

Potential for Hybridization Between an Invasive Seagrass Species and its Native Congener

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In May 2006, we were awarded \$4934 under the Center for Invasive Plant Management's Seed Grant Program. Monies granted were to be used for travel to a field station (University of Washington's Friday Harbor Laboratories), field collections, laboratory breeding, and genetic analyses of field samples. Internal grants from my previous institution (Georgia College & State University) provided matching funds to pay for most travel-related costs, as well as to fund a M.S. student to assist with collections and measurements. Therefore, CIPM monies were used primarily to fund a sub-contract for molecular analyses of genetic samples.

Our goals were to examine the impacts of an invasive seagrass species (*Zostera japonica*, or dwarf eelgrass) on the region's dominant native seagrass species (*Zostera marina*, or eelgrass). Specifically, we proposed using collections, morphological measurements, breeding trials, and molecular analyses to assess the potential for hybridization between these two closely-related species. As an ancillary experiment, we also planned to examine the impacts of dwarf eelgrass on the population genetic structure and diversity of native eelgrass beds.

Results: Morphological Measurements

Fifty plants of each type (*Z. marina*, *Z. japonica*, and putative hybrids) were collected to analyze the interpopulation and interspecific variation among individuals. As in our preliminary trials, plants were assigned to putative groups and then measured for number of blades, blade length, and blade width. Groups were compared using ANOVA followed by SNK *post-hoc* tests (SAS 1999). Results supported preliminary data, showing distinct phenotypic differences (for two diagnostic characteristics: blade width and total blade length) among the three groups (Fig. 1, Fig. 2).

Results: Breeding Trials

In May 2007, 100 *Zostera japonica* and 100 *Zostera marina* plants were collected from Padilla Bay, Washington; specific identity was confirmed based on morphological features. Plants were stored in indoor flow-through seawater tanks at Friday Harbor Laboratories. Supplementary collections (100 plants per species) were made halfway through the breeding trials to ensure a constant supply of flowering individuals.

Every 8 hours for 3 weeks, plants were checked for flowering individuals. Crossing techniques followed those of Ruckelshaus (1994), with post-pollination bagging of inflorescences. A total of 275 interspecific (*Z. japonica* x *Z. marina*) crosses were attempted, and intraspecific crosses (N = 114) were also done to serve as a control. Two weeks after pollination, numbers of seeds per shoot were counted.

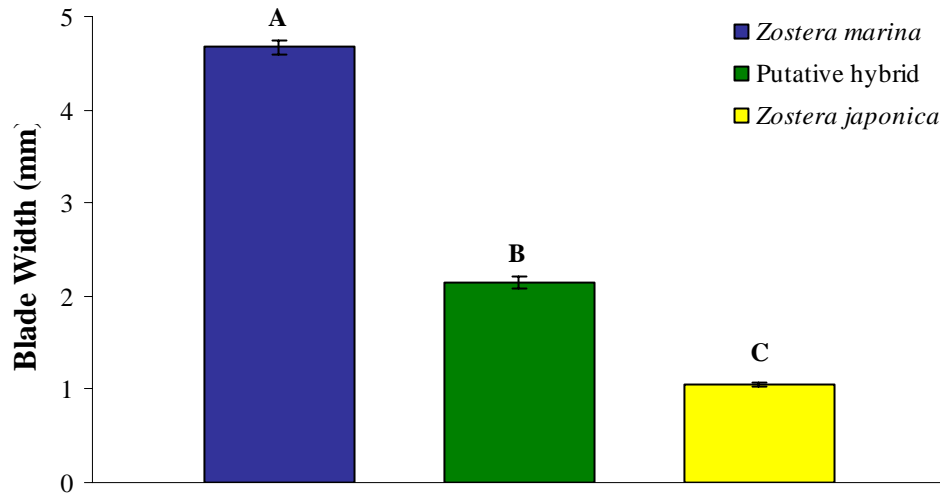


Figure 1. Mean (\pm 1 S.E.) blade width, which differed significantly among groups (SAS GLM; $P < 0.0001$). Letters above bars indicate results of a SNK post-hoc test.

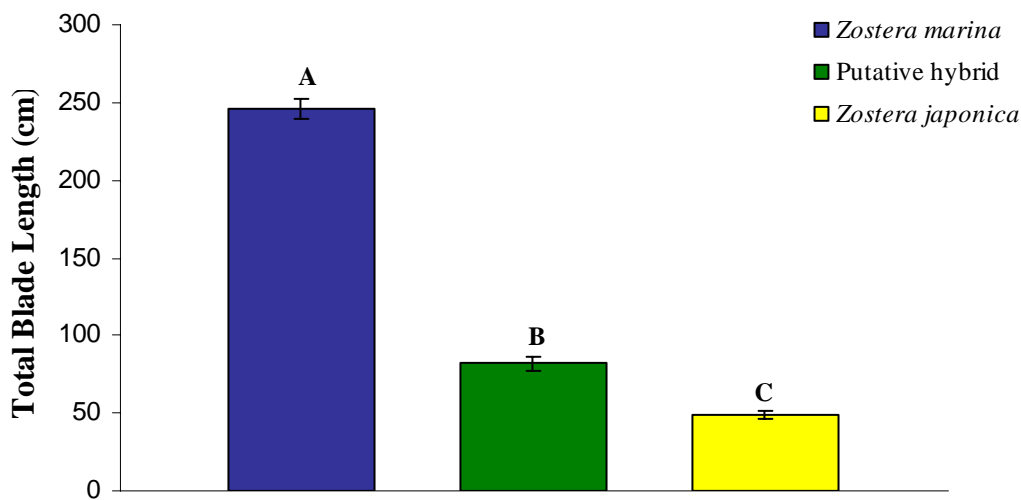


Figure 2. Mean (\pm 1 S.E.) length of all blades, which differed significantly among groups (SAS GLM; $P < 0.0001$). Letters above bars indicate results of a SNK post-hoc test.

Breeding trials yielded relatively low seed output (16% for intraspecific crosses; Fig. 3), comparable to those in other artificial mating studies with *Zostera* spp. (Ruckelshaus 1994, Rhode and Duffy 2004). Although viable seeds were produced from intraspecific matings, crosses between *Z. japonica* and *Z. marina* failed to yield any seeds. Further visual examination of inflorescences showed that, in many interspecific crosses, seeds seemed to have aborted prior to maturation. Based on these data, we conclude that, unlike in other plant systems (Levin et al. 1996, Salmon et al. 2005), the probability of harm to native eelgrass via hybridization with the invasive angiosperm is negligible.

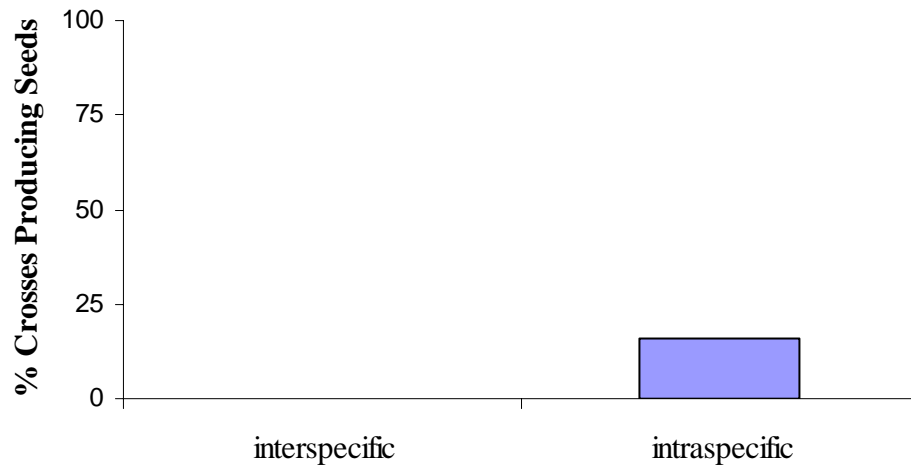


Figure 3. Percent of inter- and intraspecific crosses that produced viable seeds. Significantly more seeds were produced by intraspecific crosses (SAS GLM; $P = 0.0506$).

Results: Molecular Analyses of Putative Hybrids

One hundred plants from each group (*Z. marina*, *Z. japonica*, putative hybrids) were assayed using *Zostera marina*- and *Zostera japonica*-specific microsatellite markers to assign them a genetic identity. DNA was first extracted from dried tissue leaves using FastPrep DNA Extraction kits and then PCR-amplified for twelve microsatellite loci following standard protocols (Reusch et al. 2000). Next, fluorescently labeled microsatellite markers were assayed on an Applied Biosystems 3100 Genetic Analyzer, with presence of plants with markers from both species used as evidence of genetic exchange between the native and invasive plants.

Of the 100 putative hybrids assayed using microsatellite markers, none amplified for markers from both species. Instead, all plants amplified as *Z. marina*. This suggests that the morphological intermediates are, in fact, a phenotypic variant of native eelgrass. Based on these results, we find no evidence that *Z. marina* and *Z. japonica* are hybridizing under natural field conditions. Follow-up experiments are testing the morphological intermediates, whose range overlaps with that of the invasive, to see if they are an annual form of *Z. marina*, one that might be more sensitive to extirpation by a competing invasive. We will also examine the phenotypic stability of this morphological intermediate.

Results: Effects of *Zostera japonica* on Population Genetic Diversity of *Zostera marina*

Samples were collected from *Zostera marina* beds with (Padilla Bay) and without (False Bay, Fidalgo Bay, Friday Harbor, Samish Bay) *Zostera japonica*. Populations varied in both phenotype (Fig. 4) and genotype (Fig. 5). In addition, the site with *Zostera japonica* had reduced diversity when compared to the sites without it (Fig. 6). This suggests that a) the presence of the invasive had a negative impact on the genetic diversity of the native, or b) the native population was always genetically depauperate, rendering it more susceptible to invasion (Crutsinger et al. 2007). Ongoing studies are examining additional *Zostera* beds to elucidate the precise reasons for, and mechanisms behind, genetic diversity reductions.

Dissemination of Results

This summer, my collaborator, our students, and I spoke about our research to more than 200 members of the general public during the annual Friday Harbor Laboratories Open House. We will distribute copies of this report, and its data, to marine scientists and managers in Washington State, including Dr.

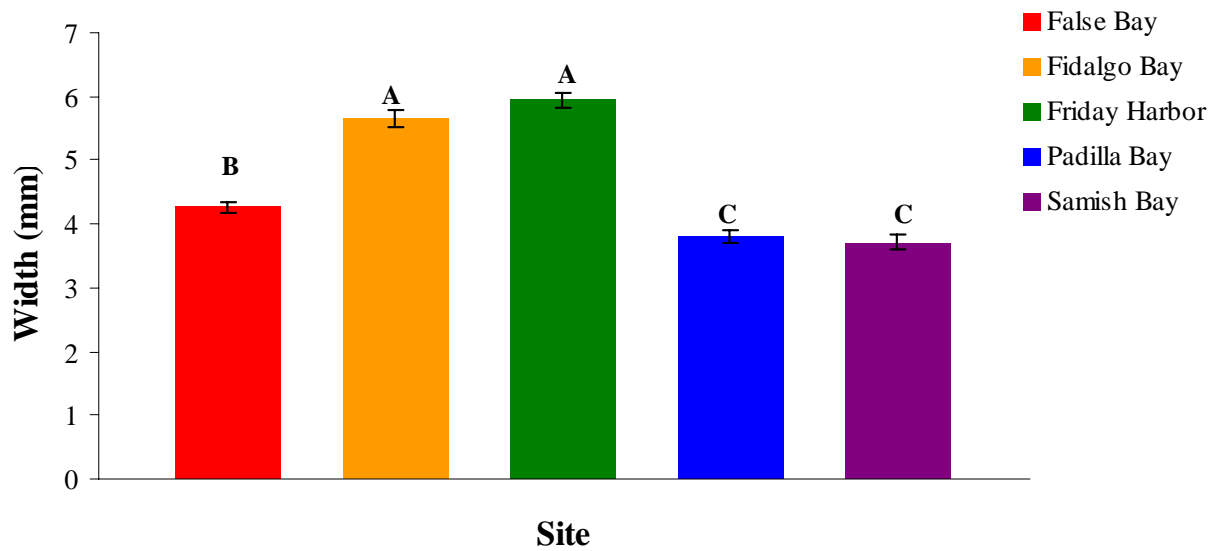


Figure 4. Mean (\pm 1 S.E.) blade width in five *Zostera marina* populations. Note that width is a diagnostic morphological character for seagrasses. Width varied significantly among populations (SAS GLM; $P < 0.0001$). Letters above bars indicate results of a SNK post-hoc test.

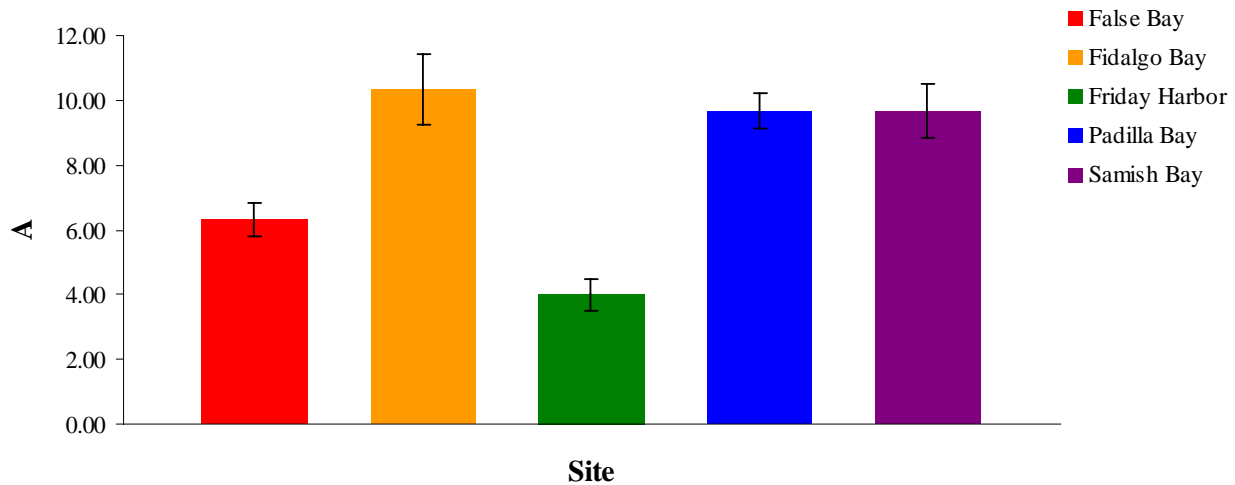


Figure 5. Mean (\pm 1 S.E.) allelic diversity in five *Zostera marina* populations. Number of alleles differed significantly among populations (SAS GLM; $P < 0.05$).

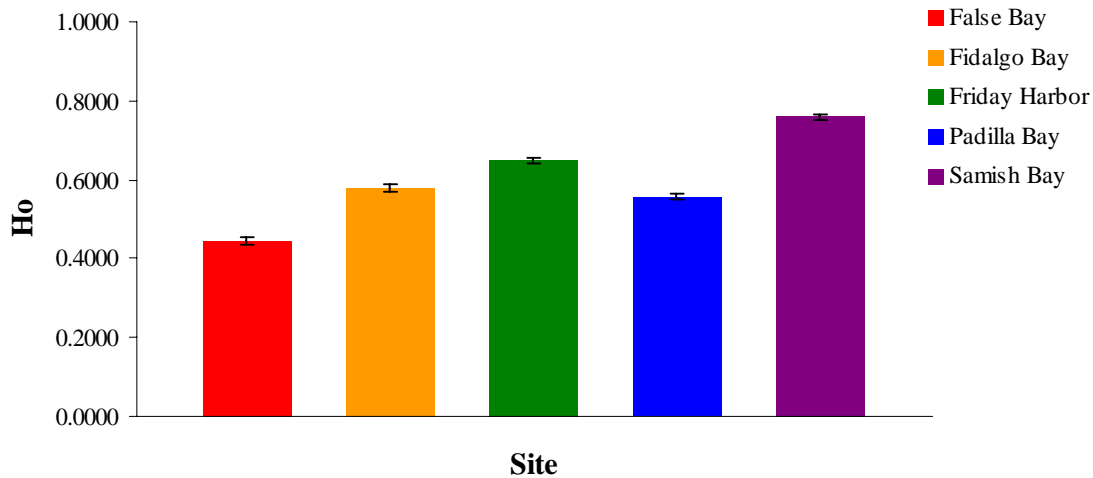


Figure 6. Mean (\pm 1 S.E.) observed heterozygosity in five *Zostera marina* populations. Padilla Bay, where the invasive was present, had less heterozygosity than other populations (except False Bay; SAS GLM; $P < 0.05$).

Douglas Bulthuis, Director of the Padilla Bay National Estuarine Research Reserve, and Dr. Sandy Wyllie-Echeverria, University of Washington faculty. We will continue to share our results with a broader scientific audience during talks at the Northwest Fisheries Science Monster Jam Seminar Series (November 2007; <http://www.nwfsc.noaa.gov/events/monster.cfm>), Northwest Scientific Association, Southeastern Ecology and Evolution Conference, and Ecological Society of America. In addition, these results will be published in peer-reviewed form. Breeding data will be published in *Castanea*, *Aquatic Botany*, or the *Botanical Journal of the Linnean Society*. Genetic results will be published in *Conservation Biology*, *Conservation Genetics*, or *Molecular Ecology*. The financial contributions of the Center for Invasive Plant Management will be acknowledged in all talks and papers related to this work.

Future Studies

With Seed Grant funding from the Center for Invasive Plant Management, we have generated much exciting data on the effects of this emerging invasive on its native congener in the waters of the Pacific Northwest. In addition to the primary research goals of this grant, two graduate students and three undergraduate students have benefited educationally from their participation in this work. I am now working to create a case-study, based on our results, for the American Museum of Natural History's NCEP (Network of Conservation Educators and Practitioners) database (<http://ncep.amnh.org/>); I have been involved in developing and testing NCEP modules since early 2006.

Results of our experiments are especially timely. Ongoing demographic monitoring by managers and environmental advocacy groups suggests that, after a lag period of more than 50 years, *Zostera japonica* is entering the log phase of its invasion, occupying new niches and increasing its geographic spread. Future studies with my collaborators and students will sample additional beds with and without *Z. japonica* to look for reduced genetic diversity in the presence of the invasive species. We will also address related questions, including the abiotic and biotic factors which make ecosystems more susceptible to *Z. japonica* recruitment, establishment, and spread.

Literature Cited

- Crutsinger, G. M., L. Souza, and N. J. Sanders. 2007. Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters* **10**:1-8.
- Levin, D. A., J. F. Ortega, and A. R. Jansen. 1996. Hybridization and the extinction of rare plant species. *Conservation Biology* **10**:10-16.
- Reusch, T. B. H., W. T. Stam, and J. L. Olsen. 2000. A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Molecular Ecology* **9**:127-140.
- Rhode, J. M., and J. E. Duffy. 2004. Seed production from the mixed mating system of Chesapeake Bay (USA) eelgrass (*Zostera marina* L; Zosteraceae). *American Journal of Botany* **91**:192-197.
- Ruckelshaus, M. 1994. Ecological and genetic factors affecting population structure in the marine angiosperm *Zostera marina* L. dissertation. University of Washington.
- Salmon, A., M. L. Ainouche, and J. F. Wendel. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Molecular Ecology* **14**:1163-1175.
- SAS. 1999. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, North Carolina, USA.