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Ecological and Evolutionary Dynamics of Zostera japonica and Spartina alterniflora Invasions in the Eastern Pacific

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Ecological and Evolutionary Dynamics of *Zostera japonica* and *Spartina alterniflora* Invasions in the Eastern Pacific

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Ecological and Evolutionary Dynamics of *Zostera japonica* and *Spartina alterniflora* Invasions in the Eastern Pacific

Abstract

Dwarf eelgrass (*Zostera japonica*) and smooth cordgrass (*Spartina alterniflora*) are ecologically important invaders of intertidal mudflats in the eastern Pacific. *S. alterniflora* and *Z. japonica* invasions alter estuarine nutrient dynamics, cause sediment and infaunal community changes, and modify waterfowl foraging habitats. Ecological and evolutionary mechanisms of the invasion success of *Z. japonica* and *S. alterniflora* were addressed with a combination of experimental and observational field methods and population genetic approaches. The following specific objectives were addressed. 1) Genetic correlates of *S. alterniflora* invasion success were assessed by comparing the multilocus microsatellite genotypes of individuals from multiple populations in the invasive (eastern Pacific) and native (western Atlantic and Gulf of Mexico) ranges. 2) Dispersal and colonization patterns of *S. alterniflora* in Willapa Bay and Grays Harbor (Washington, USA) were assessed using multilocus microsatellite genotype data. 3) The roles of competition and disturbance in the invasion success of *Z. japonica* and the concomitant decline of its native congener, *Z. marina*, in the Pacific Northwest (USA) were assessed using experimental and observational field data. 4) The interaction of landscape variation in hydrodynamic stress and competition with *Z. japonica* in the seedling recruitment of invasive *S. alterniflora* was assessed using experimental and observational data. Invasive *S. alterniflora* populations contained high frequencies of novel genotypes and were comparable in their genetic diversity to populations in the native range. The genetic structure of invasive *S. alterniflora* in Willapa Bay was consistent with colonization by multiple founding foci and local dispersal. Native *Z. marina* and invasive *Z. japonica* both experienced substantial reductions in aboveground biomass in response to interspecific competition, relative to intraspecific competition. However, when both species were subjected to disturbance, *Z. japonica* productivity and fitness improved dramatically, while *Z. marina* performance correspondingly declined. Finally, *Z. japonica* had a consistently negative influence on *S. alterniflora* seedling recruitment, but the relative strength and importance of this interaction varied spatially with hydrodynamic stress. These results demonstrate that both ecological and evolutionary genetic processes can play important roles in the success of invasive species.

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INTRODUCTION

Invasive exotic species rank among the most significant threats to biodiversity and the maintenance of ecosystem services (Vitousek et al. 1997). Although the ecological, economic, and social costs of biological invasions have been well-described (e.g., Wilcove et al. 1998, Pimentel et al. 2000, Sala et al. 2000), a general understanding of the primary determinants of invasion success is only beginning to emerge. Historically, invasion studies have focused on the population biology of invasive species. Invasion success was typically attributed to persistence through early Allee effects (Lewis and Kareiva 1993, Sakai et al. 2001, Davis H. G. et al. 2004a, Davis H. G. et al. 2004b) and subsequent rapid population expansion driven by various mechanisms, including release from natural enemies, competitive superiority relative to native species, and the availability of unexploited ecological niches (Lodge 1993, Lonsdale 1999, Tilman 1999, Shea and Chesson 2002).

A more complex portrait of invasion dynamics, which incorporates the spatiotemporal dynamism of both invading populations and their invaded environments, is emerging from the traditional population biological paradigm. It is becoming clear that spatial environmental heterogeneity can wield a major influence on invasive spread, although theoretical and empirical understanding of this influence is still limited (With 2002, Hastings et al. 2005). With respect to species interactions, theoretical models predict that invasion speed should be a decreasing function of the competition strength between resident and invading species (Okubo *et al.* 1989). There is evidence, however, that the strength of competition between residents and invaders depends on spatially and temporally varying levels of resource supply (Tilman 1988, Davis M. A. et al. 2000, Shurin et al. 2004). The predation strength faced by invaders has also been shown to depend on spatially varying habitat characteristics (Fagan and Bishop 2000, Byers 2002). In addition, there is strong evidence that the relative strength of positive and negative species interactions can vary spatially in accordance with physical environmental characteristics (Callaway and Walker 1997, Bruno et al. 2003).

A compelling body of evidence is also accumulating for the importance of evolutionary processes, and the interaction of ecological and evolutionary processes, in invasions. Recent empirical studies of invasive populations have documented rapid responses to selection (Maron et al. 2004, Yeh 2004) and have provided mixed support for the evolution of increased competitive ability (Blossey and Notzold 1995) resulting from the reallocation of resources previously applied to herbivore defense toward growth and reproduction in enemy-free space (Willis et al. 2000, Siemann and Rogers 2003, van Kleunen and Schmid 2003, Vila et al. 2003, Rogers and Siemann 2004). In addition, there is strong evidence that hybridization between species can increase the invasiveness of taxa by generating novel genotypes, masking deleterious alleles, generating increased genetic variation, and fixing heterotic genotypes (e.g., Rieseberg 1991, Ellstrand and Schierenbeck 2000, Gaskin and Schaal 2002).

This dissertation addressed ecological and evolutionary mechanisms of the invasion success of smooth cordgrass (*Spartina alterniflora*) and dwarf eelgrass (*Zostera japonica*) in the Pacific Northwest (USA). *S. alterniflora* and *Z. japonica* are ecologically important invaders of intertidal mudflats in eastern Pacific estuaries. Their invasions alter estuarine nutrient dynamics, cause sediment and infaunal community changes, and modify waterfowl foraging habitats (Posey 1988, Baldwin and Lovvorn 1994, Daehler and Strong 1996b, Hahn 2003, Larned 2003, Ayres et al. 2004). The following specific objectives were addressed in the four chapters of this dissertation. 1) Genetic correlates of *S. alterniflora* invasion success were assessed by comparing the multilocus microsatellite genotypes of individuals from multiple populations in the invaded (eastern Pacific) and native (western Atlantic and Gulf of Mexico) ranges. 2. Dispersal and colonization patterns of *S. alterniflora* in Willapa Bay and Grays Harbor (Washington, USA) were assessed using multilocus genotype data. 3) The roles of competition and disturbance in the invasion success of *Z. japonica* and the concomitant decline of its native congener, *Z. marina*, in the Pacific Northwest (USA) were assessed using experimental and observational field data. 4) The interaction of landscape variation in hydrodynamic stress and competition with *Z. japonica* in the seedling recruitment of invasive *S. alterniflora* was assessed using experimental and observational data.

Understanding the ecological and evolutionary dynamics of *S. alterniflora* and *Z. japonica* establishment and spread may inform management responses to invasion. Invasive *Spartina* species are globally distributed and are associated with significant ecological and economic impacts (Daehler and Strong 1996). *S. alterniflora* is classified as a noxious weed in Washington State and is the target of aggressive eradication programs in Washington and Oregon (USA). In San Francisco Bay (California, USA) efforts are focused on controlling the hybrid offspring of *S. alterniflora* and its native congener Pacific cordgrass (*S. foliosa*). In addition to the Pacific coast of North America, *S. alterniflora* has also been introduced to western Europe (Baumel et al. 2003, Ainouche et al. 2004), China (Chung *et al.* 2004), India (Shah and Badrinath 1985), Australia (McEnnulty et al. 2000), and New Zealand (Hicks and Silvester 1990). *Z. japonica* has invaded several estuaries between the Coquille River Estuary (Washington, USA) and the southern Strait of Georgia (British Columbia, Canada; Larned 2003). It is gaining increased recognition as an invasive species and will shortly be added to the Global Invasive Species Database, which catalogs ecologically and economically important invasive species (M. Brown and S. Pagad, personal communication). Studies that elucidate the ecological and evolutionary underpinnings of invasion success or failure may inform the control of specific invasive populations, and provide general insights into population and community ecology and the dynamics of biological invasions.

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Chapter 1. Intraspecific hybridization and the success of an invasive species

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1.1. ABSTRACT: Interspecific hybridization is an important stimulus for the rapid evolution of invasive species, and recent theory suggests that intraspecific hybridization between differentiated source populations may similarly facilitate the evolution of invasiveness. To evaluate the relationship between intraspecific hybridization and invasion success, extent of hybridization was quantified in three invasive cordgrass populations that vastly differed in their rates of spread. Invasive smooth cordgrass (*Spartina alterniflora*) has spread widely in Willapa Bay, Washington, but is rare in Grays Harbor, Washington and San Francisco Bay, California. 472 individuals from the three invasive, Pacific coast populations and ten populations in the native Atlantic and Gulf coast range were genotyped with nine polymorphic microsatellite markers. Intraspecific hybridization was quantified by 1) characterizing population structure in the native range to identify differentiated native population groups, 2) identifying unique microsatellite markers for each native population group, and 3) quantifying the percentage of genotypes in each invasive population that contained unique markers from more than one native population group. In addition, genetic diversity was quantified in the native and invasive populations. The degree of hybridization in the invasive populations correlated with their trends in population expansion, while no correlation emerged between genetic diversity and spread. These findings provide the first direct evidence for the role of intraspecific hybridization in invasions.

1.2. INTRODUCTION

Invasive exotic species rank among the most significant threats to biodiversity and the maintenance of ecosystem services (Vitousek et al. 1997). Although the ecological, economic, and social costs of biological invasions have been well-described (e.g., Wilcove et al. 1998, Pimentel et al. 2000, Sala et al. 2000), a general understanding of the determinants of invasion success is just emerging. Historically, studies of the population biology of invasive species have focused on ecological aspects of invasion dynamics. Invasion success has been attributed to persistence through early Allee effects (Lewis M. A. and Kareiva 1993, Sakai et al. 2001, Davis et al. 2004a, Davis et al. 2004b) and subsequent rapid population expansion driven by various mechanisms, including release from natural enemies, competitive superiority relative to native species, and the availability of unexploited ecological niches (Lodge 1993, Lonsdale 1999, Tilman 1999, Shea and Chesson 2002).

A compelling body of evidence is accumulating for the importance of evolutionary processes in plant invasions. Recent studies of invasive populations have documented rapid responses to selection (Maron et al. 2004, Yeh 2004) and have provided mixed support for the evolution of increased competitive ability (Blossey and Notzold 1995) resulting from the reallocation of resources previously applied to herbivore defense toward growth and reproduction in enemy-free space (Willis et al. 2000, Siemann and Rogers 2003, van Kleunen and Schmid 2003, Vila et al. 2003, Rogers and Siemann 2004). In addition, there is strong empirical evidence that hybridization between species can increase the invasiveness of taxa by generating novel genotypes, masking deleterious alleles, generating increased genetic variation, and fixing heterotic genotypes (e.g., Rieseberg 1991, reviewed in Ellstrand and Schierenbeck 2000, Gaskin and Schaal 2002). Although recent reviews have argued that intraspecific hybridization between differentiated source populations can similarly facilitate the evolution of invasiveness, empirical evidence for this mechanism is lacking (Ellstrand and Schierenbeck 2000, Vila et al. 2000, Lee 2002, Lambrinos 2004).

This study assessed the roles of genetic diversity and intraspecific hybridization in the extent of Pacific coast invasions of smooth cordgrass (*Spartina alterniflora*). Native to the Atlantic and Gulf of Mexico coasts of the Americas, *S. alterniflora* is an ecologically damaging invader of coastal bays and estuaries (Daehler and Strong 1996, Ayres et al. 2004). Invasive Pacific coast populations (Willapa Bay and Grays Harbor, Washington and San Francisco Bay, California) have recently established with different histories of introduction and spread. The rapidly expanding Willapa Bay population is 60-100 years old and occupies 7300 ha of intertidal mudflats. The slowly spreading Grays Harbor population is ca. 25 years old and occupies 1.1 ha (Murphy 2004). The San Francisco Bay population is approximately 30 years old and has also spread very little. In San Francisco Bay, *S. alterniflora* has hybridized with its native congener, Pacific cordgrass (*S. foliosa*). Although *S. alterniflora* × *foliosa* hybrids have spread rapidly and are abundant, pure *S. alterniflora* is rare in San Francisco Bay and can be distinguished from *S. alterniflora* × *foliosa* hybrids by randomly amplified polymorphic DNA (RAPD) markers (Ayres et al. 1999, Daehler et al. 1999, Ayres et al. 2004). No interspecific hybridization has occurred in Willapa Bay or Grays Harbor, where *S. foliosa* is absent. Polymorphic microsatellite markers were used to identify native geographic sources of invasive populations and to examine the correlation of genetic diversity and intraspecific hybridization with the extent of invasions.

1.3. MATERIALS AND METHODS

1.3.1. S. alterniflora ecology and invasion history

First reported in Willapa Bay, WA in the 1940s, *S. alterniflora* may have been accidentally introduced ca. 1894 in oyster shipments from New Jersey, New York, and Chesapeake Bay (Sayce 1988). It was first reported in Grays Harbor, WA in the 1980s (L. Holcomb, personal communication). *S. alterniflora* was deliberately introduced to San Francisco Bay, CA in the 1970s, when the US Army Corps of Engineers planted seed purchased from an environmental consulting firm in Maryland (Faber 2000). According to communication with the firm's nursery manager, the firm obtained *S. alterniflora* seed stock from marshes in Maine and Virginia during the 1970s (L. Hunter-Cario, personal communication). Because of its propensity to be inadvertently spread through use as packing material and ballast, additional introductions of *S. alterniflora* to Willapa and San Francisco Bays and Grays Harbor are not unlikely. *S. alterniflora*'s use as a shipping material has been implicated in its introduction to southern England and western France (Ainouche et al. 2004) and in the introduction of its congener *S. densiflora* to Humboldt Bay, CA (Kittelson and Boyd 1997).

S. alterniflora is a hexaploid obligately-outcrossing, wind-pollinated grass of coastal mudflats (Davis et al. 2004a). Considered an ecological foundation species in its native range, the rhizomatous perennial is classified as a noxious weed on the North American Pacific coast (Washington 2002, Oregon Administrative Rules 2003, Oregon Department of Agriculture 2003). Invasions displace fish, shellfish, and shorebird habitats; increase sediment accretion; reduce tidal flows; and alter algal primary productivity and benthic community structure (Daehler and Strong 1996). Although the impacts of *S. alterniflora* invasions are well-studied (e.g., Sayce 1988, Daehler and Strong 1996, Ayres et al. 1999, Ayres et al. 2004, Davis et al. 2004b, Civille et al.), the genetic aspects of invasions are poorly understood.

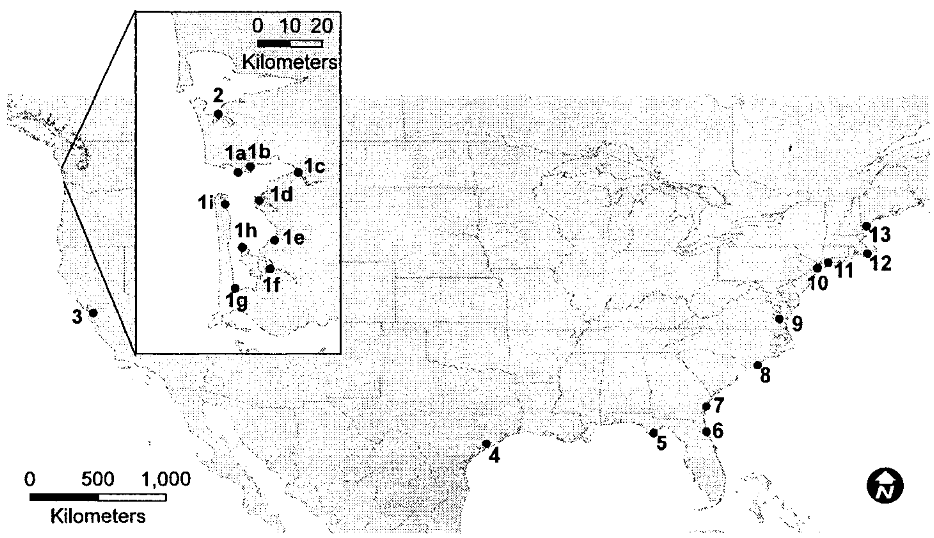
Information on the genetic variation of introduced *S. alterniflora* is limited to a single study that failed to detect RAPD marker variation in the oldest and largest invasive North American population, located in Willapa Bay (Stiller and Denton 1995). Although numerous studies have described the invasion dynamics of hybrid *S. alterniflora* × *foliosa* in San Francisco Bay, the genetic diversity of the invasive parental population has not been assessed (Ayres et al. 1999, Daehler 1999, Anttila et al. 2000). The genetic structure and diversity of *S. alterniflora* in its native range has also been assessed with RAPD markers. O'Brien and Freshwater (1999) concluded that genetic variation was continuously distributed on the Atlantic and Gulf coasts and identified three geographically-correlated genetic groups (New England/New Jersey, North Carolina/South Atlantic, and Gulf coast).

1.3.2. Sample collection

Leaf material was collected from 535 individuals in salt marshes on the US Pacific, Atlantic, and Gulf of Mexico coasts (Figure 1.1). Because of *S. alterniflora's* clonal nature, leaf material was collected from plants separated by at least 10 m to avoid resampling genetic individuals (genets). Despite efforts to sample distinct individuals, duplicate multilocus genotypes were detected within populations, indicating that some genets had been sampled more than once. Duplicate genotypes were excluded from genetic data analyses to avoid biasing estimates of population differentiation, resulting in a net total of 472 individual multilocus genotypes. Sampling locations and sample sizes, following duplicate genotype exclusion, are listed in Figure 1.1. In the invasive range, samples were collected from Willapa Bay, Grays Harbor, and San Francisco Bay. Because San Francisco Bay marshes are primarily comprised of native *S. foliosa* and hybrid *S. alterniflora* × *foliosa*, the taxonomic (i.e., non-hybrid *S. alterniflora*) identity of sampled individuals was verified with species-specific RAPD markers using previously developed methodology (Ayres et al. 1999, Daehler et al. 1999, Anttila et al. 2000). In the native range, samples were collected from Wells National Estuarine Research Reserve (NERR), Maine; Long Island, New York; Raritan Bay, New Jersey; Waquoit Bay NERR, Massachusetts; Chesapeake Bay NERR, Virginia; North Carolina NERR, North Carolina; Sapelo Island NERR, Georgia; Guana Tolomato Matanzas NERR, Florida (Atlantic coast); Apalachicola NERR, Florida (Gulf coast); and Matagorda, Texas.

Figure 1.1. Map of study locations. Sample sizes are listed in parentheses after each population name.

1) Willapa Bay (*1a = Toke Point, 1b = Cedar River, 1c = Willapa River, 1d = Palix River, 1e = Nemah River, 1f = Naselle River, 1g = Tarlatt Slough, 1h = Diamond Point, 1i = Stackpole Slough;* 200); 2) Grays Harbor (22); 3) San Francisco Bay (24); 4) Texas (29); 5) Florida Gulf (16); 6) Florida Atlantic (31); 7) Georgia (21); 8) North Carolina (15); 9) Virginia (14); 10) New Jersey (21); 11) New York (31); 12) Massachusetts (22); 13) Maine (28).



1.3.3. DNA extraction and microsatellite genotyping

Genomic DNA was extracted from fresh *S. alterniflora* leaf tissue using the Dneasy® Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). Individuals were genotyped using nine previously developed disomic, polymorphic microsatellite markers (GenBank accession numbers Spar.16, Spar.15, Spar.17, Spar.13, Spar.20, Spar.07, Spar.18, Spar.14, Spar.19), using PCR protocols described in Blum et al. (2004) and Sloop et al. (Sloop et al. In press). Forward primers were 5' fluorescently labeled with one of the following dyes: 6-FAM™ (6-Carbofluorecein), HEX™ (Hexachlorofluorescein), NED™ (Applied Biosystems (ABI) proprietary yellow), or PET™ (ABI proprietary red). A 3730xl 96-capillary DNA analyzer and Genemapper 3.0 software (ABI, Foster City, CA, USA) were used to size the PCR products.

1.3.4. Genetic data analyses

Although *S. alterniflora* is hexaploid, only disomic markers were utilized for this study and genotype data were subsequently analyzed as diploid data. Loci were assumed to be disomic because 1) only one or two alleles amplified per locus in any individual among all 472 individuals sampled, despite the presence of up to 12 alleles at each locus within each population; and 2) peak heights in genotyping traces were nearly always symmetrical at heterozygous loci, suggesting equal allelic dosages. To assess genetic diversity, expected (H_E) and observed (H_O) heterozygosity were calculated with GENETIC DATA ANALYSIS software (GDA, Lewis and Zaykin 2001). Because sample sizes differed between populations, Hurlbert's rarefaction index (Hurlbert 1971) was also applied in Fstat V. 2.9.3.2 to calculate allelic richness (Goudet 1995, El Mousadik and Petit 1996, Petit et al. 1998), an allelic diversity metric that corrects for biases incurred by sample size differences. Exact tests for departures from Hardy-Weinberg and linkage equilibrium for each population were calculated with GDA (Lewis and Zaykin 2001). When appropriate, Bonferroni-adjusted probability values for multiple comparisons were calculated to assess statistical significance.

Because different methods of characterizing genetic structure can produce varying results, genetic structure was assessed with a combination of methods. Both traditional, fixation index-based methods and alternative approaches based on factorial correspondence analysis (FCA) and the neighbor-joining method (Saitou and Nei 1987) were employed. For the fixation-index approach, Weir and Cockerham's (1984) estimator θ was used to calculate pairwise values of Wright's F_{ST} in Fstat V. 2.9.3.2 (Goudet 1995). Significance values for pairwise population comparisons were calculated with probability tests in Fstat. Traditional F_{ST}-based methods provide a quantitative and easily interpretable estimate of the strength of population differentiation. However, they measure differentiation between populations that are defined *a priori*, in a manner that may not accurately reflect population substructure.

In contrast, the two clustering approaches utilized to describe population structure were unbiased by *a priori* sample origin. For the first clustering approach, FCA was conducted in Genetix 4.3 (Belkhir 2004) by transforming data into a contingency table of allele frequencies for each sample. The χ^2 distance centered on the marginal distribution of the contingency table was used to measure the similarity of each pair of samples in k-dimensional space (k = number of alleles). Populations were ordinated along the factorial axes with the largest eigenvalues to evaluate their spatial relationships. For the second clustering approach, Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) were calculated between pairs of sampling locations and used to generate an unweighted neighbor-joining phylogram by implementing the SEQBOOT, GENEDIST, NEIGHBOR, and CONSENSE computer programs in PHYLIP (Phylogeny Inference Package) V 3.6 (Felsenstein 1989, 2004). Bootstrapping values were computed using 1000 randomized data sets.

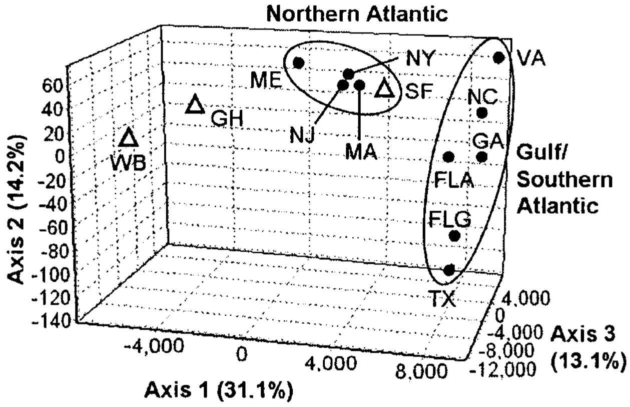
Once native population groups had been defined, unique, "regional marker" alleles for each population group were identified. Regional markers occurred within a single native population group and in at least one invasive population. Only allele frequencies > 0.05 were evaluated, to avoid potential biases incurred by the failure to detect rare alleles. Genotypes in invasive populations that contained regional markers from more than one native population group were identified as intraspecific-hybrid genotypes. The percentage of intraspecific-hybrid genotypes was quantified in each invasive population. Thus, regional markers provided an important inference from these data and methods regarding intraspecific hybridization.

1.4. RESULTS

1.4.1. Native population structure

Both the FCA and neighbor-joining phylogram indicated that the native sampling locations formed two distinct population groups. The FCA revealed that the Northern Atlantic populations between New Jersey and Maine comprised a single group, and the sampling locations between Texas and Virginia formed an additional Gulf/Southern Atlantic group. The Northern Atlantic populations were distinct from the Gulf/Southern Atlantic populations (Figure 1.2). The invasive San Francisco Bay population grouped with native sampling locations in the Northern Atlantic group, indicating its similarity to the populations in this group, while the Willapa Bay and Grays Harbor populations were not similar to either native population group. The first three axes in the FCA explained 58.3% of the total genetic variance.

Figure 1.2. Genetic differentiation among native and invasive smooth cordgrass populations based on factorial correspondence analysis (FCA) of allele frequencies at nine microsatellite loci. Circles represent native populations and triangles represent invasive populations. Population abbreviations: TX = Texas, FLG = Florida Gulf coast, FLA = Florida Atlantic coast, GA = Georgia; NC = North Carolina, VA = Virginia, NJ = New Jersey, NY = New York, MA= Massachusetts, ME = Maine, WB = Willapa Bay, GH = Grays Harbor, SF = San Francisco Bay.



The neighbor-joining phylogram also supported the divergence of Northern Atlantic and Gulf/Southern Atlantic populations (Figure 1.3). The strongly supported grouping of the sampling locations between New Jersey and Maine (91% of 1000 bootstrap samples) and the low bootstrap values for the other branches in the phylogram corroborated the genetic structure revealed by FCA.

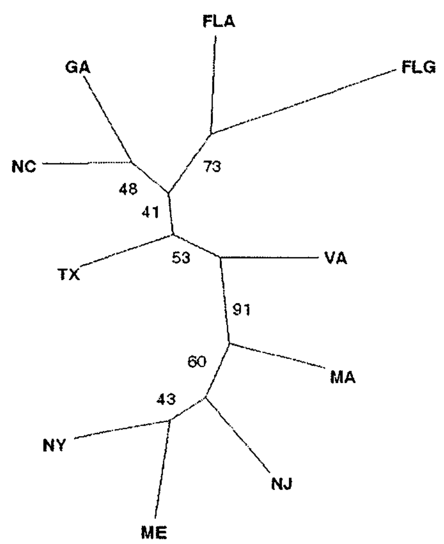


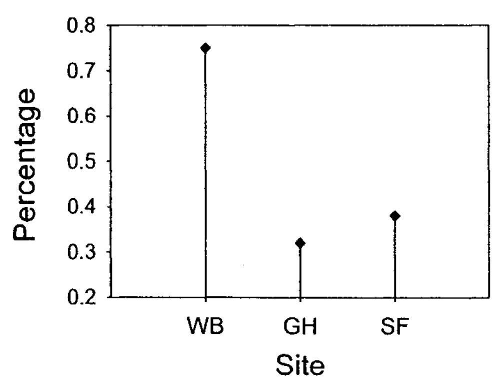
Figure 1.3. Unrooted neighbor-joining phylogram of native *S. alterniflora* populations based on Cavalli-Sforza (1967) chord distances calculated from allele frequencies. Numbers at nodes represent the percentage of 1000 bootstrap simulations that supported the associated grouping. See Figure 1.2 for an explanation of population abbreviations.

Pairwise F_{ST} comparisons of the native and invasive populations indicated that most populations within the Gulf/Southern Atlantic group were not significantly differentiated. In contrast, most pairs of populations within the Northern Atlantic group were significantly differentiated by F_{ST} (F_{ST} = 0.120-0.211). The Willapa Bay population was strongly and significantly differentiated from all other populations but Grays Harbor, from which it was significantly but weakly differentiated (F_{ST} = 0.087). The Grays Harbor population was strongly differentiated from all native populations except the Gulf coast of Florida and Virginia (F_{ST} = 0.256-0.484), while the San Francisco Bay population was differentiated from all native populations except the Gulf coast of Florida, Georgia, Virginia, and New Jersey (F_{ST} = 0.070-0.328). The Willapa Bay and Grays Harbor populations were also were strongly and significantly differentiated from the San Francisco Bay population (F_{ST} = 0.328 and 0.296, respectively). Table 1.1 contains a complete list of pairwise F_{ST} values.

1.4.2. Intraspecific hybridization

The frequencies of intraspecific-hybrid genotypes in the three invasive populations correlated with their trends in expansion, suggesting that intraspecific hybridization may influence their rates of spread. Based on the population groupings determined by FCA and the neighbor-joining phylogram, nine regional marker alleles were identified. Six markers were identified for the Gulf/Southern Atlantic group, and three markers were identified for the Northern Atlantic group (Table 1.2). Intraspecific hybrids were indicated by the presence of markers from both the Northern Atlantic and Gulf/Southern Atlantic population groups within the same multilocus genotype. The frequency of intraspecific-hybrid genotypes correlated with the differential spread of the three invasive populations: 75% of the rapidly spreading Willapa Bay population was comprised of hybrid genotypes, in comparison to 32% and 38% of the small and slowly spreading Grays Harbor and San Francisco populations (Figure 1.4).

Figure 1.4. Percentage of intraspecific-hybrid genotypes per invasive smooth cordgrass population. WB = Willapa Bay, GH = Grays Harbor, and SF = San Francisco Bay.



Because only allele frequencies > 0.05 were evaluated to identify regional markers, four of the regional markers appeared in very low (< 0.05) frequencies in one or two populations outside their diagnostic population group. To examine whether these markers significantly influenced estimates of intraspecific hybridization, these four markers were excluded and only the remaining "robust" markers were applied to characterize hybrids. The "robust" markers produced slightly reduced estimates of intraspecific hybridization (WB = 64%, SG = 23%, SF = 33%), but the overall trend persisted.

Although the sample size in Willapa Bay was nearly an order of magnitude larger than the sample sizes in the native populations, all alleles detected in the three invasive populations were also detected in the native range. The lack of alleles unique to the invasive populations (i.e., not detected in native populations) suggested that the native population sample sizes were sufficiently large to approximate the allele frequencies of the native populations. Table 1.3 contains a complete list of allele frequencies for all populations.

1.4.3. Genetic diversity

Genetic diversity did not correlate with the rates of spread of invasive populations. Among the invasive populations, the highest mean values of allelic richness and expected and observed heterozygosity were measured in the smallest invasive population, San Francisco Bay (Table 1.4). In the invasive populations, sample-size corrected mean allelic richness ranged from 2.14 in Willapa Bay to 3.24 in San Francisco Bay (Table 1.4). Average expected heterozygosity values ranged from 0.30 (Grays Harbor) to 0.60

(San Francisco Bay), and average observed heterozygosity values ranged from 0.11 (Grays Harbor) to 0.44 (San Francisco Bay). In the native range, allelic richness ranged from 1.77 (Georgia) to 3.62 (Maine), average expected heterozygosity ranged from 0.32 (Florida Gulf) to 0.61 (Long Island), and average observed heterozygosity ranged from 0.33 (Maine) to 0.59 (Georgia). No loci consistently deviated from HW equilibrium across populations, using a Bonferroni-corrected nominal significance value of $P = 0.0064$. However, eight of nine loci deviated from Hardy-Weinberg equilibrium in Willapa Bay. Table 1.5 contains a comprehensive summary of genetic variation by locus and population, including results for tests for Hardy-Weinberg equilibrium. All pairs of loci were in linkage equilibrium in the majority of populations, using a Bonferroni-corrected nominal significance value of $P = 0.0014$. A complete summary of linkage disequilibrium tests results is presented in Table 1.6.

1.5. DISCUSSION

The results of this study provide the first direct evidence of a correlation between intraspecific hybridization and the spread of an invasive species. Previous studies have detected genotypes from multiple source populations within invasive populations (Saltonstall 2002, Kolbe et al. 2004), but this is the first to empirically examine the relationship between extent of intraspecific hybridization and rates of spread. Strikingly, 75% of the rapidly spreading Willapa Bay population was comprised of intraspecific hybrid genotypes, in comparison to <40% of the slowly spreading San Francisco Bay and Grays Harbor populations. The lower extent of intraspecific hybridization in San Francisco Bay and Grays Harbor may reflect a shortage of opportunities for sexual reproduction. In San Francisco Bay, *S. alterniflora* rarely flowers (D. Ayres, personal communication) and is swamped by pollen from *S. alterniflora* × *foliosa* hybrids. As a consequence, very little pure *S. alterniflora* seed is produced each generation, providing few opportunities for the assembly of novel *S. alterniflora* genotypes through recombination. The Grays Harbor population is comprised of patchy, isolated clones, whose seed production is likely constrained by pollen limitation (Davis et al. 2004b). Thus, the majority of population growth is likely to occur through clonal growth, rather than recruitment by seed. Although interactions with *S. foliosa* and *S. alterniflora* × *foliosa* hybrids in San Francisco Bay, various control measures in the three invaded estuaries, and environmental variation have likely also influenced the progression of these invasions, substantial differences in the extent of intraspecific hybridization suggest a potentially important role for evolutionary processes.

No correlation between genetic diversity and the rates of spread of invasive populations emerged in this study. Among the invasive populations, values of diversity metrics were highest in San Francisco Bay, a very small and slowly spreading population. H_E and H_O were lowest in Grays Harbor, also a small and slowly spreading population. The lower genetic diversity in Grays Harbor may reflect the patchy distribution of the population; in contrast to Willapa Bay and San Francisco Bay, which contain contiguous *Spartina* stands, the Grays Harbor's population consists largely of isolated clones which may experience higher rates of inbreeding. Previous studies have provided similarly inconsistent evidence for the relationship between genetic diversity and invasion success. Despite the genetic bottlenecks typically associated with introduction, many successful invasive populations contain surprisingly high levels of genetic variation (Barrett and

Richardson 1986, Stepien et al. 2002, Johnson and Starks 2004). Yet others contain extremely low levels of variation (e.g., Hurka 2003, Xu et al. 2003). In some cases, genetic bottlenecks appear to have increased the invasiveness of introduced populations by purging deleterious alleles (Parisod et al. 2005) or by reducing the intensity of intraspecific competition (Tsutsui et al. 2000). Notably, the majority of studies that have assessed genetic variation in invasive populations have quantified selectively neutral variation, which is reported to correlate poorly with variation in quantitative traits (Reed and Frankham 2001). Thus, while quantitative genetic variation is expected to increase adaptive potential, the presumably selectively neutral microsatellite variation measured in this study may be a poor indicator of invasion success.

Intraspecific hybridization may be an unappreciated mechanism for a pattern frequently observed in invasive taxa: the correlation of multiple introductions with invasion success (Ehrlich et al. 1988, Barrett and Husband 1990). This correlation has primarily been attributed to the demographic consequences of multiple introductions. Both the size of founding populations and their frequency of introduction have been shown to correlate with the probability of establishment (Crawley 1986). Multiple introductions can increase genetic variation in invasive populations, which could attenuate inbreeding depression or influence the ability of introduced populations to evolve in response to novel selective pressures (Novak and Mack 1993, Facon et al. 2003, Kolbe et al. 2004). Intraspecific hybridization may also be a mechanism for the invasion success associated with repeated introductions. Interspecific-hybrid plant lineages frequently demonstrate greater ecological amplitudes than their parental species, invading ecological communities uninhabited by either parental species or occupying a significantly larger range of habitats (Stace 1975, Thompson 1991, Daehler and Strong 1997, Neuffer and Hurka 1999). Intraspecific-hybrid plant lineages could possess similarly increased ecological amplitudes. Intraspecific hybridization and other rapid evolutionary processes may explain why many introduced species initially exhibit lag times prior to becoming invasive (Mack 1985, Pyšek and Prach 1993, Kowarik 1995, Ewel et al. 1999), and why many species become invasive only after repeated introduction. Additionally, the results of this study suggest that in some cases, intraspecific hybridization may be a more important mechanism than genetic diversity in the invasion success associated with multiple introductions.

Intraspecific hybridization is facilitated by the anthropogenic dispersal of invasive species, a feature that suggests it may be a common factor in invasions. Modern transportation is increasing the frequency of long-distance dispersal events in natural populations. As a consequence, rates of contact of historically allopatric populations and species have been greatly accelerated. Although transportation has long been recognized as an important vector for the introduction of non-native species, its role in the evolution of invasiveness of introduced species also merits consideration. Intraspecific hybridization facilitated by anthropogenic dispersal is potentially an important mechanism for the evolution of invasiveness, and many more examples of this phenomenon are likely to emerge as additional cases of cryptic hybridization are unveiled by molecular analyses. Separating cause and effect in the intraspecific hybridization of invasive species is the next challenge for invasion biologists.

1.6. ACKNOWLEDGEMENTS

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Table 1.2. Allele frequencies for regional marker alleles in North American smooth cordgrass populations. Alleles in bold text were identified as "robust" marker alleles (see text for explanation). See Figure 1.2 for an explanation of population abbreviations. A complete list of allele frequencies is presented in the supplemental materials.

		Gulf/Southern Atlantic						*Northern Atlantic*				*Pacific*		
Locus	**Allele**	**TX**	**FLG**	**FLA**	**GA**	**NC**	**VA**	**NJ**	**NY**	**MA**	**ME**	**WB**	**GH**	**SF**
Spar.16	**374**	**0.19**	-	-	-	-	-	-	-	-	-	-	-	**0.07**
	380	0.04	-	-	-	-	-	0.24	0.21	0.15	0.52	0.41	0.29	0.17
	382	0.02	-	0.02	-	-	-	0.41	0.18	-	0.36	0.09	0.37	0.33
Spar.15	**279**	-	-	**0.05**	-	-	-	-	-	-	-	**0.44**	**0.11**	-
	283	**0.09**	-	-	-	-	-	-	-	-	-	-	-	**0.13**
Spar.20	**177**	-	-	-	-	-	-	**0.25**	**0.45**	**0.63**	**0.86**	**1**	**1**	**0.19**
Spar.07	**277**	**0.12**	-	-	-	**0.23**	-	-	-	-	-	-	**0.05**	-
	301	-	**0.22**	**0.07**	**0.26**	-	-	-	-	-	-	-	-	**0.11**
Spar.19	332	-	0.53	0.25	0.44	0.25	-	-	-	-	0.04	0.27	0.13	0.06

Table 1.3. Allele frequencies for North American smooth cordgrass populations. Alleles in bold were identified as marker alleles diagnostic of either Northern Atlantic or Southern Atlantic/Gulf regions. See Figure 1.2 for an explanation of population abbreviations.

		Gulf/Southern Atlantic						*Northern Atlantic*				*Pacific (Invasive)*			
Locus	**Allele**	**TX**	**FLG**	**FLA**	**GA**	**NC**	**VA**	**NJ**	**NY**	**MA**	**ME**	**WB**	**GH**	**SF**	**Overall**
Spar.16	362	-	-	-	0.32	-	-	-	-	-	-	0	-	-	0.01
	366	-	-	0.02	0.18	-	-	-	-	-	-	-	-	-	0.01
	368	-	-	-	-	-	-	-	0.02	-	-	-	-	-	0
	370	0.04	-	-	-	-	-	-	-	-	-	-	-	-	0
	372	0.04	-	0.47	0.5	0.6	-	0.24	0.08	0.08	-	-	-	0.07	0.1
	374	**0.19**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.1**	**0.02**
	376	0.21	1	0.45	-	0.4	0.64	0.12	0.18	0.4	-	0.04	0.11	0.11	0.17
	378	-	-	0.02	-	-	-	-	0.16	0.08	0.13	-	-	-	0.02
	380	**0.04**	**-**	**-**	**-**	**-**	**-**	**0.24**	**0.21**	**0.15**	**0.52**	**0.41**	**0.29**	**0.17**	**0.26**
	382	**0.02**	-	0.02	**-**	**-**	**-**	**0.41**	**0.18**	**-**	**0.36**	**0.09**	**0.37**	**0.33**	**0.13**
	384	0.35	-	0.03	-	-	0.36	-	0.13	0.15	-	0.46	0.24	0.26	0.26
	386	0.1	-	-	-	-	-	-	0.05	0.15	-	-	-	-	0.02
	388	0.04	-	-	-	-	-	-	-	-	-	-	-	-	0
Spar.15	261	-	-	-	0.02	-	-	-	-	-	-	-	-	-	0
	263	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0
	265	0.2	-	0.13	0.02	-	0.08	-	-	-	-	-	-	-	0.02
	267	-	-	0.07	0.14	0.07	-	-	0.15	0.15	0.04	-	-	-	0.03
	269	0.14	0.06	0.02	-	0.3	0.04	0.14	0.27	0.05	0.02	-	-	0.22	0.06
	271	0.05	-	0.48	0.02	-	-	0.81	0.32	0.43	0.34	0.55	0.89	0.26	0.43
	273	0.16	-	0.02	0.24	0.07	-	0.05	0.08	0.33	0.54	0.01	-	0.3	0.1
	275	0.09	-	0.05	0.55	0.03	0.85	-	0.16	-	0.04	-	-	0.04	0.07
	277	0.09	-	-	-	0.2	-	-	0.02	-	0.04	0	-	0.04	0.02
	279	**-**	**-**	**0.05**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.44**	**0.11**	**-**	**0.2**
	281	0.02	-	0.02	-	-	-	-	-	-	-	-	-	-	0
	283	**0.09**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.13**	**0.01**
	285	-	-	0.05	-	0.33	0.04	-	-	-	-	-	-	-	0.02
	287	0.11	-	0.02	-	-	-	-	-	0.05	-	-	-	-	0.01
	289	0.04	0.94	0.07	-	-	-	-	-	-	-	-	-	-	0.02
	291	-	-	0.03	-	-	-	-	-	-	-	-	-	-	0

		Gulf/Southern Atlantic						*Northern Atlantic*				*Pacific (Invasive)*			
Locus	**Allele**	**TX**	**FLG**	**FLA**	**GA**	**NC**	**VA**	**NJ**	**NY**	**MA**	**ME**	**WB**	**GH**	**SF**	**Overall**
Spar.17	373	0.71	1	0.98	0.71	0.6	0.71	0.07	0.17	0.33	0.02	0	-	0.17	0.2
	376	0.29	-	0.02	0.27	-	-	0.57	0.37	0.15	0.54	0.36	0.28	0.38	0.32
	379	-	-	-	-	0.4	-	-	-	0.08	-	0	-	-	0.01
	382	-	-	-	0.03	-	0.29	0.37	0.3	0.35	0.27	0.63	0.73	0.46	0.44
	385	-	-	-	-	-	-	-	0.09	-	0.07	-	-	-	0.01
	388	-	-	-	-	-	-	-	-	0.1	0.09	-	-	-	0.01
	391	-	-	-	-	-	-	-	0.07	-	0.02	-	-	-	0.01
Spar.13	286	-	-	-	-	-	-	-	0.02	-	0.05	0	-	-	0.01
	289	-	-	-	-	-	0.04	-	-	-	-	-	-	-	0
	292	-	-	0.05	-	-	-	-	0.02	-	-	0.17	-	-	0.08
	295	1	1	0.95	1	1	0.96	1	0.97	1	0.95	0.83	1	1	0.92
Spar.20	169	0.21	-	-	-	-	0.04	-	-	-	-	-	-	-	0.01
	171	0.79	0.5	0.11	0.48	0.07	-	-	0.03	0.08	-	0	-	-	0.1
	173	-	0.5	0.63	0.52	0.63	0.5	0.75	0.07	0.08	-	0	-	0.48	0.18
	175	-	-	0.19	-	-	0.46	-	0.03	0.23	-	-	-	0.19	0.05
	177	**-**	**-**	**-**	**-**	**-**	**-**	**0.25**	**0.45**	**0.63**	**0.86**	**1**	**1**	**0.19**	**0.6**
	179	-	-	-	-	0.3	-	-	0.37	-	0.14	-	-	0.13	0.05
	181	-	-	0.07	-	-	-	-	0.03	-	-	-	-	0.02	0.01
	185	-	-	-	-	-	-	-	0.02	-	-	-	-	-	0
Spar.07	275	0.04	-	-	-	-	-	-	-	-	-	-	-	-	0
	277	**0.12**	**-**	**-**	**-**	**0.23**	**-**	**-**	**-**	**-**	**-**	**-**	**0.05**	**-**	**0.02**
	279	0.29	-	-	0.18	0.03	-	-	-	-	-	0.01	-	-	0.03
	281	0.24	-	-	-	-	-	-	0.13	-	-	-	-	-	0.03
	283	0.14	-	0.13	-	0.2	-	-	0.15	-	-	0.13	-	-	0.09
	285	0.12	-	-	0.16	-	0.11	0.33	0.28	0.03	-	0.65	0.41	0.23	0.36
	287	0.05	-	0.08	-	-	-	0.25	0.3	0.68	0.94	0.17	0.41	0.57	0.23
	289	-	-	0.02	-	0.1	0.79	-	0.04	0.18	-	-	-	0.09	0.05
	291	-	0.5	0.22	0.03	-	-	-	-	-	-	-	-	-	0.04
	293	-	-	0.05	-	0.2	-	0.42	-	-	-	0.02	-	-	0.03
	295	-	-	0.15	0.29	0.1	-	-	0.06	0.1	0.06	0.03	0.14	-	0.06
	297	-	0.28	0.17	-	0.13	0.11	-	-	-	-	-	-	-	0.03
	299	-	-	0.08	0.05	-	-	-	0.06	0.03	-	-	-	-	0.01
	301	**-**	**0.22**	**0.07**	**0.26**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.11**	**0.03**
	303	-	-	-	0.03	-	-	-	-	-	-	-	-	-	0
	305	-	-	0.03	-	-	-	-	-	-	-	-	-	-	0

Locus	Allele	TX	FLG	FLA	GA	NC	VA	NJ	NY	MA	ME	WB	GH	SF	Overall
		Gulf/Southern Atlantic						*Northern Atlantic*				*Pacific (Invasive)*			
Spar.18	176	-	-	0.23	-	-	-	-	-	-	-	-	-	-	0.02
	179	-	0.5	0.47	0.55	0.47	0.54	0.53	0.82	0.28	0.68	0.62	0.77	0.48	0.56
	182	0.55	-	0.24	0.45	0.43	-	0.48	0.15	0.2	0.32	-	-	0.52	0.17
	185	0.19	0.5	0.07	-	0.07	-	-	0.03	0.13	-	0.38	0.23	-	0.21
	188	0.04	-	-	-	-	0.46	-	-	0.38	-	-	-	-	0.03
	191	0.05	-	-	-	0.03	-	-	-	0.03	-	-	-	-	0.01
	194	0.05	-	-	-	-	-	-	-	-	-	-	-	-	0
	197	0.12	-	-	-	-	-	-	-	-	-	-	-	-	0.01
Spar.14	226	0.8	0.5	0.16	0.29	0.07	0.07	0.38	0.38	0.3	0.76	0.99	1	0.3	0.68
	228	0.2	0.5	0.75	0.5	0.77	0.93	0.62	0.57	0.55	0.24	0	-	0.59	0.28
	230	-	-	0.09	0.21	0.03	-	-	0.05	0.15	-	-	-	0.11	0.03
	232	-	-	-	-	0.13	-	-	-	-	-	0.01	-	-	0.01
Spar.19	318	0.02	-	-	-	-	-	-	-	-	-	-	-	-	0
	324	-	-	0.03	-	-	-	-	-	-	-	-	-	-	0
	326	-	-	-	-	0.04	-	-	-	-	-	-	-	-	0
	328	0.43	-	0.13	-	0.14	-	0.07	0.02	-	-	0.22	-	0.06	0.15
	330	0.13	-	0.03	0.38	0.29	0.07	-	0.14	-	-	0.01	0.07	0.31	0.07
	332	**-**	**0.53**	**0.25**	**0.44**	**0.25**	**-**	**-**	**-**	**-**	0.04	**0.27**	**0.13**	**0.06**	**0.19**
	334	0.27	0.43	0.3	-	0.04	0.46	0.29	0.14	0.2	0.07	0.02	-	0.29	0.12
	336	0.05	-	-	0.13	0.18	0.39	0.5	0.42	0.1	0.61	0	-	0.23	0.12
	338	0.09	-	0.28	0.06	0.07	-	0.07	0.1	0.67	0.22	0.49	0.8	0.04	0.32
	340	0.02	-	-	-	-	-	-	0.19	-	0.04	-	-	-	0.02
	342	-	-	-	-	-	0.07	0.07	-	0.03	0.02	0	-	-	0.01
	348	-	0.03	-	-	-	-	-	-	-	-	-	-	-	0

Table 1.4. Summary of variation at nine microsatellite loci in North American smooth cordgrass populations. A = allelic richness, H_E = expected heterozygosity, H_O = observed heterozygosity.

Population	A	H_E	H_O	Population	A	H_E	H_O
Texas	5.22	0.55	0.34	New York	5.33	0.61	0.51
Florida Gulf	1.89	0.32	0.56	Massachusetts	4.22	0.58	0.54
Florida Atlantic	5.44	0.53	0.43	Maine	3.44	0.41	0.33
Georgia	3.44	0.54	0.59	Willapa Bay	4.11	0.39	0.23
North Carolina	4	0.56	0.4	Grays Harbor	2.22	0.3	0.11
Virginia	2.78	0.4	0.44	San Francisco	4	0.6	0.44
New Jersey	2.78	0.48	0.43				

Table 1.5. Summary statistics. A = allelic richness; H_E = expected heterozygosity; H_O = observed heterozygosity; f = inbreeding coefficient; P(HWE) = probability of Hardy-Weinberg equilibrium. Bold values are statistically significant given a Bonferroni-corrected nominal significance value of 0.006. Sample sizes are given in parentheses after each sampling location. See Figure 1.2 for an explanation of population abbreviations.

Pop	Locus	A	He	Ho	f	P(HWE)	Pop	Locus	A	He	Ho	f	P(HWE)
TX (29)	Spar07	4.95	0.82	0.62	0.24	0.01	NY (31)	Spar07	5.49	0.8	0.41	0.5	0
	Spar13	1.98	0	0	0	1		Spar13	4.07	0.06	0.06	0	1
	Spar14	1	0.32	0.18	0.45	0.04		Spar14	1.32	0.54	0.5	0.07	0.44
	Spar15	6.35	0.89	0.46	0.48	0		Spar15	4.39	0.78	0.77	0.01	0.44
	Spar16	1.92	0.8	0.66	0.18	0.01		Spar16	3.57	0.86	0.81	0.06	0.74
	Spar17	3.85	0.42	0.05	0.88	0		Spar17	2.12	0.75	0.33	0.56	0
	Spar18	1.91	0.65	0.31	0.53	0		Spar18	2.45	0.31	0.35	-0.2	1
	Spar19	4.19	0.73	0.57	0.22	0.04		Spar19	4.37	0.75	0.62	0.19	0.04
	Spar20	5	0.33	0.21	0.38	0.07		Spar20	4.84	0.66	0.71	-0.1	0.29
FLG (16)	Spar07	1	0.64	1	-0.6	0	MA (22)	Spar07	4.71	0.54	0.32	0.42	0.02
	Spar13	1	0	0	0	1		Spar13	4.12	0	0	0	1
	Spar14	1	0.52	1	-1	0		Spar14	1	0.57	0.59	0	0.81
	Spar15	1.63	0.13	0.13	0	1		Spar15	3.72	0.71	0.86	-0.2	0.2
	Spar16	2	0	0	0	1		Spar16	3.13	0.8	0.68	0.15	0.03
	Spar17	2	0	0	0	1		Spar17	3.93	0.75	0.55	0.28	0.01
	Spar18	2	0.52	1	-1	0		Spar18	2.83	0.76	0.64	0.17	0.27
	Spar19	2.33	0.54	0.93	-0.8	0		Spar19	2.99	0.48	0.47	0.01	0.68
	Spar20	2	0.52	1	-1	0		Spar20	3.13	0.56	0.6	0.07	0.54

Pop	Locus	A	He	Ho	f	P(HWE)	Pop	Locus	A	He	Ho	f	P(HWE)
FLA (31)	Spar07	2.78	0.88	0.87	0.01	0.13	ME (28)	Spar07	2.76	0.11	0	1	0.03
	Spar13	1.24	0.09	0.1	0	0.05		Spar13	3.52	0.1	0.11	0	1
	Spar14	1.42	0.41	0.23	0.46	0		Spar14	1.45	0.37	0.33	0.11	0.61
	Spar15	5.1	0.74	0.77	0	0.38		Spar15	3.15	0.6	0.54	0.12	0.02
	Spar16	3.15	0.58	0.45	0.23	0		Spar16	1.82	0.6	0.71	-0.2	0.71
	Spar17	3.41	0.05	0.05	0	1		Spar17	1.99	0.64	0.39	0.39	0.01
	Spar18	2.52	0.68	0.35	0.48	0		Spar18	1.95	0.44	0.21	0.52	0.01
	Spar19	4.21	0.77	0.45	0.43	0.01		Spar19	3.47	0.59	0.61	0	0.32
	Spar20	6.07	0.56	0.65	-0.2	0.11		Spar20	1.51	0.25	0.07	0.72	0
GA (21)	Spar07	2.89	0.81	0.58	0.29	0	GH (22)	Spar07	3.66	0.67	0.05	0.93	0
	Spar13	2.27	0	0	0	1		Spar13	1.98	0	0	0	1
	Spar14	1	0.64	0.89	-0.4	0		Spar14	1	0	0	0	1
	Spar15	3.5	0.64	0.86	-0.4	0.01		Spar15	1.74	0.21	0.23	-0.1	1
	Spar16	2	0.63	0.88	-0.4	0.01		Spar16	1	0.73	0.26	0.65	0
	Spar17	2	0.44	0.35	0.21	0.65		Spar17	1.95	0.41	0.35	0.15	0.58
	Spar18	2.92	0.51	0.89	-0.8	0		Spar18	1	0.36	0.09	0.75	0
	Spar19	3.33	0.67	0.88	-0.3	0.02		Spar19	2.39	0.35	0	1	0
	Spar20	4.71	0.51	0	1	0		Spar20	3.25	0	0	0	1
NC (15)	Spar07	2	0.86	0.8	0.07	0	SF (24)	Spar07	4.63	0.62	0.32	0.49	0
	Spar13	2	0	0	0	1		Spar13	2.9	0	0	0	1
	Spar14	1	0.4	0.13	0.68	0		Spar14	1	0.56	0.57	0	1
	Spar15	4.38	0.77	0.6	0.23	0		Spar15	4.46	0.79	0.7	0.12	0.92
	Spar16	2.55	0.5	0.67	-0.4	0.31		Spar16	3.79	0.79	0.74	0.07	0.08
	Spar17	2.9	0.53	0	1	0.05		Spar17	2	0.65	0.08	0.88	0
	Spar18	2.72	0.61	0.13	0.79	0		Spar18	2.71	0.51	0.23	0.56	0.01
	Spar19	5.03	0.83	0.64	0.23	0		Spar19	4.31	0.77	0.58	0.25	0
	Spar20	5.43	0.52	0.6	-0.2	0.11		Spar20	3.35	0.7	0.79	-0.1	0.12

Pop	Locus	A	He	Ho	f	P(HWE)	Pop	Locus	A	He	Ho	f	P(HWE)
VA (14)	Spar07	2	0.37	0.21	0.43	0	WB (200)	Spar07	2.95	0.53	0.12	0.77	0
	Spar13	2	0.07	0.07	0	1		Spar13	2.04	0.29	0.24	0.17	0.03
	Spar14	1.36	0.14	0.14	0	1		Spar14	1.88	0.02	0.01	0.67	0
	Spar15	2.4	0.29	0.08	0.74	0		Spar15	2.07	0.5	0.38	0.24	0
	Spar16	2.95	0.48	0.73	-0.5	0.17		Spar16	1.05	0.61	0.35	0.42	0
	Spar17	2	0.44	0	1	0.02		Spar17	1.99	0.47	0.34	0.27	0
	Spar18	1.6	0.52	0.79	-0.6	0.1		Spar18	1.08	0.47	0.34	0.28	0
	Spar19	3.19	0.64	0.93	-0.5	0		Spar19	3.13	0.64	0.28	0.57	0
	Spar20	2.5	0.61	1	-0.7	0		Spar20	3.05	0.01	0.01	0.5	0
NJ (21)	Spar07	3.67	0.68	0.5	0.27	0							
	Spar13	2.56	0	0	0	1							
	Spar14	1	0.48	0.67	-0.4	0.15							
	Spar15	2.25	0.33	0.19	0.43	0.07							
	Spar16	1.97	0.73	0.52	0.28	0.01							
	Spar17	2	0.56	0.47	0.17	0.07							
	Spar18	2	0.51	0.45	0.12	0.67							
	Spar19	3.77	0.68	0.71	-0.1	0.01							
	Spar20	2.97	0.38	0.4	0	1							

Table 1.6. Results of pairwise tests for linkage disequibrium among microsatellite loci. Values <0.0014 indicate a Bonferroni-corrected significant probability of linkage disequilibrium. See Figure 1.2 for an explanation of population abbreviations.

Locus Pair	TX	FLG	FLA	GA	NC	VA	NJ	MA	NY	ME	WB	GH	SF
Spar16/15	0.24	1	0.06	0.04	0.04	0.13	**<0.0014**	0.01	0.37	0.4	**<0.0014**	0.75	0.19
Spar16/17	0.13	1	0.46	0.36	0.2	0.45	0	0.31	0.77	0.8	**<0.0014**	0.15	0.68
Spar16/13	1	1	0.59	1	0.29	0.18	1	0.07	0.47	0.87	0.67	1	0.08
Spar16/20	0.11	1	0.3	0.31	0.1	0.06	0.01	0.08	0.64	0.95	1	1	0.39
Spar16/07	0.87	1	0.01	0.04	**<0.0014**	0.12	0	0.08	0.27	0.24	0.03	**<0.0014**	0.04
Spar16/18	0.74	1	0.28	0.12	0.05	0.12	**<0.0014**	0.08	0.57	0.4	0.03	0.65	0.77
Spar16/14	0.01	1	0.27	0.76	0	0.04	0.01	0.16	0.3	0.77	1	1	0.33
Spar16/19	0.05	1	0.03	0.51	0.85	0.06	**<0.0014**	0.01	0.17	0.63	0.45	0.47	0.16
Spar15/17	0.3	1	0.67	0	0.4	0.34	0	0.19	0.47	0.89	0.01	0.31	0.42
Spar15/13	1	1	0.66	1	1	1	0.07	0.26	0.54	0.54	0.04	1	0.92
Spar15/20	0.01	1	0.64	**<0.0014**	**<0.0014**	0.01	0.03	0.11	0.14	0.75	0.01	1	0.26
Spar15/07	0.59	0.37	0.17	**<0.0014**	**<0.0014**	0.02	**<0.0014**	0.24	0.43	1	**<0.0014**	**<0.0014**	0.42
Spar15/18	0.53	1	0.15	0.01	**<0.0014**	**<0.0014**	0.02	0.25	0.42	0.05	0.06	0.15	0.33
Spar15/14	0.11	1	0.57	**<0.0014**	**<0.0014**	0.16	0	0.7	0.16	0.65	0.02	1	0.03
Spar15/19	**<0.0014**	1	0.16	**<0.0014**	0.01	0.01	0.1	1	0.39	0.41	0.08	1	0.08
Spar17/13	1	1	1	0.66	1	1	0.07	0.26	0.49	0.43	0.12	1	1
Spar17/20	0.06	1	0.11	0.01	0.54	0.04	0.27	0.11	0.87	0.5	0.01	1	0.38
Spar17/07	0.97	1	0.2	0.06	0.02	0.14	0.01	0.04	0.09	1	0.24	**<0.0014**	0.64
Spar17/18	0.72	1	0.38	0.24	1	0.07	0.03	0.03	0.42	0.44	0.02	0.52	0.23
Spar17/14	0.21	1	1	0	0.1	0.07	0.03	0.63	0.9	0.54	0.01	1	0.78
Spar17/19	0.16	1	1	0	1	0.04	0.08	0.52	0.84	0.64	0.28	0.8	0.28
Spar13/20	0.07	1	0.24	1	0.1	1	1	1	0.53	1	1	1	0.14

Locus Pair	TX	FLG	FLA	GA	NC	VA	NJ	MA	NY	ME	WB	GH	SF
Spar13/07	1	1	0.08	1	**<0.0014**	1	1	0.83	0.57	1	0.33	**<0.0014**	1
Spar13/18	1	1	0.02	1	1	0.14	0.68	0.75	0.3	1	0.29	1	1
Spar13/14	0.04	1	1	1	1	1	0.16	0.86	0.54	0.87	0.5	1	1
Spar13/19	0.03	1	0.1	1	1	1	1	0.05	0.63	0.43	0.32	1	1
Spar20/07	0.01	1	0.57	**<0.0014**	**<0.0014**	0	0.16	0.34	0.25	1	0.05	**<0.0014**	0.3
Spar20/18	0.09	1	0.65	0.2	0.01	**<0.0014**	0.81	0.53	0.45	0.87	1	1	0.04
Spar20/14	0.01	1	0.42	**<0.0014**	0.02	0.02	0.44	0.46	0.61	0.92	0.01	1	0.55
Spar20/19	0	1	0.98	**<0.0014**	0.26	**<0.0014**	0.07	0.07	0.95	0.36	0.03	1	0.55
Spar07/18	0.33	1	0.33	0.05	**<0.0014**	**<0.0014**	**<0.0014**	0.26	0.07	0.42	0.14	**<0.0014**	0.6
Spar07/14	0.03	1	0.1	**<0.0014**	**<0.0014**	0.07	0.77	0.5	0.37	1	0.03	**<0.0014**	0.94
Spar07/19	0.01	0.19	0.6	**<0.0014**	0	0	0.33	0.35	0.95	0.23	**<0.0014**	**<0.0014**	0.62
Spar18/14	0.14	1	0.17	0.19	0.19	0.01	**<0.0014**	0.23	0.94	0.54	0.55	1	0.22
Spar18/19	0	1	0.09	0.2	0.19	**<0.0014**	0.01	0.76	0.97	0.31	0.01	0	0.76
Spar14/19	0.04	1	0.45	**<0.0014**	0.5	0.02	0.08	0.01	0.37	0.29	0.02	1	0.53

Chapter 2. Strong spatial genetic structure in a recently introduced marine invader (*Spartina alterniflora*)

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2.1. ABSTRACT: Coastal bays and estuaries are among the ecological systems most heavily impacted by exotic species invasions. Rates of invasions into coastal habitats are accelerating, and predicting their ecological and evolutionary impacts requires information on the genetic structure and dispersal patterns of marine invaders. Cordgrasses (*Spartina* spp.) are globally introduced and among the most prolific and damaging of invasive marine plants. Their floating seeds and inflorescences are indicative of long-distance dispersal capacity. Historical data suggest that the circa 100 year old Willapa Bay (Washington, USA) smooth cordgrass (*Spartina alterniflora*) population was founded by multiple introductions to several regions of the estuary in association with oyster aquaculture practices. Willapa Bay is also popularly hypothesized to be the source of a sequential invasion of a geographically proximate estuary, Grays Harbor. 220 individuals from Willapa Bay and Grays Harbor were genotyped at nine polymorphic microsatellite loci to allow an assessment of dispersal and the consistency of genetic data with the following hypotheses. 1) The Willapa Bay population was established by multiple founding foci. 2) Willapa Bay is the source of the Grays Harbor population. 3) High rates of dispersal and gene flow within Willapa Bay, facilitated by tidal seed dispersal, have prevented the development of genetic structure. Bayesian cluster analyses revealed fine-scale genetic structure within Willapa Bay of a magnitude exceeding that exhibited by most marine populations over scales of thousands of kilometers. The observed spatial genetic structure was consistent with the historical record of widespread, early colonization of multiple foci in the bay. The strong genetic structure within Willapa Bay was also indicative of spatially limited gene flow, and suggested that the majority of seed dispersal occurs over local (within-estuary) spatial

scales. However, the genetic data were also consistent with the colonization of Grays Harbor by individuals from Willapa Bay, suggesting the ability of *S. alterniflora* to occasionally disperse longer distances. These results demonstrate that genetic structure can develop rapidly in invading species, and that propagule dispersal can be spatially limited in marine invaders adapted for long-distance dispersal.

2.2. INTRODUCTION

Coastal bays and estuaries are among the ecological systems most heavily impacted by exotic species invasions (Cohen & Carlton 1995, 1998, Grosholz 2002). Exposed to high densities of invading organisms, these sheltered coastal regions are buffered from the severe environmental stresses of the outer coast and provide relatively benign habitats for arriving propagules. Commercial shipping activities represent the primary vectors of introduction of exotic species into marine habitats. In addition to transporting massive loads of propagule-rich ballast water, the hulls of shipping vessels provide highly mobile substrates that sustain and vector the dispersal of diverse invertebrate fouling communities (Carlton 1985, Carlton & Geller 1993, Carlton 1996, Ruiz et al. 2000). Bays and estuaries also experience high levels of natural and anthropogenic disturbance, which can facilitate the establishment of invasive species or modify their impacts.

Understanding the dispersal dynamics and population structure of invasive species is critical to predicting their spatial spread (Wadsworth et al. 2000). Invasion dynamics may be particularly cryptic in marine environments, where oceanographic processes, ballast water exchange, and larval behavior can strongly and interactively influence patterns of dispersal and establishment. The logistical difficulties of directly tracking propagule (seed, vegetative fragment, or planktonic larvae) transport in the water column have made characterizing the dispersal of marine invaders a challenging task, especially when geographic barriers to dispersal are absent. Molecular marker approaches, increasingly applied to the description of marine population structure and dispersal, have the potential to provide insights into marine invasion dynamics that are of both basic and applied significance. By revealing population structure, identifying source populations, and estimating dispersal distances, recent molecular studies have increased our understanding of marine (including estuarine) invasion dynamics and may inform the development of more effective control programs (Geller et al. 1997, Jousson et al. 1998, Martel et al. 2004, Roman & Palumbi 2004).

Cordgrasses (*Spartina* spp.) are among the most prolific and damaging of marine invasive plants. The rhizomatous perennial *S. alterniflora* was examined as a model species because it is the most widely distributed and damaging invasive cordgrass. Native to the Atlantic and Gulf coasts of the Americas, its ecology and distribution are well-documented in both its native and invasive ranges (e.g., Sayce 1988, Daehler & Strong 1996, Ayres et al. 1999, Ayres et al. 2004, Davis et al. 2004, Richards et al. 2005, Travis & Hester 2005, Civille et al. in press). Globally invasive, *S. alterniflora* impacts biodiversity and fisheries by radically altering habitat structure, food web composition, and nutrient cycling. In addition, invasions increase sediment accretion, reduce tidal flows, and alter algal primary productivity and benthic community structure, which may translate to higher food web impacts (Daehler & Strong 1996). In 2004, the largest invasive *S. alterniflora* population, located in Willapa Bay (Washington, USA), occupied 7300 total hectares of mudflats (Murphy 2004).

Historical data suggest that the Willapa Bay population was founded by multiple introductions to several regions of the estuary in association with oyster aquaculture practices (Civille et al. in press). In the 19^{th}, century, oysters were translocated from western Atlantic estuaries to the eastern Pacific estuaries of Willapa Bay and Grays Harbor (Washington, USA), and subsequently moved within Willapa Bay. Spatial patterns of *S. alterniflora* establishment in Willapa Bay are coincident with the location of commercial oyster beds (Civille et al. in press). Although *S. alterniflora* had established in several locations in Willapa Bay by 1945, it was not observed in Grays Harbor until the 1980s, leading to popular speculation that Grays Harbor had been secondarily invaded by *S. alterniflora* from Willapa Bay (Civille et al. in press, L. Holcomb personal communication). 220 individuals from Willapa Bay and Grays Harbor were genotyped at nine polymorphic microsatellite loci to assess the consistency of genetic data with the following hypotheses. 1) The Willapa Bay population was established by multiple founding foci. 2) Willapa Bay is the source of the Grays Harbor population. 3) High rates of dispersal and gene flow within Willapa Bay, facilitated by tidal seed dispersal, have prevented the development of genetic structure. Because native *S. alterniflora* populations are strongly geographically differentiated, multiple introductions could result in the development of strong spatial genetic structure within Willapa Bay. However, if rates of gene flow are high between regions of the bay, as suggested by *S. alterniflora*'s tidal seed dispersal, then weak or no spatial structure would be predicted to emerge. If Willapa Bay is the source of the Grays Harbor population, then the Grays Harbor population should be very similar to Willapa Bay *S. alterniflora*.

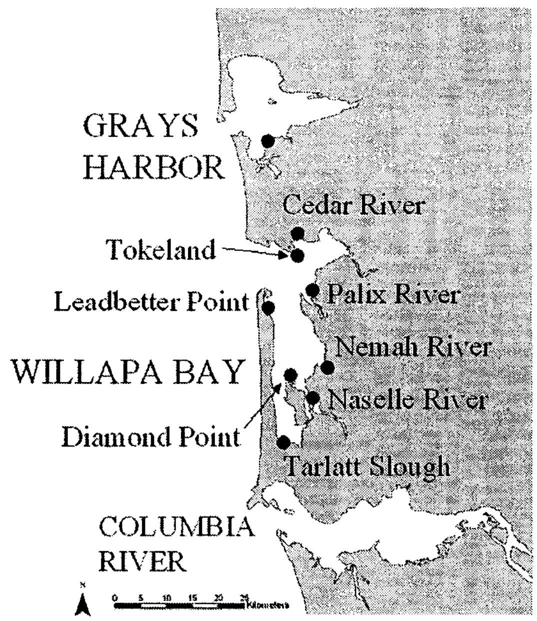
Nine previously developed microsatellite markers were applied to assess the genetic structure and diversity of *S. alterniflora* in Willapa Bay and Grays Harbor. A previous study of introduced *S. alterniflora* in Willapa Bay detected little randomly amplified polymorphic DNA (RAPD) variation among individuals, leading the authors to conclude that the entire population had likely been founded by a single clone (Stiller & Denton 1995). In comparison to dominant RAPD markers, microsatellites are codominant, produce data of higher reproducibility, and are characterized by higher rates of polymorphism. These characteristics lend them greater power for investigating individual relatedness and fine-scale population structure (Chakraborty & Jin 1993).

2.3. MATERIALS AND METHODS

2.3.1. Sample collection and DNA isolation

Leaf samples were collected in 2003 and 2004 from intertidal mudflats accessed on foot or by airboat in Willapa Bay and Grays Harbor (Washington State, USA; Figure 2.1). Leaf material was collected from individuals from nine regions in Willapa Bay (Cedar River, Diamond Point, Naselle River, Nemah River, Palix River, Tarlatt Slough, Tokeland, and Leadbetter Point; n=20-30) and southern Grays Harbor (n=22). Samples were collected from isolated clones or from stands of contiguous marsh sampled at random intervals of at least 10 m.

Figure 2.1. Map of sampling locations



2.3.2. DNA extraction and microsatellite genotyping

Genomic DNA was extracted from fresh *S. alterniflora* leaf tissue using the Dneasy® Plant Mini Kit (Qiagen Inc., Valencia, CA, USA). The kit protocol was revised to incorporate 200 mg wet weight fresh leaf material and 170 mL buffer AP1. Individuals were genotyped using nine previously developed disomic, polymorphic microsatellite markers (GenBank accession numbers Spar.16, Spar.15, Spar.17, Spar.13, Spar.20, Spar.07, Spar.18, Spar.14, Spar.19), using PCR protocols described in Blum et al. (2004) and Sloop et al. (in press). Forward primers were 5' fluorescently labeled with one of the following dyes: 6-FAM™ (6-Carbofluorecein), HEX™ (Hexachlorofluorescein), NED™ (Applied Biosystems (ABI) proprietary yellow), or PET™ (ABI proprietary red). A 3730xl 96-capillary DNA analyzer and Genemapper 3.0 software (ABI, Foster City, CA, USA) were used to size the PCR products.

2.3.3 Genetic data analyses

Although *S. alterniflora* is hexaploid, only disomic markers were utilized for this study and genotype data were subsequently analyzed as diploid data. Disomic markers were previously identified based on genotyping data for 472 individuals from the native and invasive ranges of *S. alterniflora* (Bando Chapter 1). Only one or two alleles amplified per disomic locus in any of the 472 individuals, despite the presence of up to 16 alleles per locus per population. Only one or two alleles amplified per locus in the samples included in this study, although up to 6 alleles were present at each locus in each population. In addition, peak heights in genotyping traces were nearly always symmetrical at heterozygous loci, suggesting equivalent allelic dosages. To assess genetic diversity, expected (H_E) and observed (H_O) heterozygosity were calculated with GENETIC DATA ANALYSIS software (GDA, Lewis & Zaykin 2001). Because sample sizes differed between populations, Hurlbert's (1971) rarefaction index was also applied in Fstat V. 2.9.3.2 to calculate allelic richness, an allelic diversity metric that corrects for biases incurred by sample size differences (Goudet 1995, El Mousadik & Petit 1996, Petit et al. 1998). Exact tests for departures from Hardy-Weinberg and linkage equilibrium for each population were calculated with GDA (Lewis & Zaykin 2001). When appropriate, Bonferroni-adjusted probability values for multiple comparisons were calculated to assess statistical significance.

A model-based genetic clustering method was applied to multilocus genotype data in *structure* V. 2 (Pritchard *et al.* 2000) to infer population structure. The *structure* algorithm uses a Bayesian clustering approach to estimate the number of subpopulations (*K*) represented by a pool of individual multilocus genotypes, without regard for *a priori* groupings, such as sampling locations. To investigate all potential patterns of genetic structure, *K* was allowed to range from 1 to 10. Three independent runs were conducted for each value of *K*, using a burn-in period of 1×10^6 iterations followed by 9×10^6 iterations. The resulting values for the log probability of the data (Pr X | *K*; Equation 12 in Pritchard et al. 2000) were plotted against *K* to determine the value of *K* at which the rate of change of the log probability of the data most steeply declined. This *K*-value represented the uppermost hierarchical number of subpopulations (Evanno *et al.* 2005). An admixture model in the *structure* program was used to probabilistically assign individuals to genetic clusters and to estimate the fraction of each individual's genome that was derived from each cluster.

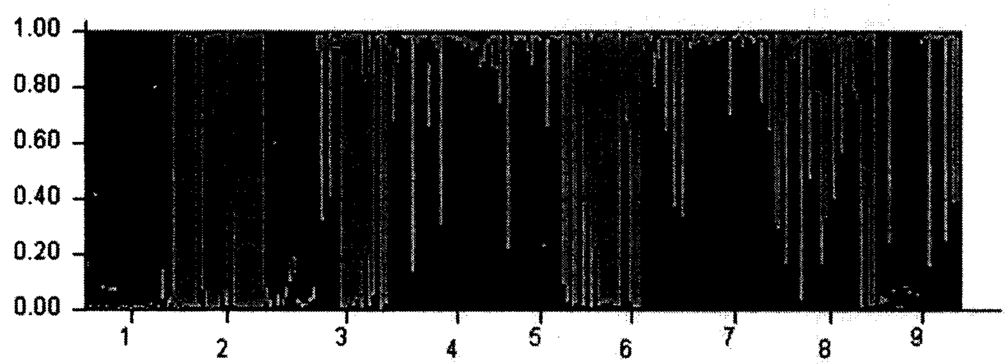
Migration rates between sampling locations were estimated with BayesAss+ 1.3 software (Wilson & Rannala 2003). BayesAss+ applies a Bayesian method to individual multilocus genotype data to estimate recent (i.e., within the last several generations) migration rates. The BayesAss+ algorithm estimates the posterior probability distributions of population allele frequencies and inbreeding coefficients, and relies on Markov Chain Monte Carlo techniques to estimate posterior probabilities (Wilson & Rannala 2003). The program allows population genotype frequencies to deviate from Hardy-Weinberg equilibrium.

2.4. RESULTS

The F_{ST} calculations and Bayesian cluster analyses revealed that many of the sites within Willapa Bay were strongly differentiated. In contrast to previous analyses of RAPD data, which failed to detect genetic structure in Willapa Bay *S. alterniflora* (Stiller & Denton 1995), analyses of microsatellite genotype data revealed significant genetic structure. A global F_{ST} estimate demonstrated significant and strong differentiation among marshes in the bay (F_{ST} = 0.17, 99% confidence limits 0.108–0.223). Significant pairwise F_{ST} values were as high as 0.27 (mean = 0.14; Bonferroni-corrected 5% nominal significance value = 0.0014). Pairwise F_{ST} values and their significance probabilities are presented in Table 2.1.

The Bayesian cluster analyses revealed that the mostly likely number of native and invasive genetic clusters (*K*) was three, given the observed distribution of allele phenotypes (Figure 2.2). The Cedar River and Diamond Point sites in the northern bay formed two distinct subpopulations. The Naselle and Nemah Rivers and Tarlatt Slough in the southern bay formed a third distinct subpopulation, with evidence of some gene flow and admixture with the Diamond Point subpopulation. The Leadbetter and Grays Harbor sites contained immigrants from all three subpopulations. The Tokeland and Palix individuals derived their ancestry from two subpopulations and displayed a higher degree of admixture than the other sites. The Grays Harbor population did not form a distinct subpopulation relative to Willapa Bay.

Figure 2.2. Distribution of membership in subpopulations as determined by the *structure* program. Populations: 1 = Cedar River, 2 = Diamond Point, 3 = Leadbetter Point, 4 = Naselle River, 5 = Nemah River, 6 = Palix River, 7 = Tarlatt Slough, 8 = Tokeland, 9 = Grays Harbor.



Pairwise migration rates estimated by BayesAss+ were highly asymmetrical between some sites. The highest migration rates occurred from Tarlatt Slough to the Naselle and Nemah Rivers (0.20 and 0.17, respectively). No migration was observed in the opposite direction, from the Nemah and Naselle Rivers to Tarlatt Slough. Table 2.2 displays the migration rates between each pair of sampled marshes, as calculated by the BayesAss+ program.

No loci deviated from HW equilibrium in more than three sites when a Bonferroni-corrected nominal significance value of 0.006 was applied (Table 2.3). No pairs of loci were in significant linkage disequilibrium in any sites except at the Palix River, applying a Bonferroni-corrected nominal significance value of 0.0014 (Table 2.4). In Willapa Bay, H_O ranged between 0.085 (Cedar River) and 0.377 (Leadbetter Point). H_O was substantially lower in Cedar River than all other Willapa Bay sites; the next lowest H_O value in the bay was 0.207 (Naselle River; Table 2.3). Observed heterozygosity was also low in Grays Harbor ($H_O = 0.109$). By comparison, H_O ranged from 0.331 (Maine) to 0.641 (Georgia) in a previous analysis of ten populations between Maine and Texas in the species' native range (Bando Chapter 1). H_E ranged from 0.280 (Tarlatt Slough) to 0.377 (Leadbetter) in Willapa Bay, and was 0.304 in Grays Harbor (Table 2.3). Allelic richness was 2.10 in Grays Harbor and ranged from 1.84 (Naselle River) to 2.07 (Diamond Point) in Willapa Bay (Table 2.3). Allelic richness was previously measured from 1.765 to 3.623 in populations in the native range (Bando Chapter 1).

2.5. DISCUSSION

Many of the sampling locations in Willapa Bay were highly differentiated on the basis of their observed microsatellite genotypes, using both F_{ST} estimates and Bayesian approaches (Pritchard *et al.* 2000). The magnitude of genetic differentiation (global $F_{ST} = 0.17$) exceeded that observed in many marine species (Waples 1998). However, there was no clear geographic pattern to the genetic structure in Willapa Bay. The Cedar River and Diamond Point sites represented distinct subpopulations and were among the earliest sites invaded by *S. alterniflora* in Willapa Bay, possibly as a result of oyster aquaculture practices (Civille *et al.* in press). The genetic structure may reflect the signature of multiple founding foci. Multiple founding events would be consistent with historic and genetic data that suggest Willapa Bay has sustained serial introductions of *S. alterniflora* from several source populations in the Atlantic and Gulf coasts of North America (Civille et al. in press, Bando Chapter 1). There appears to be a strong relationship between oyster culture and the introduction and spread of *S. alterniflora* in Willapa Bay. In the 1890s, barrels of oysters were brought to the bay from estuaries on the Atlantic coast of the U.S. It has been hypothesized that these oyster shipments were the primary vector for the introduction of *S. alterniflora* to Willapa Bay (Civille *et al.* in press). Once planted in Willapa Bay, oysters were subsequently moved between areas in the bay, including the locations sampled in this study. The accidental dispersal of *S. alterniflora* propagules during the course of these oyster translocations may underlie some of the observed spatial genetic structure within the bay.

The presence of genetic structure within Willapa Bay indicated that gene flow is spatially limited, and suggests that most dispersal occurs over small spatial scales within the bay. The prevalence of local dispersal was corroborated by the migration rates estimated between marshes by BayesAss+ (Wilson and Rannala 2003). The highest migration rates occurred between Tarlatt Slough and the Naselle and Nemah Rivers, three proximate sites in the south bay. Interestingly, migration rates were highly asymmetrical: migration rates were high (17 -20%) from Tarlatt Slough to the Nemah and Naselle Rivers but zero in the opposite direction. Asymmetrical migration rates could contribute to the development of genetic structure in Willapa Bay and may be driven by several potential mechanisms, including the following. i. Seed production varies spatially within the estuary. ii. Dispersal differs between regions of the estuary due to variation in oceanography or other transport mechanisms. iii. Local adaptation and selection on seedlings (e.g., for salinity tolerance) results in the differential survival of immigrant and resident seedlings in marshes.

The Grays Harbor population did not form a separate genetic cluster from the population in Willapa Bay, which is consistent with their proximity and with the hypothesis that the Grays Harbor population is derived from the Willapa Bay population (Civille *et al.* in press). Observed heterozygosity was substantially lower in Grays Harbor than in all but one site in Willapa Bay, suggesting a smaller effective population size in Grays Harbor. Thus, while the Grays Harbor population may reflect the ability of Willapa *S. alterniflora* to disperse outside of Willapa Bay, these long-distance dispersal events are likely infrequent, with the majority of propagule dispersal occurring within the bay.

Invasive *Spartina* species are globally distributed and are associated with significant ecological and economic impacts (Daehler and Strong 1996). *S. alterniflora* is classified as a noxious weed in Washington State and is the target of aggressive eradication programs in Washington and Oregon (USA). In San Francisco Bay, CA, efforts are focused on controlling the hybrid offspring of *S. alterniflora* and its native congener Pacific cordgrass (*S. foliosa*). In addition to the Pacific coast of North America, *S. alterniflora* has also been introduced to western Europe (Baumel et al. 2003, Ainouche et al. 2004), China (Chung *et al.* 2004), India (Shah & Badrinath 1985), Australia (McEnnulty *et al.* 2000), and New Zealand (Hicks & Silvester 1990). Because of limited funding and logistical constraints, control operations for *S. alterniflora* and many other invasive species are based on long-term adaptive management, rather than immediate eradication. Understanding the dispersal dynamics of invasive *Spartina* species may inform the management and control of invasive populations. For example, if seed dispersal is fairly localized, then eradicating proximate meadows or timing mechanical controls in proximate areas to precede seed set may be a necessary and effective method of limiting the recolonization of treated areas. The fine-scale genetic structure detected by microsatellite analyses indicate that gene flow is spatially limited and suggests that most seed dispersal occurs over relatively small spatial scales. Thus, control of adjacent seed-producing meadows may be critical to the local eradication of *S. alterniflora*.

Marine plants adapted for long-distance hydrochorous dispersal, such as *S. alterniflora*, may be useful models for generalizing the dispersal dynamics of sessile marine organisms with planktonic larvae, without the confounding effect of species-specific variation in larval behavior. *S. alterniflora* is a more comparable analog for planktonic larval dispersal than are seagrasses and marine macroalgae, which are often characterized by local retention mechanisms, such as nonbuoyant propagules (Albrecht 1998, Stiger & Payri 1999). Even seagrass genera with large, buoyant inflorescences or fruits are only estimated to disperse distances of tens of kilometers (Orth et al. 1994, Harwell & Orth 2002, Lacap et al. 2002). In contrast, *S. alterniflora* seeds frequently disperse in wrack (Bando, pers. obs), which is highly mobile due to its buoyancy and recalcitrance. *S. alterniflora* seedheads in wrack can float for approximately two months on the open ocean before being shredded and waterlogged by wave action, while loose seeds are estimated to float for up to 30 days (K. Sayce, unpublished data).

Our understanding of the dispersal and genetic structure of marine organisms is just emerging from its historic "black box" approach. Understanding the spatial partitioning of neutral genetic variation within invading species is important for understanding their evolutionary potential, because it indicates the scale of evolutionary independence of subpopulations and the degree to which subpopulations are free to evolve in response to local variation in selective pressures (Grosberg & Cunningham 2001) The fine-scale (within-estuary) genetic structure described in this study may represent local adaptive potential and reflect the dispersal dynamics of invasive *S. alterniflora* as well as the spatial and temporal patterns of its spread. These results demonstrate that genetic structure can develop rapidly in invading species, even those with high dispersal capacity. In addition, these results demonstrate that propagule dispersal of marine organisms adapted for high dispersal capacity can be spatially limited even in the absence of larval settling behavior. By allowing us to elucidate spatial and temporal patterns of range expansion, population genetic studies continue to increase our understanding of population structure in the sea, and may ultimately lead to the design of more effective invasive species control programs.

2.6. ACKNOWLEDGEMENTS

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2.7. LITERATURE CITED

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TABLES

Table 2.1. Pairwise F_{ST} values for Willapa Bay and Grays Harbor sites based on microsatellite allele frequencies. NS=P>0.0014; * P≤0.0014 (5% nominal significance level adjusted for multiple comparisons); ** P≤0.00028 (1% nominal significance level adjusted for multiple comparisons)

	CR	DP	LB	NA	NE	PA	TS	TK	GH
CR	-	0.32 (NS)	0.26 (NS)	0.36 (NS)	0.31 (NS)	0.39 (NS)	0.46 (NS)	0.44 (NS)	0.29 (NS)
DP		-	0.07**	0.19**	0.15**	0.098**	0.27**	0.17**	0.21**
LB			-	0.13**	0.05**	0.10**	0.16**	0.17**	0.13**
NA				-	0.02 (NS)	0.12**	0.10*	0.14**	0.03**
NE					-	0.12**	0.11*	0.15 (NS)	0.07 (NS)
PA						-	0.12**	0.07**	0.19**
TS							-	0.15*	0.15**
TK								-	0.21**
GH									-

Population Abbreviations:

WILLAPA BAY:

CR = Cedar River

NA = Naselle River

TS = Tarlatt Slough

DP = Diamond Point

NE = Nemah River

TK = Tokeland

LB = Leadbetter Point

PA = Palix River

GRAYS HARBOR:

GH = southern Grays Harbor

Table 2.2. Asymmetrical migration rates estimated by BayesAss+ based on multilocus genotype data.

		Source								
		CR	**DP**	**LB**	**NA**	**NE**	**PA**	**TS**	**TK**	**GH**
Destination	**CR**	**0.96**	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.01
	DP	0.01	**0.93**	0.03	0.00	0.00	0.01	0.00	0.00	0.00
	LB	0.00	0.05	**0.85**	0.00	0.01	0.04	0.02	0.01	0.01
	NS	0.00	0.01	0.01	**0.70**	0.05	0.01	0.20	0.01	0.02
	NE	0.01	0.01	0.01	0.01	**0.75**	0.01	0.17	0.02	0.02
	PA	0.00	0.01	0.01	0.00	0.01	**0.89**	0.05	0.02	0.00
	TS	0.00	0.00	0.00	0.00	0.00	0.00	**0.98**	0.00	0.00
	TK	0.00	0.02	0.01	0.01	0.02	0.02	0.13	**0.79**	0.01
	GH	0.00	0.01	0.01	0.00	0.01	0.01	0.02	0.01	**0.93**

Table 2.3. Summary statistics for Willapa Bay *S. alterniflora* genotyped at nine microsatellite loci.

Site	Locus	A	He	Ho	P(HW)	Site	Locus	A	He	Ho	P(HW)	Site	Locus	A	He	Ho	P(HW)
Cedar River (CR)	Spar.07	2.00	0.44	0.00	0.022	**Naselle River (NA)**	Spar.07	2.00	0.48	0.23	0.010	**Tarlatt Slough (TS)**	Spar.07	2.00	0.13	0.05	0.068
	Spar.13	2.42	0.05	0.05	1.000		Spar.13	1.98	0.18	0.19	1.000		Spar.13	1.92	0.49	0.45	1.000
	Spar.14	2.35	0.05	0.05	1.000		Spar.14	2.30	0.08	0.00	0.019		Spar.14	1.99	0.00	0.00	1.000
	Spar.15	1.35	0.27	0.10	0.008		Spar.15	1.81	0.34	0.27	0.280		Spar.15	2.00	0.24	0.09	0.026
	Spar.16	1.70	0.53	0.11	0.041		Spar.16	1.00	0.50	0.38	0.408		Spar.16	1.00	0.42	0.29	0.278
	Spar.17	2.00	0.54	0.25	0.013		Spar.17	2.00	0.49	0.26	0.019		Spar.17	1.69	0.37	0.38	1.000
	Spar.18	1.92	0.23	0.16	0.267		Spar.18	2.00	0.46	0.38	0.414		Spar.18	1.97	0.30	0.27	0.551
	Spar.19	1.35	0.19	0.00	0.000		Spar.19	1.49	0.28	0.14	0.065		Spar.19	1.00	0.55	0.36	0.037
	Spar.20	2.17	0.10	0.05	0.023		Spar.20	1.96	0.00	0.00	1.000		Spar.20	3.52	0.00	0.00	1.000
	Mean	1.92	0.27	0.09	0.683		Mean	1.84	0.31	0.21	0.342		Mean	1.90	0.28	0.21	0.251
Diamond Point (DP)	Spar.07	3.46	0.57	0.00	0.000	**Nemah River (NE)**	Spar.07	2.00	0.52	0.18	0.010	**Tokeland (TK)**	Spar.07	2.00	0.22	0.08	0.003
	Spar.13	2.00	0.03	0.03	1.000		Spar.13	2.41	0.30	0.35	1.000		Spar.13	2.00	0.27	0.23	0.466
	Spar.14	2.00	0.00	0.00	1.000		Spar.14	1.99	0.00	0.00	0.019		Spar.14	2.00	0.00	0.00	1.000
	Spar.15	1.24	0.48	0.55	0.453		Spar.15	1.97	0.54	0.59	0.280		Spar.15	1.94	0.40	0.46	0.632
	Spar.16	1.00	0.65	0.37	0.000		Spar.16	1.00	0.51	0.44	0.408		Spar.16	1.00	0.42	0.25	0.062
	Spar.17	2.91	0.45	0.38	0.437		Spar.17	2.00	0.34	0.29	0.019		Spar.17	2.23	0.46	0.43	1.000
	Spar.18	2.00	0.49	0.41	0.455		Spar.18	2.00	0.44	0.25	0.414		Spar.18	2.00	0.50	0.42	0.456
	Spar.19	1.00	0.62	0.45	0.010		Spar.19	1.00	0.45	0.29	0.065		Spar.19	1.00	0.56	0.27	0.002
	Spar.20	2.97	0.00	0.00	1.000		Spar.20	2.00	0.00	0.00	1.000		Spar.20	2.87	0.00	0.00	1.000
	Mean	2.07	0.37	0.24	0.337		Mean	1.82	0.34	0.27	0.234		Mean	1.89	0.32	0.24	0.248
Leadbetter Point (LB)	Spar.07	3.54	0.58	0.23	0.000	**Palix River (PA)**	Spar.07	2.00	0.34	0.10	0.015	**Grays Harbor (GH)**	Spar.07	3.87	0.64	0.05	0.000
	Spar.13	2.00	0.33	0.20	0.065		Spar.13	2.00	0.44	0.41	1.000		Spar.13	1.88	0.00	0.00	1.000
	Spar.14	1.96	0.00	0.00	1.000		Spar.14	2.00	0.00	0.00	1.000		Spar.14	2.00	0.00	0.00	1.000
	Spar.15	1.97	0.46	0.50	0.702		Spar.15	2.00	0.46	0.41	0.800		Spar.15	1.00	0.21	0.24	1.000
	Spar.16	1.00	0.70	0.23	0.000		Spar.16	1.00	0.50	0.59	0.641		Spar.16	1.00	0.74	0.28	0.000
	Spar.17	2.95	0.30	0.17	0.031		Spar.17	2.87	0.50	0.54	0.530		Spar.17	3.42	0.40	0.32	0.532
	Spar.18	1.98	0.35	0.30	0.593		Spar.18	2.00	0.50	0.39	0.126		Spar.18	1.99	0.37	0.10	0.004
	Spar.19	1.00	0.67	0.33	0.000		Spar.19	1.00	0.58	0.28	0.253		Spar.19	1.00	0.37	0.00	0.000
	Spar.20	2.99	0.00	0.00	1.000		Spar.20	2.88	0.00	0.00	1.000		Spar.20	2.71	0.00	0.00	1.000
	Mean	2.16	0.38	0.22	0.424		Mean	1.97	0.37	0.30	0.180		Mean	2.10	0.30	0.11	0.649

Table 2.4. Results of pairwise tests for linkage disequibrium among microsatellite loci. Values <0.0006 indicate a Bonferroni-corrected significant probability of linkage disequilibrium. See Table 2.1 for an explanation of population abbreviations.

Locus Pair	CR	DP	LB	NS	NM	PA	TS	TK	GH
Spar16/15	1.0000	0.7610	0.9880	0.0200	0.7260	0.7950	0.0900	0.1050	0.8940
Spar16/17	0.0072	0.0984	0.1413	0.2216	0.4941	0.9719	0.5731	0.0009	0.1753
Spar16/13	1.0000	0.6653	0.2625	0.2400	0.8278	0.2125	0.3459	0.1569	1.0000
Spar16/20	1.0000	1.0000	1.0000	0.4072	0.6419	0.4278	0.2772	0.1266	1.0000
Spar16/07	1.0000	0.1966	0.7713	0.0056	0.0125	**<0.0006**	0.0403	0.0222	0.6681
Spar16/18	1.0000	0.0006	0.5372	0.0366	0.0263	0.4475	0.3863	0.0506	0.7944
Spar16/14	1.0000	1.0000	1.0000	0.5841	0.5978	0.4269	0.1153	0.1309	1.0000
Spar16/19	1.0000	0.0434	0.8584	0.0431	0.2141	0.9847	0.0484	0.0259	0.5069
Spar15/17	0.0338	0.4244	0.8644	0.0319	0.4706	0.8116	1.0000	0.4313	0.3509
Spar15/13	1.0000	0.4194	0.0022	0.6069	0.7419	0.7722	0.4831	0.3700	1.0000
Spar15/20	0.1109	0.4572	0.7009	0.2813	0.8013	0.7094	1.0000	1.0000	1.0000
Spar15/07	1.0000	0.4597	0.6009	0.5456	0.9178	**<0.0006**	0.1466	0.8334	0.1366
Spar15/18	0.5116	0.3072	0.0309	0.0288	0.3538	0.0341	0.6109	0.8694	0.1641
Spar15/14	0.1106	0.4600	0.7125	0.7419	0.8159	0.3722	1.0000	1.0000	1.0000
Spar15/19	0.1925	0.9147	0.3647	0.0959	0.4775	0.2850	0.7606	0.8494	1.0000
Spar17/13	0.2538	0.1209	0.1441	0.3753	0.7463	0.4603	0.1194	0.2075	0.5559
Spar17/20	0.0494	0.4344	1.0000	1.0000	0.5322	1.0000	1.0000	1.0000	0.5509
Spar17/07	0.1419	0.8184	0.1097	0.1938	0.4681	0.0016	0.0359	0.1641	0.0069
Spar17/18	0.0013	0.3066	0.4672	0.1834	0.0856	0.2234	0.8531	0.1616	0.4347
Spar17/14	0.0503	0.4300	1.0000	1.0000	0.4366	0.6972	1.0000	1.0000	0.5491
Spar17/19	0.0134	0.4150	0.2959	0.0131	0.1513	0.8491	0.8691	0.6166	0.3891
Spar13/20	1.0000	1.0000	0.0559	1.0000	1.0000	1.0000	1.0000	0.4916	1.0000
Spar13/07	1.0000	1.0000	0.1109	0.2688	0.8853	**<0.0006**	0.1047	0.6900	1.0000
Spar13/18	0.0616	0.6175	0.1153	0.5975	0.3025	0.6584	0.4334	0.3306	1.0000
Spar13/14	1.0000	1.0000	0.0578	0.3425	1.0000	1.0000	1.0000	0.5038	1.0000
Spar13/19	0.1125	1.0000	0.0284	0.1325	0.3222	0.8678	0.4800	0.1813	1.0000
Spar20/07	1.0000	1.0000	1.0000	1.0000	1.0000	**<0.0006**	0.0650	1.0000	1.0000
Spar20/18	0.2647	0.4731	0.5956	0.4116	0.1047	0.2806	0.5403	0.6972	1.0000
Spar20/14	0.0497	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Spar20/19	0.1016	1.0000	1.0000	0.0622	0.2556	1.0000	1.0000	1.0000	1.0000
Spar07/18	0.5109	0.0084	0.6419	0.4491	0.0625	**<0.0006**	0.1013	0.7184	0.0319
Spar07/14	1.0000	1.0000	1.0000	0.2275	1.0000	**<0.0006**	0.0725	1.0000	1.0000
Spar07/19	1.0000	0.1859	0.1563	0.0244	0.0616	**<0.0006**	0.0947	0.0359	0.5581
Spar18/14	0.2600	0.4556	0.5978	0.4703	0.1106	0.4284	0.5634	0.6838	1.0000
Spar18/19	0.0672	0.0597	0.4084	0.0328	0.0013	0.1138	0.6909	0.2288	0.0022
Spar14/19	0.0969	1.0000	1.0000	0.0209	0.1069	1.0000	1.0000	1.0000	1.0000

Chapter 3. The roles of competition and disturbance in a marine invasion

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3.1. ABSTRACT: Two hypotheses for the decline of native species are the superior exploitation of disturbance by exotic species and the competitive displacement of native species by their exotic counterparts. Theory predicts that functional similarity will increase the intensity of competition between native and invasive species. Ecologically important "foundation" species, *Zostera marina* and other seagrasses have globally declined during the past century. This study used transplant and vegetation removal experiments to test the hypotheses that disturbance and competitive interactions with an invasive congener (*Z. japonica*) are contributing to the decline of native *Z. marina* in the northeastern Pacific. Interspecific competition reduced *Z. marina* and *Z. japonica* aboveground biomass by 44% and 96%, respectively, relative to intraspecific competition. Disturbance substantially enhanced *Z. japonica* productivity and fitness, and concomitantly decreased *Z. marina* performance, effects that persisted two years following substrate disturbance. These results demonstrate that disturbance and competitive interactions with *Z. japonica* reduce *Z. marina* performance, and suggest that *Z. japonica*'s success as an invasive species stems dually from its ability to persist in competition with *Z. marina* and its positive response to disturbance. These results highlight the importance of understanding the interconnected roles of species interactions and disturbance in the decline of seagrass habitats, and provide a rationale for amending conservation policy in Washington State. In the interest of conserving native eelgrass populations, the current policy of protecting both native and invasive *Zostera* spp. should be refined to differentiate between native and invader, and to rescind the protection of invasive eelgrass.

3.2. INTRODUCTION

Understanding factors that control the establishment and spread of invasive species is a major goal of ecology and invasion biology. Competitive interactions with native species influence the establishment and spread of invasive populations (e.g., Corbin and D'Antonio 2004) and are important mechanisms of invader impacts to native taxa (e.g., Morrison 2000, Brown et al. 2002, Branch and Steffani 2004). The consequences of competition with resident species for invasion success are the basis of the hypothesized link between native species richness and invasion resistance. According to the diversity-invasion resistance hypothesis, speciose communities are less invasible than depauperate communities because fewer unexploited resources are available to invaders (Elton 1958, Case 1990, Stachowicz et al. 2002). Recent niche theory has corroborated the link between species richness and invasion resistance by linking resource competition between native and invasive species to reductions in invader "niche opportunities", or conditions favoring establishment (Shea and Chesson 2002). Unless resources are spatially or temporally partitioned, species with similar resource and habitat requirements are predicted to compete intensely (Dudgeon et al. 1999). Thus, strong competition is expected to arise between functionally similar native and invasive species.

Many invasive species are, however, weak competitors whose establishment and spread is facilitated by disturbance. Often a key determinant of the invasibility of natural communities (Elton 1958, Moyle 1986, Mack 1989, Minchinton and Bertness 2003), disturbance can facilitate invasion success by removing competitively superior native species, by mediating interactions between native and invasive species, or by physically or chemically altering the environment in ways that favor invaders (Lake and Leishman

2004). Perennial, native eelgrass (*Zostera marina*) is the dominant macrophyte of low intertidal and shallow subtidal flats in the northeastern Pacific (i.e., the Pacific Northwest coast of North America). On low intertidal flats, it co-occurs with annual, invasive dwarf eelgrass (*Z. japonica*). *Z. japonica* is rapidly invading both unvegetated mudflats and vegetated flats historically dominated by intertidal *Z. marina* populations. Like many kelps, mangroves and corals, *Zostera* spp. and other seagrasses are "ecosystem engineers" (sensu Jones et al. 1994, 1997) that physically modify substrate and water column characteristics and provide the structural foundation for their associated communities (Orth 1977, Heck and Orth 1980, Fonseca et al. 1982). Assessing their relative competitive abilities and responses to disturbance are important to understanding their interaction, which has direct implications for the management and conservation of intertidal seagrass habitats in the Pacific Northwest.

The occurrence of *Z. japonica* in the Pacific Northwest was first recorded in 1957, in the southern Washington estuary of Willapa Bay (Harrison and Bigley 1982). It was likely introduced with Pacific oyster (*Crassostrea gigas*) aquaculture stock shipments, which have been imported from Japan to Willapa Bay since the 1920s (Phillips 1984). Willapa Bay is one of the leading oyster-growing regions in the U.S., producing approximately 15% of the national harvest (Dumbauld et al. 2000, B. Dumbauld, pers. comm.). *Z. japonica* currently occupies the majority of intertidal mudflats in Willapa Bay and has also spread to several estuaries between the Coquille River Estuary, Washington, and the southern Strait of Georgia, British Columbia (Larned 2003). In invaded estuaries, native *Z. marina* and introduced *Z. japonica* can overlap in tidal elevational ranges and occupy vast mixed-species meadows.

In the species' native ranges, *Z. marina* is widely distributed in the Pacific and Atlantic basins, and *Z. japonica* occurs between northeastern Russia and tropical Vietnam (den Hartog 1970). Little is known of the interaction of *Z. marina* and *Z. japonica* in either their native or introduced habitats (but see Nomme and Harrison 1991a, b). Although native Asian populations of the species co-occur, the Pacific Northwest population of *Z. marina* has evolved in the absence of *Z. japonica* and could experience fitness reductions as the result of novel interactions with its congener.

Understanding the interaction between *Z. marina* and *Z. japonica* is germane to the management and conservation of seagrass habitats, which have globally declined over the past century. These declines have generated considerable ecological and economic concern, as seagrasses perform vital ecosystem services, including primary production, habitat generation for ecologically and economically important finfish and shellfish species, and shoreline buffering from erosion and eutrophication (Phillips and Menez 1988, Alberte et al. 1994, Williams and Davis 1996). *Z. marina* is the most abundant seagrass in the Northern Hemisphere (Baden et al. 2003) and the dominant native macrophyte of Pacific Northwest mudflats. It has experienced an unquantified decrease from its historical distribution in Washington State (Levings and Thom 1994). Seagrass declines have been attributed to a number of factors, including coastal development, sediment and nutrient loading, and disease (Short and Wyllie-Echeverria 1996, Williams and Davis 1996, Green and Short 2003). This study is the first to experimentally assess the roles of disturbance and competition with an invasive seagrass in the decline of a native seagrass.

In response to regional native seagrass declines, the state of Washington has adopted a "no-net-loss" policy for the management of eelgrass habitats (Pawlik and Olson 1995). This policy is based on the documented habitat values of native eelgrass beds to fish and wildlife, including the importance of intertidal beds as foraging habitats for shorebirds and waterfowl (Fresh 1994, Hershman and Lind 1994, Wyllie-Echeverria et al. 1995). At present, the policy does not distinguish between invasive *Z. japonica* and native *Z. marina*. Hence, the species are conferred equal protection, despite the paucity of information on their interaction and on the ecological value of *Z. japonica* habitats (but see Posey 1988). However, if interactions with *Z. japonica* are contributing to *Z. marina* declines, the protection of invasive eelgrass may conflict with the conservation of native eelgrass habitats. Data on the impacts of *Z. japonica* invasions to *Z. marina* are needed to formulate appropriate management and policy responses to the spread of *Z. japonica*.

This study used manipulative competition and disturbance experiments to evaluate the relative competitive abilities of *Z. marina* and *Z. japonica* and to compare the species' responses to disturbance. The specific objectives were to 1) compare the relative effects of intra- and interspecific competition on growth and productivity, and 2) determine the effects of disturbance on aboveground biomass and reproductive output (a proxy for fitness). Nomme and Harrison (1991 a, b) implicitly evaluated the interaction between *Z. marina* and *Z. japonica* by 1) comparing *Zostera* spp. morphology in monospecific patches (sods) transplanted into three different tidal zones, and by 2) conducting multivariate analyses of individuals in naturally established mixed and monospecific beds. In their study, monospecific *Zostera* spp. beds were located in different tidal zones than mixed beds, potentially confounding the effects of species interactions with that of tidal elevation. In contrast, this study employed experimental manipulations that controlled for tidal elevation and explicitly tested for interaction between the two congeners. The results of this study provide a basis for re-evaluating current management policies for seagrass species, and demonstrate how understanding species' interactions and autecology can contribute to species conservation and invasive species management.

3.3. MATERIALS AND METHODS

Field experiments were conducted from July 2002 to June 2004 at Stackpole Slough in the Willapa National Wildlife Refuge in northwestern Willapa Bay, Washington (124°06' W, 46°24' N). Willapa Bay is a shallow, 260 km^2 estuary that contains approximately 18,800 ha of intertidal mudflats and extensive *Zostera* spp. beds. Annual *Z. japonica* typically occupies tidal elevations of +1 to +3 m relative to mean lowest low water (MLLW), and perennial *Z. marina* typically occupies elevations of -1 to +2 m MLLW (Dumbauld and Wyllie-Echeverria 2003). This study evaluated the interaction between the two species in their range of tidal elevational overlap (+1 to +2 MLLW). In this zone of overlap, *Z. marina* forms a taller canopy and grows in lower shoot densities than *Z. japonica*. Background data on percent cover, shoot lengths, and shoot densities were collected in the mixed eelgrass zone of Stackpole Slough in 2001 along 21 random transects perpendicular to the shoreline. The average shoot lengths of *Z. marina* and *Z. japonica* were 76.77±28.29 cm and 31.84±12.99 cm, respectively (all values herein are mean ± one standard deviation). Background shoot densities per 0.25m^2 were 14.30±14.60 for *Z. marina* and 82.70±70.25) for *Z. japonica*. Average percent cover in 0.25m^2 quadrats was 48.4 ±34.88% for *Z. marina* and 54.80±34.62% for *Z. japonica*.

Previous studies have demonstrated that variation in seagrass habitat structure can influence infaunal community structure (Webster et al. 1998), habitat use by fishes (Jenkins and Sutherland 1997), and algal recruitment and mortality (Inglis 1994). Thus, morphological and structural differences between *Z. marina* and *Z. japonica* may translate to differences in the structure and ecological functions of their associated communities (but see Posey 1988).

3.3.1. Competition Experiment

3.3.1.1. Experimental Design

In July 2002, 27 0.25m^2 study plots were established in an intertidal mixed-eelgrass meadow on a transect parallel to the shoreline. Nine blocks of three 0.5 × 0.5 m plots were cleared by hand of all existing above- and belowground vegetation. In addition, 0.5 m-wide buffer zones were cleared around plot margins to prevent the encroachment of surrounding vegetation. Plots within blocks were spaced by 2 m and blocks were separated by 5-10 m. A shallow layer of water (1-3 cm) covered all plots during low tides. Three treatments were randomly assigned to plots within each block: monospecific *Z. japonica*, mixed-species, and monospecific *Z. marina*.

Transplants were collected from a mixed-eelgrass meadow adjacent to the study site. Eight individuals were transplanted into each plot in an even array, with four individuals of each species planted for the mixed-species treatment. Each transplanted individual consisted of a terminal ramet, its adjacent ramet (short shoot), and their shared root-rhizome complex. The ramet density of transplants in the experimental plots (16/0.25m^2) was within the natural range of *Zostera* spp. ramet densities in Willapa Bay. Mean leaf lengths, recorded at the time of transplanting, were 70.9±25.5 cm for *Z. marina* transplants and 27.1±14.6 cm for *Z. japonica* transplants (n=108). Transplants were rinsed of sediments and anchored in the substrate with 20 cm-long ground staples.

The 27 plots were monitored for transplant survival and maintained at 2-week intervals by clearing any vegetation that had encroached from the vegetated outer mudflat into the 0.5-m buffer zones surrounding study plots. No transplants encroached from the study plots into the buffer zones. Transplants were allowed to recover from transplant shock for 1 month prior to marking leaves for leaf growth rate estimates. Leaves were marked on 21-22 August 2002 and all above-ground biomass was harvested on 7 September for leaf growth rate and dry-weight (DW) biomass measurements.

Although this experiment was conducted using a randomized complete block design (RCBD), block effects were non-significant for biomass and leaf growth rate responses and treatment effects were subsequently analyzed in a completely randomized design (CRD) framework. Variations in biomass and leaf growth rates between treatments were analyzed for both species with one-way analysis of variance (ANOVA) in JMP 5.0 (SAS 2002).

3.3.1.2. Leaf Growth Rates and Above-ground Biomass

Leaf growth rates were measured with a variation of Zieman's (1974) leaf-marking technique, in which each non-growing leaf sheath and its enclosed growing leaves were punctured with sewing pins. Transplant leaves were marked with two adjacent pinholes to aid in the differentiation of leaf-marking scars from other leaf damage. Approximately 2 weeks after leaf marking, transplants were revisited and all aboveground biomass was clipped, bagged, and transported to a laboratory for measurement. Leaves were wiped clean of epiphytes and leaf growth rates were measured as the distances between pinhole scars on the leaf sheaths and leaves, divided by the number of days between marking and measurement. One leaf was randomly selected from each shoot for measurement. After leaf growth measurements were conducted, the contents of each plot were separated by treatment and species. Samples were dried in paper bags in a drying oven for 14 days at 60° C for DW biomass measures.

3.3.1.3. Interaction Intensities

Relative interaction intensities were calculated for each species to evaluate the symmetry of inter- and intraspecific competition. Relative competition intensity (RCI) and log response ratio (ln RR) were calculated as metrics of competitive intensity based on experimental biomass measurements. RCI is the most commonly used interaction strength metric in experimental plant ecology (Goldberg et al. 1999) and is most commonly applied with biomass as a response variable. RCI was calculated as $RCI=(X_{monospecific} - X_{mixed})/X_{monospecific}$ where X=final individual biomass. To address the morphometric differences between the two study species, standardized interaction intensities were also calculated as $\ln RR=\ln(X_{monospecific}/X_{mixed})$. A RCI of 0 indicates no effect of interspecific interaction; a positive RCI indicates competition (i.e., reduced performance); and a negative RCI indicates facilitation (enhanced performance).

3.3.2. Disturbance Experiment

3.3.2.1. Experimental Design

The destructive vegetation sampling in September 2002 of the 27 plots utilized in the transplant experiment constituted the vegetation removal treatment for the disturbance experiment. The disturbance associated with vegetation removal was roughly analogous to several forms of disturbance common to mudflat habitats (e.g., shellfish dredging, crushing by watercraft, and trampling), though perhaps more severe because all above- and below-ground vegetation were removed. The plots were allowed to naturally revegetate for two years. In June 2004, all aboveground biomass was collected from the original 27 0.25-m^2 plots to assess the recovery of *Z. marina* and *Z. japonica* following disturbance. In addition, 27 0.25-m^2 control plots were randomly sampled from a transect parallel to and spaced 2 m from the experimental plots. In the shallow bathymetry of Willapa Bay mudflats, a 2-m distance on a tidal plain is insufficient to produce a detectable difference in tidal elevation, provided that channels are avoided. Disturbance and control plots were thus located at equivalent tidal elevations. The following variables were measured for each species in each plot: 1) number of shoots, 2) number of flowering shoots, 3) average number of inflorescences per short, 4) maximum number of inflorescences per flowering shoot, and 5) average shoot length of five randomly selected shoots (or all shoots if fewer than five were present). After these measurements were completed, samples were dried for 14 days at 60° C for DW biomass measures.

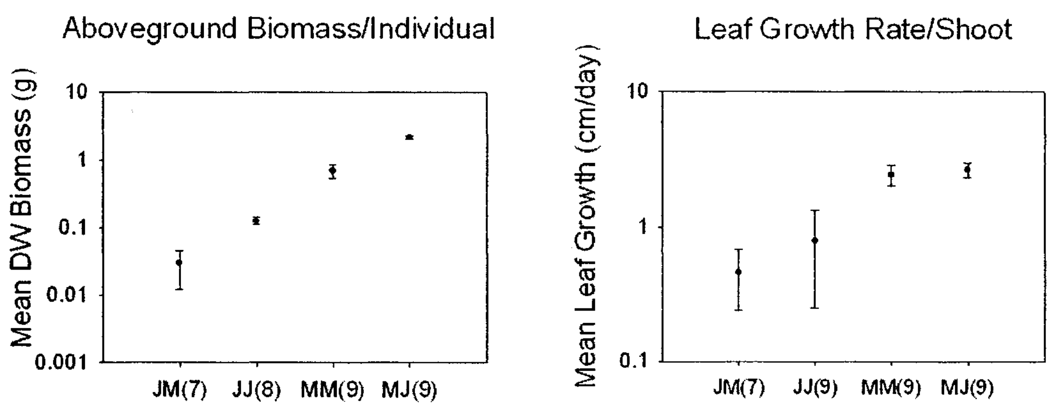
3.4. RESULTS

3.4.1. Competition Experiment

3.4.1.1. Above-ground Biomass

The final mean above-ground biomass of *Z. marina* individuals was 55% higher in the monospecific treatment plots than in the mixed-species treatment plots (ANOVA; $F_{1, 17}$=9.01; P<0.01; Figure 3.1). Mean above-ground biomass of *Z. japonica* was 165% higher in the monospecific plots than in the mixed-species plots (ANOVA; $F_{1, 14}$=7.80; P=0.015; Figure 3.1).

Figure 3.1. Competition experiment. Mean values ± 1 SD for *Z. marina* and *Z. japonica* response variables in the presence and absence of congeners. Sample sizes (number of plots) are in parentheses. JM=*Z. japonica* in mixed plots, JJ=*Z. japonica* in monospecific plots, MM=*Z. marina* in monospecific plots, and MJ=*Z. marina* in mixed plots.



3.4.1.2. Leaf Growth Rates

Mean daily leaf growth rates of *Z. marina* in the mixed-species treatment did not differ from that in the monospecific treatment plots (ANOVA; $F_{1, 17}$=1.43; P=0.25; Figure 3.1). Similarly, the mean leaf growth rate of *Z. japonica* did not differ between mixed (0.46±0.22 cm, n=7) and monospecific treatment plots (0.78±0.53 cm, n=9) (ANOVA; $F_{1, 17}$=1.43; P=0.25; Figure 3.1).

3.4.1.3. Interaction Intensities

In both species, relative competition intensity (RCI) and log response ratios (ln RR) demonstrated that interspecific competition had much stronger negative effects on above-ground biomass than intraspecific competition. RCI values indicated that interspecific competition reduced *Z. marina* biomass by 35% (RCI=0.35) and *Z. japonica* biomass by 62% relative to intraspecific competition. Similarly, ln RR values indicated that interspecific competition reduced *Z. marina* and *Z. japonica* biomass by 44% and 96%, respectively.

3.4.2. Disturbance Experiment

3.4.2.1. Productivity

Disturbance resulted in a 14-fold reduction in *Z. marina* biomass ($F_{1, 53}$=163.4; P<0.0001), a 4-fold reduction in shoot density ($F_{1, 51}$=79.2; P<0.0001), and a 50% reduction in average shoot length ($F_{1, 48}$=78.4; P<0.001; Table 3.1). In contrast, disturbance was associated with an 11-fold increase in *Z. japonica* biomass ($F_{1, 52}$=21.6; P<0.0001, a 12-fold increase in shoot density ($F_{1, 51}$=33.1; P<0.0001), and no effect on shoot length ($F_{1,48}$=0.05; P=0.83; Table 3.1). Thus, while the biomass of the native decreased 14 fold, that of the invader increased 11 fold, giving the invader a massive [(14×11)-fold] advantage in disturbed plots.

3.4.2.2. Reproductive Output

Disturbance had no effect on the number of *Z. marina* flowering shoots produced per plot ($F_{1,52}$=1.52; P=0.22) or the average number of inflorescences per flowering shoot ($F_{1,9}$=3.56; P=0.81). However, disturbance was associated with a 6-fold decrease in the maximum number of inflorescences per *Z. marina* flowering shoot ($F_{1,9}$=14.0; P<0.01). In contrast, disturbance resulted in a 19-fold increase in *Z. japonica* flowering shoot production ($F_{1,53}$=6.25; P=0.016), but had no effect on average number of inflorescences per shoot ($F_{1,21}$=0.062; P=0.81) or the maximum number of inflorescences per flowering shoot ($F_{1,21}$=0.0058; P=0.94; Table 3.1).

3.5. DISCUSSION

Although *Z. japonica* is common and abundant in Willapa Bay and other invaded estuaries, its impacts on native communities and ecosystems remain poorly understood. The consequences of *Z. japonica* invasion for native eelgrass, and the functional differences between native and invasive eelgrass, are questions of immediate relevance to management. Previous studies have demonstrated that *Z. japonica* invasions alter estuarine nutrient dynamics (Hahn 2003, Larned 2003), cause sediment and infaunal community changes (Posey 1988), and modify waterfowl foraging habitats (Baldwin and Lovvorn 1994). *Z. japonica*'s impacts to fish and shellfish have yet to be evaluated, but structural differences between *Z. japonica* and *Z. marina* beds may translate to differences in planktonic larval retention and the quality of epibenthic nursery and feeding habitats (e.g., Jenkins and Sutherland 1997, Webster et al. 1998). This study is the first to empirically demonstrate the strength and asymmetry of interspecific competition between *Z. marina* and *Z. japonica,* and the differential effects of disturbance on the performance of each species.

The competition experiment revealed that both species experienced substantial reductions in aboveground biomass in response to interspecific competition, relative to intraspecific competition. The relative reduction in biomass was greater for *Z. japonica*, indicating that *Z. marina* is a better competitor in the absence of disturbance. However, when the species were subjected to disturbance, *Z. japonica* productivity and fitness improved dramatically, while *Z. marina* performance correspondingly declined. Thus, disturbance and some competitive ability (i.e., the ability to persist in competition with *Z. marina*) appear to underlie the invasiveness of *Z. japonica. Z. japonica*'s positive response to disturbance is particularly relevant to its invasion success, as tidal flats are dynamic systems that experience high levels of natural and anthropogenic disturbance, including bioturbation, coastal development, boating, shellfish culture, and in some regions, invasive smooth cordgrass (*S. alterniflora*) control. The additive and/or synergistic effects of disturbance and competition with *Z. japonica* could have profoundly negative effects on intertidal *Z. marina* and its associated community

Previous studies that have assessed the effects of competition and disturbance on plant invader success have frequently demonstrated that exotic dominance is determined by the interaction of competitive traits with disturbance. Gerlach and Rice (2003) demonstrated that star thistle (*Centaurea solstitialis*) invasiveness stemmed from the species' ability to persist in competition with annual grasses, combined with its plastic growth and reproductive responses to disturbance. In contrast, the competitive dominance of Scotch broom (*Cytisus scoparius*; Paynter et al. 2003) and some invasive perennial grasses (MacDougall and Turkington 2004) appears to depend on the absence of disturbance. Ultimately, an invader's success may depend on the interaction of competitive abilities, disturbance regime, and the availability of limiting resources (MacDougall and Turkington 2004).

Possible mechanisms for the performance reductions observed in association with competition and disturbance include light and nutrient limitation. Impaired water clarity, epiphyte loading, and overgrowth by macroalgae are all plausible outcomes of disturbance that would result in eelgrass declines driven by light limitation (Hauxwell et al. 2003). Competition for nutrients may be a common phenomenon among seagrasses, which are often nutrient-limited (Short 1983, Dennison et al. 1987), and simultaneously exploit nutrients in sediments and the water column (Iizumi and Hattori 1982, Thursby and Harlin 1982, Short and McRoy 1984, Williams and Ruckelshaus 1993). *Z. japonica* has a higher surface area-volume ratio and a higher NO_3 influx rate per above-ground biomass unit than *Z. marina* (Larned 2003), which suggests that it is a superior competitor for water-column nutrients.

Considering the responses of foundation species to varying disturbance intensities is necessary to formulate effective management plans for habitats that experience dynamic disturbance regimes. The disturbance experiment demonstrated the dramatic and persistent enhancement of *Z. japonica* fitness, and reduction of *Z. marina* fitness, following disturbance. The competition experiment demonstrated that interspecific competition between *Z. marina* and *Z. japonica* was stronger than intraspecific competition. Stronger inter- than intraspecific competition is destabilizing, and may accelerate the displacement of *Z. marina* by *Z. japonica*. Previous studies have suggested that *Z. marina* and *Z. japonica* perform similar ecological functions (e.g., Posey 1988). The disparities in *Zostera* species' responses to disturbance highlight the importance of studying the autecology and interactions of native and invasive species that appear to be functionally similar.

These experimental results, combined with the simultaneous irruption of *Z. japonica* and decline of *Z. marina* in the Pacific Northwest, strongly suggest that disturbance and interactions with *Z. japonica* are factors in *Z. marina* declines. Estuarine mudflats are subject to high levels of natural and anthropogenic disturbance. Since *Z. japonica* is able to persist in competition with *Z. marina*, and since disturbance confers a substantial and persistent fitness advantage to *Z. japonica*, the invader is likely to dominate disturbed areas, to the detriment of its native congener. Although this study did not mechanistically link the results of the competition and disturbance experiments to naturally occurring *Z. marina* declines, they are consistent with observed patterns of decline. The results of this study suggest that the current Washington State policy of conferring blanket protection to *Zostera* spp. is inconsistent with the goal of protecting native eelgrass. The effective conservation of intertidal *Z. marina* habitats may require refining this policy to differentiate between native and invasive eelgrass species. Although additional information is needed to determine the relative costs and benefits of controlling *Z. japonica*, the information at hand suggests that at the very least, the protection of invasive eelgrass should be rescinded in the interest of conserving native intertidal eelgrass habitats.

3.6. ACKNOWLEDGEMENTS

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TABLES

Table 3.1. Disturbance experiment. Mean values (standard deviations in parentheses) for *Zostera marina* and *Z. japonica* response variables in disturbed and control plots.

	Zostera marina		*Zostera japonica*	
Response Variable	**Disturbed**	**Control**	**Disturbed**	**Control**
Biomass (g)	1.46 (1.90)**	20.2 (7.39)**	3.36 (3.39)**	0.29 (0.52)**
# Shoots	5.19 (5.05)**	22.3 (8.48)**	123 (1.1)**	10.2 (12.0)**
#Flowering Shoots	0.11 (0.33)	0.37 (1.00)	5.56 (10.9)*	0.30 (1.03)*
Ave # Inflorescences/Shoot	2.00 (1.00)	6.94 (4.35)	2.33 (1.06)	2.17 (0.76)
Max # Inflorescences/Shoot	1.50 (1.29)*	8.83 (3.71)*	3.42 (1.89)	3.33 (1.53)
Average Shoot Length	43.1 (13.4)**	92.3 (23.0)**	27.2 (11.0)	27.9 (9.78)

* 0.001<P<0.05; ** P<0.001

Chapter 4. Invasion dynamics in a heterogeneous landscape: the interaction of spatial environmental variation and competition in seedling recruitment of an invasive plant

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4.1. ABSTRACT: Although theory predicts that spatial habitat heterogeneity will influence the outcome of local species interactions and their regional population consequences, few studies have empirically examined the interaction of habitat heterogeneity and competition in the spread of invasive species. We used field manipulations to investigate the covariation of hydrodynamic stress and smooth cordgrass (*Spartina alterniflora*) seedling recruitment in the presence and absence of a simultaneously invading competitor, dwarf eelgrass (*Zostera japonica*). *Z. japonica* and *S. alterniflora* are two ecologically and economically problematic Pacific Coast invaders. Hydrodynamic stress and *Z. japonica* presence strongly and interactively decreased *S. alterniflora* seedling growth and survival. Because *S. alterniflora* invasions increase sedimentation, reduce tidal flows, and alter primary productivity and community structure, *Z. japonica*'s effects on *S. alterniflora* population dynamics indirectly influence estuarine structure and function. These results demonstrate the importance of simultaneously considering species interactions and the consequences of spatial environmental variation in invader spread.

4.2. INTRODUCTION

Exotic species invasions rank among the foremost threats to native biodiversity and the maintenance of ecosystem services (Vitousek et al. 1997). Several decades of research have catalogued a myriad of ecological, economic, and social consequences of biological invasions, including declines and extinctions of native species, reduced agricultural production, and threats to human health (Wilcove et al. 1998, Pimentel et al. 2000, Sala et al. 2000). Despite mounting scientific and political interest in predicting and controlling species invasions, our understanding of the proximate mechanisms underlying successful invasion remains limited. This lack of understanding has often restricted predictions of the spread and impacts of invaders and the development of effective control strategies (Suarez et al. 1999).

One major complication to predicting the rate and spatial extent of invasions is that the physical and biological landscape encountered by invaders is rarely uniform. There is growing appreciation among invasion biologists that spatial environmental heterogeneity can have major effects on invasive spread, although theoretical and empirical understanding of this influence is still limited (With 2002, Hastings et al. 2005). With respect to species interactions, theoretical models predict that invasion speed should decrease as a function of the competition strength between resident and invading species (Okubo et al. 1989, Hart and Gardner 1997). This strength of competition between residents and invaders often depends on spatially and temporally varying levels of resource supply (Tilman 1988, Davis et al. 2000, Davis et al. 2000, Shurin et al. 2004). The predation strength faced by invaders has also been shown to depend on spatially varying habitat characteristics (Fagan and Bishop 2000, Byers 2002).

In addition, there is evidence that the relative strength of positive and negative species interactions can vary spatially (Callaway and Walker 1997, Bruno et al. 2003). Much of this evidence comes from studies of plant community interactions in wetlands, systems characterized by strong gradients in physical stress that are associated with tidal inundation and wave energy (Bertness and Callaway 1994). A wealth of work in New England salt marshes has demonstrated that positive species interactions predominate in more physically stressful locations, while negative species interactions predominate in more benign locations (Bertness and Callaway 1994, Brooker and Callaghan 1998, Bertness and Ewanchuk 2002). Few studies, however, have attempted to assess how physical characteristics and species interactions interact to influence patterns of invasion in these systems.

We evaluated how the invasion success of the intertidal grass *Spartina alterniflora* is influenced by variation in physical stress and by interaction with another invasive intertidal grass, dwarf eelgrass (*Zostera japonica*). *S. alterniflora* and *Z. japonica* are ecologically important invaders of intertidal mudflats in the Pacific Northwest US. Both *S. alterniflora* and *Z. japonica* invasions alter estuarine nutrient dynamics, cause sediment and infaunal community changes, and modify waterfowl foraging habitats (Posey 1988, Baldwin and Lovvorn 1994, Daehler and Strong 1996, Hahn 2003, Larned 2003, Ayres et al. 2004).

The above-ground vegetation of both species creates drag, which attenuates the physical stress associated with wave energy (Fonseca et al. 1982, Bertness and Callaway 1994). While this habitat modification could potentially facilitate the recruitment and spread of *S. alterniflora*, no studies have tested this hypothesis. In addition, no studies have assessed the degree to which the relative strength of positive and negative interactions between the two species varies with levels of physical stress. We present the results of manipulative field experiments designed to quantify the effect of *Z. japonica* cover on *S. alterniflora* seedling recruitment across field sites that vary in levels of physical stress associated with hydrodynamic energy. The implications of our results for predicting the coexistence and spread of the two species in invaded habitats are discussed.

4.3. MATERIALS AND METHODS

4.3.1. Study system

We conducted field experiments in 2001, 2003, and 2004 to evaluate the interaction between two co-occurring invasive macrophytes, *Z. japonica* and *S. alterniflora*, on intertidal mudflats of Willapa Bay, Washington. Willapa Bay is a shallow, 260 km^2 embayment in southern Washington State, USA (46°40'N, 124°02'W) that contains approximately 18,800 hectares of intertidal mudflats. The bay's low intertidal and subtidal mudflats are dominated by native eelgrass (*Z. marina*) between approximately +1.8 to –6 m MLLW. Though currently dominated by invasive eelgrass (*Z. japonica*), the bay's intertidal mudflats above +1.8 m MLLW were historically unvegetated and fringed at their landward margin by native marsh. The native low marsh consists primarily of *Salicornia virginica, Jaumea carnosa, Triglochin maritimum* and *Plantago maritimum*. *Distichlis spicata, Deschampsia caespitosa*, *Juncus balticus* and *Potentilla pacifica* comprise the native high marsh dominants.

Over the last century, Willapa Bay's intertidal mudflats have been invaded by two non-native clonal macrophytes: smooth cordgrass (*S. alterniflora*) and dwarf eelgrass (*Z. japonica*). *S. alterniflora* is a perennial halophyte that is native to the North American Gulf and Atlantic coasts and is classified as a noxious weed in Washington state. It was probably accidentally introduced to Willapa Bay from New York State in the 1890s as packing material for Atlantic oysters, *Crassostrea virginica* (Sayce 1988). Recruitment by seed is the primary means of introduction of *S. alterniflora* into new habitats (Daehler 1999). Seedlings establish on open mudflats and expand vegetatively, eventually coalescing into dense, monospecific meadows.

Z. japonica is an annual Asian seagrass that is facultatively perennial in Pacific Northwest habitats (Phillips 1984) and often overwinters in Willapa Bay and other sheltered estuaries. It was probably inadvertently introduced from Japan as packing material for Pacific oysters (Harrison and Bigley 1982) and was first documented in Willapa Bay in 1957 (Phillips 1984). The ecological impacts of *Z. japonica* invasion are largely unknown, although the species negatively affects biomass and growth rates of its native congener, *Z. marina* (Bando Chapter 3). *Z. japonica* and other seagrasses are known to reduce local flow rates, decrease sediment particle size, and increase sediment accretion (Orth 1977, Fonseca et al. 1982, Irlandi and Peterson 1991).

Approximately one-third of the bay's intertidal mudflats have been invaded by *S. alterniflora* (Brown et al. 2001). While quantitative estimates of *Z. japonica* distribution are unavailable, the species is highly abundant and dominates higher-elevation intertidal mudflats where *S. alterniflora* and native marsh are absent. The upper tidal elevational range of *Z. japonica* overlaps with the lower range of *S. alterniflora*, and the two species often co-occur in Willapa Bay. The absence of native macrophytes at the tidal elevations relevant to this study allowed us to target the effects of *Z. japonica* on *S. alterniflora* seedling survival.

4.3.2. Site characteristics

Experimental sites represented the common range of hydrodynamic conditions experienced by macrophytes on tidal flats in Willapa Bay, WA. We estimated hydrodynamic energy at the sites indirectly, using the dissolution rates of zinc anodes. McGehee (1998) demonstrated that anode dissolution rate is proportional to current flow both in the laboratory and in subtidal field conditions. We affixed weighed zinc anodes to PVC posts 10 cm above the tidal flat (n=10 per site). Anodes were deployed between May and October 2003. Zinc dissolution rates over the five months indicated that the sites differed in the storm and tide-driven flow they experienced. Two sites (Leadbetter and Pickernell) experienced relatively high flow, while two sites (Palix and Tarlatt) experienced relatively low flow (Table 4.1). Sediment grain size is expected to be proportional to hydrodynamic energy, with fine-grained clay and mud sediments restricted to low energy conditions (Postma 1967). Sediment properties at the sites were consistent with this prediction (Table 4.1). Established *S. alterniflora* meadows and *Z. marina* beds were present at all four sites.

4.3.3. 2001 Leadbetter study

A pilot study in July-September 2001 evaluated the effects of naturally established *Z. japonica* cover on the growth and condition of transplanted *S. alterniflora* seedlings. The experiment was conducted on a large intertidal mudflat vegetated by *Z. japonica* in the Willapa National Wildlife Refuge on the northwestern boundary of Willapa Bay. The site is located seaward of a *S. alterniflora* meadow and landward of a mixed eelgrass (*Z. japonica* and native *Z. marina*) meadow. Forty pairs of ½ × ½ m plots were established on the mudflat. Treatment and control plots were randomly designated within each pair of plots. Control plots were left vegetated and the top layer of sediment was gently pressed and turbated to mimic the disturbance associated with *Z. japonica* removal.

Transplanted individuals were collected by hand-pulling *S. alterniflora* seedlings. Seedlings were differentiated from tillers of adult plants by their discrete taproots, lack of rhizomes, and isolation (>1 m) from adult clones. All transplant material was collected within 300 m of the study site and transplanted within 24 hours of collection into control and removal plots marked with PVC stakes. The removal treatment consisted of carefully clearing all above-ground *Z. japonica* cover by hand. Seedlings were washed of sediments and a single seedling was transplanted into the center of each plot. Removal plots were maintained by removing *Z. japonica* aboveground regrowth by hand twice monthly for 3 months. Control plots were maintained on the same schedule by disturbing the top layer of sediments to mimic the disturbance caused by *Z. japonica* removal. Seedling condition and growth were assessed 3 months after transplanting. Condition was recorded on a scale of 1 to 4, with 1=missing or dead (leaves brown and decaying), 2=poor (leaves wilted and yellow), 3=fair (leaves wilted but mostly green), and 4=good (leaves turgid and green). Paired t-tests were applied to compare seedling growth and condition in the presence and absence of *Z. japonica* cover.

4.3.4. 2003-2004 multi-site study

In 2003, a seedling transplant experiment was established at four sites in Willapa Bay to evaluate the effect of naturally established *Z. japonica* cover on *S. alterniflora* seedling survival. Sites were chosen to encompass the range of substrate and hydrological conditions commonly encountered by recruiting *S. alterniflora* individuals (Table 4.1). Treatment and control plots (n=10 per site) were established on open mudflats at four sites along a transect parallel to the shoreline. Experimental plots were established at Leadbetter State Park (mixed), Tarlatt Slough (mud), Pickernell Slough (mixed) and Palix River (mud). *Z. japonica* aboveground biomass was removed by hand from treatment plots. Control plots were pressed and their surface sediments were turbated to mimic the effects of *Z. japonica* removal. Two locally collected seedlings were transplanted into plots at each site. Treatment plots were maintained by removing *Z. japonica* cover monthly. Control plots were maintained by mimicking disturbance associated with *Z. japonica* removal on the same schedule. *S. alterniflora* seedling performance was assessed monthly at each site for 3 months. The survival and biomass of seedlings were analyzed using a two-way ANOVA with substrate type and *Z. japonica* cover treatment as factors

4.4. RESULTS

4.4.1. 2001 Leadbetter study

The presence of *Z. japonica* cover was associated with poorer average *S. alterniflora* seedling condition (ranked from 1 (lowest) to 4 (highest) at the end of the experiment; $X_{control}$=3.33, $X_{removal}$=2.69; paired t=2.85, df=39, P=0.003). The presence of *Z. japonica* cover reduced *S. alterniflora* seedling growth, although this effect was not statistically significant ($X_{removal}$±1 S.E.=14.0±3.36 cm; $X_{control}$=11.9±1.05 cm, paired t=0.84, df=24, P=0.20).

4.4.2. 2003-2004 multi-site study

S. alterniflora transplant survivorship differed strongly across sites. None of the 120 transplants survived the first year at Pickernell, the site with the highest measured rates of current flow, and only 3.33±1.95% (mean±S.E.) survived at Leadbetter, the next highest flow site. Survivorship was markedly greater at the two lowest flow sites, with 43.33±8.10% survivorship at Palix and 55.00±6.29% survivorship at Tarlatt, the lowest flow site.

Z. japonica removal increased *S. alterniflora* survivorship after one year, but the magnitude of the increase in survivorship depended on site (Figures 4.1 and 4.2; two-way ANOVA: removal $F_{1,72}$=10.72, P=0.002; site $F_{3,72}$=40.01, P<0.001; removal × site interaction $F_{3,72}$=3.69, P=0.02). Using planned comparisons within the two-way ANOVA, the difference in survivorship between removal and control plots was significant only at the Palix site ($F_{1,72}$=19.53, P< 0.001).

Figure 4.1. Survivorship of experimental *S. alterniflora* transplants over one year (June 2003-June 2004) at two high energy sites within plots cleared of *Zostera japonica* (○) and vegetated control plots (●). Values are means (n=10 plots)±1 S.E.

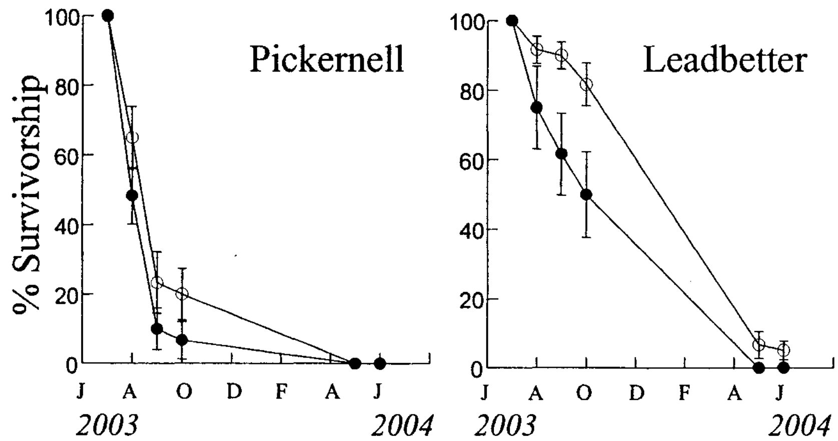
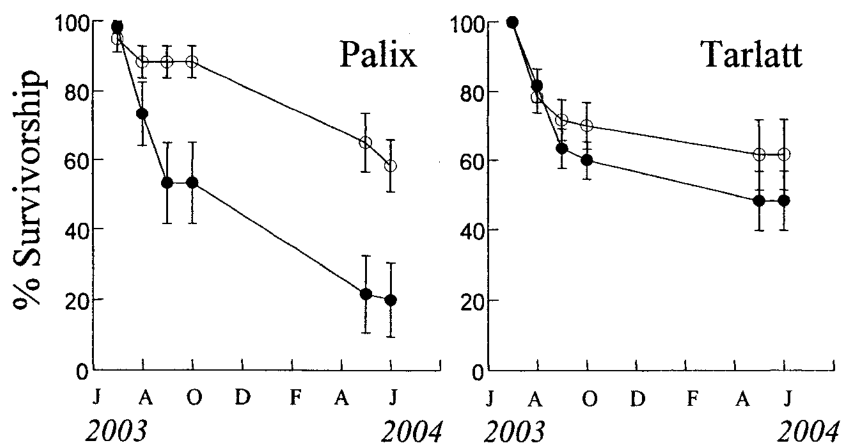
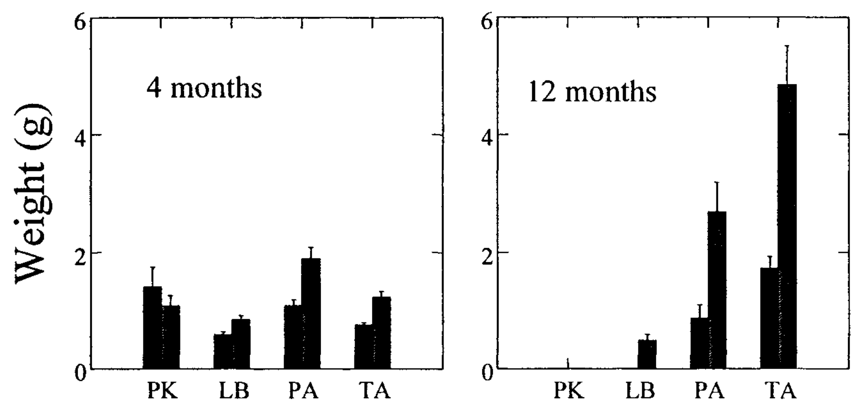


Figure 4.2. Survivorship of experimental *S. alterniflora* transplants over one year (June 2003-June 2004) at two low-energy sites within plots cleared of *Zostera japonica* (○) and vegetated control plots (●). Values are means (n=10 plots)±1 S.E.



The growth of *S. alterniflora* transplants varied across the four sites and was affected by the presence of *Z. japonica*. After four months, removing *Z. japonica* from plots resulted in a 50% or greater increase in *S. alterniflora* above-ground biomass at all sites except Pickernell (Figure 4.3; two-way ANOVA: removal $F_{1,250}$=5.64, P=0.02; site $F_{3,250}$=21.01, P<0.001; removal × site $F_{3,250}$=2.04, P=0.11). After 12 months there were only enough surviving seedlings to make comparisons at the two low-energy sites, but the effect of *Z. japonica* removal on *S. alterniflora* biomass was more pronounced, increasing *S. alterniflora* biomass more than twofold (Figure 4.3; two-way ANOVA: site $F_{1,108}$=11.09, P=0.001; removal $F_{1,108}$=26.67, P<0.001; site × removal $F_{1,108}$=0.88; P=0.35). The above-ground biomass of 1-year *S. alterniflora* seedlings at Palix was three times higher in removal plots (2.69±0.50 g=mean± 1 S.E.) than in control plots (0.87±0.24 g). *Z. japonica* removal caused a similar increase in above-ground biomass at Tarlatt. There, the above-ground biomass of seedlings was increased nearly threefold by removal of *Z. japonica* ($X_{treatment}$± 1 S.E.=4.84±0.67 g, $X_{control}$±1 S.E.=1.73±0.20 g).

Figure 4.3. Above-ground biomass of experimental *S. alterniflora* transplants after four months and 12 months at four sites. Values are means ± 1 S.E. of all surviving transplants within a treatment at each site; sample sizes vary across sites and treatments. Hashed bars are plots cleared of *Zostera japonica*; solid bars are vegetated control plots. PK = Pickernell; LB = Leadbetter; PA = Palix; TA = Tarlatt.



4.5. DISCUSSION

While the influence of *Z. japonica* on *S. alterniflora* seedling recruitment in this study was consistently negative, the relative strength and importance of this interaction was spatially variable. At sites with the highest hydrodynamic energy, *S. alterniflora* seedling recruitment after one year was nearly zero regardless of the presence of *Z. japonica*. This is consistent with the low observed natural abundance of seedlings at these sites relative to the two low energy sites (Table 4.1). The strong recruitment inhibition at these sites is likely related to both the direct and indirect influences of physical stress. There was greater storm intensity and sediment movement at the two high tidal energy sites. Nearly all of the PVC poles marking plots were lost over the winter as these sites, while no PVC was lost at either Palix or Tarlatt. In addition, seed bags that were placed on the sediment surface in October 2003 as part of a separate experiment were covered by 2-5 cm of sediment the following spring at both Pickernell and Leadbetter, where most seedlings perished, but were covered by only 0-2 cm at Palix and Tarlatt, where seedling survivorship was high.

Substrate characteristics related to tidal energy also likely influenced recruitment success. Sediment grain size is typically proportional to tidal energy, with coarse sediments occurring in high-energy areas and fine-grained sediments restricted to low energy conditions (Postma 1967). Nitrogen and organic matter content were inversely correlated with sediment grain size and positively correlated with *Spartina* spp. growth in previous studies (Gibson et al. 1994, Talley et al. 2000). In a common-garden experiment in Willapa Bay, *S. alterniflora* seedlings grown in sandy sediment performed poorly relative to seedlings grown in muddy sediment at both high and low energy sites (J. Lambrinos and K. J. Bando, unpublished data). In addition, nitrogen supplementation produced a greater biomass increase in adult *S. alterniflora* plants at sandy sites than at muddy sites in Willapa Bay (C. Tyler et al., unpublished data).

In contrast to the two sandy, high-energy sites, the 1-year survival of *S. alterniflora* transplants was considerably greater at the two muddy, low energy sites. At these more benign sites, the competitive influence of *Z. japonica* became apparent, although the influence was only statistically significant at the Palix site. These results suggest that the negative impact of *Z. japonica* on *S. alterniflora* may be ameliorated at the most benign sites, perhaps because nitrogen and other sediment macronutrients are less limiting.

To date, *S. alterniflora* has invaded one-third of Willapa Bay's intertidal mudflats. Unchecked, its spread will have profound economic consequences for the Willapa Bay region, which produces one-sixth of the U. S. commercial oyster harvest. Successful control of the species in Willapa Bay and other estuaries is likely to hinge on understanding the factors regulating its dispersal, spread, and colonization of new habitats. *Z. japonica* and *S. alterniflora* invasions are rapidly and dramatically altering the structure and function of Pacific Northwest estuarine habitats. Interactions between *Z. japonica* and *S. alterniflora* are likely to influence community structure because of the low above-ground diversity of the mudflat community and because the interacting species are "ecosystem engineers" (sensu Jones et al. 1994) that create structure for benthic invertebrates. In low-diversity communities, interactions between a few species may have particularly strong implications for community structure and ecosystem function.

Theoretical and empirical studies have illustrated that spatial and temporal resource heterogeneity can influence the strength and outcome of species interactions and permit the coexistence of competing species (Chesson 2003, Miller and Zedler 2003, Palmer 2003, Corbin and D'Antonio 2004). However, our understanding of the consequences of spatial heterogeneity for invader spread rates is still limited. Although the two most commonly applied types of spread models, reaction-diffusion and integrodifference equation models, are spatial in the sense that population densities are spatially variable, both types of models assume a homogeneous physical landscape and generally have not considered how spatial habitat heterogeneity influences invader spread (With 2002). In addition, few studies to date have explicitly addressed the impacts of natural enemies, such as predators and competitors, on rates of invader spatial spread (Hastings et al. 2005). In a rare study that directly examined the consequences of interactions with natural enemies on spread rates, Lonsdale (1993) showed that herbivores substantially reduced the spread rate of the invasive shrub *Mimosa pigra*. Both reaction-diffusion and integrodifference equation models have demonstrated that competition can slow invader spread, with invasion speed decreasing as a function of interaction strength (reviewed in Hastings et al. 2005).

Spatial habitat heterogeneity can influence invasion patterns through a variety of mechanisms that affect dispersal, colonization, or population growth (With 2002, Hastings *et al.* 2005). For instance, the spread of the invasive grass *Cortaderia jubata* in California is influenced by strong spatial variation in physical habitat structure that affects *C. jubata* seed dispersal and the strength of herbivory pressure on *C. jubata* seedlings (Lambrinos in press). Only a few theoretical studies, however, have addressed how mechanisms combine in heterogeneous landscapes to influence invasions. Some studies, for instance, have attempted to assess how spatial variability in both dispersal and demographic traits combine to influence spread (Cruywagen et al. 1996, Shigesada 1997, With 2002) In this study, habitat heterogeneity influenced *S. alterniflora* seedling recruitment both directly through physical effects on seedling growth and survival, and indirectly by mediating species interactions that influence seedling growth and survival. Interestingly, the strong competitive inhibition of *S. alterniflora* recruitment by *Z. japonica* at the low-energy sites tended to mitigate the strongly positive influence on *S. alterniflora* recruitment success caused by reduced physical stress at these sites. This suggests that the spatial correlation of different invasion mechanisms may have an important influence on invasion patterns. Facilitating and resisting mechanisms could potentially interfere with or amplify each other across a landscape. In addition, it is likely that different mechanisms act across different spatial scales of heterogeneity. In

Willapa Bay, for instance, substrate and water flow characteristics are fairly uniform across large regions of the estuary. *Z. japonica* cover, on the other hand, can vary dramatically over the scale of meters. It is unclear what influence this difference has on *S. alterniflora* recruitment patterns.

Although gaps clearly remain in our understanding of the interaction between competition and landscape structure in the spread of invasive species, prior studies on the effects of spatial competition and habitat heterogeneity suggest that the environmentally variable interaction of *S. alterniflora* and *Z. japonica* has consequences for their spatial spread. The suppression of *S. alterniflora* seedling recruitment by *Z. japonica* cover was stronger on muddy than sandy substrates. Because *S. alterniflora* primarily colonizes new sites by establishing on mud substrates from seed, this effect could slow the spread of *S. alterniflora* in Willapa Bay and its colonization of additional estuaries where *Z. japonica* is present. The results of this study suggest that priority effects influence the establishment and spread of *Z. japonica* and *S. alterniflora*. When *Z. japonica* invasion precedes *S. alterniflora* invasion, *Z. japonica* cover may limit *S. alterniflora* seedling recruitment and slow the rate of *S. alterniflora* invasion of new sites. However, *S. alterniflora* also spreads vegetatively by tillering, which permits it population growth even when seedling survival is negligible. Although *Z. japonica* can inhibit seedling establishment, it cannot prevent the vegetative expansion of established *S. alterniflora* clones. Furthermore, when *S. alterniflora* invasion precedes *Z. japonica* invasion, its dense canopy inhibits *Z. japonica* establishment by attenuating light (K. J. Bando, unpublished data).

Theoretical studies have assessed how dispersal and demographic rates influence population persistence in a landscape divided between favorable and unfavorable patches. Spatially-explicit metapopulation models generally demonstrate that persistence requires a threshold proportion of favorable patches that is partly determined by the rate of dispersal (Lande 1987, Sondgerath and Schroder 2001, Smith et al 2002). The exceptionally poor recruitment success of *S. alterniflora* at the high-energy sites in this study suggests that similar sites throughout Willapa Bay may act as population sinks. However, we currently have a poor understanding of the degree to which *S. alterniflora* populations in Willapa Bay are linked by dispersal.

Considering the role of habitat heterogeneity may be important for designing management and control regimes in spatially varying habitats, particularly because disturbance is often associated with the establishment and spread of invaders. Invasive species that modify the physical structure and ecological character of communities are useful models for exploring community assembly theory in natural systems. Priority effects may be particularly important in benthic marine systems that are characterized by only a few macrophyte or macroalgal species, whose interactions have important implications for community structure. Successful control of *Z. japonica* in Willapa Bay and other estuaries is likely to hinge on understanding the factors regulating its dispersal, spread, and colonization of new habitats. *Z. japonica* and *S. alterniflora* invasions are rapidly and dramatically altering the structure and function of Pacific Northwest estuarine habitats. Understanding the mechanisms underlying their patterns of distribution and abundance will improve our ability to predict and control the spread of these important invaders.

4.6. ACKNOWLEDGEMENTS

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TABLES

Table 4.1. Site characteristics

	Pickernell	*Leadbetter*	*Palix*	*Tarlatt*
Substrate	mixed sand + mud	mixed sand + mud	mud	mud
Pore water salinity (parts per thousand)	27.8±0.2	27.2±0.2	27.6±0.02	27.5±0.2
Zinc wt loss (g)	1.00±0.10	1.10±0.19	0.62±0.09	0.38±0.14
Natural *Spartina alterniflora* seedling abundance (seedlings/m^2)	9±5	10±9	152±118	517±428
Zostera japonica cover (% cover)	93±2	93±3	76±6	83±3