res 28:31151–31162. https://doi.org/10.1007/s11356-021-12925-7

Zhang X, Zhou Y, Liu P, et al (2015) Temporal pattern in biometrics and nutrient stoichiometry of the intertidal seagrass *Zostera japonica* and its adaptation to air exposure in a temperate marine lagoon (China): Implications for restoration and management. Mar Pollut Bull 94:103–113. https://doi.org/10.1016/j.marpolbul.2015.03.004

Zhang X, Zhou Y, Xue DX, Liu JX (2016) Genetic divergence of the endangered seagrass *Zostera japonica* Ascherson & Graebner between temperate and subtropical coasts of China based on partial sequences of matK and ITS. Biochem Syst Ecol 68:51–57. https://doi.org/10.1016/j.bse.2016.06.008

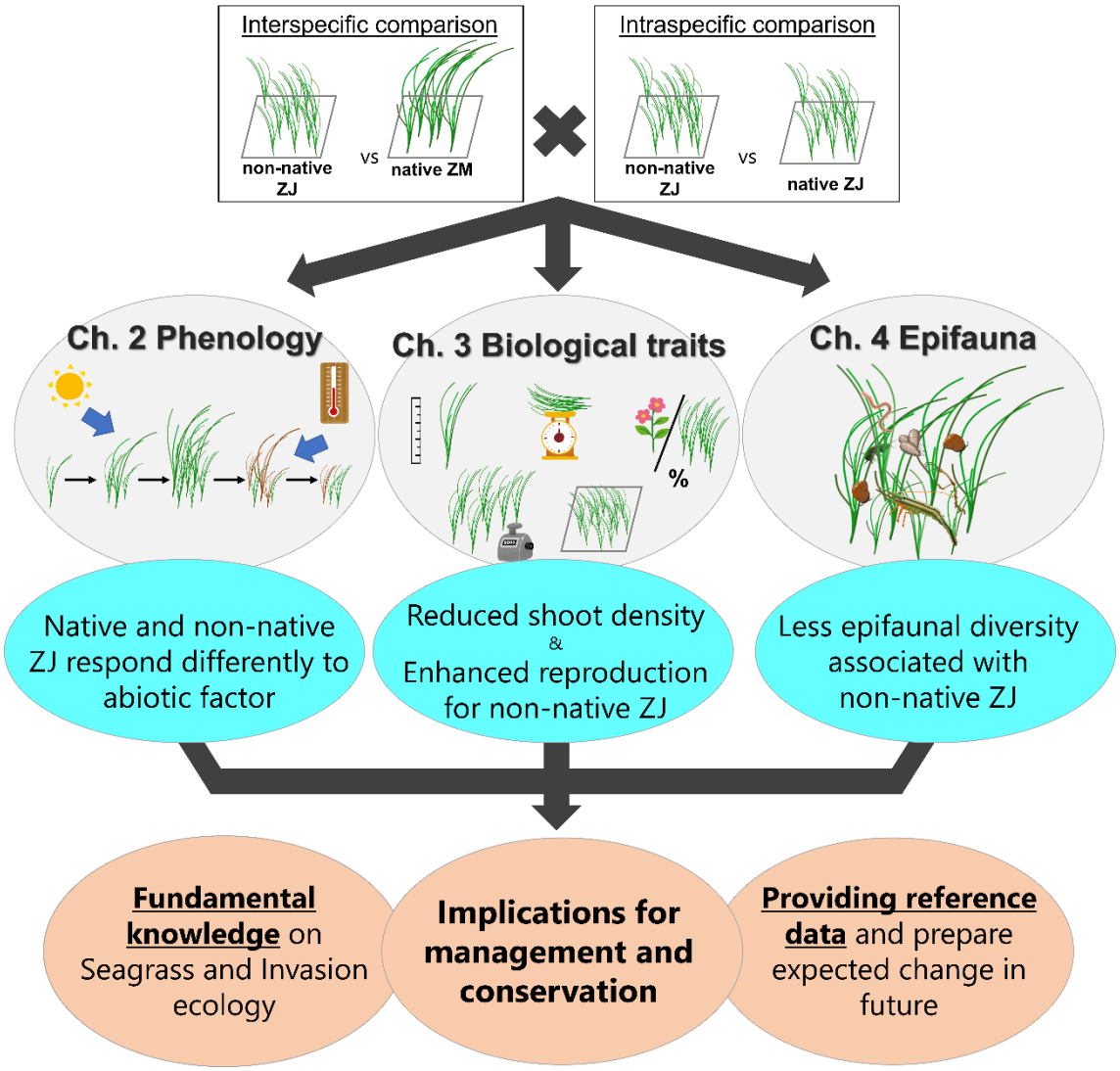

Figure 5.1. Schematic graphics summarizing the proposed approach, focused seagrass aspects and their major findings, and implications provided from this thesis.