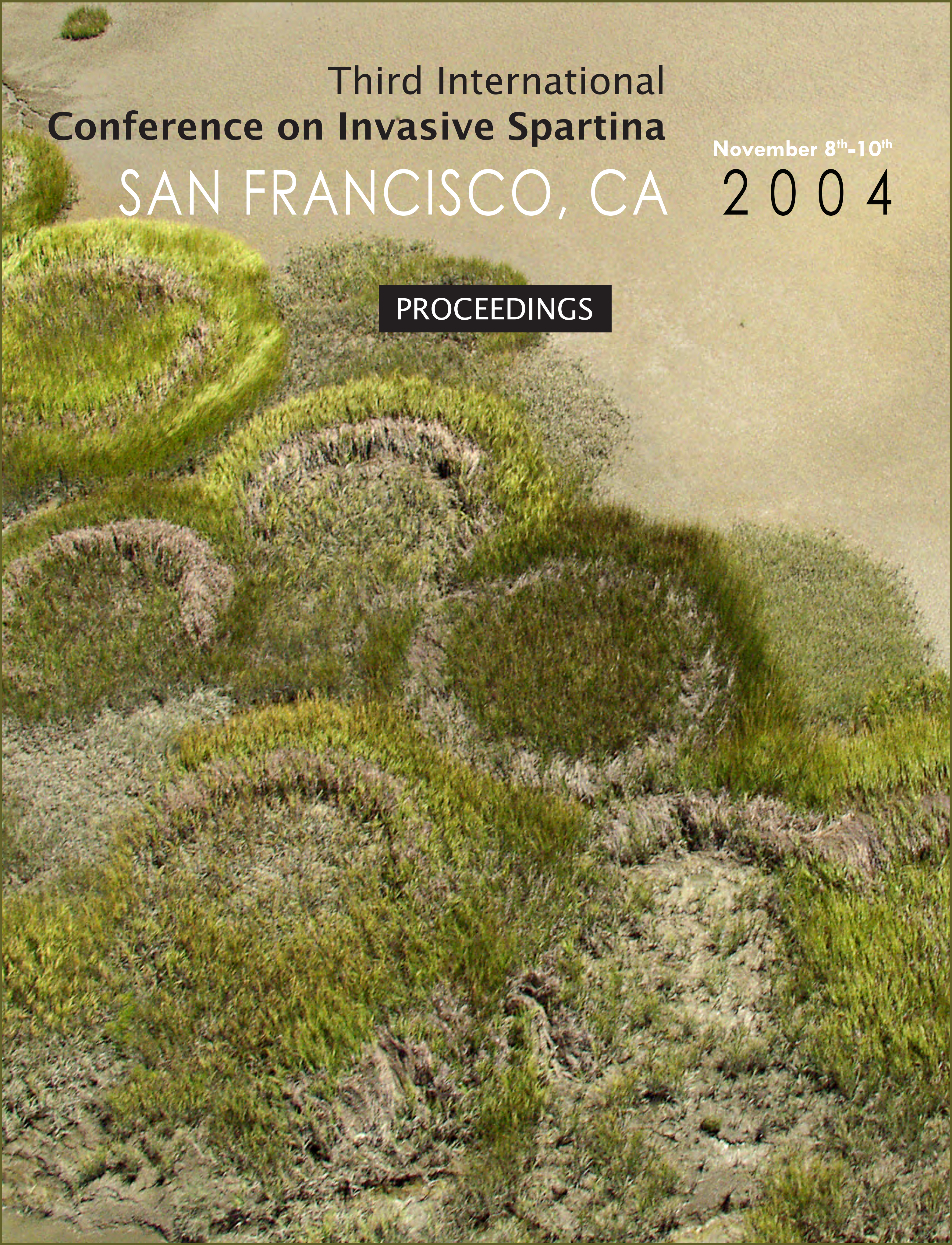

Third International
Conference on Invasive Spartina

SAN FRANCISCO, CA

November 8th-10th
2004

PROCEEDINGS

Proceedings of the Third International Conference on Invasive Spartina

SAN FRANCISCO, CALIFORNIA

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FORWARD & ACKNOWLEDGEMENTS

The Third International Conference on Invasive Spartina convened to provide a forum for the best and latest *Spartina* research, and an opportunity to discuss new developments in Spartina science with marsh land managers and technical experts who had extensive experience with this invasive genus. The theme of the conference, "Linking science and management," reflected the desire of the organizers to improve both fields through increased exposure and interaction with each other, continuing and extending a focus on uniting managers and researchers that was established at earlier international conferences on this subject. The first international *Spartina* conference was held in 1990 in Seattle, Washington, and the second in 1997 in Olympia, Washington. We are pleased to have hosted the Third International Conference on Invasive Spartina here in San Francisco, California in 2004.

The success of this event was the result of hard work and support of a number of individuals and organizations. The Conference Organizing Committee helped to guide the development of the conference and addressed organizational issues as they arose. The Conference Organizing Committee included:

Ms. Marcia Brockbank, *Program Manager, San Francisco Estuary Project*
Dr. Mike Conner, *Executive Director, San Francisco Estuary Institute*
Prof. Edwin Grosholz, *Department of Environmental Science and Policy, University of California, Davis*
Mr. Paul Hedge, *Project Manager, National Oceans Office, Australia*
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Ms. Peggy Olofson, *Director, San Francisco Estuary Invasive Spartina Project*
Dr. Kim Patten, *Director, Long Beach Research and Extension Unit, Washington State University*
Dr. Bobbye Smith, *Regional Science Liaison to Office of Research and Development, USEPA Region 9*
Ms. Erin Williams, *Non-native Invasive Species Program Coordinator, U.S. Fish and Wildlife Service*

The Science Program Committee developed the conference program, selected presentations and special guest presenters, and provided support throughout the conference. The Science Program Committee included:

Dr. Lars Anderson, *Exotic and Invasive Weed Research Laboratory, U.S. Department of Agriculture*
Dr. Debra Ayres, *Department of Evolution and Ecology, University of California, Davis*
Dr. Peter Baye, *San Francisco Estuary Invasive Spartina Project*
Ms. Janie Civille, *Department of Evolution and Ecology, University of California, Davis*
Dr. Joshua Collins, *Wetlands Program, San Francisco Estuary Institute*
Prof. Edwin Grosholz, *Department of Environmental Science and Policy, University of California, Davis*
Dr. Kim Patten, *Long Beach Research and Extension Unit, University of Washington*
Dr. Drew Talley, *Romberg Tiburon Center, San Francisco Bay National Estuarine Research Reserve*

Major financial support for the conference was provided by the California State Coastal Conservancy, San Francisco Bay-Delta Science Consortium (Agreement #46000001642), and University of California, Davis. Additional support for the conference was provided by the National Science Foundation, California Sea Grant, San Francisco Bay Joint Venture, San Francisco Estuary Project, San Francisco Estuary Institute, and U.S. Environmental Protection Agency, Region 9.

A luncheon and exhibit on the second day of the conference provided an opportunity for participants to network while learning about emerging treatment technologies. We are grateful to the following companies for their sponsorship of this luncheon: Aquatic Environments, Inc., BASF Corporation, Cygnet

Enterprises West, Inc., Helena Chemical Company, Monsanto Company, Nufarm Turf & Specialty, Wilbur-Ellis Company, and Wilco Industries.

Production of this proceedings volume has been a long and fragmented process, because of the sporadic availability of time and funding. We thank the authors for both their time and effort in preparing these excellent papers, and their patience as we worked through several years of staff changes, competing priorities, and funding loss. We are especially grateful to Debra Ayres, who ultimately coordinated the peer review of the 36 papers in the first three sections of this volume; to Drew Kerr, who reviewed and edited the 16 papers in the last section; and to Stephanie Ericson, who worked countless hours for over four years to edit, organize, and layout the entire volume.

Peggy R. Olofson
Organizing Committee Chair and
Director, San Francisco Estuary Invasive Spartina Project

TABLE OF CONTENTS

INTRODUCTION

> “Maritime *Spartina* species define and maintain the shoreline along broad expanses of temperate coasts where they are native. The large *Spartina* species grow lower on the tidal plane than other vascular plants; tall, stiff stems reduce waves and currents to precipitate sediments from turbid estuarine waters. With the right conditions, roots grow upward through harvested sediments to elevate the marsh. This engineering can alter the physical, hydrological, and ecological environments of salt marshes and estuaries. Where native, *Spartinas* are uniformly valued, mostly for defining and solidifying the shore. The potential to terrestrialize the shore was the rationale of many of the scores of *Spartina* introductions. In a time of rising sea levels, these plants are valued as a barrier to the sea in native areas and in China and Europe where they have been cultivated. In contrast, in North America, Australia, Tasmania, and New Zealand, and in some parts of China, nonnative *Spartinas* are seen as a bane both to ecology and to human uses of salt marshes and estuaries” (Strong and Ayres 2009).

Three conferences on *Spartina* were held during the last two decades to detail current understanding of the biological, ecological, and political repercussions of invasive *Spartina* and its control. The first international conference on invasive *Spartina* was held in Seattle, Washington, USA in 1990 (Mumford et al. 1991). In addition to organizers, there were 31 attendees, 17 presentations, and discussion on outcomes of four regional strategy workgroups (all on the Pacific coast of the United States). While most of the presenters were local to Washington State, *Spartina* researchers also came from the U.S. East Coast, the United Kingdom and New Zealand. Many questions were posed at the conference. Attendees deliberated on potential changes to the habitat (sedimentation rates and detrital breakdown) and fauna (invertebrates, fish, and birds) as a result of invasion, and during and following control. As well, questions arose on the efficacies of biological, chemical and mechanical control methods, and potential constraints on method — environmental, political and/or economic. It was agreed that models of *Spartina* spread should be developed to understand population dynamics that could inform control strategies. Attendees recommended these basic steps to address invasive *Spartina* control: identify a lead agency, appoint a single person to be a “Spartina Czar,” inventory the extent of invasion, identify routes of spread, enlist public support, evaluate various control methods, and continue workgroup discussions.

The second conference was held in Olympia, Washington in 1997 (Patten 1997). There were 130 attendees from five countries (United States, Canada, Australia, New Zealand, and United Kingdom) at the 29 presentations. Like the first conference, it included presentations on invasive *Spartina* biology, distribution, impacts, and control. However, the 1997 conference also had presentations addressing the “political ecology of *Spartina* control” (Perkins 1997). Topics of these papers included public activities, and risk assessment associated with control measures; “the hysteria over the cordgrass” (Cohen 1997) and “the crisis of civil dialogue” (Markham 1997) were noted. New topics at the 1997 conference included the documentation of hybrids between *S. alterniflora* and *S. foliosa* in San Francisco Bay (Daehler and Strong 1997), the use of GIS mapping and associated databases to inventory and model future spread of *Spartina* (Harrington et al. 1997), the first empirical data on drift of *Spartina* seed (Sayce et al. 1997), increases in shorebird populations after *Spartina* die-back in the United Kingdom (Gray et al. 1997), and comparisons of benthic invertebrates between *Spartina* stands and mudflat (Luiting et al. 1997). Attendees suggested that additional research be conducted to develop models of population dynamics and genetic shifts, to monitor spread and environmental impacts of invasive *Spartina*, to improve mapping and modeling in order to help prioritize control and evaluate costs. They also proposed carrying out research to quantify sediment accretion rates in invaded areas compared with non-invaded habitats, to evaluate physiological tolerance and vulnerabilities of *Spartina*, assess environmental impacts associated with *Spartina* removal and specific control techniques.

The third international conference on invasive *Spartina* was held in San Francisco, California in 2004. Hosted by the California State Coastal Conservancy and the U.S. Environmental Protection Agency, the meeting drew 147 presenters and attendees from seven countries, including China, France, the United States, Canada, Australia, New Zealand, and the United Kingdom. Fifty-seven oral and poster presentations were shared; 40 on science and 17 on control or management. Of the 11 papers on *Spartina* biology in this volume, half were focused on genetics, with *Spartina* hybridizations being the primary context for genetics studies. Thirteen papers on distribution and spread of *Spartina* included spatio-temporal analysis using GIS techniques, predictions of spread due to competition, climate change, evolution, and restoration; dynamics of seed set and seedling recruitment; and two simulation models describing spread. Studies on impacts to the ecosystem ranged from increased sedimentation under *Spartina* canopies, to changes in invertebrate communities and food webs, to shifts in bird communities, populations, and behaviors due to the *Spartina* invasion.

Of the 17 papers on control and management, seven papers were focused on control *per se*, while five detailed the oftentimes complex and bureaucracy-laden routes of *Spartina* control in San Francisco Bay, Washington State, Tasmania and New Zealand — mostly benefiting from the perspective of hindsight. Two papers considered the long and hard task that lies ahead to restore estuaries that have undergone major increases in marsh elevation due to *Spartina* invasion — what Hacker and Dethier (this volume) called the "legacy of invasion."

A primary reason for hosting the 2004 *Spartina* conference in San Francisco was to give participants a first-hand look at the invasion, through helicopter and "mud-level" field trips — with an eye toward assessing the extent of the invasion, and determining whether control was, in fact, feasible or even necessary. These questions were discussed by an expert panel at the end of the conference. Overwhelmingly, the panel agreed that control was both possible and urgently needed. With this unequivocal recommendation, the California State Coastal Conservancy proceeded to fund and coordinate an aggressive regional program to eradicate introduced *Spartina* (including hybrids) from the San Francisco Estuary. By fall 2010, the program had successfully reduced the area of invasive *Spartina* from more than 800 net acres in 2005, to less than 100 net acres, a reduction of 90 percent. The program is ongoing, with full effective eradication expected before 2020.

The papers that follow in this conference proceedings volume detail the advances that have been made in our understanding of *Spartina* biology and demography, the profound environmental effects that result from these invasions worldwide, and suggest the scope of unresolved issues.

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CHAPTER ONE

Spartina Biology

CALIFORNIA CORDGRASS (*SPARTINA FOLIOSA*), AN ENDEMIC OF SALT MARSH HABITATS ALONG THE PACIFIC COAST OF WESTERN NORTH AMERICA

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California cordgrass (*Spartina foliosa*) is an endemic of the California Floristic Province in western North America. This paper reviews its historic and contemporary distribution, profiles its habitat characteristics including key adaptive traits, and makes the case that *S. foliosa* should be recognized as a foundation species within salt marshes characteristic of the California Floristic Province. This information is then considered in the context of introgressive hybridization between *S. foliosa* and the closely related *Spartina alterniflora*. Clearly, the hybrid between *S. foliosa* and *S. alterniflora* is progressively spreading genes of *S. alterniflora* into pure stands of *S. foliosa* throughout the San Francisco Bay estuary. While this raises a concern about the potential "extinction" of *S. foliosa* as a distinct genotype, it is suggested that a greater problem may be the ecological implications of this introgression for salt marshes in the San Francisco Bay estuary. Rather than eradication *per se*, it is suggested that focused containment may be the best strategy for minimizing the impact of this hybrid on salt marshes in the California Floristic Province of this region. Further, continued adaptive management studies that evaluate the impacts of this species on salt marsh restoration are recommended.

INTRODUCTION

In this review, I will examine what we know about California cordgrass and attempt to frame this discussion in the context of conservation management challenges that invasive *Spartina alterniflora* pose to the San Francisco Bay estuary. The approach will be to discuss what is known of the historic and contemporary distribution of *Spartina foliosa*, to focus on the suite of adaptive traits that makes *S. foliosa* such an important component of low marsh habitats in west coast estuaries, and to profile *S. foliosa* as a "foundation species" in this environment. I will then summarize this information in the context of threats posed by hybridization of *S. foloiosa* with the invasive *Spartina alterniflora*.

HISTORIC AND CONTEMPORARY DISTRIBUTION OF *SPARTINA FOLIOSA*

MacDonald and Barbour (1974) conducted a survey of salt marsh vegetation along the North American Pacific coast ranging from Point Barrow, Alaska to Cabo San Lucas, Baja California. In general, they found three dominant salt marsh vegetation communities over this extensive range: an arctic, boreal, and north temperate assemblage is dominated by northern grasses such as *Deschampsia caespitosa* and sedge species such as *Carex lygnbyei,* which ranges from the Seward Peninsula down to Drake's Estero in Marin County; a south temperate and subtropical assemblage ranges from San Francisco Bay to Laguna San Ignacio in Baja California, which is the assemblage occupied by *S. foliosa*; and south of this region, tropical mangrove forests and scrub assemblages dominate estuarine tidal wetlands.

The historic distribution of *S. foliosa* occurs in this intermediate zone, also known as the California Floristic Province (Raven and Axelrod 1978). The California Floristic Province occurs along a cismontane region from southern Oregon down through northern Baja California. It is characterized by a Mediterranean-type climate consisting of long, hot and dry summers punctuated by short, wet and cold winters. The California Floristic Province has recently been recognized as one of twenty-five global "biodiversity hot spots," with over 48% of the plant species being endemics (Myers et al. 2000; Calsbeek et al. 2003). *Spartina foliosa* is among these endemic species.

The particular distribution of *S. foliosa* occurs in two major disjunct regions: (1) northern California (San Francisco Bay region) and, approximately 565 kilometers away, (2) a series of geographically proximal populations from Orange County in southern California through south-central Baja California. The historic northern California distribution has been confused by one case of misidentification and two other cases of relatively recent northern range extensions. The case of misidentification was first recognized by P. Faber (personal communication) who perceived that the extensive stands of *Spartina* long recognized in Humboldt Bay wetlands were actually *Spartina densiflora* (a caespitose species from South America) rather than *S. foliosa*. Later, Spicher and Josselyn (1985) published a confirmation of this observation. Barnhart et al. (1992) speculate that *S. densiflora* may have been introduced to Humboldt Bay as long ago as the 1860s as part of shingle or dry ballast deposited during the height of shipping activities in this formerly bustling port. Spicher and Josselyn (1985) also reported a local population of *S. foliosa* at Bodega Bay; however, this population was almost certainly not present when Barbour (1970) and MacDonald and Barbour (1974) reported on salt marsh vegetation in Bodega Bay wetlands. In the 1990s, a large population of *S. foliosa* was observed colonizing the accreting delta of Lagunitas Creek in southern Tomales Bay (Baye 2004, personal communication). Howell (1949, 1970) in his flora of Marin, however, does not mention this locality for *S. foliosa* nor do any historic herbarium collections suggest that either the Bodega Bay or Tomales Bay populations occurred prior to the latter part of the twentieth century. MacDonald and Barbour (1974) specifically remark upon the absence of *S. foliosa* in Tomales Bay.

On the other hand, Howell (1949, 1970) and historic herbarium collections do confirm that *S. foliosa* has historically been found in Drake's Estero in southern Point Reyes as well as the relatively nearby Bolinas Lagoon in Marin County. Other than these two outer coastal estuaries, all other historic northern California populations of *S. foliosa* are found within the lower portion of the San Francisco Bay estuary, ranging from tidal wetlands of South San Francisco Bay up through San Pablo Bay and the lower tidal wetlands of the Petaluma River, Sonoma Creek, and the Napa River. *Spartina foliosa* continues up into the Carquinez Straights but is largely unknown from Suisun Bay. In this eastern region, it is apparently constrained by low brackish marsh vegetation dominated by *Schoenoplectus acutus* and *Schoenoplectus californicus* and its distribution is dynamic depending on decadal shifts in the salinity gradient in this portion of the estuary. It is somewhat remarkable that there are no historic or current occurrences of *S. foliosa* from the Golden Gate south along the central California coast to Point Conception, including likely tidal wetlands such as Elkhorn Slough and Morro Bay. Peter Baye (personal communication 2004) suggests that before the construction of jetties, these central Californian lagoons were not tidal during summers (due to low flows) so it is possible that they did not provide suitable habitat.

In summary, *S. foliosa* in northern California was historically concentrated within the lower, saline reaches of the San Francisco Bay estuary where it was an important constituent of the extensive salt marshes that historically dominated tidal wetlands in this region. It appears to be slowly moving north along the coast and is now present in Bodega Bay. Its eastern distribution in the San Francisco Bay estuary is dynamic and dependant on long-term shifts of the salinity gradient in the Carquinez Straight and lower Suisun Bay subregion.

The second historic center of distribution for *S. foliosa* is relatively well documented by MacDonald and Barbour (1974), Trnka and Zedler (2000), and Wiggins (1980). The northernmost historic locality is Mugu Lagoon (Orange County), considerably south of Point Conception. There is then a fairly continuous occurrence of *S. foliosa* wherever conditions are suitable down to the Tijuana Estuary in San Diego County (e.g. Anaheim Bay, Bolsa Chica Bay, Newport Bay, Santa Margarita River, Los Peñasquitos Lagoon, San Elijo Lagoon, San Diegito Lagoon, Mission Bay, and San Diego Bay). This distribution continues in a relatively consistent fashion along the coast of Baja California in Estero de Punta Banda, Bahia de San Quintin, Laguna Guerrero Negro, Ojo de Liebre, Laguna San Ignacio, and Bahia de la Magdalena. It is of interest that Bahia de la Magdalena is at a boundary between tropical and subtropical marine ecosystems. It may be that *S. foliosa* is able to extend further south than most California Floristic Province plant endemics because it is responding as much to these marine influences as to Mediterranean climate influences. South of Laguna Guerrero Negro, *S. foliosa* begins to co-occur with mangroves and mangrove-like shrubs such as *Rhizophora mangle*, *Laguncularia racemosa*, *Conocarpus erecta*, and *Avicennia germinans*. MacDonald and Barbour (1974) point out that these same mangrove associates co-occur with smooth cordgrass (*S. alterniflora*) in marshes near Tabasco on the Gulf of Mexico. Thus, the closest geographic distance between California cordgrass and smooth cordgrass is at *S. foliosa*'s southern distributional limit. There are only about 1,200 kilometers separating the Gulf coast of Mexico from the Pacific coast of Baja California.

HABITAT CHARACTERISTICS AND ADAPTIVE TRAITS

Throughout its entire range, *S. foliosa* occupies a distinctive zone associated with coastal salt marshes. In most cases, it is the only vascular plant species present in this zone, which roughly corresponds to bay or channel margins at or near the mean high tide line (Schoenherr 1995). Every day, *S. foliosa* habitat is inundated by salt water for one to several hours. Further, it is exposed to the forces of wave action and high velocity channel flows. This combination of high salinity, prolonged inundation, and daily hydrological disturbance is obviously beyond the tolerance limits for other salt marsh vascular plant species. *Spartina foliosa* is able to tolerate these extreme conditions because of a unique suite of adaptive traits that include: (1) C4 photosynthesis, a physiological drought-adaptive mechanism which means that about half as much water is needed for the same amount of carbohydrate produced; (2) epidermal salt glands which also help to conserve water by reducing salt concentrations in cell sap; (3) possession of deep rhizomes (about 15 cm) which regenerate new segments each season and create extensive, branching mats that penetrate anaerobic sediments and anchor shoreline habitat; (4) stems and leaves that are composed of aerenchymous tissue that allows oxygen to travel from aerial portions of the plant down to the rhizomes that are embedded in anaerobic sediments; and (5) stems and leaves that are about one meter tall, enabling vegetative structures to remain above extreme high tide levels so that oxygen can reliably be transported to the roots.

These vegetative adaptive traits are also complemented by a number of important reproductive adaptations that include: (1) wind pollination; (2) asynchronous flowering; i.e. protogyny (i.e. females flower first, then males come into bloom) which promotes out-crossing yet allows some overlap in flowering time so that individuals can partially self-fertilize (reproductive insurance); (3) production of large numbers of small seeds that can be dispersed by flotation (hydrochory); (4) seeds with awns and other structures that make them available for attachment to bird feathers and potential avian dispersal (Vivian-Smith and Stiles 1994); (5) vegetative rhizome fragments that can be transported by water to further spread individual genotypes; (6) phenology cued into optimal conditions for flowering (late summer), seed set (fall) and dispersal during winter storm events; and (7) timing of dispersal coinciding with fresh conditions in the estuary during winter flooding, such that fresh water conditions promote germination and establishment.

The unique suite of vegetative and reproductive adaptations of *S. foliosa* have resulted in its successful occupation of an important low marsh niche within west coast tidal wetlands found in the California Floristic Province (and beyond through south-central Baja California). In fact, as I will argue below, *S. foliosa* could be viewed as a "foundation species" i.e. a species that has a profound effect on tidal wetland functions such as succession, productivity, and habitat structure. These functions ultimately facilitate the occupation of such tidal wetlands by a myriad of microbial, algal, plant, invertebrate, fish, and bird species.

SPARTINA FOLIOSA AS A FOUNDATION SPECIES

The concept of a foundation species has been articulated by Dayton (1972), Bruno and Bertness (2001), and Bruno et al. (2003) in the context of recognizing facilitation as an essential element of contemporary ecological theory. Unlike keystone species, which are proportionately rare in communities and yet exert a disproportionate influence over community structure through processes such as predation or ecological engineering, foundation species are habitat-forming dominant species that provide the framework for the assembly of an entire community. California cordgrass appears to be an excellent example of a foundation species.

The importance of S. foliosa as an initiator of tidal wetland succession in San Francisco Bay tidal wetlands has long been appreciated. For example, Howell (1949) makes the following comment: "Pacific (i.e. California) cord grass is generally the first plant to appear on tidal flats where it frequently establishes broad pure stands. Later it is succeeded by Salicornia and a more diversified salt marsh association as higher ground is built up around it. In this later association Spartina still occurs as a narrow fringe along tidal sloughs and also occasionally as a localized colony in low areas". In recently restored tidal wetlands in the bay today, such as Carl's Marsh in Sonoma County or Pond 2A in Napa County, this exact pattern has been observed. Spartina foliosa is usually the first species to colonize barren mud flats by floating seeds and rhizome fragments. As clones grow and establish, rhizomatous mats trap sediment and facilitate the incremental rise of a marsh plain. As elevations become suitable, other vascular plant species colonize the emerging marsh plain and eventually displace S. foliosa to the accreting edges of the marsh, margins of drainage channels, or it persists in low depressions in the marsh where duration of inundation apparently detracts other marsh plain species from becoming established (personal observation).

Once established, dense low marsh stands of S. foliosa provide a key role in marsh productivity. Each year, S. foliosa rhizomes put out fresh shoots that rapidly grow into tall, mature stems and leaves. A considerable phase of carbon fixation then takes place before flowering begins in mid summer (June). After fruiting in the fall, stems die back and much of the vegetative matter of the stand contributes to the detritus base of the wetland food web. Further, stem wrack is washed up to the marsh/upland transition zone, providing habitat and nutrients for a variety of organisms that inhabit this interface. Along with this direct contribution to the wetland food web, S. foliosa also provides an important indirect contribution because its rhizomatous mass provides key habitat for a diverse assemblage of algae, including nitrogen-fixing cyanobacteria. Dawson and Foster (1982) describe this phenomenon as follows: "The mud beneath and between California cord grass is covered with various algae, including films of golden-colored, unicellular diatoms, the brownish-green Enteromorpha, red Polysiphonia, the brownish-green, siphonous Vaucheria, and blue-green algae. All of these kinds of algae can be important contributors to marsh productivity and, in addition, some of the blue-greens can convert nitrogen gas into other nitro-nutrients for the algae as well as the flowering plants."

Stands of *S. foliosa* also provide habitat structure that is important for a number of species that occupy tidal wetlands. The relationship between California cordgrass and California and light-footed clapper rails (both subspecies of *Rallus longirostris*) is an excellent case in point. Both for cover and nesting habitat, California cordgrass is essential for the success of this species. Boyer and Zedler (1996) also point out that insects occupy stands of *S. foliosa* and these undoubtedly provide food resources for passerine birds (e.g. marsh wrens and song sparrows) that live in salt marsh habitats.

Finally, because of its importance as a foundation species, particularly in terms of succession, *S. foliosa* is also an essential element for tidal wetland restoration projects. Seeds, rhizome fragments, and plugs of *S. foliosa* can be used to revegetate formerly diked wetlands that are restored to tidal action. An interesting example is the restoration of Muzzi Marsh in Marin County which, because of an altered hydrology, is still dominated by large meadows of California cordgrass. One of the goals of this project was to recover habitat for California clapper rails and, indeed, this species has colonized Muzzi Marsh and now hosts a thriving, nesting population (Page and Evens 1987). In San Francisco Bay, it is likely that the use of *S. foliosa* material in marsh restoration projects will have to be much more carefully controlled because of the possibility that hybrid source material may contaminate seed sources. In fact, one of the greatest challenges of the invasion of *S. alterniflora* and its introgression with *S. foliosa* revolves around the desperate need and opportunity to restore historic tidal wetlands in lower San Francisco Bay in the face of the potential for the hybrids to occupy restoration sites and send the successional trajectory into a completely unknown realm.

SUMMARY AND IMPLICATIONS OF HYBRIDIZATION

Spartina foliosa has great significance as a foundation species, shaping the structure and function of salt marsh assemblages within the California Floristic Province. The ecological dilemma is that its genetically-compatible sister species, *S. alterniflora* (Baumel et al. 2002), possesses similar adaptive traits combined with greater robustness, fertility, and ecological amplitude than *S. foliosa*. Fortunately, a majority of the range and individual populations of *S. foliosa* lie in the southern center of its distribution from Point Mugu in Orange County through Bahia Magdalena in Baja California. Unfortunately, since 70% of the acreage of salt marsh habitats in California occurs in the San Francisco Bay estuary, it is likely that the largest extant populations of *S. foliosa* are at risk due to contamination by hybridization. As pointed out by Daehler and Strong (1997), Ayres et al. (2003), and Baye (2004), given the rapid spread *S. foliosa* x *S. alterniflora* hybrids, the array of short form and tall form recombinants, the male superiority of these hybrids (Antilla et al. 1998, 2000), and the greater ecological amplitude of these hybrid genotypes, it is probable that within the San Francisco Bay estuary, *S. foliosa* will ultimately be transformed into a new entity that combines traits of both *S. foliosa* and *S. alterniflora*.

Just as *S. foliosa* is a foundation species in San Francisco Bay tidal wetlands, and a myriad of species have life history traits that are adapted to this species, it is likely that the hybrid entity will take its place as a foundation species as well, triggering a cascade of responses by species whose ecological roles are entwined with *S. foliosa*. It is also likely that some species will benefit from this emergent hybrid entity while others will not. These implications are currently under investigation, as revealed by several papers in this volume. In any case, given the fact that the hybrid can spread by wind-born pollen, it is hard to imagine that the future demography of *S. foliosa* in San Francisco Bay will not be influenced by *S. alterniflora* to some degree. The fact that hybrids appear to be more successful than pure *S. alterniflora* suggests that *S. foliosa* is making an important contribution to the adaptive success of this entity. In that sense, *S. foliosa* is not so much going “extinct” as it is being transformed into a more fertile and ecologically successful new organism that combines traits of both species, an alternative condition that Arnold (1997) describes in a more positive light as “genetic enrichment”.

In a review of the role of natural hybridization and evolution, Arnold (1997) points out that there are both socio-cultural and scientific underpinnings to the view that all hybridization is “completely maladaptive”. Yet, we are discovering that hybridization is an important evolutionary mechanism. Stebbins (1950, 1957) argued that a high degree of genetic variability is required for rapid rates of adaptation and speciation. His idea was that genetic recombination from hybridization between differently adapted species, rather than mutation, is the most likely source of such variation. The challenge for hybrid speciation under natural conditions is to (1) produce a fit recombinant and (2) keep this genotype intact in the face of potential genetic swamping by the parents (Arnold 1996, Rieseberg 1997, Turelli et al. 2001). The hybridization event between *S. foliosa* and *S. alterniflora* has been mediated by human causes rather than shifting climate or some other “natural” event. As a result, the more robust but perhaps less locally adapted *S. alterniflora* does not exist in high enough numbers to swamp out the hybrid. On the other hand, the far more abundant and locally adaptive *S. foliosa* appears to lack the pollen fertility to swamp out the invading hybrids (Antilla et al. 1998). What seems to be happening is that *S. alterniflora* genes are introgressing into *S. foliosa* populations faster than pure *S. alterniflora* individuals are colonizing other parts of the bay (Antilla et al. 2000). In that sense, over many generations, if pure *S. alterniflora* can be constrained or even eradicated, it is more likely that *S. foliosa* will assimilate *S. alterniflora* genes into its populations than the contrary (i.e. *S. alterniflora* genes are introgressing into pure *S. foliosa* populations). Ultimately, fertile recombinant genotypes appear to be poised to generate a new evolutionary unit within San Francisco Bay whose ultimate fate and impact on the ecology of the wetlands of this estuary, and elsewhere, are both unknown and potentially beyond our control.

While it is still possible, one question should be addressed that could have legal implications for the potential management of this situation. That is, is there any evidence that the northern *S. foliosa* populations are genetically distinct from southern populations of this species? If it could be established that northern populations represent a distinct population segment, then one could make the case that San Francisco Bay area *S. foliosa* should be protected under the Endangered Species Act. How that might be done is well beyond the scope of this paper, but it might help focus a strategy of containment and conservation of *S. foliosa* that would provide additional leverage to help protect the areas of San Francisco Bay salt marshes that are so far not impacted by the *Spartina* hybrid.

In the long term, it certainly is possible that this hybrid evolutionary unit will spread beyond San Francisco Bay. In fact, in 2001, a hybrid individual was found in Bolinas Lagoon (35 kilometers away), and in 2002, five hybrids were found in Drake's Estero, some 45 kilometers from the nearest San Francisco Bay population of hybrid entities (Zaremba and McGowan 2004). Hybrids within the bay have increased some 317%, particularly in the Central Bay (Zaremba and McGowan 2004). Given that the Bay is a magnet for migratory shorebirds and waterfowl, and that many species are flying south during fall migration, it is hard to imagine that the hybrid won't eventually colonize the southern center of distribution for *S. foliosa.* On the other hand, even given dispersal to southern California, it is possible that the hybrid will not fare as well as *S. foliosa* in the hotter and drier southern habitats. Nonetheless, it is advisable for southern California and Baja California wetland managers to remain vigilant to this possibility and to act quickly to eliminate hybrids, as was the case in Bolinas Lagoon and Drake's Estero.

As known by wetland ecologists for decades, *S. foliosa* is a vital force in shaping salt marsh communities in San Francisco Bay, southern California, and Baja California. The introduction of *S. alterniflora* into San Francisco Bay has most likely begun a new chapter in the evolutionary fate of *S. foliosa*, and in so doing, is reorganizing its relationship to a host of other tidal wetland species in this region. We need to forge a realistic strategy to deal with this unexpected development. Massive eradication efforts could have their own unexpected consequences, especially if directed towards the hybrid. From the perspective of *S. foliosa*, the fact that its genome is being transformed into a new hybrid entity is not, in my view, extinction per se, although this perspective can certainly be argued and it is not an insignificant concern. The more compelling issue, however, is how the hybrid will affect future salt marsh and mud flat habitats. Given that large-scale wetland restoration projects are planned for the South Bay and elsewhere, it might be worth taking a pluralistic approach to managing this crisis. Design restoration activities to test different approaches, from aggressive prevention to passive observation. Give these trials enough time to evaluate the ecological impacts of wetland restoration projects that are influenced by hybrids. To the degree possible, as in the eradication effort at Bolinas and Drake's Estero, aggressively contain hybrid colonists wherever they can be caught in time. Like it or not, humans are part of nature, and nature is full of surprises. In the long term, perhaps the best we can do is manage this complexity with respect, active curiosity (including various experimental approaches), and perhaps, if not fatalism, at least a degree of tolerance and humble appreciation for the evolutionary creativity inherent in this remarkable situation.

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LOCAL AND GEOGRAPHIC VARIATION IN *SPARTINA*-HERBIVORE INTERACTIONS

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Spartina alterniflora is consumed by a variety of herbivores, and the nature of these plant-herbivore interactions varies on both local and geographic scales. The palatability of *S. alterniflora* to herbivores varies within single marshes as a function of elevation. Tall-form plants, which occur at low elevations close to creek banks, are more palatable to herbivores than are short-form plants, which occur at middle elevations on the marsh platform. The proximate causes of this local variation in palatability include variation in leaf nitrogen content and chemical defenses. Differences in these leaf traits are driven by variation in sediment biogeochemistry across the elevational gradient. The palatability of *S. alterniflora* to herbivores also varies geographically. High-latitude (New England) plants along the Atlantic Coast of the United States are more palatable to herbivores than are low-latitude (South Atlantic Bight) plants. The proximate causes of this latitudinal variation in palatability include variation in leaf nitrogen content, toughness, and chemical defenses. Differences in palatability and leaf traits persisted over 5 clonal generations in a common-garden greenhouse environment, and thus are probably genetically determined. A number of processes may contribute to driving this variation in leaf traits. The most likely ultimate causes are latitudinal variation in herbivore pressure and latitudinal variation in plant phenology. Understanding spatial variation in interactions between herbivores and *S. alterniflora* within its native range may shed insights into how these interactions develop when *S. alterniflora* is introduced to new regions, and may inform biocontrol efforts.

Keywords: common-garden experiment, elevation, herbivory, latitude, leaf traits, salt marsh, *Spartina alterniflora*

INTRODUCTION

Early salt-marsh studies discounted the importance of herbivory to salt marsh ecology because herbivores did not appear to play a major role in energy flow through the marsh food web (Smalley 1960; Teal 1962). More recent work, however, has shown that herbivores can affect the biomass, distributions and reproduction of a variety of salt marsh plants (Srivastava and Jefferies 1996; Bortolus and Iribarne 1999; Pennings and Bertness 2001; Silliman and Zieman 2001; Rand 2003; Silliman and Bortolus 2003; Ho and Pennings 2008). Thus, a general understanding of the ecology of salt-marsh plants requires consideration of plant-herbivore interactions.

In order to obtain a general understanding of plant-herbivore interactions, it is necessary to consider spatial variation. Even within the limited confines of salt marsh habitats, interactions between plants and herbivores are not exactly the same everywhere. They vary with marsh elevation (Silliman and Bertness 2002; Goranson et al. 2004), local plant community composition (Rand 1999, 2003, 2004), and latitude (Pennings et al. 2001; Pennings and Silliman 2005; Pennings et al. 2007). Thus, a general understanding of plant-herbivore interactions in salt marshes must incorporate spatial variation on a variety of scales.

Here, I address variation in interactions between herbivores and *Spartina alterniflora* at local and geographic scales. At the local scale I focus on the intertidal gradient across the marsh. Along this gradient, soil biogeochemistry varies markedly, producing strong variation in plant morphology and palatability to herbivores. At the geographic scale I focus on latitudinal variation along the Atlantic Coast of the United States. Latitudinal differences in palatability of salt marsh plants to herbivores are striking and general across the plant and herbivore community. Understanding variation in plant-herbivore interactions at both these scales provides a framework for synthesizing results of different studies done in different locations, allows tests of general ecological theory, and may shed insights into ecological processes obtained when *Spartina* is introduced into new geographic regions.

LOCAL VARIATION

Spartina alterniflora varies in palatability to herbivores within individual marshes because of variation in soil biogeochemistry that mediates plant traits. Salt marsh habitats are physically stressful for plants. Periodic flooding of marsh soils leads to high levels of sulfides, low redox levels, low levels of oxygen, and low bioavailability of nitrogen, a suite of factors that are inimicable to vigorous plant growth (Ponnamperuma 1972; Mendelssohn and Morris 2000). The high salinity of salt marsh soils further limits plant growth by reducing soil water potential, damaging cellular processes, and interfering with nitrogen uptake (Drake 1989; Mendelssohn and Morris 2000).

Flooding and salinity levels vary across the marsh landscape (Pennings and Bertness 2001). Flooding is least at the terrestrial border of the marsh, which is rarely flooded, and increases at lower marsh elevations; however, stress caused by flooding may be reduced at low elevations immediately adjacent to creekbanks, where there is a high level of exchange between pore water and the water column (Howes et al. 1981; Howes and Goehringer 1994). Salinity levels are low at the terrestrial border, close to those of the water column at the creekbank, and may peak in the high marsh if conditions favor evaporation of water and concentration of salts (Pennings and Bertness 1999). Because of this variation in soil biogeochemistry across elevation, almost all salt marsh plants exhibit strong spatial variation in height and other morphological traits (Richards et al. 2005). For *S. alterniflora*, which occurs at middle to low marsh elevations, plants at middle elevations are short (to < 50 cm) and plants at low elevations, especially close to creekbanks, are tall (to > 200 cm). This spatial variation in morphology is strongly driven by variation in the abiotic environment, although genetic variation among plants also affects morphology (Valiela et al. 1978; Anderson and Treshow 1980; Gallagher et al. 1988; Proffitt et al. 2003).

Given strong variation in plant height and morphology across the intertidal zone, it is not surprising that palatability to herbivores also varies across intertidal gradients. Low-marsh plants are more palatable than mid-marsh plants to a variety of herbivores, including geese (Buchsbaum et al. 1984), hemiptera (Denno et al. 1980; Denno et al. 1996), grasshoppers (Goranson et al. 2004) and snails (Silliman and Bertness 2002).

The plant traits mediating this variation in palatability have not been explicitly examined; however, low- and mid-marsh plants differ in two important ways that are likely to affect palatability to herbivores. First, low-marsh plants have a higher nitrogen content than do plants occurring in the mid-marsh (Gallagher et al. 1980; Bowdish and Stiling 1998). Second, the concentration of phenolics (a class of chemical compounds that often deter feeding by herbivores) is lower in low-marsh plants than in mid-marsh plants (Buchsbaum et al. 1984). Variation in these two traits likely explains much of the preference of herbivores for low-marsh versus mid-marsh *S. alterniflora*.

The palatability of *S. alterniflora* to herbivores is also affected by past herbivory (Denno et al. 2000). Feeding by herbivores may reduce plant nutritional content and/or induce defenses against herbivores. In low-latitude marshes, gastropods that damage *S. alterniflora* are much more abundant in the mid marsh than the low marsh (Silliman and Bertness 2002). To the extent that this pattern is general among herbivores, herbivore feeding damage in the middle marsh zone could reinforce patterns of plant palatability created by biogeochemical differences across elevation.

Variation in palatability to herbivores across marsh elevation is not unique to *S. alterniflora*. A number of salt marsh plants vary in palatability to herbivores as a function of marsh elevation and/or salinity (Hemminga and van Soelen 1988; Levine et al. 1998; Moon and Stiling 2000; Goranson et al. 2004). Given that the physical stress gradients in salt marshes affect both nitrogen availability and plant size, it is likely that most salt marsh plants vary in nitrogen content and toughness across elevation, and it would be surprising if variation in these factors did not affect palatability to herbivores. The details of how physical stress mediates plant palatability in salt marshes, however, appear to be species-specific, depending on both the plant and the herbivore involved, and therefore cannot be reduced to a simple generalization that applies across all species (Goranson et al. 2004).

LATITUDINAL VARIATION

Spartina alterniflora also varies in palatability to herbivores across latitude. Paired feeding preference assays comparing *S. alterniflora* from New England versus the South Atlantic Bight found that four species of herbivores strongly preferred to eat the high-latitude plants versus the low-latitude plants (Pennings et al. 2001). Results were significant in 19 of 21 assays, and did not depend on season, year, species of herbivore, or geographic origin of the herbivore. Similar results were obtained for nine other salt marsh plant species that were studied, and held true across a suite of herbivore taxa, indicating that the preference for high- versus low-latitude plants was general across the entire plant and herbivore community.

What plant traits explain the preference for high-latitude plants? A comparison of plant traits suggested that high-latitude *S. alterniflora* plants had a higher nitrogen content and were less tough than low-latitude plants (Siska et al. 2002). In addition, given a choice between polar extracts of high- and low-latitude plants, consumers consistently preferred to eat the high-latitude extracts, suggesting that there were latitudinal differences in polar chemistry. (Consumers did not show consistent preferences for non-polar extracts.) Phenolics, which are polar compounds, were higher in low- versus high-latitude plants. Thus, this study suggested that all three plant traits that were examined—nitrogen, toughness and secondary chemistry—might make high-latitude plants more palatable than low-latitude plants.

Differences in palatability of *S. alterniflora* plants across latitude appear to be constitutive rather than solely a plastic response to the environment. When *S. alterniflora* plants were grown in a common-garden greenhouse environment over five clonal generations and two growing seasons, latitudinal differences in palatability, toughness and nitrogen content persisted undiminished (Salgado and Pennings 2005). (Palatability of extracts was not examined in this study.) This study did not rule out the possibility that *S. alterniflora* has induced defenses against herbivores, but did indicate that plastic responses alone, whether to the biotic or abiotic environment, would not be sufficient to explain the latitudinal patterns in palatability. However, constitutive differences in defense are strong, and could explain part or all of the latitudinal gradient in palatability. The conclusion that there is latitudinal variation in genetic resistance to herbivory is consistent with other studies showing constitutive latitudinal variation in *S. alterniflora* traits (Seneca 1974; Seliskar et al. 2002) and genetic differentiation of *S. alterniflora* populations across latitude (O'Brien and Freshwater 1999).

The ultimate factors producing these latitudinal differences in plant traits are yet to be determined; however, because all ten plant species studied showed similar latitudinal differences in palatability (Pennings et al. 2001), it is probable that the ultimate explanations lie in general ecological and biogeographic processes rather than idiosyncratic aspects of *Spartina* biology. Because these studies were all done at sites exposed to full-strength seawater, latitudinal differences in soil edaphic conditions were minor compared to the differences that occur across elevation within single marshes, and thus probably not important. It is possible that latitudinal differences in substrate type (peat at high latitudes; mineral soils at low latitudes) might select for differences in plant palatability in some way. The most likely hypotheses, however, are that the differences in plant traits are produced by latitudinal differences in herbivore pressure or in plant phenology.

Considerable evidence indicates that herbivore pressure on *S. alterniflora* and other marsh plants is greater at low versus high latitudes. In low-latitude marshes, gastropods can strongly suppress growth of *S. alterniflora* (Silliman and Zieman 2001), but gastropods have no effect on *S. alterniflora* growth in high-latitude marshes (Pennings and Silliman 2005). Omnivorous grasshoppers are larger and are more likely to eat leaves rather than seeds or arthropods in low- versus high-latitude marshes (Pennings and Silliman 2005; Wason and Pennings 2008). Grazing damage to *S. alterniflora* from snails and grasshoppers (but not *Prokelisia* spp.) is greater in low- versus high-latitude marshes (Pennings, unpublished data). Thus, it is possible that greater herbivore pressure at low versus high latitudes could select for increased plant defenses at low latitudes, creating a geographic difference in plant palatability. Alternatively, the shorter growing season at high latitudes might select for leaves that are higher in nitrogen but less tough (Reich and Oleksyn 2004; Wright et al. 2004). Differences in these leaf traits driven by phenology would likely affect palatability to herbivores even if herbivore pressure did not select for the traits.

CONCLUSIONS

Spartina alterniflora varies in palatability to herbivores at both local and geographic scales. We have a basic understanding of how variation in leaf traits correlates with variation in palatability at both spatial scales, but how much different leaf traits affect the feeding preferences of different herbivores remains to be determined. Similarly, we have some understanding of how variation in soil biogeochemistry, herbivore pressure and phenology may be the ultimate factors mediating local and geographic variation in leaf traits, but much remains to be learned about the relative importance of these ultimate factors at different spatial scales.

Given that local and geographic patterns in palatability of *S. alterniflora* exist, these patterns are important for at least three reasons. First, they provide a unifying framework to link studies of plant-herbivore interactions done in different zones of different marshes. Although different results may be obtained in different studies, much of the variation among studies may be reconciled by understanding the underlying differences in plant palatability at local and geographic scales. Second, this variation allows tests of general theories of plant-herbivore interactions, which posit that plant-herbivore interactions will vary predictably with physical stress (Goranson et al. 2004; Huberty and Denno 2004) and latitude (Pennings et al. 2001). Third, these patterns may lend insight into some aspects of introductions of *S. alterniflora* into new geographic locations. For example, plants introduced from relatively high-latitude sites are likely to be more vulnerable to herbivores than plants introduced from low-latitude sites.

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SPECIATION, GENETIC AND GENOMIC EVOLUTION IN *SPARTINA*

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The genus *Spartina* offers several examples of reticulate evolution through interspecific hybridization and polyploidy. These processes appear to have critical impact on adaptation and invasive abilities. Here we examine how molecular analyses have helped our undertsanding of the evolutionary patterns of *Spartina*, with particular focus on *Spartina anglica*, which is a well-known example of recent and successful polyploid species of hybrid origin that has now colonized several continents. Molecular phylogenies have provided new insights on relationships and genomic divergence among species. *S. anglica* is characterised by morphological plasticity and large ecological amplitude, contrasting with a weak inter-individual genetic variation in both its native (western Europe) and introduced (*e.g.*, Australia) ranges. However, the homeologous sub-genomes of *S. anglica* exhibit consistent epigenetic and expression plasticity, which would represent key processes explaining the ecological success of this species.

INTRODUCTION

Hybridization and polyploidy are among the most prominent evolutionary processes involved in diversification and speciation in plants. This is particularly well illustrated in the genus *Spartina* where reticulate events and genome duplication (allopolyploidy) have occurred recurrently (Ainouche et al. 2004a). Recent hybridization and polyploidisation events have resulted in the expansion of particularly successful genotypes with notorious ecological impacts, and represent excellent opportunities to explore the early evolutionary processes that accompany the establishment and expansion of a new species.

In this paper, we will examine how recent molecular analyses have helped our understanding of the evolutionary patterns in *Spartina*, with particular focus on *Spartina anglica* which is a well-known example of recent and successful polyploid species of hybrid origin that has now colonized several continents.

HYBRIDIZATION AND POLYPLOIDY AS MAJOR PROCESSES IN THE EVOLUTION OF SPARTINA

All *Spartina* species are polyploids; although an extensive screening of the chromosome numbers at the population level still needs to be performed in various species, no diploid species are known in the genus, where the main ploidy levels recorded in the existing literature are tetraploid (2n = 40), hexaploid (2n = 60, 62) or dodecaploid (2n = 10, 122, 14), with a basic chromosome number x = 10 (Marchant 1963, 1968). *Spartina* is a member of the tribe Chloridoideae of the Grass family and it is composed of 17 perennial species that are usually salt tolerant and thus colonize coastal or inland salt marshes in both the Northern and Southern hemispheres. Most of the species originate from the New World (Mobberley 1956). Only four taxa are native to the Old-world: *S. maritima*, *S. x neyrautii*, *S. x townsendii* and *S. anglica*, the three latter being of recent (19th century) hybrid origin.

Using nuclear (*ITS* and *Waxy*) and chloroplast (*trnT-trnL*) DNA sequences, Baumel et al. (2002a) have shown that the genus has evolved through two well-supported lineages. The first lineage comprises the American tetraploid species *S. patens*, *S. bakeri*, *S. cynusoroides*, *S. gracilis*, and the endemic *S. arundinacea* from the South-Atlantic and Indian oceans, that appears closely related to the southeastern American *S. ciliata.* The second lineage is composed of the hexaploid species including the eastern-American *S. alterniflora*, that appears weakly divergent from its sister species *S. foliosa* from California, the Atlantic Euro-African *S. maritima* that is more differentiated at both the molecular and morphological levels. The tetraploid *S. argentinensis* is basal to this hexaploid lineage (Baumel et al. 2002a).

Recent and well-documented hybridization events involve *S. alterniflora* that has been introduced in California and in western Europe, and it has, in both cases, hybridized with native species (Fig. 1). The patterns and outcomes of these hybridizations agree with the phylogenetic relationships and the molecular divergence found between species: fertile introgressant hybrids involving the sister parental species *S. alterniflora* and *S. foliosa* on one hand, and sterile hybrids (*S. x neyrautii* and *S. x townsendii*) followed by alloploid speciation (*S. anglica*), involving related, but more divergent parental species (*S. alterniflora* and *S. maritima*) on the other hand.

In California, *S. alterniflora* was deliberately introduced in the mid-1970s in San Francisco Bay where it now co-occurs with the native *S. foliosa* (Daehler and Strong 1997). D. Strong and his co-workers have extensively studied the ecological and evolutionary consequences of this introduction (Ayres and Strong 2010). Hybridization between these two outcrossing, wind-pollinated species occurs during the overlap of their flowering periods and has been shown to occur bi-directionally (Antilla et al. 2000). Recurrent backcrosses have resulted in hybrid swarms that display most frequently the chloroplast haplotype of *S. foliosa* and up to 90% of the nuclear markers specific to *S. alterniflora* (Ayres et al. 1999; Antilla et al. 2000). These hybrids are rapidly spreading, and they are now considered as a conservation threat to the native S. *foliosa* populations.

Reticulate events recently recorded in California also involve *S. densiflora* that has been introduced from Chile: Baumel et al. (2002a) reported an unexpected phylogenetic incongruence between different molecular data sets for the phylogenetic placement of a *S. densiflora* sample from Humboldt Bay (California), and have consequently interpreted this incongruence as possibly resulting from hybridization with *S. foliosa* or *S. alterniflora*. Although the history of *S. densiflora* in both its native and introduced range needs further molecular investigation, the recent discovery of hybrids between *S. densiflora* and *S. foliosa* in the San Francisco Bay (Ayres and Lee 2010) confirms that hybridization may take place even between distantly related *Spartina* species.

In western Europe, *S. alterniflora* has been accidentally introduced by shipping ballast at the end of the 19th century. In Southampton Bay (England) hybridization with *S. maritima* resulted in a first generation hybrid *S. townsendii*, that is still growing vegetatively near Hythe. Chromosome doubling gave rise to a new fertile allopolyploid species, *S. anglica* that has rapidly expanded in range (Gray and Raybould 1997). *Spartina anglica* and *S. x townsendii* have

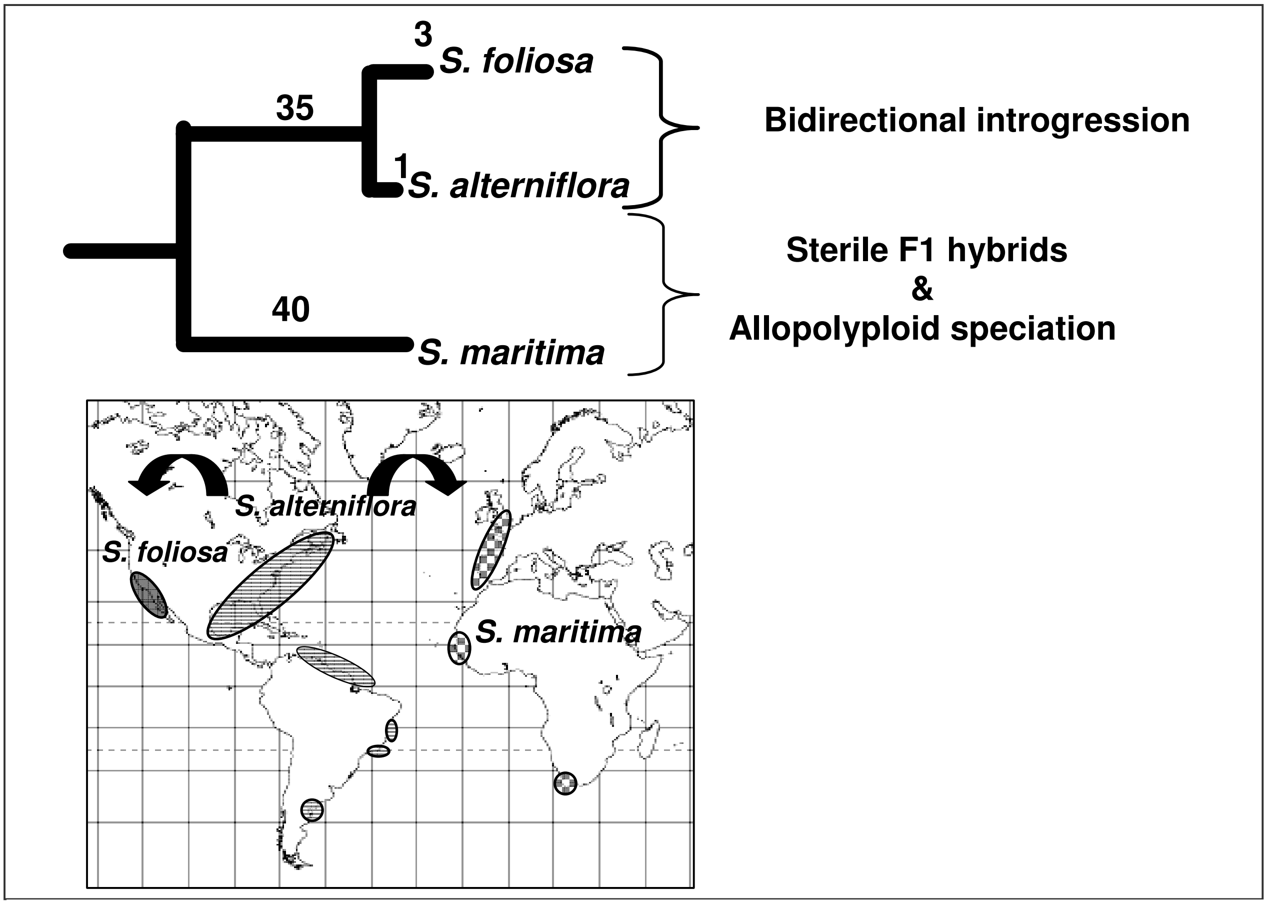

Fig. 1. Phylogenetic relationships of three Spartina species involved in recent hybridizations. Branch lengths are proportional to the number of nucleotide changes (above the branches) recorded from the analysis of two nuclear (ITS and Waxy) and one chloroplast (trnT-trnL spacer) DNA sequences (data from Baumel et al. 2002a). The map displays the natural range of these species and the two arrows indicate the recent introductions of *Spartina alterniflora*.

the same chloroplast genome as *S. alterniflora,* which was considered the maternal genome donor when it hybridized with *S. maritima* (Ferris et al. 2001).

Another sterile hybrid has been described in southwest France (Basque region) on the Spanish border and has been called *S. x neyrautii* (Foucault, 1897). As the two hybrids *S. x neyrautii* and *S. x townsendii* display marked morphological differences, they were first considered as reciprocal hybrids (Marchant 1977). However, Baumel et al. (2003) found that *S. x neyrautii* also has the same chloroplast genome as *S. alterniflora*, which indicates that hybridization occurred in the same direction (with *S. maritima* as the male parent) in England and in southwest France. Both parental species (*S. maritima* and *S. alterniflora*) have been found genetically depauperate in western Europe (Raybould et al. 1991a; Baumel et al. 2003; Yannic et al. 2004), so the two hybrids have inherited similar parental genotypes that may be distinguished by a few molecular markers (Baumel et al. 2003). Moreover, Salmon et al. (2005) have found that these two taxa display very similar genetic and epigenetic dynamics.

THE YOUNG INVASIVE *S. ANGLICA*

The neoallopolyploid *S. anglica* is a perfect example of the fitness that can be immediately gained following duplication of a hybrid genome: This species displays larger ecological amplitude than its parents; as a pioneer species, it is able to colonize a vacant niche further down the shore, and to tolerate several hours of immersion under sea water (Thompson, 1991). *Spartina anglica* is characterized by higher physiological tolerance to anoxic soil conditions, and it is able to enhance the sediment oxygenation (Lee 2003). This species now has a worldwide distribution (north-western Europe, China, Australia, North America), as a result of both natural propagation and deliberate introductions for land reclamation. This propagation is facilitated by high seed production and impressive vegetative features such as strong rhizomes, that increase the sediment accumulation. The rapid spread of the introduced plants has lead to various attempts to control or eradiate the species (Hedge et al. 2010; Hacker and Dethier 2010). However, after a variable period of rapid expansion, *S. anglica* populations seem to experience “dieback” in older colonized sites, which is probably caused by age-related decline of vigor or by competition with other species after niche elevation (Gray 2010; An et al. 2010).

Spartina anglica benefits from the reunion of two divergent (homeologous) genomes from *S. alterniflora* and *S. maritima* (Baumel et al. 2002a), therefore it is characterized by high levels of intergenomic heterozygosity at nuclear loci (Guenegou et al. 1988; Raybould et al. 1991b). However, contrasting with most other known cases of recent alloploid formation that have been explored with molecular markers (Soltis et al. 2004; Abbott and Lowe 2004), *S. anglica* has undergone a genetic bottleneck as a result of either a unique hybridization event, or multiple events involving similar parental genotypes (Ainouche et al. 2004b). The populations of *S. anglica* display consistent morphological variation, particularly in different successional stages where pioneer populations have been found to exhibit phenotypes with smaller plants and smaller inflorescence than in mature populations (Thompson et al. 1991a). This variation has been found to be caused mainly by phenotypic plasticity rather than by genetic differentiation (Thompson et al. 1991b, c).

The British populations appear genetically depauperate for allozyme markers (Raybould et al. 1991b). Baumel et al. (2001) have explored the populations of *Spartina anglica* in France using RAPD (Random Amplification of Polymorphic DNA) and ISSR (InterSimple Sequence Repeat) markers, and have found that most populations are composed of the same major genotype that is identical to the first generation hybrid *S. x townsendii*. Table 1 summarizes molecular investigations that have been conducted in populations from both the native range of the species (western Europe) and more recently colonized continents (Australia). Most of the introduced populations of *S. anglica* in the world are believed to have originated from Pool Harbor, southern England (Hubbard 1965). In southern England, *S. x towsendii* has been sampled in Hythe (for comparison with *S. anglica* genotypes), and the populations of *S. anglica* have been sampled in Lymington, the former site where *S. anglica* was recorded, Fawley (near Hythe), Keyhaven, Sand Bank, and Studland (Pool Harbour). Samples from Ireland (Bull Island) were also analyzed. Forty-three out of 45 *S. anglica* samples from England and Ireland have the same multilocus RAPD genotype (Table 1); only two individuals exhibit different genotypes: one individual, sampled in Fawley differs from the major genotype by the absence of one RAPD marker, whereas two other individuals from Lymington are distinguished by the loss of two DNA fragments. A few ISSR, IRAP and REMAP markers are polymorphic (Table 1), which allowed Baumel et al. (2002b) to distinguish 13 genotypes that were encountered at low frequency in some populations; these genotypes are very similar to the major one, as most of them differ by only one (lost) marker.

The French populations have been sampled in various locations of the Atlantic coast (Baumel et al. 2001), including Baie des Veys (Normandy) the first colonized site in France where *S. anglica* was recorded in 1906. Most individuals sampled in France also belong to the predominant genotype. One small population from Kerdruc (southern Brittany) was found fixed for two mutations, with the absence of one RAPD (Baumel et al. 2001) and one ISSR marker (this study). The few slightly different other genotypes (Table 1) were found to be randomly distributed, and at low frequency, among the other French populations. In Saint-Armel (southern Brittany), *S. anglica* grows sympatrically with its parental species *S. maritima*.

Table 1: Inter-individual molecular variation in native and introduced *Spartina anglica* populations investigated using three multilocus methods: RAPD (Random Amplification of Polymorphic DNA, 14 primers), ISSR (Inter Simple Sequence Repeats, 4 primers), IRAP (Inter-Retrotransposon Amplified Polymorphism, 7 primer pairs), REMAP (Retrotransposon Microsatellite Amplified Polymorphism, 4 primer pairs). The mean individual number sampled per population = 10. N= number of samples analyzed per region; pm = polymorphic markers; m=number of markers recorded; mg = number of genotypes that differ from the "major" genotype identified by Baumel et al. (2001) and identical to *S. x townsendii*.

	RAPD			**ISSR**			**IRAP/REMAP**		
	N	*pm/ m*	*mg/N*	*N*	*pm/m*	*mg/N*	*N*	*pm/m*	*mg/N*
England & Ireland	45	3/146	2/45	20	0/14	0/20			
				40^{b}	3/92	4/40	40^{b}	10/296	9/40
France				20	2/92	2/20	5	2/296	2/5
	129^{a}	6/146	6/129	129^{a}	1/14	1/129			
Australia	20	0/146	0/20	160	0/21	0/160	-	-	-

^{a} = data from Baumel et al. (2001); ^{b} = data from Baumel et al. (2002b).

Intermediate, morphologically variable individuals may be encountered (Guenegou and Levasseur, personal communication), but no genetic exchange was found between the two species according to cytological, allozyme (M.T. Misset and G. Allard, unpublished data) and molecular (Baumel et al. 2001) data.

In Australia, 160 samples were collected in various sites from Victoria (Corner Inlet including the mouth of the Albert River and Anderson Inlet, Gippsland) and Tasmania (Franklin River in Port Sorell Bay, Duck River near Smithton, Perkins Bay, Tamar River and Little Swanport estuary). In Victoria, various attempts at eradication have been performed using the herbicide Fusillade; we have sampled non-treated zones and also collected rare surviving plants from treated areas. Morphological variation is also encountered in the Australian populations where two distinct phenotypes (short plants of 10 – 20 cm high on the one hand and taller plants measuring up to 30 cm on the other hand) were collected in the Albert River (Victoria). In Little Swanport, 20 seedlings have been collected in order to analyse samples resulting unambiguously from sexual reproduction. We found that all the individuals investigated in Australia and Tasmania display the same multilocus RAPD or ISSR genotype (Table 1), which is identical to the major genotype encountered in Europe and to *S. x townsendii*.

It appears that populations of *S. anglica* in both its native range and more recently colonized areas are mainly composed of the major genotype that is identical to the first generation hybrid *S. x townsendii*. Some variation may be encountered, however, that result from a few mutations occurring in local populations and corresponding in most cases to fragment loss. Ayres and Strong (2001) also noted fragment loss in *S. anglica*. They encountered variation in RAPD and ISSR analyses including samples of *S. anglica* from England and Australia, and observed variable band-loss at five *S. maritima*-specific loci. However, Salmon et al. (2005) have reported preferential fragment loss of *S. alterniflora*-specific markers using AFLP (Amplified Fragment Length Polymorphism) in both *S. x townsendii* and *S. anglica*. It should be noted that the estimation of diversity may be either underestimated or overestimated according to both sampling (number of individuals analysed and number of markers examined over the genome) and the technique used: We can see (Table 1) that although globally limited, more variation can be detected from ISSR and IRAP–REMAP markers, that target repetitive (Simple Sequence Repeats and Retrotransposons), potentially more variable, parts of the genome. Moreover, considering that these PCR-based markers are dominant, variation resulting from segregating heterozygous genotypes cannot be ruled out. Another potential source of variation is the structural dynamic that may affect recently formed allopolyploid genomes (Wendel 2000), but in the case of *S. anglica*, this dynamic does not seem to affect the genotype initially formed in England to the extent that was reported in the literature in other allopolyploid model systems (Baumel et al. 2002b; Ainouche et al. 2004b).

We have recently explored the epigenetic alterations that may affect the allopolyploid genome of *S. anglica* (Salmon et al. 2005). Epigenetic mechanisms cause expression changes that do not result from the modification of the DNA sequence, and have been shown to result from various, non-exclusive processes including DNA or histone methylation, histone acetylation, and chromatin compaction

Table 2: Comparisons of cDNA AFLP patterns in *S. maritima*, *S. alternilflora*, the hybrid *S. x townsendii* and the allopolyploid *S. anglia*. The following selective primer combinations have been employed: EcoRI + ACG / MseI + CAA, EcoRI + ACG / MseI + CAC, EcoRI + ACA / MseI + CAA EcoRI + ACA / MseI + CAC EcoRI + AAC / MseI + CAA EcoRI + AAC / MseI + CA, EcoRI + AGA / MseI + CAG, EcoRI + AGA / MseI + CCA, EcoRI + ACC / MseI + CAA.

		Fragments inherited in the hybrid and the allopoloïd	*Lost fragments in the hybrid and present in the allopolyploid*	*Lost fragments in the allopolyploid only*	*Lost fragments in the hybrid and the allopolyploid*
Monomorphic fragments (173)		127	30	14	2
Polymorphic fragments (61)	*S. maritima*	28	2	13	4
	S. alteniflora	10	0	4	0
TOTAL		165 (70.5%)	32 (13.7%)	31 (13.2%)	6 (2.5%)

(Liu and Wendel 2003). Epigenetic changes have been found associated with phenotypic instability in experimentally re-synthesized allopolyploids (Comai et al. 2000; Kashkush et al. 2002). Using Methylation Sensitive AFLP (MSAP), Salmon et al. (2005) have compared the methylation patterns of the parental genomes of *S. maritima* and *S. alterniflora*, to those of the first generation hybrid *S. x townsendii* and the resulting allopolyploid *S. anglica*. Thirty percent of the parental methylation patterns were found altered in the hybrid and the allopolyploid, indicating that hybridization, rather than genome doubling have triggered most of the methylation changes of *S. anglica.* This high level of epigenetic changes might explain the morphological plasticity of *S. anglica* and calls for further investigations of the potential regulation of duplicate gene expression.

In this perspective, transcriptomic changes in the hybrid and the allopolyploid have been first analyzed using cDNA AFLP in order to examine whether the parental genomes have different expression patterns in *S. x townsendii* and *S. anglica*. RNA was extracted from young leaves collected in individual plants cultivated in the greenhouse, in *S. maritima* (collected in Guerande and Saint Armel, Brittany France), *S. alterniflora* (collected in Landerneau, Brittany, France), *S. x towsendii* (Hythe, England) and *S. anglica* (collected in Saint Lunaire and Saint Armel, Brittany, France). Inter-individual or inter-population variation of cDNA AFLP patterns was checked for each species and only stable, species-specific cDNA fragments were taken into account for comparisons. Of the over 234 unambiguous fragments scored, 173 were found monomorphic and 61 were polymorphic (*i.e.*, present or absent) between the parental species. As AFLP markers are dominant, polymorphic fragments are expected to be present in the hybrid and the allopolyploid if expression of genome additivity is respected. Loss of parental fragment is interpreted as a result of gene silencing, and appearance of fragments that are absent in both parental species is interpreted as novel gene expression. This genome-wide screening of duplicate gene expression has been previously successfully employed to test genome expression plasticity in various allopolyploids (Comai et al. 2000; Lee and Chen 2001; Kashkush et al. 2002; Soltis et al. 2004; Adams et al. 2005). About seventy percent of the parental fragments are inherited by both the hybrid and the allopolyploid (Table 2). Among the 61 polymorphic fragments, 38 (28 from *S. maritima* and 10 from *S. alterniflora*) were found to be additive in the hybrid and the allopolyploid. Seventeen fragments (13 from *S. maritima* and 4 from *S. alterniflora*) are lost in *S. anglica*. All the fragments present in the hybrid and the allopolyploid are already present in one (or both) the parental species. When considering both monomorphic and polymorphic fragments between the parental species, the allopolyploid has lost 37 parental fragments of which 31 were still present in the F1 hybrid *S. x townsendii* and 6 were lost in the hybrid and the allopolyploid, which indicates an overall silencing in *S. anglica* of 15.8 % of the examined loci, that occurred mostly following genome duplication in the polyploid. Although more loci have to be screened in *Spartina* to verify the extent of the changes overall the genome, it appears from this first screening that 34.4 % of the loci are silenced in *S. anglica*, when considering only the parental fragments that are polymorphic. This represents a consistent amount of changes compared to those recorded in other polyploids using similar methods. Kashkush et al. (2002) have reported about 2% alteration of parental transcripts in experimentally re-synthesized allopolyploid wheat. Expression alteration has also been found in allopolyploid *Arabidopsis* (Comai et al. 2000; Lee and Chen 2001). Recently, Soltis et al. (2004) found about 5% cDNA parental fragment loss and 4 % novel expression in the allotetraploid *Tragopogon mirus* and *Tragopogon miscellus*. Adams et al. (2003) showed differential expression patterns between homeologous genes in different tissues of allotetraploid cotton (*Gossypium*), suggesting that sub-functionalization was consequent to allopolyploid formation. Over 2000 transcripts were screened by cDNA AFLP (Adams et al. 2004) and about 5% of the duplicated genes were inferred to have been silenced or down-regulated in the experimentally re-synthesized allotetraploid *Gossypium*.

SUMMARY AND CONCLUSIONS

Allopolyploid species represent two important outcomes of hybridization and genome duplication that are critical for their evolutionary success: Hybridization leads to the merger of two more or less differentiated genomes that have previously evolved independently and polyploidization entails an immediate duplication and functional redundancy at all loci. These events entail various molecular interactions and adjustments that have received much attention in the recent literature, as they are now considered as the key processes that explain the evolutionary success of polyploidy in Eucaryotes (reviewed in Liu and Wendel 2003; Osborn et al. 2003). Heterosis and dosage effect in duplicated genomes are likely to increase the metabolic plasticity of the duplicated genes, thereby affecting the fitness of the newly formed species (Riddle and Birchler 2003).

The particularly successful *Spartina anglica* is characterised by morphological plasticity and large ecological amplitude, contrasting with a weak inter-individual genetic variation, but consistent epigenetic and expression plasticity of the duplicated homeologous genomes. Further investigations and identification of the sequences that are affected by epigenetic regulation and expression changes should deepen our understanding of the ecological success of this young species. *Spartina* offers particular opportunities to learn about the evolutionary and ecological consequences of polyploid speciation and reticulate evolution at both the short term (in nascent species such as *S. anglica*) and long term (in the parental, older polyploid species) of the evolutionary time scale.

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EVOLUTION OF INVASIVE *SPARTINA* HYBRIDS IN SAN FRANCISCO BAY

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Rapid evolution in contemporary time occurs when genetically variable individuals face strong selection pressures. Just such a conjunction is taking place in the intertidal marshes of the San Francisco estuary. Three decades ago, exotic smooth cordgrass, *Spartina alterniflora*, was planted in the Bay for erosion control and marsh restoration. The natural structure of the Pacific estuaries leaves broad expanses of open mud flats, critical foraging grounds for millions of birds. Fringing the upper mud flat margin is the native cordgrass *S. foliosa* that, due to small stature and sparse growth, is unable to colonize the mud flats or modify their geomorphology, unlike the alien congener. Shortly after the introduction, the two species hybridized; in the last 20-odd years a broad array of genotypes has arisen through reciprocal hybridization and introgression. Our research has found that some hybrids are taller, have more rapid rates of lateral expansion, have higher tolerance to salinity, have higher rates of self-pollinated seed set, and are better sires on the native species than either parental species. We predict that these traits will result in 1) rampant colonization of open mud—both in the intertidal and in restoration sites, 2) invasion of the middle elevation *Salicornia*-dominated saline marsh plains, 3) isolated self-compatible plants founding new populations; and 4) hybrid pollen siring the lion's share of seed in surrounding *S. foliosa* plants in native marshes. Natural selection will favor these traits in a positive feedback that will result in an accelerating population growth rate of the fittest, most invasive hybrid genotypes. Indirect evidence that evolution has ready occurred is our finding of super-exponential growth of hybrid cordgrass cover in the Bay. Effective management and prediction of hybrid cordgrass invasion must incorporate a dynamic viewpoint of *Spartina* population parameters.

Keywords: Invasive *Spartina*, hybridization

INTRODUCTION

Spartina alterniflora, smooth cordgrass, native to the Atlantic coast was introduced into San Francisco Bay in the 1970s. It hybridized with native California cordgrass, *S. foliosa* resulting in an interbreeding swarm of highly genetically variable plants (Ayres et al. 1999; Anttila et al. 2000). One of the chief constraints on evolution is a lack of heritable variation in fitness traits. Conversely, high variation in heritable fitness-related traits may fuel rapid evolution. Current approaches to understand successful plant invasion consider both the invader and the invaded system; invasive plants possess traits that allow them to invade open niches in vulnerable systems. With these concepts in mind, we assessed genetic variation in hybrid cordgrass, measured variation in physical traits of cordgrass hybrids (*S. alterniflora* x *foliosa)* that would confer invasive ability in the open mud of Pacific estuaries and in the high salinity tidal plains dominated by pickleweed (*Sarcocornia virginica*, former name *Salicornia*), and evaluated rate of hybrid spread.

METHODS

Genetic variation. In previous work we documented the genetic variability of *S. alterniflora* x *foliosa* hybrids using nuclear DNA markers and chloroplast DNA sequences (Ayres et al. 1999; Anttila et al. 2000).

Phenotypic variation. We focused on several traits that we propose confer superior invasion ability. These traits were 1) stem height, where tall stems allow plants to survive tidal inundation and grow further down the intertidal plain; 2) rapid lateral expansion that anchors plants in soft mud substrates and allows encroachment into the space of other cordgrass plants; 3) high seed and pollen production to favor colonization of open mud flat habitat and seed siring on other plants; and 4) tolerance to salinity and brackish water that permits growth in the *Sarcocornia* higher marsh (high salinity) and tidal creeks (brackish water).

At Cogswell Marsh, a former salt pond restored to tidal action in 1980, we compared variation in vegetative and sexual reproduction among established plants (Zaremba 2001). Seventy-two plants were haphazardly selected from a 1998 aerial photograph; their genotypes were determined using nuclear DNA analysis (Ayres et al. 1999; Zaremba 2000). Heights of the three tallest stems (excluding inflorescences) in three randomly placed quadrats were measured in 1998 and 1999 (results reported for 1998 only). Diameters were staked and re-measured in 1998, 1999 and 2000. Inflorescences were randomly collected (three per plant) and assessed for seed set and pollen viability and quantity (see Zaremba 2001 for a full description of methods). In a greenhouse at UC Davis, we compared growth of an array of hybrids under three salinity regimes (Pakenham-Walsh et al. this volume; Pakenham-Walsh 2003).

Spread rate. We developed physical indicators of hybridity (tall, wide, red, stems; late flowering) in greenhouse and field studies to identify hybrids by sight in the field (Ayres et al. 2004). We combined aerial photographs, ground surveys, and genetic confirmations to estimate areal cover by hybrid cordgrass and *S. alterniflora* in 2001. We estimated cordgrass hybrid cover from the original 1976 population, and in the 1990s from early studies (Callaway and Josselyn 1992; Daehler and Strong 1996) to plot cordgrass cover over time.

RESULTS

Genetic variation. Diverse hybrid nuclear DNA genotypes were present (Ayres et al. 1999), evidence that widespread crossing has occurred among cordgrass genotypes. Using chloroplast sequences, we found that hybridization was bi-directional; that is, both *S. alterniflora* and *S. foliosa* have been seed parents to hybrids (Antilla et al. 2000). We also determined that *S. foliosa* has been the dominant seed parent of hybrids.

Phenotypic variation. A diverse array of hybrid genotypes was present in the sampled population at Cogwesll Marsh. Nine of the 72 plants were genetically determined to be (non-hybrid) parental species; four plants of native *S. foliosa* and five plants of *S. alternilfora*. (Note: plants in Figs. 1-3 are arranged according to the magnitude of the trait and the order is NOT the same in all graphs.)

Height. Individual plant height varied from < 30 cm to >120 cm (Fig. 1). Both *S. foliosa* and *S. alterniflora* plants ranged from 30 to 70 cm in height. While the tallest 23% of the plants were hybrids, most hybrids fell within the height range of the parental species, and three plants were shorter than the shortest *S. foliosa* plant.

Lateral spread. Plant areal increase varied from plants that shrunk in area after two growing seasons (1998–1999, and 1999–2000) to a plant that expanded over 200 m^2 (Fig. 2; data are presented for 52 plants for which we had a complete data set). Plants of the parental species had low to moderate growth rates while the fastest growing 30% of plants were hybrids.

Salinity tolerance. We found that under conditions of high salinity several hybrids exceeded the growth of both parental plants by two to three times, and by as much as six times under low salinity conditions (Pakenham-Walsh 2003; Pakenham-Walsh, Ayres and Strong in this proceedings volume).

Sexual reproduction. Hybrid inflorescences averaged over twice as many flowers as those of *S. foliosa* (460 vs 200), but hybrid inflorescences varied from fewer than 125 flowers to inflorescences with over 800 flowers (Fig. 3, Ayres et al. 2008). Fertile seed per inflorescence was one and a half times higher in hybrids than in *S. foliosa*, but varied in hybrid plants from zero seed set (including a plant with over 800 flowers per inflorescence) to five times higher seed set than the native. We determined from genetic analyses that between 50% and 80% of the seeds from the four *S. foliosa*

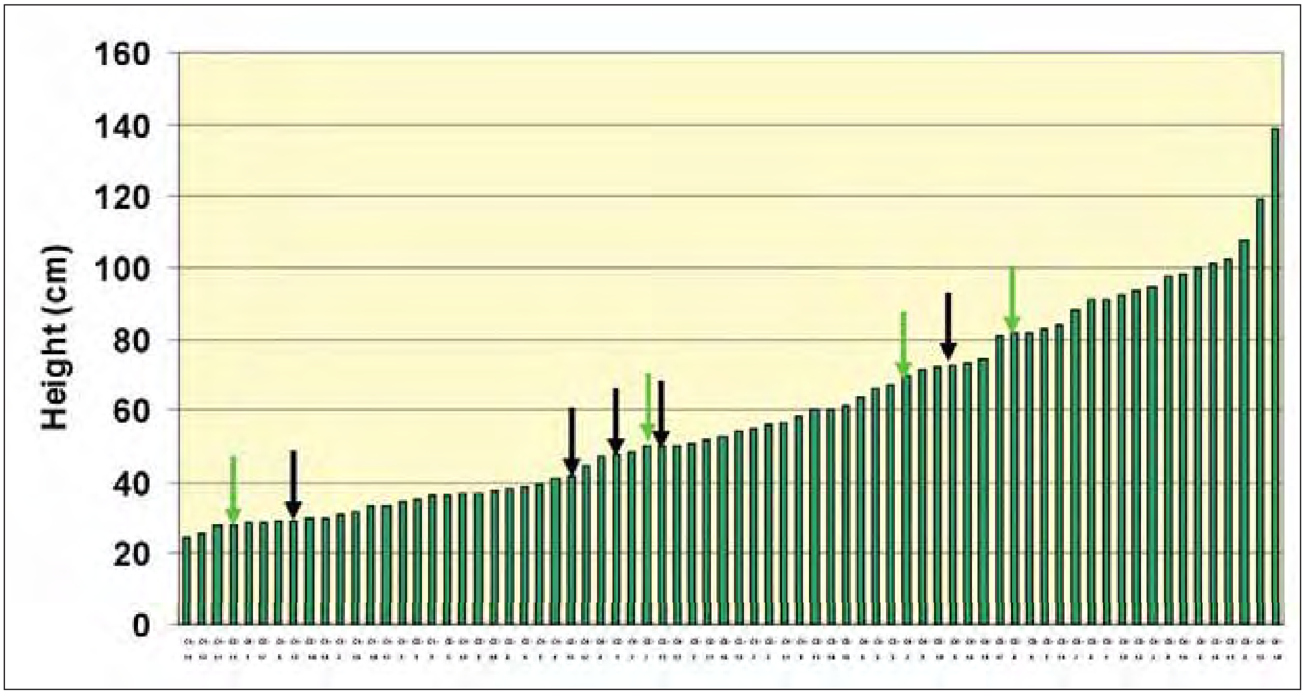


Fig. 1. Stem height in 1998 of cordgrass plants (individual plants are on the x-axis) at Cogswell Marsh. Grey arrows are *S. foliosa* and black arrows are *S. alterniflora* plants (by genetic analyses); the rest are hybrids.

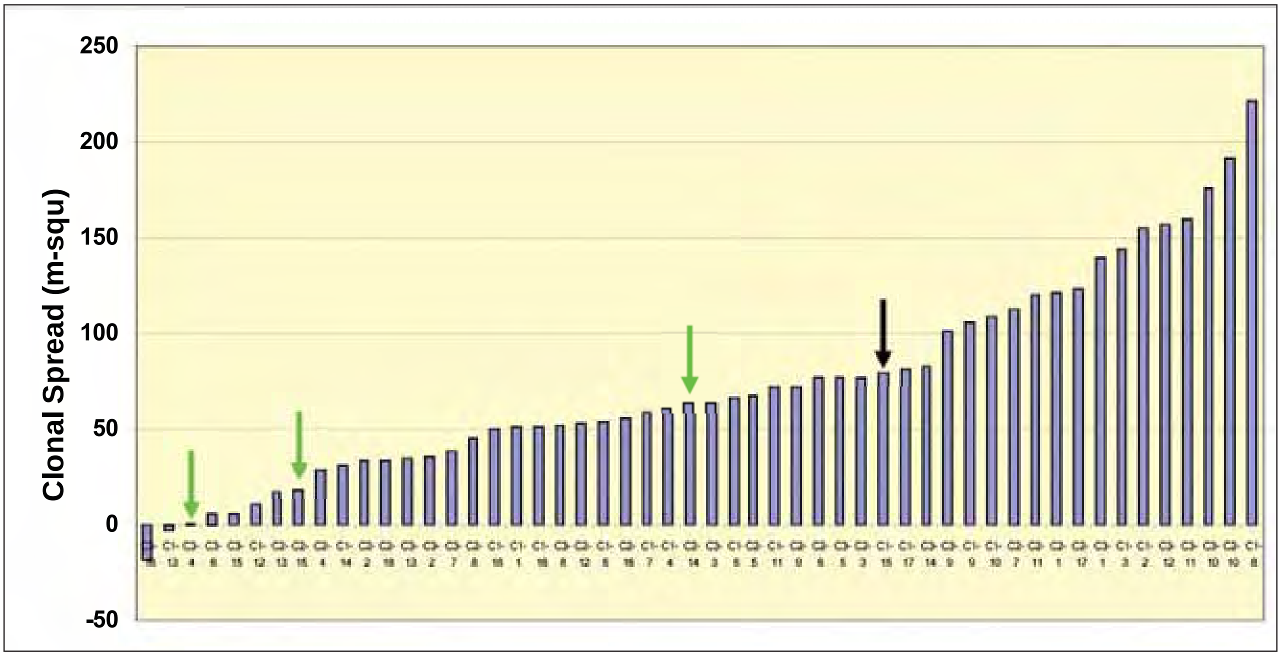


Fig. 2. Clonal spread in area of individual plants (along the x-axis) at Cogswell Marsh from 1998 to 2000 (complete data was only available for 52 plants). Grey arrows are *S. foliosa* and black arrow is *S. alterniflora* plants (by genetic analyses); the rest are hybrids.

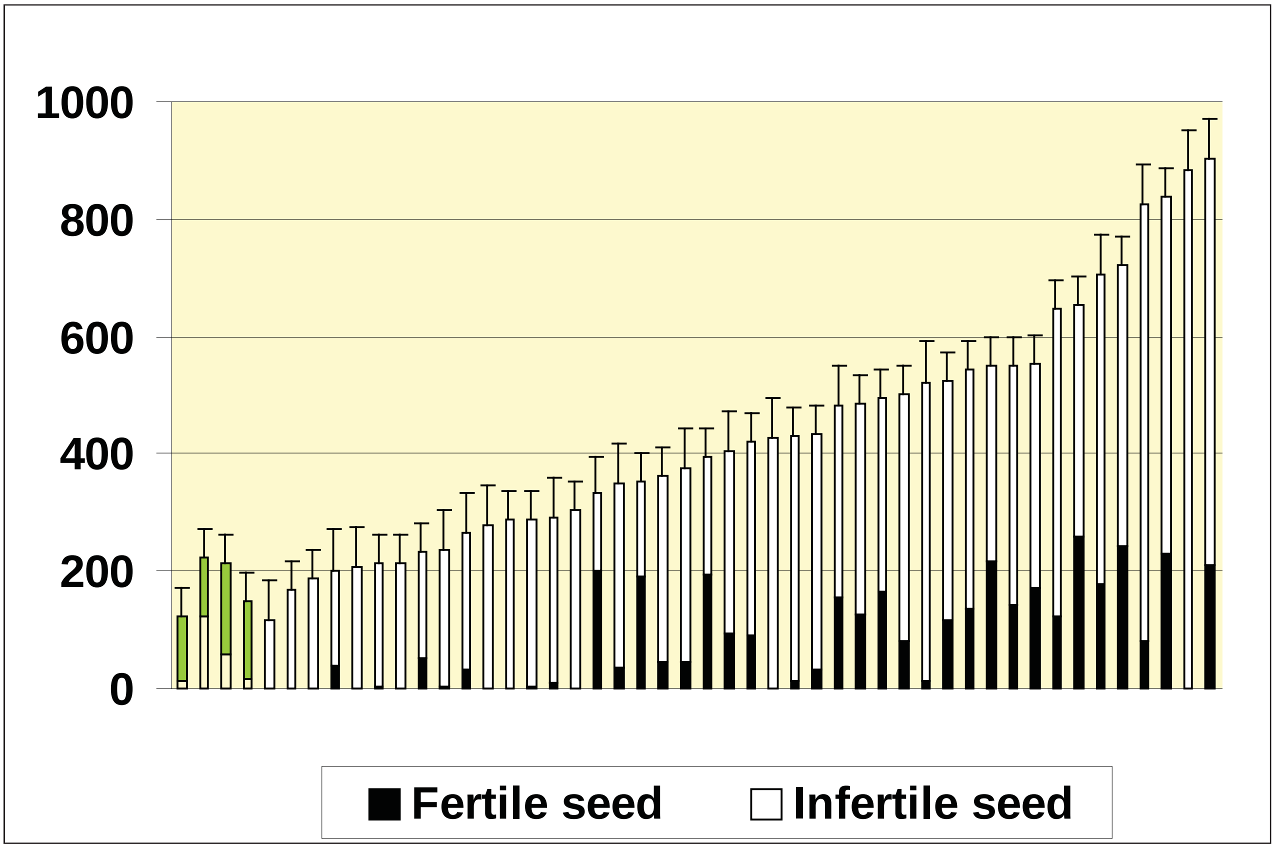


Fig. 3. Sexual reproduction of 48 cordgrass plants at Cogswell Marsh in 1998 (many plants did not flower); individual plants are along the x-axis. Each bar is the average number of flowers on an inflorescence; the black portion represents those florets that contained a fertile seed, the open portion represents those flowers without a seed. The *S. foliosa* plants are along the left side, with grey bars; the single *S. alterniflora* did not flower.

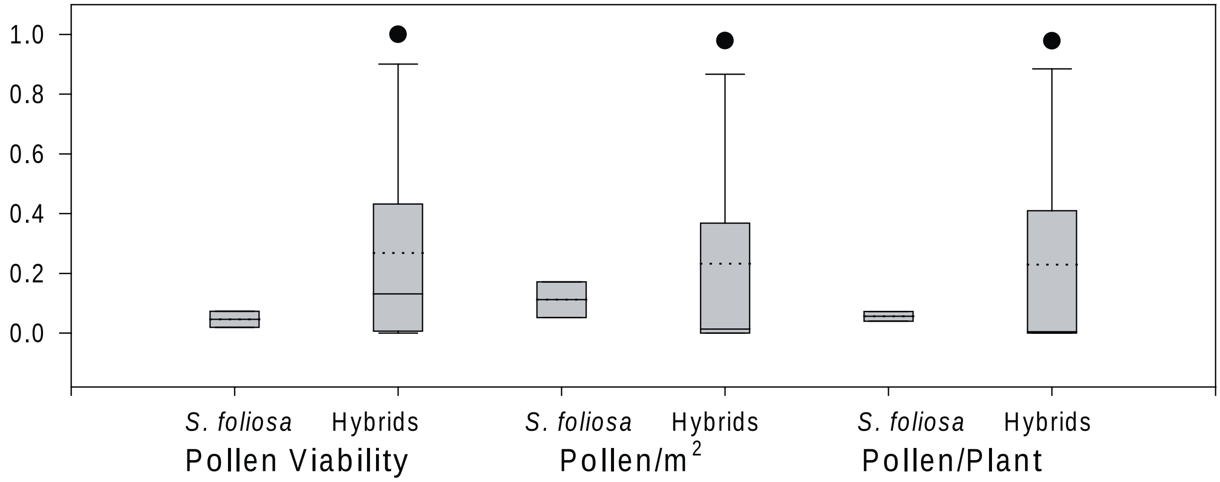
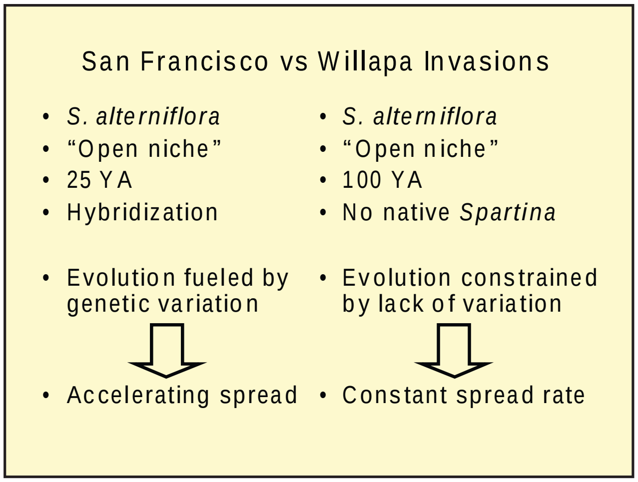


Fig. 4. Relative pollen viability and production of *S. foliosa* and cordgrass hybrids; box-whisker plots show 25th, 50th and 75th per-centiles solid lines in the "box", whiskers are the 5th and 95th percentile; solid circles are at the 2.5 and 95.6 percentile; dotted line is the mean. This figure first appeared in Ayres et al. 2008.



San Francisco vs Willapa Invasions

• *S. alterniflora*	• *S. alterniflora*
• "Open niche"	• "Open niche"
• 25 YA	• 100 YA
• Hybridization	• No native *Spartina*
• Evolution fueled by genetic variation	• Evolution constrained by lack of variation
⇩	⇩
• Accelerating spread	• Constant spread rate

Fig. 5. Comparison of *S. alterniflora* introductions into 2 Pacific coast USA estuaries, San Francisco Bay, CA, and Willapa Bay, WA. We posit that spread rates are a function of the amount of genetic variation.

plants in the marsh were hybrids. The net result was that over 95% of the seed produced from all 42 plants in our study (ca. 40 million seeds in 1998) was hybrid.

Hybrid pollen had 3.5 times the viability of *S. foliosa* pollen (8.24% vs 2.38% viable grains, respectively), and hybrid plants produced twice the number of pollen grains as native plants (9.5 million vs. 4.3 million grains per plant) (Fig. 4, Ayres et al. 2008). Viable pollen production ranged from a single hybrid individual (plant C1-5) that produced 58% of the total viable pollen to zero. The cumulative effects of the scarcity of *S. foliosa* plants, and differences in pollen production and viability resulted in hybrid plants producing over 400 times more viable pollen than the native plants.

Spread Rate. Combining the results of our 2000–2001 cordgrass survey with literature-based estimates of populations size, we found that spread rates of hybrid and *S. alterniflora* have increased over time (Ayres et al. 2004), from 10% (during 1973 to 1992) to 40% (from 1993 to 2001) per year. This pattern is apparently not typical of *S. alterniflora* invasion in general as analyses of spread in Willapa Bay, Washington found a constant growth rate of 12% over the past 100 years (Civille et al. 2005; Taylor et al. 2004, and in this proceeding volume).

DISCUSSION AND CONCLUSIONS

We have shown that hybrids are genetically variable due to interbreeding among hybrids and backcrossing to the native (Ayres et al. 1999; Anttila et al. 2000). In the present study, we demonstrate that hybrids are also phenotypically highly variable in features of vegetative and sexual reproduction, with a subset of hybrid individuals having superior growth and contributing disproportionately to seed and pollen production.

We theorize that the open niche of native marshes, intertidal mud flats, and restoration sites provides strong selection for vigorous vegetative growth and seed production. Hybridization has provided large amounts of genetic and phenotypic variation for these traits. We suggest that the combination of natural selection and variability in fitness traits sets up a scenario of positive feedback whereby strong natural selection favors a subset of highly fit hybrids that drives growth rate, followed by further selection on hybrid plants for invasion ability and enhanced growth rates (Fig. 5). The accelerating growth rate we have observed in cordgrass populations in San Francisco Bay is indirect evidence that this has already occurred.

This outcome contrasts with that in Willapa Bay, where *S. alterniflora* was introduced into the same open niche of intertidal mudflat habitat 100 years ago. There is no native *Spartina* at Willapa, and therefore hybridization has not occurred. We propose that evolution there has been constrained by a relative lack of genetic diversity in the population of *S. alterniflora*), resulting in constant spread rates for the last 100 years (Fig. 5).

We conclude that if cordgrass is rapidly evolving in its invasion ability, it will be difficult to precisely predict its eventual ecological range or where the tidal marsh system is or will be vulnerable.

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EVOLVING INVASIBILITY OF EXOTIC *SPARTINA* HYBRIDS IN UPPER SALT MARSH ZONES OF SAN FRANCISCO BAY

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Keywords: *Spartina, Sarcocornia virginica*, invasive, hybridization

INTRODUCTION

Invasion by hybrid cordgrass (*Spartina alterniflora* x *Spartina foliosa*) is profoundly altering habitat structure within the intertidal zone of San Francisco Bay, California. Spartina hybrids exhibit wide ecological tolerance compared to native *S. foliosa* and are invading the naturally unvegetated lower intertidal zone. Heterogeneous hybrid genotypes exhibit traits of both salinity tolerance and competitive vigor, which may enable invasion of higher marsh zones historically dominated by the highly salt-tolerant native pickleweed species *Sarcocornia virginica*. The hybrid cordgrass swarm possesses a high degree of genetic variation, with bi-directional introgression due to overlapping flowering periods of hybrids and both parental species (Ayres et al. 1999; Antilla et al. 2000). Hybridization has been shown to play a role in large and rapid adaptive evolution (Rieseberg et al. 2003), promoting the ability for niche separation between hybrids and parental species (Rieseberg et al. 1999).

Drawing on field observations indicating higher relative fitness of hybrid *Spartina* compared to native *S. foliosa* in the higher marsh zones, we conducted two experiments to address the threat of hybrid colonization of *Sa. virginica* habitat. A greenhouse experiment investigated salinity tolerance of hybrids. A field experiment examined competitive suppression of hybrids by *Sa. virginica*.

METHODS

For the greenhouse experiment, clonal fragments of eighteen hybrid genotypes were collected from Cogswell Marsh, Hayward, California. In addition to the hybrids, one *S. alterniflora* and two *S. foliosa* genotypes (21 plants total) were grown at three salinity levels (10, 25 and 40 ppt) with three replications for one growing season in Davis, California (Fig. 1). Total dry biomass (roots and above-ground material) was determined at the end of the season, and effects of genotype, salinity and their potential interaction determined with statistical analysis.

The field experiment was conducted at Cogswell Marsh, a former salt pond opened to tidal action in 1980. Four plot treatments (pure *Spartina* sp., pure *Sa. virginica*, clipped *Sa. virginica* and unclipped *Sa. virginica;* five replicates) were set up at each of eight hybrid clone locations and one *S. foliosa* clone (Fig. 2). End-of-season shoot density and above-ground biomass were determined for each plot, and effects of genotype and plot type were determined with statistical analysis.

Fig. 1., left. 40 ppt. (left) vs. 10 ppt. (right) in salinity experiment.

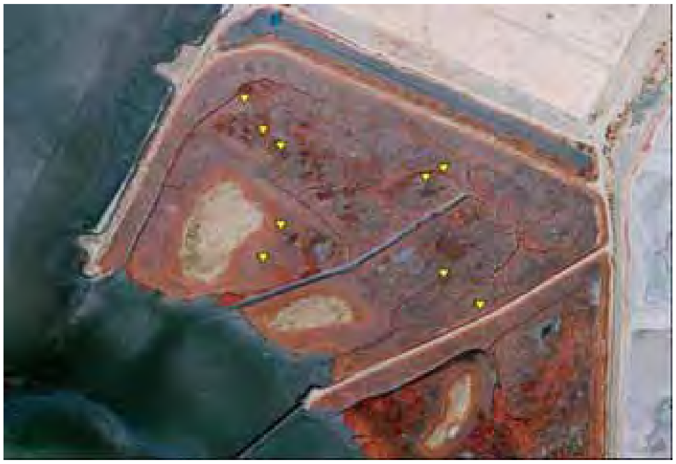

Fig. 2., right. Location of nine *Spartina* genotypes at Cogswell Marsh.

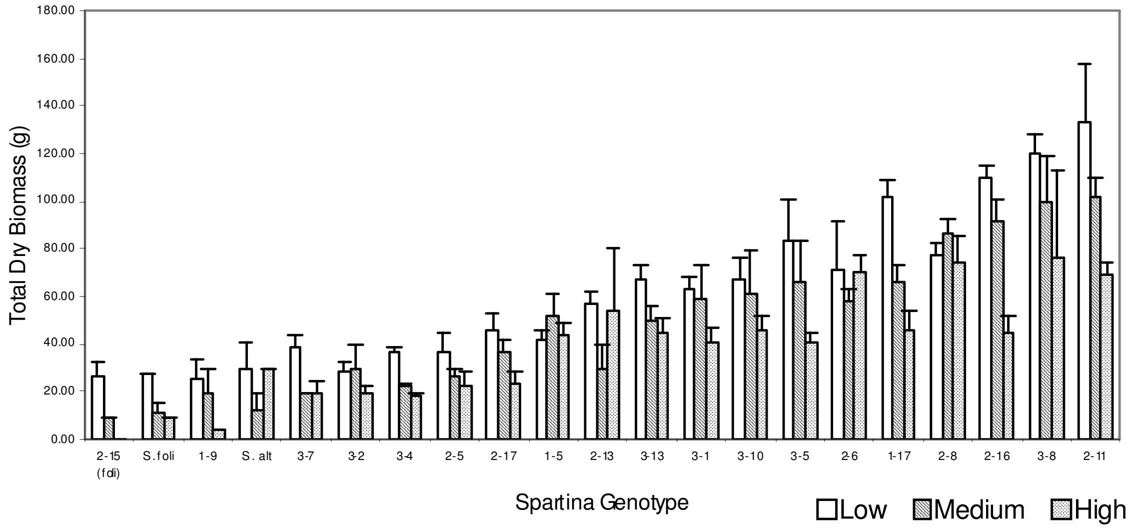

Fig. 3. Total biomass of *Spartina* genotypes at each salinity level, in increasing order of total biomass achieved by each genotype (low + medium + high salinities).

RESULTS

Hybrid *Spartina* genotypes exhibited great variability in morphological traits, response to salinity stress and competitive suppression by *Sa. virginica*. Salinity reduced total biomass of most *Spartina* genotypes. Several hybrid cordgrass genotypes exhibited more robust growth than the parental species at both low and high salinity levels (Fig. 3).

Results of the field experiment indicated that shoot density of hybrid cordgrasses was highly sensitive to *Sa. virginica* removal, increasing an average of 105% (range = 39-211%; Table 1). End-of-season biomass of cordgrass genotypes increased an average of 64% in response to *Sa. virginica* removal (range = 2-144%; Table 1). Genotypes in Table 1 are listed in order of appearance on Fig. 3.

CONCLUSIONS

Certain hybrids (e.g., genotypes 1-5 and 2-8) showed a combination of competitive vigor in the field and relatively stronger performance in higher salinity conditions, exhibiting the potential to successfully expand into higher marsh zones. The native *S. foliosa* has weaker competitive abilities and tolerance for salinity. Our results support a positive association between hybridization and invasion ability. The genetic heterogeneity of *Spartina* hybrids will lead to the evolution of even more invasive hybrid populations.

Table 1. Genotype means and percent change between plots with *S. virginica* "clipped" vs. unclipped, end-of-season shoot density and aboveground dry biomass.

Spartina	**Shoot Density (#/m^2)**			**Dry Biomass (g/m^2)**		
Genotype	**Unclipped**	**Clipped**	**% Change**	**Unclipped**	**Clipped**	**% Change**
2-15 (*S. foli*)	41	84	104	107	206	93
1-9	107	235	119*	212	330	56
2-17	84	117	39	234	243	4
1-5	67	136	103*	695	1360	96*
3-13	77	173	126*	277	426	54
3-1	74	117	59	464	471	2
1-17	65	104	60	512	1251	144*
2-8	62	139	124*	501	556	11
3-8	46	142	211*	159	342	115

* Significant difference ($P < 0.05$)

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VARYING SUCCESS OF *SPARTINA* SPP. INVASIONS IN CHINA: GENETIC DIVERSITY OR DIFFERENTIATION?

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Biological invasions are widely envisaged as a component of global change, which not only threaten native biodiversity but also cause a considerable economic loss to the invaded areas. However, for some species little is known about why they are successful invaders. At the molecular level, many researchers have reported that high genetic diversity contributes to the success of plant invasions whereas other studies have shown that reduced genetic variation makes invasive species more successful. Obviously, these are contradictory explanations. Our studies of *Spartina* in China show that *S. alterniflora* with higher population differentiation (*Fst)* was more successful in invading coastal China than *S. anglica* which had low *Fst*, although the latter species, having fixed heterozygosity, had much higher genetic diversity than the former. Greater number of chromosomes or higher genetic diversity (P and H) does not necessarily mean higher adaptability and more successful invasion for exotic species, while higher *Fst* was associated with higher invading ability in *S. alterniflora*. However, factors other than genetic variation and population genetic structure may be more important in determining invasion success.

Keywords: Average heterogeneity, genetic diversity, plant invasion, population differentiation, *Spartina*

Invasive plant species threaten the integrity of natural ecosystems and reduce the growth of economic crops throughout the world by displacing native plant communities (Kennedy et al. 2002), establishing monocultures and competing with economic species in new habitats (Callaway 2002). At the population level, the leading theories for the successful invasion of plants are their escape from the natural enemies that hold them in check, freeing them to utilize their full potential for resource competition (Keane & Crawley 2002); allelopathic effects, where the phytotoxins released by exotic plants damage native species (Callaway & Aschehoug 2000; Bais et al. 2003); and the occurrence of a suitable niche existing in the new location (Sakai et al. 2001). At the molecular level, most researchers have reported that high genetic diversity contributes to the success of plant invasions (Ellstrand & Schierenbeck 2000; Novak & Mack 2001), although reduced genetic variation can make invasive species more successful (Tsutsui et al. 2000). Here we suggest that the different molecular parameters are correlated with success or failure of *Spartina* invasions in China.

Historically, no native species of the genus *Spartina* existed in China. For the purpose of ecological engineering, two *Spartina* species, *S. anglica* and *S. alterniflora*, were introduced to China in the last century. However, their fates differed. *Spartina anglica* originated in 1870s in east coastal England and is a hybrid between *S. maritima* as paternal species and *S. alterniflora* as the seed parent (Ferris et al. 1997). In 1963, 44 individuals (actually ramets) of *S. anglica* were produced by seed in China; 21 of them were planted in the field and the others were used to produce more offspring, three of which had marvelous reproductive capacity (Chung 1985). From 3 individuals, 9,100,000 clones were produced in 1966, and were planted in 40 hectares (ha) of costal regions in China. Thirteen years later, about 31,600 ha of *S. anglica* low marshes were established in coastal China, and 36,000 ha in 83 counties along coastal China (Fig. 1a) in 1985 (Qin & Chung 1992). Its southern limit in China was at 21º27'N whereas the southern limit in its native range is located between 42ºand 43º N in Europe (Chung 1993).

The populations of *S. anglica* in China have generally lower height, lower biomass and lower net production (Chung 1985) than their counterparts in its native range (Chater & Jones 1951; Gray & Benham 1991; Hubbard 1969) (Table 1). Starting in 1993, *S. anglica* became shorter in height, and lower in biomass and net biomass production. Pronounced dieback occurred in Chinese populations of *S. anglica* like the older populations in England (Gray et al. 1991). In 2000, *S. anglica* was found only in six counties (Fig. 1b). In 2002, the populations of *S. anglica* were found only in three counties, and the total area was less than 50 ha. Plant performance continued to be suppressed, e.g., height, biomass and net production were reduced; and the suppression still continues. Most of the *S. anglica* populations have been competitively replaced by *S. alterniflora*, *Phragmites australis*, *Typha* spp. and *Scirpus* spp.

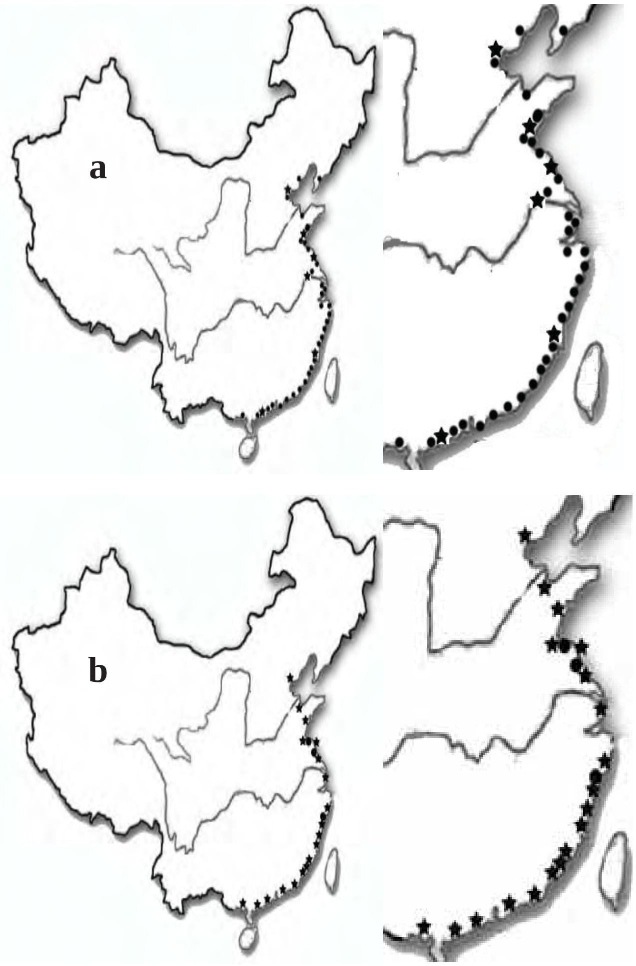

Fig. 1. Distribution of *Spartina anglica* and *Spartina alterniflora* in 1985 (a) and 2000 (b) along coastal China. Circles represent *Spartina anglica* and stars denote *Spartina alterniflora*.

Table 1. Variation of *Spartina anglica* between native range and China

i s	i r							i			
	s s[1]										
i ri i	1	2		4		6			H	d	s
	86	10	0.8	0.8	1.6	0.8	0.8	0.126	0.4202	0.0 84	0.0 20
rs	1 11 1							1 66 1 8		2000 2002	
i ss 2	41 .8 12 1. [2]							24 . 8	8 1.0	21 .28	02.6
r d i 2 yr	4 4 00							110.	601.8	6.	112.4
H i	0 0[4]							1 80		0	

d r r ri s s r s 1 . 2001
2 H bb rd 1 6 r y d 1 1 d 4 r d s 1 1.

Seven multilocus genotypes (G1-G7) were identified using RAPD markers for *S. anglica* in its native Europe (Baumel et al. 2001). G1 is the basic type that accounts for 86% of the samples (Table 1). G2 – G7 are varieties of G1 and only account for 10% - 0.8% of the total. The study showed that *S. anglica* has low genetic variation due to a genetic bottleneck. As an exotic species, *S. anglica* in China has even lower genetic variation with lower percentage of polymorphical loci (P), mean coefficient of dissimilarity (*Gd*) and coefficient of population differentiation (*Fst*) (Table 1) although AFLP markers can provide higher diversity than RAPD markers. Lower variation may indicate that the species partially lost some of its variation during the human-aided invasions in China, since only small parts of native population were collected in the introduction process.

S. alterniflora is a native species of north Atlantic America. In 1979, 60 individuals and hundreds of seeds were collected from Florida, Georgia and North Carolina, consisting of three distinct populations that differed in height, biomass production and protein (Chung 1985; Qin & Chung 1992), namely F-type (Florida-type), G-type (Georgia-type) and N-type (North Carolina-type). In 1981, about 400 square meters (m^2) of planted area were established for each population, and in 1985 the area expanded to 260 ha with human help (Qin & Chung 1992) (Fig. 1a). In 1990, there were 1,300 ha of *S. alterniflora* monoculture along coastal China, but only G-type remained as the two other types were replaced by the G-type through intraspecific competition (Guan et al. 2003). In 2000, the total area reached 120,000 ha (Guan et al. 2003) (Fig. 1b).

Unlike *S. anglica*, the exotic *S. alterniflora* has high reproductive and dispersal capacity, and is competitive; it took only 19 years to increase its area from 0.08 ha to 21,300 ha in Jiangsu Province (Shen & Liu 2002). It excluded almost all the other native plants that were originally dominant in wetlands, including *Phragmites australis*, *Typha* spp., *Scirpus* spp., *Suadae* spp., and even invaded fishponds and young mangrove swamps (Qian & Ma 1995). Many native species, including plants, some endangered birds (Ma et al. 2003), and molluscs of economic importance in coastal China are threatened by *S. alterniflora* invasions (Qian & Ma 1994). *Spartina alterniflora* is one of nine notorious invasive pest plants in China since the species directly causes millions of dollars of economic loss per year (Chen 1998). *Spartina alterniflora* is still rapidly replacing the native plants, although the Chinese government and scientists are doing their best to control or eradiate the species by physical, chemical, biological and integrated methods (Lin 1997; Liu & Huang 2000).

S. alterniflora populations in China have similar percent of polymorphic loci (P) to native populations (Travis et al. 2002), but we found that the species in China has much higher average heterogeneity (H), coefficient of population differentiation (*Fst*), and much lower mean coefficient of dissimilarity (*Gd*) (Table 2). The introduced *S. alterniflora* has accumulated much population genetic differentiation,

Table 2. Genetic variation of *Spartina alterniflora* in native range and China based on AFLP markers. percentage of polymorphical loci (P), heterogeneity (H), mean coefficient of dissimilarity (*Gd*) and coefficient of population differentiation (*Fst*).

i r				i			
	H	d	s		H	d	s
0. 0 0	0.11 4	0.2	0.064	0.280	0. 4	0.1 4	0.20 6

d r r r is . 2002 .

and considerably increased its invading capacity into coastal China since its arrival in 1979. Rapid and genetic differentiation may aid *S. alterniflora* in being a successful invasive species.

Although *S. anglica* arrived in China 16 years earlier than its seed parent *S. alterniflora*, the species has much lower genetic differentiation in coastal China's environments (Tables 1 and 2). *Spartina anglica* experienced a genetic bottleneck during its formation, which might have contributed to its dieback in China as in its native Europe (Gray et al. 1991; Thompson 1991).

S. anglica has 120-124 chromosomes while *S. alterniflora* has 62 (Gray & Benham 1991). *Spartina anglica* should have high heterosis and potentially higher adaptability than either parental species. *Spartina anglica* has replaced *S. maritima* in coastal England (Gray et al. 1991). However, *S. alterniflora* has rapidly occupied previous *S. anglica* habitats in China. We suggest that the species with low population differentiation (*Fst*) may be outcompeted by a species with high differentiation in changing environments like coastal areas of China, where accidental typhoons, irregular tidal cycles and human-caused disturbances often happen. Further, a greater number of chromosomes or higher genetic diversity (P and H) does not guarantee a higher adaptability and more successful invasion for exotic species. However, the generality of this finding needs to be tested further in more taxa.

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SPARTINA DENSIFLORA X *FOLIOSA* HYBRIDS FOUND IN SAN FRANCISCO BAY

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INTRODUCTION

In the 1970s, *Spartina densiflora* and *S. foliosa* were planted during the restoration of Creekside Park in Marin County to a tidal salt marsh. In 2001 we discovered cordgrass plants that spread by rhizomes like *S. foliosa*, but had dense, evergreen stems like *S. densiflora*. *Spartina foliosa*, California cordgrass, is native to the state. Plants grow laterally by rhizomes, creating meadows of sparse, evenly-spaced, deciduous stems. The species occupies lower tidal environments (above mean sea level to mean high water). *Spartina densiflora*, dense-flowered cordgrass, is native to South America. Lack of rhizomes create a bunch-type grass, with dense, largely evergreen stems. The species occupies higher tidal areas than *S. foliosa*, occurring with *Sarcocornia viginica*. The intermediate appearance of the Creekside Park plants suggested that the two species had hybridized.

MOLECULAR AND CYTOLOGICAL DYNAMICS OF S. DENSIFLORA X FOLIOSA HYBRIDS

We developed and used RAPD (Random Amplified Polymorphic DNA) nuclear DNA markers specific to either *S. foliosa* or *S. densiflora* to identify and type hybrids (F1 or introgressed). We used species-specific chloroplast DNA sequences (Anttila et al. 2000) to determine the maternal parent of hybrid plants, as chloroplasts are maternally inherited in *Spartina* (Ferris et al. 1997). Chromosome numbers in root tips were counted in the parental species and in seven hybrid plants. We estimated genome size in most plants using flow cytometry (Grotkopp 2004; Galbraith 1982), compared the genome sizes with the corresponding chromosome counts, and used genome size to rapidly assess the ploidy of hybrids (see Ayres et al. 2008 for details).

We found 35 hybrid plants. All exhibited a F1 pattern of nuclear bands; that is, generally they contained all 13 *S. densiflora*-specific bands and all nine *S. foliosa*-specific bands. A few plants lacked one or two bands. Most plants (17 out of 20 analyzed) had *S. densiflora* cpDNA. Most hybrids were intermediate between *S. densiflora* and *S. foliosa* in chromosome number and genome size (Table 1); both chromosome number and genome size are consistent with haploid gametes of each parental species (31 from *S. foliosa* + 35 from *S. densiflora*) uniting to form a F1 hybrid with 66 chromosomes. However, two plants were triploids, with the cp DNA of *S. foliosa*. Chromosome counts and genome size assessments are consistent with a 2n contribution by *S. foliosa* and a 1n contribution by *S. densiflora*, with loss of one and three chromosomes, respectively, in the two triploid individuals. Due to chromosomal mis-matching, viable gamete formation is probably rare in all hybrids. Even so, the presence of triploid plants is important as it indicates that several avenues exist

Table 1. Molecular and cytological dynamics of *S. densiflora* x *foliosa* hybrids.

	S. foliosa	*S. densiflora*	2n hybrids	3n hybrids
Type/number of hybrids (RAPDs)			33 F1	2 F1
cp DNA	Sf	Sd	17 Sd: 1 Sf	2 Sf : 0 Sd
Chromosome number	62	70	65/66	94/96
Genome size- pg (SD)	4.46 (SD= 0.10)	5.16 (SD = 0.06)	4.83 (SD = 0.06)	7.0 (SD = 0.01)
Chromosome math	31 (S.f. 1n) + 35 (S.d. 1n) = 66 (S.d x f 2n)			
Triploid math	62 (S.f. 2n) + 35 (S.d. 1n) - (1 or 3 chromosomes) = 94 or 96 (S.d x f 3n)			
Genome size math	{0.5 *4.46 (S.f. 2n)} + {0.5 *5.16 (S.d. 2n)} = 4.81 pg (S.d x f 2n)			
Triploid math	{1*4.46 (S. f. 2n)} + {0.5 *5.16 (S.d. 2n)} = 7.0 pg (S.d x f 3n)			

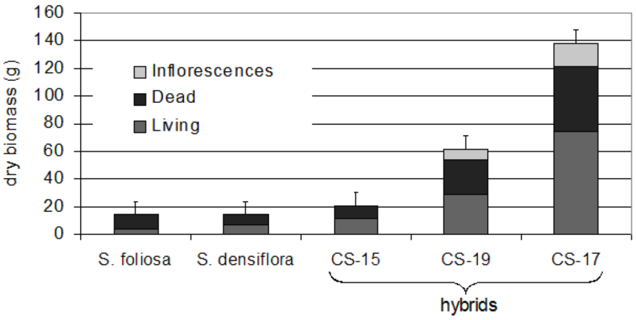

Fig. 1. Final aboveground biomass of some plants in greenhouse salinity experiment. CS-17 and CS-19 (diploid plants) are some of the transgressive hybrids, while CS-15 (triploid plant) has performance comparable to the parental species under the same high salinity stress.

which may give rise to a new alloployploid species. Thus, this new hybridization possibly offers us a chance to observe the origin of a new species.

ECOLOGY OF *S. DENSIFLORA X FOLIOSA* HYBRIDS

This is the second *Spartina* hybridization in the San Francisco estuary in the past three decades; the other, between *S. alterniflora x foliosa*, has resulted in a backcrossing swarm of invasive hybrids. Introductions of *Spartina* have resulted in major biological invasions in salt marshes around the world. Two of these invaders are hybrids between native and introduced species (*S. alterniflora x foliosa*; *S. anglica*). Given the history of *Spartina* invasions and hybridizations, we investigated whether *S. densiflora x foliosa* hybrids have the potential to spread and invade surrounding marshes in the Bay. This would require that hybrids tolerate marsh salinity and tidal inundation, and produce viable seed. A greenhouse experiment was performed with ten hybrid genotypes to assess their salinity tolerance against the parental species (*S. foliosa* – low elevation, low salinity; and *S. densiflora* – higher elevation, higher salinity). Salinity was increased by 10 parts per thousand per week (ppt/week), and we measured several fitness indicators for 10 weeks. In the field, we combined mapping data with elevational measurements (using a Trimble Total Station) to determine the relative tidal elevations of hybrids, native species, and *S. densiflora*. We also collected inflorescences from the field to measure their reproduction (seed set).

We found that some hybrids have high salinity tolerance based on final aboveground biomass and flower production at the end of 10 weeks (Fig. 1). Tolerance to high salinity could allow hybrids to grow in higher marsh environments, largely occupied by *Sarcocornia virginica*. Hybrids had an elevational range similar to *S. densiflora* at Creekside Park. *Spartina foliosa* occupies the lowest range, while the dominant *Sarcocornia virginica* is found in the highest. The hybrids occupy the same “middle” range as their *S. densiflora* parent, which suggests that the hybrids might not tolerate tidal inundation as well as their *S. foliosa* parent. Finally, hybrids set no seed.

SUMMARY AND CONCLUSIONS

We found that the salinity tolerance of some of hybrid genotypes exceeded that of both parental species; that hybrids occurred higher in the marsh than *S. foliosa* and within a narrower elevation range than either parental species; and hybrids were apparently sterile as they produced no seed in the field and produced only shriveled anthers in the greenhouse experiment. We conclude that despite hybrid superiority in salinity tolerance, *S. densiflora* x *foliosa* hybrids will not be invasive due to sterility. Even so, sterility may be overcome if fertile tetraploids or hexaploids evolve from hybrid individuals.

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FUNGAL SYMBIOSIS: A POTENTIAL MECHANISM OF PLANT INVASIVENESS

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We propose that fungal endophytes provide a mechanism for the habitat expansion of invasive plants including *Spartina* species. We have determined that *Spartina* spp. are symbiotic with endophytic fungi and have begun to assess the role of fungal endophytes in the invasiveness of these plants. Preliminary data indicate that the fungal endophytes in *Spartina* spp. in Puget Sound are native to that region. The role of symbiosis in the invasion of invasive species and the adaptation of plants to high stress habitats is discussed.

Keywords: symbiosis, fungi, lifestyle, plant ecology, fungal ecology, *Colletotrichum, Curvularia, Spartina*

INTRODUCTION

It is estimated that thousands of plants are introduced into non-native habitats every year, however, only a small percentage of these plants become invasive. Interestingly, invasions may result from intercontinental or intracontinental plant movements and may involve either vast or very short distances. Several theories have been proposed to explain the invasiveness of plants (Daehler 2003; Callaway and Ridenour 2004). However, none adequately explain why plants can achieve high densities in new habitats (Daehler, 2003; Silvertown 2004). It does appear that invasion is context dependent and may involve a number of biotic and abiotic factors (Daehler 2003). Although there have been numerous studies on invasive plants, few include fungal symbiosis as a component of plant biology. Yet, all plants in natural ecosystems are thought to be symbiotic with mycorrhizal and/or endophytic fungi. These fungi differ in distribution, biology and physiology. Mycorrhizal fungi are limited to colonizing roots and grow out into the rhizosphere effectively expanding root systems by transporting nutrients and water that would otherwise be unavaiable to root systems (Read 1999).

Endophytic fungi reside entirely within plant tissues and may occur in specific tissues (roots, crowns, stems, leaves, seed coats or seeds) or throughout the plant. Although mycorrhizal fungi do not colonize all plants, it is thought that all plants are colonized by endophytic fungi. Fungal endophytes can be divided into two major groups (Rodriguez et al. 2009b): 1) a small number of fastidious species that are restricted to a small number of monocot hosts (Clay and Schardl 2002) and 2) a large number of tractable species with broad host ranges (Stone et al. 2004). Both groups of fungal endophytes are known to be important to the structure, function, and health of plant communities. In fact, without fungal symbioses, plant communities do not appear to survive many environmental stresses (Arnold et al. 2003; Dingle and McGee 2003; Ernst et al., 2003; Redman et al. 2002b).

FUNGAL SYMBIOTIC LIFESTYLES

Collectively, fungal symbionts express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism. These lifestyles are based on positive, neutral or negative fitness benefits to the host and symbiont (Table 1, Lewis 1985). Mutualistic fungi have been shown to increase plant growth and productivity (Marks and Clay 1996; Varma et al. 1999; Read 1999; Redman et al. 2002a; Rodriguez et al. 2009a), and confer tolerance to abiotic and biotic stresses including drought, metals, salt, temperature, pathogens and herbivores (Bacon and Hill 1996; Bacon 1993; Read 1999; Carroll 1986; Redman et al. 2001; Redman et al. 2002b; Latch 1993; Rodriguez et al. 2008). We have demonstrated that endophytic fungi also have the ability to switch lifestyles in response to host genotypes and that the host range of fungal endophytes is typically greater than previously thought (Redman et al. 2001). For example, fungi from the genus *Colletotrichum* are classified as plant pathogenic fungi. However, several species have the ability to asymptomatically colonize plants not previously known to be hosts and express non-pathogenic lifestyles including mutualism. When *Colletotrichum* species express mutualistic lifesytles they confer disease resistance against fungi that express pathogneic lifestyles in the respective host plant. Therefore, one fungal isolate may be a virulent pathogen on one host and a disease protecting mutualist on another.

Symbiotically conferred disease resistance is correlated with the activation of host defense systems (Redman et al. 1999). When exposed to virulent pathogens, non-symbiotic plants slowly activated defense systems over a four-day period and by day five the plants were dead. Symbiotic plants activated defense systems within 24 hours of exposure to a virulent pathogen and completely terminated pathogen

Table 1. Fitness impacts of Symbiotic Lifestyles

Lifestyle	*Fitness Impact*	
	Host	Symbiont
Parasitism	-	+
Commensalism	0	+
Mutualism	+	+

- = fitness decreased, + = fitness increased,
0 = fitness not affected

ingress. It appears that when *Colletotrichum* species are expressing non-pathogenic lifestyles they are acting as biological triggers allowing plants to recognize virulent pathogens more quickly (Rodriguez et al. 2004). The effectiveness of an endophyte as a biological trigger appears to be a function of currently undefined genetic compatibility with the host.

ADAPTIVE SYMBIOSIS

Mutualistic fungi are known to confer a variety of fitness benefits. However, it is not known if symbiotically conferred stress tolerance reflects an adaptive response by the host and/or symbiont. We have studied the ecological significance of endophytic fungi in plants thriving in geothermal soils and found that the symbiosis is responsible for the ability of both host and symbiont to survive thermal stress (Redman et al. 2002b; Marquez et al. 2007). In over 200 individuals analyzed, the plant *Dichanthelium lanuginosum* is colonized by one fungal species (*Curvularia protuberata)* that is known to be a pathogen in other plant species (Farr et al. 1989). The fungal endophyte colonizes roots, crowns, stems, leaves and seed coats but not seeds. When seed coats are removed and seeds' surfaces are sterilized it is possible to propagate plants devoid of the fungus. This approach allowed us to compare the ability of symbiotic and non-symbiotic plants to mitigate the impacts of thermal stress. Neither of the symbiotic partners tolerated temperatures above 40°C, however, the symbiosis allowed both to survive root temperatures up to 70°C. Recently we have observed that isolates of *C. protuberata* from non-geothermal plants do not confer temperature tolerance suggesting that this is an adaptive response by fungi in the geothermal habitat (Rodriguez et al. 2008).

Comparative studies with *Colletotrichum* and *Curvularia* species support the hypothesis that symbioses adapt to habitat stresses (Rodriguez et al. 2008). These fungi were assessed for the ability to confer heat tolerance (an abiotic stress in geothermal habitats) and disease resistance (a biotic stress in agricultural habitats) to host plants. *Colletotrichum* isolates expressing non-pathogenic lifestyles conferred disease resistance but not heat tolerance. Conversely, *Curvularia* isolates conferred heat tolerance but not disease resistance. This observation suggests that at least some of the benefits conferred by mutualistic endophytes appear to reflect habitat-specific adaptation.

Colletotrichum species that express a mutualistic lifestyle typically are able to asymptomatically colonize genetically divergent plant species (Redman et al. 2001). For example, *C. magna* is a virulent pathogen on cucurbits but is a disease protecting mutualist on tomato (*Lycopersicon esculentum*)*,* beans (*Phaseolus vulgaris*) and strawberry (*Fragaria ananassa*)*,* three genetically divergent species. Therefore, it appears that genetically distant plant species can gain novel biological function simply by forming symbioses with different fungi. This could allow individual plant species to make quantum evolutionary leaps and expand into new habitats or become dominant members of existing communities.

SYMBIOSIS AS A POTENTIAL MECHANISM OF INVASIVENESS

Based on the broad host range of class 2 fungal endophytes, their ability to confer abiotic and biotic stress tolerance, and the apparent adaptive nature of symbioses, we propose the following hypothesis: The conversion of at least some non-native plants into invasive species requires the establishment of symbioses with endophytic fungi that confer tolerance to abiotic and biotic stresses. These symbioses allow invasive species to circumvent the ecological factors that keep native species under control and plant communities balanced. In order to properly challenge this hypothesis (designated the "symbiotic communication hypothesis") several questions must be addressed such as: Do non-native species carry non-native endophytes when transported? Are the non-native endophytes capable of releasing non-native plants from ecological controls? If the non-native plants are devoid of endophytes what are the dynamics of colonization by native fungi? If an adaptive response is required before non-native plants become invasive, what are the temporal dynamics of adaptation?

Another mystery of plant invasions is that there is a time lag time between introduction and spread which may be more than 100 years (Binggeli 2000). Several hypotheses have been proposed to explain invasive lag time but none have adequately addressed this phenomenon. We have demonstrated that subtle differences in host genotypes are sufficient to eliminate symbiotically conferred stress tolerance and may result in the expression of pathogenic rather than mutualistic lifestyles by endophytic fungi (Redman et al. 2001). Therefore, we propose that the lag phase commonly observed between introduction and invasion may reflect one or more of the following:

1) Endophytes carried with non-native plants adapt to new habitat stresses and confer stress tolerance allowing non-native plants to outcompete native plants;

2) Non-native plants establish symbioses with native fungi that communicate more efficiently than with native plants;

3) Non-native plants bring fungi that switch lifestyles and are pathogenic on native plants;

4) Non-native plants alter the rhizosphere such that native root pathogens normally present in low abundance become abundant and are more virulent on native plants than the non-native species.

To begin testing the symbiotic communication hypothesis we have begun to assess the fungal endophytes in several native and invasive plant species in Puget Sound and native habitats of the invasive species. The list of plant species we are analyzing includes *Spartina anglica, S. alterniflora* and *S. patens.* A minimum of three populations for each plant species was analyzed and at least 10 plants/per population were sampled. All of the *Spartina* plants analyzed (N=100) contained class 2 endophytes which appear to be native fungi based on a biogeographic study (Rodriguez *et al.* in preparation). These endophytes are currently being taxonomically defined by sequence analysis of nuclear ribosomal DNA and classical morphological methods. The host range of the endophytes is also being assessed and culture conditions identified for fungal conidiation. In addition, we have developed methods to germinate *Spartina* seeds devoid of endophytic fungi and are beginning to perform comparative studies to evaluate symbiotic and non-symbiotic plants. In 2005-2007 we performed greenhouse experiments to understand the symbiotic dynamics of *Spartina anglica* in the presence and absence of abiotic stress. These studies indicated that *Spartina*, like native coastal plants, establish associations with endophytes that confer salt tolerance Rodriguez *et al.* 2008; Rodriguez *et al.,* in preparation). Therefore, we surmise that endophytes play an important role in the invasion of coastal areas by *Spartina* species. Studies are underway to determine if endophytes contribute to the invasion processes in non-coastal habitats that impose different degrees of abiotic stress.

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IS ERGOT A NATURAL COMPONENT OF *SPARTINA* MARSHES? DISTRIBUTION AND ECOLOGICAL HOST RANGE OF SALT MARSH *CLAVICEPS PURPUREA*

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This study was undertaken to better characterize the geographic distribution and host range of *Claviceps purpurea* from grass hosts in salt marsh habitats. *Claviceps purpurea* contains three intraspecific groups, each with a habitat association: G1 from terrestrial habitats, G2 from moist habitats and G3 from salt marshes. Twenty-six G3 isolates, representing 11 distinct populations were characterized based on the presence of an *Eco*RI restriction site in the 5.8S ribosomal DNA, and genetic similarity to isolates representing the other two *C. purpurea* intraspecific groups (G1 and G2). *Distichlis spicata* was identified as the first non-*Spartina* host to G3 *C. purpurea*. In addition, all isolates originating from *Spartina densiflora*, *S. foliosa*, *S. alterniflora*, and *S. anglica* were identified as belonging to G3 based on genetic analysis. Salt marsh *Claviceps purpurea* can be found along the Atlantic and Pacific Coasts of North America, Argentina, Ireland and England. A global distribution suggests that salt marsh (G3) *C. purpurea* is a natural component of *Spartina*-dominated salt marsh habitats.

Keywords: Ergot, *Claviceps purpurea*, *Spartina*, host range

INTRODUCTION

Claviceps purpurea (Fr.) Tul, the cause of ergot, is a well known pathogen of cereal grains and forage. The pathogen has a global distribution, and a wide host range within the Poaceae. This broad host range has led many researchers to seek evidence of taxonomic substructure based on morphology (Loveless 1971), alkaloid production (Eleuterius and Meyers 1977; Kobel and Sanglier 1978), and genetic characteristics (Jungehülsing and Tudzynski 1997).

In a recent study, Pazoutová et al. (2000) synthesized previous research on *C. purpurea* morphology, alkaloid chemistry and genetics, and identified three distinct groups within the species. Rather than divisions based on host range, the groups were defined by habitat association. The largest group, G1, was associated with terrestrial grasses, and G2 with grasses in moist environments, whereas G3 was found only in salt marsh habitats. Isolates associated with G1 and G3 share an *Eco*RI restriction site in the 5.8S ribosomal DNA (rDNA), which G2 isolates lack. The three groups can be differentiated by RAPD analysis as well (Pazoutová et al. 2000).

The ecological host range of maritime *C. purpurea* (G3) appears very narrow, compared to G1 and G2. G3 has been isolated exclusively from cordgrass species of the genus *Spartina*. Two host species to G3 have been identified, each in only one location: common cordgrass, *S. anglica*, in the United Kingdom and smooth cordgrass, *S. alterniflora*, from the state of New Jersey on the Atlantic coast of the United States (Pazoutová et al. 2000, 2002*b*).

Whereas only two populations of G3 have been identified using molecular markers, the host to this intraspecific group, *Spartina*, is a widespread genus including both terrestrial and halophytic salt marsh species. Most members of the genus, including *S. alterniflora*, share a common native geographic range along the Atlantic Coast of North and South America. Introduced populations of *S. alterniflora* have established on the Pacific Coast as well, in the San Francisco Bay, California (Daehler and Strong 1994) and Willapa Bay, Washington (Stiller and Denton 1995). Thus, the geographic range of G3 may be much greater than is presently recognized and it is reasonable to think that diversity in populations of the pathogen may reflect the diversity of the host species. The objectives of this study were to assess the geographic distribution and ecological host range of G3 *C. purpurea*.

MATERIALS AND METHODS

Isolates included in this study, and their origins, are listed in Table 1. Isolates were sterilized and cultured according to the methods described in Pazoutová et al. (2000). Isolates not collected in the field were obtained as pure cultures from S. Pazoutová, including G1 isolates 374 and 428 (Pazoutová et al. 2000), G2 isolates 236 and 434 (Pazoutová et al. 2000), G3 isolates 500 and 538 (Pazoutová et al. 2002*b*), and G1 isolates165, 204 and 478. DNA was extracted, and RAPD and AFLP markers were developed according to the methods described in Pazoutová et al. (2000). *Eco*RI analysis was also performed using the methods described in Pazoutová et al. (2000). All monomorphic and polymorphic RAPD and AFLP markers were scored using a binary system (zero = absent and one = present). Percent similarity between isolates was calculated by dividing the number of shared markers by the total number of markers and multiplying by 100.

RESULTS

Distichlis spicata was identified as the first non-*Spartina* host to G3 *C. purpurea*. In addition, all isolates originating from *Spartina densiflora*, *S. foliosa*, *S. alterniflora*, and *S. anglica* were identified as belonging to G3 based on genetic analysis. G1 and G3 isolates contained an *Eco*RI restriction site within the 5.8S rDNA, while G2 isolates lacked this site, consistent with distinctions among these groups described by Pazoutová et al. (2000). Using RAPD and AFLP markers, between-group diversity was extraordinarily high with only less than 5 % of markers shared by all members of G1, G2 and G3. Within-group similarity for G3 isolates was approximately 40%.

DISCUSSION

The maritime group of *C. purpurea* (G3) has been distinguished from other intraspecific groups in this species by having an *Eco*RI site in the 5.8S ribosomal DNA, and a unique banding pattern based on RAPD analysis of nuclear DNA. Based on these characters, and AFLP analysis, the field collections examined in this study from *Spartina* spp. and *Distichlis spicata* are representative of the maritime group. The results of this study revealed G3 *C. purpurea* on three new species, *S. foliosa*, *S. densiflora*, and *D. spicata*.

In addition, this study has identified salt marsh (G3) *C. purpurea* on *S. alterniflora* where it is invasive: in the San Francisco Bay, CA and Willapa Bay, WA. *Spartina alterniflora* was intentionally introduced into the San Francisco Bay as seed in the 1970s; *S. alterniflora* was unintentionally introduced to Willapa Bay, Washington over a century ago (Stiller and Denton 1995; Civille et al. 2005). The impacts of *C. purpurea* on *S. foliosa* populations are not known but there is evidence that the pathogen can reduce seed production in this species (A. Fisher, 2007). In Willapa Bay, the impacts of *C. purpurea* on seed production are not known. However, rates of infection are so low, that even if the pathogen reduces seed production on individual infloresences, *C. purpurea* is unlikely to be a significant factor for *S. alterniflora* fecundity.

Spartina anglica, host to maritime *C. purpurea* on the coast of the United Kingdom and now identified as a host in Ireland, has a hybrid origin, being derived from native *S. maritima* and introduced *S. alterniflora* (Raybould et al. 1991). The first incidence of *C. purpurea* on *S. anglica* and *S. townsendii*, the sterile F1 hybrid of *S. maritima* and *S. alterniflora*, was reported in Ireland in 1975 (Boyle 1976). While unsure of its origin, Boyle argued that this and a herbarium specimen of *C. purpurea* on *S. anglica* collected in 1971 were the first definitive reports of *C. purpurea* on salt marsh *Spartina* spp. in Great Britain and Ireland.

Distichlis spicata has been previously identified as a host to *C. purpurea* (Sprague 1950), and is currently the only known host to G3 outside the genus *Spartina*. Perhaps not surprisingly, *Distichlis* and *Spartina* are closely related genera, belonging to the same subfamily (Chloridoideae) and tribe (Cynodonteae) (Peterson et al. 2001). This suggests a phylogenetic correspondence between host and pathogen. The range of *D. spicata* includes the Atlantic Coast of North America, the Gulf Coast states, Cuba, and the Pacific Coast of North America, from British Columbia south to Mexico, and South America (Hitchcock 1971). *Distichlis spicata* is typically found in salt marshes and seashores on moist and alkaline soils, and borders some *S. alterniflora* marshes in Willapa Bay, WA.

In Argentina, G3 *C. purpurea* was collected from *S. densiflora. Spartina densiflora* is native to the Atlantic and Pacific coasts of southern South America (Mobberley 1956). Based on alkaloid profile, Samuelson and Gjerstad (1966) proposed that *C. purpurea* from *Spartina* spp. in coastal

Table 1. Origin and host plant of *C. purpurea* isolates.

^{a}Origin	Host	Group ID
Březno, Czech Republic	*Dactylis glomerata*	G1
Altamont, AL, USA	*F. arundinacea*	G1
Lauderdale, AL, USA	*Festuca arundinacea*	G1
L'Anse aux meadows, Newfoundland, Canada	*Leymus mollis*	G1
Zubri, Czech Republic	*Poa pratensis*	G1
MacDoel, CA, USA	*Secale cereale*	G1
Aberdeen, ID, USA	*Secale cereale*	G1
Hohenheim, Germany	*Secale cereale*	G1
Phillipsreuth, Germany	*Dactylis* spp.	G2
Vlčí Pole u Bousova, Czech Republic	*Molinia coerulea*	G2
Willapa River, WA, USA	*Distichlis spicata*	G3
Palix River, WA, USA	*S. alterniflora*	G3
Dolphin Island, AL, USA	*S. alterniflora*	G3
Point Reyes NS, CA, USA	*S. alterniflora*	G3
St. Augustine, FL, USA	*S. alterniflora*	G3
Marsh Landing, GA, USA	*S. alterniflora*	G3
Flax River, NY, USA	*S. alterniflora*	G3
Rhode Island, USA	*S. alterniflora*	G3
Southriver, NJ, USA	*S. alterniflora*	G3
Marchwood, UK	*S. alterniflora*	G3
Dublin, Ireland	*S. anglica*	G3
Argentina Celpa Marsh, Argentina	*S. densiflora*	G3
Bolinas Lagoon, CA, USA	*S. foliosa*	G3
Palo Alto, CA, USA	*S. foliosa*	G3
Mountain View, CA, USA	*S. foliosa*	G3
San Mateo, CA, USA	*S. foliosa*	G3
Point Reyes NS, CA, USA	*S foliosa*	G3

Argentina was a separate species, *C. maritima*, or 'feather ergot.' The host at the time was misidentified as *S. maritima* (Eleuterius and Meyers 1977). Eleuterius and Meyers (1977) proposed it was more likely to have been *S. alterniflora*, based on floristic surveys of the region, but since *S. densiflora* is now known to be a host in South America, it could be a candidate as well.

Our results support the recognition of three discrete groups within *C. purpurea*. Results of RAPD and AFLP analysis showed high intergroup variability between G1, G2, and G3. Using a collection of isolates from a different set of hosts, Jungehülsing and Tudzynski (1997) also found high intraspecific variability using RAPD markers. In their study, parsimony analysis grouped samples from the same host species together, suggesting some degree of host specificity. More sampling is necessary to reveal the ecological and physiological range of G3, though there is evidence that the pathogenicity profile of G3, following greenhouse inoculations, is not limited to salt marsh species (Pazoutová et al. 2002*a*). Laboratory experiments notwithstanding, our results imply a degree of specificity in the ecological range of G3.

Our results confirm the presence of three intraspecific groups within *C. purpurea* and support from both RAPD and AFLP profiles strongly suggests widespread geographic dispersal of salt marsh *C. purpurea* throughout the range of both native and invasive *Spartina* species.

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MECHANISMS OF SULFIDE AND AOXIA TOLERANCE IN SALT MARSH GRASSES IN RELATION TO ELEVATIONAL ZONATION

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Sharply-defined ecotones commonly separate species living in high intertidal and low intertidal estuarine zones. Low intertidal regions are characterized by anoxic sediments and toxic levels of hydrogen sulfide. These conditions exclude high marsh species. In contrast, low marsh species are believed to possess physiological adaptations to resist the anoxia and sulfide. However, these adaptations are poorly understood. One of the most important characteristics of waterlogged sediments is the lack of oxygen. Many wetland plants have been shown to transport atmospheric oxygen internally to support respiration in submerged tissues. This ability may allow plant survival in low intertidal marsh areas and is often implicated as a factor in determining species zonation in estuaries. In this study, oxygen transport and metabolic characteristics related to anoxia tolerance and rhizosphere oxidation were investigated in the emergent estuarine species *Spartina alterniflora*, *S. anglica*, *S. densiflora*, *S. patens*, and *Distichlis spicata* (Poaceae). Plants were grown in greenhouse experiments under simulated estuarine conditions. All species showed a strong ability to respire anaerobically. The high intertidal marsh species *S. densiflora*, *S. patens*, and *D. spicata* were found to have high aerobic respiration rates, low oxygen transport rates, and an apparent high sensitivity to sulfide. The low intertidal marsh species *S. alterniflora* and *S. anglica* had lower aerobic respiration rates, moderate to high oxygen transport rates, and a lower sensitivity to sulfide. *Spartina anglica* appeared to have the greatest ability to transport oxygen and was more resistant to mudflat-related stressors compared to the other plants in this study. Evidence is presented that aerobic respiration rates and sulfide sensitivity may be important factors for differences in estuarine zonation between species.

Keywords: *Distichlis spicata*, hypoxia, oxygen transport, respiration, sediment, *Spartina*, sulfide

INTRODUCTION

The introduction of four species of *Spartina* grasses (Poaceae) into Washington estuaries has led to many devastating ecological and economic impacts. Nearly 8,100 hectares (ha) of intertidal mudflat in Willapa Bay, Washington, USA, has been affected by introduced *S. alterniflora* (Hedge et al. 2003). Similarly, *S. anglica* was introduced in northern Puget Sound, Washington in 1961 to presvent shoreline erosion, but quickly spread via tidal currents and now affects over 3,300 ha in the Puget Sound area (Hacker et al. 2001). Other *Spartina* introductions into Washington estuaries include *Spartina densiflora* Brong. (WSDA news release 11 Jan 2002) and *S. patens* (Aiton) Muhl. (Frenkel 1987). These populations remain small and are closely monitored to prevent spread.

Introduced *Spartina* flourishes in West Coast estuaries because it can occupy an open niche: low intertidal mudflats and tidal channels characterized by highly reducing conditions (an oxidation reduction potential less than -300 millivolts [Eh<-300 mV]; Brix and Sorrell 1996). Native vegetation cannot colonize these areas. *Spartina* survives in these low intertidal areas because it has a suitable physiology to cope with anoxic mudflats and their associated toxins (Maricle et al. 2006; Maricle and Lee 2007). In contrast, native species like *Distichlis spicata* (L.) Greene are confined to the mid- and upper-intertidal zones (Emmett et al. 2000) where sediment redox potentials are substantially higher.

In their native range, *Spartina* grasses dominate many salt marshes (Teal and Teal 1969). North American East Coast estuaries are dominated by monotypic stands of *Spartina alterniflora* and *S. patens*, often divided by a sharp elevational ecotone (Bertness 1991). It has also been noted that *S. anglica* can grow lower in the intertidal zone than either of its parent species (Thompson 1991). Sharp elevational zonation patterns are common in salt marsh communities (Adam 2002), suggesting differences in ecological amplitude between species that may be due to physiological factors interacting with abiotic gradients. Plants more tolerant of reducing sediments are able to grow lower in the intertidal range, while less tolerant plants are restricted to higher intertidal regions (Maricle et al. 2006).

Grasses of the genus *Spartina* grow extremely well in estuarine sediments that normally do not have sufficient oxygen to support other plant species. A key to their success in these environments is thought to be oxygen transport to the roots and rhizosphere, facilitated by a system of gas spaces (aerenchyma) connecting leaves to root tissues (Teal and Kanwisher 1966, Hwang and Morris 1991, Arenovski and Howes 1992, Howes and Teal 1994). The presence and functioning of aerenchyma is well documented in plants tolerant of flooded conditions, and generally results in a supply of oxygen for aerobic respiration as well as radial oxygen loss to the environment (reviewed by Jackson and Armstrong 1999).

Once oxygen has reached submerged tissues in emergent plants like *Spartina*, it has at least three possible fates (Fig. 1). Oxygen can be released into the rhizosphere, support mitochondrial respiration, or be used in sulfide oxidation processes. The strength of these competing oxygen sinks can be important in the ecophysiology of wetland plants since it influences how oxygen is budgeted in submerged tissues (Sorrell 1999).

Anoxic estuarine sediments represent a strong external oxygen sink and they can overwhelm plant oxygen transport processes. Therefore, *Spartina* grasses also must exhibit a strong capability for anaerobic respiration to sustain metabolism when oxygen supplies are low. The enzyme alcohol dehydrogenase (ADH) catalyzes the final reaction in fermentative ethanol synthesis. The ability to respire under hypoxic conditions is important for life in waterlogged soils, so ADH activity in these plants appears to be an adaptation for anoxia tolerance (Crawford 1967).

To understand the mechanisms conferring success in low intertidal zones, aspects of oxygen transport and metabolic characteristics related to anoxia tolerance and rhizosphere oxidation were investigated in greenhouse *Spartina* plants. The four *Spartina* species introduced into Washington estuaries provide a good system for studying estuarine zonation since they represent a range from high marsh to low marsh species. The low marsh species *Spartina alterniflora* and *S. anglica* were studied and compared to the high marsh species *S. densiflora*, *S. patens*, and the native *Distichlis spicata*.

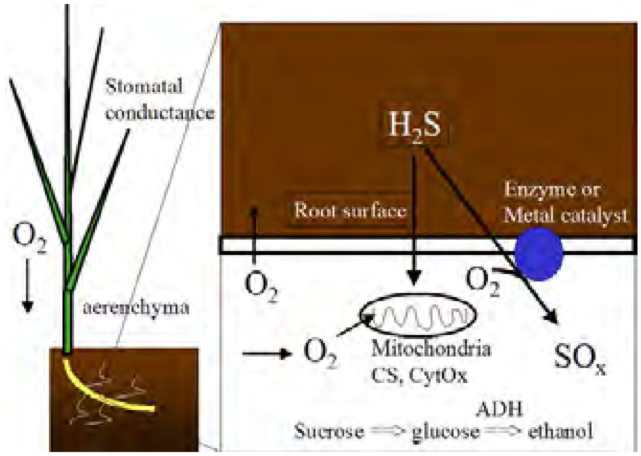

Fig. 1. Atmospheric oxygen can be transported through wetland plants to submerged tissues. Once oxygen reaches the roots (inset), it has several possible fates. Measurements of the indicated processes and enzyme activities may indicate how oxygen is allocated in the roots of flood-tolerant plants.

Plants are aerobes. Therefore, survival in waterlogged soils requires a supply of oxygen to support tissues submerged in anoxic sediments (Crawford 1982). However, many additional physiological processes can be affected by the supply of oxygen to submerged tissues in wetland plants. Measures of the mechanisms shown in Fig. 1 may allow one to estimate how much oxygen is used in aerobic respiration and how strong external oxygen sinks may be. In the present study, rates of oxygen transport were measured and compared to rates of aerobic respiration. Highly reducing mudflat conditions may inhibit aerobic respiration processes and induce alternative anaerobic respiration pathways. Therefore, rates of aerobic respiration were measured as well as activities of the aerobic respiration enzyme cytochrome *c* oxidase and the anaerobic respiration enzyme alcohol dehydrogenase. Tolerance of estuarine mudflat conditions may also require mechanisms to detoxify hydrogen sulfide, a phytotoxin produced under anaerobic conditions. Consequently, rates of sulfide oxidation processes were also measured in root tissues. The results of this study are expected to provide a physiological explanation to help define differences between high marsh and low marsh functional types and their relationship to estuarine zonation.

MATERIALS AND METHODS

Spartina plants were collected from field sites and subsequently maintained under greenhouse conditions. *Spartina alterniflora* plants were collected in Willapa Bay, Washington and *S. anglica* was collected from northern Puget Sound, Washington. Additionally, *S. patens* plants were obtained from the Gulf Coast of northwest Florida and *S. densiflora* plants were obtained from the Odiel Salt Marshes, southwest Spain. The native *Distichlis spicata* was collected in northern Puget Sound, Washington.

Greenhouse temperatures were 26°C during the day and 18°C at night. Natural lighting provided a photosynthetic photon flux density (PPFD) averaging 200 micromoles quanta per square meter per second (200 μmol quanta m^{-2} sec^{-1}) during daylight hours with peaks around 1,100 μmol quanta m^{-2} sec^{-1} on sunny days. Daughter tillers from field-collected plants were potted individually in a 50/50 (vol./vol.) sand/potting soil mixture and were watered to saturation twice weekly with modified Hoagland nutrient solution (Epstein 1972). Freshly potted plants were selected for uniformity in size and randomized between flooded and drained treatments. At least four replicate plants were grown in each treatment. Plants were allowed 60-80 days in their respective treatment (drained or flooded) before testing or harvest. All statistical analyses were performed between species and treatments with a two-factor (species and treatment) analysis of variance (Statview 5; 1998 SAS Institute Inc., Cary, NC; α=0.05).

Individual plants were tested for their ability to transport oxygen internally after the method of Maricle and Lee (2002). A fiber optic oxygen-sensing probe (FOXY-R probe; Ocean Optics Inc., Dunedin, FL) was used to measure dissolved oxygen concentrations in sealed flasks containing roots of intact plants suspended in water. Flask water contained penicillin G and streptomycin sulfate at 1 gram per liter (1 g L^{-1}) each and 50 mg L^{-1} chloramphenicol to prevent bacterial respiration. During testing, plants were placed under a 250 watt (W) metal halide light (Hydrofarm gardening products; Petaluma, CA). At plant level, PPFD was about 150 μmol quanta m^{-2} s^{-1} and air temperature was 28°C. Flask dissolved oxygen concentrations started at 100 μM (±10 μM) and were measured over a period of 2-3 hours to observe consumption or release of oxygen by plants to the surrounding medium.

Parallel measurements of oxygen consumption were conducted on plants where aerenchyma transport capabilities were blocked. Placing the shoot of the plant in a 100% N_2 atmosphere prevents the entry of oxygen into the aerenchyma system (Armstrong 1964, Teal and Kanwisher 1966). Plants were maintained in the dark during this measurement to prevent the release of photosynthetic oxygen. Rates of oxygen consumption were compared between plants under a 21% O_2 atmosphere and a 100% N_2 atmosphere; the difference between the two flux rates equals the amount of oxygen transported internally through the plant's aerenchyma system (Lee 2003). Rates of internal oxygen transport were standardized to g fresh root weight. Oxygen consumption in the dark under 100% N_2 represents total oxygen demand by the plant and was taken to be the dark respiration rate (Maricle and Lee 2007).

At harvest, root samples were obtained from each plant, flash-frozen in liquid nitrogen, and stored at –80°C. Cytochrome *c* oxidase (CytOx) and sulfide oxidase (SOx) activities were determined in extracts from root tissue samples. Roots were ground in liquid nitrogen and cold extraction buffer was added at 2 milliliters per gram (2 mL g^{-1}) (Maxwell and Bateman 1967). This mixture was homogenized with a mortar and pestle, filtered through Miracloth (Calbiochem; San Diego, CA), and centrifuged at 1,000 g for 20 minutes at 4°C. The supernatant was used in CytOx and SOx assays. Alcohol dehydrogenase (ADH) was extracted from root tissue samples after John and Greenway (1976). Roots were ground in liquid nitrogen, and cold extraction buffer was added at 5 mL g^{-1}. The resulting mixture was homogenized with a mortar and pestle, filtered through Miracloth, and centrifuged at 10,000 g for 10 min at 4°C. This supernatant was used in ADH assays. All enzyme assays were performed spectrophotometrically at 25°C (Maricle et al. 2006).

CytOx activity was determined as the rate of cytochrome *c* oxidation, measured as a decrease in absorbance at 550 nanometers (nm) (Smith 1955). Rates of CytOx activity were corrected for background rates of cytochrome *c* oxidation, then standardized to g fresh root weight. ADH activity was assayed spectrophotometrically in the ethanol-forming direction (John and Greenway 1976). Enzyme activity was determined as the rate of NADH oxidation, measured as a decrease in absorbance at 340 nm. Rates of NADH oxidation in the presence of acetaldehyde were corrected for background rates of ethanol formation, then standardized to g fresh root weight (Maricle et al. 2006).

A colorimetric method was developed to measure the activity of sulfide oxidation processes. 50 microliter (μL) aliquots of extract were added to a series of 1 mL buffer solutions of 100 μM Na_2S. 40 μL of Cline reagent (Cline 1969) was added to the solutions after 0, 10, and 20 min to determine the amount of sulfide present. Background rates of sulfide oxidation were measured by adding 50 μL phosphate buffer instead of enzyme extract to a series of tubes containing 100 μM Na_2S as described above. SOx activity was measured as a decrease in sulfide concentration over time. Total rates of SOx activity were corrected for background rates of spontaneous sulfide oxidation, then standardized to g fresh root weight. Rates of nonenzymatic sulfide oxidation were determined using 50 μL aliquots of boiled enzyme extract. Enzymatic rates of sulfide oxidation were calculated from the difference between the rates of total sulfide oxidation and nonenzymatic sulfide oxidation (Maricle et al. 2006).

RESULTS AND DISCUSSION

Soil waterlogging is the most common cause of plant oxygen deficiency (Vartapetian and Jackson 1997). Impacts of flooding on plant productivity can also have significant impacts on commodity crops. Excessive rains in the spring of 1993 resulted in a 33% reduction in Midwest crop production (Bray et al. 2000). Despite these kinds of economic losses, many observers may have overlooked the cattails and other wetland plants flourishing in nearby flooded ditches. Differences in flooding tolerance have long been recognized between plant species, but the specific physiology governing the differences has remained largely unknown.

Internal oxygen transport is often regarded to be an important factor conferring plant success in waterlogged areas, and therefore is thought to be important in estuarine zonation (e.g., Gleason and Zieman 1981, Bertness 1991). The oxygen transport rates measured in this study exhibited

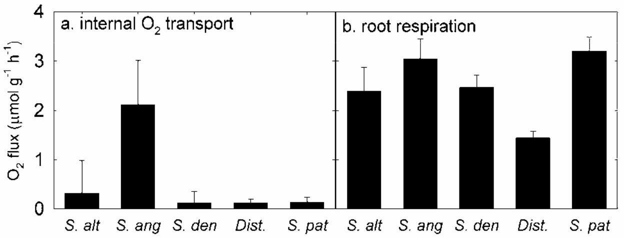

Fig. 2. (a.) Internal oxygen transport rates, and (b.) root respiration rates (µmol g^{-1} h^{-1}) of *Spartina* and *Distichlis* grown under flooded soil treatments. Shown is the mean of 5-14 plants ± SE. Species are labeled as follows: *S. alt = Spartina alterniflora*, *S. ang = S. anglica*, *S. den = S. densiflora*, *Dist. = Distichlis spicata*, *S. pat = S. patens.*

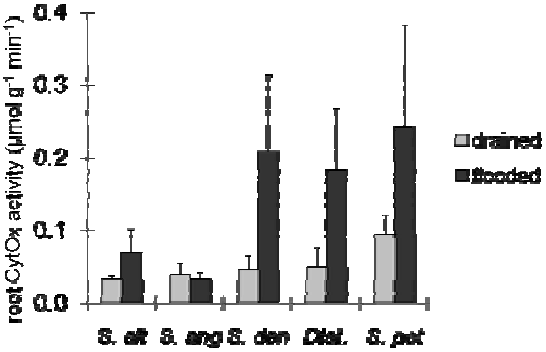

Fig. 3. Cytochrome *c* oxidase (CytOx) activities (µmol g^{-1} min^{-1}) of *Spartina* and *Distichlis* grown under drained and flooded soil treatments. Shown is the mean of 8-15 plants ± SE. Species are labeled as in Fig. 2.

a variability among individuals, with some apparent differences between species. The highest rates of transport were exhibited by *S. anglica*, with moderate to low rates in all other species (Fig. 2a). Compared to the other species in this study, *S. anglica* showed the greatest ability to transport oxygen (analysis of variance [ANOVA], $p<0.010$). The other species had lower rates of oxygen transport. However, the high marsh species that are sensitive to hypoxia transported oxygen at similar rates compared to the low marsh species *S. alterniflora*. Clearly there are factors, possibly physiological, in high marsh species that prevent them from surviving in low marsh habitats.

Spartina anglica was the only species with oxygen transport rates greater than zero under lab conditions (Fig. 2a). Therefore, the superior ability of *S. anglica* to tolerate anoxic sediments may be at least partly due to increased rates of internal oxygen transport. However, conditions in this study did not completely replicate the highly reducing character of estuarine mudflats. A much larger redox gradient can exist between plant roots and the rhizosphere when plants grow in estuarine sediment compared to roots suspended in water. Rates of oxygen transport in the field may be substantially larger than those measured in the lab. Consequently the relative capabilities of each species are resolved in this study, but not the maximum rates possible in the field.

In some cases, low rates of aerobic respiration might be related to the ability to tolerate anoxic conditions. Low oxygen demand is clearly an advantage when living in low-oxygen conditions. Aerobic respiration rates differed among species in the present study (Fig. 2b), with the high marsh species high marsh species *D. spicata* exhibiting the lowest respiration rates compared to all other species ($p<0.001$).

The mean cytochrome *c* oxidase (CytOx) activity for all species ranged from 0.033 to 0.241 µmol g^{-1} min^{-1} across waterlogging treatments (Fig. 3). CytOx catalyzes the terminal electron transfer to oxygen in aerobic respiration and may therefore serve as an indication of aerobic capacity. Root CytOx activities significantly increased in response to flooding in the high marsh species *S. densiflora*, *S. patens*, and *D. spicata* (ANOVA, $p\leq0.035$) but not the low marsh species *S. anglica* or *S. alterniflora* (ANOVA, $p\geq0.142$). Increased activities of CytOx may allow increased oxygen scavenging in oxygen-deficient tissues. The high marsh species had significantly higher CytOx activities compared to the low marsh species (ANOVA, $p<0.001$).

Differences in mitochondrial "dark" respiration rates (measured under 100% N_2) supported measured CytOx activities (Figs. 2-3). Overall rates of oxygen uptake ranged from 0.23 µmol g^{-1} h^{-1} in the low marsh species *S. anglica* to 2.94 µmol g^{-1} h^{-1} in the high marsh species *D. spicata* (Fig. 2b). Respiration rates were similar to values reported for other estuarine and terrestrial plants (Byrd et al. 1992). High marsh species had significantly greater CytOx activities than low marsh species (Fig. 3). This suggests that while differences among marsh species are correlated with habitat preference, these species still maintain an aerobic poise to metabolism despite potential oxygen limitation.

ADH activities were low in all plants studied, indicating metabolism adapted for flooding tolerance (Crawford 1967). Many previous studies have established differences in root ADH activity between marsh species found in high intertidal (less anoxia tolerant) vs. low intertidal (more anoxia tolerant) environments. Roots of the high marsh species *Spartina patens* have been shown to increase ADH activity following flooding (Burdick and Mendelssohn 1987, Naidoo et al. 1992, Pezeshki et al. 1993), while roots of the low marsh species *S. alterniflora* are more resistant to flooding and generally do not show increases in ADH activity under similar conditions (Naidoo et al. 1992). However, a strong rhizosphere oxygen demand can result in high ADH activities even in anoxia tolerant low marsh species like *S. alterniflora* that may transport substantial oxygen (Mendelssohn et al. 1981). In the present study, mean ADH activities ranged from 0.71 to 5.35 µmol g^{-1} min^{-1} across species and flooding treatments (Fig. 4). Root ADH

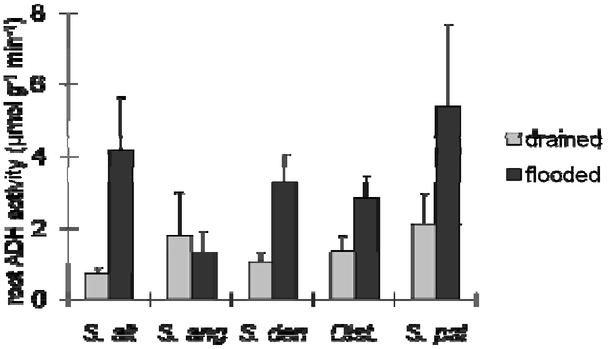

Fig. 4. Alcohol dehydrogenase (ADH) activities (μmol g^{-1} min^{-1}) of *Spartina* and *Distichlis* grown under drained and flooded soil treatments. Shown is the mean of 7-13 plants ± SE. Species are labeled as in Fig. 2.

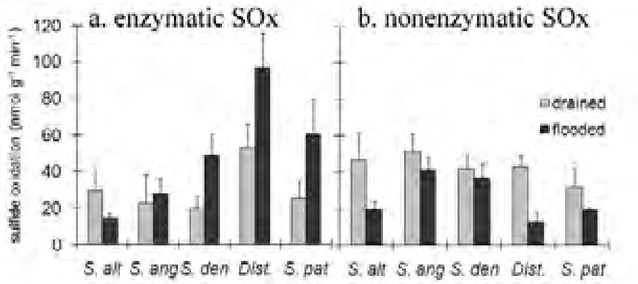

Fig. 5. Sulfide oxidase (SOx) activities (nmol g^{-1} min^{-1}) of *Spartina* and *Distichlis* grown under drained and flooded soil treatments. Shown are (a.) enzymatic and (b.) nonenzymatic rates of sulfide oxidation. The mean of 4-10 plants are shown ± SE. Species are labeled as in Fig. 2.

activities in *S. anglica* were significantly lower than all other species (ANOVA, $p \leq 0.046$). Flooded soil conditions resulted in significantly higher ADH activities in all species (ANOVA, $p \leq 0.018$) expect the low marsh species *S. anglica* (ANOVA, $p=0.564$).

Most plants can survive short term absence of oxygen (≤60 min) without cell death. In these cases, normal ATP stores are quickly depleted in active cells, and mitochondrial swelling is usually observed within minutes (Drew 1997). Large reserves of stored carbon can help the cells respire anaerobically, but longer absences of oxygen can result in cell death. Irreversible damage to mitochondria and cell viability generally occurs following 15 hours of anaerobiosis (Perata and Alpi 1993). However, a supply of oxygen from internal aeration can allow marsh plants like *Spartina* to respire aerobically despite growing in waterlogged substrates. The superior oxygen transport abilities of *S. anglica* may have helped to account for its low root ADH activities observed in this study.

The plants studied showed varying degrees of sulfide oxidation capacity. Total sulfide oxidation was partitioned into enzymatic and nonenzymatic processes. The mean enzymatic sulfide oxidase (SOx) activity ranged from 14.4 to 97.2 nmol g^{-1} min^{-1} across species and waterlogging treatments (Fig. 5a). Enzymatic SOx activities were highest in the high marsh species *S. patens* and *D. spicata* and were significantly lower in *S. densiflora* and the low marsh species *S. alterniflora* and *S. anglica* (ANOVA, $p \leq 0.050$). This difference may suggest that high marsh species are more sensitive to sediment sulfides and thus require greater enzymatic protection. Enzymatic SOx activities did not change in response to flooding across species (ANOVA, $p \geq 0.960$).

Nonbiotic factors such as metal ions can contribute to sulfide oxidation (Lee et al. 1999). Such nonenzymatic processes were also found to be important in sulfide oxidation in the present study. Mean nonenzymatic sulfide oxidation rates ranged from 12.6 to 51.1 nmol g^{-1} min^{-1} across species and waterlogging treatments (Fig. 5b). Nonenzymatic rates of sulfide oxidation were not different between species or flooding treatment (ANOVA, $p \geq 0.141$).

CONCLUSIONS

The upper regions of salt marshes are characterized by oxidized soils, since tidal flooding is rare. However, episodic flooding at the highest tides can result in occasional anoxic and sulfidic conditions. Therefore, plants from the high marsh are not forced to withstand chronic anoxia. The high marsh species *S. patens*, *S. densiflora*, and *Distichlis spicata* were found to have high aerobic respiration rates and high aerobic enzyme activity. This aerobic oxygen demand may be too high to allow survival in anoxic low marsh conditions, where plants had lower aerobic demand. Anaerobic pathways (root ADH activities) increased after flooding in all three high marsh species suggesting a high sensitivity to soil waterlogging. Internal oxygen transport rates were low in these plants since they are adapted to life in sediments where soil oxygen is not normally limiting to root respiration.

Additionally, higher SOx activities were found in high marsh species compared to low marsh species. These trends suggested that high marsh species were more sensitive to sulfide and required greater protection of aerobic respiration. This idea is consistent with the finding that these species exhibited higher activities of CytOx, the site of sulfide inhibition of aerobic respiration (Bagarinao 1992). High rates of aerobic respiration and apparent sulfide sensitivity may substantially account for the exclusion of these species from low marsh zones.

The low zones of salt marshes are characterized by frequent tidal flooding. This leads to highly reduced sediments, often containing high levels of sulfides. Plants inhabiting low marsh regions must be able to tolerate highly reducing and sulfidic sediment conditions. *Spartina alterniflora* is the dominant low marsh species in many North American East- and Gulf Coast estuaries (Bertness

1991). *Spartina anglica* can grow lower in the intertidal range than *S. alterniflora* (Frenkel 1987, Sayce and Mumford 1990), and therefore any other species in this study. Low marsh species exhibited low aerobic respiration rates and CytOx activity, which when coupled to high rates of internal oxygen transport may pose a significant advantage for survival in anoxic sediments. The ability to supply oxygen to submerged tissue is crucial to survival in the low marsh, as no plant tissue can endure anoxia indefinitely (Crawford 1982). Low marsh species must also possess an ability to respire anaerobically since demand for oxygen from highly reduced sediments may overwhelm transport processes. ADH activities measured in *Spartina* roots indicated a well-developed capacity for fermentation. However, increases in root ADH were not observed in the low marsh species *S. anglica*. High rates of oxygen transport in *S. anglica* may be adequate to supply oxygen to roots and external sinks, as suggested by its low root ADH activities. Low marsh species may be more resistant to sulfides when compared to high marsh species. Lower aerobic respiration rates and lower CytOx activities may relax needs for intense sulfide oxidation requirements. Alternatively, higher rates of oxygen transport in some low marsh species may help to oxidize rhizosphere sulfides, resulting in reduced SOx activity. However, the acute toxicity of dissolved sulfides around roots still necessitates moderate SOx activities in low marsh species.

The results of this study suggest metabolic characteristics related to respiration and sulfide tolerance may affect zonation of grasses in estuaries. While internal oxygen transport is important for survival in estuarine sediments, this study may indicate that zonation within estuaries is dependent on more than just oxygen transport. Aerobic respiration rates and sensitivity to sediment sulfides may play a large role in influencing estuarine zonation as well.

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EFFECTS OF SALINITY ON PHOTOSYNTHESIS IN C_4 ESTUARINE GRASSES

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The effects of salinity on gross and net photosynthesis rates were measured in estuarine C_4 grasses. Net CO_2 fixation was most affected by increasing salinity in *Spartina patens* and *S. alterniflora*, moderately affected in *Distichlis spicata* and *S. densiflora*, and unaffected in *S. anglica. Spartina anglica* exhibited a decrease in internal carbon dioxide (CO_2) with increasing salinity, suggesting some decrease in stomatal conductance. The results suggest *S. anglica* has a superior level of salt tolerance compared to other species in the study, which could have implications for community structure in field sites or for invasive potential of *S. anglica*. The maximum quantum efficiency of CO_2 fixation, measured under limiting light, decreased with increasing salinity in *S. alterniflora* and *S. patens*, indicating an increase in leakage of CO_2 from the CO_2 pump. While carbon fixation decreased under increasing salinity in most species, fluorescence yield data showed there was little effect on the use of solar energy in photochemistry. This indicates additional sinks are induced under salinity for use of photochemically generated energy (e.g., increase in the CO_2 pump, photorespiration, or Mehler reaction). Also, in *S. patens* and *D. spicata,* which had moderate decreases in photosynthesis, non-photochemical quenching (NPQ) mechanisms increased with salinity indicating some of the excess light energy was lost as heat. Therefore, excess excitation energy was diverted away from the photosynthetic reaction centers to prevent photoinhibition. Variable to maximal fluorescence (F_V/F_M) ratios were not significantly decreased by increasing salinity, suggesting there was no damage to photosystem II (PSII) reaction centers in any species.

Keywords: *Spartina*, *Distichlis*, chlorophyll fluorescence, gas exchange, salt stress

INTRODUCTION

High and fluctuating salinity levels are characteristic of salt marshes. Elevated soil salinity can cause low water potentials, which can decrease stomatal conductance, reducing incoming CO_2, and thus reducing photosynthetic rates (Willmer 1983). One aim of the present study was to determine how stomatal conductance and photosynthesis in C_4 salt marsh grasses are affected by environmental salinity.

Gross and net photosynthesis rates were measured under three salinity levels in the estuarine C_4 grasses *Spartina alterniflora*, *S. anglica*, *S. patens*, *S. densiflora*, and *Distichlis spicata* in growth chamber studies. Differences in these parameters were anticipated as a result of changes in photosynthesis as affected by salinity. Primary production by *Spartina* is an important input into the estuarine system (Peterson et al. 1985), so understanding the photosynthesis and hence production of this material may be important for understanding estuarine ecology and trophic interactions.

MATERIALS AND METHODS

Plants were collected at field sites in Washington (*S. alterniflora*, *S. anglica*, and *D. spicata*), Florida (*S. patens*), and Spain (*S. densiflora*). Tillers were potted in a 50/50 volume to volume (v/v) sand/potting soil mixture, and plants were watered to saturation twice weekly with modified Hoagland solution (Epstein 1972). Growth chamber conditions were 14L/10D (light/dark hours) with 26°C days and 18°C nights. Photosynthetic photon flux density (PPFD) was 300 micromoles per square meter per second (μmol m^{-2} s^{-1}) at bench level. Flooded treatment plants were placed in large plastic tubs in a randomized block design. Water was maintained at 2 centimeters (cm) above the soil surface and was completely replaced weekly. Salinity levels were increased 15 parts per thousand (‰) per week until flooded treatments included 0, 15, and 30‰ salt (Instant Ocean salts; Aquarium Systems, Mentor, Ohio). Drained treatments contained 0‰ salt. Plants were held for 30 days under final treatment conditions before testing. There were at least three replicate plants per species/treatment combination.

Chlorophyll fluorescence was measured with an OS-500 modulated fluorometer (Opti-Sciences, Inc.; Tyngsboro, MA). Gross photosynthetic rates of O_2 evolution were calculated from fluorescence yield measurements after Krall and Edwards (1992). The second-youngest leaf on each plant was tested and light-response curves were generated for PPFD of 15-2000 μmol m^{-2} s^{-1}.

A FastEst gas exchange system (Maricle et al. 2007) was used to measure leaf gas exchange on high (30‰) and low (0‰) salinity plants. Intact leaves were enclosed in a leaf chamber at 25°C and 25% relative humidity. Measures of CO_2 uptake gave net photosynthesis rates, and measures of external water vapor allowed calculations of stomatal conductance and the ratio of internal to atmospheric CO_2 concentration (c_i/c_a). A CO_2 analyzer (Li-Cor 6251; Lincoln, Nebraska) was used to measure leaf photosynthetic carbon uptake. Simultaneous measures of chlorophyll fluorescence (Walz PAM 101; Effeltrich, Germany) allowed calculations of gross photosynthetic rates of O_2 evolution. Light-response curves were generated for PPFD of 0-1100 μmol m^{-2} s^{-1}. A 20-minute dark period was allowed before measuring F_V/F_M at 0 μmol m^{-2} s^{-1}. The initial slope of each plant's light-response curve (under limiting light) was taken to be the quantum efficiency of CO_2 fixation or O_2 evolution (Genty et al. 1989).

Light-response curves were compared between species and treatments using repeated measures analysis of covariance (ANCOVAR). Parameters like quantum efficiency, c_i/c_a, F_V/F_M, and NPQ were compared between species and treatments using analysis of variance (ANOVA). Treatments were blocked by tubs in all analyses.

RESULTS AND DISCUSSION

Maximum rates of gross photosynthesis (rates of O_2 evolution) were quite high in this study (Fig. 1), consistent with productivity data presented by Long and Woolhouse (1979) for *Spartina* species. There were no significant differences in gross photosynthesis rates between species, treatment, or their interactions (ANCOVAR, $p \geq 0.439$).

Maximum quantum efficiencies of O_2 evolution, measured under limiting light, were not significantly decreased by salinity in any species (ANOVA, $p \geq 0.107$; Table 1). Values for gross quantum efficiencies in *Spartina* and *Distichlis* were slightly higher than previously published net quantum efficiency measures for C_4 monocots.

Values for quantum efficiencies of CO_2 fixation were slightly lower than gross quantum efficiencies (Table 1) and were similar to those reported by Ehleringer and Pearcy (1983) for C_4 monocots. Quantum efficiencies of CO_2 fixation decreased in *S. alterniflora* and *S. patens* with increased salinity (ANOVA, $p \leq 0.058$), but not in any other species (ANOVA, $p \geq 0.161$).

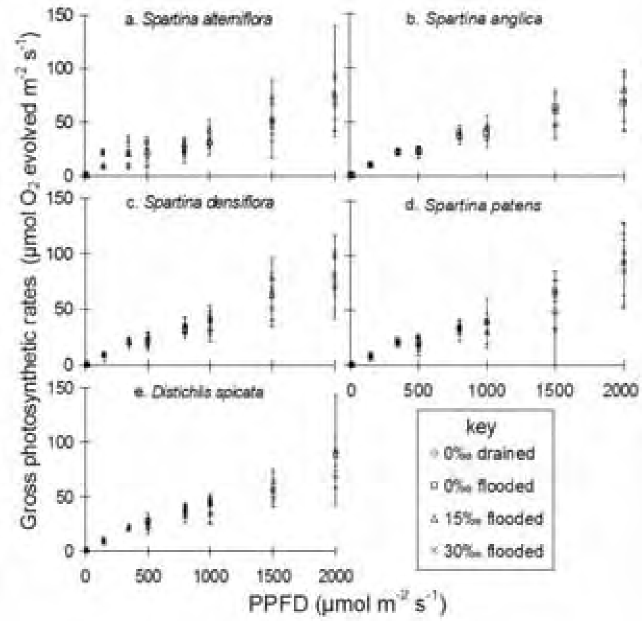

Fig. 1. Light-response curves showing gross photosynthesis rates (μmol O_2 evolved m^{-2} s^{-1}) of *Spartina* and *Distichlis* plants in flooded or drained soil conditions and salt up to 30‰. Points are means of 3-23 plants ± SD.

There were large differences between gross and net photosynthesis rates in most species (Table 2). This resulted in a surplus of harvested light energy not used in CO_2 fixation. This energy must be dissipated in order to prevent damage to photosynthetic reaction centers. Net rates of photosynthesis were lower in 30‰ salt compared to 0‰ salt in *S. patens*, *S. alterniflora*, and *D. spicata* (ANOVA, $p \leq 0.063$), but not in *S. anglica* or *S. densiflora* (ANOVA, $p \geq 0.624$).

Maintaining gross photosynthesis rates (as measured by fluorescence yield) in the light while rates of CO_2 fixation decrease with increasing salinity indicates additional

Table 1. Photosynthesis data collected for high- and low-salinity, flooded-treatment plants in the gas-exchange system. Shown is the net quantum efficiency of CO_2 fixation, the gross quantum efficiency of O_2 evolution, the maximum amount of nonphotochemical quenching and c_i/c_a values at 1100 μmol m^{-2} s^{-1}, and the maximum F_V/F_M ratio of dark-adapted plants. The mean ± SD (n) is given for each species/treatment combination.

Species	Treatment salinity	Net QE (CO_2 photon^{-1})	Gross QE (O_2 photon^{-1})	Max NPQ (unitless)	c_i/c_a (unitless)	Max F_V/F_M (unitless)
S. alterniflora	0‰	0.065 ± 0.020 (3)	0.066 ± 0.010 (3)	1.99 ± 0.24 (3)	0.53 ± 0.10 (3)	0.74 ± 0.01 (3)
	30‰	0.026 ± 0.016 (3)	0.046 ± 0.010 (3)	1.80 ± 0.36 (3)	0.60 ± 0.15 (3)	0.67 ± 0.08 (3)
S. anglica	0‰	0.025 ± 0.018 (4)	0.049 ± 0.009 (4)	1.64 ± 0.33 (4)	0.61 ± 0.11 (4)	0.72 ± 0.04 (4)
	30‰	0.027 ± 0.021 (4)	0.056 ± 0.009 (4)	1.63 ± 0.50 (4)	0.41 ± 0.08 (4)	0.72 ± 0.03 (4)
S. densiflora	0‰	0.049 ± 0.048 (4)	0.056 ± 0.014 (4)	1.78 ± 0.24 (4)	0.57 ± 0.17 (4)	0.74 ± 0.01 (4)
	30‰	0.034 ± 0.025 (4)	0.063 ± 0.006 (4)	1.44 ± 0.12 (4)	0.35 ± 0.14 (4)	0.73 ± 0.01 (4)
S. patens	0‰	0.067 ± 0.018 (3)	0.068 ± 0.003 (3)	1.19 ± 0.08 (3)	0.56 ± 0.14 (3)	0.71 ± 0.01 (3)
	30‰	0.033 ± 0.022 (3)	0.057 ± 0.007 (3)	1.70 ± 1.16 (3)	0.53 ± 0.19 (3)	0.72 ± 0.01 (3)
D. spicata	0‰	0.038 ± 0.002 (3)	0.050 ± 0.014 (3)	0.79 ± 0.23 (3)	0.53 ± 0.04 (3)	0.60 ± 0.10 (3)
	30‰	0.023 ± 0.015 (4)	0.057 ± 0.006 (4)	1.39 ± 0.37 (4)	0.53 ± 0.19 (3)	0.69 ± 0.04 (4)

Table 2. Gross and net photosynthetic rates collected for high- and low-salinity, flooded-treatment plants at moderate PPFD (1100 μmol m^{-2} s^{-1}). The mean gross photosynthetic rate of O_2 evolution and net photosynthetic rate of CO_2 fixation ± SD (n) is given for each species/treatment combination.

Species	Salinity	Gross PS rates (μmol O_2 m^{-2} s^{-1})	Net PS rates (μmol CO_2 m^{-2} s^{-1})
S. alterniflora	0‰	40.7 ± 1.0 (3)	11.0 ± 0.8 (3)
	30‰	34.4 ± 9.0 (3)	2.9 ± 2.6 (3)
S. anglica	0‰	40.4 ± 5.5 (4)	2.2 ± 1.7 (4)
	30‰	41.7 ± 2.6 (4)	5.9 ± 4.4 (4)
S. densiflora	0‰	38.3 ± 12.9 (4)	5.3 ± 7.2 (4)
	30‰	46.3 ± 1.7 (4)	3.3 ± 11.4 (4)
S. patens	0‰	48.2 ± 2.5 (3)	16.1 ± 11.4 (3)
	30‰	42.0 ± 1.6 (3)	3.0 ± 2.1 (3)
D. spicata	0‰	41.7 ± 13.2 (3)	10.3 ± 2.7 (3)
	30‰	41.0 ± 3.3 (4)	6.3 ± 7.0 (4)

electron sinks are induced. Some possibilities include (Demmig-Adams and Adams, 1992): CO_2 pump activity may increase to compensate for bundle sheath CO_2 leakage; this would use additional ATP generated by the Mehler reaction, and was potentially reflected in lower PSII yields in *S. alterniflora* and *S. patens* under high salinity (Table 1). Alternatively, an increase in photorespiration could help sustain gross photosynthesis rates if CO_2 levels drop in the bundle sheath allowing O_2 to react with RuBP. A consequence of photorespiration is producing products like PGA and ammonia that need reductive power from photochemistry. Increased reduction rates of nitrate, sulfate, or phosphate within chloroplasts could utilize excess light energy. Further work will be needed to see if nitrate or sulfate reduction or photorespiration rates increase with increasing salinity. Finally, energy not used in photochemistry may be dissipated by nonphotochemical quenching (NPQ) mechanisms, where excess light energy is lost as heat. Maximum amounts of NPQ increased with salinity in *S. patens* and *D. spicata* (ANOVA, $p \leq 0.073$), but in no other species (ANOVA, $p \geq 0.570$; Table 1).

Under high light, rates of light-harvesting (gross photosynthesis) will invariably be larger than carbon fixation (net photosynthesis) rates. Therefore excess energy must be safely dissipated before reaction centers are damaged (Demmig-Adams and Adams 1992). Dark-adapted F_V/F_M ratios of chlorophyll fluorescence can indicate damage to the PSII reaction center (Krause and Weis 1991). F_V/F_M ratios were not significantly reduced by salinity in any species in this study (ANOVA, $p \geq 0.165$; Table 1), suggesting excess energy was efficiently dispersed before reaction centers were damaged. Prevention of photoinhibition is likely to be important in determining plant resistance to environmental stresses that reduce carbon fixation relative to light harvesting rates (Demmig-Adams and Adams 1992).

The c_i/c_a values of the plants in this study ranged from 0.35 to 0.61 (Table 1). The c_i/c_a value of *S. anglica* significantly decreased with increasing salinity at moderate PPFD (ANOVA, p=0.032). The c_i/c_a values of all other species did not change in response to increasing salinity (ANOVA, $p \geq 0.421$). In previous studies, c_i/c_a values did not change with increasing salinity in C_4 grasses (Bowman et al. 1989, Meinzer et al. 1994). C_3 leaf c_i/c_a values tend to be higher than corresponding C_4 values and are more sensitive to increasing salinity (Brugnoli and Lauteri 1991). Increasing salinity may decrease C_3 c_i/c_a values by as much as 0.48 (Farquhar et al. 1982). In the present study, c_i/c_a decreased by 0.20 in *S. anglica*, and by 0.22 in *S. densiflora* (nonsignificant change) with 30‰ salinity, but only as much as 0.07 in the other species (Table 1). We believe this to be the first report of salinity-induced decreases in C_4 c_i/c_a values. One factor contributing to salt sensitivity in C_4 plants may be the susceptibility of CO_2 leakage from bundle sheath cells under increasing salinity. Increasing salinity appeared to decrease c_i/c_a in *S. anglica*, but it was the most resistant species to salinity in terms of CO_2 fixation. This suggests CO_2 pump activity does not increase in *S. anglica* with increasing salinity. Rates of photorespiration or nitrate reduction may increase instead to use excess light energy. Excess energy dissipation in response to salinity may be an area for future investigation in marsh halophytes.

This work illustrated how light harvesting and CO_2 uptake relate to sediment salinity in C_4 marsh grasses. Excess energy dissipation may also increase in times of salinity stress. Additionally, NPQ mechanisms increase in many plants as a result of external salinity. Portable flourometers can easily be used in the field to determine *in vivo* NPQ and thus *in vivo* salt stress in some plants. However, photochemistry does not appear to be affected by salt, so fluorescence yield measurements in the light or dark-adapted F_V/F_M measures will not reflect salt stress. In contrast, gas-exchange methods appear to be far more sensitive to salinity.

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CHAPTER TWO

Spartina Distribution and Spread

A TALE OF TWO INVADED ESTUARIES: *SPARTINA* IN SAN FRANCISCO BAY, CALIFORNIA AND WILLAPA BAY, WASHINGTON

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Maritime *Spartina* species grow lower on the tidal plane than other vascular plants and maintain the shoreline on temperate coasts where they are native. All but two of the 14 known species are native to the Atlantic. *Spartina alterniflora* was introduced a century ago into Willapa Bay, Washington, far north of the native limit of this genus. This Atlantic native spread exponentially through tidelands there at a remarkably constant approximately 12% per year over the 55-year history of aerial photographs. In 2000, it covered approximately 1,670 of 6,000 hectares (ha) or 27% of the intertidal habitat of Willapa Bay. However, the rate of spread was slowed greatly by an Allee effect due to poor pollen dispersal. Without the Allee effect, the invasion would have covered the lion's share of Willapa Bay long ago. Large-scale chemical control is now greatly reducing *S. alterniflora* in Willapa Bay. The San Francisco Bay region is the northern limit of *Spartina foliosa*, one of two *Spartina* species native to the Pacific. Introduced *Spartina* played virtually no role in San Francisco Bay until 1975, when the U.S. Army Corps of Engineers planted *S. alterniflora*, which hybridized with the native *S. foliosa* soon afterwards. While *S. alterniflora* has become virtually extinct, the hybrids have had a truly phenomenal rate of spread. The few hybrids that formed in the late 1970s spread to about 1,500 ha when the San Francisco Estuary Invasive *Spartina* Project (ISP) began their control effort. The rapid spread of hybrids is probably due to the evolution of self-pollination, thus eliminating the Allee effect. Ultimate success of the ISP will depend upon a sophisticated combination of biochemical systematics with ecological field research that determines dynamics of cryptic hybrids that could survive control efforts.

Key Words: Hybrid, rate of spread

Spartinas are cordgrasses (Strong and Ayres 2009). The species of estuarine cordgrasses that we have studied are wind-pollinated, largely self-incompatible and outbreeding. They are protogynous; the female flowers appear before the male flowers. In order to set much viable seed, each plant requires pollen to be carried on the wind from a different, earlier-flowering plant that has progressed to the later stage of having male, pollen-bearing flowers.

Cordgrasses are ecosystem engineers. Their tall dense stems slow water movement and cause sediment to settle and be bound by thick, fibrous roots. Roots grow upward through the settling sediment to form thick peat that elevates the surface of the marsh. Cordgrasses that invade areas with no emergent vegetation greatly increase local photosynthetic rates. Their roots greatly increase subsurface carbon which remains in the anoxic sediment long after the plants have been removed. Invasive cordgrasses have transformed vast expanses of open intertidal mudflat into meadows that elevate with time. Estuarine cordgrasses disperse primarily by floating seed that does not accumulate in soil. No evidence indicates a seed bank more than one year old for estuarine cordgrasses.

All but one of the 14 nominal species of cordgrass are native to the Americas: *Spartina maritima* is endemic to the south of England and France (Daehler and Strong 1997). The Pacific has but two natives, *Spartina densiflora* in Chile and S*partina foliosa*, California cordgrass, that thrives in Baja California and in San Francisco Bay. People purposefully and inadvertently spread cordgrasses, and four non-native *Spartina*s have been introduced to San Francisco Bay.

Spartina patens is the least successful San Francisco invader and is known from only two plants in Suisun Bay (Ayres et al. 2004). Native to Atlantic marshes of North America, *S. patens* spread rapidly at one site in Oregon during the mid-20th century and it has appeared recently in Spain. With somewhat greater success, *S. densiflora* spread to several sites in San Francisco Bay after at least two introductions in Marin County, where it was brought from the huge, century-old Humboldt Bay, California infestation. This species has also been introduced into Spain.

Spartina anglica arose in England in the 19th century as a hybrid of *S. maritima* and *S. alterniflora*, after the latter species was introduced from its native Atlantic shores of North America. *Spartina anglica* spread widely after introduction to Puget Sound, Washington, the Netherlands, Tasmania, Australia, and New Zealand. However, *S. anglica* has not spread to other places in San Francisco Bay post-introduction in 1977 to Creekside Park in Greenbrae, Marin County.

In misguided attempts at marsh restoration in the mid-1970s, the fourth species, *Spartina alterniflora,* was introduced twice into south San Francisco Bay. Seed from a Maryland marsh was sown in New Alameda Creek, located between Fremont and Union City (Faber 2000). The second known introduction, a planting sponsored by the Army Corps of Engineers at Alameda Island, occurred about 40 kilometers (km) north of the first (Ayres et al. 2003). As a result of lack of pollen from conspecific plants and pollen swamping from *S. alterniflora* x *S. foliosa* hybrids, *S. alterniflora* has become quite rare in San Francisco Bay in the two or three decades since introduction.

One or both of the *S. alterniflora* introductions resulted in hybridization with the native California cordgrass to produce a backcrossing swarm of *S. alterniflora* x *S. foliosa* hybrids in San Francisco Bay. The chloroplast DNA of both parental species is found among the plants in the swarm indicating that both parental species have served as seed parents of hybrids. While hybridization probably has occurred multiple times F1 hybrids are rare in the field. With great effort in the greenhouse we have produced a few F1 hybrids.

A subset of hybrid genotypes are extremely fit in one or a combination of the following traits: vegetative growth rate, numbers of viable seed, volume of pollen, and/or self-compatibility. These hybrid traits can be transgressive, which means that hybrids exceed both parental species in the magnitude of the trait. We entertain the hypothesis that the most important transgressive trait of hybrids is self-compatibility, which allows a plant to pollinate itself and set seed at low density after invasion.

A great deal of hybrid *S. alterniflora x S. foliosa* seed is carried on the currents and tides around San Francisco Bay and new marshes are invaded every year. Hybrid seed floats to open mud flats, germinates, and grows rapidly. This seed also spreads into vegetated marshes comprised of *S. foliosa* and other native marsh plants. Hybrid invasion of vegetative marshes leads to severe ecological and genetic competition with native *S. foliosa*. Hybrid cordgrass is increasing at greater than exponential rates in San Francisco Bay. In 2002, approximately 1,500 ha of salt marsh was dominated by the swarm in San Francisco Bay (Ayres et al. 2004). The most recent pair of censuses yields a doubling time of about three months for coverage of the hybrid swarm in the Bay.

The loss of native cordgrass due to competition and interbreeding is accelerating. We can conceive of no natural limitation to this loss, which is a runaway, unregulated process that could lead to the extinction of *S. foliosa* in San Francisco Bay. Similarly, dispersal of hybrids to salt marshes in Baja California could lead to the extinction of *S. foliosa* there (Ayres et al. 2003). In contrast, Willapa Bay, Washington has no native cordgrass and no potential for hybridization. *Spartina alterniflora* was introduced there more than 100 years ago (Civille et al. 2005) and probably arrived as a hitchhiker on the numerous trains from New York harbor that brought oysters for outplanting in Willapa Bay at the end of the 19th and beginning of the 20th centuries. The first hard historical evidence of the invasion is from a photo and publication in 1941. The large patch size of the plant implies that it had already been growing for several decades. The first aerial photos, from 1945, show several large colonies, further evidence that introduction occurred decades earlier. The multiple, widely separated colonies imply multiple introductions rather than spread from a single focus. Coverage of *S. alterniflora* has increased at a rate very close to exponential between 1945 and 2000, about 12% per year. This gives a doubling time of about six years. Approximately 1,670 ha (27%) of the 6,000 ha of intertidal habitat of Willapa Bay had been colonized by *S. alterniflora* by 2000. Large-scale chemical control is now greatly reducing *S. alterniflora* in Willapa Bay (Strong and Ayres 2009).

In an echo of the slow spread of *S. alterniflora* in San Francisco Bay, we have found that very little viable seed is set at the leading edge of the invasion of Willapa Bay (Davis et al. 2004a). Seed settles at low densities onto open mud and recruits are widely separated from one another. Decades later, when the circular clones coalesce to form continuous meadows, seed set increases by an order of magnitude. This is a weak Allee effect. A strong Allee effect would result if no seed is set by plants at low densities, indicating that *S. alterniflora* would have become extinct at the low densities of the initial invasion. The weak Allee effect has slowed the rate of invasion. Were there no Allee effect, *S. alterniflora* would have to be able to self-pollinate, and single plants would set seed at the same rate as plants growing in high densities. Without an Allee effect, the rate of increase in coverage of *S. alterniflora* would have been about 30% per year, and the doubling time would have been as short as 2.5 years instead of the actual six years (Taylor et al. 2004). Thus, without the Allee effect, the approximately three ha of *S. alterniflora* shown in the 1945 aerial photos would have grown to completely cover the entire 19,000 ha of intertidal area of Willapa Bay by about 1977.

The dearth of viable seed set at the leading edge of the invasion of *S. alterniflora* in Willapa Bay and the weak Allee effect are a result of a lack of pollen produced by low-density plants (Davis et al. 2004b). We found ninefold more pollen on stigmas of high-density plants in old marshes than on those of low-density plants at the leading edge of the invasion. Only in old marshes, where plants had grown together to form dense meadows, was there sufficient pollen on stigmas for much seed set. Experimental pollination augmentation of low-density plants, but not of high-density plants, increased seed set. Experimental pollen exclusion from high-density plants, but not from low-density plants, decreased seed set.

In summary, the cordgrass invasion of San Francisco Bay is by hybrids of *S. alterniflora* and *S. foliosa*, not by *S. alterniflora* alone. The hybrid has a truly phenomenal rate of spread, and this rate is accelerating. This is evidence that the hybrids are much more invasive than *S. alterniflora* alone.

The first 40 or 50 years of spread in Willapa Bay produced less than 10 ha of *S. alterniflora*. Beginning with a very small number of hybrids in the 1970s or 1980s, the overall coverage of the hybrid has grown to about 1,500 ha.

At a rate of increase of about 12% per year, similar to *S. alterniflora* in Willapa Bay, San Francisco Bay would have only about 3.4 times the area of the initial introductions some 30 years later. If the two introductions of *S. alterniflora* had amounted to about one ha of *S. alterniflora,* there would be only 3.4 ha now. I speculate that without hybridization, this conference would not have occurred.

Transgressive traits confer greater fitness upon a subset of hybrids than either parent exhibits. We entertain the hypothesis that the key trait is increased self-compatibility of some hybrids. This would erase the weak Allee effect that we see in Willapa Bay as increased self-compatibility allows single plants to set abundant seed.

The presence of hybrids means that control of non-native cordgrass in San Francisco Bay will require research and understanding beyond that required in Willapa Bay. Biochemical systematics are needed to detect hybrids at low frequency, in order that hybrids can be removed before the marsh is overrun by them. Strategies concerning which and how much hybrid cordgrass to eliminate for effective control will require understanding of the dynamic mechanisms of hybrid spread. Research on these subjects is just beginning.

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SPARTINA IN CHINA: INTRODUCTION, HISTORY, CURRENT STATUS, AND RECENT RESEARCH

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Spartina spp., with their strong ability for survival, growth, and expansion, attracts ecologists' attention throughout the world. Four *Spartina* species: *Spartina anglica*, *S. alterniflora, S. patens* and *S. cynosuroides* were introduced into China in 1963, 1979, 1979 and 1998, respectively, for the purposes of agricultural and ecological engineering. So far the first three species still survive in coastal China with varying abundance. *Spartina anglica* is dying back, whereas *S. alterniflora* has been rampantly invading. *Spartina patens* appears to be a potential invasive species in China. We examine the history of *Spartina*'s introductions into China, discuss the fate of the introduced species and their impacts on native ecosystems, and review the studies conducted in China over the past 40 years (from 1963-2003), especially those carried out in the Institute of *Spartina* and Tideland Development at Nanjing University.

Keywords: Ecological engineering, genetic structure, invasive impacts, management, *Spartina*

INTRODUCTION

Four *Spartina* species have been introduced into China as bio-engineers for agricultural and ecological engineering since 1963. They were used to accelerate the development of coastal tidelands for croplands, protect the dykes from typhoons and control erosion of tidelands from tidal waves (Chung and Zhuo 1979; Chung 1982, 1985). The species have had different fates in coastal areas of China resulting from both human activities and natural stresses. Seeds of *S. patens* and *S. cynosuroides* were introduced into China, together with *S. alterniflora*. But, *S. patens* failed to germinate in the laboratory and *S. cynosuroides* failed to survive in the field. In 1998, seeds of *S. patens* were introduced into China again and successfully germinated after ion radiation treatment (Zhou 2003), then tissue culture was used to produce offspring at a large scale. So far, about 20 hectares (ha) of *S. patens* has been transplanted in Tianjing, Jiangsu and Zhejiang Provinces (Zhou et al 2003).

Spartina alterniflora has rapidly spread to other coastal areas in China, outcompeted native plants and become one of most harmful invading plants in China. In contrast, *S. anglica* is experiencing a dieback and may disappear from the coasts. Although *S. anglica* is a serious invasive species in other countries (Daehler and Strong 1996; Kriworken and Hedge 2000; Baumel et al. 2001; Hacker et al. 2001), it is now not an invasive species in China. More recently, *S. patens* has shown the potential to be an invasive species (Zhou et al. 2003). The four species have had different different fates including the failure of *S. cynosuroides* introduction and different invasive processes in coastal China. Here we give an brief account of the experiences from Chinese scientists and studies that have been conducted in China since the introduction of these species to this country. It is hoped that the information presented here can be of some use to the scientists who are working on use, management, control and eradication of the species that are invading both Atlantic and Pacific coastal areas worldwide.

HISTORY OF *SPARTINA* INTRODUCTION IN CHINA

Spartina anglica

Spartina anglica was the first *Spartina* species introduced into China from another country. In 1963, 35 plants from Essex in England and 100 plants from Hojer in Denmark were introduced to the ecological laboratory of Nanjing University by Prof. Chung-Hsin Chung (Chong-Xin Zhong) with the help of the Chinese Committee of Science and Technology. The *Spartina* survivors — 21 from England and 50 from Denmark — were sent to Sheyang Experimental Station (35°N) on the Yellow Sea coast after a simple survivial check in the laboratory. The survivors of the English *Spartina* successfully produced 435,000 new ramets from July 1963 to April 1964, which were planted in the field in 1964; by 1966 the area of the plantation had increased to 32 ha. However, the Danish population soon died off. Almost all of the *S. anglica* in China are descendants of the original 21 English plants, excluding the plantations in Xiaoshan and Wenlin of Zhejiang Province. In 1964, 507 seeds and 18 plants from Poole Harbor in England were introduced to China, of which 440 seeds with potential germination capacity were treated and sowed in the laboratory; 157 of them germinated, a germination rate of 30.97%, and 44 seedlings survived. The 18 plants died off in the lab. One year later, 30,601 individuals were obtained from the 44 surviving seedlings (Fig. 1), and most of them were planted in Xiaoshan and Wenlin Experimental Stations along the coast of the East Sea (29^{o}N). In the experiment, one rhizome segment produced 9.10 million individuals during 29 months, and four ramets produced 7.60 million individuals during 33 months. Most of the 16.70 million individuals were planted in mid-1966 in two East Sea stations, whose area was 73 ha. The total area of *S. anglica* plantation was 78 ha by August 1966 in the two stations. But, from 1973 to 1980, most of the plantations in the two stations were reclaimed for croplands; thus almost all the individuals of *S. anglica* died off there.

Four *Spartina* species: *Spartina anglica*, *S. alterniflora, S. patens* and *S. cynosuroides* have been introduced into China in 1963, 1979, 1979 and 1998, respectively, for the purposes of agricultural and ecological engineering.By 1978, all the coastal provinces of China planted *S. anglica*. There were 6,330 ha, 3,750 ha and 200 ha of *S. anglica* plantations in Jiangsu, Zhejiang and Shandong Provinces, respectively. Meanwhile, 5 ha, 2/3 ha, 1/2 ha and 1/3 ha existed in Hebei, Liaoning, Tianjing and Guangdong Provinces, respectively. Less than 1/3 ha of the species was established in Shanghai, Fujian and Guangxi Provinces. In the field, *S. anglica* had a strong reproductive capacity by asexual propagation through ramets and rhizomes (Fig. 2). By 1980, all 83 counties of coastal China had *S. anglica* with a total area of 31,590 ha; by 1985 area had increased to 36,000 ha, although the sexual reproduction of the species was very low.

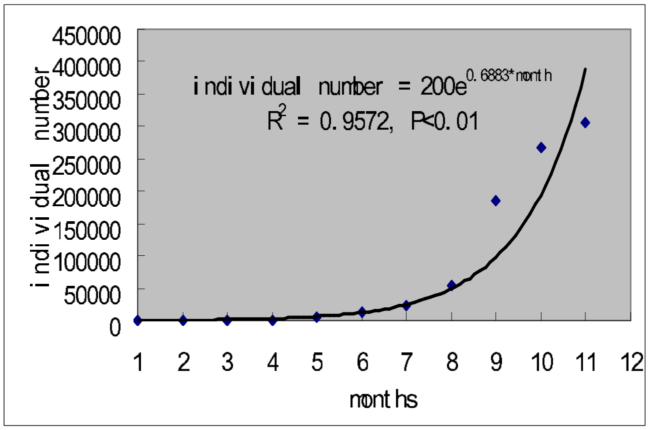

Fig. 1 Asexual reproduction of exotic *Spartina anglica* from seedlings. (Data from Chung et al 1985.)

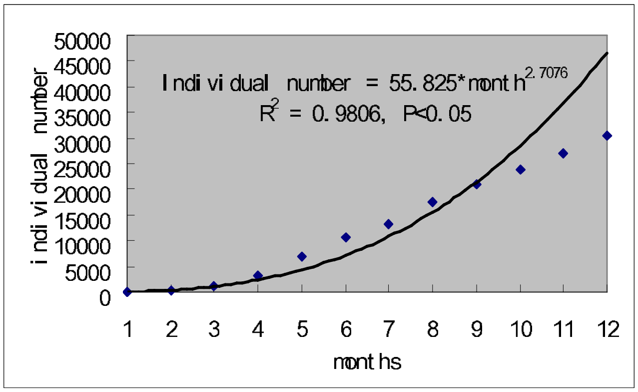

Fig. 2 Reproduction of *Spartina anglica* under natural conditions by asexual propogation. (Data from Chung et al 1985.)

Spartina alterniflora

Similarly, seeds and individuals of *S. alterniflora* were also introduced to China by Professor Chung at the end of 1979 from North Carolina, Georgia and Florida, USA. Seedlings were successfully obtained from the seeds and new ramets were reproduced from the exotic individuals in the laboratory and garden of Nanjing University in 1980, and then were sent to Luoyuanwan Station (26^{0}30'). The

Table 1. Growth of one-year *Spartina alterniflora* from three different origins. (Data from Xu & Zhuo 1985.)

Sources	Culm height (cm)		Leaf length (cm)		Leaf width (cm)		Ear length (cm)		Biomass (DW g/m^2)	Leaf color
	Range	Max	Range	Max	Range	Max	Range	Max		
North Carolina	110-170	217	45-70	95.5	1.3-1.5	2.0	14-30	48	297.9	Black green
Georgia	140-240	275	50-70	90.0	1.4-1.7	2.1	18-35	42	457.2	Light green
Florida	70-100	128	40-60	82.0	1.5-1.7	2.1	12-15	30	268.2	Green

Table 2. Height and biomass growth of *Spartina alterniflora* from different origins at the field plots (Xu & Zhuo 1985).

Introductive Sources	Culm height (cm)	Above-ground biomass (g DW/m^2)	Below-ground biomass (g DW/m^2)	Total bio-mass (g DW/m^2)
North Carolina	170 (35)	1289 (136)	1235 (160)	2524 (274)
Georgia	240 (50)	2745 (502)	1366 (25)	4111 (486)
Florida	110 (25)	972 (139)	928 (100)	1900 (83)
ANOVA & T-test	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$

* All the individuals were planted in the spring of 1981.

Table 3. Isozyme patterns of 3 genetic-types of *Spartina alterniflora.* (Data from Chen & Chung 1991.)

Elevation (m)	Inundation frequency (days/month)	Inundation time (hours/day)	Survivorship (%)	Increase rate of individuals per clump (times)	Height (cm)
0.0	30	5.0 - 8.0	16.7	0	12.5
0.5	30	3.5 - 7.0	66.7	1.5	27.6
1.0	30	1.5 - 6.0	66.7	4.8	37.3
1.5	30	0.5 - 4.5	100.0	10.8	90.2
2.0	26 - 30	0.5 - 3.5	100.0	13.7	101.4
2.5	18 - 28	0.5 - 2.0	100.0	11.8	98.2
3.0	14 - 22	0.5 - 2.0	100.0	8.0	72.3

* Seedling samples were collected in the field plots of Luoyuan Estuary.

plants were planted in the field in the spring of 1981 into an area of 1,000-1,300 m^2. However, most of the *Spartina* salt marsh of coastal China was developed from 0.5 kg of seeds from North Carolina.(Chung et al. 1985) Three morphs of *S. alterniflora* were identified by Xu & Zhou (1985) and Qin et al. (1985) based on their morphological data (culm height, leaf size, ear length and biomass), respectively, collected from the garden of Nanjing University (Table 1) and field plots in Luoyuan Bay of East Sea (Table 2). Both the experiments showed that Georgia *Spartina* had the tallest and strongest culms, and the highest biomass. Meanwhile, Chen and Chung (1991) reported that there were three genetic types in their studies of isozymes of EST, MDH and POD of the seedlings (Table 3). Since the people liked to plant higher and stronger individuals of the species, most of the *S. alterniflora* plantations in China are now tall form (Georgia population) *S. alterniflora*. The tall form *Spartina* had significant variations in survivorship, culm height and biomass along the elevational gradients of the Chinese coast (Chen & Chung 1991).

IMPORTANT HUMAN FORCES DRIVING *SPARTINA* INVASIONS

In 1962, the Chinese Government decided to introduce *Spartina* from its native habitats for agricultural goals according to the scientists' recommendations. In 1963, *S. anglica* was introduced from Europe by the Chinese government. In 1969, the first workshop was held by the government of Zhejiang Province to accelerate development of *S. anglica* marshes. From 1973 to 1978, several workshops were held by the governments of Jiangsu and Shandong provinces for the same purposes. Here we give some of the events that are associated with *Spartina* in chronological order:

- 1978: *Spartina* research group of Nanjing University received the National Award for Natural Science of China.
- 1978: The Institute of *Spartina* and Tideland Development at Nanjing University was founded by China's Ministry of Education, together with China's Committee of Science and Technology, China's Ministry of Agriculture and China's Ocean Bureau. The institute consisted of 17 members.
- 1978-1979: Two workshops were held by China's Committee of Science and Technology to accelerate introduction of *S. anglica*.
- 1979: Three other *Spartina* species were introduced by Professor Chung-Hsin Chung, funded by the Chinese government.
- 1985: The first Chinese *Spartina* research monograph "Research Advances in *Spartina* — Achievement of the Past 22 Years", was published as a special issue of the Journal of Nanjing University; the monograph contained three reviews, 26 reports and 24 short communications.
- 1985-1990: Several honors were awarded to Prof. Chung by different Chinese government departments for his distinguished achievement in introduction of *Spartina*.
- 1992: A monograph, Applied Studies on *Spartina* (eds. Qin, P. and C.H. Chung), was published by Ocean Press; it contained 30 papers.
- 1993: Prof. Chung received an award from the Society of Wetland Scientists.
- 1995: Prof. Chung received a Distinguished Fellow Award from Ohio State University.
- 1996: The *Spartina* Research Group of Nanjing University received a Distinguished Contribution Award from the International Society of Ecological Engineering.
- 1998: *S. patens* was again introduced into China as a salt-tolerant economic species.

CURRENT STATUS OF *SPARTINA* INVASIONS IN CHINA

In 1985, *S. anglica* plantation, with human aid, reached to an area of 36,000 ha, and was distributed in 83 coastal counties in China. The species' range had increased by 330 times from 1966 to 1985. Most of the plants came from 21 English individuals. After that time, however, the species has declined without human plantings. Only 50 ha of the plantations still existed in 2000 (Table 4). The species has been also experiencing dieback because it cannot produce

Table 4. Area changes and their causes of *Spartina* in China. (Partial data from Chung et al. 1985.)

Year	*Spartina anglica*		*Spartina alterniflora*	
	Area (ha)	Causes	Area (ha)	Causes
1966	110	Planting and natural reproduction		
1973	2,000	Planting and natural reproduction		
1978	10,295	Planting and natural reproduction		
1980	31,590	Planting and natural reproduction	0.13	Planting
1985	36,000	Planting and natural reproduction	260	Planting and natural reproduction
1988	-	Stop planting	1,300	Planting and natural reproduction
1995	-	Natural reproduction	-	Almost stop planting
2000	<50	Reclamation and dieback	112,000	Planting and natural reproduction

any seeds and it has become shorter at all elevations.

On the other hand, the range of *S. alterniflora* increased by 10,000 times from 1980 to 1988. Alhough planting the species on a large scale was stopped in 1995, it still increased by 86 times from 1988 to 2000. The species is still invading coastal areas in China by natural dispersal and has become a threat to the native ecosystems. From 1995 to 2000, ca. 200,000 ha of *S. alterniflora* marshes and tidelands were reclaimed, which did not stop the invasion of the species.

Many native species, including plants, some endangered birds (Ma et al. 2004), and mollusks of economic value in coastal areas are threatened by *S. alterniflora* invasions. It excluded almost all the other native plants that were originally dominant in wetlands, including *Phragmites australis*, *Typha* spp., *Scirpus* spp., *Suadae* spp., and even invaded fishponds and young mangrove swamps (Qian and Ma 1995). For example, in intertidal zones of the Yangzi River estuary, *S. alterniflora* invaded *Scirpus mariqueter* communities. Through competition with the native species, *S. alterniflora* has greatly decreased native species abundance, and even excluded them (Li et al. in this volume). Li et al. (this volume) also compared the structure of nematode communities among *Spartina* marsh and *S. mariqueter* and *Phragmites* marshes of Dongtan wetlands on Chongming Island, and found significant differences in trophic structure of nematode communities between the marshes.

Spartina alterniflora is one of 16 notorious invasive pest plants in China as the species directly causes millions of dollars of economic loss per year (An et al, 2007). *Spartina alterniflora* is still rapidly spreading, although the Chinese government and scientists are doing their best to control or eradiate the species by physical, chemical, biological and integrated methods (Lin 1997; Liu and Huang 2000).

RESEARCH BEFORE 1995

Since *S. anglica* arrived at Nanjing University of China, a series of studies (all the papers published before 1986 in the cited literature) had been carried out by the *Spartina* Research Group, which focused on *S. anglica* by 1985. For example:

- 1963 to 1966: Asexual propagation from rhizome and ramets; seed germination, tolerance of individuals to cold, hot, drought, water logging, silt sediment and salt stress; growth under different elevation; sexual and asexual reproduction from established individuals; transplanting and management techniques.
- 1967 to 1973: Field transplanting; impacts of *S. anglica* growth on soil properties and planting techniques in inland riparian wetlands.
- 1974 to 1978: Green manure for cropland, forage for sheep, goat and pig; height growth; fertilized flowers and seed production; biomass production, net biomass production; impacts of harvest on growth and reproduction and impacts of grazing by cows, sheep and goats.
- 1979 to 1985: Silt sediment rate of *Spartina* plantation; uses in protection of dykes; prevention of tideland erosion; purification of polluted water; impact on beneath fauna; Gram negative bacteria composition; anatomy of roots and stems; germination of seeds produced in China and impacts of puncture and air pressure on seed germination; physiology; tissue culture; introduction to riparian wetlands in Yellow River of Northwestern China; biochemical composition and nutrient; impacts of fertilizer on growth and biomass production; forage for fish; structures of salt glands; ultra-structures of leaf cells; DNA contents; ecotype identification and methods to increase the seed production.

Table 5. Survivorship and growth of *Spartina alterniflora* at different elevation levels. (Revised from Xu and Zhuo 1985.)

Elevation (m)	Inundation frequency (days/month)	Inundation time (hours/day)	Survivorship (%)	Increase rate of individuals per clump (times)	Height (cm)
0.0	30	5.0 - 8.0	16.7	0	12.5
0.5	30	3.5 - 7.0	66.7	1.5	27.6
1.0	30	1.5 - 6.0	66.7	4.8	37.3
1.5	30	0.5 - 4.5	100.0	10.8	90.2
2.0	26 - 30	0.5 - 3.5	100.0	13.7	101.4
2.5	18 - 28	0.5 - 2.0	100.0	11.8	98.2
3.0	14 - 22	0.5 - 2.0	100.0	8.0	72.3

After 1985, most studies (in the cited literature between 1986 and 1994) focused on biology and ecology of *S. alterniflora*, including survivorship and growth at different elevations (Table 5); growth rate and growth form/type from three different origins; above- and below-ground biomass and biomass allocation of three growth-type populations; seed germination at different saline stresses; effect of N-fixed 4088 strain of bacteria on seed germination and individual growth; transportation and distribution of phosphorus (P) in organs under salt stress; physiology and biochemistry of leaf and root; effects of seed soaking on seed germination; enzyme activity, sugar contents, amino acid and cold tolerance of the seedling under different salt stress; translocation of mineral elements; contents, allocation and uses of Selenium (Se) of plants; flavonoids and immunity activity; isozyme differentiation; micro-morphology of pollen grains; ultrastructure of mesophyll cells; anatomy of seeds; biomass dynamics, species structures, energy storage and energy flow of ecosystem; the relationships between *Spartina* and clamworms; soil enzyme activity in *Spartina* salt marsh; extraction method, toxicology, nutrient contents, functions and uses of biomineral beverages/tea.

Overall, most of the studies on *Spartina* before 1995 were involved in ecology after introduction, ecological engineering, multi-dimensional uses, physiology, biochemistry and the population biology of the species.

RECENT STUDIES AND ADVANCES

Since 1995, two research groups were reconstructed with two professors, six associate professors, three assistant professors and their students at Nanjing University. Meanwhile, a couple of Chinese scientists joined in the research from other institutions. Our studies on *S. alterniflora* focused on: multidimensional uses, invasive consequences and management, competition with different species, wave reduction, ecological services and evaluation, energy flow and community dynamics, evaluation of ecological engineering, purification of wastewater, sediment process, remote sensing, expending process and outbreak of populations, soil property of salt marsh, rare waterfowl conservation, cDNA library, flavonoids of biomass, decomposition dynamics of plants, genetic diversity and genetic differentiation, greenhouse gas release of salt marsh. The work on *S. anglica* covered salt-induced transcript, microbial composition, mechanism of dieback and genetic structures of declining populations. Furthermore, the studies on *S. patens* included tissue culture of seed, ion implantations of plants, effect of ion beam on seed germination and uses of the species. Most of the studies are involved in structures and functions of *Spartina* salt marshes; genetic diversity and dieback of *S. anglica*; genetic variation, outbreak, management and control of *S. alterniflora*, restoration of degraded coastal ecosystems invaded by *S. alterniflora*; and effects of *S. alterniflora* invasion on release of carbon-, nitrogen- and sulfer-based trace gases. Some of the important results are summarized below:

- Rhizome and ramets of *S. anglica* have strong asexual reproductive capacity in both the laboratory and coastal ecosystems, but the seeds have very low germination rate. The species grows well in rapid sedimentation conditions at 2.5 meters (m) to 4.2 m in elevation above sea level if its leaves are not completely covered by the sediments, has broad temperature range from -25°C to 42°C and can survive in open coasts with violent impacts from typhoons and tides. *Spartina anglica* can grow at salinities of 0-6% (W/W), with the optimal salinity of 0.4-1.4%. Best growth occurs at 3.0±0.3 m in elevation with a tolerance range from 2.3 m to 4.0 m. It decreased the dissoluble ions of soil by 2.6% and in reclaimed tidelands the species decreased saline concentration and pH by 38.2% and 2.4%, respectively.
- Fertile flowers and seed production of *S. anglica* decreased with increasing density. On average, 24.6% of flowers can be fertilized and produce seeds in the second year after planting in the field when the individual density is low. But, in the fourth year, the crowded ramet populations did not flower. Our observations show that the species has lost its sexual propagation ability. But, the trade-off between density and seed production needs more experiments both in the field and under controlled conditions.
- During the first four years of invasion, individual density, leaf area index, biomass (both above- and below-ground biomass) and net assimilation production of *S. anglica* significantly ($p<0.05$) increased with time. There were significant relationships (linear or exponential, $p<0.05$) between density and each of these parameters. Maximal height and growth of culms depended on the elevation of the sites.
- *Spartina anglica* significantly decreased species number of the benthic fauna of native ecosystems from 16 to 10 species, especially the economically important species, such as *Cylina sinensis*, *Mactra veneriformis* and *Morella meretrix*, and significantly shifted the species composition. There were only five common species between the invasive plantation and native ecosystems. But, the exotic species favored the inhabitation of *Perineris aibunhitensis*, and the clamworm increased to 89 individuals per square meter (ind/m^2) (at the top 20 centimeters (cm)) from 3 ind/m^2; meanwhile, the number of clamworm species decreased from five to two.
- *Spartina alterniflora* produced a large number of seeds which germinated in the field in the first year of planting. In the laboratory, seeds could germinate at salinities between 5-7% and at low temperature (0-5°C). Drought made the seeds lose their germination ability. Three morphs (F, N and G) were identified by culm height, stem extension, ear length, leaf size and color. N-fixed bacteria could enhance seed germination, individual growth and thus combine a beneficial assemblage with *S. alterniflora*. It could grow well at 1%, high concentration of NaCl, say <3%, can accelerate P absorption by the roots and >99% of 32P is stored in the root during the first 4 days at 10,000 parts per million (ppm) of P solution.
- *Spartina alterniflora* has rapidly occupied the niches and replaced all other plant species in coastal ecosystems of China, greatly shifted the landscapes and species composition of the tidelands, and thus drastically damaged the native ecosystems. This in turn threatens agriculture, aquaculture of the regions, and rare and endangered species in coastal reserves.
- Freshwater irrigation, even with polluted freshwater and wastewater, can be used to control, even eradicate, *S. alterniflora* populations. "Harvesting above-ground parts of *Spartina* and then culturing mollusks" is another use-

ful method to eradicate *Spartina*, but more experiments are needed to verify its effectiveness (Chen et al. 2007).

- *Spartina alterniflora* has lower genetic diversity but higher genetic differentiation capacity whereas *S. anglica* has higher genetic diversity and lower differentiation capacity (An et al. this volume). These genetic differences may have contributed to the different invasion dynamics, and different fates, for the two species of *Spartina*.

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SPREAD OF INVASIVE *SPARTINA* IN THE SAN FRANCISCO ESTUARY

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We mapped the location and extent of all non-native *Spartina* in the San Francisco estuary in 2000 and 2001 and mapped a sample of 28 sites in 2003. We incorporated aerial photographs, ground surveys, and genetic analyses into a GIS. These sites demonstrated an average increase of 317 percent coverage of *S. alterniflora* x *foliosa* hybrids, radiating from points of the deliberate introduction of *S. alterniflora*. Extrapolating to the entire estuary, this suggests a potential increase from ca 190 hectares(ha) (470 acres[ac]) recorded in 2001 to as much as 793 ha (1,960 ac) in 2003. Hybrids now occupy approximately four percent of the total area of marsh and mudflats in the bay. Spread was greater in tidal marshes or formerly diked baylands and mudflats than in creeks, sloughs, and urbanized marsh (riprap, boat ramps). Genetic testing found no new invasion sites. Manual control methods applied in 2002-2003—digging or covering with geo-textile fabric—were effective at removing or killing small populations or single plants of *Spartina* species.

Keywords: invasive *Spartina*, *S. alterniflora*, *S. densiflora*, *S. patens*, *S. alterniflora x foliosa* hybrids, monitoring

INTRODUCTION

The San Francisco Bay Estuary contains the largest and most ecologically important expanses of tidal mudflats and salt marshes in the contiguous western United States with a diverse array of native plants and animals. Over the years, many non-native species of plants and animals have been introduced to the Estuary threatening to change the structure, function, and value of the Estuary's tidal lands. In recent decades four species of non-native *Spartina* have begun to spread rapidly in the Estuary. Though valuable in their native settings, these introduced *Spartina* species are highly aggressive in this new environment and frequently become the dominant plant in areas they invade. (Callaway and Josselyn 1992; Cohen and Carleton 1995; Daehler and Strong 1996; Goals Project 1999; Ayres et al. 2003; California Coastal Conservancy 2003; Ayres et al. 2004).

In 2000 the California Coastal Conservancy established the San Francisco Estuary Invasive *Spartina* Project (ISP) to provide a regionally coordinated approach to controlling or eradicating non-native *Spartina* in San Francisco Bay. The ISP includes a monitoring program to map non-native *Spartina* and to assess the effectiveness of treatment methods. In 2000-2001 the ISP mapped the entire Estuary using the methods outlined in Collins et al. (2001). In 2003 the ISP Monitoring Program mapped a subset of 28 sites, monitored sites treated in 2002 and 2003, used genetic testing to confirm identifications at known and suspected invasion sites, and compared methods for monitoring cordgrass in the Estuary.

Five species of *Spartina* are currently found in the San Francisco Bay Estuary including the native, *S. foliosa*. The four non-native species currently found in the estuary are *S. alterniflora*, *S. densiflora*, *S. anglica*, and *S. patens*. Hybrids between Atlantic smooth cordgrass *S. alterniflora*, and the native Pacific cordgrass *S. foliosa* (hereafter termed "hybrids") now threaten the ecological balance of the Estuary and are likely to cause the extinction of native Pacific cordgrass, choke tidal creeks, dominate newly restored tidal marshes, and displace thousands of acres of existing shorebird habitat (Ayres and Strong, this vol.; Stralberg et al. this vol.; Ayres et al. 2003; Ayres et al. 2008). Invasive cordgrasses from the San Francisco Estuary could spread to other California estuaries through seed dispersal on the tides.

The 2000-2001 survey found 195 net hectares (ha) (483 acres (ac)) of non-native *Spartina* distributed throughout nearly 16,187 ha (40,000 ac) of tidal marsh and 11,736 ha (29,000 ac) of tidal flats (Ayres et al. 2004). Net area is the coverage if all non-native *Spartina* plants were contiguous while gross area would be all the marsh areas that have some non-native *Spartina* plants. Of this total, 190 ha (470 ac) were hybrids, 5 ha (13 ac) were *S. densiflora*, 0.23 ha (0.58 ac) were *S. patens*, and 0.04 ha (0.09 ac) were *S. anglica*. The hybrids have increased in area 100-fold since the 1970s, from just over one ha of planted *S. alterniflora* in 1978 (Ayres et al. 2004). It is hypopthesized that the proliferation of hybrids is accelerating the rate at which areas are covered due to the evolution of greater invasiveness (Ayres et al. 2004; Ayres and Strong this vol.; Hall et al. 2006 and this vol.). In addition to the physical displacement of native marsh plants, the hybrid invasion threatens the genetic integrity and continued existence of the native *Spartina foliosa* (Ayres et al. 2003).

METHODS

Overall Description

A total of 31 estimates of area covered by three species of non-native *Spartina* were made at 28 separate sampling sites in 2003 (Table 1). The three species were *S. patens*, *S. densiflora* and hybrids. A sample of sites was selected to provide coverage for the entire bay shoreline stratified by "region" as defined by the Wetlands Goals Project (Goals Project 1999) (latitude) and "site type." Field sites were selected across the latitudinal extent of the non-native *Spartina* spp. invasion in the Estuary (Fig. 1). The regions were North Bay (NB) (2 sites), Central Bay (CB) (21 sites), and South Bay (SB) (5 sites). The site types were I) tidal, micro-tidal, and formerly diked bayland, and back barrier marsh (9 sites); (II) fringing tidal marsh, mud flats and estuarine beaches (7 sites); (III) major tidal sloughs, creeks or flood control channels (5 sites); and (IV) urbanized rock, riprap, docks, boat ramps and marinas (7 sites). At least two marshes of each site type were selected from each of the three regions of the Bay. Within each site type six to seven marshes were selected.

The three non-native species differed in their distributions among the three bay regions. *Spartina. patens* occurred at one location, Southampton, in the North Bay where no other non-native species occurred. *Spartina densiflora* occurred at seven locations: one in the North Bay and six in the Central Bay. At three of the Central Bay sites *S. densiflora* and hybrids both occurred. Two of these three sites are adjacent to each other, that is, Blackie's Creek runs through Blackie's Pasture. Hybrids occurred at 23 separate sites from the Central Bay and the South Bay. It was not possible to sample equal numbers of sites within each type and region due to the unequal frequencies of appropriate sites, the requirement to avoid clapper rails in some locations and to sample particular sites at particular tide heights, and limited numbers of trained field staff and Global Positioning System (GPS) units. Nevertheless, as noted below, the sampling in 2003 encompassed the full known regional extent of non-native *Spartina* species distribution.

Mapping

In 2003 distribution and abundance of non-native *Spartina* were mapped at the 28 sample sites and compared to the distribution and abundance at those locations in 2001. Overall methods followed Collins et al. (2001) with some modifications.

At each sampling site observers mapped the location and areal extent of non-native *Spartina* using a GPS data

Table 1. Change in Area Covered Between 2001 and 2003 Using Field Measurements and Field Estimates*at 28 sites for three Spartina taxa.

Sites	Taxa	*Area in 2001 (ac)*	*Area in 2003 (ac)*	*Area in 2001 (square meters)*	*Area in 2003 (square meters)*	*Change in Area 2001 to 2003*
Site Type I						
Bunker Marsh*	*Hybrids*	0.39	1.40	1580.11	5665.59	259%
Citation Marsh*	*Hybrids*	0.51	1.93	2059.74	7803.41	279%
Cogswell Marsh (North Quadrant)*	*Hybrids*	0.37	1.32	1503.16	5334.08	255%
Piper Park West	*S. densiflora*	0.02	0.08	99.92	318.40	219%
Southampton Marsh	*S. patens*	0.31	0.05	1244.49	196.99	-84%
Point Pinole	*S. densiflora*	0.00	0.00	2.99	6.39	114%
Palo Alto Baylands	*Hybrids*	0.12	0.15	478.26	618.49	29%
Corte Madera Marsh Reserve 1	*S. densiflora*	0.011	0.014	44.95	60.75	35%
Corte Madera Marsh Reserve 2	*Hybrids*	0.01	0.05	26.46	214.92	712%
Pickleweed Park	*S. densiflora*	0.49	0.03	1964.07	118.73	-94%
Site Type II						
Emeryville West	*Hybrids*	0.07	1.59	282.60	6430.15	2175%
Coyote Creek Marsh	*Hybrids*	0.09	0.06	353.25	233.15	-34%
Alameda Island – N. Elsie Roemer*	*Hybrids*	0.29	0.98	1171.89	3948.94	237%
Blackie's Pasture 1	*S. densiflora*	0.021	0.020	84.12	82.66	-2%
Blackie's Pasture 2	*Hybrids*	0.01	0.08	41.37	330.90	700%
Ideal Marsh*	*Hybrids*	0.26	0.88	1070.72	3541.00	231%
Richmond Inner Harbor – Steege Marsh	*Hybrids*	0.01	0.02	32.19	100.46	212%
Bayshore Park	*Hybrids*	0.05	0.25	168.19	1018.84	506%
Site Type III						
Blackie's Creek 1	*Hybrids*	0.03	0.02	108.33	93.97	-13%
Blackie's Creek 2	*S. densiflora*	0.00	0.00	12.06	8.46	-30%
Colma Creek	*Hybrids*	2.36	7.16	9551.11	28957.46	203%
Corte Madera Creek	*S. densiflora*	1.19	2.70	4802.16	10948.30	128%
San Leandro Creek	*Hybrids*	2.16	3.83	8760.60	15481.79	77%
San Mateo Creek	*Hybrids*	0.20	0.78	825.41	3172.16	284%
Site Type IV						
Coyote Pt Marina	*Hybrids*	0.35	0.74	1408.81	3004.87	113%
Oakland Inner Harbor	*Hybrids*	2.74	5.40	11087.72	21685.73	96%
Yosemite Slough	*Hybrids*	0.02	0.12	75.47	476.02	531%
India Basin	*Hybrids*	0.10	0.01	408.20	41.47	-90%
Pier 94	*Hybrids*	0.04	0.03	171.72	129.50	-25%
Pier 98/Heron's Head (treat took place in 2002)	*Hybrids*	0.002	0.009	8.83	37.40	324%
Loch Lomond Marina	*Hybrids*	0.005	0.016	19.63	64.98	231%

Site and Marsh Types:
Type I. Former Diked Bayland/Microtidal/Tidal/Back Barrier Marsh
Type II. Fringing Tidal Marsh/Mudflats/Estuarine Beaches
Type III. Major Tidal Slough, Creek or Flood Control Channel
Type IV. Urbanized rock, riprap, dock, ramp, marina.

entry unit (Trimble Geo-Explorer III). Plant data were entered into the unit as points, lines or polygons depending on the extent of the invasion. The GPS units automatically collect data on date, time, location, area and polygon perimeter and line length. Field staff manually entered site name, species identification, clone identification, clone diameter, line width, sample name, percent cover class, other applicable data and comments.

GPS data were downloaded using Pathfinder™ software, exported to ArcView™, differentially corrected, and reviewed for errors. The data were then exported to Excel™ and once again reviewed for data entry errors. Final data were summarized and mapped in ArcView™ and statistically analyzed using SYSTAT™.

Three of the four non-native taxa of *Spartina* found in the San Francisco Estuary—*S. Alterniflora* hybrids, *S. densiflora*, and *S. patens*—were mapped in 2003. The one location where *S. anglica* was found in 2001 was not included in the 2003 monitoring program. *Spartina* species were identified in the field using plant morphology (Zaremba 2001). Leaf samples were collected for genetic analysis to confirm field identifications of *S. alterniflora* and hybrids.

Aerial Photo Interpretation

In addition to field-collected data, aerial photo interpretation was used at five of the 28 sites for large *Spartina* hybrid infestations, not single clones. Color infrared photos were taken at 1:6000 feet scale at low tide during the peak of the growing season between August-October when the plants were still green to allow for accurate yearly comparison. Photos were scanned at 1200 dpi and orthorectified then imported into ArcView™ 3.3 for review and analysis. Polygons were digitized around the *Spartina* meadows and polygons were given a cover class (<1% seedlings, <1% mature, 1-10%, 10-30%, 30-60%, 60-90%, 90-100%). Areas of the polygons were calculated using the ArcView™ Xtools extension and then area of the *Spartina* infestation of the polygon was calculated as the total polygon area multiplied by the percent cover of non-native cordgrass.

Fig. 1. Non-native Spartina Baywide Distribution Map in 2001(light) relative to the 2003 Monitoring Sites (dark).

Five of the 28 sites compared between 2001 and 2003 were only surveyed with aerial photography in 2001. Field derived areas for these sites were estimated using empirical relationships in the data (Zaremba et al. 2004).

The total calculated non-native *Spartina* areas from 28 sites were compared over time to determine spread (percent change in area covered) between years. Field and aerial photo interpretation measurements were compared at 5 sites to assess the utility of the aerial photo interpretation methodology. While not a robust study given the small sample size, assessing the aerial photo interpretation method was an important to the ISP Monitoring Program.

Genetic Analyses

Genetic analyses to confirm species identification were done at each of the 28 sites and at additional sites requested by concerned landowners, managers or stakeholders (Fig. 2).

Additional genetic surveys were done at a few sites along the coast north of the Golden Gate in Point Reyes National Seashore and Bolinas Lagoon. In order to confirm species identification, leaves from ambiguous plants and at least 3-5 plants per monitoring site were collected according to standard methods used previously (Ayres et al. 2004) and conforming to recommendations in Collins et al. (2001). Where needed, transects were run the length of marshes sampling every 10 meters to determine (1) if there were any “hidden” *S. alterniflora* hybrids, or (2) the percent invasion of a particular marsh. From each individual clone of unknown species identification and from each 10-meter sample along the transects, a single plant leaf was collected for genetic analysis using RAPD (Random Amplified Polymorphic DNA) nuclear markers (Daehler and Strong 1997; Ayres et al. 1999).

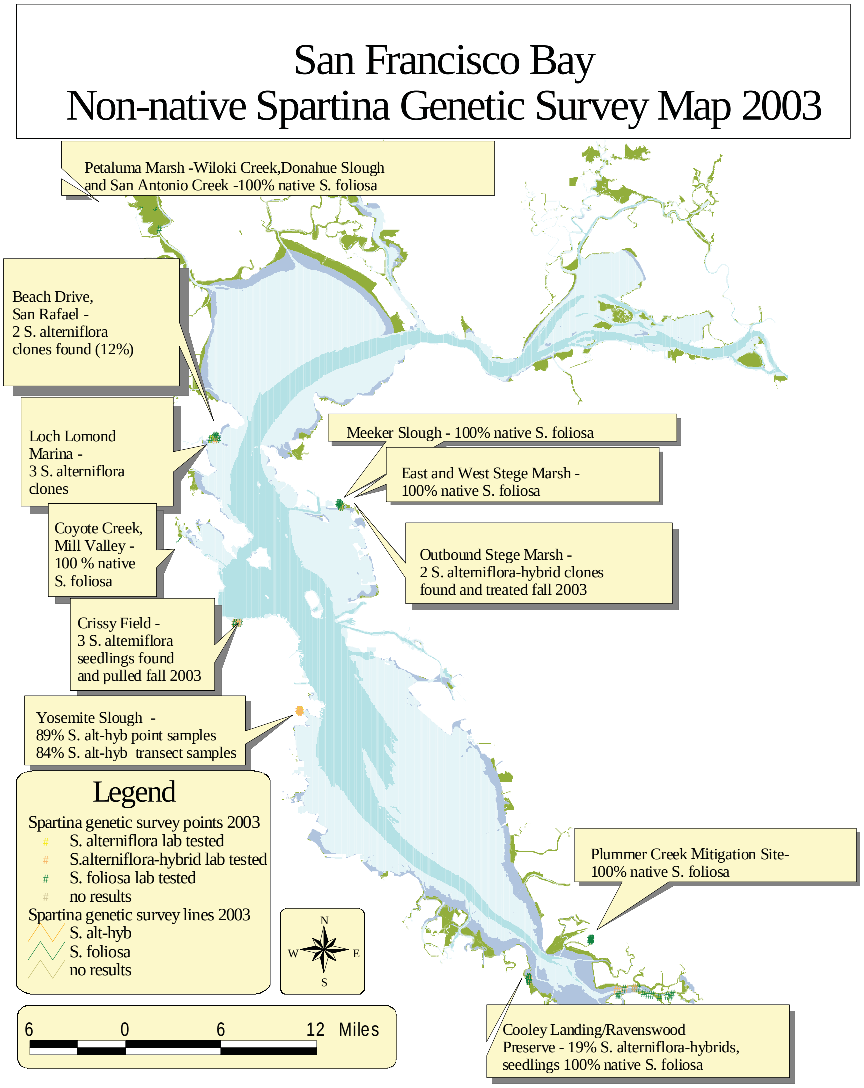

Fig. 2 - Baywide Map of Genetics Sampling Sites

Efficacy Monitoring

ISP staff monitored the efficacy of 13 different treatments at eight sites. Some treatments were done in 2002 with assessment of efficacy in 2003. Treatments done in 2003 will be assessed in 2004. Treatment monitoring data were collected consistent with methods described in Collins et al. (2001). Prior to treatment the extent of non-native *Spartina* was mapped to determine the total area of non-native *Spartina*. For large treatment sites mapped as polygons with a percent cover of non-native *Spartina* the sampling scheme described by Collins et al. (2001) was modified from a graphical information system (GIS) based sampling approach to a field-based approach. A stratified random sub-sampling method was applied. Transects were run across the length of the treatment area parallel to the shoreline at selected elevations and then random points were sampled along the transects. For smaller treatment sites where individual clones were mapped as points all the mapped plants up to a maximum of 30 plants per site were monitored. If more than 30 plants were mapped, 30 were randomly selected to monitor pre-treatment and post-treatment to determine the treatment efficacy. Measurements of overall plant vigor including plant height, density per 0.25 meter, plant vigor (high/medium/low), tide wash (yes/no), plant species percent cover (native and non-native plant species) per 0.25 meter, percent flower *Spartina* per 0.25 meter, sediment type, high/medium/low marsh, and burn (yes/no) were collected from each sampling location. The entire treatment area was mapped again one year post-treatment and the same treatment efficacy data were collected.

Table 2. Change in area covered by non-native *Spartina* species between 2001 and 2003 averaged over all sites surveyed, comparing aerial photo interpretation measurements (APIM) with field measurements and field estimates.

Species	*Average Site Change in Area from 2001-2003*	
	*Field Measurement + Field Estimates (N=28)***	*APIM (N=5)**
S. Alterniflora Hybrids	317.00%	213.05%
S. densiflora	52.83%	n/a
S. patens	-84.2%	n/a
All Species	244.41%	213.1%

* Five of the 28 sites had only aerial photo interpretation measurements for 2001. For these five sites, a 2001 field estimate was calculated using a regression curve based on a correlation between the existing 2001 and 2003 field measurements. This 2001 field estimate was used in the place of the 2001 field survey data to calculate the change in area from 2001-2003.

** For five sites of the 28 sites, 2001 aerial photo interpretation measurement was used in the place of the 2001 field survey data to calculate the change in area from 2001-2003.

Data Analyses

Data were subjected to a variety of quality assurance methods (Zaremba et al. 2004). Cross-tabulation of categorical names was done to check for typographical errors and duplications. Summary statistics were then calculated for quantitative variables to check for unreasonable ranges and outliers. Descriptive statistics were calculated and raw data transformed as needed to meet requirements of parametric statistical tests.

We tested the hypothesis that there was an increase in area covered by non-native *Spartina* using a t-test between area covered in 2001 and 2003. The hypothesis that a particular species, site type or bay region (latitude) had an influence on the change in area covered between 2001 and 2003 was tested with an Analysis of Variance (ANOVA). We tested the hypothesis that site latitude had a significant effect on change in area covered between 2001 and 2003 by using an Analysis of Covariance (ANCOVA). The accuracy of field identification of *S. alterniflora*, *S. foliosa* and their hybrids was tested statistically with a Chi-square test of frequencies of correct field identification of *S. alterniflora*, *S. alterniflora* hybrids, and *S. foliosa*. This test used the genetic analysis as the true (or theoretically expected) frequencies and the field observations as the observed frequencies.

Based on data averaged over all species and all sites sampled, the non-native species and hybrids of *Spartina* increased 244% in area covered (paired sample t-test, $p = 0.003$) between 2001 and 2003 (Table 2). Field surveys (field measurement plus estimates) showed an increase of non-native *Spartina* of 15% in the North Bay, 292% in the Central Bay and 177% in the South Bay from 2001 to 2003. These measurements showed that non-native *Spartina* (all species) increased from 2001-2003 by 172% at Type I sites (tidal, micro-tidal, and formerly diked bayland, and back barrier marsh), by 504% at Type II sites (fringing tidal marsh, mud flats and estuarine beaches), by 108% at Type III sites (major tidal sloughs, creeks or flood control channels), and by 169% at Type IV sites (urbanized rock, riprap, dock, ramp, marina).

S. patens occurred at only one site in the North Bay, a Site Type I. It decreased in area covered by 84%.

S. densiflora decreased in nearly as many sites (3) as it increased (4). Statistical tests were precluded for *S. densiflora* because of low sample sizes but the average percent change in area covered was 53% with a range from -94% to +219%. *Spartina densiflora* was mapped at only one sample location in the North Bay (Pt. Pinole), where it increased by 114%. *Spartina densiflora* increased by 43% in the Central Bay. *Spartina densiflora* increased in Type I and Type III sites but apparently decreased slightly at Type II sites. No *S. densiflora* were noted at Type IV sites.

Hybrids make up most of the non-native coverage and their proportion among the non-natives increased in 2003 compared to 2001 accounting for 83% of the non-native coverage in 2001 and increasing to 90% of non-native cordgrasses in 2003. Hybrids increased in cover three-fold between 2001 and 2003 (paired sample t-test, $p < 0.001$) with a range from -90% to +2175% depending on site. Hybrids increased in area covered in 19 of 23 marshes sampled, a statistically significant proportion of the sites (non-parametric sign test, $p = 0.003$).

Hybrids in the South Bay were responsible for the 177% increase in non-native invasive *Spartina* between 2001-2003. Hybrids increased by 403% in the Central Bay. Hybrids were not found in any sample sites of the North Bay. Hybrids had higher mean and total area covered in the Central Bay than in the South Bay in both 2001 and 2003. The slight trend of percent change in hybrid area covered with latitude (south-to-north trend) was not statistically significant ($p = 0.18$).

While hybrid acreage increased greatly from 2001 to 2003 across all site types, site types I and II had higher percent increases, on average, than site types III and IV (non-parametric sign test; $p = 0.254$). Analysis of the area covered by hybrids (log-transformed square meters) in 2003 found no statistical difference among site types (ANOVA, $p = 0.377$).

Aerial Photos versus Field Measurements

The estimates of the area covered by non-native *Spartina* hybrids differed between the aerial photo

Table 3. Change in Area Covered by Non-native *Spartina* between 2001 and 2003 for Five Sites Comparing Aerial Photo Interpretation Measurements (APIM), Field Estimate and Field Measurement Measurements.

Site Name	*Site Type*	*Species*	*Area in 2001 (square meters)*		*Area in 2003 (square meters)*		*Change in Area 2001 to 2003*		*Difference Between Methods of Estimating Areas*
			APIM	Field Estimate*	APIM	Field Measurement	APIM 2001 vs APIM 2003	Field Estimate 2001 vs Field Measurement 2003	Field Measurement 2003 vs APIM 2003
Bunker Marsh	I	*Hybrids*	5,100.86	1,580.11	8,501.62	5,665.59	67%	259%	-33%
Citation Marsh	I	*Hybrids*	244.83	2,059.735	2,448.35	7,803.41	900%	279%	219%
Cogswell Marsh (No. Quad.)	I	*Hybrids*	700.12	1,503.16	700.12	5,334.08	0%	255%	662%
Alameda Island - North Elsie Roemer	II	*Hybrids*	2,882.37	1,171.89	3,610.10	3,948.94	25%	237%	9%
Ideal Marsh	II	*Hybrids*	2,229.01	1,070.72	3,863.62	3,541.00	73%	231%	-8%
Average	Avg	*Avg*	2,231.43	1,477.12	3,824.74	5,258.60	213%	252%	170%

*No field measurement collected in 2001. 2001 field estimate was calculated using a regression curve based on a correlation between the existing 2001 and 2003 field measurements.

interpretation and field measurement methods. The aerial photo interpretation measurements of area covered by hybrids were on average 170% less than the field measurements (Table 3). However, both methods recorded at least a doubling of area invaded in two years. The aerial photo interpretation measurements indicated that the hybrids increased 322% at Site Type I and 49% at Site Type II.

This discrepancy affects the estimates of total acreage involved but does not substantially change the estimates of rate of increase of the invasion between 2001 and 2003, which is approximately doubled or more, whether averaged by region or by site type. The relationship of aerial photo measurements of area to field measurements of area was tested statistically using a t-test and linear regression (on log-transformed data) of field estimates on photo estimates for five sites in 2003. No difference in mean area covered was found between methods (t-test, $p = 0.616$). However, no regression relationship was found ($p = 0.797$). This was likely due to high variability among the small sample size of aerial photo measurements. Thus, aerial photo interpretation measurements should be used with caution to estimate cover.

Accuracy of Field Identification

Over the course of the 2003 monitoring season, 12 landowners, managers or concerned stakeholders requested surveys for the presence of non-native *Spartina* (Fig. 2). In addition, genetic tests were performed to confirm species identification by field staff at each of the inventory monitoring sites (Table 1). A total of 68 plant samples were identified to species in the field using plant morphology and analyzed using genetic tools (Daehler and Strong 1997; Ayres et al. 1999). Fifty-five of the plants were field identified as *S. alterniflora* hybrids, and 13 as *S. foliosa*. Forty-nine of the plants were confirmed genetically to be *S. alterniflora* hybrids, and 18 were confirmed to be *S. foliosa*. There was not a statistically significant difference between field classification and subsequent genetic (or true) identification at the 0.05 level (Chi Square). More native *S. foliosa* were called hybrid than vice versa. 89% of the hybrids and 100% of the *S. foliosa* were correctly identified.

Efficacy

Sites treated in 2002 were monitored in 2003 as well as limited monitoring for the few sites that were treated in 2003 (Table 4.) Manual Methods such as digging of *S. densiflora* at Piper Park or trampling and covering in Point Reyes National Seashore (PRNS) were effective on a small scale. Return visits were required to make sure no new shoots had emerged.

DISCUSSION

Spread

Based on the ISP's 2003 survey results, the average increase in area (calculated by site) of all non-native *Spartina* species was 244% over a period of two years. For *S. alterniflora* and hybrids, the increase was 317%. Applying this rate of increase, which represents an average across all site types with differing and variable environmental conditions and stages of invasion, to the 190 ha (470 ac) recorded in the 2001 survey suggests that hybrids could have spread to as high as 793 ha (1,960 ac). In contrast, *S. alterniflora* spread only 25% in 11 years in Willapa Bay,

Table 4. Change in Area of Non-native Spartina at Pre- and Post-treatment Sites Monitored in 2003.

Site name	*Treat-ment Year(s)*	*Pre-Post Treat-ment*	*Area*			*% Change 2002-2003*	*% Change 2001-2003*	*Treatment & Comment*
			2001 Ac/m^2	2002 Ac/m^2	2003 Ac/m^2			
Piper Park **(entire treatment area including east strip marsh)**	2002	Post	0.03/ 123.0	0.02/ 97.12	0.01/ 50.47	-48.0%	-59.0%	Dig Winter 2003; 2002-3 treatment, volunteers did not finish the entire treatment area. Nor did they dig every plant in the primary treatment area thus not 100% kill.
Piper Park **(primary treatment area w/out east strip marsh)**	2002	Post	0.03/ 122.17	0.02/ 85.92	0.01/ 23.00	-73.2%	-81.2%	Dig Winter 2003; 2002-3 treatment, primary treatment area, however volunteers did not dig every plant thus not 100% kill.
PRNS-Drakes/Limantour Estero	2002	Post	n/a	0.06/ 233.53	0.00005/ 0.20	-99.9%	n/a	Trample & Cover Summer 2002; One clone at Creamery Bay had a patch that grew out from under the tarp.
PRNS-Drakes Estero	2003	Pre	n/a	n/a	0.005/ 19.63	n/a	n/a	Trample & Cover Fall 2003.
Bolinas Lagoon North	2002	Post	n/a	0.002/ 7.07	0.00001/ 0.03	-99.6%	n/a	Dig Winter 2002; Return visits found occasional new sprouts.
Bolinas Lagoon South	2003	Pre	n/a	n/a	38.47/ 0.01	n/a	n/a*	Trample & Cover Summer 2003.
Emeryville Crescent	2002 2003	Pre & Post	0.05/ 215.68	n/a	0.63/ 2547.52	n/a	1081.2%	Mow & Cover Summer 2003; Small scale 2002 mowing treatment had no effect thus mostly seeing spread.
Emeryville Crescent – Mowed Portion	2002 2003	Pre & Post	0.05/ 205.2775	n/a	0.38/ 1539.58	n/a	650.0%	Mow 2003.
Emeryville Crescent – Mowed & Covered Portion	2002 2003	Pre & Post	0.00/ 3.53	n/a	0.08/ 306.15	n/a	8566.7%	Mow & Cover Summer 2003.
Richmond Inner Harbor – Steege Marsh	2003	Pre	0.01/ 32.19	n/a	0.02/ 100.46	n/a	n/a*	Trample & Cover Fall 2003.
Alameda Island – North Elsie Roemer	2003	Pre & Post	0.53/ 2143.97	n/a	0.53/ 2125.05	n/a	-0.88	Mow/Mow & Spray 2002/Fall 2003; GIS based area calc 2003. Area calculations may be "imperfect".
Pier 94	2003	Pre	0.04/ 171.72	n/a	0.08/ 318.94	n/a	n/a*	Dig, Trample & Cover Summer 2003.

n/a* Pre-treatment data only. No percent change results.

Washington from 800 ha (1977 ac) to 1,000 ha (2471 ac) between 1988 and 1999 (Daehler and Strong 1996). The formation of hybrids between *S. alterniflora* and *S. foliosa* may have greatly increased the spread of hybrid *Spartina* relative to *S. alterniflora*; e.g., hybrids expanded 150% in Cogswell Marsh, Hayward, California between 1999 and 2000, including some individual hybrid clones that increased 300% (Zaremba, 2001). Ayres and Strong (2000) reported a remarkable 740% increase of *S. alterniflora*-hybrids from 5% to 42% at San Lorenzo Marsh between 1997 and 2000. It has been proposed that the formation of hybrids expains the rapid rate of expansion in the San Francisco Estuary relative to other estuaries where hybridization does not occur. Ayres et al. (2004) speculated that the recent rapid spread of hybrids in the Estuary may be a result of selection for hybrids with exponential clonal growth and seed production. Theoretical models found greater-than-exponential spread rates can occur when clonal growth and seed production are under selection (Hall et al. this vol.; Hall et al. 2006).

Cover by non-native *Spartina* increased at all site types from 2001 to 2003, however, site types differed in the rate at which cover increased. The large variations in spread rates among site types likely reflects the proximity of individuals sites to seed source in addition to habitat suitability.

Fringing tidal marshes, mudflats, and estuarine beaches (Site Type II) experienced the greatest increase in cover (504% increase). Tidal and microtidal marshes, formerly diked baylands, and back barrier marshes (Site Type I) and urbanized shorelines (Site Type IV) increased by about 170%. Cover in tidal sloughs, creeks, and flood control channels (Site Type III) increased by 108%. The differences among site types may be related to successional processes linked to sedimentation consistent with *S. alterniflora* and *S. townsendii* invasions in New Zealand and *S. anglica* in England (Daehler and Strong 1996), and predictions by Callaway and Josselyn (1992).

One interpretation of the spread rate differences among sites is that invasion rates of susceptible habitat are initially high and then slow until more susceptible habitat is created by sediment accretion. Fringing marshes, mudflats and beach habitat (Site Type II) experienced the highest rate of spread from 2001 to 2003, suggesting a new invasion front was being exploited by hybrids. Presumably, after the initial rapid colonization of suitable “empty-niche” habitat, the rate of spread would slow to match the rate at which the *Spartina* augments sedimentation and creates additional habitat to invade.

Established *S. foliosa* marshes and open mudflats of a newly opened restoration sites (Site Type I) are initially highly susceptible to invasion by pollen and by seed, respectively. The formerly diked bayland Citation Marsh experienced a 900% increase in hybrid cover in three years. Then, the rate of cover increase slowed as suitable habitat was filled in.

Site Type IIIs may be initially slower to colonize because they include deep channels and creeks, where sediment must accrete before they are at suitable elevation for more extensive colonization. Once the channel beds have sufficiently accreted, colonization of remaining channel banks and bottoms is rapid, with clonal colonies quickly coalescing into meadows. Rate of spread in urbanized shoreline (site type IV) may likewise be relatively low due to poor or little sediment availability.

Among the three non-native *Spartina* taxa, hybrids spread the most rapidly (317% in three years). Hybrids have high rates of vegetative spread, produce large quantities of pollen, have successful seed set, and readily backcross to the native *S. foliosa*. *Spartina foliosa* exposed to pollen of hybrids produces hybrid seeds, further accelerating the hybrid invasion. The rate of colonization is particularly rapid in the early stages of invasion. For example, Emeryville West showed an increase in cover of 2,175%, between 2001 and 2003. All habitats in the Bay appear to be susceptible to invasion by this *S. alterniflora* hybrid.

S. densiflora, which has a cespitose growth form and invests more reproductive effort in seed production than vegetative spread, didn’t spread to the same degree as did hybrids: 52% averaged over all site types. Site Type I experienced the greatest increase in cover of *S. densiflora* (68%).

S. patens apparently decreased in cover by 84%, but it is found only at Southampton Marsh where there is less than an acre of total cover. The apparent decrease is likely due to mapping error not a true decrease in cover.

Of the three Bay regions, the Central Bay, nearest to the original introduction sites in the Fremont and San Bruno marshes, had the largest increase in cover, 292%. Hybrids, the fastest spreading species (392%), dominate this region of the Bay. Clearly, the hybrids are the dominant invasive *Spartina* taxa and are well established, and both their acreage and rate of spread is greatest in the heart of the Bay.

Some new non-native *Spartina* populations were found in the sites surveyed at the request of land owners, managers, and stakeholders. In the South Bay, a survey of the Plummer Creek Mitigation Site found only native *Spartina*. However, 19% of seedlings from a *Spartina* spp. population at the Cooley Landing/Ravenswood Preserve Restoration Project were genetically tested and determined to be hybrids. Both of these sites are near extensive hybrid populations, and considered to be at risk. Genetic surveys were performed at a few small sites in the Central Bay (Crissy Field, Steege Marsh and Beach Drive) as part of a management regime to identify and remove newly established non-natives.

GIS

Three difficulties emerged during the 2003 monitoring: imprecision of measuring small clonal patches or small areas due to the limits of precision of the GPS units, differences between aerial photo interpretation estimates and ground-truth data, and low power to detect small changes in area coverage because of broad cover class intervals (Zaremba et al. 2004). Field mapping with three-meter- resolution GPS units is imprecise for small areas such as new invasion sites, which necessarily contain small populations, and the physically smaller *Spartina* species, *S. densiflora* and *S. patens*. These possible sources of error could account for the apparent decrease in cover of *S. patens* at Southhampton Marsh or *S. densiflora* at Pickleweed Park or Blackie’s Pasture and Creek.

Remote sensing using aerial infra-red photography has potential for synoptic mapping of the spread of invasive *Spartina* compared with labor-intensive field mapping. However, among the five sites where we compared these methods in 2003 there were two sites where aerial estimates were 8-33% less than field estimates and three sites where aerial estimates were 9-661% greater than field estimates. Averaged over all five sites, the field measurements were 170% greater than the aerial photo measurements. Aerial photo interpretation methods may prove useful to monitor the spread of non-native *Spartina* in grossly invaded areas, but it is not currently precise enough to map without field truthing. The examples of both higher and lower aerial photo interpretation cover estimates indicate that the current method of digitizing polygons around the marsh or *Spartina* patch with a cover class may be too coarse to estimate cover precisely.

Broad cover-class boundaries also contribute to imprecision. For example, if a polygon area is within a cover class, e.g. 10-30%, then it is assigned the midpoint of the class, 15% in this example. In both 2001 and 2003 Cogswell Marsh fell within cover class 10-30%. Either there was no change in cover between 2001 and 2003 or the difference was not large enough for 2003 to be assigned to the next larger class. Thus, a trend of expansion or contraction will be detected only when it crosses a cover class boundary. However, the cover classes are useful for determining an approximate infestation level and can be used to prioritize removal efforts.

Accuracy of Field Identification

Genetic tests were also performed to confirm species identification by field staff at each of the Inventory Monitoring Sites. The genetic tests confirmed that field staff were accurately identifying both *S. alterniflora* hybrids (89%) and *S. foliosa* (100%). The samples labeled as "unknown" or "unidentifiable" by field staff were found just as likely to be hybrids as *S. foliosa* (55%-45%).

Trained field biologists can accurately identify the majority of non-native *Spartina* plants, but genetic testing of ambiguous hybrid specimens is necessary to reduce the risk of overlooking non-natives and their hybrids.

Efficacy

Post-treatment monitoring was done at five sites that were treated in 2002 and pre-treatment monitoring was done at seven sites. Manual removal (digging) of *S. densiflora* at Piper Park was effective; however, a number of divots remained visible in the marsh plain several months after the clones were removed. It is uncertain how long these features will remain in the marsh and it is unclear what effect they may have on the quality of the marsh habitat. We observed that volunteer removal efforts are excellent for public outreach, but it was frequently necessary for organizers and ISP staff to complete work begun by volunteers.

Clones in Bolinas Lagoon and Point Reyes that had been dug or trampled and covered were reduced by 95%. However, the clone that was dug out in Bolinas Lagoon continued to produce some shoots around the clone edge that required pulling. A single clone at Pier 98, which had been partially dug out and covered with geo-textile fabric by volunteers, had a number of remaining shoots that required further control. Clones must be entirely covered for the treatment to be effective. Roots and rhizomes should be confirmed dead as was done at Point Reyes before uncovering treated clones. Preliminary post-treatment monitoring of the Emeryville treatment site that was mowed in 2002 indicated no noticeable treatment effect.

CONCLUSIONS AND RECOMMENDATIONS

Data from 28 sites showed that non-native *Spartina* species are spreading at a rapid rate in the San Francisco Bay Estuary. Between 2000-2001 and 2003, the average percent increase in area covered by all non-native *Spartina* species in the Estuary was 244%, and by hybrids, 317%. Some sites had several hundred percent increases. While hybrids increased in area at all site types, spread was greatest for fringing tidal marshes, mudflats, and estuarine beaches (Site Type II). There was a slight trend in increase of hybrid cover with latitude (south to north trend). Aerial photo area measurements were compared with field measurements at five sites where both methods were used in 2003. There was no difference in the mean coverage between methods, but given the small sample size and that no regression relationship was found, aerial photo interpretation measurements should be used with caution to estimate field measurements for a single site. The increasingly rapid rate of spread of *Spartina,* in particular hybrids, continues to threaten existing habitat and species assemblages and potentially threatens the success of ongoing and planned restoration projects within the San Francisco Estuary and outer coast marshes. Successful control will only be achieved with dedication of adequate resources, attention to following protocols completely, and follow-up monitoring and periodic re-treatment as needed.

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REMOTE SENSING, LIDAR AND GIS INFORM LANDSCAPE AND POPULATION ECOLOGY, WILLAPA BAY, WASHINGTON

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Keywords: *Spartina alterniflora*, Willapa Bay, photogrammetry, mapping

The spread of *Spartina alterniflora* Loisel. (smooth cordgrass, hereafter referred to as *Spartina*) in Pacific Northwest estuaries presents a unique opportunity to examine ecological interactions between an invasive clonal organism and local abiotic factors. Willapa Bay is a large, shallow, tidal basin on the southwest coast of Washington State. The bay is approximately 360 square kilometers (km^2) at mean high tide, with well over 190 km^2 of soft mud and sand tideflats exposed at mean low tide (Civille 2005). The mixed diurnal tides of the northern Pacific coast distinguish Washington estuaries from those on the Atlantic coast where S. *alterniflora* is the predominant native salt marsh species. *Spartina* species are the principle components of Atlantic and Gulf coast estuaries, but the introduction of *Spartina* to the Willapa Bay estuary and its open mudflats has led to the rapid conversion of thousands of intertidal hectares into dense stands of upper tidal meadows (Sayce 1988; Daehler and Strong 1996; Civille et al. 2005). The rapid expansion of this robust clonal grass across the open habitat of intertidal mudflats is a textbook example of unimpeded colonization, and presents challenges not only to management efforts, but to ecologists as well.

The initial questions relevant to this research were generated by biologists and land managers in the state of Washington, who needed to understand *Spartina* expansion for environmental impact analyses, adaptive management plans, and to direct control efforts in the most efficient manner (Sayce 1988; Aberle 1990; Aberle 1993; Civille 1993). These questions included: where were plants spreading fastest, where was intertidal habitat being lost most rapidly, and whether stopping seed production was more effective than removing as many new plants as possible (Moody and Mack 1988). Ecologists studying invasive population growth dynamics ask similar questions. What is regulating the growth of *Spartina* on Willapa Bay mudflats? Are these regulators density dependent or independent? With the seemingly limitless expanse of habitat available for colonization, why is it that some areas are filling in more quickly than others? Are there factors intrinsic to the biology and mating system of *Spartina* that influence its spread, or are abiotic factors more important in shaping the colonization fronts? Does competition between *Spartina* plants limit the population, and will availability of resources affect their growth? Have evolutionary changes taken place in the Willapa Bay population that allow *Spartina* to compete more effectively? Many of these questions have been addressed in experimental and theoretical work by members of the *Spartina* Biocomplexity Group at the University of California at Davis, (Davis et al. 2004a; Davis et al. 2004b; Taylor 2004; Taylor and Hastings 2004; Civille 2005; Davis 2005; Civille 2006) and were presented at this conference by their primary authors (Davis et al.; Taylor et al.; this volume).

Spartina was probably brought to the Willapa estuary through the transplanting of eastern oysters to bolster the flagging oyster industry of Willapa Bay in the 1890s and early 1900s. The California gold rush had fueled the depletion of native oysters from the bay, and an effort was made by the United States Fisheries Bureau to replant the bay with Chesapeake stock. The completion of the transcontinental railway allowed the trip to be made in about a week (Townsend 1896; Scheffer 1945; Civille et al. 2005), and eastern oysters continued to be brought via railcar to Willapa until around 1917. The climate was apparently too cool for eastern oysters, and although they grew well, they were never able to reproduce successfully in Willapa at commercially viable levels. *Spartina*, however, was able to establish in small colonies that were close to beds used to rear eastern oysters (Civille et al. 2005).

The first written and photographic evidence of *Spartina* presence in the estuary was gathered at the Willapa National Wildlife Refuge by T.H. Scheffer in 1940 (Scheffer 1945). A photograph taken by Scheffer shows several “green islands,” and was donated to the California Academy of Science Herbarium to document his findings. If an average height of one meter (m) is assumed, the large plant in the photo is about 42 m in diameter, covering approximately 1,367 square meters (m^2) of mudflat. The first aerial photographs of the bay were taken by the Army Corps of Engineers in 1945, and *Spartina* plants of a similar size to those in Scheffer's photo can be seen in these photos at widely separated locations around the bay (Civille et al. 2005).

A time series analysis of *Spartina* spread across the Willapa tidal flats was conducted using 55 years of synoptic aerial photography, spanning from 1945 to 2000. The methodology used for the study was a combination of photogrammetry, image analysis and geographic information system (GIS) techniques (Wilkie 1996). Over 2,000 photos (black and white, true color and color infra-red) were digitized, examined for *Spartina* plants, then geo- or orthorectified to fit the curvature of the earth and assigned geographic coordinates. The resulting corrected images were then classified into thematic representations of *Spartina* meadows and clones. The final year of the series, comprised of color infrared images acquired in 2000, was ortho-rectified to remove radial and scale distortions, then classified through hierarchical supervised and unsupervised non-parametric methods into 330 thematic raster files, with less than one meter positional error and a 0.3 m minimum mapping unit (Civille 2005). All other spatial data were georectified to this baseline layer. These tasks were accomplished with a large format scanner (28 x 43 centimeter [cm] platform), Photoshop (Adobe), and Imagine (ERDAS), a robust digital photogrammetry software package. The resulting raster files were then converted into GIS vector coverages in ArcInfo (ESRI), and combined with a bathymetry digital elevation model (DEM) to obtain z (elevation) values for each polygon.

A vector-based coverage model was chosen for our analysis, rather than raster-based, because polygons are automatically assigned a unique identifier, area and perimeter by ArcInfo software (Flynn and Pitts 2000). This allowed us to extract demographic history for each plant (greater than 300,000 individuals in 1997 alone) that we detected on the photos. These data were used to parameterize mathematical population models (Taylor et al. 2004), and establish the ages of plants used in genetic and reproductive fitness experiments (Davis et al. 2004). Regions, which are a higher order organizational object in ArcInfo, were applied to the coverages, allowing us to analyze clonal growth and meadow formation year-to-year. Each contributing polygon (clone) within a region (large meadow), maintains its own unique identifier and also has a region identifier, which are maintained through data extraction and statistical analyses (Civille 2005).

Spartina elevation data were analyzed with two separate bathymetry (elevation) layers; one that was collected by the National Oceanic and Atmospheric Administration (NOAA) with depth soundings in 1953 (channels updated in 1986); and another that was acquired in the spring of 2002. The new bathymetry data was collected with LiDAR (Light Detection And Ranging) techniques, and is the first of its kind to be gathered for an entire estuarine intertidal zone with tidal control (Cracknell 1999; Irish and Lillycrop 1999; Flowers 2002). Tidal exposure times were calculated for the Willapa estuary during winter months when plant aboveground growth has died back, leaving mudflat elevations as the primary targets reflected by the laser. Data collection was scheduled for predicted tidal elevations lower than 30 cm Mean Low Water (MLW), although the actual tidal elevations at the time of acquisition varied from this target (Civille 2005). The LiDAR data was collected through a collaborative effort with the NOAA Coastal Services Center in Charleston, South Carolina, and data was acquired and compiled by photogrammetrists at Spencer B. Gross in Portland, Oregon.

To determine whether individual *Spartina* plants were growing more rapidly in different abiotic conditions, we extracted areas from polygons that overlapped one another in separate years. These were assumed to be the same individual increasing (or diminishing) in area through time. A stratified random selection of 408 of these co-occurring clones was extracted from the 1994 and 1997 layers, then statistically analyzed according to a simple relative areal growth index ***[(Area1997-Area1994)/Area1994]***. This index relates the amount of areal growth to the initial size of the plant. An ANOVA was performed on the 1994-1997 data set and significant differences in relative growth rates were observed between mud and sand substrates and tidal elevation of the clones (Fig. 1). Clones in soft mud substrates with proximity to fresh water sources exhibited significantly higher areal growth rates than those on sand. Clones at higher elevations in general are growing more quickly than those at lower elevations, although differences were not significant in all elevational classes. *Spartina* plants are noticeably absent from sand substrates lower than

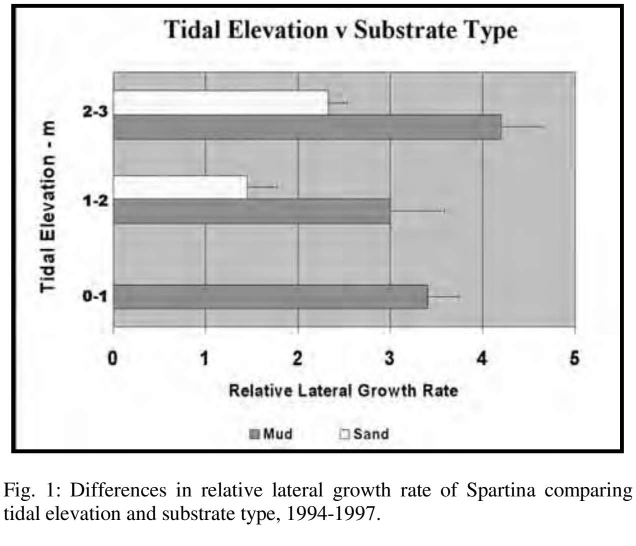

Fig. 1: Differences in relative lateral growth rate of Spartina comparing tidal elevation and substrate type, 1994-1997.

one m above MLW, although present in large numbers at these elevations on mud.

Seedling recruitment is another demographic property that is critical to the successful spread of S. *alterniflora* in Willapa Bay. New individuals observed in 1997 (polygons less than three m in diameter without earlier counterparts) out-numbered those that were present in 1994 by an order of magnitude: over 46,000 single new plants versus 4,500 of similar size in 1994. By 2000, the number of new individuals recruiting into the population rose by another order of magnitude to more than 155,550 new plants. Again, recruitment was greatest on those parts of the bay with soft mud substrates and in proximity to fresh water sources (Civille 2005). The lateral growth and merger data are providing important insights into abiotic factors that control *Spartin*a growth, and were used to populate and validate theoretical model development and exploration (Taylor et al. 2004; Taylor and Hastings 2004).

The high resolution spatial datasets have provided demographic and ecological information at a level of detail and bay-wide scale that could never have been collected by individual ecologists with transects and sampling hoops across such difficult and remote terrain. The photographic record that has been maintained and cataloged provides unique opportunities to look into the history of this invasion, and may provide insight into the mechanisms and characteristics of other invasions.

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IMPLICATIONS OF VARIABLE RECRUITMENT FOR THE MANAGEMENT OF *SPARTINA ALTERNIFLORA* IN WILLAPA BAY, WASHINGTON

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The spatial expansion of *Spartina alterniflora* populations in Willapa Bay, Washington has been driven primarily by seedling recruitment. We measured this recruitment at the landscape scale using a series of precision GPS guided airboat censuses. Recruitment is highly variable across both space and time. Over the four years of observation, yearly average estuary-wide recruitment varied from a low of 12 per hectare (ha) to a high of 500/ha. Variability across individual census plots was even greater, ranging from 0 per ha to over 4000/ha. Several factors influence this variability. 1) Local recruitment tracks variation in local seed production. 2) Tidal elevation and hydrological conditions influence the spatial pattern of seed deposition and retention, and this is reflected in micro-spatial recruitment patterns. 3) Substrate characteristics independent of elevation and hydrology significantly influence seedling survivorship and growth. These results have broad implications for the management of invasive *Spartina* populations. Poor recruitment years afford windows of opportunity for effective control, but could equally lead to complacency and an inadequate response during recruitment pulses. The strong relationship between local seed production and local recruitment suggests that control strategies that do not explicitly account for inter-regional dispersal can still be successful over the short-term, even if long-term management requires an explicit understanding of regional dispersal pathways. Finally, the strong differences in recruitment and growth patterns caused by substrate differences may allow the categorization of sites by recruitment risk.

Keywords: heterogeneity**,** invasion, spread, wetland

INTRODUCTION

Spartina alterniflora has spread over 60 square kilometers (km^2) of the approximately 190 km^2 of intertidal habitat in Willapa Bay, Washington (Civille et al., 2005; Civille et al. 2010). This large-scale invasion threatens to disrupt many important ecosystem services such as habitat for oysters and migratory birds, nutrient cycling, and storm water runoff control (Sayce 1988). A consortium of local residents, state agencies and the Federal government is currently attempting to manage the invasion with the ultimate goal of eradicating *S. alterniflora* from the estuary.

There have been few attempts to comprehensively manage such a large plant invasion. Property ownership, funding allocations, and the idiosyncratic interests of stakeholders usually force management to occur on an ad hoc and project-specific basis. However, given the large extent of many invasions there is a growing appreciation for the need to manage invasive plants at explicitly landscape scales (With 2002).

The management of large-scale invasions takes time. Some invasions will likely require ongoing management, while the eradication of others will take years. In the case of *S. alterniflora* in Willapa Bay, coordinated control activities began over 10 years ago.

The effective management of landscape-scale invasions, therefore, requires an understanding of how basic properties of invasions vary across space and time. Here we report that two basic components of *S. alterniflora* demography, seed production and seedling recruitment, vary considerably across different habitats in the bay and across years. We discuss the consequences of this variability for the management of *S. alterniflora* in Willapa Bay.

METHODS

Seedling Recruitment

To test the degree of spatial and temporal variation in seedling recruitment we established 44 permanent plots on tidal mudflat that was free of *S. alterniflora*. Plots covered the full spatial extent of the estuary and spanned a range of substrate characteristics, distance to established *S. alterniflora*, and tidal elevation. Plots were 15 m wide and varied in length depending on local site characteristics. We recorded the location of each plot using GPS equipment (Trimble Pathfinder Pro XRS). Beginning in June 2001 we

Fig. 1. Seedling census using airboat and GPS guided navigation.

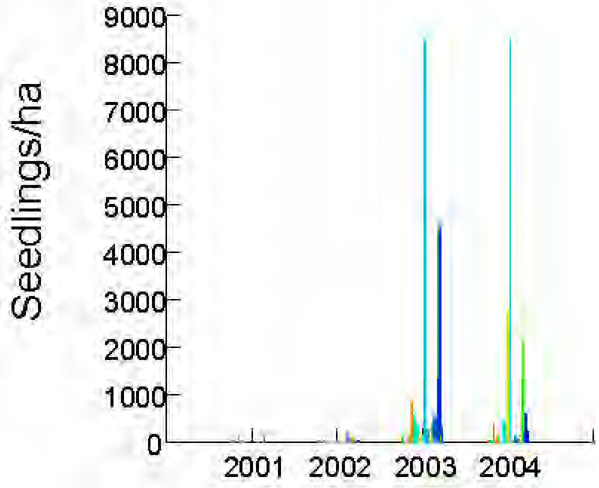

Fig. 2. Recruitment of *S. alterniflora* seedlings across 44 permanent plots in Willapa Bay, Washington.

used airboat surveys to visually census each plot for new seedling recruits (Fig. 1). Using the GPS as a navigational guide we traversed the borders of each plot in the airboat. A team of three would count the total number of seedlings observed during each airboat pass. We used average counts of the three observers as the estimate of the number of seedlings per plot. Seedling counts among observers never varied more than 10%, and usually were within one or two seedlings of each other. We conducted yearly censuses each June from 2001-2004.

Seed Production

To test for variability in seed production, we established 30 50 m x 50 m permanent plots in established *S. alterniflora* meadows across the full spatial extent of the invasion. Plots spanned a range of meadow ages. Beginning in the fall of 2002 we collected 15 inflorescences each from 10 individual clones within the permanent plots. We also estimated *S. alterniflora* cover and inflorescence density within each plot. We placed the inflorescences in wet, cold (10^{O}C) storage to break dormancy. For each clone we calculated the germination rate per inflorescence. Using these values and the estimates of inflorescence density at each site we calculated an estimate of mean viable seed production per square meter (m^2) at each site.

RESULTS

Recruitment is episodic

Over the course of the study, the yearly variation in seedling recruitment was dramatically episodic (Fig. 2). Yearly average estuary-wide recruitment varied from a low of 12/ha to a high of 500/ha. Site-specific yearly variation was even greater. For instance, recruitment at the Tarlatt Slough site was 82 seedlings/ha in 2002 and 4,509 seedlings/ha in 2003.

Recruitment varies with substrate

The spatial variation in recruitment was equally variable. Within a given year, recruitment density across sites ranged from 0/ha to over 4,000/ha. One factor contributing to this variation was substrate type. Muddy sites had more than five times greater recruitment than sandy or mixed sites (Fig. 3). Most potential sites for *Spartina* colonization within Willapa Bay are sheltered from strong wave energy (maximum fetch less than 1 kilometer [km]). Most of the sandy sites in this study are associated with regions of high tidal energy such as channel edges. A few sites were located in relatively high fetch areas (approximately 4 km) or on bars near the mouths of rivers.

Control influences local recruitment

Meadows that received control treatments in 2003 had significantly reduced seed production relative to sites that

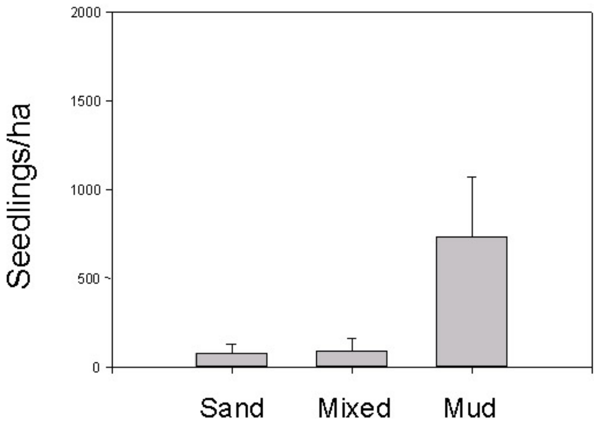

Fig. 3. Recruitment of *S. alterniflora* seedlings in plots varying in substrate texture. Values are means ± 1 S.E.

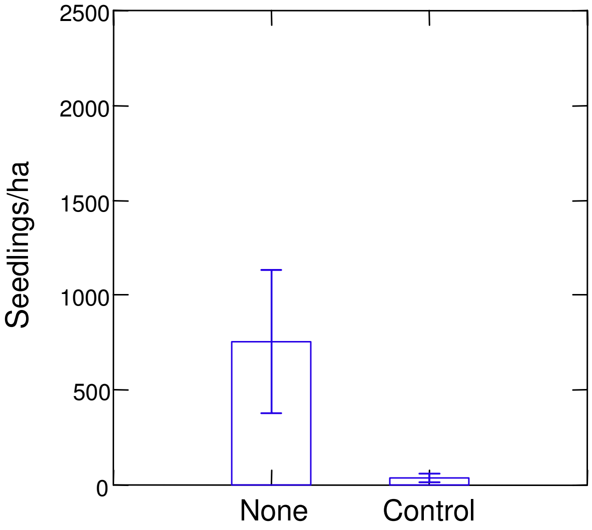

Fig. 4. The influence of control efforts in 2003 on local seedling recruitment in 2004. Control methods included a variety of mechanical methods and herbicide application. Values are means ± 1 S.E.

did not receive control. The local reduction in seed production influenced local (sub-region) seed recruitment the following year (Fig. 4). Areas such as the Palix and Cedar rivers that did not receive control in 2003 had high seed production that fall and correspondingly high seedling recruitment the following year. In contrast, sites that received control in 2003 such as Tarlatt Slough and Diamond Point experienced low rates of seed production that fall and had correspondingly low recruitment the following year.

DISCUSSION

Causes of variability

Spartina alterniflora recruitment in Willapa Bay is highly variable across years and across sub-regions of the bay. The yearly variation observed in this study corroborates longer-term data from historical aerial photographs suggesting that there have been strong episodic bursts of *S. alterniflora* colonization (Civille et al. 2005). A significant component of this yearly variation in recruitment appears to be directly related to levels of seed production the previous year. For the limited two years that we are able to make comparisons, the average estuary-wide recruitment tracks average estuary-wide seed production the previous year. In addition, there is evidence that *S. alterniflora* seed set is significantly influenced by pollen availability, and that pollen availability is significantly reduced by wet and cool conditions (Davis et al. 2004, and this volume). Yearly average bay-wide recruitment is positively correlated with the number of August degree days (the cumulative number of growing degree days in August) and negatively correlated with total August precipitation in the previous year (Lambrinos, unpublished data).

Substrate characteristics appear to play an important role in the site-to-site variability in recruitment. *Spartina alterniflora* seedlings are far less likely to establish at sandy sites than at muddy sites. The mechanisms generating this pattern are still unclear. Spartina seeds may be less frequently deposited at more erosive sandy sites. In river systems seed and sediment deposition are closely related (Goodson et al. 2003). In addition, seedlings may be less likely to establish at sandy sites because of higher erosion and physical stress. Alternatively, seedlings could perform poorly in sandy sediments because of nutritional limitations. Increasing sediment sand corresponds with decreasing sediment nitrogen (N) content at these sites (Tyler et al. 2007). Evidence from transplant experiments and common garden experiments indicate that *S. alterniflora* seedlings grow poorly on sand relative to mud irrespective of the ambient hydrological conditions (Lambrinos and Bando 2008).

Undoubtedly, another factor in the site-to-site variability in recruitment is seed supply. *Spartina alterniflora* can potentially disperse long distances (Howard and Sytsma 2010). However, the fact that regions that received control in 2003 had correspondingly reduced recruitment levels in 2004, while no reductions in recruitment occurred in regions that did not receive control suggests that a sizeable portion of recruitment is derived from local seed sources.

Implications for management

The strongly episodic and spatially variable nature of *S. alterniflora* recruitment in Willapa Bay has important implications for management. Years of poor recruitment have probably slowed the rate of the invasion in Willapa Bay. Both vegetative growth and recruitment by seed contribute to the spatial expansion of the invasion. However, most of the expansion in the bay has occurred through seedling recruitment and subsequent growth of clones, not from the lateral growth of established meadows (Civille et al. 2005). With moderate and low annual budgets, the optimal control strategy in Willapa Bay is to target nascent populations (Taylor and Hastings 2004; Taylor et al., this volume). However, a "clone only" strategy may be incompatible with other management objectives such as restoring habitat for migratory birds. Periods of low recruitment may provide opportunities to target meadows with little reduction in control effort against nascent clones. In contrast, periods of high recruitment would be poor times to allocate treatment resources to established meadows.

At the same time, periods of low recruitment may foster complacency. This is unlikely to occur with a comprehensive and long-term management program such as the one currently being implemented in Willapa Bay. However, poor recruitment years may hamper implementation of such comprehensive management plans. For instance, if initial infestations are followed by years of low recruitment there may be little impetus to expend resources on control because the spatial expansion of populations is limited. Even if control is initiated at a particular site, the need to develop a regional control strategy may not be obvious. Untreated infestations may then serve as significant seed sources during subsequent good seed production years.

The poor seedling recruitment at sandy sites compared to muddy sites has allowed the *S. alterniflora* management program in Willapa Bay to focus control efforts spatially. The sandy Long Beach Peninsula has been given a low priority for control, and control resources will only be spent here during the final stages of the control program (K. Murphy, pers. comm.). The ability to prioritize estuaries in terms of susceptibility to *S. alterniflora* recruitment is an important consideration given the limited resources usually available and the wide spatial extent of most estuaries.

The ability to spatially allocate control resources is also aided by the fact that a significant proportion of local recruitment is derived from local seed production. Control efforts in one area are unlikely to be completely swamped by recruitment coming from distant or hydrologically disjunct un-controlled areas. This suggests that *S. alterniflora* control in different sub-regions of an estuary can be managed somewhat independently (e.g., controlled sequentially) and still be successful. Long-distance dispersal is certainly not trivial, however, and long-term management will require extensive monitoring and a better understanding of dispersal pathways.

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POLLEN LIMITATION IN A WIND-POLLINATED INVASIVE GRASS, *SPARTINA ALTERNIFLORA*

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Limits to, and the consequences of, pollen availability in wind-pollinated plants have been little studied. Reproductive failure or depression because of a lack of available mates could lead to population demographic consequences for many species. Of particular interest is how pollen limitation affects the rate of spatial spread of invasive plants. We performed a manipulative pollen addition and exclusion study to investigate the role of pollen limitation in an invasive perennial estuarine grass, *Spartina alterniflora*. We found pollen impoverishment at the low density leading edge of a large invasion, causing an eight fold reduction in seed set among low density plants, though not among the high density plants. We found pollen loads on stigmas to be determined by pollen availability in the air. Furthermore, the amount of airborne pollen is dictated by the spatial pattern of plants, with much more pollen available over continuous meadows than in areas of low plant density. The delay of appreciable numbers of seed persists for decades until vegetative growth coalesces plants into continuous meadows, and this has slowed the rate of spread of the invasion.

Keywords: Allee effect, invasive species, pollen limitation, *Spartina alterniflora*

When Willapa Bay, Washington was first colonized by *Spartina alterniflora* in the 1890s, the very slow rate of spread gave little reason for concern. However, by the 1980s, there were vast areas of seedling recruitment and large meadow formation on mudflats previously unoccupied by any other species of emergent plant. In 1995, the problem had grown so daunting that the Washington State Legislature declared the *Spartina* invasion an "environmental emergency." As with many invasive organisms, *S. alterniflora* maintained an initially low profile due to a "lag time" between colonization and rapid spread (e.g., Kowarik 1995). In this paper, we examine the ultimate mechanistic cause responsible for the *Spartina* lag phase and the consequences of its cessation.

Invasive *S. alterniflora* recruits onto open Pacific mudflats as seeds drift in on the tides yearly, leaving no seedbank (Woodhouse 1979). Seedlings then grow rhizomatously into widely spaced circular clones. Eventually, these isolated clones grow together to form continuous meadows. Individuals within a low-density population, such as isolated clones of *S. alterniflora*, may suffer from depressed reproduction due to a lack of sexual partners. This syndrome, known as an Allee effect (Allee 1931), will cause populations to go extinct if the population density drops below a threshold. A less well-known manifestation is the weak, as opposed to strong, Allee effect. Under the weak Allee effect, the per capita rate of growth (the growth rate for individuals within the population) is depressed when population density is low, but never becomes negative as occurs with a strong effect. We surmise that the Allee effect in Willapa Bay *Spartina* is weak because existing individuals are perennial; while they may make very few seeds when they are isolated, they survive until population density becomes high with meadow formation and the Allee effect ends.

To find whether density has an effect upon the number of seeds a plant can produce and the viability of those seeds, we collected five inflorescences from 20 individual *S. alterniflora* plants at five different sites within Willapa Bay in the year 2000 (Long Island, Peninsula, Palix River, Shoalwaters, Porter Point). At each site we collected half the inflorescences from low density clones (all individuals isolated within bare mud) and half from high density meadows. We assessed both seed set (# seeds / # florets / inflorescence) and germination percentage (# germinations / # florets / inflorescence). We found that considerably fewer isolated clones (37%) than meadow plants (92%) could set any seed at all. Furthermore, for the plants that could set at least some seed, the meadow plants had a much higher proportion of seeds (0.30) than the isolated clones (0.08) (Davis et al. 2004a). Finally, the seeds of the isolated clones were much less viable with a proportion of 0.13 germinating as opposed to a proportion of 0.34 of the meadow plants' seeds germinating. There was no obvious effect of proximity of neighboring clones on isolated individuals. While some low density areas were more crowded than others, there was no obvious trend towards relatively higher or lower seed set as long as the clones were not touching one another. This suggests that the clones need to merge together before the step-wise increase in seed set is achieved by meadow individuals. This work is reported in full in Davis *et al.* (2004a).

We then investigated whether pollen limitation could be the mechanism behind the Allee effect in Willapa Bay *Spartina*. While this may appear to be an intuitive hypothesis, pollen limitation is generally assumed not to occur in wind-pollinated plants, such as grasses, conifers and oak trees. There are many studies of pollen limitation in animal-pollinated plants (Burd 1994; Larson & Barrett 2000), very few of wind-pollinated plants, and none before this one of wind-pollinated invasive plants. Pollen limitation in animal vectored plants can diminish reproduction and constrain invasion success (Barthell et al. 2001; Parker 1997). Using simulation and analytic models, we asked whether the Allee effect in Willapa Bay *Spartina* could have slowed the rate of invasion and found it did produce a lag time (Taylor et al. *these proceedings*; Taylor et al. 2004).

We conducted an observational and manipulative study to assess the role of pollen limitation in the ability of Willapa Bay *S. alterniflora* to set seed. To see if there is a difference in pollen deposition rates on stigmas, we collected stigmas from isolated clones and high density meadows over three different sites (Cedar River n = 20; Bone River n = 40; Palix River n = 94) and screened one lobe (half) of stigmas for pollen load. We found that meadow plants' stigmas captured nine times the pollen that the isolated clones' did. Isolated plants on average had less than one pollen grain per stigma lobe while meadow plants had more than six. Although plants at individual sites differed in their pollen loads, in every case, the meadow plants always had more (Davis et al. 2004b). Interestingly, the Cedar River collections had very little pollen with even meadow plants having on average less than one pollen grain per stigma lobe, probably because it rained on the day of collection and the previous two days suggesting pollen flow is inhibited on wet days and possibly rainy years.

To find how pollen deposition changes over a windward-to-leeward gradient and to see if the amount of pollen in the air is correlated with the amount of pollen deposited on stigmas, we set out pollen traps and collected adjacent stigmas. We set 10 traps along each of six transects. Oriented along the direction of the prevailing wind: two in windward isolated clones (I & II), one along the windward meadow edge (III), one in the windward meadow (IV), one in the leeward meadow (V) and one 100 m from the leeward side of the meadow across an unvegetated channel (VI) (Davis et al. 2004). We found extremely high correlation between the pollen loads on the traps and stigmas ($r = 0.99$) supporting the hypothesis that the amount of pollen on stigmas is controlled by pollen abundance on the wind. On the pollen traps, we found very little pollen anywhere in the isolated clones or along the meadow edge. Pollen loads increased inside the windward end of the meadow and increased yet more dramatically at the leeward end of the meadow. However, the amount of pollen dropped precipitously across the unvegetated channel suggesting that the gap was too far for nearly all of the airborne pollen to cross (Davis et al. 2004).

To conclusively determine whether isolated clones are making fewer seeds than the meadow plants because of pollen scarcity, we performed a manipulative experiment by supplementing and excluding pollen received by inflorescences and leaving control inflorescences open to ambient pollen availability. Twenty-four plants received all treatments and a further 20 plants received pollen addition and control treatments only. We found the pollen exclusion treatment reduced seed set in the meadow plants by more than sixfold, but caused no reduction in the isolated clones. The pollen addition treatment had no effect on the seed set of the meadow plants, but did raise that of the isolated clones by more than threefold. We were not able to saturate the plants' inflorescences with pollen, as an inflorescence has receptive stigmas for about 10 days (~3 days per stigma) and we were able to supplement on only three consecutive days. This is likely the reason why the isolated clones' pollen addition seed set was not as high as the meadow plants' ambient control (Davis et al. 2004). These results indicate that isolated clones, colonists at low density, are extremely pollen-limited whereas seed set in the high density meadow is not limited by pollen. This work is reported in full in Davis et al. (2004b). We found that an Allee effect contributed to the lag time in spatial spread of *S. alterniflora* in Willapa Bay. Furthermore, this Allee effect appears to be the result of pollen limitation of the colonizing clones at the leading edge of the invasion. In the studies outlined here, and also in two years of unpublished data, we consistently found that isolated individuals set very few seeds. However, these studies were done in the single location of Willapa Bay, so similar results may or may not be duplicated in other locations. We found a wide range in self-compatibility of *Spartina alterniflora* from the native range (Davis 2005), so the ability of isolated plants to use their own seeds in different locations may be largely influenced by the source of the colonizing individuals. It would be advisable for managers of other invaded areas to confirm the lack of seeds in isolated *Spartina alterniflora* before discounting their potential as significant seed sources.

Lag times offer a transient opportunity for management of invasive species. However, lag times can induce a false sense of security about the threat from exotic plants so they often go unnoticed and uncontrolled until the invader begins to spread aggressively (Parker 2004). These studies raise the possibility of pollen limitation in other wind-pollinated species. It is an open question whether this is a common phenomenon, or whether it is more likely to be found among populations that are actively declining or expanding.

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INVASIVE HYBRID CORDGRASS (*SPARTINA ALTERNIFLORA X FOLIOSA*) RECRUITMENT DYNAMICS IN OPEN MUDFLATS OF SAN FRANCISCO BAY

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Hybrid *Spartina* are currently expanding their range in the San Francisco Bay (SFB) at a rate exceeding exponential growth. A subset of transgressive hybrid *Spartina* plants that positively exceed the fitness trait values of their parents are competitively and reproductively superior to both parents and other hybrids and likely drive the invasion. In order to colonize the vast open SFB mudflats and found new populations hybrid cordgrass plants have to evolve self-compatibility and exhibit rapid vegetative and lateral growth. The mudflat tidal cycle covers or exposes plants for up to six hours, so new seedlings have to be robust and fast growing to survive and establish. A small number of hybrid and native *Spartina* have colonized the open mudflats along the eastern shore of SFB. To discern mudflat seedling recruitment dynamics we investigated (1) the numbers and locations of recruiting seedlings at three SFB sites in 2003 and 2004 via GPS/GIS, and (2) the genetic relationship of established adult plants and parentage of seedlings using microsatellite markers. Our results identify all sampled seedlings as hybrids, and show a dramatic increase in seedling recruitment numbers in 2004. Molecular investigations reveal surrounding, inter-related adult plants as the most likely sires for most seedlings, and also show evidence of increased self-fertilization in isolated plants. We found seedling recruitment to be spatially heterogeneous along shorelines, with local pockets of recruitment at invaded sites and highly local aggregations of seedlings in proximity to their seed (and pollen) parents. These results give support to transgressive hybrid plants as the drivers of the invasion.

Keywords: Hybrid *Spartina*, seedling recruitment, self-compatibility

INTRODUCTION

In light of the accelerating spread of *Spartina* hybrids in SFB (Ayres et al. 2004) we propose that highly invasive individuals at the forefront of the invasion possess extreme phenotypes for traits important in hybrid cordgrass survival and spread. We posit these traits are i) self-compatibility, which enables single individuals to found new populations; ii) height, which confers the ability to grow low on the open mud of the intertidal plane; iii) lateral spread, which anchors plants into the shifting substrate and allows colonization of occupied or unoccupied neighboring patches; iv) tolerance to high (40 parts per trillion (ppt)) salinity which allows plants to growth higher on the intertidal plane in the range of *Salicornia virginica*, and v) the timing and abundance of flowering, which is important in siring ability on early flowering native *S. foliosa* and in seed production (Ayres et al. 2008). These traits cause the selective superiority of those individuals that possess all or most of them with regard to other hybrids and both parent species, and we propose that these hybrid individuals will drive the invasion of SFB open mudflats. We have found that *Spartina* hybrids show highly variable self-compatibility, while both hybrid parent species (*S. alterniflora* and *S. foliosa)* are marginally self-compatible (H. Davis and D. Ayres, unpublished data) (Fig. 1). Hybrid individuals thus greatly differ in their ability to successfully set self-fertilized seed, and certain hybrid plants are transgressive, exceeding both parent species and other hybrids in their ability to self-fertilize (Fig. 1).

RESULTS AND DISCUSSION

We performed GPS surveys in 2003 and 2004 at three sites along the eastern shores of SFB (Elsie Roemer Marsh, Robert's Landing, Hayward Shoreline) and found a dramatic increase in seedling establishment from 2003-2004 (Fig. 2). At Hayward shoreline all genetically surveyed seedlings were hybrids. Overlapping GPS points and the relatively larger size of some individuals at Hayward suggest some seedling survival from 2003 to 2004 (Fig. 2). Microsatellite parentage analysis (Sloop et al. 2005; Blum et al. 2004; Gerber et al. 2003) of a sub-sample of GPS-surveyed seedlings and surrounding plants suggests that seedlings originate most likely from local seed sources, rather than from seeds swept in by the tides (Sloop et al. 2009). Our parentage data show a large likelihood of establishing seedlings as the progeny of the surrounding clones. In 2003 38% of all establishing seedlings were most likely self-fertilized at Hayward Shoreline. Microsatellite analysis also revealed that at Robert's Landing the majority of 2003 seeds collected from isolated hybrid plants were self-fertilized.

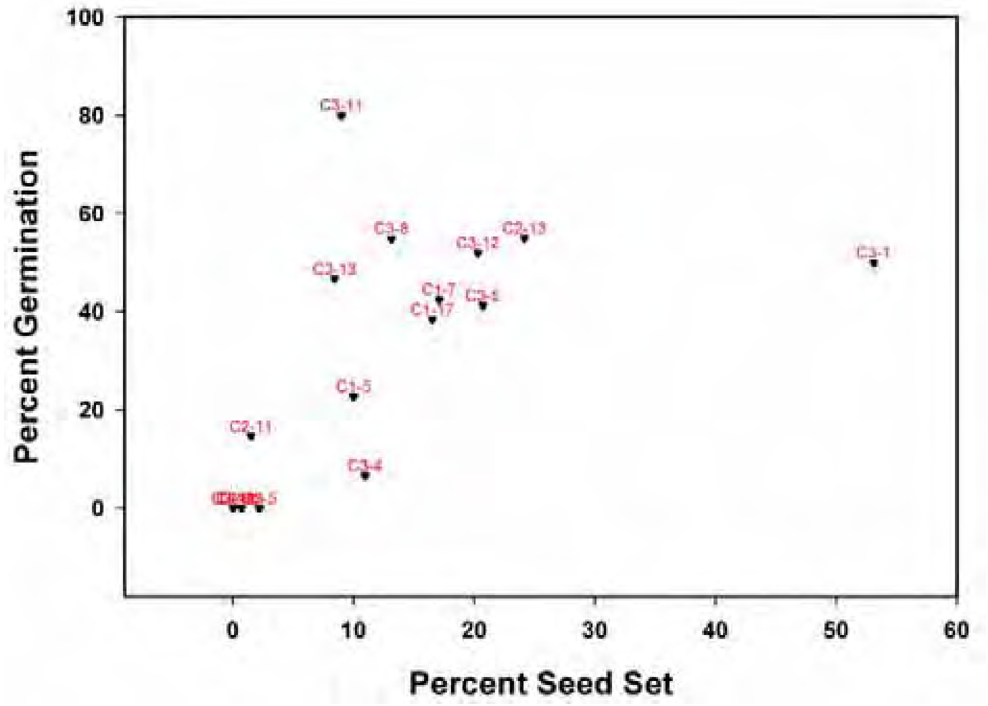

Fig.1: Percent seed set vs. % germination of self-fertilized hybrid seeds from Cogswell Marsh, Hayward. Average germination/seed set rates for self-fertilized *S. foliosa* and *S. alterniflora* are 25%/12% and 45%/25% respectively. Hybrid C3-1 represents a transgressive individual exceeding both parents and other hybrids in germination and seed set of self-fertilized seed.

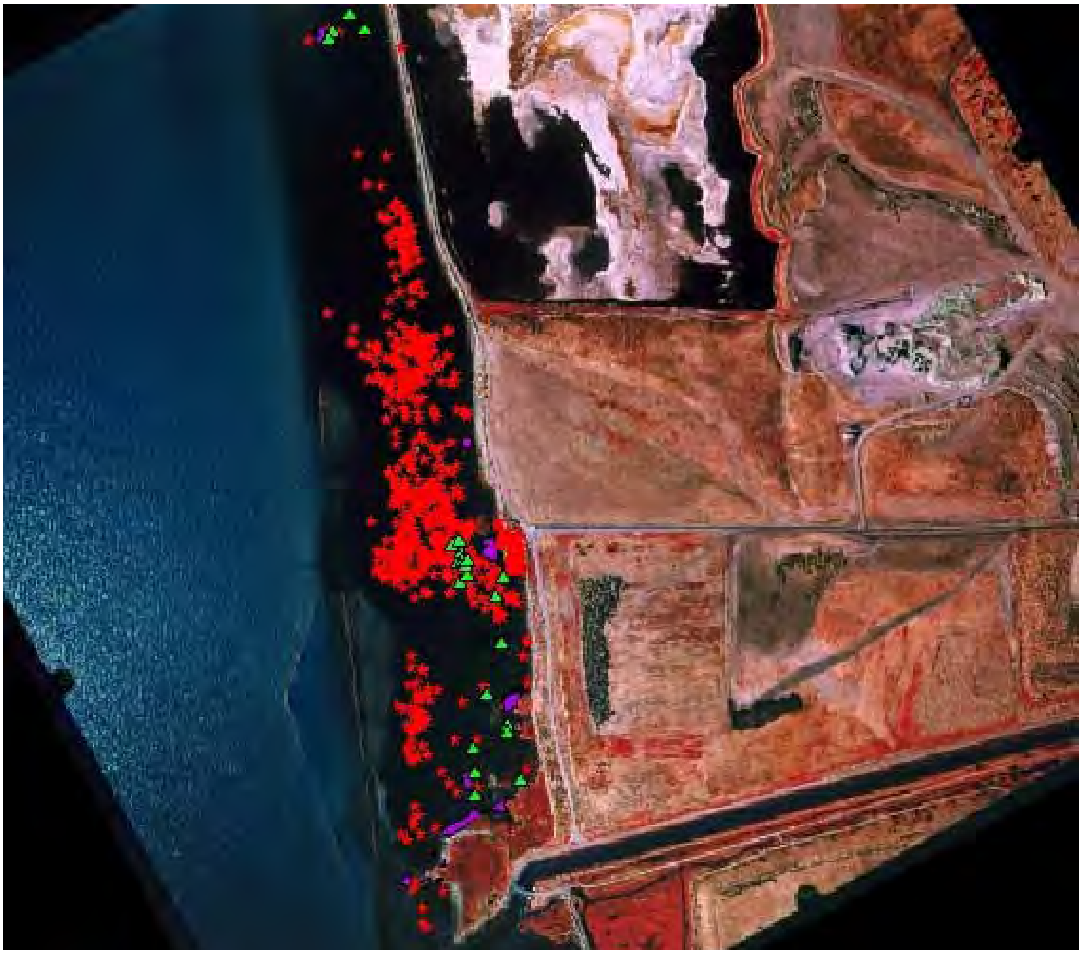

Fig. 2: Temporal variation in seedling recruitment at Hayward shoreline. Distribution of seedlings in Spring 2004 (stars) increased >500-fold from Spring 2003 (triangles). Overlap of stars and triangles suggests survival of 2003 seedlings.

Spartina hybrid seedling occurrence is highest at invaded mudflat sites. While recruitment is mainly local, seed exportation on the tide will likely intensify as populations grow and expand. We predict that transgressive hybrid plants, with the greatest reproductive fitness will drive the spread, colonizing new open mudflat sites all around SFB at an ever-increasing rate. This will not only further threaten the persistence of the native California cordgrass *S. foliosa* (Ayres et al. 2003), but will likely irreversibly change the character of the SFB ecosystem. Open mudflats will turn into hybrid *Spartina* meadows, which over time, after the accretion of sediment, will change from intertidal to terrestrial systems.

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THE INFLUENCE OF INTERTIDAL ZONE AND NATIVE VEGETATION ON THE SURVIVAL AND GROWTH OF *SPARTINA ANGLICA* IN NORTHERN PUGET SOUND, WA, USA

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Spartina anglica colonizes mudflats, tidal channels, salt marshes, and gravel beaches in northern Puget Sound, Washington, USA. We measured *Spartina* seedling survival and growth along an intertidal gradient from mudflat to salt marsh at Alice Bay, Skagit County, Washington. *Spartina* seedlings were transplanted into plots with full and reduced competition in open mudflat, a low marsh intertidal zone dominated by *Salicornia virginica*, and a middle marsh zone dominated by *Distichlis spicata*. In the mudflat, where little native vegetation was present, two separate treatments were monitored: unmanipulated (*in situ*) seedlings and seedlings transplanted within the mudflat. The *in situ* seedlings had 100% survival whereas seedlings transplanted within the mudflat had 60% survival. The mean relative growth rate (RGR) of *in situ* seedlings in the mudflat was 16 milligrams per milligram per day (mg mg^{-1} day^{-1}) compared to RGR of 8 mg mg^{-1} day^{-1} for the transplanted seedlings. In the *Salicornia virginica* zone, *Spartina* survival was 94% and RGR averaged 13 mg mg^{-1} day^{-1} with reduced competition. *Spartina* seedling survival was 90% and mean RGR was 11 mg mg^{-1} day^{-1} when grown in full competition with *Salicornia*. In the *Distichlis* zone, *Spartina* survival was 57% and the RGR was 5 mg mg^{-1} day^{-1} without competition. *Spartina* seedling survivorship was zero when grown in competition with *Distichlis.* In general, *Spartina* survival and RGR were highest at lower intertidal elevations and with reduced competition. The spread of *S. anglica* into upper intertidal levels appears limited by competition as well as physical conditions along the intertidal gradient.

Keywords: competition**,** *Distichlis spicata, Salicornia virginica, Spartina anglica*, zonation

INTRODUCTION

Vegetation zonation in salt marshes is determined by both competitive (Bertness 1991a; Pennings and Callaway 1992) and facilitative (Bertness and Shumway 1993, Bertness and Hacker 1994; Callaway and Pennings 2000) biotic interactions as well as physical conditions including soil waterlogging, oxygen availability (Howes et al. 1981), sulfide toxicity (King et al. 1982) and porewater salinity (Callaway et al. 1990, Pennings and Callaway 1992). In northwestern Washington, salt marsh communities often form a fringe of vegetation along the upper edges of mudflats. These sites have high porewater salinities (>30 grams per kilogram [g kg^{-1}) and are typically isolated from riverine freshwater. The lowest intertidal zones in these marshes are typically colonized by mats of *Salicornia virginica* with intermittent patches of *Spergularia canadensis*, *Triglochin maritimum*, and *Puccinellia maritima*. Frequently, individuals of these species will colonize the open mudflat several meters off the seaward edge of contiguous salt marsh.

The introduction of *Spartina anglica* into northwestern Washington has altered the zonation of salt marsh vegetation by creating a community of emergent vegetation below the *Salicornia virginica* zone on sediment that was previously open mudflat. Throughout northern Puget Sound, *Spartina* can form dense stands in areas that were formerly open mud flats (e.g. Triangle Cove and Livingston Bay, Island County). However, *Spartina* is only found sporadically growing among native plants in higher intertidal zones (e.g. at Maylor Marsh and English Boom, Island County).

Based on the tendency of *Spartina* to colonize open mudflats as opposed to closed mats of native vegetation, we hypothesized that *Spartina* is an inferior competitor and that survival and growth rates would be highest in the lowest intertidal zones and in experimental plots where competition from native plants was limited.

MATERIALS AND METHODS

We conducted a seedling transplant experiment in four locations at Alice Bay (48º 33’ 20” N, 122º 29’11” W; Skagit County). A series of 10 random plots were placed along 30-meter (m) transects in the mudflat. We also monitored the growth of 10 naturally occurring, unmanipulated (*in situ*) seedlings in the mudflat to control for the effects of transplanting. Ten paired plots were

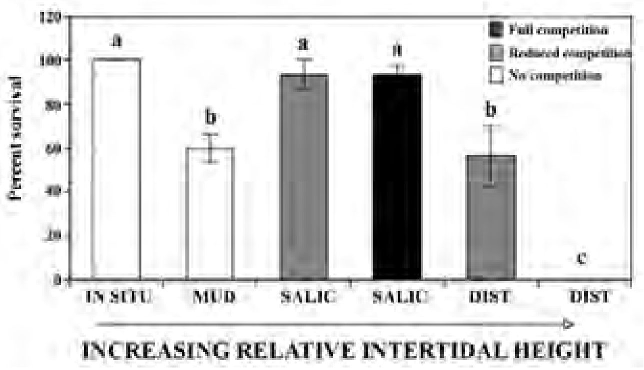

Fig. 1. Percent survival (mean±SE; n=10 plots) of *Spartina* seedlings planted within each intertidal zone. IN SITU: unmanipulated mudflat seedlings; MUD: transplanted mudflat seedlings; SALIC: *Salicornia* zone; DIST: *Distichlis* zone. Different letters indicate significant differences ($p < 0.05$) between treatment groups using Tukey's multiple comparisons test. Due to the lack of variation in the IN SITU and the *Distichlis* full competition plots, these treatments could not be compared using ANOVA. Instead, comparisons of these treatments were made using 95% confidence intervals.

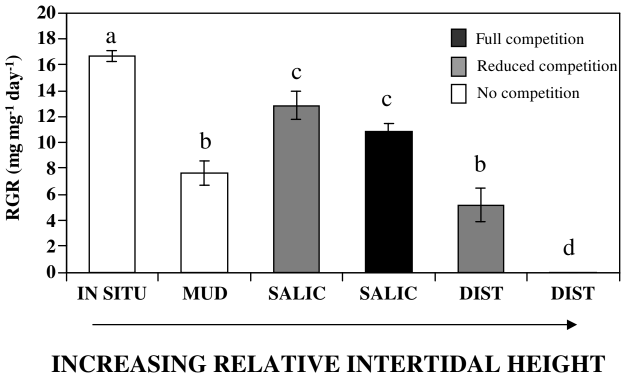

Fig. 2. *Spartina* seedling RGR (mean±SE; n=10 plots) across intertidal zones (abbreviations as in Figure 1). Different letters indicate significant differences ($p < 0.05$) between treatment groups using Tukey's multiple comparisons test. Due to a lack of variance, the *Distichlis* full competition treatment was omitted from the ANOVA and comparisons were made with 95% confidence intervals.

established in the *Salicornia virginica*-dominated salt marsh zone immediately adjacent to the mudflat. Ten paired plots also were placed in the *Distichlis spicata*-dominated zone located immediately above the *Salicornia* zone. Plots were randomly placed along 30 m transects in near monocultures of *Salicornia* and *Distichlis*. In the *Salicornia* and *Distichlis* zones each plot consisted of a subplot with native vegetation intact (full competition) and native vegation removed (reduced competition). In June 2001, three seedlings were transplanted into each plot or subplot within each zone. Seedlings were watered immediately after being transplanted and were tagged for identification. Reduced competition plots were cleared of all native vegetation (ca. 0.25 m diameter) and maintained throughout the study.

In late September-early October 2002, each *Spartina* plant was harvested from the experimental plots. Plants were transported to the lab, washed repeatedly, dried for 72 hours at 70ºC, and weighed. Biomass within each subplot was averaged for the three replicate seedlings in each subplot. Relative growth rates (RGR) of *Spartina* were calculated for all competition treatments at each intertidal level (Eqn. 1; Hunt 1978)

$$RGR = lnW_2 - lnW_1 / T_2 - T_1 \qquad \textit{Eqn. 1}$$

where W_1 and W_2 are the plant weights at times 1 (T_1) and 2 (T_2). Initial weight (W_1) was estimated using a regression of tiller number in relation to dried seedling biomass (Hellquist 2005).

Survival data were transformed using the arcsin square root transformation. Survival and RGR data were analyzed using analysis of variance in the mixed model procedure of SAS 9.1 (SAS Institute 2000). Mixed models are appropriate when random factors (split plots) are present in an experimental design (SAS Institute 2000). The Satterthwaite adjustment was applied to compensate for heterogeneity of variances. Unless otherwise noted (see Figs. 1 and 2), Tukey's pairwise comparisons were made ($p < 0.05$) across treatments.

RESULTS

All seedlings survived over the course of the experiment within the unmanipulated, *in situ* mudflat treatment. Seedlings transplanted within the mudflat had a 40% decrease in survival (Fig.1). Percent survival was high (> 90%) and not different between the full and reduced competition plots in the *Salicornia* zone. However, all seedlings died with full competition in the *Distichlis* zone. Fifty percent of the seedlings survived in reduced competition subplots within the *Distichlis* zone. Survival in the *Salicornia* zone was higher than in both *Distichlis* treatments (Fig. 1).

The RGR of *Spartina anglica* generally decreased moving from the low *Salicornia*-dominated zone to the higher *Distichlis*-dominated zone (Fig. 2). The unmanipulated, *in situ* plants had the greatest RGR. The mudflat *in situ* seedlings had a growth rate over two times greater than the seedlings transplanted within the mudflat. In the *Salicornia* zone there were no differences in RGR between full competition and reduced competition subplots. *Distichlis* zone seedlings grew slower than within the *in situ* and *Salicornia* treatments. The lowest RGRs were in the *Distichlis* zone and in the transplanted mudflat seedlings (Fig. 2).

DISCUSSION

The high RGR of seedlings on mudflats corresponds to similar findings by Dethier and Hacker (2005). Transplanting seedlings clearly reduced survivorship and RGR of *Spartina* seedlings on the mudflat where there was no competition from native vegetation (Fig. 1). Dislodging the roots during transplantation in the mudflat made seedlings vulnerable to uprooting via tidal disturbance. Tidal uprooting can be an important source of *S. anglica* seedling mortality in open mudflats (Groenendijk 1986). In this study only transplanted seedlings in the mudflat experienced mortality that could be attributed to tidal disturbance. Seedling survival did not differ between competition treatments in the *Salicornia* zone, but was greatly reduced by full competition in the *Distichlis* zone. Reduced survival in the *Distichlis* zone appeared to be due to competition with native vegetation as well as rodent herbivory. The apparent asymmetric affect of *Salicornia* and *Distichlis* on *Spartina* seedlings may be the result of interacting substrate conditions in the lower intertidal zones that are favorable to *Spartina* growth.

Although *Spartina* survival in the *Salicornia* treatments was not different, *Spartina* tiller production and total biomass were reduced in the presence of *Salicornia* (Hellquist 2005). Decreased survival and RGR by *Spartina anglica* when grown in competition with native vegetation (e.g. *Distichlis*) is consistent with previous studies of *Spartina*. In greenhouse and field studies, *Puccinellia maritima* has been shown to have a competitive effect on *S. anglica* (Scholten and Rozema 1990; Thompson et al. 1993; Huckle et al. 2000). Competitive suppression by neighboring vegetation also has been documented for *S. alterniflora* in the northeastern United States (Bertness 1991b) and *S. maritima* in Spain (Castellanos et al. 1994)

Spartina anglica colonizes a variety of habitats including mudflats, low and high salinity salt marshes, as well as cobble and gravel beaches in Puget Sound (Hacker et al. 2001). At Alice Bay, a mudflat with abundant *Spartina* adjoins a protected salt marsh that is truncated by a dike. *Spartina* is a sporadic colonizer among *Salicornia,* but was absent from the virtual monospecific stands of *Distichlis spicata* higher along the intertidal gradient. Our experiment shows that at Alice Bay, in the absence of competitors, *Spartina* is capable of surviving along the entire salt marsh gradient. In high salinity salt marshes *Spartina* may be restricted to lower intertidal zones such as the *Salicornia* zone due to interspecific competition and a lack of available open substrate (Hacker et al. 2001). Although not tested here, propagule pressure also can play a role in species invasions (e.g. Von Holle and Simberloff 2005) and may influence the distribution of *Spartina* across intertidal zones.

Although these data and additional growth parameter data (Hellquist 2005) indicate that biotic factors play a role in the distribution of *Spartina* in high salinity marshes (defined as > 29 g kg^{-1} by Hacker et al. 2001) the importance of abiotic factors such as tidal uprooting (Groenendijk 1986) and soil physical characteristics also contribute to *Spartina* colonization (Hacker et al. 2001; Dethier and Hacker 2005; Hellquist 2005). Physical factors have been shown to be important in controlling seed germination of *S. anglica* in Puget Sound (Dethier and Hacker 2005). Physical factors may play a more important role during seed germination and initial establishment (Dethier and Hacker 2005), but biotic interactions may become more important during seedling maturation (Hellquist 2005)

An understanding of the physical and biotic factors responsible for plant zonation in salt marshes is necessary to better identify anthropogenic pressures that threaten salt marshes and to establish effective restoration programs (Bertness and Pennings 2000). These data as well as Dethier and Hacker (2005) and Hellquist (2005), suggest that removal of *Spartina* in the mudflats will be an effective way to slow the spread of *Spartina* by controlling fast-growing populations that can serve as seed sources. Successful control in high salinity marshes is probably aided by slower invasion rates mediated by biotic and physical factors (Hacker et al. 2001; Hellquist 2005). Knowledge of *Spartina* growth patterns, the influence of physical factors, and the importance of biotic interactions will provide a valuable context to prioritize sites for *Spartina* control (Dethier and Hacker 2005; Hellquist 2005).

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WILL *SPARTINA ANGLICA* INVADE NORTHWARDS WITH CHANGING CLIMATE?

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Spartina anglica's successful invasion has depended on its ability to occupy mudflats at lower elevations than existing saltmarsh vegetation but has slowed, with dieback in the south and successional replacement in the north. The elevational niche of *S. anglica* in the UK was shown to extend below that of its main competitor *Puccinellia maritima* by 68 centimeters (cms). The niche of the two species overlapped by 20 cms and within this zone their distribution depends on the outcome of their competitive interaction. This interaction was investigated in the light of projected climate changes and the fact that the two species utilize different photosynthetic pathways. A competition experiment under elevated levels of temperature and carbon dioxide is described and its implications for the future development of *Spartina*-dominated marshes is discussed.

Keywords: *Spartina anglica*, elevational niche, *Puccinellia maritima*, climate change, competition, C3 and C4 grasses

THE ORIGIN AND SPREAD OF *S. ANGLICA*

Spartina anglica CE Hubbard is arguably the best known example worldwide of an invasive allopolyploid species. It originated on the south coast of England some time between 1870, when the sterile F1 hybrid *S. townsendii* was first noticed, and 1892 when the first fertile specimen was collected. The allopolyploid was subsequently named as *Spartina anglica* and its spread both naturally and by deliberate planting, has been extremely well documented — Gray et al. (1991) provide a summary. The parental species, *S. maritima,* a native of the old world, and *S. alterniflora*, accidentally introduced from North America into Southampton Water, probably in ships' ballast, were in contact for a relatively short period (30-40 years?). Nevertheless the patterns of isoenzyme variation have unambiguously established the hybrid origin of *S. anglica* (Raybould et al. 1991a), and analysis of the DNA sequence of the chloroplast leucine tRNA gene intron has shown that *S. alterniflora* was the female parent in the original cross (cpDNA is maternally inherited in most grass species) (Ferris et al. 1997). This is not surprising in view of the relative frequency of the two species in the area of hybridization during the middle of the last century.

Following hybridization and chromosome doubling (neither of which events have been repeated experimentally) the hybrids and their parents appear to have been quite rapidly isolated both genetically and ecologically. *Spartina maritima* is found in the mid-level and high marsh zones of well established salt marshes and is today largely confined to the east coast of England in the counties of Essex and Suffolk (Raybould et al. 1991b). The nearest extant population to the site of origin is on the Isle of Wight, about 10 miles away. *Spartina alterniflora* has been reduced to a single clonal population in Southampton Water and is rapidly being lost as the lower zones of the salt marshes are eroded along this coast. (There are a small number of other clones of the species elsewhere but these have been deliberately introduced from known sources since the date of the original hybridization.) Thus, somewhat ironically in view of the main theme of this conference, *S. alterniflora* in the UK is regarded as a rare and threatened species of conservation interest, and its presence was recently instrumental in the refusal for permission to extend and develop the nearby port. The sterile hybrid *S. townsendii* is also largely confined to the original hybridization site, although its distribution is uncertain because it was introduced to many estuaries along with the fertile form.

By contrast *S. anglica* has colonized, or been planted in, almost all English and Welsh estuaries, is found in Scotland, and occurs in suitable habitats around the coast of Europe from 48 to 57.5° N. It has famously been introduced to many other parts of the world and in several areas has become a serious invader of native biotopes. As detailed below, *S. anglica* mostly occurs in the lowest zones of salt marshes, and at its peak occupied almost 25% of the total saltmarsh area in Britain (10,000 hectares (ha) of the 44,000 ha surveyed by Charman (1990)).

The initial rapid genetic isolation, and current ecological isolation, of the species involved in the evolution of *S. anglica* makes a fascinating contrast to the situation in San Francisco Bay, where hybridization between the native plant *S. foliosa,* and its ecological replacement, are a feature of the invasion of the non-native *S. alterniflora.*

THE CURRENT STATUS OF *S. ANGLICA* IN EUROPE

Invasions of European mudflats by *S. anglica* (hereafter refered to simply as '*Spartina*') has been characterised by a different sequence of events at different latitudes. In the south of England, and from the southwest Netherlands southwards, large monospecific stands developed rapidly following initial introduction and have in many places been followed in the early years of the last century by 'dieback,' the swards breaking up and retreating in area. By contrast in northern England north of about 54° N and in the Dutch and German Waddensea, invading *Spartina* is replaced successionally by other species, most commonly the grass *Puccinellia maritima* (hereafter '*Puccinellia*'). For example in Morecambe Bay a 100-ha marsh formerly dominated by *Spartina* is now a *Puccinellia*-dominated sward from which *Spartina* has almost disappeared (Gray and Raybould 1997). Although confounded in some cases by latitudinal variation in sediment type (the northern marshes tend to be more sandy) this contrast between northern and southern marshes may be related to the effects of differences in climate and daylength on the two species (see below).

Although the *Spartina* invasion has not completely halted it has certainly slowed, and with dieback widespread in the south and a slow rate of colonization in the north, there is probably a net loss of area of *Spartina* marsh at the present day. Small isolated foci of recolonization may be found in the south where, in response to rising relative sea levels, sea defences have been removed to create new mudflats and salt marsh, a process termed 'managed realignment'. Active invasion by *Spartina* of such newly created intertidal mudflats can be observed at Tollesbury in Essex, a county which has lost more than 25% of its salt marsh in the last thirty years. For the moment it seems the *Spartina* invasion is 'on hold.'

INTERACTIONS BETWEEN *SPARTINA* AND *PUCCINELLIA*

The most parsimonious explanation for *Spartina*'s success as an invader is that it has been able to grow on intertidal mudflats at lower elevations than the existing perennial saltmarsh vegetation. This is undoubtedly due to a range of adaptive morphological and physiological features the net effect of which enable *Spartina* to withstand higher frequencies of tidal submergence. The 'elevational niche' of *Spartina* in Britain has been measured by surveying transects across salt marshes and recording the highest and lowest levels of the sward (in meters above the standard UK datum height) and relating this to tidal constants (Gray et al. 1991, 1995). Comparison of this niche with that of other saltmarsh species confirms that *Spartina* extends on average 68 centimeters (cm) below *Puccinellia*, the species with the next lowest elevational limit. There is also a niche overlap between the two species by 20 cm. Within this overlap zone the distribution of the two species is likely to be principally determined by interspecific competition, and Scholten and Rozema (1990) provide clear evidence of this using an elegant removal experiment. The outcome of interspecific competition was shown to be critically dependent on variation in local marsh elevation.

Measurement of *Spartina*'s elevational niche also indicated that its upper limit varies with latitude. The regression equation describing this limit (Gray et al. 1991) was:

$$\textit{Upper Limit} = 4.74 + 0.483(R) + 0.068(F) - 0.099(L)$$

where R = spring tide range (m), F = fetch in the direction of the transect (kilometers) and L = latitude (decimal degrees N). This equation, which accounted for 90.2% of the variation in the species' upper limit, shows that *Spartina* does not extend so far upshore in more northerly latitudes. A possible explanation for this is that the competitive interaction between *Spartina* and *Puccinellia* increasingly favours the latter as one goes northwards, enabling it to invade the *Spartina* zone at lower elevations.

The increasing competitive advantage of *Puccinellia* at higher latitudes may be linked to the different effects of temperature on *Spartina* and *Puccinellia*, which are revealed by differences in their seasonal growth patterns. Field measurements on a salt marsh at 52° N showed that *Puccinellia* shoot weight increased in March when air temperatures rose above 5° C, with growth peaking in June and July, whereas *Spartina* did not begin to grow until May, when temperatures reached 9° C, and reached maximum growth in October (Dunn et al. 1981). This later development and growth of *Spartina* can be related to the species' utilization of the C4 photosynthetic pathway. One of only eight C4 species in the UK flora, *Spartina* is only partially adapted to cooler climates (C4 photosynthesis, in which the first product of carbon dioxide fixation is oxaloacetate instead of phosphoglycerate as in C3 species, is most common in semi-arid tropical and subtropical regions) (Long 1983,1990).

THE EFFECTS OF CLIMATE CHANGE

The studies outlined above suggest that the northward invasion of *Spartina* is being prevented, or slowed, by the species inability to grow at low temperatures, and that, at its northern limits it is replaced by *Puccinellia* (a C3 species with a circumpolar distribution from 70° N southwards). These two pioneer saltmarsh species bioengineer vast tracts of intertidal mudflats and must be regarded as keystone species in saltmarsh development. The obvious and intriguing question is: will increases in air temperatures predicted under various climate change scenarios enable *Spartina* to invade northwards into marshes currently dominated by *Puccinellia*? Higher temperatures will both increase growth rate and extend the period of growth during the early months of the year. *Spartina* is unlikely to be affected by increased atmospheric carbon dioxide as, where water supply is not limiting, photosynthesis in C4 plants is carbon dioxide saturated. However higher atmospheric carbon dioxide is likely to increase photosynthesis, and hence growth, in C3 plants such as *Puccinellia* due to higher conversion efficiency.

The different effects of elevated temperatures and carbon dioxide on the two species were modelled by Long (1990). The model predicts the primary production of *Spartina* and *Puccinellia* to be expected in the year 2050, assuming a 3 degree rise in temperature and a doubling of atmospheric carbon dioxide. It was validated by comparing the predictions of production under 1978 conditions with empirical field data. The increase in annual net production of *Spartina* from 1.3 kilograms per square meter (kg/m^2) in 1978 to 2.1 kg/m^2 in 2050 was largely attributable to temperature-driven increases in leaf area, enabling the point where the leaf area index is sufficient to intercept 30% of the incoming solar radiation to be reached 50 days earlier. The increase in *Puccinellia*, from 1.4 kg/m^2 in 1978 to 2.5 kg/m^2 in 2050, was largely attributed to higher conversion efficiency in a high carbon dioxide environment but the model indicated that *Puccinellia* would gain from higher temperatures in spring and autumn.

Long's model, as he acknowledges, excludes several factors which may change with climate and will impact on plant growth. These include salinity, nutrient availability and water level. Also excluded is a consideration of the competitive interactions between the two species under changing conditions.

A COMPETITION EXPERIMENT BETWEEN THE TWO SPECIES

In order to gain insight into how changing climate may affect the distribution of *Spartina* and *Puccinellia* the two species were grown in a competition experiment under controlled conditions of carbon dioxide and temperature. The experiment and full results are described in Gray and Mogg (2001) and only a summary is given below.

Plants of the two species were originally sampled from a salt marsh in Morecambe Bay (at 54° 10' N) and grown in common conditions for 18 weeks before 160 tillers of each species were removed, size-matched and transplanted into pots containing standard soils. The experimental design was a replacement series (de Wit 1960), each series comprising five pots of four tillers (4 *Spartina*, 3 *Spartina* + 1 *Puccinellia*, 2 *Spartina* + 2 *Puccinellia*, 1 *Spartina* + 3 *Puccinellia*, and 4 *Puccinellia*). Eight series were set up in large pots and eight in smaller pots to give two density levels and, following a three-week establishment period, the pots were transferred to the experimental conditions. These comprised eight hemispherical glasshouses (the 'solardomes') in which atmosphere and temperature are controlled to a high degree of precision. The eight domes allowed two replicates of each treatment and the four treatments were: ambient temperature + ambient CO^2; ambient temperature + a CO^2 concentration of 340 parts per million; temperature elevated by 3° C and tracked continuously above ambient + ambient CO^2; and elevated temperature + elevated CO^2. The pots were maintained with non-limiting water supplies, fertilized, and harvested after 11 months growth when each plant was separated into above and below ground material before weighing. The results are summarized in Table 1.

The main conclusions to be drawn from the experiment are (1) most yield variables were significantly affected by treatment, either by temperature or carbon dioxide level or the interaction between these treatments and density (*Spartina* height and inflorescence number being exceptions), (2) the main competitive interaction effects were due to the competitive superiority of *Puccinellia*, which had a significant negative effect on *Spartina*'s height and above ground weight and displayed strong intraspecific competitive effects on tiller number and biomass, (3) *Spartina* responded to higher temperature as predicted, but also, at low plant density, to carbon dioxide concentration. In both cases the response was mainly an increase in below-ground growth including rhizomes, (4) *Puccinellia* responded to higher atmospheric carbon dioxide and higher temperature mainly by increasing above-ground growth, (5) *Spartina* had an unexplained poor performance in the ++ treatment, an effect seen at low density and therefore unlikely to be explained by competition with *Puccinellia*,

Table 1. Individual plant means for six yield variables at final harvest.

Trait	*Species*	*Amb*	*+T*	*+CO^2*	*++*	*Sig mn effects*
Till no.	Spa	4.10	6.17	6.00	3.48	D*, CT*
	Pucc	31.46	17.69	25.76	25.98	P**, CTD*
Fl. Till	Spa	0.13	0.11	0.28	0.01	ns
	Pucc	1.79	0.42	1.34	1.53	D***, DP*
Ht(cm)	Spa	15.37	16.69	19.98	17.88	P*
	Pucc	44.47	44.08	48.24	54.98	D***, CD*
S wt(g)	Spa	0.45	0.70	0.89	0.37	D*, P*
	Pucc	1.77	1.28	1.61	2.70	D***, CDT**
BG wt(g)	Spa	0.61	0.97	1.38	0.60	CT*, CTD**
	Pucc	0.42	0.24	0.36	0.37	CTP**
Biom(g)	Spa	1.04	1.67	1.98	0.97	CT**, CTD**
	Pucc	2.19	1.53	1.97	3.08	D***, CTP*

The treatments (see text) were: Amb = ambient temperature and CO^2, +T = elevated temperature and ambient CO^2, +CO^2 = ambient temperature and elevated CO^2, ++ = elevated temperature and elevated CO^2. The variables were: Till no. = number of tillers, Fl. Till = number of flowering tillers, Ht(cm) = plant height, S wt(g) = shoot weight, BG wt(g) = weight of below-ground material, Biom(g) = total biomass. The significance levels are from a generalised ANOVA of log transformed values in which D = effect of density (pot size) (1df), C = effect of elevated CO^2 (1df), T = effect of elevated temperature (1df), and P = effect of the proportion of *Puccinellia* (which is a measure of competitive interaction effects) (3df). Only main interaction effects are given. Significance * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns no significant effects detected. (after Gray and Mogg 2001).

and (6) the pervasive effects of plant density on most variables underlines the importance of varying density in such experimental designs (Gibson et al. 1999).

A comparison of plant growth in ambient conditions with that in the ++ treatment provides broad agreement with Long's (1990) model predictions. The mean biomass of individual *Puccinellia* plants increased by about 100% in pure stands, a similar order of magnitude to the 80% increase in cumulative net primary production in the model. Increases in *Spartina* yield of 72% and 95% in elevated temperature and carbon dioxide respectively compare favourably with the model prediction of 62%, but the small increase in ++ conditions (+5%) was unexpected. The response of *Puccinellia* is broadly in line with that found in other C3 species (Bazzaz 1990) as is *Spartina*'s response to elevated temperature. However the increased yield of *Spartina* in higher carbon dioxide is at variance with other work on C4 grasses, including *Spartina patens* (Curtis et al. 1989).

DISCUSSION AND SPECULATION

As mentioned earlier, plant performance will be affected by several other factors related to projected climate change, some of which are difficult to predict, and it is generally risky to make predictions on the basis of photosynthetic pathway or CO^2 response alone (Dukes and Mooney 1999). Nevertheless it is interesting to speculate on the implications of the above experiment for the future changes in *Spartina* distribution. Indeed there are some aspects of the interaction between *Spartina* and *Puccinellia* which encourage us to believe that our predictions have a better than average chance of being somewhere near the mark – a relatively simple two-species system, predominately vegetative competition related to elevation as a resource, a strong seasonal element, wide dispersal of propagules, locally shared resource levels and so on (see Gray and Mogg 2001 for a fuller discussion).

Locally, competition will be influenced by the balance between increasing temperatures and carbon dioxide concentration. The average global warming of 1 to 3.5° C over the next century predicted as a result of increased greenhouse gases is likely to vary spatially and to be higher in northern latitudes in winter (Houghton 1996). In general however we may expect *Spartina* to extend its range northwards of 57° N as temperatures and carbon dioxide rise. Seed set in northern populations, which is currently limited by the length of the growing season (for *Spartina* this is the number of days above 9° C) may also increase and add to the plant's capacity to colonize new mudfats. The increased biomass below ground should enable the species to survive in the lower parts of its elevational niche. However, it seems likely that the competitively superior *Puccinellia* will replace *Spartina* at appropriate elevations. It is even possible that the high performance of *Puccinellia* under elevated temperature and carbon dioxide indicates that it will replace *Spartina* at lower elevations in northern latitudes. The future management of northern marshes, especially the extent to which they are grazed (a process which favours *Puccinellia* over *Spartina*) will influence the interaction between the two species.

In conclusion, our experiment suggests that changing climatic conditions could kick-start *Spartina*'s stalled invasion, enabling it to colonize mudflats and marshes northwards of its present distribution. Much will depend on changes in other ecosystem processes and on other features of the changing climate. Higher rainfall, through its effect on salinity, and changes in storm frequency and wind direction are likely to be important factors. A key factor will be the impact of rising relative sea levels and their influence on local sediment availability and accretion.

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COMPETITION AMONG MARSH MACROPHYTES BY MEANS OF VERTICAL GEOMORPHOLOGICAL DISPLACEMENT

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This paper describes a theory of biogeomorphology that addresses how intertidal macrophytes can modify landscape elevation. Competitive interactions among marsh plant species are mediated by the influence of vegetation on sediment accretion and their modification of the relative elevation of the marsh surface. A model described here demonstrates the effects of feedback between physical processes like sediment accretion and biological processes such as those that determine species zonation patterns. Changes in geomorphology, primary productivity and the spatial distribution of plant species are explained by competitive interactions and by interactions among the tides, biomass density, and sediment accretion that move marsh elevation towards an equilibrium with mean sea level (MSL). This equilibrium is affected positively (relative elevation of the marsh surface increases) by the biomass density of emergent, salt marsh macrophytes and negatively by the rate of sea-level rise. It was demonstrated that a dominant, invading species is able to modify its environment to exclude competitively inferior species. However, the outcome depends on a number of variables including the rate of sea-level rise and the fundamental biomass distributions of species across the intertidal gradient. The model predicts that a marsh can move toward alternative states, depending on the rate of sea-level rise and species biomass distributions within the tidal frame.

Keywords: sedimentation, marshes, *Spartina*, sea level, models, geomorphology, competition

INTRODUCTION

Physical ecosystem engineering is a process common to organisms that possess the ability to physically modify their habitats (Jones et al. 1997). This trait is common to intertidal marsh macrophytes that have the potential to raise the relative elevation of their habitat and modify the geomorphology of the coastal landscape, potentially to the benefit and detriment of other species (Zedler & Kercher 2004). Historically, coastal wetlands have maintained an elevation in equilibrium with mean sea level by the accumulation of mineral sediment or organic matter (Redfield 1972; Stevenson et al. 1986). Commonly, stable intertidal salt marshes occupy a broad, flat expanse of landscape often referred to as the marsh platform at an elevation within the intertidal zone that approximates that of local mean high water (MHW) (Krone 1985). Marsh species typically segregate along gently sloping gradients across the marsh platform (Hacker & Bertness 1999; Silvestri et al. 20005). The elevations of the marsh platform relative to sea level determine inundation frequency, duration and, consequently, wetland productivity and species distributions.

Recent work in a North Inlet, SC marsh has shown that the relative elevation of the sediment surface is a critically important variable that ultimately controls the productivity of the salt marsh plant community (Morris et al. 2002). Productivity has a positive feedback on the rate of accretion of the marsh surface. This feedback is key to predicting the responses of coastal wetlands to rising sea level, including changes to the geometry of the land margin and to the total area of wetland habitat. It is also fundamental to understanding the spread of invasive marsh macrophytes, such as *Spartina* hybrids in San Francisco Bay, that have the ability to physically alter their environment in a manner that benefits the invading species (Cuddington & Hastings 2004).

The objective of the study reported here is to explore the behavior of a model that explicitly treats the modification of habitat elevation by a hypothetical group of marsh macrophytes competing for habitat space within the intertidal zone. The model accounts for the species-specific effect of marsh vegetation on mineral sediment accretion, and for feedbacks among sea-level rise, relative elevation, species replacement, and primary production. From this work it should be possible to generalize about the types of adaptations that enable macrophyte species to exploit a particular range of habitat within the intertidal zone and that endow some species with superior competitive abilities. The concept of geomorphological displacement is described whereby one species displaces another by modifying the relative elevation of its habitat.

MODEL DESCRIPTION

The model described here is based on fieldwork that was carried out in a salt marsh at North Inlet, South Carolina (Morris et al. 2002). The model was initially developed and calibrated for a single species, *Spartina alterniflora*, which forms a monoculture over the majority of this marsh within a narrow range between 0.22 and 0.481 m relative to NAVD88 (Morris et al. 2005). At North Inlet, the mean high water level was 0.618 m (2001 through May, 2003) with a mean tidal range of 1.39 m. Details about the North Inlet marsh can be found in a variety of sources (Eiser & Kjerfve 1986; Dame et al. 2000; Morris 2000; Morris et al. 2005). Below, the model is generalized for two or more species and its predictions for the case of two hypothetical species are discussed.

The Elevation of the Marsh Surface

The elevation of the marsh platform equilibrates at a position where erosion and deposition are equal. It is clear that sediment deposition must approach zero at elevations near that of the highest high tide and should increase as the concentration of suspended sediment and hydroperiod increase. This logic is based upon a consideration of abiotic processes alone and predicts that intertidal marshes approach an equilibrium elevation that approximates MHW (Krone 1985). A rise in relative sea level will increase flooding of the marsh and inundation time thereby increasing the opportunity for sediment deposition and re-establishing the elevation of the marsh relative to the new MSL (Pethick 1981, Krone 1985, French 1993). Sedimentation rate is the product of settling velocity and time of inundation. Since depth below mean higher high water (MHHW) and time of inundation are roughly proportional, the net rate of change in the elevation of the marsh surface (dY/dt), is proportional to the depth (D) of the marsh surface: $dY/dt \propto D$. Strictly speaking, sedimentation rate should be proportional to depth, whereas other processes that affect elevation, like compaction and decomposition, may or may not be depth-dependent. However, compaction can be taken to be a constant that is accounted for in the local rate of sea-level rise.

Feedbacks between the marsh vegetation and the sediments are also important. The rate of change of elevation of the marsh platform is a positive function of the standing density of plant biomass (B). For simplicity it is assumed that this relationship is linear: $dY/dt \propto q + kB$, where parameters *q* and *k* are proportional to the settling velocity and the efficiency of the vegetation as a sediment trap, respectively. The product of *k*B represents the positive effect that aboveground biomass density has on the trapping of suspended sediments (e.g. Leonard & Luther, 1995; Christiansen et al 2000). The values of *q* and *k* are likely to vary locally and regionally as a function of sediment availability and tidal range (e.g. Stevenson et al. 1986). Note that *k* also may vary by species. This implies that the efficiency of sediment trapping may vary among species (e.g. Rooth and Stevenson 2000). Combining these concepts, the absolute change in elevation of the marsh surface, for depths (D) within the intertidal zone, can be approximated by:

$$dY/dt = (q + kB)D, \text{ for } D>0 \qquad (1)$$

The *k*B term implicitly accounts for the contribution of organic matter accretion when the model is calibrated to the total accretion rate. It assumes that organic matter accretion is proportional to the standing biomass density of the vegetation. We have recent unpublished results that show that this assumption overly simplistic, but the qualitative behavior of the model with respect to total sediment accretion and sea-level rise is not changed by breaking out organic matter accretion as a separate term.

The Vertical-Distribution of Standing Biomass

Biomass density, B (g/m^2), is variable and changes with a number of environmental conditions including the relative elevation of the marsh platform. For any intertidal species, there exist upper and lower limits of relative elevation. For the hybrid in San Francisco Bay as for *S. alterniflora* on the Atlantic coast, the lower limit is probably set by the hypoxia resulting from tidal flooding, while the upper elevation is determined by salt stress, desiccation, and competitive pressure from other species. The dominant representatives from different plant communities along a topographic gradient will have different biomass distributions (*a*, *b* and *c* values in Eq. 2) that may or may not overlap, and interspecific competition or facilitative interactions may modify the shapes of the curves where overlap occurs (eg. Bertness 1991; Emery et al. 2001; Bertness & Ewanchuk 2002; Pennings et al. 2005). These distributions can be described by a family of curves:

$$B_i = a_iD + b_iD^2 + c_i \qquad (2)$$

where *a*, *b*, and *c* are coefficients that determine the upper and lower depth limits, and magnitude of B and where the subscript *i* refers to a specific dominant species or community type. Depth, D (cm), is positive for depths less than MHHW. These curves can be viewed as dimensions of a species' fundamental (in the absence of competitors) or realized (in the presence of competitors) niche, sensu Hutchinson (1957). The values of the coefficients *a*, *b*, and *c* will also differ regionally as a function of tide range, salinity, or climate. In examples discussed below, the values of *a*, *b*, and *c* where chosen to represent hypothetical species with biomass distributions that span different ranges along an intertidal gradient.

MHHW is used as the zero datum (D_0) for convenience since it is approximates the elevation where biomass density and sedimentation rate approach zero. However, because MHHW is based upon an arithmetic average of the higher high water height of each tidal day observed over the National Tidal Datum Epoch, D_0 may differ from MHHW regionally, depending on factors such as wind tides. The departure of D_0 from MHHW is probably greatest in microtidal estuaries where wind tides often dominate astronomical tides. Moreover, MHHW will vary due to low frequency variability in mean sea level and with the 18.6-yr lunar nodal cycle (Stumpf and Haines, 1998). The lunar nodal cycle changes the tidal amplitude by about 5 cm and is simulated below by allowing D_0 to vary by 5 cm at a frequency of 1/18.6 yr.

Feedbacks Among Biomass Distribution, Relative Elevation and Sea-Level Rise

Substituting for B in Eq. 1 yields the following equation that describes the absolute change in elevation of the marsh surface as a function of depth:

$$dY/dt = [q + k_i(a_iD + b_iD^2 + c_i)]D, \text{ for } D>0 \qquad (3)$$

Equation 3 was solved numerically for a hypothetical marsh landscape with one or two species differing in their biomass distributions. The time-zero marsh elevation and rates of sea-level rise were set arbitrarily and model simulations of 120-yr duration were generated to allow time for convergence on a dynamic steady state. A rate of sea-level rise of either 0.2 or 0.8 cm/yr was specified, and a sinusoidal function was used to simulate changes in tidal amplitude of ± 2.5 cm over the 18.6-yr lunar nodal cycle, centered about a mean tidal amplitude of 0.6 m. The coefficient values for *q* and *k* were set at 0.00018 yr^{-1} and 1.5 × 10^{-5} cm m^2 g^{-1} yr^{-1} (0.15 cm^3 g^{-1} yr^{-1}). These parameter values were taken from a calibration of the model to North Inlet data (Morris et al. 2002). In addition, species distributions were varied in order to explore effects on equilibrium elevation and species persistence. Species distributions (Eq. 2) were described by specifying a maximum biomass and optimum elevation, operationally defined here as the elevation that results in the greatest biomass density, and species range, defined as the upper and lower depth limits.

RESULTS AND DISCUSSION

The Single Species Example

When the model was solved for a single species with a distribution between 15 and 30 cm above mean sea level (Fig. 1A), and a rate of sea-level rise of 0.2 cm/yr, the marsh surface elevation equilibrated at about 26 cm above mean sea level and a depth of 34 ± 2.9 cm (mean ± amplitude) below MHHW (Fig 1B). This elevation is above the optimum elevation specified in Fig. 1A, which is a condition for stability (Morris et al. 2002). The constraint on productivity imposed by high pore-water salinities that develop at super-optimal elevations is an important factor in maintaining relative elevation, because a rise in relative sea level brings about an increase in flooding, decreases pore water salinity (Morris 1995), and increases biomass density (Morris 2000). The increase in biomass density will enhance sediment deposition by increasing sediment trapping efficiency (Gleason et al. 1979, Leonard & Luther 1995, Yang 1998).

MHHW varies over the course of the 18.6-yr lunar nodal cycle by ± 2.5 cm. Consequently, the depth of marsh surface below MHHW varies at the same frequency (Fig. 1B), resulting in a cycle of standing biomass with a range of 296 g/m^2 (Fig. 1C). This is consistent with empirical measurements from North Inlet, SC where we have seen changes in *S. alterniflora* productivity of 248 g m^{-2} yr^{-1} over the lunar nodal cycle. It should be noted that MHHW varies independently of MSL owing to long-period astronomical forcing, e.g. the lunar nodal cycle, but MHHW will also vary directly with changes in MSL. Consequently, MHHW is a better tidal datum for purposes of defining the growth ranges of intertidal species than is MSL.

Result of Adding a Competitor with Wider Distribution and High Biomass

When a competing species was added to the model with a distribution like that of species 2 in Fig. 2A, species 1 was eliminated within about 10 yr (Fig. 2C). Examples are common of this type of interaction, such as the invasion of

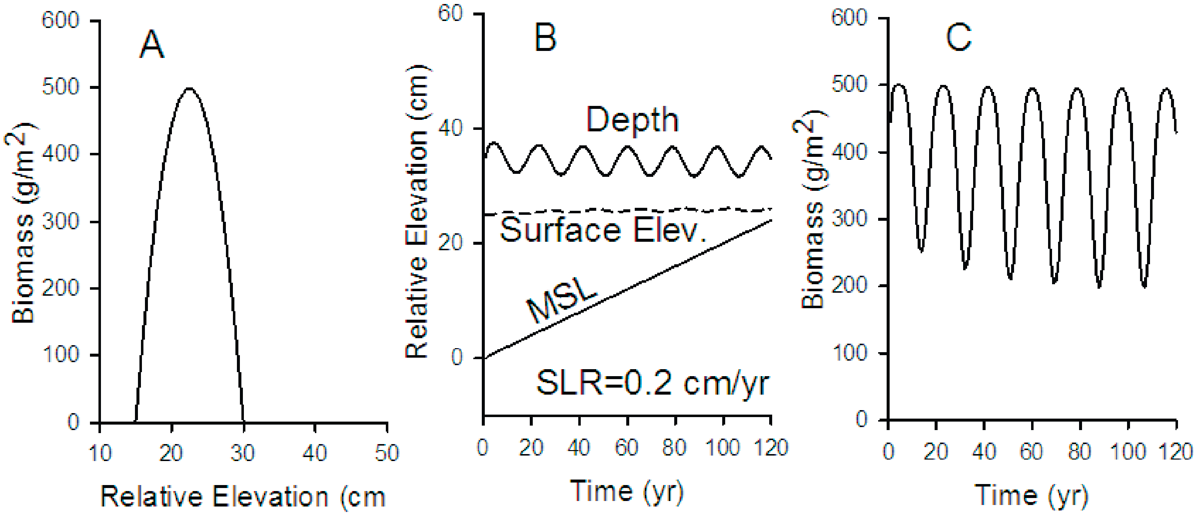

Fig. 1 Model results with a single species (S1), distributed as above (A), and a rate of sea-level rise of 0.2 cm/yr, as shown in B. Panel C shows the predicted biomass trajectory as (B) the marsh surface equilibrates at a relative surface elevation of about 25 cm above mean sea level (MSL) and a depth below MHHW of about 35 cm.

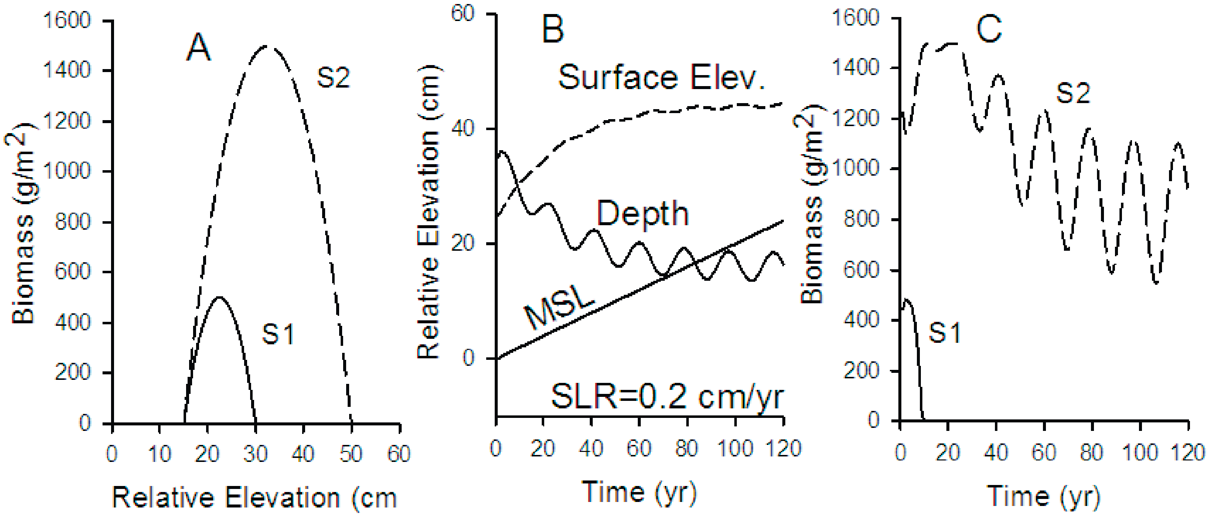

Fig. 2 Model results with two species with biomass distributions as above (A) and a rate of sea-level rise of 0.2 cm/yr. Shown in panel C is the predicted biomass trajectory of both species as (B) the marsh equilibrates at a higher relative surface elevation of about 45 cm above mean sea level (MSL) and a depth below MHHW of about 15 cm.

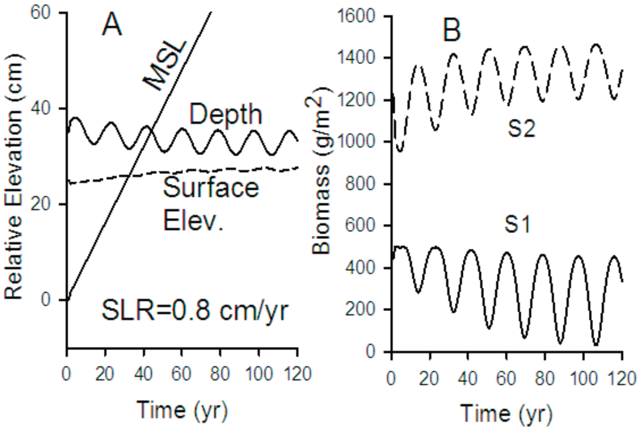

Fig.3. Model results with two species with biomass distributions as in the previous example (Fig. 2A) when the rate of sea-level rise was increased to 0.8 cm/yr; parameter values were otherwise identical to those in the previous example (Fig. 2). Shown in panel B is the predicted biomass trajectory of both species as (A) the marsh surface equilibrates at a relative surface elevation of about 25 cm above mean sea level (MSL) and a depth of about 35 cm below MHHW.

Phragmites australis into brackish *Spartina patens* marsh (Windham 1999). Note that competition occurs in this model indirectly by virtue of a change in relative elevation, favoring species 2. Species responses are defined exclusively by their habitat distributions (Eq. 2) and not by direct interference. For example, species 2 displaced species 1 when species 2 was given a habitat range wider than species 1, a higher optimum elevation, and a greater biomass density at its optimum elevation. In this example, the marsh equilibrated at a higher surface elevation, about 45 cm (Fig. 2B), than in the preceding case. The equilibrium surface elevation (Fig. 2B) was significantly higher than the optimum elevation for species 2 (Fig. 2A), which is a consequence of the relatively high biomass density of species 2. Thus, as the amplitude of a species' biomass distribution increases, the equilibrium elevation of the marsh surface will increase.

This prediction is important for predicting and understanding changes in salt marshes following the introduction of an alien species. An invader with a greater biomass and a wider habitat range can raise the elevation of the marsh above the range of the native species, although there are several qualifications that must be noted. Firstly, the model discussed here is zero- dimensional or plot-scale. In two dimensions, the geomorphology of the marsh landscape will adjust to changes in its relative elevation, and it is possible that the resulting topographic gradients will support a dynamically stable zonation of species, i.e. marshes transgress and species migrate and segregate across topographic gradients. Secondly, there are conditions, such as the rate of sea-level rise, explored below, that lead to the persistence of a weaker species.

Facultative Behavior and the Rate of Sea-Level Rise

A facultative interaction between species is possible by virtue of the additive effects on sediment accretion of overlapping species distributions. The additive effect on accretion rate can maintain the elevation of the marsh surface within both species' ranges at a high rate of sea-level rise. This was demonstrated by raising the rate of sea-level rise from 0.2 to 0.8 cm/yr (Fig. 3). The increase in rate of sea-level rise resulted in stabilization of the relative surface elevation at about 28 cm (Fig. 3A) at an elevation that is greater than the optimum that had been specified for species 1 (22.5 cm) and suboptimal for species 2 (32.5 cm, Fig. 2A). At this elevation both species were able to coexist (Fig. 3B).

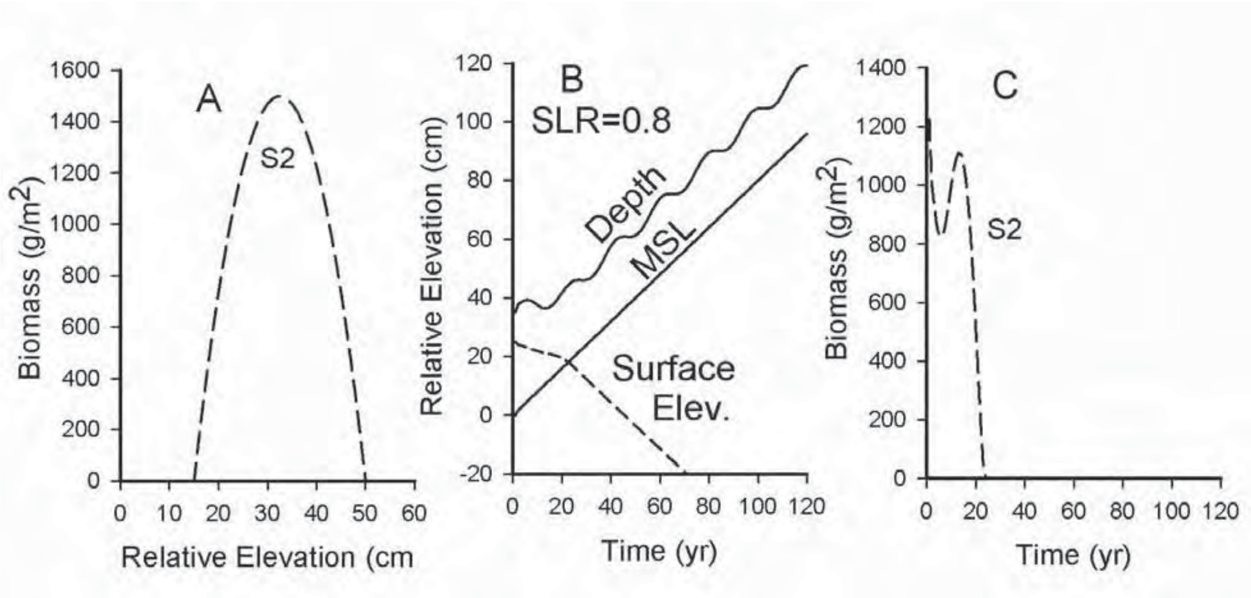

Fig.4. Model results with one species with a biomass distribution as above (A) and a rate of sea-level rise of 0.8 cm/yr. Shown in panel C is the predicted biomass trajectory of species 2 as (B) the marsh surface equilibrates at an elevation below MSL and outside of species 2's range. Parameter values were otherwise identical to those in the previous examples (Fig. 2 and 3).

The biomass of species 2 varied with depth at the frequency of the lunar nodal cycle, but 180° out-of-phase, whereas the lunar nodal cycle and biomass of species 1 were in phase (Fig. 3B). The biomass of species 2 decreased with a rise in MHHW, i.e. as equilibrium depth increased, because the marsh surface elevation, 28 cm, was suboptimal for growth. Conversely, the biomass of species 1 increased with a rise in MHHW because surface elevation was super-optimal for its growth. Thus, species can coexist theoretically when there is a periodic change in flood duration and when one species responds to the change positively and the other negatively. This is one of several conditions that promote a facultative interaction between species.

Facultative interactions among marsh macrophytes involving amelioration of soil salinity have been described (Bertness & Ewanchuk 2002), and simulation results shown here suggest that biogeomorphological interactions could also be facultative. When two species with overlapping distributions (Fig. 2A) were present, and the rate of sea-level rise was raised to 0.8 cm/yr, the resulting interaction could be described as facultative (Fig. 3), because neither species persists in the absence of the other (Fig. 4). When species 1 was removed from the simulation, the equilibrium elevation quickly dropped below MSL and below the lower limit for species 2. Species 1 suffered the same fate when species 2 was removed. However, at a low rate of sea-level rise, 0.2 cm/yr, species 1 did not survive (Fig. 2). Thus, the outcome of competition and the emergence of facultative behavior depend on the rate of sea-level rise.

The Rate of Sea-Level Rise and Alternative Stable States

It can also be demonstrated that the marsh will move toward alternative stable states or habitat preemption by one species or another, depending on the rate of sea-level rise and the species' biomass distributions. When the rate of sea-level rise was 0.8 cm/yr and the distribution of species 1 was modified by raising its maximum biomass to equal that of species 2 (Fig. 5A), only species 1 persisted throughout the simulation, while species 2 was intermittent (Fig. 5C). The marsh surface equilibrated at an elevation of about 23 cm (Fig. 5B), which was at the lower limit of species 2 and above the optimum elevation for species 1 (Fig. 5A). Keeping the species distributions as in Fig. 5A and lowering the rate of sea-level rise to 0.2 cm/yr resulted in a different outcome. At this lower rate of sea-level rise, only species 2 persisted (Fig. 6C). The marsh surface equilibrated at an elevation of about 46 cm (Fig. 6A), which is above the upper limit of species 1 and above the optimum of species 2 (Fig. 5A).

CONCLUSIONS

This paper describes a theory of biogeomorphology that addresses how intertidal macrophytes can modify landscape elevation and affect the outcomes of species interactions by means of vertical geomorphological displacement. Competition by geomorphogical displacement is indirect and is a function of the fundamental and realized niches that describe species biomass distributions in the tidal frame. The outcomes of competitive interactions are a great deal more complex than described here when there are direct interferences or facultative interactions of the sort described by Bertness and Ewanchuk (2002). These types of interactions can modify a species' realized distributions dynamically and would result in truly complex behavior.

Marsh primary production and standing biomass are sensitive to hydroperiod and have positive effects on sediment accretion. In that part of the tidal frame that is higher

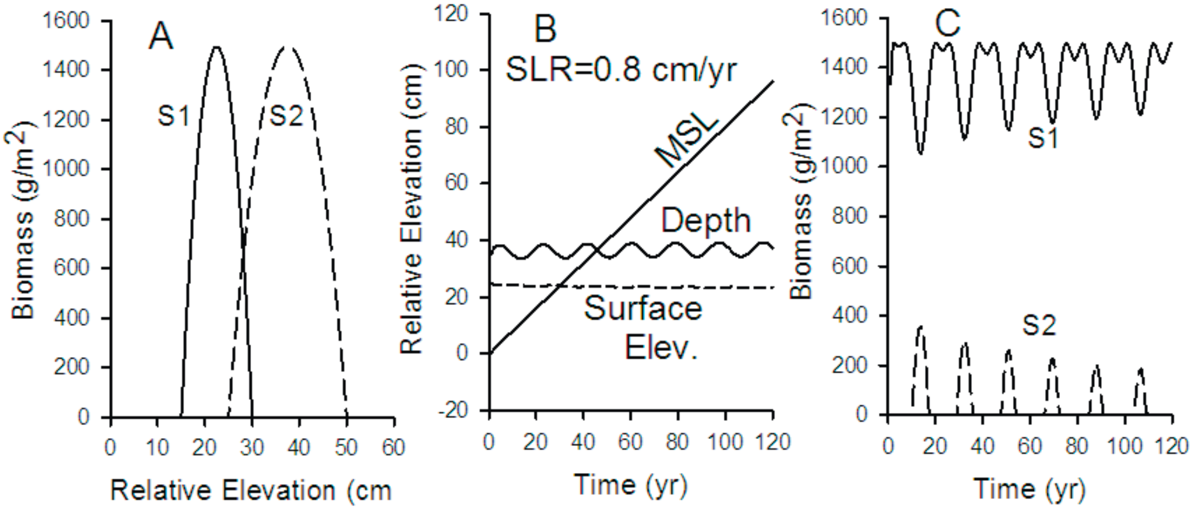

Figure 5. Model results with two species with biomass distributions as above (A) and a rate of sea-level rise of 0.8 cm/yr (B). Shown in panel C is the predicted biomass trajectory of both species (species 2 approaches extinction) as the marsh surface equilibrates at a relative elevation of about 20 cm (B) above mean sea level (MSL).

than a species 'optimum' elevation, rising sea level increases primary production, which stimulates sedimentation and maintains equilibrium with MSL. Optimum elevation is in quotes because, while that is the elevation that favors maximum growth, it borders on instability. Several species or communities of plants may coexist if they partition the habitat space, and if a topographic gradient of sufficient slope exists. Alternatively, coexistence is unlikely following an invasion by a species with greater niche breadth and higher productivity as in Fig. 2A S2. Locally, sediment accretion may drive species replacements or succession. Because of the sensitivity of species' productivities to hydroperiod, anomalies in mean sea level and astronomically forced changes in MHHW can alter the dynamics of competing species on decadal or shorter time scales.

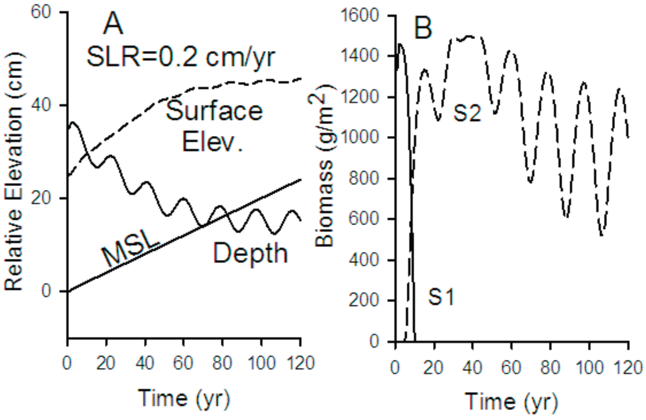

Fig. 6. Model results with two species with biomass distributions as in the previous example (Fig. 5A). Shown in (B) is the predicted biomass trajectory of both species (s1 became extinct) as the marsh surface equilibrates at a relative elevation of about 45 cm (A) above mean sea level (MSL). The rate of sea-level rise was decreased to 0.2 cm/yr (A); parameter values were otherwise identical to those in the previous example (Fig. 5).

Species differ in their tolerance to flooding, hypoxia, desiccation, and salt stress (Pennings & Callaway 1992, Kuhn & Zedler 1997). Consequently, the fundamental distributions of plant species within the intertidal zone are determined by physical factors that set upper and lower depth limits, and optimum depths. The realized distributions may or may not resemble closely the fundamental distributions, depending on the strength of competitive or facilitative interactions among species (Ungar 1998, Bertness 1991). For a given rate of sea-level rise, the relative shapes of these distributions ultimately determine the equilibrium elevation, species replacements and persistence. One species may replace another by modifying the elevation of its habitat to the detriment of competitors. This is characteristic of species with relatively high biomass densities such as the *Spartina* hybrid swarms that have become established in San Francisco Bay (Daehler & Strong 1997, Ayres et al. 2004).

These insights are important to our fundamental understating of the ecology of marsh ecosystems and should also be relevant to the management community responsible for forecasting and controlling the course of species invasions. On a fundamental level, the work demonstrates how processes that operate at different temporal scales can interact to modify ecosystem structure and function. For example, the outcome of interspecific competition among marsh macrophytes, a biological process that operates on relative short time scales, can be affected by the rate of sea-level rise, a process that occurs on very long time scales.

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MODELING THE SPREAD OF INVASIVE *SPARTINA* HYBRIDS IN SAN FRANCISCO BAY

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The emergence of highly fit hybrids between native and introduced species is an increasingly widespread problem that can impact entire ecosystems. In San Francisco Bay, a swarm of hybrid cordgrass (*Spartina foliosa* x *alterniflora*) is covering vast areas of intertidal mudflat, threatening the native *S. foliosa* with extinction. Here we outline a modeling approach for assessing the relative importance of elevated hybrid fitness traits in explaining the rapidity of this invasion. We demonstrate that elevated growth rate, seedling survival and pollen production of hybrids relative to the native species interact to predict the observed faster-than-exponential spread of cordgrass through the Bay.

Keywords: invasion, model, hybridization, *Spartina*

INTRODUCTION

An exotic introduced into a new range is often phenomenally successful; this may be due to exploitation of a previously unfilled niche, a release from natural enemies, or increased competitive ability relative to the native biota (Williamson 1996). However, in many ecosystems, the emergence of hybrids between native and exotic species is the greatest threat to local biodiversity, particularly if the hybrids have superior fecundity or suvivorship compared with the native (Wolf et al. 2001). An example of this is the invasion of hybrid cordgrass *Spartina foliosa* x *alterniflora* in San Francisco Bay. Since the introduction of *S. alterniflora* in the 1970s, hybrids have emerged whose growth rates, fecundity and tolerance to environmental conditions exceed that of both parental lineages (Ayres et al. 2003). The hybrids impact on many organisms in the Bay by covering open mudflat crucial to feeding shorebirds, and changing the tidal profile through sediment accumulated in their root mass. Contrary to classical ecological theory, which suggests that a species with unlimited resources can grow at most exponentially (e.g., Turchin 2003), the area covered by hybrid cordgrass in the Bay has increased *super-exponentially* (i.e. the growth rate per unit area is increasing through time; Fig. 1). If this invasion continues unchecked, it seems likely that the native *S. foliosa* is doomed to extinction through introgression (Ayres et al. 2003).

In order to design effective control strategies, it is vital to determine the key hybrid traits responsible for driving this invasion. Increased vegetative growth rates, elevated pollen production and seedling survival of hybrid *Spartina* relative to the native have all been proposed as contributing to the rate and extent of this invasion (Ayres et al. 2004). Mathematical models are a useful tool for investigating the relative importance of these mechanisms. In this paper we outline the modeling approach used to describe the dynamics of this hybridization. We use the model to test how increased vegetative growth rate, seedling survival and pollen production of hybrids affect the rate of population expansion in areas of high and low recruitment. We find that depending on the level of local recruitment, increased vegetative growth rate or seedling survival of hybrids can result in super-exponential population growth. Elevated hybrid pollen production alone results in an increase in the frequency of hybrids in the population, but does not predict super-exponential growth unless coupled with elevated hybrid growth rate or seedling survival.

MODEL AND METHODS

Here we outline the key modeling assumptions and sketch the derivation of the model. The derivation of the full quantitative genetic, integro-difference equation model is

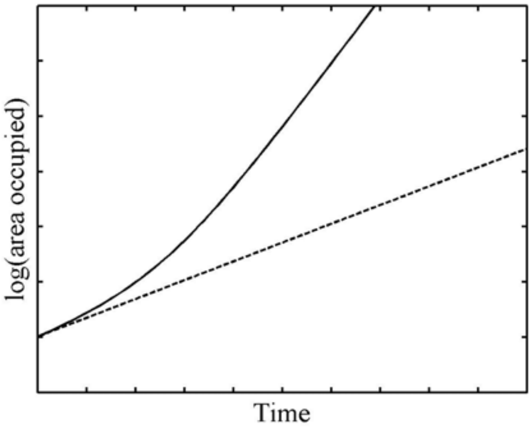

Fig. 1: super-exponential (bold line) vs exponential (dashed line) population growth. After a sufficiently long time, the area occupied by an invasive growing super-exponentially differs from an exponentially growing population by orders of magnitude.

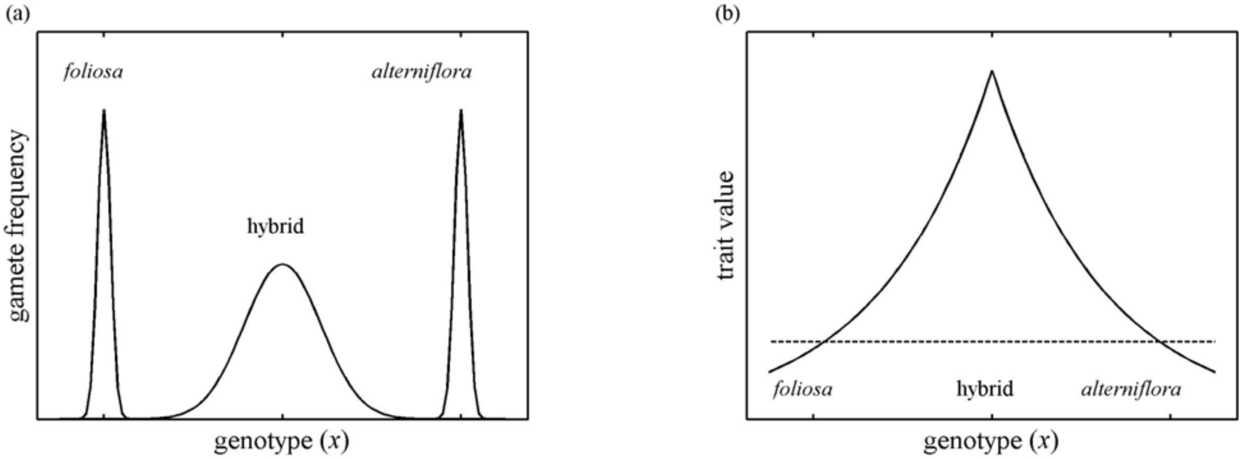

Fig. 2 (a) The distribution of gamete genotypes as a function of parental genotypes. The broad distribution of hybrid genotypes reflects the higher genetic diversity of the hybrids compared with the pure lineages. (b) Functional form of the genotype-dependent (bold line) and genotype-independent (dashed line) trait values (growth rate, seedling survival or pollen production).

outlined in Hall et al. 2006. In order to elucidate the role of genetic recombination in driving this invasion, we make several simplifying assumptions about the population dynamics. Specifically, we assume spatial and environmental homogeneity, and focus on the spread of plants into open mudflat (i.e., we ignore density-dependent effects such as crowding). In the spirit of many quantitative genetics models (e.g., Bulmer 1980), we assume that a large number of loci interact independently and additively to determine phenotypic fitness, and ignore the effects of environment on phenotype.

Plants are classified by their genotype value, x, scaled such that pure *S. foliosa* has genotypes clustered around x=-1, pure *S. alterniflora* around x=+1, with hybrids spanning the intermediate range of genotype values. Plants of genotype x produce gametes whose genotype is drawn from a Normal distribution with mean $x/2$. The variance of this distribution is genotype-specific, under the assumption that hybrids can produce genetically more diverse offspring than either parental lineage (Fig. 2a). When pollen of genotype y successfully combines with an ovule of type z, the resulting seed has genotype $y+z$.

The population-level model is formulated in terms of the total area occupied by plants of genotype x in year t, denoted $P_t(x)$. The area occupied by plants in year t+1 is given by the sum of vegetative growth of adult plants in year t and the area occupied by new seedlings. Mathematically this is expressed by

$$\boldsymbol{P_{t+1}(x)=G(x)\,P_t(x)+S(x)\,N_t(x),}$$

where $G(x)$ is the (genotype-dependent) vegetative growth rate, $S(x)$ is the survival of seedlings of genotype x, and $N_t(x)$ is the total number of seeds produced in year t of type x. The number of seeds produced of type x is the product of the frequency of pollen produced by male parents of genotype y (proportional to the genotype-specific pollen production rate M), the number of ovules from female parents of genotype z, and the probability that pollen of type y and ovules of type z combine to produce a seed of type x, summed over all possible genotype values of the male and female parents.

The above model is used to test the effects of elevated hybrid growth rate, seedling survival and pollen production (G, S and M respectively) on the total area occupied by *Spartina* of all genotypes, and the frequency of hybrid genotypes in the population. For comparison, the model is also run under a null scenario whereby all genotypes are equally fit. The functional dependence of an elevated trait value on a plant's genotype is depicted in Fig. 2b. Two levels of seedling recruitment are also considered: low (as might be expected at a site subject to strong tidal action) and high (e.g. in a sheltered inlet or marsh restoration site).

RESULTS

When the model is run under the assumption that all *Spartina* genotypes are equally fit, the population does not undergo super-exponential growth. However, even in the absence of any fitness advantage to the hybrid, the frequency of pure *S. foliosa* plants in the population suffers a slow decline, suggesting that given sufficiently long time, the hybrid swarm could dominate.

If the baseline level of seedling recruitment is low, an elevated vegetative growth rate of hybrids can produce the observed faster-than-exponential population growth. Elevated seedling survival of hybrids also results in super-exponential growth, but at a much less dramatic rate. Conversely, in regions where seedling recruitment is high, elevated hybrid seedling survival produces faster population expansion than elevated hybrid growth rate. Elevated hybrid growth rates and seedling survival both result in a rapid decline in the frequency of native genotypes: essentially as the area covered by hybrid cordgrass increases, the probability of hybrid pollen fertilizing the native also increases. This ‘pollen-swamping' suggests that ultimately all new recruits to the *Spartina* population will be hybrid, resulting in extinction of the native through introgression.

In both recruitment scenarios, the effect of elevated rates of hybrid pollen production on the rate of invasion is negligible, assuming the hybrids have no other fitness advantage. However, the frequency of native *S. foliosa* genotypes declines at a much faster rate than in the null model (where all genotypes produce the same amount of viable pollen). If elevated hybrid pollen production is coupled with an increased hybrid growth rate or seedling survival, the resulting invasion proceeds faster than it would for elevated hybrid growth rates or seedling survival alone. The effects of the differing life-history processes on the rate of invasion of hybrid *Spartina* genotypes are summarized in Table 1.

Table 1: Effects of elevated life-history traits and parental compatibility on the rate of invasion of hybrid *Spartina*.

Trait	Effect on growth rate
All genotypes equally fit	Exponential growth; increase in frequency of hybrids
Elevated growth rate (*G*)	Super-exponential; effect greatest when seedling recruitment low
Elevated seedling survival (*S*)	Super-exponential; effect greatest when seedling recruitment high
Elevated pollen production (*M*)	Exponential growth; rapid increase in hybrid frequency; super-exponential only in conjunction with elevated *S* or *M*

DISCUSSION

Here we have shown that a model which allows for elevated fitness traits (growth, seedling survival, pollen production) in hybrid *Spartina* can explain its explosive propagation through San Francisco Bay. As well as predicting a huge increase in the total area covered by *Spartina* in the Bay, the model also shows a sharp decline in the frequency of pure *S. foliosa* genotypes over the next century, as an increasingly higher percentage of seedlings have hybrid origin. If this invasion continues unchecked, the native *S. foliosa* will become extinct in the Bay. Other models of invasion and hybridization predict the ultimate extinction of the species (Huxel 1999; Wolf et al. 2001). The work presented here extends previous modeling efforts by linking the population dynamics and genetics of the invasion, making transparent the roles of genetic recombination and selection acting on favourable phenotypic traits.

Hybrid *Spartina* needs to be eradicated to ensure the persistence of the native *S. foliosa* in San Francisco Bay. Super-exponential population growth of hybrids may be driven by different life-history processes depending on the level of local recruitment. In accordance with recent modeling work on the control of *S. alterniflora* in Willapa Bay, Washington (Taylor and Hastings 2004), this suggests that control efforts at sites with differing levels of seedling recruitment should be targeted at different stage classes of *Spartina*. At low recruitment sites, control should be targeted at the removal of fast-growing isolated clones. At sites with potentially a high level of recruitment, such as marsh restoration sites, control should focus on the removal of seedlings, or herbicide treatment of hybrid *Spartina* meadows during the pollination season.

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MODELING THE SPREAD AND CONTROL OF *SPARTINA ALTERNIFLORA* IN A PACIFIC ESTUARY

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Results from both a spatially-explicit simulation model and a spatially-implicit analytical model show that an Allee effect can slow the spread of an invasive plant, *Spartina alterniflora* at sites within a Pacific coast estuary. The average rate of spread with the Allee effect is about 20% per year. Removing the Allee effect results in an average rate of spread of about 30% per year. The analytical model which partitions the population according to density classes, instead of the more usual age or stage classes was used in conjunction with a genetic algorithm to investigate density-based eradication strategies. We ask whether it is more efficient to first remove low-density plants at the edge of an invasion site that produce fewer propagules but spread rapidly by rhizomes or to remove high-density plants that spread slowly but produce most of the new recruits. We explored the consequences of the Allee effect and different annual budget levels on the optimal strategy. We found that the optimal strategy was dependent on annual budget levels. At low budgets, it was necessary to remove the low-density areas first to achieve eradication but if a high budget is available then the optimal strategy is to prioritise high-density areas. Without an Allee effect the optimal strategy would always prioritize the removal of the fast-growing low-density areas. Given uncertainty in future budgets, we recommend a strategy that prioritizses the removal of low density plants over the high density plants. The reproductive Allee effect in this system is not sufficiently strong to outweigh the importance of the rapid vegetative spread.

Keywords: Allee effect, *Spartina alterniflora*, Willapa Bay, invasive species, control strategy, eradication, pollen limitation

This paper describes two models that we developed to study the invasion of *Spartina alterniflora* into Willapa Bay in Washington State, USA. The first is a spatially explicit simulation model that we used to investigate population level consequences of an Allee effect caused by pollen limitation. The second is a derived non-spatial analytical model that we used to investigate eradication strategies.

As is described by others at this conference (Davis et al.; Civille et al. this issue), *S. alterniflora* (smooth cordgrass, hereafter referred to as *Spartina*) was introduced to Willapa bay just over 100 years ago. It was introduced into a small number of locations around the bay and then spread itself around the whole estuary. Individuals become established as seedlings then grow, vegetatively, into circular clones. Eventually the clones merge together to form meadows. By 2002, many of the mudflats of Willapa Bay had been completely converted to cordgrass meadow and almost all of the mudflats have been invaded to some degree, although there are still extensive areas of mostly unvegetated mudflat. A typical mudflat is in transition, a meadow has already formed, uncoalesced clones are scattered at the edge of the meadow and the rest of the mudflat is uninvaded.

When we measured seedset in these clones we found that seed production in the meadows is vastly greater than (more than ten times) seed production in the uncoalesced clones (Davis et al. 2004a). This reduced seed production when density is low is an example of an Allee effect, defined generally as a positive relationship between any component of fitness (in this case seed production) and conspecific density (Stephens et al. 1999). Later experiments demonstrated that pollen limitation is the mechanism that causes the Allee effect in the Willapa Bay population of *S. alterniflora* (Davis et al. 2004b).

SIMULATION MODEL OF *SPARTINA* SPREAD

We calculated, from data collected in the field, that the meadows produce an average of approximately 500 seeds per square meter (m^{-2}) and that the clones only produce approximately 12 seeds m^{-2}. Data from GIS maps from 1994 and 1997 (Civille 2005) was used to estimate seed dispersal distances, establishment probability and vegetative growth rates and, using these parameters, we built a spatially explicit simulation model of the spread of *Spartina*. Full details of the model and its parameterization are given in Taylor et al. 2004.

The model represents one square kilometer of mudflat, arranged as a square lattice of 1000 by 1000 cells; each cell is one square meter and can either be occupied by *Spartina* or vacant. The simulated invasion starts from a single occupied cell in the center of the lattice, representing a single 1-m^2 clone, and is run for 100 years. In each year the plants grow vegetatively into empty neighboring sites and produce a random number of seeds (distributed according to a Poisson distribution), the seeds disperse exponentially away from the parents and have a small probability of becoming established clones. When two clones become adjacent to one another, that patch of *Spartina* is classified as a meadow. We have two levels of seed production; cells that are classified as clones produce a small number of seeds; cells that are classified as meadows produce a larger number. We created a variant of the model in which we removed the Allee effect by setting the seed production of clones to the same as the seed production of meadows.

We found that the Allee effect dramatically slows the invasion. From 380 runs of the model, with parameters varied over their estimated ranges, we calculated an average increase in area occupied of about 20% per year with the Allee effect; the area occupied by *Spartina* doubles approximately every 5 years. Removing the Allee effect and repeating those runs gave an average of 30% increase per year, a doubling time of less than 4 years (Taylor et al. 2004) (Fig. 1). This result is consistent with theoretical investigations of the consequences of an Allee effect in invasions (Lewis and Kareiva 1993, Wang and Kot 2001, Wang et al. 2002).

ANALYTICAL MODEL OF *SPARTINA* SPREAD

We then created a non-spatial analytical model of this same process (Taylor et al. 2004). We kept some of the structure of the simulation model by using as the main variables the area occupied by seedlings (S), the area occupied by clones (C) and the area occupied by meadows (M). Other variables are the number of individual clones (N_C) and the number of meadows (N_M).

The main equations of this model are:

$$S_{t+1} = f_C C_t + f_M M_t \qquad (1)$$

$$C_{t+1} = S_t + (1-\eta) g_c C_t \qquad (2)$$

$$M_{t+1} = \eta g_c C_t + g_M M_t \qquad (3)$$

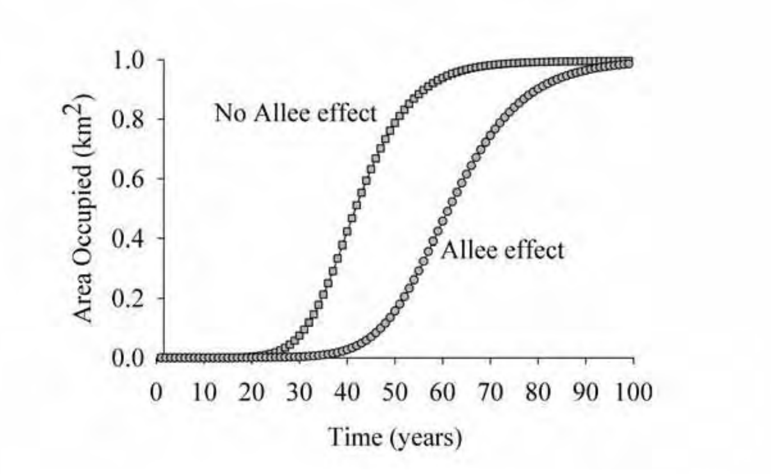

Fig. 1: Mean area occupied by *Spartina* as predicted by 380 runs of the simulation model with and without the Allee effect. The upper line (squares) shows the predicted area occupied when there is no Allee effect and the lower line (circles) shows the predicted area occupied with the Allee effect. A modified form of this figure was originally published in Taylor et al. 2004, *Ecology* 85(12).

Equation 1 says that seedlings are created from the seeds produced by clones and seeds produced by meadows. The fecundity of the clones (f_C) is much smaller than the fecundity of the meadows (f_M) when there is an Allee effect. We remove the Allee effect in the same way as in the simulation model, by setting f_C equal to fM. Equation 2 says that the area occupied by clones increases by seedlings becoming clones and also by vegetative spread of existing clones (at growth rate g_C) and decreases by a proportion of the area occupied by clones (η) coalescing into other patches of *Spartina* and becoming meadow. Equation 3 describes the dynamics of area occupied by meadow which increases by the clones coalescing into meadow at rate η and also by vegetative growth of existing meadow (growth rate g_M). These equations are only a partial description of the model. The full model includes equations for the dynamics of the number of clones (N_C) and the number of meadows (N_M) and also describes how the parameters (η, f_C,f_M,g_C, and g_M) change with the area occupied by clones and meadows and number of clones and meadows. A full description of this model is provided in (Taylor et al. 2004). Several comparisons of the analytical model with the simulation model give us confidence that the two models predict the same dynamics. We use the analytical model to find optimal density-based eradication strategies. Full details of this work are given in Taylor and Hastings 2004, and we give a brief summary here.

MANAGEMENT STRATEGIES

Efforts to control or eradicate *Spartina* in Willapa Bay began in the early 1990s and it soon became obvious that eradicating this plant this was not going to be easy or cheap. *Spartina* can be removed mechanically by mowing or digging and it can be killed with herbicide but all of the methods require specialized equipment and repeated treatments (Patten, this issue). Large scale efforts to control this invasion began in 2003 and current plans predict that the invasion will be eradicated within ten years if the current level of control is maintained (Murphy 2003).

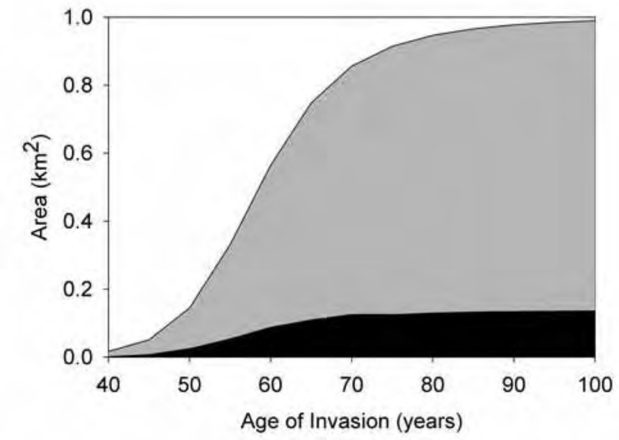

Fig. 2: Grey area shows area occupied by *Spartina* invasion of different ages as predicted by the analytical model and black area shows minimum area that needs to be removed each year for eradication to succeed within ten years.

We used the model to explore two questions about control. First, how much *Spartina* has to be removed each year in order to clear this invasion? Second, is it better to target the high density meadows that are producing most of the seed but not spreading vegetatively very quickly; or is it more effective to target the low density clones that produce very little seed (because of the Allee effect) but spread vegetatively relatively much more quickly because they are surrounded by open mud?

We assume a fixed annual budget and that there is a fixed cost per square meter of removing *Spartina*; this means that a fixed maximum area can be removed each year. We define a control strategy as (1) T_t : the actual area of *Spartina* removed in each year t and (2) X_t the proportion of the area removed that is meadow in each year t. For a control program that lasts ten years there will be ten values of T, one for each year and ten values of X. We only consider control of clones and meadows (not seedlings) so $(1 - X_t)$ is the proportion of the area removed that is clones. We model control of clones by subtracting $T_t(1 - X_t)$ from the left hand side of equation 2 and we model control of meadows by subtracting T_tX_t from the left hand side of equation 3 (Taylor and Hastings 2004).

In order to be considered successful the control strategy has to completely eradicate the invasion within ten years. For a successful strategy, there are two other objectives. The first is to minimize the total cost which is the same as minimizing the total area removed over the ten years (since we are assuming a fixed cost per square meter). The second is to minimize the risk that this one site of the invasion poses to other sites in Willapa Bay. We assume that the risk of seeds escaping and colonizing other mudflats in the bay is linearly proportional to the total number of seeds produced during the ten years of control. In order to minimize both cost and risk, we minimized the product of the two quantities. We used a genetic algorithm to find the optimal strategy; for details of the optimization see Taylor and Hastings (2004). In the case when the annual

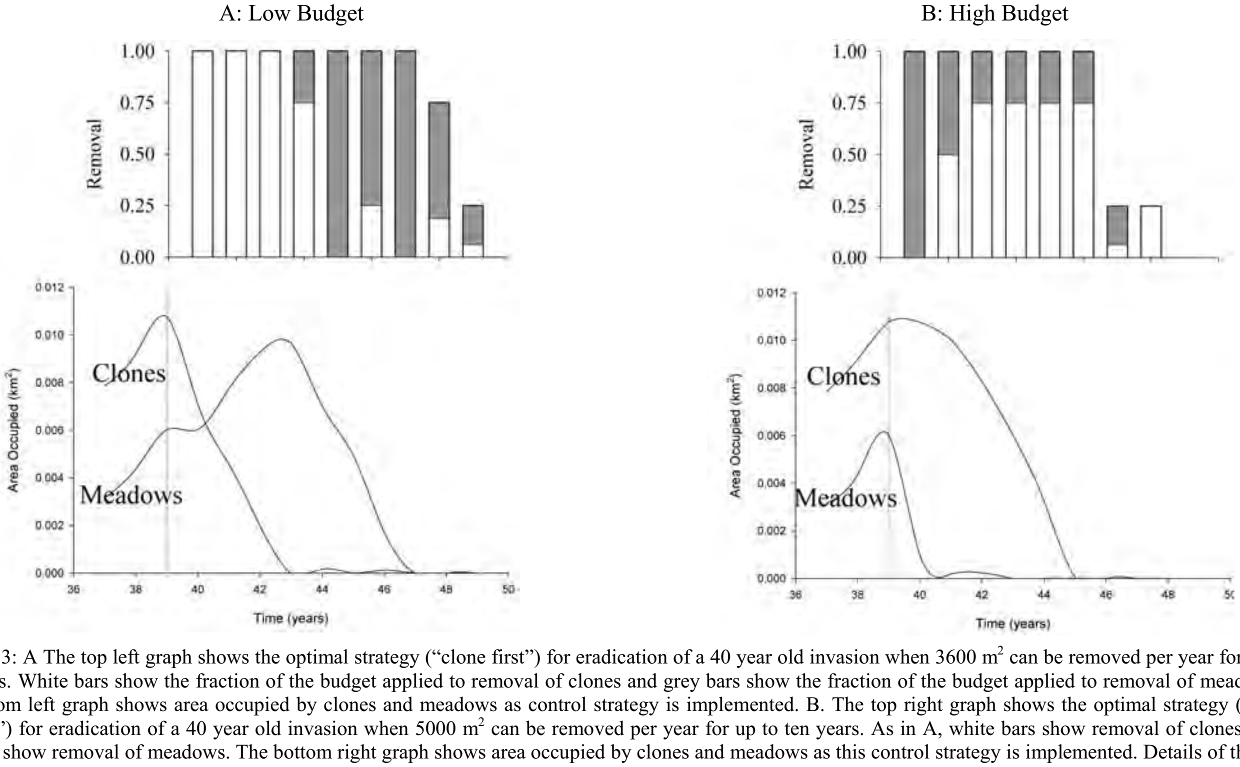

Fig. 3: A The top left graph shows the optimal strategy (“clone first”) for eradication of a 40 year old invasion when 3600 m^2 can be removed per year for up to ten years. White bars show the fraction of the budget applied to removal of clones and grey bars show the fraction of the budget applied to removal of meadows. The bottom left graph shows area occupied by clones and meadows as control strategy is implemented. B. The top right graph shows the optimal strategy (“meadow first”) for eradication of a 40 year old invasion when 5000 m^2 can be removed per year for up to ten years. As in A, white bars show removal of clones and grey bars show removal of meadows. The bottom right graph shows area occupied by clones and meadows as this control strategy is implemented. Details of the optimization are given in text and in Taylor and Hastings (2004).

budget is lower than is necessary to eradicate the invasion, the optimization algorithm is unable to find a strategy that removes all the *Spartina* but instead finds the strategy that minimizes the area occupied at the end of the control period.

We calculated the minimum annual budget in terms of area removed that is necessary to eradicate an invasion within ten years when starting control after 40 to 100 years of unchecked invasion. The results are shown in Fig. 2. The necessary annual budget depends on the starting time and size of the invasion but roughly speaking it is necessary to remove annually at least the equivalent of about 15 to 20% of the starting area of the invasion.

We found that the optimal allocation of resources (removal of clones versus meadows) depends on the annual budget level (Fig. 3). For a 40 year old invasion that occupies ~17,000 m^2, if the budget is low (in this case removing at most 3600m^2 per year; an area equivalent to about 22% of the initial invasion), the optimal strategy is to remove clones first allowing the meadows to initially expand and then remove meadows once the clones are largely removed. If the budget is high (removing at most 5000 m^2 per year; an area equivalent to 30% of the initial invasion), the optimal strategy is to remove the meadows first, allowing the clones to expand and start removing clones in a later year. We also found that the clone-first strategy is optimal when minimizing cost only and ignoring the risk posed by escaping seeds and that the clone-first strategy is the only possible way to eradicate an invasion when the budget is low. The meadow-first strategy can only be used when the budget is high and it is optimal because it substantially reduces the risk of escaping seed although it does not substantially reduce the cost. We also found that the clone-first strategy is optimal when there is no Allee effect since; in this case, the clones produce as much seed as the meadows but grow faster vegetatively (Taylor and Hastings 2004).

CONCLUSIONS

We conclude that the Allee effect caused by pollen limitation has dramatically slowed down the spread of this plant and affects the optimal eradication strategy. The optimal control strategy illustrates the importance of vegetative spread in this plant. To control an invasion with limited or uncertain resources, the best and only viable strategy is to prioritize the removal of the fast growing, low density clones even though they produce very few of the new seedlings. With higher budgets and taking into account the risk of seed escaping to colonize other sites, the optimal strategy is to prioritize removal of the slower-growing, high density meadows.

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HYBRID CORDGRASS (*SPARTINA*) AND TIDAL MARSH RESTORATION IN SAN FRANCISCO BAY: IF YOU BUILD IT, THEY WILL COME

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Keywords: invasive species, salt ponds

Restoration sites built on former salt ponds (Fig. 1) present an ideal combination of biotic and abiotic conditions for *Spartina* regeneration by seed. First, they are unvegetated so seedlings grow unhindered by competition with established plants. Second, they are graded to present a range of intertidal elevations enhancing the opportunity for colonization at elevations that are neither too saline, nor receive too much tidal innundation for cordgrass seedling germination and growth. Third, tidal waters enter through levee breaches (see Fig. 1), muting the force of the waves and reducing seedling loss due to physical removal by tidal action. And finally, many of the restored marshes in the southern and eastern regions of the Bay are near marshes invaded by hybrids, or are actually fed by tidal water which travels though hybrid *Spartina*-choked channels (i.e. Alameda Creek and Alameda Flood Control Channel; se e # in Fig. 2), thus ensuring a tidally-borne seed bank rich in floating hybrid *Spartina* seed.

The result is that recent restoration sites in the area have been quickly colonized by large numbers of hybrid *Spartina* (Table 1). This pattern can be expected to continue as more new marshes are opened until hybrid cordgrass is controlled. Hybrid cordgrass thus presents a substantial challenge to marsh restoration that is particularly relevant in light of the planned restoration of 25 square miles of salt ponds in the South Bay (Fig. 2).

Fig. 1. Cogswell Marsh in Hayward, CA is a former salt pond opened to tidal action (note levee breaches along the shoreline and within marsh denoted by ^) in 1980. Non-working salt ponds are at the left and top of the photo (photos provided by California Coastal Conservancy).

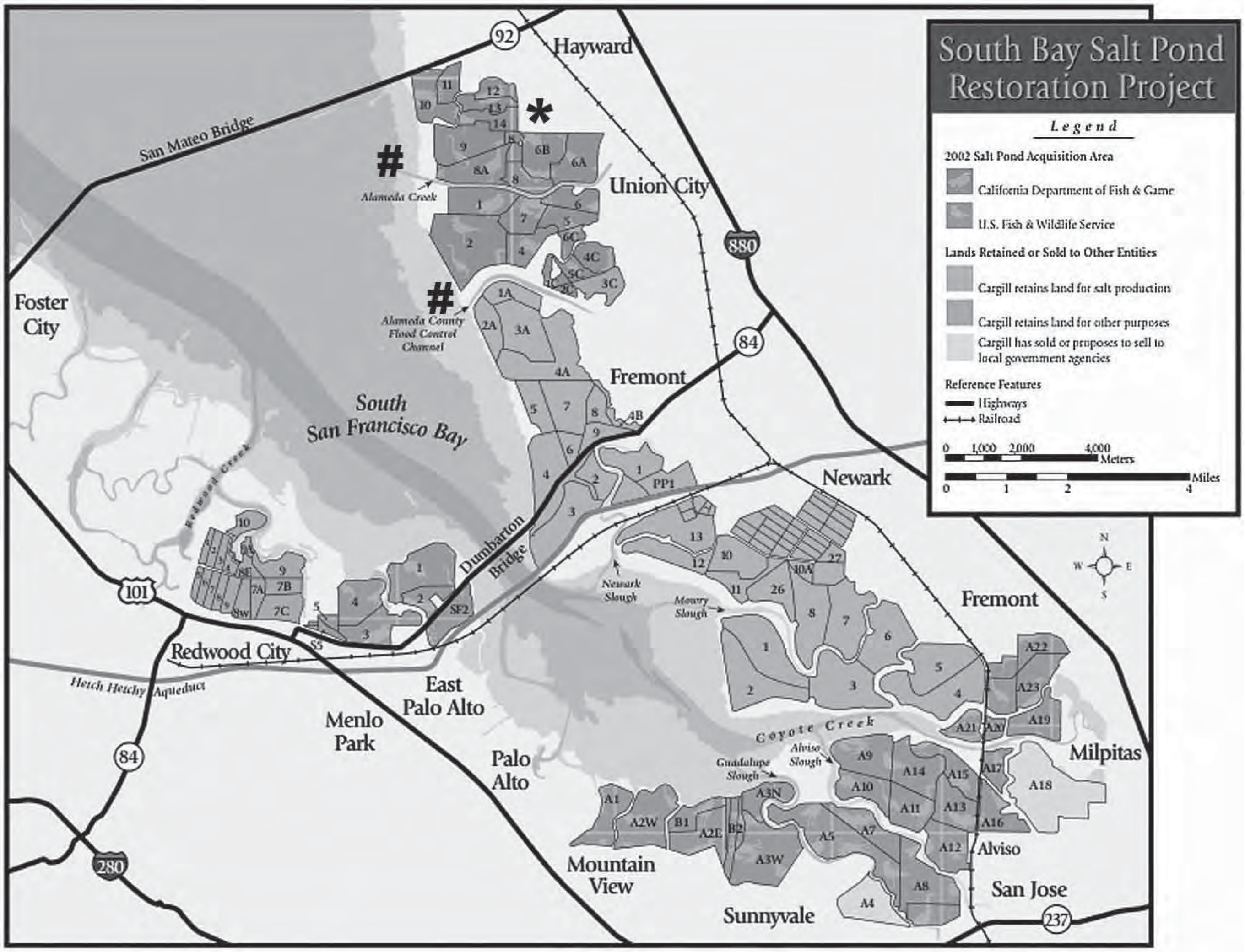

Fig. 2. Twenty-five-square mile South Bay Salt Pond Restoration Project which will result in large-scale restoration of wetlands from the San Mateo Bridge to the southern end of SF Bay. Pound sign (#) denotes hybrid *Spartina* invaded creeks and channels in the area; asterisk (*) is the Eden Landing restoration site (map from http://www.southbayrestoration.org/images/map.gif).

Table 1. Percentage of hybrid plants as of 2004, determined by genetic analysis, in restored tidal marshes opened to tidal action. Question marks indicate uncertainty in opening dates and subsequent invasion by hybrid cordgrass.

Marsh	Year Opened	Acreage	% hybrid plants
Cogswell	1980	250	94
Oro Loma	1997	364	73
Arrowhead	1998	70	100
Eden Landing (*Fig. 2)	2004?	836	??
South Bay Salt Ponds	??	15,100	??

CHAPTER THREE

Ecosystem Effects of Invasive *Spartina*

ASSESSMENT OF THE POTENTIAL CONSEQUENCES OF LARGE-SCALE ERADICATION OF *SPARTINA ANGLICA* FROM THE TAMAR ESTUARY, TASMANIA

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Spartina anglica is a vigorous exotic perennial salt marsh grass typically inhabiting the upper intertidal zone of temperate estuaries. Following its introduction into the Tamar Estuary, Tasmania, patterns of sediment deposition and erosion have been significantly altered. This paper presents the background to an interdisciplinary approach used to determine the potential impacts of the wide scale eradication of *S. anglica*, from what is now Australia's largest infestation. Analysis of these interdisciplinary lines of inquiry have provided a greater understanding of the biogeomorphological responses to restoration attempts within intertidal zones and have provided a sound basis on which to formulate and implement future management of *Spartina*.

Keywords: *Spartina anglica***,** Estuaries, salt marsh, intertidal zone, geomorphology, sward, sedimentation, erosion, accretion, eradication, surveying

INTRODUCTION

Spartina anglica, (rice grass or cordgrass) is a vigorous exotic perennial salt marsh grass typically inhabiting the upper intertidal zone of temperate estuaries. *Spartina anglica* influences marsh development through the ability of the canopy to promote sediment deposition (Shi et al. 1995), and the ability of the dense rhizomes and roots to increase the erosion resistance of the substrate (Brown 1998a; Brown et al. 1998b; Van Eerdt 1985). These properties, along with its ability to establish and spread rapidly are largely the reason for its deliberate introduction to temperate estuaries throughout the world (Chapman 1960; Lee and Partridge 1983; Ranwell 1967).

Accelerated accretion rates following the establishment of *S. anglica* have been observed in the Netherlands (Van Eerdt 1985), China (Chung 1990), New Zealand (Lee and Partridge 1983) and in Poole Harbour, United Kingdom (Long et al. 1999). However, compaction and settling have been found to be important components of sedimentation that may, in some instances result in negligible change in topographic height of mudbanks (Lee and Partridge 1983). Furthermore, studies of Louisiana marshes suggest that root growth and below-ground accumulation of organic matter, rather than inorganic matter governs the maintenance of salt marshes in the vertical plane (Hatton 1983; Nyman 1995; Turner 2004).

This paper provides the background to an interdisciplinary and precautionary study that was conducted between 2002 and 2007 as part of a PhD research project aimed at assessing the impacts of the wide scale eradication of *S. anglica* from the Tamar Estuary, Tasmania. Methodology and results of this study have been reported elsewhere (Sheehan & Ellison, 2007; Sheehan, 2008).

Background

The Tamar Estuary (Fig. 1) extends some 71 kilometers (km) inland from Bass Strait through to its tidal extent at the city of Launceston. The Tamar is a semi-diurnal mesotidal system with tides ranging from 1.3 meters (m) at Low Head, to 4 m at Launceston. The Tamar is well supplied with sediment from the South and North Esk Rivers, which, in the narrow and poorly flushed estuary, tend to accumulate as fine-grained silt deposits in the upper reaches of the system both within the channel and on adjacent mudflats and shoals. The average rate of siltation has been estimated at 30,000 cubic meters (m^3) per year, though this may vary between 10,000 and 90,000 depending on variation in river flow (Foster et al. 1986). Sedimentation in the Tamar's upper reaches has been an issue of long standing concern, both for reasons of shipping channel maintenance and environmental quality.

Introduction of Spartina

Spartina anglica was introduced in 1947 at Windermere (Fig. 1) in an attempt to stabilize sediments and safeguard against further siltation of the shipping channel at a time when commercial water traffic travelled the length of the estuary (Phillips 1975; Pringle 1993; Ranwell 1967). Mudflats and shoals in the upper Tamar were prone to severe erosion during the combination of flood tide and north-west winds. It was thought that stabilizing the mudflats would promote vertical accretion, defining the channel to enhance scour, and hence reducing the likelihood of further siltation.

In 1997, surveys showed that *S. anglica* covered some 420 hectares (ha) (4.2 km^2) of intertidal zone within the

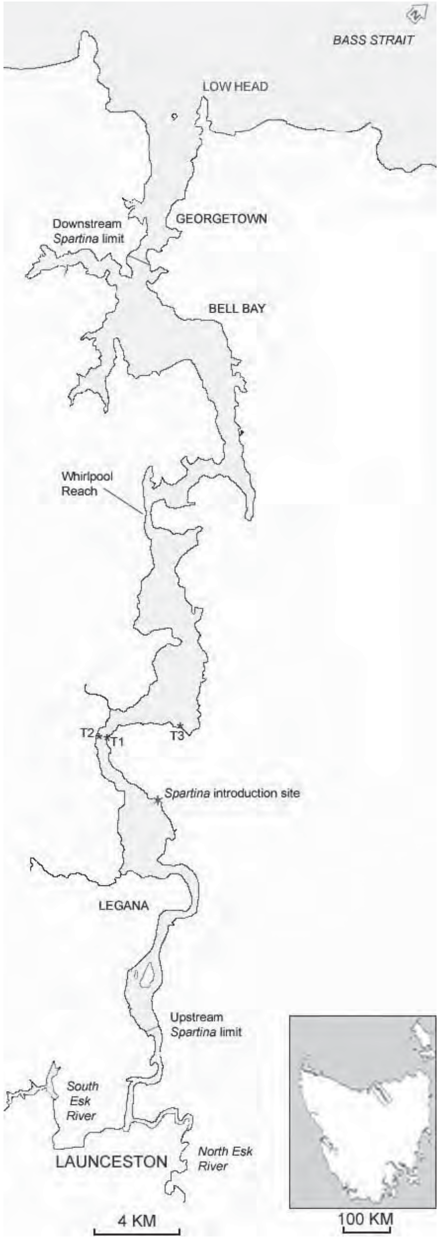

Fig. 1: Tamar Estuary, Tasmania.

Tamar; however, recent surveys indicate that it continues to spread throughout the lower estuary (Hedge 1998).

Although the introduction of *S. anglica* has improved navigability in the Tamar Estuary (Pringle 1993; Wells 1995), it is now considered a pest species as the current generation of natural resource managers and land-holders consider its progressive invasion to be a major threat to the integrity of inter-tidal coastal ecosystems and wetlands of international importance (Doody 1990; Wells 1995).

The present management strategy for *S. anglica* within the Tamar is one of containment rather than eradication due to the complex nature of the Estuary as outline above. There is, however, community and industry pressure to eradicate *S. anglica* from the banks of the Tamar.

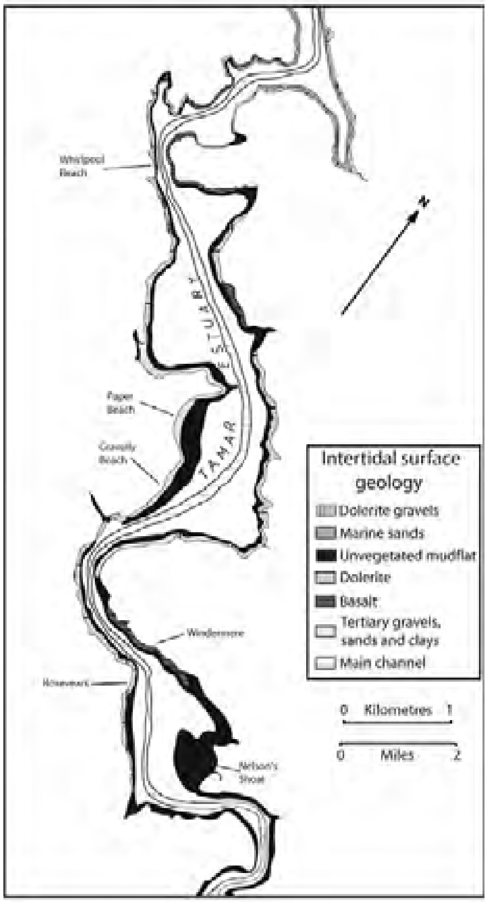

Fig. 2: Physiographic units of the Tamar Estuary intertidal zone.

GEOMORPHOLOGY OF THE INTERTIDAL ZONE

The intertidal zone within the Tamar consists of four distinct physiographic units (Fig. 2):

1) Narrow mudflats of recent alluvial deposits largely of terrestrial origin entering the system via the North Esk and the South Esk Rivers. These have accumulated predominantly in the upper estuary, however this unit can also be found in the mid and lower intertidal zones of the mid estuary or in larger embayments that receive sediment from minor tributaries;

2) A laterally extensive area of outcropping substrate which has weathered *in situ* to form a narrow boulder beach throughout the mid and some parts of the lower Tamar. This formation consists largely of Dolerite but also some Miocene basalt shorelines at Whirlpool reach;

3) Extensive beaches within the mid Tamar consisting of a thin veneer of Tertiary sands and gravels underlain by clays. The clays are likely to be lacustrine deposits of the Miocene while the sand and gravel are indicative of high-energy fluvial environments, which would have been present during the Pleistocene; and

Fig. 3: Native salt marsh communities of the lower salt marsh being progressively invaded by *S. anglica* clones, establishing seaward of the native vegetation.

Source: T. Coles

a) b)

Fig. 4: The intertidal zone of the Tamar at Paper Beach in 1956 (a), showing the sand and gravel substrate, and in 2004 (b) *Spartina* marsh extending some 140 m seaward.

Fig. 5: One of the few remaining sandy intertidal zones of Tamar, the upper intertidal zone is typically sand and gravels grading into mudflat.

4) Marine sands, limited to the lower estuary close to the mouth at Low head.

VEGETATION OF THE INTERTIDAL ZONE

Prior to the establishment of *S. anglica***,** much of the intertidal zone, particularly in the mid Tamar, was unvegetated. *Enteromorpha* and other algae were found extensively on mudflats at all elevations within the intertidal zone throughout the estuary. The absence of higher native salt marsh plants is probably due to their inability to colonize the various intertidal geologies and promote the accumulation of fine silts. Native salt marsh vegetation, such as *Sarcocornia quinqueflora*, *Sclerostegia arbuscula*, and *Suaeda australis* is limited to a narrow fringe below the high water mark and in sheltered embayments, with extensive salt marshes occurring only near Bell Bay, on relatively sandy substrates in the lower estuary (Fig. 3). The ability of *S. anglica* to establish at elevations lower than native salt marsh plants provides a valuable competitive advantage. *Spartina anglica* has successfully colonized in isolated clumps, coalesced to form laterally extensive swards seaward of the native salt marsh, and progressively move landward into the native vegetation.

Fig. 6: *Spartina anglica* colonization of a dolerite boulder beach at East Arm.

Fig. 7: Mature *Spartina* marsh at Lone Pine point, previously a Dolerite Boulder beach.

Fig. 8: A prograding *Spartina* marsh, with the coalescence of isolated clumps characteristic of *Spartina* colonization.

IMPACTS OF SPARTINA INVASION

The introduction of *S. anglica* to the Tamar Estuary has brought about a dramatic and rapid change to the physiography of the intertidal zone, illustrated well at a photo point established in 1956 at Paper beach (Fig. 4a and b). The colonization of what was essentially a vacant niche has transformed the gently grading sandy intertidal zones, as shown in Fig. 5, and hard rock intertidal zones (Fig. 6) into laterally extensive muddy terraces (Figs. 7 and 8).

The ecological impacts of *Spartina* invasion in temperate estuaries of South Eastern Australia are poorly understood. Studies of benthic macro-invertebrate communities in the Little Swanport Estuary, Tasmania suggest that *Spartina* significantly increases macro-invertebrate species richness and total species abundance when compared to previously non-vegetated intertidal areas (Hedge 1997). Additionally, macro-invertebrate communities of *Spartina* marshes showed remarkable similarity to those of native salt marshes (Hedge and Kriwoken 2000). It is suggested therefore that *Spartina* invasion has provided a niche of soft substrate and dense protection, favoring macro-invertebrates and some birds such as the purple swamphen (*Porphyrio porphyrio*). It is likely that this subsequently caused a displacement of species that formerly inhabited or utilized the intertidal zone. Species assemblage and utilization of the intertidal zone prior to *Spartina* is not well documented, but likely was relatively rich in fish and bird species.

DISCUSSION

This paper has provided background for the research project that assessed the alteration to the intertidal zone of the Tamar Estuary. Using transect based topographic surveys and coring, the volume of material trapped under *Spartina* was calculated to be 1,193,441 m^3, comprised of approximately 17% *Spartina*-derived organic matter and 83% silts and clays. Based on historical profiles, sedimentation rates since the introduction of *S. anglica* have been estimated at between 8.7 and 52.4 millimeters per year (mm yr-1).

From the analysis of 80 cores from four sites, *Spartina*-trapped sediment was found to contain levels of cadmium, copper, lead and zinc elevated above background levels. However, these generaly were below trigger values of the ANZECC/ARMCANZ (2000) interim sediment quality guidelines. It is considered unlikely that released sediments would impact on water quality or health of biota with respect to trace metals or organic contamination.

Behaviour of sediment with respect to erosion rates, sediment redeposition and causative hydrodynamics were also monitored within a test area from which *Spartina* cover was removed. It has been demonstrated that the eradication of *S. anglica* will result in elevation loss from the *Spartina* marsh surface at a rate six times greater than in vegetated marshes. The study assumed that this elevation loss is caused by liberation of sediments. The rate of elevation loss or erosion is likely to increase once the dead *S. anglica* root mat decomposes and the surface cohesion and sediment-binding capacity is diminished. Elevation loss increases by a factor of 1.06 with every 10 m from the high water bank. Erosion rates in the outer 40 m of the marsh were also significantly greater than the remainder of the marsh at both sites, suggesting that a process other than *S. anglica* removal is contributing to that retreat of the lower marsh.

Analysis of these interdisciplinary lines of inquiry have allowed for a greater understanding of the biogeomorphological responses to restoration attempts within intertidal zones and have provided a sound basis on which to formulate and implement future management of *Spartina*. *Spartina* eradication is recommended for the lower estuary (type 2 marshes) only, where trapped sediment volumes are significantly smaller and tidal flushing is greatest. This would enable significant areas of sand/gravel intertidal zones to recover and reduce the likelihood of further downstream expansion of *Spartina* swards. Retaining type 1 marshes in the upper estuary will prevent the remobilization of sediments that contain the highest concentrations of contaminants, and will retain the marshes for the ecological role they currently perform.

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SPARTINA INVASION CHANGES INTERTIDAL ECOSYSTEM METABOLISM IN SAN FRANCISCO BAY

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In San Francisco Bay, Atlantic smooth cordgrass, *Spartina alterniflora,* and its hybrids have invaded unvegetated mudflats and native marshes formerly dominated by *Sarcocornia pacifica* and *Spartina foliosa*. This recent, rapid invasion has dramatically changed ecosystem processes and food web structure. We measured sediment fluxes of carbon dioxide (CO_2) to determine microalgal gross primary production (GPP), sediment respiration and net sediment metabolism in native intertidal areas (mudflats, *S. pacifica* and *S. foliosa*) and in adjacent areas invaded by hybrid *Spartina*. Sediment microalgal GPP and microalgal chlorophyll *a* were substantially higher in all native habitats than in the hybrid. At the same time, sediment respiration rates were generally higher in native vegetation than in hybrid-dominated habitats, but substantially lower on the mudflats than elsewhere. Net sediment metabolism switched from autotrophy to heterotrophy following invasion of mudflats. However, the higher respiration rate in native vegetation, especially *S. pacifica*, relative to hybrid areas, suggests slower decomposition of hybrid detritus. This result is corroborated by litterbag decomposition rates and indicates a build-up and/or export of refractory organic matter following hybrid invasion. The switch from a microalgal-dominated system to a refractory detritus-dominated system has clear implications for support of higher trophic levels within the intertidal zone of San Francisco Bay.

Keywords: ecosystem metabolism, carbon cycling, *Spartina alterniflora*, *Sarcocornia* sp., *Spartina foliosa*, benthic microalgae

INTRODUCTION

One of the most serious threats to natural ecosystems and the maintenance of ecosystem services is the invasion of non-native plant species (Drake et al. 1989; Vitousek et al. 1997). These threats may include the extinction of native species, loss of functional native diversity, changes in nutrient cycling and organic matter storage and loss of habitat. One of the most dramatic invasions in coastal systems in western North America has been the invasion of *Spartina alterniflora* (smooth cordgrass), a native of western Atlantic marshes. This invasion has fostered large-scale alterations in ecosystem processes in commercially and ecologically important estuaries in both California and Washington.

S. alterniflora was first introduced into San Francisco Bay by the U.S. Army Corps of Engineers in 1973 for marsh restoration (Faber 2000). Subsequent hybridization with the native cordgrass, *Spartina foliosa*, resulted in a highly successful hybrid population (henceforth hybrid *Spartina*) that has colonized ~500 acres, in south and central portions of the Bay (Daehler and Strong 1997; Ayres et al. 2004). Hybrid *Spartina* occupies a wide tidal range that includes historically unvegetated mudflats and native marshes once dominated by *S. foliosa* and *Sarcocornia pacifica* (Callaway and Josselyn 1992).

The *Spartina* invasion has greatly altered the cycling of carbon (C) in the intertidal zone. Hybrid *Spartina* forms dense clones, with an average aboveground biomass of 1.8 ± 0.3 kilograms per square meter (kg m^{-2}) at the end of the growing season (fall; Tyler et al. 2007). Much of the aboveground portion senesces during the winter, but the substantial belowground roots and rhizomes (mean = 4.2 ± 0.8 kg m^{-2}) persist year-round (Tyler et al. 2007). The carbon to nitrogen (C:N) ratio of *Spartina* is higher than the native vegetation, particularly when compared to microalgae, and the slow decomposition of this refractory detritus has resulted in substantial deposits of belowground organic matter (Neira et al. 2005). We do not know how overall ecosystem production and respiration are changed as a result of this invasion, but this is clearly important in understanding the overall impact of invasion on estuarine ecosystem function.

The lower productivity and low stature of native southern California salt marsh plants, which results in more light reaching the sediment surface, may promote microalgal productivity that is generally greater than that of the vascular plants (Zedler 1984). In contrast, primary production in Atlantic and Gulf coast marshes is generally dominated by *S. alterniflora* (Zedler 1980). Studies of salt marsh food webs on both the Atlantic and Pacific coasts show that microalgae may contribute up to 50% of the C assimilated by invertebrates and higher trophic levels (Page 1995; Deegan and Garritt 1997; Kwak and Zedler 1997; Page 1997). The impact of *Spartina* invasion on microalgal productivity within vegetated marshes of San Francisco Bay is not well understood. However, because algae are more readily assimilated than detrital *Spartina*, the invasion of *Spartina* may ultimately reduce the availability of primary production to higher trophic levels (i.e., consumers).

The results presented here are a preliminary analysis of the impact of invasive *Spartina* on microalgal production, sediment respiration and net sediment metabolism. Future analysis of these data, in combination with detailed estimates of vascular plant production and decomposition rates will ultimately give us a more complete understanding of how *Spartina* influences overall ecosystem functioning in the intertidal areas of San Francisco Bay.

METHODS

Sediment CO_2 fluxes and benthic chlorophyll *a* (Chl *a*) were measured in native- and hybrid-dominated areas at four sites in South San Francisco Bay in December 2003 and March, June and September 2004 (Fig. 1). Two sites, Cogswell marsh (Hayward Regional Shoreline) and San Mateo marsh, represent areas formerly dominated by pickleweed, *S. pacifica*. The Elsie Roemer Bird Sanctuary on Alameda Island and the San Lorenzo marsh at Robert's Landing are mudflats currently being invaded by hybrid *Spartina*. Also at San Lorenzo, areas of native *S. foliosa* are overrun with hybrid *Spartina*.

Sediment CO_2 fluxes were measured in polycarbonate cores as described in (Neubauer et al. 2000). Briefly, polycarbonate cores (9.3 centimeters inside diameter [cm I.D.]) were inserted 5 cm into the sediment (headspace approximately 800 cubic centimeters [cm^3]). Incurrent and excurrent tubes were fitted into the sealed lid and connected to a LiCor LI820 Infrared CO_2 Gas Analyzer. Air flow was maintained at approximately 500 millileters per minute (ml min^{-1}) using a small electric air pump. Prior to each sampling period the Gas Analyzer was calibrated using CO_2 standards (Scott Specialty Gases, 0 parts per million [ppm] and 1,007 ppm CO_2). All measurements were conducted between approximately 10 am and 2 pm during low tide. Each core was darkened using plastic pots coated with aluminum foil for a minimum of 30 minutes prior to making measurements. We recorded CO_2 concentrations in the core every two seconds for 4-6 minutes in the dark and then removed the darkening pot. After waiting another 4-6 minutes, the CO_2 concentrations in the light were recorded.

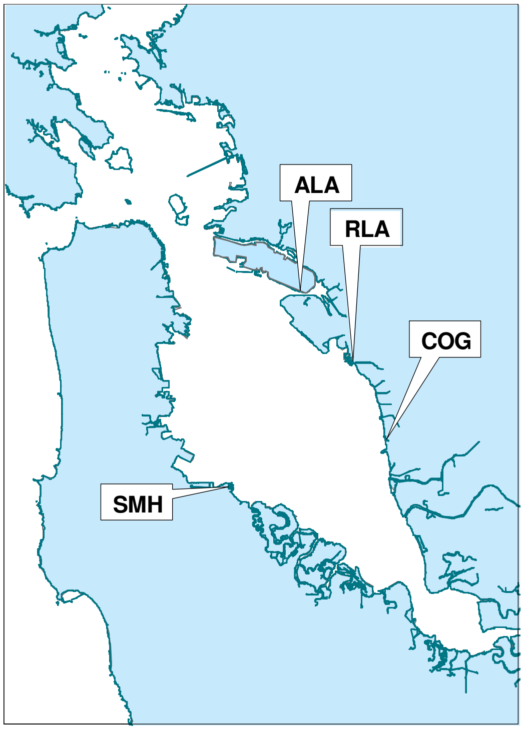


Fig. 1. South San Francisco Bay showing sampling sites: Elsie Roemer Bird Sanctuary (ALA), Robert's Landing (RLA), Cogswell (COG) and San Mateo (SMH).

Three replicates were performed in each area at each site. Fluxes were estimated based on the slope of the change in concentration over time using the equation:

$$J = \frac{dC}{dt} \bullet \frac{V}{A}$$

where J is the flux rate in micromoles per square meter per hour (μmol m^{-2} h^{-1}), A is the core area, V is the headspace volume, C is the concentration of CO_2 and t is time.

Light at the sediment surface and at the top of the canopy was recorded every two minutes during the sampling period using a Li-Cor model LI1400 meter with a 4π spherical quantum sensor. Sediment temperature adjacent to each core was measured using an analog soil temperature probe. Following each set of measurements two small (1.1 cm I.D. x 0.5 cm depth) cores were taken for Chl *a* analysis. These samples were kept frozen until sonication of the sediment with cold 90% acetone, overnight extraction in the freezer and standard spectrophotometric measurement.

Hourly gross primary production (GPP) was calculated from the difference between CO_2 fluxes in the light and the dark by assuming that sediment respiration was the same in the light and the dark. Daily GPP was calculated by multiplying hourly GPP by the numbers of hours of light on the day of measurement. Net daily sediment metabolism was calculated by summing daily GPP and hourly respiration x 24. Data were pooled across sampling dates and statistical comparisons were made (ANOVA) between GPP, sediment respiration, light at the sediment surface, and Chl *a* measured in hybrid *Spartina* and native areas for each of the three native conditions (mudflats, *S. foliosa*, *S. pacifica*). Mudflats and *S. pacifica* were represented by two sites and *S. foliosa* by a single site (San Lorenzo).

RESULTS

We found significant differences for several variables between native and *Spartina* hybrid-invaded habitats in South San Francisco Bay.

GPP

Microalgal GPP was greater in native habitats than in hybrid *Spartina* in all cases (Fig. 2A). This difference was significant for the mudflat-hybrid comparison and the *S. pacifica*-hybrid comparison, where GPP was more than two times greater in *S. pacifica*.

Sediment Respiration

Hybrid *Spartina* had significantly greater sediment respiration rates than those found on the mudflat (Fig. 2B). In contrast, both *S. foliosa* and *S. pacifica* had higher respiration rates, but only significantly so for *S. pacifica*.

Net Sediment Metabolism

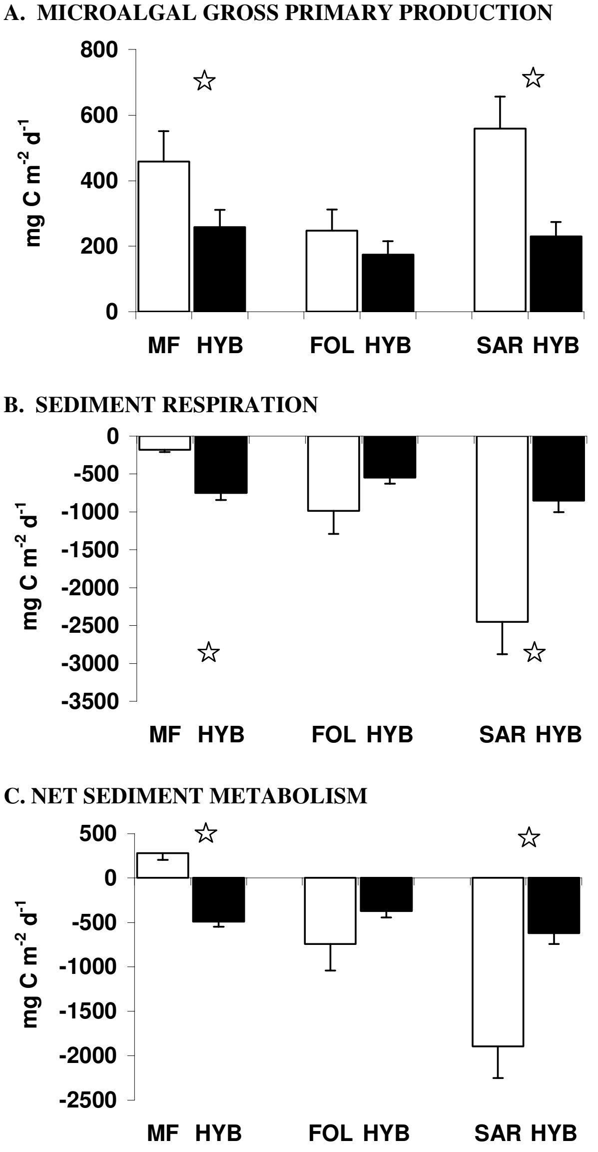
Invaded mudflats switched from net autotrophic (positive net metabolism) to net heterotrophic (negative net metabolism) upon invasion by hybrid *Spartina* (Fig. 2C). The native habitats, *S. foliosa* and *S. pacifica,* had higher net metabolism than the hybrid. Again, only *S. pacifica* was significantly different from the hybrid.

Light

Spartina hybrid invasion resulted in a significant reduction in light availability at the sediment surface relative to mudflats (18% of available light) and *S. foliosa* (61% in *S. foliosa*; 26% in hybrid; Fig. 3A). Light availability was similar in *S. pacifica* and hybrid habitats (16% and 18% of available light, respectively).

Benthic Chl a

Native habitats had higher Chl *a* than hybrid habitats in all cases, but only significantly so relative to *S. pacifica* (Fig. 3B).


A. MICROALGAL GROSS PRIMARY PRODUCTION

B. SEDIMENT RESPIRATION

C. NET SEDIMENT METABOLISM

Fig. 2. Microalgal gross primary production (2A), sediment respiration (2B) and net sediment metabolism (2C) measured in mudflats (MF), *S. foliosa* (FOL), *S. pacifica* (SAR) and adjacent hybrid areas. Stars represent significant differences between areas ($p < 0.05$) based on ANOVA.

DISCUSSION

The invasion of *S. alterniflora* and its hybrids into the native mudflats and marshes of San Francisco Bay has clearly changed both microalgal production and sediment respiration. As a result net metabolism of the sediments is dramatically different than it was prior to invasion, with the trajectory of change depending on the initial conditions.

The consistent decrease in microalgal GPP and Chl *a* upon invasion may be due to a combination of factors, including light and nutrient availability and grazing. The substantial decrease in available light relative to an unvegetated mudflat is likely important. However, light availability is similar between hybrid and *S. pacifica* habitats, suggesting the importance of another mechanism. The current estimate of GPP is likely an overestimate of actual GPP because our measurements were made at the time of peak solar insolation and at low tide. Future refinement of these results will include an adjustment of GPP based on photosynthesis-irradiance curves for each habitat type and the reduction in photosynthesis observed

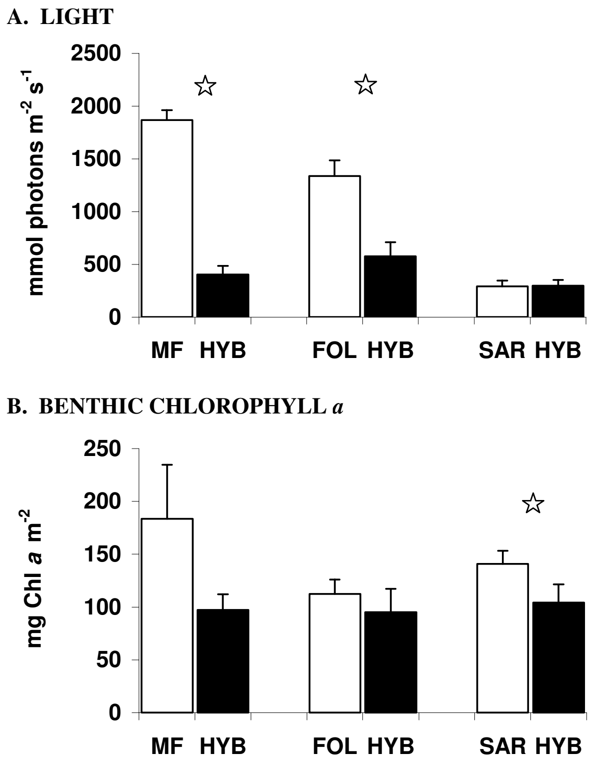


Fig. 3. Light measured at the sediment surface (3A) and benthic chlorophyll *a* (3B) measured in mudflats (MF), *S. foliosa* (FOL), *S. pacifica* (SAR) and adjacent hybrid areas. Stars represent significant differences between areas ($p < 0.05$) based on ANOVA.

during flooding, which can be 25–49% (Holmes and Mahall 1982; Pinckney and Zingmark 1993). In spite of the limitations of our current GPP estimates, there is a clear and consistent decline in microalgal abundance. This decline has very important ramifications for higher trophic levels. Infaunal invertebrate abundance and diversity declined 75% upon invasion of mudflats at the Alameda site (Neira et al. 2005). While there are a number of factors that may contribute to this loss, including a lack of available space, slower water velocities, and predation (Neira et al. 2005), the availability of microalgae as a food source is also a key contributor (Levin et al. 2006; Grosholz et al. 2009).

The massive increase in belowground biomass and organic matter that occurs when mudflats are invaded by *Spartina* is the likely cause of higher respiration rates in hybrid habitats. Both microbial and root respiration should be higher in invaded areas. However, higher respiration rates in native vegetation relative to hybrid areas are more difficult to interpret. We did not observe an increase in belowground biomass in hybrid habitats relative to *S. foliosa* or *S. pacifica*. Therefore, root respiration is likely to be similar among these three habitats. However, in separate litterbag experiments, we found that both *S. foliosa* and *S. pacifica* decompose more rapidly than hybrid *Spartina* (Tyler, unpub. data). This is consistent with the higher C:N ratio of hybrid *Spartina* relative to native vegetation. Because the hybrid detritus is refractory and thereby a poor-quality food source for microbes and invertebrates, we would expect the low sediment respiration rates that we did indeed observe. When decomposition rates are low, organic matter will remain intact and will build up in the system over time. The build up of this detritus is also supported by the finding that *Spartina* detritus does not contribute to higher trophic levels as readily as native plant detritus (Brusati 2004; Brusati and Grosholz 2007, 2008).

The difference in net sediment metabolism of hybrid sediments relative to *S. foliosa* and *S. pacifica* is driven largely by the high respiration rates found in native habitats. Hybrid invasion acts to decrease the overall metabolic rate and create an excess inventory of organic matter. The mudflats, however, switched from net autotrophy to net heterotrophy upon invasion, due to higher respiration and lower microalgal photosynthesis. In this case, the invasion acts to eliminate a valuable food source (microalgae) and replace it with a poor quality food source (*Spartina* detritus).

Future refinements to this model of ecosystem metabolism following *Spartina* hybrid invasion will include vascular plant production, above-ground decomposition of vascular plant detritus and the adjustments to microalgal GPP discussed above. From this, we will be able to estimate, on an annual basis, the very significant impact of invasive *Spartina* on ecosystem function in San Francisco Bay. At this point, we can conclude that this invasion has resulted in the replacement of mudflats and marshes containing abundant microalgae and bioavailable detritus by a habitat with lower microalgal productivity and refractory detritus. This change has very important ramifications for higher trophic levels and for the health of the estuary as a whole.

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MECHANISTIC PROCESSES DRIVING SHIFTS IN BENTHIC INFAUNAL COMMUNITIES FOLLOWING HYBRID *SPARTINA* TIDAL FLAT INVASION

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Spartina alterniflora x foliosa hybrids are perennial cordgrasses that have rapidly invaded mudflats and marshes in central and southern San Francisco Bay. Recent studies conducted by the authors at the Elsie Roemer Bird Sanctuary in Alameda (San Francisco Bay) showed a 75% reduction in macrofaunal densities and shift in macrofaunal composition. Here we identify the mechanisms that underlie such observed changes in macrofaunal community structure following tidal flat invasion by the hybrid *Spartina*. Specifically we performed a series of *in situ* manipulative experiments to examine hybrid *Spartina* canopy influence on water motion and water flow speed, larval flux, animal transport, and predation, as mediators of change for sediments and macrobenthos. Overall, hybrid *Spartina* exerted a strong influence on the hydrodynamic regime, reducing water flow that, in turn, influences flux of recruiting larvae, transport of other benthos, and the input of organic matter and sediment deposition. Habitat modification results in poor survivorship of surface-feeding taxa via sulfide toxicity, altered predation pressure and changed food availability. All these mechanisms can play key, possibly synergistic roles in structuring *Spartina*-invaded ecosystems.

Keywords: tidal flat invasion, *Spartina alterniflora*, hybrid *Spartina*, benthos, macrofauna, mechanistic processes

INTRODUCTION

San Francisco Bay is one of the most heavily invaded estuaries in the world with nearly 250 non-native or introduced species (Cohen and Carlton 1998). One of the most serious invasions has been that of the Atlantic cordgrass *Spartina alterniflora* and its hybrids (hereafter “hybrid *Spartina*”). The genetic background of the plants was confirmed by molecular genetic analysis by D. Ayres (unpublished results). Hybrid *Spartina* has invaded more than 800 hectares (ha) of mudflats and marshes in central and south San Francisco Bay (Ayres et al. 2004). This invasive cordgrass is converting mudflats at low tidal levels into dense, nearly monotypic meadows (Ayres et al. 2003, 2004). Of extreme concern is that this plant’s invasion into unvegetated tidal flats will result in a loss of open foraging area for shorebirds and fishes, flow reductions, higher sedimentation rates, changes in light penetration, and reduction of benthic algal production (Zipperer 1996; Daehler and Strong 1996; Stenzel et al. 2002; Grosholz et al. 2009).

To date very little is known about the impact of the hybrid *Spartina* invasion on sediment properties, macrofaunal communities, and ecosystem functioning. What we do know results from recent studies of three sites in San Francisco Bay, including the Elsie Roemer Bird Sanctuary in Alameda (San Francisco Bay, CA, USA) (Neira et al. 2005), which has experienced hybrid *Spartina* invasion for the past 30 years. At this site macrofaunal densities were 75% lower in hybrid *Spartina*-invaded sediments than on adjacent, uninvaded tidal flats (Neira et al. 2005). Biomass was 57% lower in invaded sediments (Levin et al. 2006). We also observed important shifts in species composition in the hybrid-invaded patches relative to tidal flats (Neira et al. 2005). Surface feeders such as *Gemma gemma* (Bivalvia), *Corophium* spp. and *Grandidierella japonica* (Amphipoda), and *Tharyx* sp. and *Eteone* sp. (Polychaeta) were negatively affected by *Spartina* invasion, exhibiting reduced densities. Subsurface-deposit feeders such as capitellid polychaetes and tubificid oligochaetes were less affected or unaffected (Neira et al. 2005). Because surface-feeding taxa are more accessible to epibenthic consumers than capitellid polychaetes and oligochaetes that live deeper in the sediment, the loss of surface feeding taxa could have profound implications for higher trophic levels and hence affect the whole ecosystem. Therefore, the aim of the following work was to experimentally document the causes of the benthic changes identified in the initial mensurative study.

METHODS

The study site was located on Alameda Island (San Francisco Bay) along the shoreline adjacent to Elsie Roemer Bird Sanctuary (37°45’35”N; 122°28’48”W). Detailed description of the study site is provided in Neira et al. (2005). Using a paired sampling design, we established 10 blocks (2x2 meters (m)), approximately 10 m inside the hybrid *Spartina* meadow, and 10 similar blocks on the adjacent open tidal flat approximately 5-10 m from the meadow edge. We performed *in situ* manipulative experiments to identify what mechanisms are responsible for observed shifts in faunal community structure following hybrid *Spartina* invasion.

Water motion: To evaluate the influence of the hybrid *Spartina* canopy on relative water motion, we used the gypsum dissolution technique (Doty 1971). Pre-weighed gypsum cards were deployed about 10 cm above the bottom for 6, 3 and 4 days during April and June 2002 in vegetated and unvegetated tidal flat habitats. It is assumed that dissolution rate of the gypsum, grams per day (g d^{-1}), is proportional to water velocity (Porter et al. 2000). Water velocity in both habitats was measured on several days near maximum ebb and flood tides just below the water surface with a Marsh-McBirney Flow Meter 2000.

Larval flux: The influence of the hybrid *Spartina* canopy on larval flux was measured in vegetated and unvegetated habitats using *Geukensia demissa* (mussel) shells as a hard substrate for settlement. Barnacle larval abundance over time was used as a proxy for larval flux. Three dowels, each with one attached *Geukensia* shell, were inserted into the sediment (1 m^2 plot) in each block of both hybrid *Spartina* habitat and tidal flat (i.e., 60 in total). Mussels remained 10-20 cm above the sediment.

Sediment deposition: We investigated the effects of *Spartina* plant canopy on short-term sediment deposition rates by deploying petri-dish sediment traps (Reed 1992) in both vegetated and unvegetated habitats (10 replicates each) during low tide. Sediment traps were composed of GF/F Whatman filters (9-cm diameter) supported on the petri dishes affixed on the sediment surface. Filters were removed and replaced 24 hours (h) later (one tidal cycle) during two successive days in July 2002. Filters were gently rinsed with distilled water to remove salts on pre-weighed aluminum dishes, oven-dried at 60°C, and re-weighed. Sediment deposition rate was calculated as mass deposited per trap and expressed in milligrams per centimeter squared per day (mg cm^{-2} $d^{-1)}$. Percent organic matter was determined by mass loss after ignition at 500°C for 4 h. Mud content was estimated after wet sieving (63 micrometers(µm)) the sediment deposited on traps and weighing both fractions (> 63 µm and < 63 µm) after drying at 60°C.

Animal transport: The influence of hybrid *Spartina* canopy on animal-transport dynamics was evaluated using passive tube traps made of polypropylene (22 cm tall x 2.7 cm diameter). To prevent escape of animals from the tube, 15 milliliters (ml) of a dense brine solution (>90 parts per thousand (ppt)) was added; the remained volume was filled with filtered seawater. Ten replicate tube traps were deployed on the unvegetated tidal flat about 10 cm above the sediment surface, supported on PVC pipe inserted into the sediment. In the hybrid *Spartina* habitat, tube traps were inserted into the sediment leaving their opening about 5 cm above the bottom. Sediment was collected from traps in plastic jars and preserved in 8% buffered formalin.

Sediment properties: In order to explore how hybrid *Spartina* ultimately affects the sediment ecosystem, we examined some key sediment properties in vegetated and unvegetated habitats (10 replicates each). Porewater salinity (top 3 cm) was measured with a hand-held refractometer, sediment redox potential (top 1 cm) was measured with a portable Mettler Toledo redox meter. Plexi-glass cores (18.1 cm^2, 0-6 cm depth) were taken for total organic content (determined after combustion at 500°C x 4h); syringe cores were taken for chlorophyll a (an estimate of sediment microalgal biomass) and determined according to Plante-Cuny (1973) after extraction with 90% acetone. Sediment porosity was determined according to Buchanan (1984). Porewater sulfide was measured concurrently by A.C. Tyler (UC Davis) based on Cline (1969) with a few modifications. Macrofauna were collected from both habitats with cores (18.1 cm^2, 0-6 cm depth) and sieved through a 0.3-mm mesh sieve.

Plant removal experiment: We also performed an experiment to determine if the invaded habitats would return to the original unvegetated condition and resemble unvegetated tidal flats. We clipped all above-ground *Spartina* plant material to the sediment level in ten replicate 2x2 m areas with adjacent controls. In both removal and controls, we compared sediment properties and macrofaunal communities among habitats after 90 days.

Sediment transplant experiment: We conducted a transplant experiment to determine whether unvegetated sediment and fauna from the tidal flat would begin to look like those in the invaded areas when moved into the area invaded by hybrid *Spartina*. In summer 2002, intact sediment (625 cm^2 x 10 cm depth) was moved from the tidal flat (about 7 m from the edge) to *Spartina*-invaded habitat (10 m inside the meadow). Tidal flat sediment removed and replaced served as a control treatment. Each treatment contained ten replicate blocks (20 in total).

Predation: In order to assess if the shifts observed in macrofaunal composition in the initial transplant experiment were the result of changes in predation pressure following *Spartina*-hybrid invasion, we performed controlled and replicated *in situ* predator exclusion experiments beginning on September 10, 2003. We used European green crabs (*Carcinus maenas*), which are common (introduced) marsh predators in San Francisco Bay (Cohen et al. 1995). Intact uninvaded tidal flat sediment and associated fauna were transplanted to hybrid *Spartina* habitat as in the transplant experiment. However, this time we enclosed one individual *C. maenas* (carapace width 40 mm) in each of eight replicated enclosures (0.3 x 0.3 m x 0.5 m height) made of galvanized hardware cloth (0.63-cm mesh). Crabs were collected from adjacent tidal flats and placed directly into enclosures. A total of eight replicate blocks with five treatments per block were established (details in Neira et al. 2006). All cages had a removable top for measurement of environmental variables such as temperature, salinity, light penetration, and redox potential. At the termination of the experiment (4 weeks), we recorded sediment temperature, salinity, light, and redox potential. For macrofaunal analyses, we collected a single-core (18.1 cm^2, 0-6 cm) from the center of each experimental and control enclosure. After sieving on a 0.3 mm mesh sieve, retained organisms were sorted, identified to species level and counted. An additional core was taken for analysis of organic matter content.

RESULTS

Relative water flow: Overall relative water velocity was reduced by more than 50% in hybrid *Spartina* habitat relative to unvegetated tidal flats. The plant canopy reduced mean flow speed from 3-4 cm s^{-1} in unvegetated tidal flat to less than 0.7 cm per second (s^{-1}) in vegetated patches. Water flux as estimated by mass loss of gypsum cards (pooled across the three measurement periods) was 14.5 ±0.9 g d^{-1} in the tidal flat and 6.6 ±0.5 g d^{-1} in the vegetated patches (paired t test, $P < 0.0001$, Table 1). Gypsum cards placed on the tidal flat were strongly eroded which revealed the dynamic and variable nature of the water regime at Elsie Roemer.

Larval flux: The hybrid *Spartina* canopy structure reduced larval flux relative to the unvegetated tidal flat. After two months, the number of barnacles per shell was nearly 10 times higher in tidal flats (32.1 ±6.4) than in *Spartina* hybrid habitat (3.6 ±1.3 (paired t test, $P = 0.022$, Table 1).

Sediment deposition: We recorded higher rates of sedimentation in the hybrid *Spartina* habitat (20.4 mg cm^{-2} d^{-1}) than on the unvegetated tidal flat (14.2 mg cm^{-2} d^{-1}) (paired t test, $P = 0.005$). The fine sediment fraction (< 63 µm) deposited on traps placed in the hybrid habitat was greater than 45%, but was less than 13% in tidal flats (Table 1).

Animal transport: We found nearly two times as many animals entrained in traps deployed in the hybrid *Spartina* habitat (4.9 ±0.6 per individual trap per day (ind trap^{-1} d^{-1})) than on the unvegetated tidal flats (2.6 ±0.5 ind trap^{-1} d^{-1}) (paired t test, $P = 0.019$, Table 1. In addition, the faunal assemblage differed between habitats (ANOSIM, P = 0.013). This may be the result of the canopy structure slowing water flow and reducing turbulence, thus allowing greater deposition of animals in the collectors placed in the vegetated patches.

Sediment properties: Most of the alterations were similar to those found during mensurative studies performed previously at this site (Neira et al. 2005). Relative to unvegetated tidal flat, sediments of unclipped hybrid *Spartina* -vegetated patches were muddier (48.6% vs 10.7% silt/clay content) with higher organic content (3.7% vs 1.3%), higher porosity 65.2% vs 48.8%) and chlorophyll *a* (94.5 mg m^{-2} vs 39.1 mg m^{-2}). Redox potential became more negative (-27 mV vs +63.9 mV) and sulfide concentrations were much higher (200-900 micromoles (µM) vs <20 µM) (Table 1).

Table 1. Summarized results of manipulative experiments performed at Elsie Roemer

Environment variables / Macrofauna	Tidal flat	*Spartina* hybrid
	(Mean ± 1 SE)	
Water column		
Water motion (weight loss of gypsum cards) (g d^{-1})	14.5 (±0.9)	6.6 (±0.5)
Water speed (cm s^{-1})	3-4	0.3-0.7
Larval flux (barnacle shell^{-1})	32.1 (±6.4)	3.6 (±1.3)
Sediment deposition rate (mg cm^{-2} d^{-1})	14.2 (±)	20.4 (±)
TOM of deposited sediment (%)	1.9 (±0.4)	13.9 (±0.8)
Mud content (<63 µm) of deposited sediment (%)	12.7 (±3.3)	45.8 (±6.1)
Animal transport/migration (ind trap^{-1})	10.5 (±2.0)	19.4 (±2.3)
Sediment habitat		
Mud content (%)	10.7 (±2.9)	48.6 (±5.5)
TOM (%)	1.3 (±0.2)	3.7 (±0.5)
Porosity (%)	48.8 (±2.3)	65.2 (±3.3)
Chlorophyll a (mg m^{-2})	39.2 (±2.6)	94.5 (±16.9)
Redox potential (mV)	+63.9 (±12.1)	-27.5 (±25.4)
Sulfide (µM)	< 20	200 - 900
Macrofaunal density (ind 18.1 cm^{-2})		
Clipping experiment (unmanipulated)	411.3 (±47.5)	239.4 (±82.8)
Transplant experiment	359.4 (±37.5)	170.3 (±24.2)

Plant removal experiment: Total density of macrofauna was drastically reduced in the hybrid *Spartina* and the experimentally clipped areas relative to unvegetated tidal flats (about 1.7 and 6.6 times, respectively). The surface feeding species, *Corophium* spp., *Grandidierella japonica* (Amphipoda) and *Gemma gemma* (Bivalvia) were the most reduced, whereas subsurface deposit feeders such as capitellid polychaetes and tubificid oligochaetes were less affected or not affected. After 90 days, changes in the sediment properties were exacerbated in clipped areas. Percent muddy was 66.9 ±5.6, organic matter content was 5.7 ±0.6 and Eh was –161.5 ±23.9. Sulfide concentrations ranged between 300-1800 µM in clipped areas (A.C. Tyler, pers comm.).

Sediment transplant experiment: After 40 d, sediment habitat conditions in the transplants became similar to those of hybrid *Spartina* areas. We observed higher porosity, finer particles, higher organic matter and chlorophyll *a*, as well as lower redox potential relative to controls. No differences between treatments were found for temperature and salinity. Macrofaunal densities and species richness (per core) were lower in the transplants relative to controls (Table 1). Again, *Corophium* spp., *G. japonica* and *G. gemma* were the taxa most affected, similar to the decline in surface-feeding taxa observed in unmanipulated sediments of hybrid *Spartina* habitat relative to tidal flats (Neira et al. 2005).

Predation: The sediment surface in cages containing crabs was disturbed with obvious depressions created by foraging crabs. The sediments also had lower organic matter and chlorophyll *a* content relative to those cages without crabs. No differences were found in salinity, temperature and light penetration between treatments. Total macrofaunal density in experimental enclosures containing crabs was 2.2 times lower than those without crabs. Density of selected surface-feeding species such as *G. japonica, Corophium* spp. and *G. gemma* declined by 57%, 87% and 67%, respectively in enclosures with crabs. Other taxa that also declined were *Sphaerosyllis californiensis*, turbellarians, a juvenile arenicolid species and juvenile capitellids (Neira et al. 2006).

DISCUSSION AND CONCLUSION

Our results show that composition and structure of tidal flat macrofaunal benthic communities are strongly influenced by hybrid *Spartina* invasion. Specifically, we found that *Spartina* reduces water flow. These, in turn, influence flux of recruiting larvae, transport of other benthos, the input of organic matter, and sediment deposition.

These physical changes interacted with chemical changes including increased porewater sulfide concentrations and more negative redox potential levels (Neira et al. 2006, 2007). We also found changes in growth and survival via predation (Neira et al. 2006) and food availability (Levin et al. 2006), which can play key, possibly synergistic, roles in structuring *Spartina*-invaded ecosystems. The presence of hybrid *Spartina* in open tidal flats exerts a strong influence on the composition and distribution of benthic invertebrates in Elsie Roemer (Neira et al. 2005, 2006).

The reduction of macrofaunal densities in hybrid *Spartina* relative to the naturally unvegetated tidal flats is consistent with results for invasive *S. anglica* (Jackson 1985) and *S. alterniflora* in other estuaries (Zipperer 1996), but contrasts with existing paradigms about positive vegetation effects on marine macrobenthos (e.g., Hedge and Kriwoken 2000; Netto and Lana 1999). In addition, not all systems respond in the same way to the hybrid *Spartina* invasion (Neira et al. 2005). For example, at other sites such as Roberts Landing (15-year invasion), hybrid *Spartina* habitat differed from tidal flat sediments in composition but not abundance. At a third San Francisco Bay site, in San Mateo, where a *Salicornia* marsh is being invaded (8-10 years), sediment properties were similar, and no differences were detected in densities or proportions of surface- or subsurface-deposit feeders, but the proportion of carnivores/omnivores and grazers increased in the hybrid *Spartina* habitat (Neira et al. 2005). At Elsie Roemer, most of the species shown to have reduced density in hybrid *Spartina*-invaded tidal flats are surface-feeding animals, such as amphipods (*Corophium* spp., *Grandidierella japonica*) bivalves (*Gemma gemma*) and polychaetes (*Tharyx* spp.). Thus, where hybrid *Spartina* has invaded tidal flats it is likely to cause not only changes in the habitat structure, but also to shift macrofaunal feeding modes from surface-microalgae feeders to subsurface detritivores. The shift from surface-feeding taxa can have negative ecological implications for higher trophic levels. Birds and fishes depend more on larger, surface-feeding species (Simenstad and Thom 1995), which decline in abundance with the invasion of *Spartina*. However, the full impact of the *Spartina* invasion on birds and fishes in San Francisco Bay has yet to be measured.

We can conclude that the invasion of hybrid *Spartina* has resulted in substantial changes in benthic communities by modifying the physical and chemical environment. However, we find that not all ecosystems respond identically following hybrid *Spartina* invasion in San Francisco Bay. Hybrid *Spartina* can have differing and complex effects on the sediment environment and associated fauna depending on the location, type of habitat involved, age of invasion, and local hydrodynamics. The processes underlying the variable responses to *Spartina* invasion, and the different rates of recovery following invasion, are factors that should be considered in planning for *Spartina* eradication.

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SPARTINA ALTERNIFLORA INVASIONS IN THE YANGTZE RIVER ESTUARY, CHINA: A SYNOPSIS

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Spartina alterniflora was first found in the Yangtze River estuary in the mid 1990s, and has now become the most abundant vascular plant in the estuarine marshlands. We have investigated the potential consequences of *Spartina alterniflora* invasions to the salt marshes on two large islands in the estuary, Chongming and Jiuduansha, over the past five years, focusing on effects on biodiversity and ecosystem processes of the marshlands resulting from the invasion. We here summarize the major findings from our previous work and provide the relevant literature.

Keywords: Biodiversity, ecosystem processes, plant invasions, saltmarshes, *Spartina alterniflora*, Yangtze River estuary

INTRODUCTION

The Yangtze River estuary with two national nature reserves is an important ecoregion as it is the home for many economically and ecologically important species, and serves as an important stopover site for migratory birds on the East Asian-Australasian Flyway (Chen et al. 2003; Ma et al. 2004). However, in common with other estuaries in the world (Cohen and Carlton 1998; Grosholz 2002), the Yangtze River estuary is seriously threatened by exotic species invasions. Of all the invasive exotic species, smooth cordgrass (*Spartina alterniflora*) introduced from North America, has become the most harmful exotic plant to the salt marshes, leading to multifold consequences to the estuary. Over the past five years, we have investigated the population spread of *S. alterniflora* on two major islands in the estuary, Chongming and Jiuduansha, and examined the ecosystem-level effects of its invasion. This synopsis is based on these investigations. Detailed reports were published elsewhere. (See References for the full list.)

SPREAD AND DISTRIBUTION

S. alterniflora was intentionally introduced from America to China in 1979 (An et al. this volume), and was then spread to the Yangtze River estuary in the mid 1990s both by natural dispersal and intentional introductions. For the purposes of ecological engineering, i.e., rapid sediment accretion, *S. alterniflora* was then intentionally introduced to two large islands in the estuary, Jiuduansha (in 1997) and Chongming (in 2001 and 2003). On each island a national nature reserve had been set aside for conserving native biodiversity and maintaining ecosystem integrity. In fact, this invasive plant has invaded almost all the mudflats and salt marshes in the Yangtze River estuary (Fig. 1A), *S. alterniflora* either colonized tidal mudflats (Fig. 1D) or replaced native plants like *Scirpus mariqueter* (Fig. 1E) (Chen et al. 2004) and *Phragmites australis* (Wang et al. 2006b), and has become one of the most abundant species in estuarine ecosystems during a period of just over 10 years. Our recent data obtained through remote sensing show that in 2005 *S. alterniflora* monocultures accounted for 49.4% and 37.0% of the vegetated area in Dongtan (Fig. 1B) and Jiuduansha (Fig. 1C) marshlands respectively (Li et al. 2009). Range expansion of *S. alterniflora* still continues in estuarine wetlands.

Successful invasions of *S. alterniflora* in the Yangtze River estuary were the result of interactions between biotic, abiotic and human factors (Wang et al. 2006a). The “enemy release hypothesis” that is used to explain successful invasions of exotic plants elsewhere might also apply to those of *S. alterniflora* in the Yangtze River estuary. *Spartina alterniflora* has a number of superior traits such as fast growth, a highly efficient use of resources, a high tolerance to salt and a well-developed belowground system that make it a superior competitor or invader and a potent ecosystem engineer (Li et al. 2009). It is for this latter reason that *S. alterniflora* was widely used for erosion control and sediment accretion along shorelines in the Yangtze River estuary (Wang et al. 2006a), which might have directly led to its rapid spread in the marshlands. Frequent reclamation in the Yangtze River estuary has made the estuarine ecosystem susceptible to *S. alterniflora* as areas outside the dike exhibit conditions typical of early saltmarsh succession, which favor *S. alterniflora* rather than native plants. Environmental changes (saltwater intrusion and eutrophication) caused by human activities

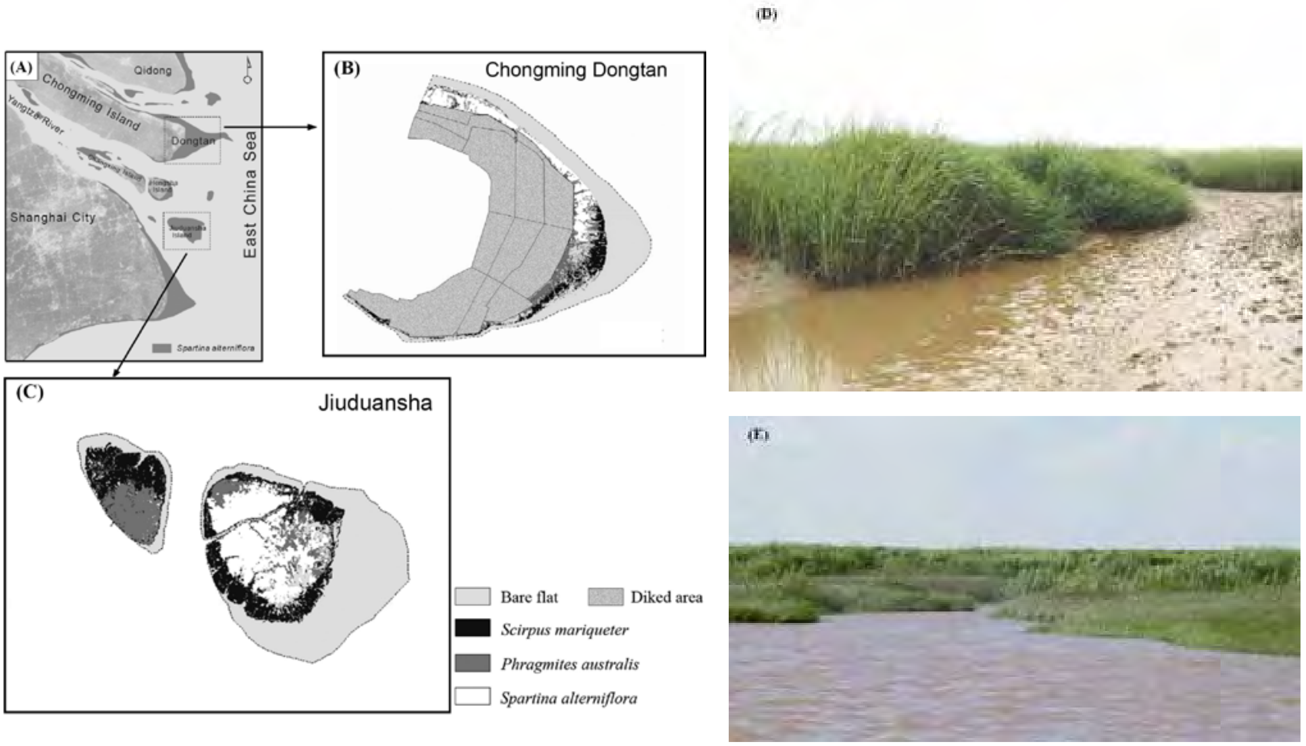

Fig. 1A) Locations of Jiuduansha and Chongming Dongtan in the Yangtze River estuary, on each of which a national nature reserve was set up in 2005 (Li et al. 2009); B) and C) Distribution of *S. alterniflora* in Chongming Dongtan and Jiuduansha marshlands in 2005; D) Conversion of tidal mudflats to *S. alterniflora* meadows; and E) Replacement of *S. mariqueter* with *S. alterniflora*.

might also facilitate further invasions in the estuary (Wang et al. 2006a, b).

PLANT COMMUNITIES

One of the threats of invasive exotic plants to native ecosystems is their competitive effects on native plant biodiversity. Our experimental studies showed that *S. alterniflora* had great competitive effects on native plant species in the estuary, e.g., *S. mariqueter,* which is endemic to the Yangtze River estuary (Chen et al. 2004; 2005b) and *P. australis* (Wang et al. 2006b), which resulted in the reduced abundance and even local extinction of these native plants. In particular, the decline of *S. mariqueter* abundance has been dramatic, which may have important consequences to shorebird communities because this sedge serves as food for shorebirds and creates favorable habitats for the birds (Ma et al. 2009; Li et al. 2009).

SOIL BIOTIC COMMUNITIES

Nematodes are important components of the soil ecosystem and play important roles in ecosystem functioning. The changes in composition of plant communities are believed to affect the structure of nematode communities (Yeates 1999). We compared the structure of soil nematode communities among native (*S. mariqueter* and *P. australis*) and *S. alterniflora* communities on Chongming, Jiuduansha Islands and Nanhui (Chen et al. 2007b). There were no significant differences among the three plant communities in total density of nematodes, the number of genera, or in the diversity indices. However, *S. alterniflora* invasions altered the trophic structure of nematode communities in soil. The relative abundance of bacterivores significantly increased, whereas that of plant feeders and algal feeders declined in *S. alterniflora* communities, compared with that in *P. australis* and *S. mariqueter* communities. Our further experimental study demonstrated that the changes in nematode communities were the result of altered litter quality due to the replacement of native plants by *S. alterniflora* (Chen et al. 2007a).

Similarly, we compared the structure of macrobenthic invertebrate communities between *S. mariqueter* and *S. alterniflora* communities at Chongming Dongtan (Chen et al. 2005a). No significant difference in total density of macrobenthic invertebrates was detected between the two plant communities although the relative abundance of some common species was altered, either increased or decreased. One of our recent studies also showed that *S. alterniflora* provided compatible habitats for the native crab *Sesarma dehaani* by offering moderate environmental conditions (e.g., mild temperature in summer) (Wang et al. 2008). Like nematodes, the relative abundance of major trophic functional groups of macrobenthic invertebrates was also altered when *S. mariqueter* was replaced by *S. alterniflora*. *Spartina alterniflora* communities had a higher proportion of suspensivores but a lower proportion of herbivores and detritivores than *S. mariqueter* communities (Chen et al. 2005a).

In addition, we compared the structure and diversity of the bacterial communities in rhizosphere soils of *S. alterniflora*, *P. australis* and *S. mariqueter* through constructing 16S ribosomal DNA (rDNA) clone libraries. Our results showed that the shift of species composition of plant communities from native plants to *S. alterniflora* caused considerable changes in bacterial composition of rhizosphere soils in estuarine salt masrhes (Wang et al. 2007).

The above changes in soil biota caused by *S. alterniflora* invasions could have great effects on ecosystem processes in the soils that are associated with these organisms as decomposers.

ARTHROPOD COMMUNITIES

Plants serve as both food and habitats for many arthropods, so subtle shifts in plant community composition may lead to considerable changes in the arthropod community. In order to understand the possible effects of *S. alterniflora* invasions on arthropods, we examined the community structure and diets of arthropods in an estuarine salt marsh previously dominated by native *P. australis* at Chongming Dongtan through net sweeping and plant harvesting methods and stable isotope analysis (Wu et al. 2009). We found that diversity indices were not significantly different between exotic and native plant communities, but the total abundance of insects estimated through plant harvesting was found to be lower in *S. alterniflora* monocultures than that in *P. australis* monocultures. Community structure of insects in *S. alterniflora* monocultures was dissimilar to that in *P. australis* monocultures and *P. australis*–*S. alterniflora* mixtures. Moreover, stable isotope analysis showed that although some native arthropods (perhaps generalists) shifted their diets, most native taxa did prefer *P. australis* to *S. alterniflora* even in *S. alterniflora* monocultures. *Spartina alterniflora* invasions resulted in reduced abundances of some arthropds, and increased the dominance of others that fed preferentially on *S. alterniflora*. The experimental evidence provided showed that invasive plants can change the community structure and diets of native arthropods, which will eventually alter arthropod food webs, and affect the integrity and functioning of native ecosystems within a nature reserve that has been set aside for conserving the native biodiversity and maintaining the ecosystem integrity.

BIRD COMMUNITIES

The conversion of mudflats to meadows resulting from *S. alterniflora* invasions had significant impact on birds of Charadriidae and Scolopacidae, which might have been attributable to the reduction in food resources and physical alterations of habitats for birds (Li et al. 2009). Nevertheless, *S. alterniflora* may also provide habitat for certain landbirds. Recent bird surveys indicated that the Japanese marsh warbler *Megalurus pryeri*, a newly-recorded species in the Yangtze River estuary, nested exclusively in *S. alterniflora* communities (Gan et al. 2006). This might be because the dense vegetation benefits the construction of nests. In addition, *M. pryeri* largely fed on the invertebrates in *S. alterniflora* communities (Gan 2009). It is likely that the rapid population increase of the Japanese marsh warbler was related to the rapid spread of *S. alterniflora* in the Yangtze River estuary. However, the effects of *S. alterniflora* on local landbird communities still remain largely unexplored, and further studies are needed.

CARBON AND NITROGEN CYCLES

S. alterniflora had great productive potential in the marshlands in the Yangtze River estuary. The results we obtained in Jiuduansha marshlands showed that exotic *S. alterniflora* had much greater primary productivity than natives *S. mariqueter* and *P. australis* (Liao et al. 2007) because the exotic had greater leaf area index (LAI) and a longer photosynthetic season than the native species (Jiang et al. 2009). At the same time, decomposition rates of *S. alterniflora* litter, particularly the belowground litter, were lower than those of *S. mariqueter* and *P. australis* litter due to the lower litter quality of *S. alterniflora* (Liao et al. 2008). Therefore, larger stocks of carbon and nitrogen were found in the ecosystems dominated by *S. alterniflora* than in those dominated by *S. mariqueter* and *P. australis*. Our further analysis of stable carbon isotopes also confirmed that the replacement of *S. mariqueter* by *S. alterniflora* significantly increased soil organic carbon and total soil nitrogen (Cheng et al. 2006), especially soil labile carbon, recalcitrant carbon, and soil recalcitrant nitrogen contents in the upper soil layers (0–60 cm) (Cheng et al. 2008). The ecosystem carbon and nitrogen cycles altered by *S. alterniflora* invasions might be of limited significance on a large scale in relation to the range of *S. alterniflora* in the Yangtze River estuary, but might have potentially far-reaching impact on the adjacent ecosystems. In fact, *S. alterniflora* functioned as the primary energy source for certain nektons in the marshlands in the Yangtze River estuary (Quan et al. 2007). Potential cascading effects on estuarine food webs of increased ecosystem productivity due to *S. alterniflora* invasions needs further exploration.

CONCLUSIONS

The invasion history of *S. alterniflora* in the Yangtze River estuary is relatively short, but this invasive plant has been found to cause considerable impact on structure and functioning of native ecosystems although it might have positive effects on certain species. However, the responses of native ecosystems to plant invasions may be of longer duration than a few decades, thus long-term monitoring is needed to understand the ecosystem changes in response to *S. alterniflora* invasions. In the meantime, most of our studies have examined the effects of *S. alterniflora* invasions on biodiversity and processes of native ecosystems. Therefore, future studies are clearly needed to examine how the change of one ecosystem component affects or is affected by those of other components, as examined by Levin et al. (2006) in San Francisco Bay (USA), and to link the changes of biodiversity caused by *S. alterniflora* invasions to the alterations of ecosystem processes. Finally, considering that most of the marshlands invaded by *S. alterniflora* that we studied are protected for conserving the native biodiversity and maintaining the ecosystem integrity in the Yangtze River estuary, *S. alterniflora* invasions need to be managed appropriately.

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THE ROLE OF *SPARTINA ANGLICA* PRODUCTION IN BIVALVE DIETS IN NORTHERN PUGET SOUND, WA, USA

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The importance of salt marsh productivity to coastal food webs is a question of ecological and economic importance. In northern Puget Sound, Washington, USA, a relatively new source of production has become prominent with the establishment of invasive *Spartina anglica* (Poaceae: English cordgrass). *Spartina anglica* has converted native coastal mudflat communities that had little or no emergent vascular vegetation into expansive cordgrass meadows. One consequence of *Spartina* productivity on invaded mudflats may be altered trophic patterns. Three bivalves with different feeding modes (*Macoma balthica*, *Mya arenaria*, and *Mytilus* sp.) that are commonly found at the edges of *Spartina* meadows were selected to investigate whether *Spartina* is contributing to bivalve diets. We compared the stable isotope ratios ($\delta^{13}C$ and $\delta^{34}S$) of the bivalves to potential food sources including macroalgae, *Spartina*, and other vascular plants. We estimated the feasible contributions of production to bivalve diets during March 2003 by analyzing the results of multiple source, mass-balanced linear mixing models as calculated by IsoSource. Our estimates indicate that *Spartina* biomass may comprise 37-60% of the diet of *Macoma*, while dead *Spartina* biomass contributes 0-46%. For *Mya arenaria* a filter-feeding clam, 40-59% of its diet may contain *Spartina* biomass, while 0-35% of its diet may consist of dead *Spartina* biomass. *Mytilus* sp., a filter-feeding mussel, had 19-44% of its diet originating from *Spartina* biomass while dead *Spartina* may be 0-46% of its diet. *Spartina* consumption by bivalves is consistent with previous isotopic studies. Although *Spartina* biomass is considered recalcitrant, the immediate proximity of the consumers to vast quantities of *Spartina* productivity may best explain the prevalence of *Spartina* in bivalve diets while other potential sources have minor estimated contributions. This study provides an initial examination of how the biomass of an invasive plant species is becoming integrated into estuarine trophic webs.

Keywords: *Spartina anglica*, stable isotopes, mixing models, IsoSource, *Macoma*, *Mytilus*, *Mya*

INTRODUCTION

Estuarine salt marshes intercept nutrients and biomass from uplands and also export nutrients and biomass into nearshore coastal ecosystems (Deegan and Garritt 1997;Teal and Howes 2000; Valiela et al. 2000). The importance of salt marsh productivity in estuarine ecosystems has been a central research question with ramifications for economically important coastal fisheries (Peterson et al. 1985, 1986; Teal and Howes 2000; Valiela et al. 2000).

Few studies have addressed the role of invasive species in altering ecosystem processes in marine and estuarine environments (Ruiz et al. 1997, Grosholz 2002) despite the wealth of research examining how invasive species alter landscapes, nutrient cycling, and species interactions (Mack et al. 2000 and references therein). *Spartina anglica* C. E. Hubbard (Poaceae; English cordgrass) was introduced into northern Puget Sound, Washington in the early 1960s in Snohomish County (Hacker et al. 2001; Hellquist 2005). *Spartina anglica* covers nearly 300 solid hectares (ha) of Puget Sound intertidal habitat (Hedge et al. 2003). *Spartina anglica* colonizes soft sediments and is capable of rapidly converting mudflat habitats into elevated *Spartina* meadows. Habitat conversion by *Spartina* is of great concern ecologically, economically, and aesthetically (Hacker et al. 2001; Hedge et al. 2003).

There are no native maritime species of *Spartina* in the Pacific Northwest of North America and thus mudflat ecosystems of Puget Sound have developed without low intertidal meadows of emergent C_4 vegetation (i.e. *S. anglica*) along their periphery. Generally, the high carbon contents of C_4 plants have been considered to be of low nutritional quality (Caswell et al. 1973). Extensive meadows of *Spartina* that colonize mudflats represent a large subsidy of low-quality productivity for consumers. At Alice Bay, Washington, *S. anglica* meadows had over 10 times the aboveground biomass as uninvaded mudflat (Hellquist 2005). In Willapa Bay, Washington, colonization of *Zostera japonica* and *Spartina alterniflora* has increased primary productivity by more than 50% in intertidal mudflats (Ruesink et al. 2006).

As noted for introduced *Zostera japonica* (Hahn 2003), the invasion of *S. anglica* could potentially alter detrital decomposition rates and detrital quality within estuaries dominated by native eelgrass, *Zostera marina*. Like *Spartina alterniflora* that has higher C:N ratios than *Z. marina* (Ruesink et al. 2006), mudflats colonized by *S. anglica* have higher C:N ratios than those with native producers (Hellquist 2005). Changes in detrital availability and quality may influence the feeding patterns of resident detrital consumers. The *S. anglica* invasion provides a unique opportunity to understand the trophic integration of an invasive producer in an estuarine ecosystem.

Estuarine food webs are complex due to the predominance of detrital pathways, the abundance of potential productivity sources for consumption, omnivory, spatial heterogenity, and opportunistic feeding of consumers that may change throughout the course of the year (Deegan and Garritt 1997; Riera et al. 1999). The use of multiple stable isotopes (especially $\delta^{13}C$ and $\delta^{34}S$) can provide valuable insight into how *Spartina* spp. may contribute to the diets of estuarine consumers (Peterson et al. 1986; Peterson and Howarth 1987; Deegan and Garritt 1997; Valiela et al. 2000; Connolly et al. 2004). For example, $^{13}C/^{12}C$ isotope ratios can distinguish C_3 from C_4 vegetation or marine algae from terrestrial C_3 vegetation (Fry and Sherr 1984, Deegan and Garritt 1997). In marine studies, $^{34}S/^{32}S$ isotope ratios are especially useful to distinguish sources of productivity because SO_4^{2-} and HS^- that are used by plants have distinct isotopic signatures (Fry et al. 1982; Trust and Fry 1992; Connolly et al. 2004).

Due to the consistency of isotopic signatures from producers to consumers, trophic relationships can be discerned from stable isotopic data that otherwise may remain virtually unknown (Fry and Sherr 1984; Peterson et al. 1985). Once isotopic data of consumers and potential dietary producers is obtained, stable isotopic mixing models can be used to estimate the proportion of sources (producers) that contribute to the composition of a mixture (consumers; Phillips 2001).

We present data that describe the potential contributions of *Spartina* biomass to the diets of three bivalves in northern Puget Sound through the use of $\delta^{13}C$ and $\delta^{34}S$ stable isotope ratios and mixing models calculated by the computer application IsoSource (Phillips and Gregg 2003). As a deposit feeder that scours surface sediments for organic matter, we expected that the clam *Macoma balthica* would have a *Spartina* contribution in its diet. For the filter feeders *Mya arenaria* (soft-shelled clam) and *Mytilus* sp. (mussel), we expected *Spartina* contributions to be minimal or entirely absent.

MATERIALS AND METHODS

Samples were collected in March 2003 at West Pass (Camano Island, Island County, Washington; 48º 15' 19" N, 122º 24' 56" W). The West Pass and English Boom shoreline is an extensive area of mudflat fringed by native salt marsh and *Spartina* meadows located in south Skagit Bay, just north of the original introduction site of *Spartina anglica* near Stanwood, Washington. This area is dominated by approximately 100 ha of *S. anglica* meadows that grow along mudflats adjacent to the West Pass channel. Producers were randomly collected along transects and included C_3 emergent vascular vegetation (*Grindelia integrifolia* and *Salicornia virginica*), submergent vascular vegetation (*Zostera marina*), macroalgae (*Fucus spiralis),* and C_4 vascular plants. C_4 plants included *S. anglica* (living leaf and standing dead tissue) and the native salt marsh grass *Distichlis spicata*.

Three bivalve species (*Macoma balthica*, *Mya arenaria*, and *Mytilus* sp.*)* were chosen based on their feeding patterns and co-occurrence with *Spartina*. *Macoma* is a surface deposit feeder, *Mya* a filter feeder, and *Mytilus* is an epifaunal filter feeder (Incze et al. 1982; Dame 1996). Both *Macoma* and *Mya* burrow in sediments immediately adjacent to *Spartina* meadows or burrow among *Spartina* roots along tidal channels. *Mytilus* was found along *Spartina* meadows typically using *Spartina* root masses and stems as anchoring substrate. Bivalves and *Spartina* were randomly sampled on the same transect at the edge of a large tidal channel that passes through the largest *Spartina* meadow at West Pass.

Following collection, bivalves were held for 24 hours to allow gut contents to clear. Plant and animal samples were frozen, cleaned to remove sediment, epiphytes, and carbonates (10% HCl acid washes) and dried in a drying oven. Samples were ground into a fine powder prior to isotopic analysis. For *Macoma* all visceral mass tissue was used for analysis, whereas for *Mya* and *Mytilus* adductor muscles were dissected for analysis. Sample sizes for *Macoma*, *Mya*, and *Mytilus* were 12, seven, and seven individuals respectively. Producer sample sizes ranged from three to seven collections.

Samples were analyzed for $\delta^{13}C$ at the Idaho Stable Isotope Laboratory, University of Idaho, Moscow, Idaho. Continuous-flow stable isotopic analyses were conducted on a Finnigan-MAT, Delta+ isotope ratio mass spectrometer (Thermo Finnigan, Thermo Electron: Waltham, Massachusetts). Samples were flash-combusted in a NC 2500 elemental analyzer (CE Instruments, Thermo Electron: Waltham, Massachusetts) interfaced with a Conflo II. Stable isotope ratios are the ratio (R) of $^{13}C/^{12}C$ or $^{34}S/^{32}S$ expressed in standard delta (δ) notation values in parts per mille (‰) where $\delta^{13}C$ or $\delta^{34}S = [(R_{sample}/R_{standard)-1}]*1000$. The $\delta^{13}C$ and $\delta^{15}N$ reference standard was acetanilide ($\delta^{13}C = -26.07‰ \pm 0.11‰$; $\delta^{15}N = -0.33 \pm 0.17‰$). Spinach was used as an internal standard ($\delta^{13}C = -26.2‰ \pm 0.2‰$).

Sample analysis of $\delta^{34}S$ was conducted by Iso-Analytical Limited (Sandbach, Cheshire, United Kingdom). The reference standard was NBS-127 (barium sulfate $\delta^{34}S_{V\text{-}CDT} = 20.3‰$; IAEA, Vienna, Austria). Calibration and correction standards were IAEA S-1 (silver sulfide $\delta^{34}S_{V\text{-}CDT}$ = -0.3‰) and Iso-Analytical IA-R025 (barium sulfate $\delta^{34}S_{V\text{-}CDT}$ = +8.53‰). IA-R025 (barium sulfate $\delta^{34}S_{V\text{-}CDT}$ = +8.53‰) and whale baleen ($\delta^{34}S_{V\text{-}CDT}$ = +16.30‰) were used as internal standards.

The IsoSource isotopic mixing model analysis application (www.epa.gov/wed/pages/models.htm; Phillips and Gregg 2003) was used to estimate the feasible contributions of *S. anglica* and six other potential sources to the diets of *Macoma*, *Mya*, and *Mytilus*. IsoSource calculates the ranges of all possible source contributions when the number of sources placed in the mixing model exceeds n+1, where n is the number of isotopic tracers (e.g. $\delta^{13}C$ or $\delta^{34}S$; Phillips and Gregg 2003). Therefore, IsoSource allows source contributions to a mixture to be estimated regardless of the number of sources and isotopic tracers available. In these circumstances unique solutions cannot be obtained due to the use of large numbers of sources in the mixing model (Phillips and Gregg 2003). Since unique solutions cannot be determined, IsoSource output provides ranges of all possible source contributions to the mixture based on mass balance calculations (Table 1; Phillips and Gregg 2003). The more narrow the range of these solutions (e.g 30%-50% vs. 10-70%), the more reliable the interpretation of source contributions to the mixture can be. Thus, IsoSource is very useful for studies of trophic relationships where numerous food sources could exist for a consumer. IsoSource has been applied to a variety of trophic studies inside and outside of estuaries (e.g. Felicetti et al. 2003; Melville and Connolly 2003; Ben-David et al. 2004; Newsome et al. 2004).

The carbon isotope ratios of the bivalves were adjusted by a 1‰ fractionation factor based on Peterson and Fry (1987), Vander Zanden and Rasmussen (1999), and McCutchan et al. (2003). Sulfur appears to have little to no fractionation associated with its movement through trophic levels. Due to the scarcity of sulfur fractionation data (McCutchan et al. 2003), the typical assumption that there is minimal fractionation of sulfur was applied (Peterson and Howarth 1987). A biomass contribution increment of 1% and a tolerance interval of 0.11‰ was used for all IsoSource analyses to insure that the full range of feasible solutions was generated (Table 1).

RESULTS

Macoma (n=12) clustered close to the outer edge of mixing polygon in close proximity to living and dead *Spartina* tissue (Figure 1A). Only living tissue of *Spartina* was considered to be a component of the diet of *Macoma* in all solutions of the mixing model with contributions ranging from 37-60% (Table 1). Five of the six productivity sources sampled could be absent in the diet of *Macoma* (Table 1).

Table 1. IsoSource results. Ranges of feasible biomass source contributions for the each bivalve summarized from 34,854 mass balanced mixing model solutions (*Macoma balthica*), 23,996 solutions (*Mya arenaria*), and 56,083 solutions (*Mytilus* sp.). Each distribution is defined by the minimum and maximum values as well as the 1st and 99th percentiles. The percentiles represent 98% of all the possible IsoSource solutions. The mean percent of each source distribution is cited to show central tendency of the distribution only and should not be considered a point estimate of the source contribution to the diet of the bivalve.

Source	Consumer	Biomass %				
		Minimum	1%	Mean	99%	Maximum
Spartina (living leaves)	*Macoma*	37	41	52	59	60
	Mya	40	44	53	58	59
	Mytilus	19	24	36	43	44
Spartina (standing dead)	*Macoma*	0	0	15	38	46
	Mya	0	0	8	27	35
	Mytilus	0	0	11	36	46
Distichlis spicata	*Macoma*	0	0	10	26	31
	Mya	0	0	6	19	24
	Mytilus	0	0	8	25	32
C_3 Vascular Plants	*Macoma*	0	0	5	15	17
Salicornia virginica	*Mya*	3	5	14	23	26
Grindelia integrifolia	*Mytilus*	5	8	20	32	35
Zostera marina	*Macoma*	0	0	9	28	33
	Mya	0	0	10	32	40
	Mytilus	0	0	13	42	52
Macroalgae	*Macoma*	0	0	9	26	31
Fucus spiralis	*Mya*	0	0	9	30	38
	Mytilus	0	0	12	40	50

Four *Mya* clustered near the center of the mixing polygon while three other individuals clustered close to *Spartina* (Figure 1B). IsoSource indicated that *Mya* could receive 40-59% of its diet from living *Spartina* tissue and 3-26% of its diet from C_3 vegetation. All other sources had wide ranges of potential producer contributions that included zero as likely dietary contributions (Table 1).

Living *Spartina* biomass and C_3 vegetation were the only dietary sources that IsoSource included within all of the feasible solutions for *Mytilus*. Living *Spartina* could contribute 19-44% to the diet of *Mytilus*, whereas C_3 vegetation may contribute 5-35% of its diet. All other potential sources included 0% as the most frequent feasible contribution (Table 1). Six of the seven individuals of *Mytilus* clustered in the center of the mixing polygon while one individual was more depleted in its $\delta^{34}S$ signature (Fig. 1C).

DISCUSSION

Our mixing models present evidence that *Spartina anglica* is consumed by bivalves. The use of *Spartina* by *Macoma* is particularly convincing based on the constrained mixing model solution and the proximity of individuals to the isotopic signatures of living and dead *Spartina* (Fig. 1A). The mixing model for *Mya* is also convincing, although the $\delta^{13}C$ values tend to be more depleted than those of *Macoma* potentially indicating smaller *Spartina* contributions to its diet compared to *Macoma*. With the exception of one outlying individual, *Mytilus* had the most consistent isotopic signatures for $\delta^{13}C$ and $\delta^{34}S$, and the lowest ranges of potential *Spartina* contributions. As a suspension feeding mussel, *Mytilus* should be drawing its food from the

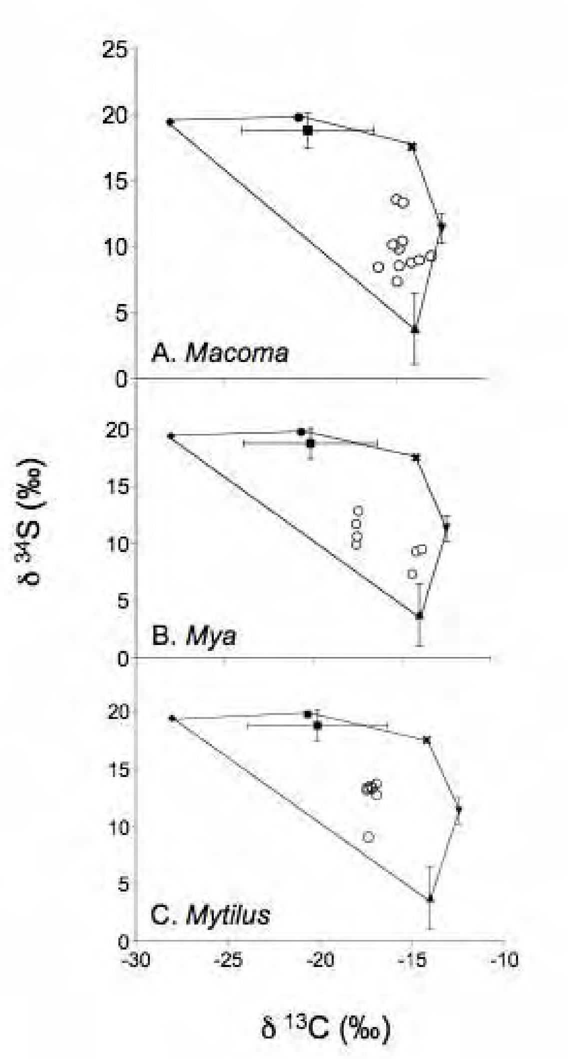

Fig. 1. Dual isotope ($\delta^{34}S$ and $\delta^{13}C$) scatterplots of the three bivalves and six producers sampled at West Pass, Island County, Washington. Each open circle is an individual consumer. A. *Macoma balthica* (n=12). B. *Mya arenaria* (n=7). C. *Mytilus* sp. (n=7). Producers (mean±SE) are the same for each panel: C_3 (pooled values of *Salicornia virginica* and *Grindelia integrifolia*: diamond), *Fucus spiralis* (closed circle), *Zostera marina* (square), *Distichlis spicata* (**x**), Standing dead *Spartina* (inverted triangle), Living *Spartina* leaves (triangle). Source sample sizes range from n=3 (*Zostera*) to n=7 (C_3 plants). Error bars are plotted for SE values > 1 ‰.

predominant sources of carbon available in the seston (Ruckelshaus et al. 1993). However, the consumption of suspended particulate organic matter (SPOM) by these bivalves at West Pass during additional 2003 sampling periods was surprisingly inconclusive despite using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ to simultaneously estimate dietary contributions (Hellquist 2005).

Previous trophic studies have provided a variety of conclusions about the importance of detrital *Spartina* in estuarine food webs. These results differ depending on consumer trophic status and location. For example, filter feeders including *Geukensia demissa* (ribbed mussel; Peterson et al. 1985, 1986; Peterson and Howarth 1987), *Crassostrea virginica* (oyster; Peterson et al. 1986) and *Mya arenaria* (Peterson et al. 1986; Peterson and Howarth 1987) have been identified as using *Spartina* biomass in their diets. The mud snail (*Ilyanassa obsoleta*) which is a deposit feeder also appears to use *Spartina* in Massachusetts and North Carolina (Peterson et al. 1986; Peterson and Howarth 1987; Currin et al. 1995). *Spartina* species also contribute to the diets of fish in Massachusetts (Peterson et al. 1986) and southern California (Kwak and Zedler 1997).

However, in a study similar to those above, the role of vascular plants in the diets of over 50 consumers was considered minimal or nonexistent in a Mississippi estuary (Sullivan and Moncreiff 1990). Instead, macroalgae and zooplankton appeared to be the major dietary components of the consumers sampled. Even *Geukensia demissa* and *Crassostrea virginica* shown by Peterson et al. (1986) to use *Spartina* biomass seemed to have little input from vascular plant productivity (Sullivan and Moncreiff 1990).

In France, *S. anglica* appears to be an unused source of productivity for bivalves (Riera et al. 1999). Despite the availability of *S. anglica* detrital matter, bivalves (*Macoma balthica* and *Mytilus edulis*) relied on suspended particulate organic matter and benthic diatoms as their primary dietary components. *Mytilus edulis* had a diet dominated by 65% phytoplankton and 35% diatoms (Riera et al. 1999) whereas the diet of *M. balthica* was estimated to be composed of 76% benthic diatoms and 24% phytoplankton. However, Jackson et al. (1986) used $\delta^{13}C$ to estimate the dietary contribution of *S. anglica* to *M. balthica* and their data suggested that 34-50% of assimilated biomass consumed by *M. balthica* was *Spartina,* for a total estimate of 0.2-0.3 grams of carbon per square meter per year (g C m^{-2} yr^{-1}) assimilated.

Relatively few studies of Puget Sound trophic dynamics have been conducted (e.g., Simenstad and Wissmar 1985; Ruckelshaus et al. 1993). In southern Puget Sound estuaries and littoral beaches, detrital carbon was shown to originate primarily from *Zostera* spp., epiphytic algae, and macroalgae (Simenstad and Wissmar 1985). Our data also indicate small contributions to bivalve diets from native marsh plants with C_3 isotopic signatures (Table 1). *Distichlis spicata*, a C_4 salt marsh plant that grows in abundance adjacent to the *Spartina* meadows at West Pass, apparently contributes very little to bivalve diets (Table 1). The small contributions of *Zostera marina* to bivalve diets is probably a function of its low abundance in the immediate vicinity of the sampling location.

This study indicates that *S. anglica* biomass is a locally important component of bivalve diets in northern Puget Sound after being in the ecosystem for just over 40 years. The extent that *Spartina* may contribute to bivalve diets in our study is somewhat unexpected due to the recalcitrant nature of *Spartina* tissue (Peterson and Howarth 1987). Of the two *Spartina* tissue types sampled, we were expecting more constrained IsoSource solutions for standing dead *Spartina* contributions to bivalve diets since we expected dead tissue to more closely match the detritus available to bivalves (Table 1). The living *Spartina* tissue samples in this study were newly emerged leaves, whereas the dead *Spartina* samples consisted of standing dead *Spartina* stems and leaves. Living and dead *Spartina* biomass were sampled to represent the end points of a range of potential *Spartina* isotopic signatures during its life history. The range of potential intermediate isotopic values that may occur during senescence is apparent as the distance between the signatures of living and dead *Spartina* (Figs. 1A-C).

It may seem contradictory that the mixing models in this study indicate the use of *Spartina* living tissue when the bivalves sampled are sediment dwelling or epifaunal detritivores. These estimates suggest that as *Spartina* decomposes, plant fragments in the early stages of decay may be consumed. Early during decay, *Spartina* would have its greatest nutritional value and would be at its least recalcitrant since C:N ratios more than double from living tissue to the standing dead tissue phase of *Spartina* (Hellquist 2005). Alternatively, the use of *Spartina* detritus by the bivalves may be the result of trophic modification of the biomass as it passes through the detrital food web (Peterson et al. 1986).

The spatial proximity of the bivalves to the edge of the *Spartina* meadow probably accounts for the higher than expected inputs of *Spartina* in the bivalve diets. Although a similar study of *S. anglica* and bivalves did not find a relationship between *Spartina* consumption and proximity to *Spartina* (Riera et al. 1999), other work has linked spatial proximity of consumers to *Spartina* with the magnitude of *Spartina* dietary contributions (Peterson et al. 1986; Peterson and Howarth 1987). Local sources of organic matter were of major dietary importance to the consumers sampled in a Massachusetts estuary (Deegan and Garritt 1997). For example, consumers in the upper estuary relied on freshwater marsh organic matter and oligohaline phytoplankton, whereas in the lower estuary marine sources were more important (e.g. benthic diatoms and salt marsh vegetation). We sampled bivalves collected from a tidal creek passing through an extensive *Spartina* meadow, where *Spartina* was the only immediate source of vascular plant productivity. It is probably not surprising that the bivalves have such a large potential contribution of *S. anglica* to their diets. *Spartina* was also a locally important dietary source for the mussel *Geukensia demissa* when sampled from within a salt marsh tidal channel (Peterson et al. 1985, 1986). In Padilla Bay, Washington the importance of locally abundant productivity sources also has been described for *Mytilus edulis* (Ruckelshaus et al. 1993).

Like *Spartina alterniflora* in Willapa Bay, Washington (Ruesink et al. 2006), *S. anglica* produces biomass throughout the growing season that senesceces during the autumn and over winter. Thus, *S. anglica* adds copious amounts of wrack to intertidal habitats. Release of biomass into the estuary as a consequence of control efforts may also contribute to the high levels of *S. anglica* in bivalve diets at West Pass. The West Pass area contains the greatest concentrations of *S. anglica* in Washington (Hacker et al. 2001) and has been subjected to intense control efforts. In 2003, West Pass and its environs were intensively controlled via applications of herbicide as well as mowing. These control treatments kill *Spartina* during the growing season and produce *Spartina* wrack much earlier than typical senescence. The less recalcitrant *Spartina* tissue that dies prematurely due to control activity enters the food web up to four to five months earlier than is typical. This plant tissue may then decay faster during the warmer summer and early fall months. By March, the *Spartina* isotopic signature would be fully incorporated into consumer tissue.

The contributions to bivalve diets in this study during March 2003 are subject to some sampling limitations. The lack of samples of benthic diatoms and SPOM unfortunately excludes productivity sources that should logically contribute to bivalve diets. However, additional stable isotope ratio data from West Pass collected later in 2003 using δ^{13}C, δ^{15}N, and δ^{34}S provide additional evidence for the consumption of *S. anglica* by bivalves (Hellquist 2005). These data indicate that there are seasonal patterns of *S. anglica* use in bivalve diets (Hellquist 2005). However, during these two additional sampling periods that include samples of sestonic and benthic production, the dietary role of these sources was unexpectedly inconclusive (Hellquist 2005).

The calculations of IsoSource greatly enhance our ability to estimate source contributions where there are more sources than elements analyzed (Phillips 2001; Phillips and Gregg 2003). However, it is crucial to remember that these mixing models should be viewed as an index of dietary consumption, and not discrete point estimates (Ben-David and Schell 2001; Phillips and Gregg 2003). The uncertainty associated with the dietary estimates generated by IsoSource (Table 1) reinforces the inexact nature of isotopic data in cases where there are multiple productivity sources with similar isotopic ratios and where consumer omnivory is prevalent.

Along with Hahn (2003), Hellquist (2005), and Ruesink et al. (2006), this research illustrates how ecosystem processes in Washington estuaries can be altered by invasive plant species including *Zostera japonica*, *S. anglica*, and *S. alterniflora*. To our knowledge, this study is the first use of multiple stable isotope ratios to examine trophic relationships in Puget Sound in relation to invasive *Spartina*. This study provides evidence that bivalves living in immediate proximity to invasive *S. anglica* are using its biomass as a source of nutrition during winter months. As a relatively new addition to the estuarine flora of northern Puget Sound, not only is *S. anglica* a productivity source that was previously absent, but it is also an ecosystem engineer that will eventually alter sediment conditions to the detriment of the bivalve populations that currently use its biomass for nutrition (Hellquist 2005).

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CONTRASTING EFFECTS OF *SPARTINA FOLIOSA* AND HYBRID *SPARTINA* ON BENTHIC INVERTEBRATES

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In San Francisco Bay, California, mudflats and native California cordgrass (*Spartina foliosa*) marshes are being invaded by a hybrid cordgrass formed by hybridization between *S. foliosa* and introduced *S. alterniflora*. We investigated differences in vegetation and sediment structure, benthic infauna, and food webs within native and invaded *Spartina* marshes between San Francisco Bay and Bodega Bay, California. The greatest impact of hybrid *Spartina* in San Francisco Bay appears to be its alteration of habitat structure rather than food webs. Habitat structure differed significantly between native and hybrid *Spartina*. Hybrid *Spartina* produced greater biomass both above and below ground, and taller stem heights. *Spartina foliosa* contained significantly higher densities and biomass of infaunal organisms in benthic cores than did mudflats, while densities and biomass of infauna in hybrid *Spartina* were lower than, or did not differ from, mudflats. Stable isotopes of carbon and nitrogen were used to examine whether macrofaunal food webs differ between native or hybrid *Spartina* and mudflats. Some consumers collected within *Spartina* showed evidence of a shift in carbon isotope ratios indicating a possible increase in *Spartina* consumption within vegetation; however, the pattern was not consistent across species and sites. Due to the differences in their effects on infauna, hybrid *Spartina* and *S. foliosa* should not be considered equivalent for marsh restoration projects.

Keywords: hybrid *Spartina*, *Spartina foliosa*, infauna, food webs, California

INTRODUCTION

In San Francisco Bay, hybrids formed between native California cordgrass (*S. foliosa*) and introduced *S. alterniflora* accrete sediment, increase elevation compared to surrounding mudflats, and significantly reduce light levels under their canopies (Neira et al. 2005). Based on tidal levels, hybrid *Spartina* would also be able to fill large areas of shallow outer coast bays if it establishes populations there (Daehler and Strong 1997). The spread of hybrid *Spartina* threatens flood control channels and habitat for migrating shorebirds, and may impact invertebrates that are food for birds and fishes.

Spartina cordgrasses may either facilitate or inhibit infauna depending on location (e.g., Capehart and Hackney 1989, Netto and Lana 1999). The invasion of hybrid *Spartina* provides an opportunity to examine how differences in structure between two closely-related ecosystem engineers affect infaunal communities. Understanding differences between *S. foliosa* and hybrid *Spartina* may help managers predict the impacts of the continued spread of hybrid *Spartina*.

This study examined whether hybrid *Spartina* is ecologically equivalent to native *S. foliosa* in its impacts on infaunal and epifaunal invertebrates. We investigated two hypotheses. First, we predicted that differences in structure between *S. foliosa* and hybrid *Spartina* will be reflected in differences in infaunal density, biomass, and taxonomic composition. Second, we predicted that, due to the greater aboveground biomass produced by hybrid *Spartina*, organisms living within the hybrid will show greater use of hybrid as a food source than those in *S. foliosa* or mudflats, based on stable isotopes of carbon and nitrogen.

METHODS

Study sites included five *S. foliosa* marshes and two hybrid *Spartina* marshes in northern California. *Spartina foliosa* sites included: China Camp State Park (38° 0.37'N, 122° 28.66'W) on San Pablo Bay; Bolinas Lagoon (38° 19.22'N, 122° 41.73'W); Shields Marsh (38° 5.35'N, 122° 50.44'W) and Tom's Point (38° 13.21'N, 122° 56.86'W) on Tomales Bay; Drakes Estero (38° 5.36'N, 122° 55.86'W). Hybrid marshes were located on the eastern side of San Francisco Bay in San Lorenzo (Roberts Landing, 37° 40.22'N 122° 09.70'W) and Alameda (Elsie Roemer Bird Sanctuary, 37° 45.58'N 122° 28.80'W). The San Lorenzo site is unique in that it contains discrete patches of both *S. foliosa* and hybrid *Spartina* (genotypes confirmed by D. Ayres, University of California, Davis, pers. comm.).

We established ten study quadrats within *Spartina* at each site, paired with ten on mudflats. These quadrats were used for vegetation, sediment, and infauna sampling. For details of methods, see Brusati and Grosholz (2006). We measured stem heights and densities, aboveground biomass, and belowground biomass at each site, as well as sediment characteristics such as organic matter content, bulk density, sediment porewater salinity, and oxidation-reduction potential. Infauna cores (5 centimeters [cm] deep x 5 cm diameter) were collected in winter and summer 2001-03. Cores were preserved in 8% formalin and organisms were counted and weighed in the laboratory. We used t-tests on transformed data to compare results between *Spartina* and mudflats.

To analyze the effects of *Spartina* on invertebrate food webs, we collected species of infaunal and epifaunal invertebrates in *S. foliosa*, hybrid *Spartina*, and mudflats. To understand how these plants are incorporated into the invertebrate food webs we analyzed organisms for naturally occuring abundances of the stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$. The carbon signal reflects a weighted average of food sources, with *Spartina* $\delta^{13}C$ = -14 ‰, which is significantly more enriched than other carbon sources in this habitat (Cloern et al. 2002). We predict that if hybrid *Spartina* is entering the food web, consumers collected within invaded areas will have a stronger *Spartina* signal than those from mudflats, with consumers from *S. foliosa* showing an intermediate signal.

RESULTS

Hybrid *Spartina* differs from *S. foliosa* in its effects on habitat structure, infaunal communities, and food webs. Hybrid *Spartina* produces more biomass, and therefore more dense structure on mudflats, than *S. foliosa* both above and below ground (Fig. 1). Alameda, where the invasion is 30 years old, has greater belowground biomass than San Lorenzo, which was invaded in the 1990s. We found few consistent differences between sediment characteristics of hybrid or *S. foliosa* and mudflats. *Spartina foliosa* generally had significantly higher infaunal densities and biomass than adjacent mudflats. Winter data are presented here, but summer samples showed similar patterns. In contrast, hybrid *Spartina* sediments never contained significantly greater densities or biomass than mudflats (Fig. 2, numbers above bars are p-values, n = 10).

Food web analysis also shows differences between *S. foliosa* and hybrid *Spartina*. Stable isotope results indicated that some species show evidence of a slight shift in $\delta^{13}C$ toward *Spartina* for individuals collected within *S. foliosa*. (Fig. 3) For example, shore crabs (*Hemigrapsus oregonensis*) and European green crabs (*Carcinus maenas*) living within *S. foliosa* at China Camp, and *H. oregonensis* in *S. foliosa* at Drakes Estero had isotopic signatures more similar to *S. foliosa* than those collected on mudflats. At Alameda, there was no significant difference in isotopic signatures of *C. maenas* or Atlantic oyster drills (*Urosalpinx cinerea*) from hybrid *Spartina* and mudflats. When results from all sites are compared, hybrid *Spartina* does not appear to produce a stronger shift in isotope signatures than *S. foliosa* (Brusati and Grosholz 2009).

DISCUSSION

Our results show that hybrid *Spartina* is not ecologically equivalent to the native cordgrass. While both *S. foliosa* and hybrid *Spartina* modify estuarine habitat, the magnitude of the changes produced by hybrid *Spartina* are greater, resulting in qualitative differences in the infauna. The strongest differences were seen in the greater height and aboveground biomass of hybrid *Spartina* compared to *S. foliosa*. The greater aboveground biomass of hybrid *Spartina* is more than would be expected from differences in stem height and density between the two species; it also reflects the fact that the hybrid's stems are considerably thicker than *S. foliosa*'s. Native *S. foliosa* faciliates infauna, possibly by stabilizing substrate or providing attachment sites for tube-building organisms. In contrast, the dense roots and

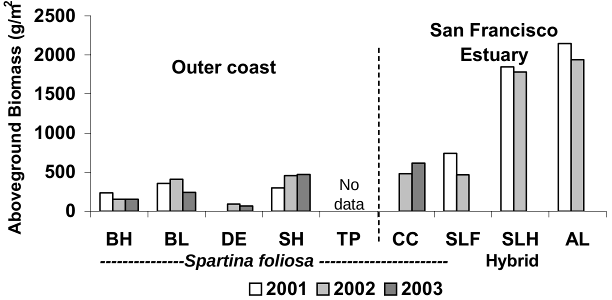

Fig. 1. Aboveground biomass of *S. foliosa* and hybrid *Spartina* 2001-03 (n = 10). Site abbreviations: BH = Bodega Harbor, BL = Bolinas Lagoon, DE = Drake's Estero, SH = Shields March, TP = Tom's Pt., CC = China Camp, SLF = Robert's Landing *S. foliosa,* SLH = San Lorenzo hybrid, AL = Alameda.

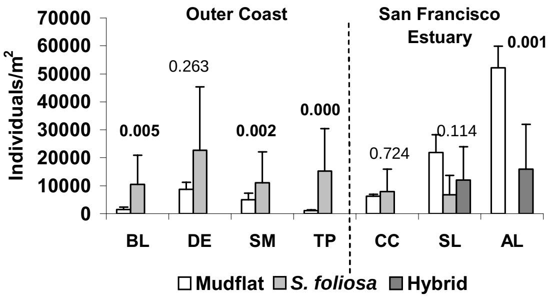

Fig. 2. Infaunal densities in *S. foliosa* exceed those in mudflats, while hybrid *Spartina* never contained greater densities than mudflats (numbers above bars are p-values, n = 10, winter 2001 data shown).

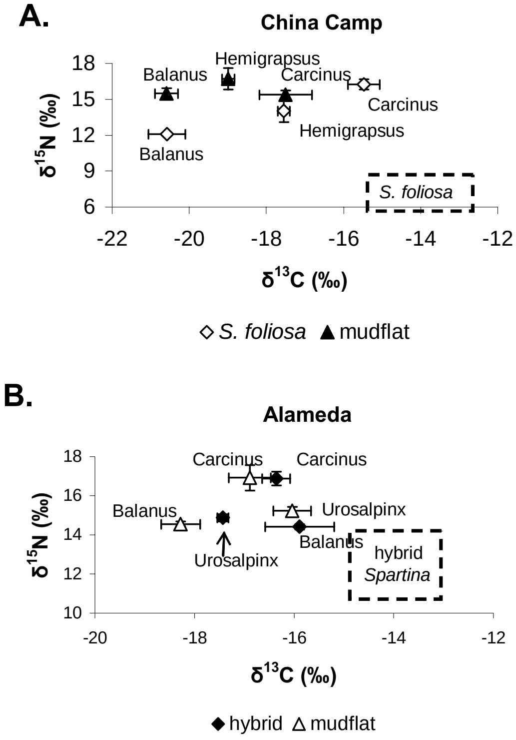

Fig. 3. Stable Isotope Results. Crabs (*H. oregonensis, C. maenas*) at China Camp showed possible incorporation of *S. foliosa* (A), while neither *C. maenas* nor oyster drills (*Urosalpinx cinerea*) at Alameda appeared to incorporate hybrid *Spartina* (B).

rhizomes of hybrid *Spartina* pre-empt space needed by infauna so that invertebrates are replaced by plant material.

Our stable isotope results show that the high biomass produced by hybrid *Spartina* does not translate into increased incorporation of *Spartina* in the infaunal food web. Hybrid *Spartina*'s thick stems may require a longer time to decompose than the thinner stems of *S. foliosa*. As a result, carbon is held in hybrid *Spartina* rather than transferred to higher trophic levels.

The change from the relatively open canopy of *S. foliosa* to the more denser stems of hybrid *Spartina* may have indirect consequences for other primary producers that are important to estuarine food webs, especially benthic microalgae. On the Atlantic coast, microalgal production was lower underneath the canopy of tall form *S. alterniflora* than under the shorter dwarf form (Sullivan and Currin 2000), suggesting a potential decrease in microalgal production in northern California as hybrid *Spartina* replaces shorter *S. foliosa*.

Comparing effects of native *S. foliosa* and hybrid *Spartina* may help resource managers predict the impact if hybrid *Spartina* establishes populations in coastal bays north of San Francisco, while data from *S. foliosa* marshes may provide reference information for evaluating restoration projects. Infauna are major food sources for shorebirds and native fishes, recycle carbon by breaking down plant detritus, and move sediment by bioturbation and suspension feeding (Levin et al. 2001). Therefore, if replacement of *S. foliosa* with hybrid *Spartina* leads to relatively lower infaunal densities, consequences for the food web may reach beyond the direct structural changes caused by the plants.

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IMPACTS OF BENTHIC INVERTEBRATES ON SEDIMENT POREWATER AMMONIUM AND SULFIDE: CONSEQUENCES FOR *SPARTINA* SEEDLING GROWTH

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Since its introduction in the 1970s, *Spartina alterniflora* and its hybrids have rapidly invaded the intertidal zone of San Francisco Bay. We know relatively little about the biotic and abiotic factors that have either facilitated or hindered this invasion. In its native range, nitrogen availability and sediment anoxia are known to limit the growth of *S. alterniflora*. Benthic invertebrates may alter porewater concentrations of both ammonium and soluble sulfide, and thereby indirectly influence *S. alterniflora* success. In order to better understand how the resident macrofaunal invertebrate community may impact the expansion of *S. alterniflora* into the intertidal zone, we examined how common invertebrates influence concentrations of ammonium and soluble sulfide in sediment porewater. We conducted laboratory microcosm experiments to determine how species from three functional feeding groups of benthic invertebrates (subsurface deposit feeders, surface deposit feeders and surface grazers) affect porewater in unvegetated areas adjacent to *Spartina*-invaded areas. In a separate experiment, we examined the growth of *S. alterniflora* seedlings in the presence of individual invertebrates. Relative to microcosms without invertebrates, where porewater ammonium was fairly high (greater than 400 micromoles per liter [µM]), we found slightly lower porewater ammonium in microcosms with *Heteromastus filiformis* (subsurface deposit feeder) or *Macoma petalum* (surface deposit feeder). Porewater sulfide was slightly higher in the presence of *H. filiformis* only. The significantly greater growth of *S. alterniflora* seedlings in the presence of *M. petalum* than in the presence of *H. filiformis* suggests that both high sulfide and high ammonium may have been detrimental to seedling success. Thus, invertebrates may indirectly influence the success of *S. alterniflora* seedlings by altering porewater chemistry.

Keywords: ammonium, benthic, functional group, invasive species, porewater, *Spartina,* sulfide

INTRODUCTION

Among the greatest threats to natural ecosystems are invasions by non-native species (Drake et al. 1989; Vitousek et al. 1997). However, the mechanisms that determine the success or failure of new invasions are often poorly understood. The invasion of Pacific Coast estuaries by *Spartina alterniflora* and its hybrids (*S. alterniflora* x native *Spartina foliosa*) provides some insight into how *Spartina* can rapidly change benthic communities through altering physico-chemical properties of the habitat. The shift from native mudflats and relatively diverse upper marsh habitats to dense *Spartina* meadows following invasion has profound effects on the functional diversity of benthic invertebrates, nutrient cycling, and other physical processes (Neira et al. 2005, 2006; Levin et al. 2006). However, we still have little information on what biotic and abiotic factors may contribute to the great success of *Spartina* in the intertidal zone of San Francisco Bay. Determining which factors most strongly influence seedling establishment will greatly improve our understanding of the dynamics of the invasion.

Previous work has shown that nutrient limitation and anoxia affect the growth and survival of native *S. alterniflora* in Atlantic coast salt marshes (e.g., Gallagher 1975; King et al. 1982; DeLaune et al. 1983; Dai and Wiegert 1997) and that nutrient limitation may be especially severe during the early stages of marsh development in both native and invaded marshes (Tyler et al. 2003, 2007). Benthic invertebrates can significantly impact the concentration of solutes in porewater and nutrient cycling through bioturbation, burrow ventilation and consumption of organic matter (e.g. Aller 1982, Peterson and Heck 1999; Christensen et al. 2000). However, research has not been done to determine whether the effects of benthic communities on the concentration of nutrients (e.g., ammonium) and toxic metabolites (e.g., soluble sulfide) in porewater can affect the success of invasive plants like *S. alterniflora*.

As part of an investigation linking benthic community structure to the establishment of *S. alterniflora* seedlings*,* we performed two pilot microcosm experiments. The objective of the first experiment was to determine whether common benthic invertebrates from three different functional feeding groups affect porewater ammonium and soluble sulfide without plants present. The objective of the second experiment was to determine whether these same species influenced the growth of *S. alterniflora* seedlings. We predicted that these three functional groups would have different effects on porewater chemistry resulting in differential growth of *S. alterniflora* seedlings.

METHODS

We conducted microcosm experiments in an environmental chamber with a constant temperature of 14°C and a 12-hour light cycle. All organisms and sediments were collected from mudflats near the edge of a hybrid *Spartina* marsh at the Elsie Roemer Bird Sanctuary, Alameda Island, San Francisco Bay, California. Microcosms consisted of clear polycarbonate tubes (9.3 centimeters inside diameter [cm I.D.] x 30 cm height [H]) filled with two layers of homogenized, defaunated sediment. The lower layer (12 cm) was seived to 500 micrometers (µm) and then frozen for two weeks to kill any organisms that passed through the seive. The surface layer (3 cm), which was seived to 300 µm, but unfrozen, inoculated the core with natural microflora. Following reconstruction, the cores acclimated in the environmental chamber for three days. Organic matter (one gram [g] dried, ground *Ulva* sp.) was added to the surface of each microcosm one day prior to organism addition. To simulate tidal inundation, we constructed an elaborate, automated system that filled each chamber with sea water (32 parts per thousand [ppt]) halfway through the light cycle and drained each chamber halfway through the dark cycle each day.

In the first experiment, we tested the effects of three functional feeding groups (subsurface deposit feeders, surface deposit feeders, and surface grazers) on porewater ammonium and soluble sulfide concentrations. Our experimental design consisted of a defaunated control and three single species treatments (n = 6) representing each of the three functional groups: the capitellid polychaete *Heteromastus filiformis* (sub-surface deposit feeder), the nassariid snail *Ilyanassa obsoleta* (surface grazer) and the tellinid clam *Macoma petalum* (surface deposit feeder) with eight replicates of each treatment (Table 1). The numbers of individuals added to each microcosm were equivalent to densities at the Elsie Roemer site (see Neira et al. 2005). We extracted porewater from depths of 2, 4, and 7 cm using a perforated stainless steel sampling probe (Berg and McGlathery 2001) at the termination of the experiment (14 days). Porewater samples were analyzed for ammonium using the indophenol blue method (Solorzano 1969) and for sulfide using a modification of the method described by Cline (1969). For statistical analyses of porewater parameters, we calculated a mean value for all three depths for each replicate and analyzed differences among treatments separately for ammonium and sulfide using ANOVA.

Table 1: Design for Experiment 1. Density is the number of individuals for that taxa per core (68 cm^2). N=6 for all treatments.

Treatment	Functional Group	Density
Defaunated control	-	-
Heteromastus filiformis	Subsurface deposit feeder	30
Ilyanassa obsolete	Surface grazer	2
Macoma petalum	Surface deposit feeder	3

In the second experiment, we used the same invertebrate treatments and defaunated control as in the first experiment, but added one *S. alterniflora* seedling to each microcosm. We used nine replicates for defaunated controls and each of the three macroinvertebrate treatments. The seedlings used in this experiment were germinated in the greenhouse using seeds from inflorescences collected in Willapa Bay, Washington. Thirty days after germination, the seedlings were transferred to environmental chambers and acclimated to experimental temperature and light regimes while salinity of the water in sediments was gradually increased from 0 to 35 ppt over a period of 10 days. Seedlings were transplanted to microcosms after this acclimation period. After 14 days, we measured change in total leaf length (sum of length of individual leaves) and seedling biomass (aboveground and belowground). We analyzed differences among treatments using ANOVA.

RESULTS

For the first experiment, preliminary analysis suggested that the affects of benthic invertebrates on porewater ammonium and sulfide concentrations differed among functional groups. Sulfide concentrations were highest in microcosms containing *H. filiformis* but differences between treatments were not significant (p = 0.381; Fig. 1A). The ammonium concentration was highest in the control and lowest in microcosms containing *M. petalum*, but again the differences were not significant (p = 0.285; Fig. 1B). The surface grazer *I. obsoleta* had no obvious effects on ammonium or sulfide.

Overall, in the second experiment, seedling establishment was relatively poor, and there was visible yellowing and dehydration of leaves in all treatments, particularly microcosms containing *H. filiformis*. However, our preliminary analysis indicates that the surface deposit feeder treatment (*M. petalum*) had a positive effect on seedling establishment (Fig. 2A, 2B and 2C). In contrast to controls that had no significant growth, the total leaf length increased approximately seven cm in microcosms containing *M. petalum*. Post-hoc Tukey tests indicated significant differences between *M. petalum* and *H. filiformis* (p = 0.048). Aboveground and belowground biomass were also higher in microcosms containing *M. petalum* than in other

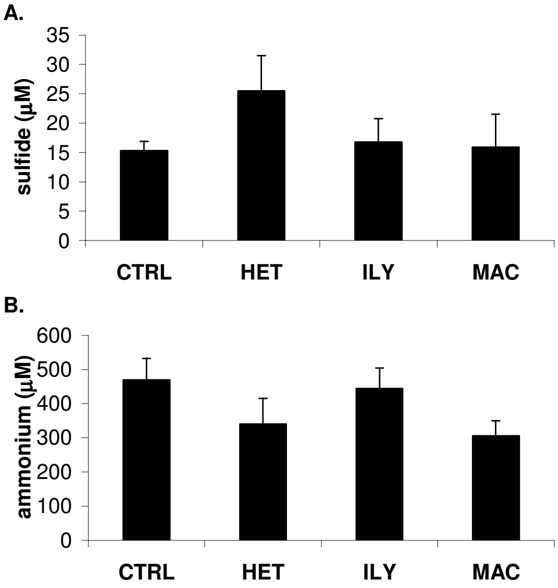

Fig. 1: Results from Experiment 1 by treatment (CTRL=defaunated control, HET=*Heteromastus filiformis*, ILY=*Ilyanassa obsoleta*, and MAC=*Macoma petalum*). **A.** Porewater sulfide concentration. **B**. Porewater ammonium concentration. N=6 for all treatments.

treatments, although these differences were not significant (Fig. 2B and 2C).

DISCUSSION

Our preliminary results suggest that under laboratory conditions benthic macroinvertebrates can affect both porewater ammonium concentrations and *Spartina alterniflora* seedling growth. We found that both *H. filiformis* and *M. petalum* lowered porewater ammonium concentrations relative to controls, while surface grazers had no obvious effect on porewater ammonium or sulfides. Benthic invertebrates can decrease porewater ammonium and soluble sulfide concentrations by flushing porewater solutes from sediments during burrow construction and irrigation (Aller 1982; Christensen et al. 2000) and by stimulating oxidation-reduction reactions (Pelegri and Blackburn 1995; Rysgaard et al. 2000), but the magnitude of change is limited by the depth of the burrow (e.g., Aller 1982; Francois et al. 2002; Michaud et al. 2006).

Despite the negative effect on porewater ammonium concentrations, *H. filiformis* appeared to have a positive effect on soluble sulfide concentrations although this was not confirmed statistically. Past work has demonstrated that invertebrates can increase sulfate reduction rates in marine sediments (Hansen et al. 1996). A variety of mechanisms including removal of inhibitory metabolites, redistribution of particles, secretion of labile mucus along burrow walls, and translocation of labile organic matter from the surface to the deeper sediment during feeding could be responsible for increased anaerobic metabolism and sulfide production (Wheatcroft et al. 1994; Marinelli and Boudreau 1996; Aller and Aller 1998). However, it is not clear which mechanisms would account for the positive effect of *H. filiformis* on soluble sulfides or why *H. filiformis* would have opposite effects on ammonium and sulfide.

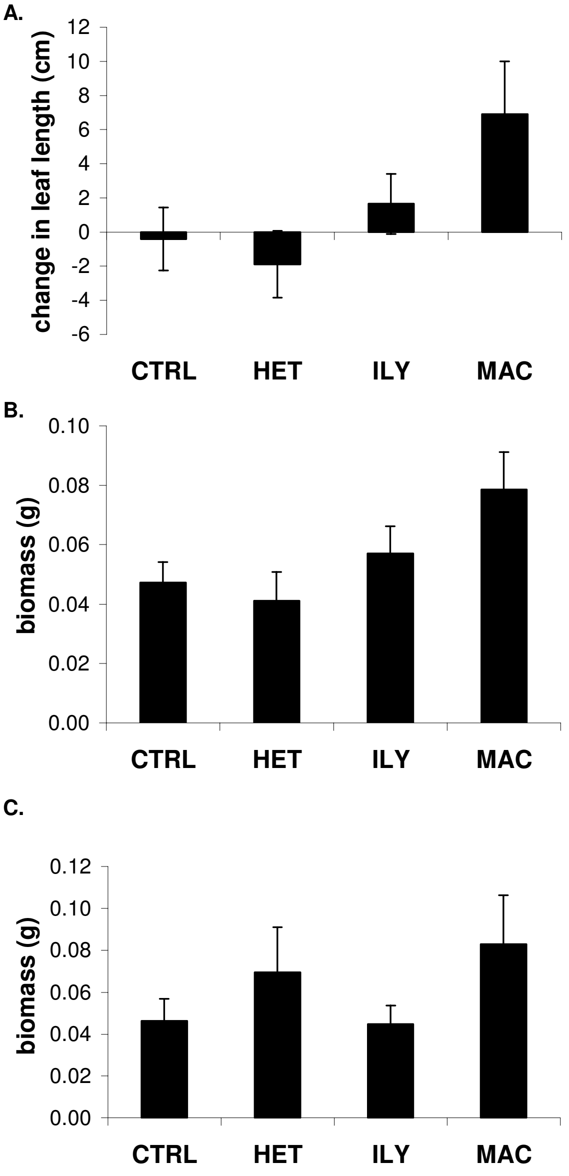

Fig. 2: Results from Experiment 2 by treatment (CTRL=defaunated control, HET=*Heteromastus filiformis*, ILY=*Ilyanassa obsoleta*, MAC=*Macoma petalum*). **A.** Change in leaf length of *Spartina alterniflora* seedlings. **B**. Aboveground biomass of *S. alterniflora* seedlings. **C.** Belowground biomass of *S. alterniflora* seedlings. * indicates treatment means that differ significantly from each other (post-hoc Tukey, p< 0.05). N = 9 for each treatment.

Significant differences between treatments for change in total leaf length suggest that the benthic community has the potential to substantially influence the establishment of *S. alterniflora* seedlings at the edge of the expanding marsh. Work from eastern U.S. marshes in the native range of *S. alterniflora* has shown growth and establishment can be limited by high porewater sulfide concentrations (Morris and Dacey 1984; Bradley and Morris 1990) and by availability of ammonium in porewater (Tyler et al. 2003). At the same time, very high ammonium can have a toxic effect on plants although tolerances vary widely between species (Britto and Kronzucker 2001). Ammonium toxicity for other marsh species has been demonstrated at concentrations above 200 µM (Tylova et al. 2008), although to our knowledge this has not been demonstrated for *S. alterniflora*. *H. filiformis* stimulated an increase in porewater sulfide concentrations, a decrease in porewater ammonium and a loss of *S. alterniflora* leaves (measured as the decrease in leaf length). In contrast, the low porewater sulfide and ammonium in the presence of *M. petalum* appeared to promote seedling success. In our experiments, concentrations of ammonium exceeded 300 µM and it is possible that it had an inhibitory effect on seedling growth. While future work is needed to clarify the mechanisms linking benthic invertebrate communities to seedling success, our results suggest that both low porewater sulfide and moderate levels of porewater ammonium are requisite for *S. alterniflora* seedling success.

The spread of *S. alterniflora* and its hybrids in West Coast estuaries may depend in part on complex interactions between infaunal community structure and local biogeochemical cycling that in turn dictate seedling success. As the invasion proceeds, changes in benthic community structure may either facilitate or inhibit the success of *S. alterniflora* and its hybrids. Long-term goals for control, eradication, and restoration depend on an understanding of the processes that control the establishment and expansion of these invaders.

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QUANTIFYING THE POTENTIAL IMPACT OF THE *SPARTINA* INVASION ON INVERTEBRATE FOOD RESOURCES FOR FORAGING SHOREBIRDS IN SAN FRANCISCO BAY

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The extensive mudflats of the San Francisco Bay estuary are critical habitats and foraging grounds for migratory shorebirds. However, San Francisco Bay has experienced invasion by hundreds of non-native introduced species, and its productive mudflats are now threatened by one of these — hybrid *Spartina alterniflora*. To assess the potential effects of the invasive cordgrass on the shorebird foraging habitat, we quantified invertebrate abundance and biomass along transects extending from the edge of the *Spartina* meadow to the lowest tide line at two locations in the bay, Robert's Landing and Elsie Roemer. We also estimated the size of areas potentially invaded by *Spartina* using tidal elevation data based on LIDAR images and tidal inundation data taken from continuous tidal height monitoring stations. We found that invertebrate biomass was greatest at higher tidal elevations nearer to the marsh, while abundance was greatest at intermediate distances from the marsh. Current estimates of tolerance to tidal inundation indicate that the area colonized by *Spartina* could increase by as much as four to eight times its present size, depending on the individual site characteristics. These data suggest that there is a high potential for losing significant amounts of valuable foraging habitat for shorebirds if *Spartina* extends its distribution to predicted tidal elevations in San Francisco Bay.

Keywords: shorebirds, San Francisco Bay, Hybrid *Spartina*, infauna

INTRODUCTION

San Francisco Bay is now home to more than 250 species of non-native plants and animals (Cohen and Carlton 1998). One of the most serious recent invasions has been the smooth cordgrass *Spartina alterniflora*, which is native to the eastern coast of the North America. Since its introduction, *S. alterniflora* has hybridized with the native cordgrass *S. foliosa* creating a hybrid that has rapidly colonized many areas of central and south San Francisco Bay (Daelher and Strong 1997, Ayres et al. 2004). The hybrid can colonize open mudflats as well as out-compete native vegetation at higher tidal heights (Ayres et al. 2004). The ecological repercussions of this invasion are far reaching with widespread impacts on community structure and ecosystem function (Neira et al. 2005; 2006, Levin et al. 2006).

One of the most serious consequences of the hybrid *Spartina* invasion is the loss of open mudflat habitat for shorebirds. San Francisco Bay, with its extensive mudflats, contains critical habitat for more than one million migratory shorebirds and is one of the most important estuaries along the Pacific Flyway (Page et al. 1999). This important habitat is now threatened by the smooth cordgrass. Shorebirds require unvegetated mudflat habitats for foraging (Goss-Custard and Moser 1988), and invasive hybrid *Spartina* threatens to colonize critical upper levels of this important habitat. As a result of the rapid spread of hybrid *Spartina,* which increased by 317% at sampled sites between 2001 and 2003 (Ayres et al. 2004), by the end of 2003 it was estimated to occupy nearly 800 hectares of bay habitat, up from less than 200 acres in 2001 (Zaremba and McGowen 2004). This rapid spread suggests that the risk for shorebird habitat loss may be substantial.

To better estimate the potential value and extent of lost foraging habitat, we measured the biomass and abundance of invertebrates at different tidal elevations at two sites in San Francisco Bay. To accurately measure tidal elevation across broad areas of the two study sites, we used LIDAR (Light Detection and Ranging), a flight-based radar system that can accurately measure elevation with high spatial resolution. We used these elevation data together with tidal height data from continuous recording stations to estimate the area likely to be invaded by *Spartina* based on current estimates of its inundation tolerance. Therefore, we were able to estimate the value and extent of habitat lost to shorebirds under different scenarios of *Spartina* spread. This information, when combined with the data on patterns of shorebird usage generated by PRBO Conservation Science (Stralberg et al. 2010) can help guide future eradication efforts by highlighting areas of particular importance for shorebirds.

METHODS

To determine the distribution and abundance of key invertebrate taxa of importance for foraging shorebirds, we sampled infauna at two sites in central San Francisco Bay with open mudflats that are being invaded by hybrid *Spartina*: Elsie Roemer Bird Sanctuary on Alameda Island (37º45'35''N; 122º28'48''W) and Roberts Landing in San Lorenzo (37º40'13''N; 122º28'48''W). At both sites, we established three transects spanning the entire tidal range of the mudflat (several hundred meters) from the edge of the *Spartina* meadow to the channel. We collected sediment core samples from the following distances from the seaward edge of the hybrid *Spartina* meadow: 1, 10, 50, 100, and 200 meters (m) and thereafter at approximately 100 m intervals to within 50 m of the channel edge. At Elsie Roemer sampling extended up to 350 m, whereas at Robert's Landing, sampling extended up to 850 m from the meadow. At each sampling location, one infauna sample was collected with a 5 centimeters (cm) diameter core taken to 5 cm depth. The location of each sample site was recorded with a shoulder mounted GPS (Global Positioning System, Trimble Pro-XR). In the lab, samples were sieved at 500 micrometers (µm) to collect organisms of importance for shorebirds. All organisms were fixed in 10% buffered formalin for at least 24 hours, then stored in 70% ethanol stained with rose bengal.

We estimated the biomass of invertebrates for the three most common phyla (>95% of the biomass): Annelida, Arthropoda (Crustacea), and Mollusca. We regressed biomass for all taxa and for each of the three common phyla on distance from the edge of the *Spartina* meadow defined as the lower limit of contiguous aboveground vegetation.

We used LIDAR images that resulted from a more extensive flyover of San Francisco Bay, but included detailed views of the two study sites. Images permitted estimations of tidal elevations to within 0.1 m at approximately 1 m spatial resolution in the x-y plane. These data were used to develop images of the study site with which we could estimate area covered by *Spartina* assuming it could spread downward in tidal elevation to a particular tidal level. We used these images together with georeferenced sampling sites to determine the abundance and distribution of invertebrates at particular tidal elevations.

We calculated inundation as a function of tidal elevations, using continuous water level data available from the National Oceanic and Atmospheric Administration (NOAA) using station # 9414750 Alameda, CA for both sites. Tidal data were measured in meters above mean low low water (MLLW) based on the updated National Tidal Datum Epoch (NTDE). We used hourly tidal data to estimate the percentage time for the year 2004 that a given elevation determined from LIDAR images would be inundated.

RESULTS

Invertebrate biomass at higher tidal elevations was approximately triple the biomass at lower elevations when both sites were analyzed together (Fig. 1) based on an exponential regression curve:

$$y = 18.593\, e^{-0.0032x},\ R^2 = 0.36 \qquad (1)$$

However, the abundance of invertebrates was greatest at intermediate tidal elevations (Fig. 2), and the least at the lowest elevations based on a polynomial regression curve:

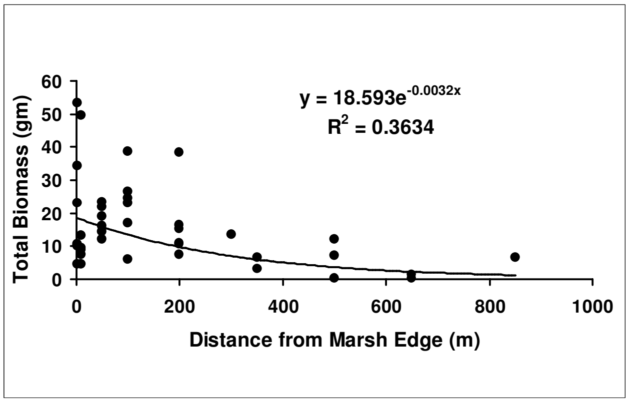

Fig. 1. Total invertebrate biomass as a function of the distance from the marsh edge at the current lower tidal limit of hybrid *Spartina*.

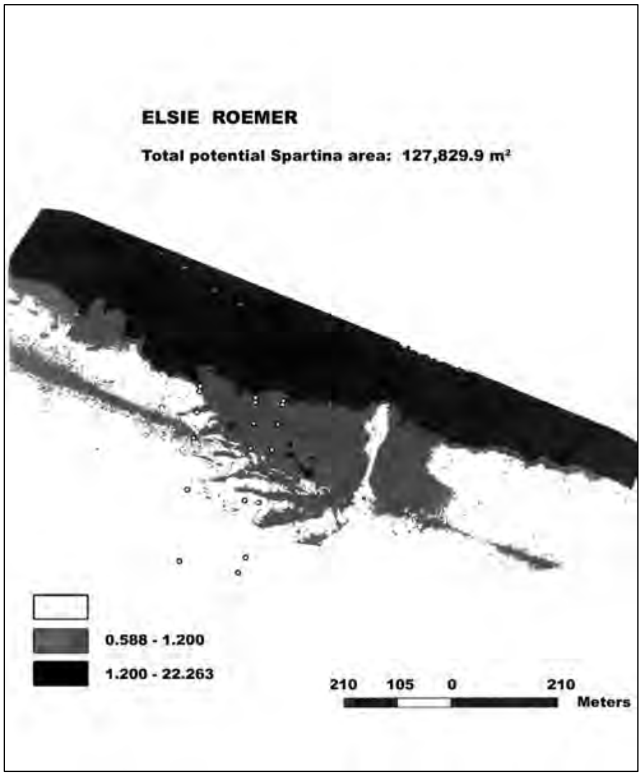

Fig. 2. LIDAR image of the Elsie Roemer site showing lower extent of hybrid *Spartina* distribution (grey) and invertebrate sampling points along transects (dots).

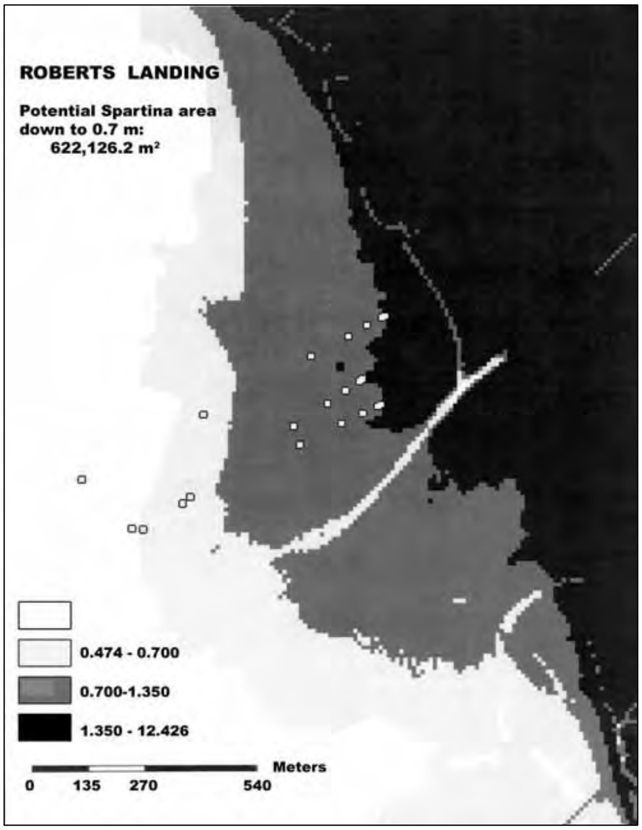

Fig. 3. LIDAR image of Robert's Landing showing lower extent of hybrid *Spartina* distribution (grey) and invertebrate sampling points along transects (dots).

$$y = -0.0006x^2 + 0.3889x + 56.787,\ R^2 = 0.1713 \qquad (2)$$

The high abundances at intermediate tidal elevations were due to high numbers of small juvenile clams (> 1 mm) that did not contribute very significant biomass. When we analyzed the data by phyla, we found that annelids, mollusks and crustaceans generally showed similar patterns. Annelids also showed a substantial decline in biomass with tidal elevation:

$$y = -0.0165x + 13.624,\ R^2=0.12 \qquad (3)$$

Molluscs and crustaceans showed a similar but more variable decline (R^2 <0.10).

The LIDAR images (Figs. 2 and 3) showed that if the distribution of *Spartina* continues to cover succeeding lower tidal zones, it will cover a substantial portion of upper tidal mudflats. At Elsie Roemer, the area invaded by *Spartina* would increase more than eightfold from 15,591 m^2 to 127,829 m^2 if it was able to extend from +1.0 m to +0.6 m above MLLW. At Robert's Landing, the area invaded by *Spartina* would increase more than four-fold from 140,971 m^2 to 622,126 m^2 as it extended its distribution from +1.1 m to +0.7 m. above MLLW.

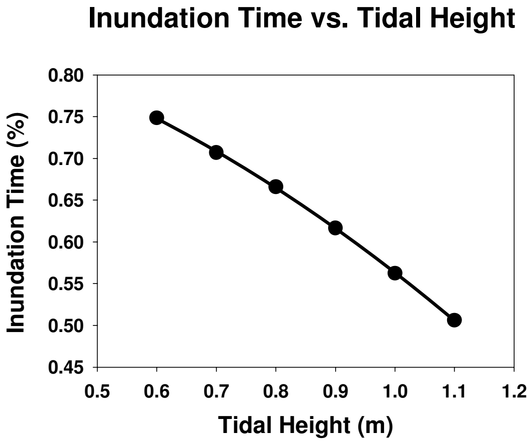

Fig. 4. Plot of inundation time (%) calculated from continuous tidal height data relative to estimated elevation from LIDAR data.

We found these tidal elevations bracketed the current predictions of 60-70% inundation time as the upper limit of inundation tolerarance for hybrid *Spartina* (J. Collins, pers. comm.), although a formal test of inundation time has yet to be conducted. Inundation time would go from approximately 50% at 1.1 m above MLLW to nearly 75% at 0.6 m above MLLW (Fig. 4).

DISCUSSION

Our data suggest that the continued expansion of hybrid *Spartina* into tidal zones below the distribution of native marsh plants like *S. foliosa* (Ayres et al. 2004) will result in significant loss of foraging habitat for shorebirds. The higher portions of the normally unvegetated tidal flats, which have the greatest exposure time and also the higher biomass of invertebrate prey, are the most available to shorebirds. It is unclear why invertebrate biomass is higher at higher tidal elevations, although it may be due to greater predation pressure at lower elevations due to fishes and invertebrate predators. It is these higher elevation areas of naturally unvegetated mudflat that are the most valuable for shorebirds and are also the most likely to be invaded in the near future by invasive hybrid *Spartina.* We note that different groups of shorebirds may be affected more than others by the loss of higher tidal zones and more restricted foraging time. Larger and longer legged shorebirds such as marbled godwits (*Limosa fedoa*) and willets (*Catoptrophorus semipalmatus*) can forage in deeper water than smaller birds like western and least sandpipers (*Calidris minutilla* and *C. mauri*), dunlin (*Calidris alpina*) and sanderlings (*Calidris alba*).

Based on our analysis of LIDAR data together with tidal inundation, our data also suggest that the area currently invaded by hybrid *Spartina* could see a potential eightfold increase at Elsie Roemer and a fourfold expansion at Robert's Landing. We emphasize that this is likely to be an upper estimate of the area invaded, given that it assumes that hybrid *Spartina* could withstand being inundated approximately 75% of the time, which is beyond current estimates of tidal inundation tolerance. However, these estimates are based in part on present distributions, which are subject to change. These estimates also do not consider that this tolerance may be under strong selection and may evolve significantly over time. In any case, our data suggest that much of the most important foraging habitat for migratory shorebirds in San Francisco Bay that lies between 0.6-1.1 m MLLW may be lost over the next several years.

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POTENTIAL IMPACTS OF *SPARTINA* SPREAD ON SHOREBIRD POPULATIONS IN SOUTH SAN FRANCISCO BAY

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San Francisco Bay holds 70% of California's mudflats and provides habitat to more wintering and migratory shorebirds than any other wetland along the Pacific coast of the contiguous U.S. The bay's mudflats are currently threatened by the spread of a non-native cordgrass, *Spartina alterniflora*, and associated hybrids, which grow at lower elevations than the native *S. foliosa* and can render large mudflat areas effectively unavailable to shorebirds for foraging. Using shorebird survey data, tide station data, and GIS-based habitat data, we analyzed the potential effect of *S. alterniflora x foliosa* hybrids on shorebird habitat in the South Bay by creating grid-based spatial models of shorebird habitat value and potential *Spartina* spread. We developed three scenarios of potential habitat value loss for shorebirds based on assumptions about the inundation tolerance of *S. alterniflora* and temporal availability of mudflat resources. Predictions of habitat value loss across seasons ranged from 27% to 80%. We identified the upper mudflats, due to their greater exposure time, and the east and south shore mudflats, due to the high numbers of birds detected there, as the areas of highest value to shorebirds in the South Bay. These areas also coincide with the areas of greatest *Spartina* invasion potential.

Keywords: *Spartina alterniflora*, mudflats, inundation tolerance, Charadrii, GIS

INTRODUCTION

The San Francisco Bay estuary holds 70% of the mudflats in California (Josselyn et al. 1990), providing habitat to over 350,000 migrating shorebirds (Charadrii) in the fall, over 325,000 in winter, and over 900,000 in the spring (based on single-day counts) (Stenzel et al. 2002). Along the Pacific coast of the contiguous United States alone (excluding Alaska), San Francisco Bay holds more shorebirds than any other wetland in all seasons (Page et al. 1999). Survey data suggest that San Francisco Bay holds over 50% of the Pacific coast populations of several shorebird species—11 of the 12 most abundant species in fall, six of 13 in winter, and seven of 13 in spring (Page et al. 1999). Within the Bay, mudflats are the most important habitats for the majority of these species (Stenzel et al. 2002).

San Francisco Bay's mudflats are now threatened by the spread of cordgrass hybrids (*Spartina alterniflora x foliosa*) (Ayres et al. 2008). The non-native *S. alterniflora* was originally introduced to the South San Francisco Bay (South Bay) in the 1970s (Callaway and Josselyn 1992) and hybridized with native *S. foliosa* (Ayres et al. 1999). *Spartina alterniflora* x *foliosa* hybrids exhibit higher tolerance to tidal submersion and salinity, as well as higher growth and germination rates than the native *S. foliosa* (Callaway and Josselyn 1992; Daehler and Strong 1997; Anttila et al. 1998; Collins 2002). Projections from sampling sites suggested that nearly 800 hectares (ha) of South Bay marshes, channels, and mudflats had been invaded by 2003, an increase of more than 300% since 2001 (Zaremba et al., this volume). Further spread of hybrid *Spartina* on the Bay's mudflats poses a great threat to shorebirds, which cannot forage in areas of dense growth (Josselyn 1983, Evans 1986, Goss-Custard and Moser 1988, White 1995).

In Willapa Bay, Washington, which was initially invaded in the late 19th century, *S. alterniflora* recently experienced explosive growth, tripling its areal extent between 1994 and 2002 and converting many mudflats to *Spartina* marshes (Buchanan 2003; Civille 2005). Surveys of Willapa Bay birds suggested a reduction in shorebird numbers by as much as 67% and foraging time by as much as 50% (Jaques 2002). Unlike San Francisco Bay, Willapa Bay is outside the range of native *Spartina* and therefore has no hybrid *Spartina*, which has been found to exhibit much more rapid rates of lateral expansion than its parental species (Ayres et al. 2008). Thus the invasion potential in San Francisco Bay is thought to be substantially greater than in Willapa Bay.

While San Francisco Bay and Willapa Bay also differ in their bathymetry and tidal inundation regimes, relationships that have been identified between tidal inundation parameters and *S. alterniflora* growth tolerance (McKee and Patrick 1988; Collins 2002) may be used to estimate the potential for hybrid *Spartina* spread in San Francisco Bay. Here we present a preliminary GIS-based analysis of the potential effects of hybrid *Spartina* on shorebird habitat in South San Francisco Bay (the South Bay), using grid-based spatial models of (a) shorebird habitat value and (b) potential *Spartina* spread.

METHODS

Many studies have demonstrated that shorebird use of mudflat habitats is spatially and temporally variable, and that this variation is closely tied to cycles of tidal inundation, as well as prey availability across the intertidal zone (Burger et al. 1977; Goss-Custard et al. 1977; Puttick 1977; Page et al. 1979; Colwell and Landrum 1993; Yates et al. 1993). While we did not have data on prey availability in San Francisco Bay, our quantification of shorebird habitat value did incorporate variation within mudflats based on tidal inundation cycles, as well as variation among mudflats based on shorebird use data from PRBO's Pacific Flyway surveys (1988-1993, Page et al. 1999). For the purpose of this exercise, we assumed that South Bay mudflats were at carrying capacity (i.e., the maximum number of birds that can be supported by a finite food supply) at the time of the surveys. By extension, we assumed that loss of habitat in one area would not be compensated for by increased use of other areas.

The spread potential of *S. alterniflora* and associated hybrids was based on percentiles of cumulative monthly tidal inundation across the mudflats. The cumulative monthly duration of inundation at a particular site is a function of mudflat elevation and tidal range, with a greater tidal range resulting in a longer duration of inundation. According to Collins' (2002) analyses of non-native *Spartina* locations in San Francisco Bay, the lower limit of *Spartina* growth appears to correspond with cumulative monthly inundation, and existing non-native *Spartina* locations suggest that the maximum cumulative duration of inundation tolerated during the month of June is approximately 70%, regardless of mean tidal range.[1] This means that the smaller the tidal range, the lower the elevation at which non-native *Spartina* would be predicted to grow. Due to uncertainty about the behavior of *S. alterniflora* hybrids, and because these plants are known to change their environment over time (Ranwell 1964; Daehler and Strong 1996), accreting sediment at rates of 1-2 cm/year in Willapa Bay (Sayce 1988) and up to 4 cm/year in Australia (Bascand 1970), we evaluated a range of inundation tolerances between 60% and 80%. We assumed that mudflat areas covered by *S. alterniflora* and associated hybrids would be effectively lost to shorebirds.

Our GIS-based analysis was restricted to mudflats mapped by the San Francisco Estuary Institute's EcoAtlas (v. 1.50b, SFEI 1998) south of the San Francisco Bay Bridge. Using EcoAtlas map layers, PRBO shorebird surveys (Stenzel et al. 2002), and tide level data from the National Oceanic and Atmospheric Administration's (NOAA) tide stations, we developed a set of grid-based data layers (ArcInfo format) that were combined to generate predictions about the potential loss of mudflat habitat and shorebird numbers.

To generate the GIS grid layers for this analysis, we completed the following steps using the ArcInfo 8.3 GRID module (ESRI 2002).

Fig. 1. Tide station locations, station IDs, and allocation of tide stations with continuous water level data to mudflat areas. Tide station information was obtained from NOAA/NOS (http://tidesandcurrents.noaa.gov/).

Elevation/Bathymetry

When this analysis was performed, bathymetry data layers of a fine enough resolution to capture mudflat topography were not available for the South Bay. Thus, to create a spread model for *S. alterniflora* and associated hybrids, we elected to estimate mudflat elevation at a 3x3 m^2 (3-m) pixel resolution, creating a digital elevation model (DEM) based on mapped mudflat boundaries, tide level data, and an assumed linear mudflat slope.

First, mean tide level (MTL) and mean lower low water (MLLW) contours were estimated from EcoAtlas (SFEI 1998) and were defined based on the boundaries between mudflat and tidal marsh and between open water and mudflat, respectively. Actual elevations along the MTL contour were not assumed to be constant, but were assigned based on MTL elevation at the closest tide station. MTL elevations were obtained from NOAA's National Oceanic Service (NOS) published benchmark sheets for seven South Bay locations that have been referenced to the new National Tidal Datum Epoch (NTDE; 1983-2001) (Fig. 1). For each mudflat area we assumed that local MTL was the same as that of the nearest NTDE-referenced tide station.

Next we used MTL and MLLW contours to determine the width of the mudflat for each 3-m pixel. We calculated the distance from each pixel to the MTL line and to the MLLW line, to obtain two separate distance grids, which were then added to obtain a single grid representing mudflat width. For

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[1] Initial estimates of 40% presented in Collins (2002) have since been revised.

each mudflat section we estimated the slope (assumed linear) by dividing the total change in elevation across the mudflat (MTL) by mudflat width (slope = rise/run). Then we created two 3-m elevation grids based on the following equations, where each 3- m pixel value was equal to the elevation at that point:

(1) elevation 1 = slope * distance to MLLW; *and*

(2) elevation 2 = MTL – (slope * distance to MTL).

We averaged these two grids to obtain the final 3-m mudflat elevation grid (DEM). Values greater than local MTL were redefined to be equal to local MTL.

Tidal Inundation and Shorebird Habitat Value

Published verified continuous (six-minute interval) water level data was available from NOS for only three of the seven tide station locations: Alameda (station ID 9414750, year 2001), Dumbarton Bridge (station ID 9414509, year 1996), and Redwood City (station ID 9414523, year 2002). Assuming that the tidal inundation regime for each mudflat area was most similar to the nearest tide station with available continous water level data, we created a tide station grid layer by allocating each 3-m pixel to a tide station (Fig. 1).

Because our shorebird data collection efforts were centered around April and September, we generated monthly inundation curves for these months using cumulative water level data from each tide station using Stata 8.0 (2003) (Fig. 2).

Water level values were in meters above MLLW. To predict the monthly tidal inundation percent of each 3-m mudflat pixel, we performed separate polynomial regression analyses (Stata 8.0, 2003) for each tide station and each month (April and September) using the appropriate NOS 6 minute water level data. Resulting regression equations (Table 1) were used to calculate grids representing April and September inundation. Separate equations were developed for each tide station so grids for each of the three tide station areas could be calculated separately and then merged to create one seamless 3-m inundation grid for each season. We then generated grids representing April and September mudflat exposure (100 - monthly inundation percent), as indices of overall shorebird habitat value (Fig. 3).

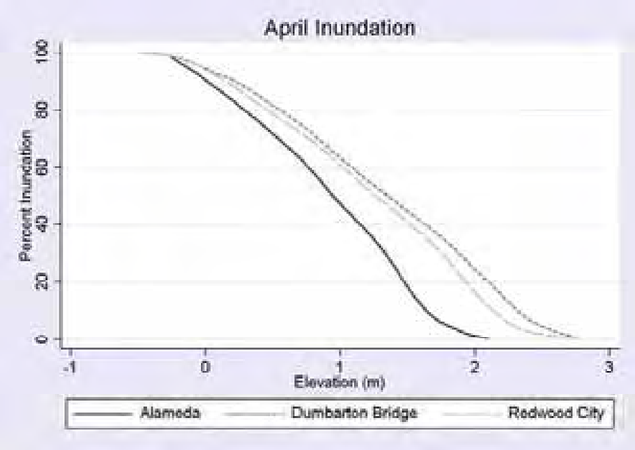

Fig. 2. Cumulative duration of tidal inundation curves for April based on water level data from Alameda, Redwood City, and Dumbarton Bridge tide stations. Elevations corresponding to 60% inundation were 0.762 m, 1.018 m, and 1.083 m, respectively.

Table 1. Coefficients for regression equations used to predict inundation from elevation. Equations were based on inundation curves obtained from 6-minute water level data from NOS tide stations and took the form $y = ax + bx^2 + cx^3 + d$, where y = percent of time inundated and x = elevation above MLLW.

Tide station	***Month***	***a***	***b***	***c***	***d***	***R2***
Alameda	April	-27.9	-24.9	7.85	91.3	0.9974
Alameda	Sept	-21.6	-28.4	7.92	96.4	0.9997
Dumbarton Bridge	April	-19.7	-13.7	2.93	94.6	0.9990
Dumbarton Bridge	Sept	-20.4	-12.1	2.36	98.2	0.9984
Redwood City	April	-20.4	-16.8	4.01	93.9	0.9974
Redwood City	Sept	-15.7	-18.2	3.79	97.9	0.9994

Potential Spartina Spread

Cumulative water level data (m above MLLW) from each tide station were used to generate monthly June inundation curves using Stata 8.0 (2003). For each tide station, June dura-

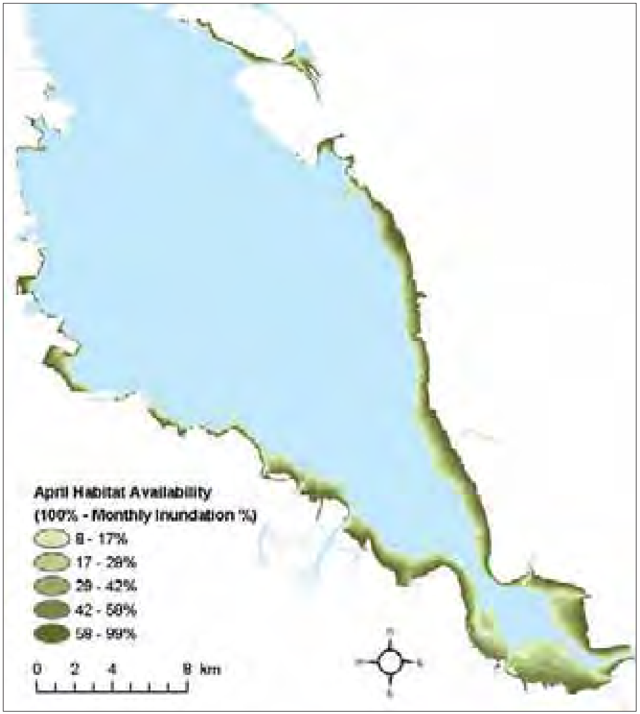

Fig. 3. April mudflat habitat availability, based on monthly mudflat exposure expressed as 100% - monthly mudflat inundation. Color shadings represent mudflat habitat quantiles, where 20% of the total area is contained in each shading category and darker colors have higher value.

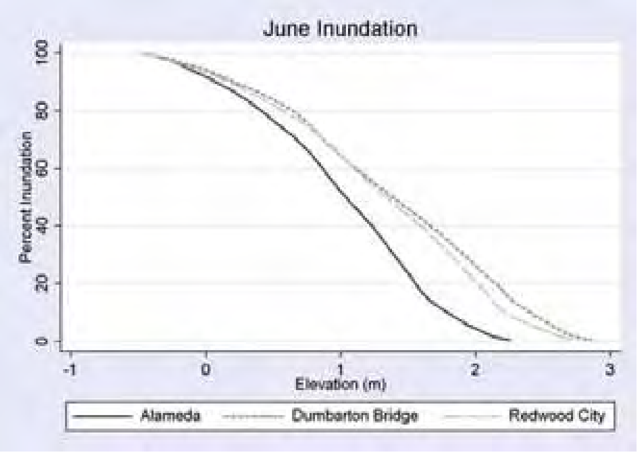

Fig. 4. Cumulative duration of inundation curves for June based on water level data from Alameda, Redwood City, and Dumbarton Bridge tide stations. Elevations corresponding to 60% inundation were 0.864 m, 1.101 m, and 1.113 m, respectively. Elevations corresponding to 70% inundation were 0.674 m, 0.882 m, and 0.874 m, respectively. Elevations corresponding to 80% inundation were 0.413 m, 0.575 m, and 0.646 m, respectively.

tion of inundation curves were used to identify the elevations at which 60%, 70%, and 80% cumulative monthly inundation were achieved (Fig. 4). Each elevation was considered a potential threshold below which *S. alterniflora* and associated hybrids would not grow (i.e., their inundation tolerance). We then calculated potential *Spartina* spread for each inundation tolerance, selecting all mudflat pixels with modeled elevations above that particular elevation threshold.

Shorebird Numbers

We used PRBO's shorebird survey data to estimate fall and spring shorebird numbers and overall biomass in kilograms (kg) for each of six South Bay mudflat census tracts (Stenzel et al. 2002). This resulted in fall and spring shorebird density grids, with densities uniformly distributed over each census tract (Fig. 5).

Table 2. Projected percent of mudflat area and shorebird habitat value lost, based on a range of *Spartina* spread scenarios (1-3). All predictions assume that mudflats are at carrying capacity, and that mudflat habitat value is inversely proportional to tidal inundation.

	Percent of mudflat invaded by Spartina	***Percent of habitat value lost to shorebirds***
Fall		
1 (60% inundation tolerance)	14%	29%
2 (70% inundation tolerance)	33%	57%
3 (80% inundation tolerance)	54%	80%
Spring		
1 (60% inundation tolerance)	14%	27%
2 (70% inundation tolerance)	33%	54%
3 (80% inundation tolerance)	54%	76%

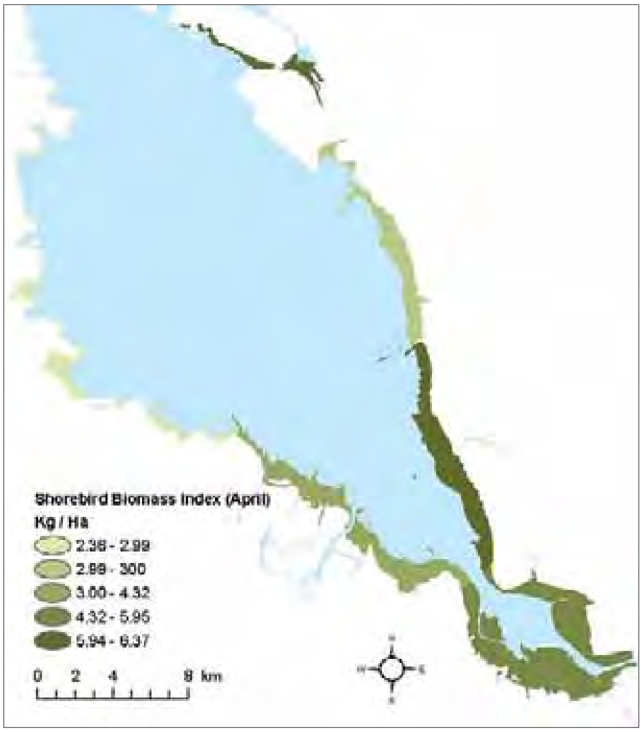

Fig. 5. Shorebird biomass density (kg/ha) based on overall means from comprehensive spring shorebird survey data (1988-1993). Species-specific biomass estimates were taken from the shorebird literature.

Potential Effects of Spartina Spread on Shorebird Numbers

Using the GIS grid layers described above, we estimated the potential effects of non-native *Spartina* spread on shorebird numbers. For each of the six South Bay census tracts and for each *Spartina* spread scenario (60%, 70%, and 80% inundation tolerance), we first calculated the percent of mudflat area that would be lost. Then we calculated the proportion of habitat value that would be lost to shorebirds if mudflat value were inversely related to the percent of mudflat inundation time.

For each of the three *Spartina* spread scenarios, we calculated a predicted loss of spring and fall shorebird biomass by multiplying the proportion of mudflat value lost in each census tract with the total estimated shorebird biomass supported by that census tract. The same was done for individual species' numbers.

RESULTS

Our *Spartina* spread model predicted that between 14% and 54% of the total South Bay mudflat area could be encroached upon by *S. alterniflora* and associated hybrids (Fig. 6, Table 2). The areas of greater *Spartina* spread poten-

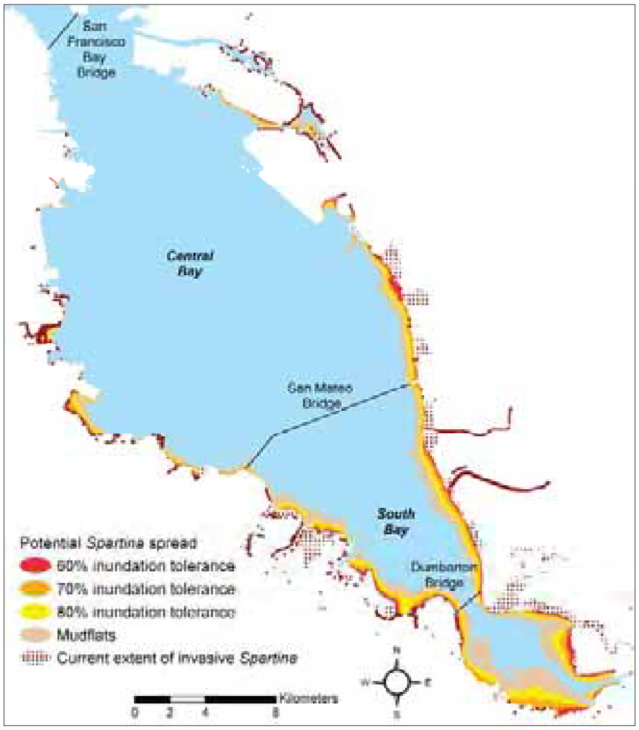

Fig. 6. Predicted extent of *Spartina* spread based on monthly inundation tolerances ranging from 60% to 80% (from Collins 2002). Sharp breaks in predictions are due to breaks in nearest tide station locations.*

tial were the upper mudflats, due to their higher elevation and lower tidal inundation frequency (Fig. 6).

Weighting the areas of potential *Spartina* spread by relative shorebird value, the predicted loss to shorebirds ranged from 27% to 80% (Table 2). The upper mudflats had the highest relative value due to their lower tidal inundation frequency (Figs. 7, 8). The east and south shore mudflats had the highest value during the fall (Fig. 7), and the east shore mudflats had the highest value during the spring (Fig. 8), based on shorebird survey numbers.

Multiplying the predicted mudflat habitat loss in each census tract by estimated current shorebird numbers yielded loss projections of 48,615 to 94,175 birds in the fall (Table 3) and 104,793 to 212,813 birds in the spring (Table 4).

Species that concentrated in the South Bay were Black-bellied Plover, Willet, Marbled Godwit, small sandpipers, and dowitchers (Stenzel et al. 2002). Overall shorebird numbers were higher in spring than in fall, driven primarily by the large number of Western Sandpipers that use San Francisco Bay as a staging area during spring migration. Because Western Sandpipers are small-bodied shorebirds, the difference between fall and spring biomass was much smaller than the difference between fall and spring numbers.

Because our models did not incorporate any species-specific differences in shorebird foraging habits, the predicted proportional change in numbers was the same for all groups. Due to migration timing and overall numbers detected on

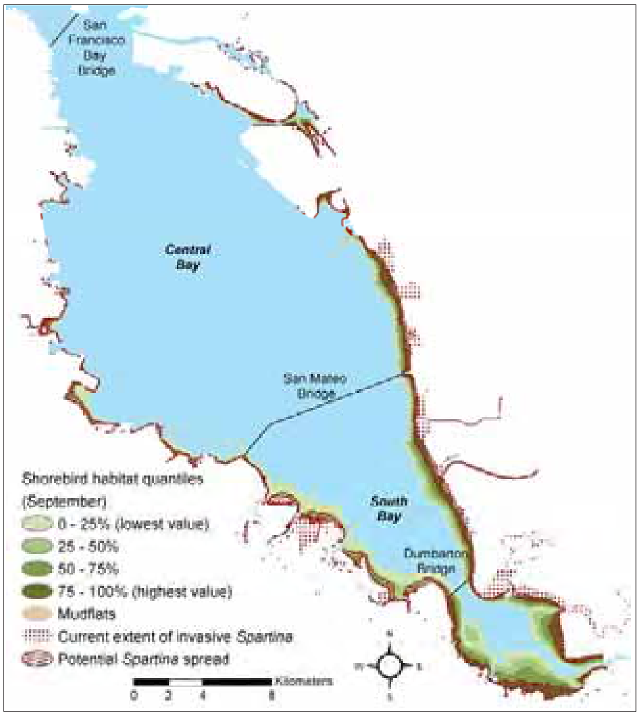

Fig. 7. Predicted extent of *Spartina* spread, based on a 70% inundation tolerance, and overlap with tidal mudflats, classified according to their potential fall season value for shorebirds. Shorebird habitat value was based on: (a) PRBO Pacific Flyway shorebird survey data (1988-1993) and (b) length of mudflat inundation during September.*

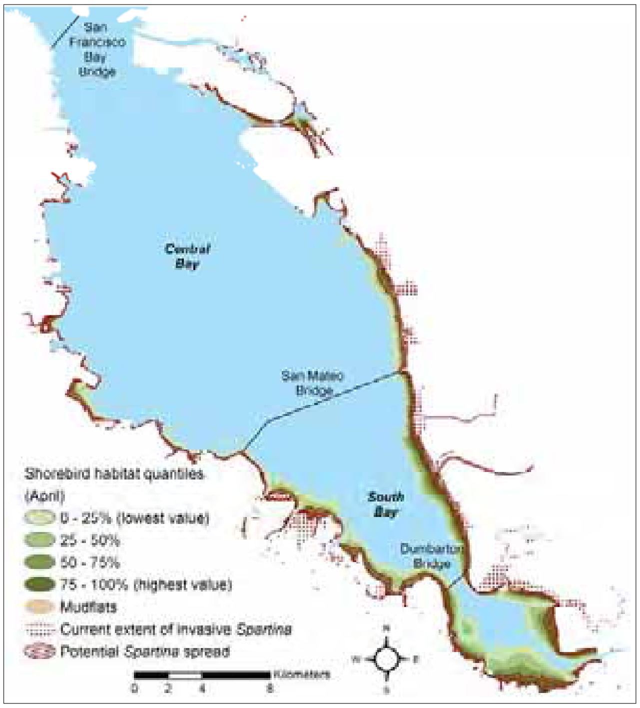

Fig. 8. Predicted extent of *Spartina* spread, based on a 70% inundation tolerance, and overlap with tidal mudflats, classified according to their potential spring season value for shorebirds. Shorebird habitat value was based on: (a) PRBO Pacific Flyway shorebird survey data (1988-1993) and (b) length of mudflat inundation during April.*

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* Current extent of invasive *Spartina* is based on pre-control mapping efforts

Table 3. Predicted loss of fall shorebird numbers by species, based on a range of *Spartina* spread scenarios. All predictions assume that mudflats are at carrying capacity, and that mudflat habitat value is proportional to tidal inundation.

	Current	*Scenario 1: 60% inundation tolerance*	*Scenario 2: 70% inundation tolerance*	*Scenario 3: 80% inundation tolerance*
Mudflat Hectares	6,062	-904	-1,988	-3,269
Bird Numbers:				
American Avocet	5,023	-1,701	-3,034	-4,101
Black-bellied Plover	8,138	-2,756	-4,916	-6,645
Dowitcher	13,377	-4,530	-8,081	-10,923
Long-billed Curlew	371	-126	-224	-303
Marbled Godwit	14,251	-4,826	-8,609	-11,636
Red Knot	1,678	-568	-1,014	-1,370
Semi-palmated Plover	1,501	-508	-907	-1,225
Willet	15,612	-5,286	-9,431	-12,747
Western and Least Sandpiper, Dunlin	160,374	-54,305	-96,880	-130,948
Total	**220,325**	**-48,615**	**-70,055**	**-94,175**

Table 4. Predicted loss of spring shorebird numbers by species, based on a range of *Spartina* spread scenarios. All predictions assume that mudflats are at carrying capacity, and that mudflat habitat value is proportional to tidal inundation.

	Current	*Scenario 1: 60% inundation tolerance*	*Scenario 2: 70% inundation tolerance*	*Scenario 3: 80% inundation tolerance*
Mudflat Hectares	6,062	-904	-1,988	-3,269
Bird Numbers:				
American Avocet	844	-263	-476	-656
Black-bellied Plover	4,595	-1,432	-2,590	-3,570
Dowitchers	33,008	-10,289	-18,608	-25,644
Long-billed Curlew	218	-68	-123	-169
Marbled Godwit	13,437	-4,188	-7,575	-10,439
Red Knot	503	-157	-284	-391
Semi-palmated Plover	725	-226	-409	-563
Willet	2,112	-658	-1,191	-1,641
Western and Least Sandpiper, Dunlin	450,817	-140,528	-254,137	-350,241
Total	**506,259**	**-104,793**	**-156,097**	**-212,813**

shorebird surveys, *Spartina* spread would have the biggest numerical impact on small shorebirds, dowitchers, and Marbled Godwits (Tables 3, 4). Willets and Black-bellied Plovers would be most affected during the fall, when their numbers are highest. In terms of total bird biomass (see Stenzel et al. 2002), the largest predicted losses were in the spring, due to higher overall biomass densities (Table 5).

DISCUSSION

The results presented herein are based on several basic assumptions, all of which should be examined in further detail in order to restrict the wide range of predicted shorebird losses. Our most fundamental assumption was that the mudflat habitats were at carrying capacity during the fall and spring survey periods. However, it is possible that only preferred areas are functioning at carrying capacity (Goss-Custard 1979). If individuals could switch to lower-quality mudflat areas without significantly affecting their overall fitness, then the potential loss of birds may have been overestimated (Goss-Custard 2003). Anecdotal evidence suggests that San Francisco Bay mudflats may reach carrying capacity during the winter, when storm-related flooding may prompt some species to move inland to the Central Valley (Warnock et al. 1995; Takekawa et al. 2002) but we do not know if mudflats and neighboring tidal and salt pond habitats are at carrying capacity during migration. Currently, we lack the data on mudflat food resources, shorebird energetics, and individual foraging behavior (especially prey preference) that would be necessary to obtain an estimate of carrying capacity.

A potential bias in the other direction, however, was that our models examined only the lower limits of *Spartina* spread and the subsequent impacts on shorebird habitat value. In reality, upward *Spartina* spread may pose an equally serious threat to shorebirds as mudflats along tidal channels and open areas within the marsh plain are colonized by invasive *Spartina* and become unavailable to foraging shorebirds.

Table 5. Predicted loss of fall and spring shorebird biomass, based on a range of *Spartina* spread scenarios (1-3). All predictions assume that muflats are at carrying capacity, and that mudflat habitat value is proportional to tidal inundation.

	Current	*Scenario 1: 60% inundation tolerance*	*Scenario 2: 70% inundation tolerance*	*Scenario 3: 80% inundation tolerance*
Fall biomass (kg)	21,416	-6,245	-12,263	-17,067
Spring biomass (kg)	25,289	-6,884	-13,560	-19,202

Another assumption of our habitat value models was that exposed mudflat areas are used evenly by shorebirds, and thus in direct proportion to their temporal availability. However, we know that individuals of many species tend to forage along the rising or receding tide line (Colwell and Landrum 1993; Durell et al. 1997), as invertebrates are more abundant and more accessible in wet substrates and tend to burrow deeper as the tide recedes and the mud dries out (Goss-Custard 1984; White 1995). For shorebirds that only foraged along the tide line, any given full tidal range mudflat should support a constant number of birds as long as some minimum mudflat area was exposed, and shorebird densities (with respect to exposed mudflat) should increase as the tide rises. In reality, it is likely that some intermediate condition exists and mudflat use patterns vary by species. In San Francisco Bay we have observed that Semipalmated Plover, Least Sandpiper, and Black-bellied Plover tend to forage higher along the tidal gradient, whereas Dunlin, dowitchers, Marbled Godwit, and other species forage closer to the tide line (PRBO unpubl. data; USGS unpubl. data).

Shorebird densities are also known to vary according to the uneven distribution of sediments, prey densities, and prey availability across the intertidal zone (Burger et al. 1977; Goss-Custard et al. 1977; Puttick 1977; Page et al. 1979; Quammen 1982, Colwell and Landrum 1993; Yates et al. 1993). This highlights the need to study the spatial distribution of shorebirds and their invertebrate prey over San Francisco Bay mudflats, especially given the dynamic nature of the largely non-native invertebrate community (Nichols et al. 1986; Cohen and Carlton 1998).

With respect to our estimates of mudflat elevation and tidal inundation, we used a simple approach that involved assuming an unrealistic linear mudflat slope. While high-resolution topographic/ bathymetric data were not available for the entire South Bay mudflat region at the time of this study, we hope that recently-obtained Light Detection and Ranging (LiDAR) data (Foxgrover and Jaffe 2005) may be used to improve our models in the future.

Finally, our model predictions were based on a static picture of mudflat spatial extent and elevation. In reality, there are several factors in addition to hybrid *Spartina* spread that may affect mudflat extent and quality, including sea level rise (Donnelly and Bertness 2001; Galbraith et al. 2002), tidal marsh restoration, and natural geomorphic processes (Foxgrover et al. 2004). Thus it would be useful to apply our models to future mudflat predictions developed by coastal geomorphologists.

CONCLUSION

The above-described models of *Spartina* inundation tolerance and shorebird habitat value allowed us to generate preliminary predictions about the potential impact of future *Spartina* spread on South Bay shorebird populations. Because mudflat habitat availability and *Spartina* spread potential are both determined by tidal inundation frequency, the areas of greatest value to shorebirds coincide with the areas of greatest *Spartina* invasion potential. In addition, the eastern shore mudflats, which had the highest recorded shorebird densities, are adjacent to one of the sites of initial *Spartina* invasion at Coyote Hills Slough, increasing their susceptibility to *Spartina* encroachment.

Due to various sources of uncertainty, our preliminary analysis resulted in a wide range of predictions: a 14% to 54% loss in mudflat area, and a 27% to 80% loss in habitat value across the three *Spartina* spread scenarios examined. At the low end, this represents a significant loss of habitat for shorebirds, which may or may not result in population declines. At the high end, our predictions suggest an extreme change in habitat availability that would almost certainly reduce the number of foraging shorebirds in the South Bay.

At the same time, other major changes are occurring in the South Bay, including the restoration of up to 5,000 hectares of commercial salt ponds to tidal, muted, and managed marsh. The loss of these salt ponds, which currently serve as valuable foraging and roosting habitat, may also reduce shorebird numbers (Stralberg et al. 2006). Thus we suspect that South Bay shorebird populations may suffer from multiple negative impacts within a relatively short timeframe if *Spartina* spread is not arrested, reducing the value of San Francisco Bay as a major migratory stopover and wintering site. Additional research and modeling will be needed to assess the cumulative effects of habitat change on shorebirds, while large-scale, integrated habitat conservation planning may help to mitigate these negative effects.

AUTHORS' NOTE:

Due to extensive *Spartina* control efforts since the writing of this paper, *Spartina* spread now poses much less of an immediate threat to shorebirds in San Francisco Bay.

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NON-NATIVE CORDGRASS AND THE CALIFORNIA CLAPPER RAIL: BIOGEOGRAPHICAL OVERLAP BETWEEN AN INVASIVE PLANT AND AN ENDANGERED BIRD

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The federally endangered California clapper rail (*Rallus longirostris obsoletus*) is a tidal marsh-dependent bird whose distribution is restricted entirely to the San Francisco Estuary. The native cordgrass, *Spartina foliosa*, has long been recognized as a critical component of clapper rail habitat within the estuary. The distribution and abundance of the clapper rail has been monitored intermittently since the mid-1970s and that effort has increased since the early 1990s. The concurrent invasion of the bay's tidal marshes by non-native *Spartina alterniflora* and its hybrids with *S. foliosa* has impacted clapper rail abundance and distribution in some areas of the estuary. In this paper we discuss: (1) habitat affinities and density estimates of rail in invaded and non-invaded marshes; (2) the distribution of non-native *Spartina* relative to that of the clapper rails over the last 15 years; and, (3) potential impacts of changing marsh ecology on rail distribution and abundance in both the near term and the long term.

Keywords: California clapper rail, *Rallus longirostris obsoletus*, *Spartina*, cordgrass, impacts, habitat characteristics, distribution, abundance

CLAPPER RAIL DISTRIBUTION, HABITAT AFFINITIES AND ABUNDANCE

Formerly more widespread along California's outer coast (e.g. Morro Bay, Elkhorn Slough, Tomales Bay), the breeding distribution of the California clapper rail is now restricted entirely to the San Francisco Estuary (Albertson and Evens 2000). Within the estuary, the clapper rail is patchily distributed through tidally influenced marshes, with population centers fairly evenly dispersed among the South Bay and the North Bay (aka "San Pablo Bay") marshes. The Central Bay also hosts significant populations, but, like the habitat, these tend to be relatively discrete and locally clustered (e.g. Arrowhead Marsh, San Bruno Marsh, Corte Madera marshes). In Suisun Bay, distribution is very spotty, densities are apparently low, and occurrence may be sporadic, especially in the northern reaches of Suisun Marsh (Collins et al. 1994; Albertson and Evens 2000; Estrella 2007). Indeed, no clapper rails were detected in the Suisun system in 2005 or 2007 (Herzog et al. 2005; Estrella 2007).

The most recent population estimates suggest that approximately 1500 California clapper rails remain in the San Francisco Estuary with approximately one-third of the population in San Pablo Bay and two-thirds in the Central and South bays, combined (Albertson and Evens 2000; Avocet Research Associates 2004; USFWS unpubl. data). This compares with estimates of 4200-6000 rails in the mid-1970s (Gill 1979). However these numbers require a caveat: Population estimates of clapper rails are fraught with uncertainty, survey coverage is sporadic, and numbers may vary widely from year-to-year.

CALIFORNIA CLAPPER RAIL HABITAT CHARACTERISTICS: PRE-INVASION

Habitat availability and the characteristics of tidal marshes differ between South, Central, and North Bay marshlands. In general, marshlands in the northern reaches are more extensive and less modified by human activity than those in the central and southern portions of the estuary. Additionally, invasion of the estuary by non-native *Spartina*—confined largely to the southern half of the estuary (http://www.spartina.org/maps.htm)—has amplified this disparity.

Synoptic surveys of North Bay marshes (north of Points San Pedro and San Pablo) in the early 1990s described habitat use in the northern reaches of the estuary (Evens and Collins 1992; Collins et al. 1994). We assume that these ecological parameters most closely resemble habitat preferences of California clapper rail's prior to the extensive modification of the estuary that began in the mid-1800s (Conomos 1979; Goals Project 1999). Additionally, earlier studies throughout the estuary described distribution, abundance, and habitat affinities of *obsoletus* prior to invasion by non-native *Spartina* (Grinnell and Miller 1944; Gill 1979; Avocet Research 1992; Evens and Collins 1992; Collins et al. 1994; Albertson 1995). Generalized habitat affinities of clapper rails in non-invaded marshes are summarized as follows:

- Distribution limited to fully tidally, saline to brackish marshlands and their foreshores within the estuary. Clapper rails do not occur upstream in the Sacramento-San Joaquin Delta and fresher portions of the system.
- Well-developed channel and slough systems that extend through or into patches of tall monocot vegetation. Channels function as important areas for foraging and as movement corridors.
- Monocot vegetation is used for nesting material; nests are built at or about Mean Higher High Water (MHHW), often at the headward reach of a tidal channel (ARA 1992). Non-native *Spartina* extends upward to nearly MHHW (Collins 2002.)
- Habitat patches typically comprise some mature and some youthful marsh.[1] The highest densities in the North Bay marshes are generally associated with the largest contiguous areas of youthful, saline marshland adjoining at least moderate amounts of historical, mature marshland (Evens and Collins 1992; Collins et al. 1994).
- Broad marshes on the bayshore, or near the mouths of tidal tributaries are favored; narrow, linear, strip marshes support much lower densities, but serve as important corridors between habitat patches and as foraging areas.
- Densities of rails are highest where patches of habitat are at least 100 hectares (ha) in size (Collins et al. 1994).
- Densities of rails tend to increase with channel density (length of channel per unit of marshland.)
- Density is positively correlated ($R^2 = 0.74$) to areal extent of contiguous marshland.
- Small parcels of marsh along the immediate margin of a tributary are more likely to support California clapper rails than small parcels that are more isolated.
- Refugial vegetation, or even man-made structures (docks, duck blinds, etc.) may provide some protection for rails, particularly during high tides and flood events. Populations of mesopredators (raccoons, skunks, feral cats, etc.), and especially the non-native red fox (*Vulpes vulpes*), threaten rail survival; aerial predators also pose a threat.
- Boardwalks, fence lines, towers, and stakes may increase predation pressure by providing perch sites for avian predators (Barn Owl, Great Horned Owl, Northern Harrier, etc.)

Numerous protocol-level population surveys have been conducted in the northern reaches of the estuary since the early 1980s (e.g. Evens and Page 1987; Collins et al. 1994; ARA 2004; Herzog et al. 2005).

At San Pablo Bay sites where rails have been detected, densities typically varied between 0.1 and 2.8 birds per hectare (Collins et al. 1992; ARA 2004; Herzog et al. 2005) with highest densities at sites where youthful marsh has recently developed, e.g. Bahia Lagoon with 1.7 to 2.8 birds/ha (ARA 2004; Herzog et al. 2005).In large mature marshes, with well-developed channel systems, but where native cordgrass is limited to a narrow fringing edge, density estimates are more typically on the order of about 0.5 birds/ha but, in the largest marshes may range up as high as 1.8 birds/ha (e.g Gallinas Creek mouth—ARA 2004; Herzog et al. 2005).

NON-NATIVE *SPARTINA* DISTRIBUTION AND ABUNDANCE AND EXTENT OF OVERLAP WITH RAILS

Within the estuary, non-native cordgrass (*Spartina alterniflora* and hybrids) is limited primarily to the marshlands and tidal flats south of Point San Pedro and Point San Pablo (Hogle 2006). Therefore, the San Pablo Bay rail subpopulations exist essentially independent of invaded habitat while the Central and South Bay rail subpopulations overlap extensively with actively colonizing non-native cordgrass (Broom 2008).

Protocol-level surveys over the three years 2005-2007 that covered 60 sites and were limited to marshes invaded by non-native cordgrass found mean baywide densities in the range of 0.58 rails/ha to 0.68 rails/ha (Broom 2008). These values are not highly disparate from those reported in the northern reaches of the estuary (Collins et al. 1994; ARA 2004; Herzog et al. 2005) Another study of 45 Central and South bay sites conducted in 2005-2006 derived an overall mean abundance 0f 0.84 (±0.163) rails/ha and found no statistically significant differences among categories (0 to >50%) of *Spartina* hybrid cover (Spautz et al. 2006). However, at several locations where *Spartina* clones have aggresively invaded the foreshore, clapper rail densities greatly exceeded those reported in San Pablo Bay, in the Central and South bays prior to the invasion by non-native cordgrass, and the mean density reported in the two aforementioned surveys. The most obvious examples:

San Bruno Marsh (Colma Creek mouth): 2.4 to 3.8 birds/ha (J. Evens, unpublished data., Spautz et al. 2006)

Arrowhead Marsh: 3.8 to 4.2 birds/ha (Spautz et al. 2006; J. Didonato, EBRPD, pers. comm.).

This positive response to youthful, recently established, intertidal marsh vegetation by *obsoletus* is not unexpected. As North Bay studies in the early 1990s reported: "*. . . highest densities of clapper rails were generally associated with the largest contiguous areas of youthful, saline marshland adjoining at least moderate areas of historical, mature marshland . . .*" (Collins et al. 1994). The colonization of marsh edge and foreshore by non-native cordgrass is apparently mimicking conditions in "native marshes" that prograde as ecological conditions change (e.g. depositional-erosion patterns). However, the Central and South Bay marshes invaded by non-native cordgrass differ from marshlands of San Pablo Bay, in that few adjoin large mature marshlands, few support a heterogeneity of marsh age classes, few exhibit a natural transitional grade from the low marsh to the high marsh plain, and vegetative cover at the marsh/upland ecotone is often sparse or absent. North

Bay marshes are also larger than marshes in the southern reach, higher in elevation, have a lower rate of subsidence relative to mean tidal level, and have more emergent marsh vegetation (Atwater et al. 1979; Josselyn 1983; Goals Project 1999; Baye et al. 2000).

ONGOING THREATS TO THE CLAPPER RAIL POPULATION

Extensive conversion of tidal lands resulting from historic and ongoing pressures of agricultural production, urbanization, and salt production has drastically reduced California clapper rail habitat in the San Francisco Estuary (Goals Project 1999; Albertson and Evens 2000). The remnant tidal marshlands of the estuary, the largest and last refuge of *R.l. obsoletus*, occupy only about 12-15% of their historic extent, yet even in such diminished capacity comprise more than 90% of all remaining California tidal marshlands (Dedrick 1989; Goals Project 1999). In addition to, and exacerbated by, habitat loss and fragmentation, the rail population is vulnerable to a redundancy of threats—predation by introduced red fox (*Vulpes vulpes*); depredation of nests and eggs by non-native rodents (*Rattus* spp.) and inflated populations of native mammals (e.g. striped skunk, raccoon); contamination (Lonzarich et al. 1992; Schwarzbach et al. 2006); diminution of peripheral refugial vegetation; and sea-level rise. The ecological alteration of the estuary's tidal marshes by non-native vegetation, especially *Spartina alterniflora* and its hybrids, and possibly *Lepidium* (Spautz and Nur 2004), poses yet another potential threat to an already beleaguered species.

Ecologists have found that non-native hybrid cordgrass exceeds the narrower niche of the native tidal marsh plant and predicted that as the invasion progresses, hybrid *Spartina* will likely spread to higher and lower elevations (Cohen 2001; Callaway and Josselyn 1992; Daehler and Strong 1996; Collins 2002; Baye 2004). As cordgrass beds expand, they are expected to "constrain the tidal network" (Collins 2002) and, as the culms reach mature densities, the stands are apt to become effective sediment traps, promoting infilling (Baye 2004). Additionally, the lower elevation limits of non-native cordgrass "correspond to the slump blocks and lower banks of large channels" (Collins 2002), prime clapper rail foraging areas.

One of the forecasts of a study on the geomorphic effects of non-native cordgrass invasion was that it will tend "*to isolate the headward reaches of first order channels from their networks,"* [in effect] "*shortening and simplifying intertidal channel networks and the shoreline of the Estuary as a whole*" (Collins 2002). This narrow elevational niche overlaps almost precisely with habitat that is critical for the California clapper rail (Collins et al. 1994; Albertson and Evens 2000). Given the importance of channel complexity to clapper rails, a reduction in channel density and complexity predicted for marshes invaded by non-native *Spartina* (Collins 2002; Ayres et al. 2003; Baye 2004) is likely to reduce the availability of habitat for clapper rails.

RAIL DENSITY AND HABITAT QUALITY

The population estimates reported above suggest that clapper rails may occur in dramatically higher densities in some invaded marshes than in non-invaded marshes. This apparent selection of non-native over native habitats by rails predicates a question: Do increased densities of rails mean increased viability of rail populations?

Some have interpreted the density of rails detected within non-native *Spartina* patches as a positive trend for this highly endangered taxon. However, the literature cautions that density is a misleading indicator of habitat quality or reproductive success (VanHorne 1983; Vickery et al. 1992; Pulliam 1996). Given the biogeography of tidal marshlands within the estuary, and the disparity in habitat types that exists between marshes in its northern and southern reaches (Josselyn 1983; Goals Project 1999; Baye et al. 2000) land managers and biologists should consider the possibility that non-native *Spartina* pastures might be ecological traps, or perhaps "attractive sinks," phenomena that occur most commonly in anthropogenically modified habitats (Delibes et al. 2001; Schlaepfer et al. 2002; Battin 2004; Robertson and Hutto 2006). Indeed, San Francisco Estuary and the California clapper rail fulfill most if not all of the criteria that increase vulnerability of animal populations to ecological traps summarized by Battin (2004). It is also worth considering the possibility that the increasing trends in abundance associated with invaded marshes (east bayshore) and coincident negative trends in less invaded sites (west bayshore) reported in Broom (2008), suggest the possibility that rails are being lured away from higher quality habitat (larger patch size, age heterogeneity, native *Spartina* beds) to occupy falsely attractive habitat. However, the supposition that ecological traps account for these apparent trends is open to other interpretations (Battin 2004; Robertson and Hutto 2006; Gilroy and Sutherland 2007).

To determine the impact of non-native *Spartina* beds to clapper rail long-term population viability, studies determining reproductive success, survivorship, and predation rates in both habitat types will be needed. Future studies aimed at determining reproductive success in invaded and non-invaded habitat may help address this important question of conservation biology.

SUMMARY AND CONCLUSIONS

Non-native cordgrass has invaded intertidal marshlands in the San Francisco Estuary, crucial habitat for the endemic and endangered California clapper rail. The ecological transformation of the intertidal marsh zone is apparently underway and future changes are anticipated. Densities of rails in invaded habitat appear to be somewhat higher than at non-invaded habitat, but the ultimate effects on the reproductive success and recovery of the California clapper rail poplation are unknown.

The South and Central Bay subpopulations of rails occupy tidal marshes that are undergoing an aggressive invasion by non-native cordgrass. The North Bay clapper rail populations occupy marshes that are mostly free of non-native cordgrass. The North Bay is also an area where extensive marsh restoration efforts are underway or in the planning phases (Siegel 2002). These differing situations offer opportunities for:

- Non-native cordgrass control in the South and Central bays employing "best management practices" to reduce or minimize impacts to rails, currently in progress by the San Frnacisco Bay Invasive Spartina Project.
- Monitoring of North Bay marshes for the presence of non-native cordgrass, particularly in marsh restoration sites.
- Control of non-native cordgrass in the North Bay prior to alteration of the structure and function of the habitat.
- Research focused on population dynamics of rails at different sites (invaded and "pristine") to determine reproductive success and survivorship.

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THE EFFECTS OF GRAZING GEESE ON HYBRID AND NATIVE *SPARTINA* IN SAN FRANCISCO BAY

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The invasion of *Spartina alterniflora* has become one of the most significant invasions in the already heavily invaded San Francisco Bay. The *Spartina* invasion has resulted in many changes to community and ecosystem processes at lower trophic levels, however, we have so far failed to document equivalent changes at higher trophic levels that are likely occurring as well. In this study, we report the consequences of the hybrid invasion with respect to an important vertebrate grazer, western Canada geese (*Branta canadensis moffitti*). Canada geese regularly nest during winter months in the vicinity of the invaded central and southern areas of San Francisco Bay. We found that Canada geese intensively graze native *Spartina foliosa* at several study sites in the central bay region removing up to 90% of the aboveground vegetation. However, geese completely ignore hybrid *Spartina* with virtually no evidence of grazing in hybrid areas adjacent to heavily grazed native *Spartina* areas. Experiments with captive geese demonstrated that geese repeatedly preferred native *Spartina* when presented intact clones of hybrid and native clones in side-by-side preference trials. However, when cut stems were presented in similar trials, geese showed no preference for the native over the hybrid. We conclude that the preference demonstrated in the first experiment was not the result of plant defensive chemistry, but the result of physical differences between intact clones and cut stems that geese could assess. Field exclosure experiments conducted for two years in areas where hybrid *Spartina* was overgrowing native *Spartina* showed that grazing of native *Spartina* by geese resulted in 25% greater rates of lateral spread of the hybrid into native areas. This suggests that grazing by geese may be accelerating the rate of invasion and ultimate replacement of native *Spartina* by hybrid *Spartina*.

Keywords: Canada geese, San Francisco Bay, hybrid *Spartina*, grazing, invasion rate

INTRODUCTION

The introduction of the smooth cordgrass (*Spartina alterniflora*) has been among the most significant of the nearly 250 non-native species invasions recorded in San Francisco Bay (Cohen and Carlton 1998). *Spartina alterniflora* became established in 1976 as the result of an intentional introduction by the Army Corp of Engineers for mitigation purposes (Faber 2000). Following establishment, *S. alterniflora* hybridized with the native cordgrass *S. foliosa.* Hybrid *Spartina* has rapidly colonized many areas of central and south San Francisco Bay (Daehler and Strong 1997; Ayres et al. 2004). Hybrid *Spartina* can colonize open mudflats as well as out-compete native vegetation at higher tidal heights (Ayres et al. 2004).

Recent studies have shown that the ecological repercussions of the hybrid *Spartina* invasion are far reaching with widespread impacts on community structure and ecosystem function (Neira et al. 2005, 2006, 2007; Levin et al. 2006). However, work to date has focused only on changes at lower trophic levels, despite the fact that vertebrate herbivores are known to consume *S. alterniflora* in its native region (Buchsbaum et al. 1981). Little is known regarding the consequences of hybridization for susceptibility to herbivores of any kind (Daehler and Strong 1997).

Among the most common and potentially important vertebrate herbivores of *Spartina* are western Canada geese, *Branta canadensis moffitti* (Banks et al. 2004). The western subspecies is the only one regularly found in San Francisco Bay (Mowbray et al. 2002) and they typically nest from November to April. While nesting, their foraging is generally restricted to nearby areas that typically include mudflats with native *Spartina*. Our initial observations suggested that geese were grazing intensively on areas occupied by native *Spartina*. One of the key questions of this study was to determine whether grazing by western Canada geese could influence the rate of invasion of hybrid *Spartina* in San Francisco Bay. Therefore, our goal was to measure grazing by Canada geese on native *Spartina* and compare this with grazing on hybrid *Spartina*. If we found evidence of selective grazing, the next goal would be to determine whether selective grazing was due to a measurable preference for one plant over the other. We would also determine the basis for any preference that might exist. Finally, we would quantify any influence that geese grazing might have on the rate of invasion of hybrid *Spartina* into areas occupied by native *Spartina*.

METHODS

Grazing Intensity in San Francisco Bay

To estimate the intensity of grazing by western Canada geese, we measured grazing at three sites in the central portion of San Francisco Bay: Point Isabel (37º53'30''N; 122º20'55''W), Robert's Landing (37º40'13''N; 122º28'48''W), and Oro Loma (37º37'45''N; 122º09'08''W). These sites were distributed along the eastern margin of San Francisco Bay and each contained areas of native *S. foliosa* with varying degrees of cover by hybrid *Spartina*. At each site, we laid 30 meter (m) transects along which 10 0.25 m x 0.25 m quadrats were established at randomly chosen points. Plant samples to verify genetic identification (hybrid vs. native) were taken within several quadrats and kept refrigerated until genetic assays using random amplified polymorphic DNA (RAPD) markers could be conducted at the *Spartina* genetics lab at UC Davis (Ayres, unpublished results), typically less than a week after collection. Genetic determination followed previously published protocols (Ayres et al. 1999). In some cases, the area covered by native *S. foliosa* was smaller so shorter transects with fewer quadrats were used. Within each quadrat, we measured the number of stems that had been grazed or not by geese and the heights of 15 stems to the nearest one centimeter (cm). Grazing produces characteristic damage that is easily identified. At sites where we also had data for hybrid *Spartina,* the same measurements were made along similar transects. We tested the proportion of stems grazed by geese between native and hybrid transects.

Field Grazing Exclosures

To experimentally measure the impact of grazing Canada geese on both native and hybrid *Spartina* in the field, we conducted exclosure experiments at the Roberts Landing site. At this site, we established goose exclosures in three areas (six replicates per treatment). The first area was in a continuous meadow of native *S. foliosa.* The second was in a continuous meadow of hybrid *Spartina.* The third was located where hybrid *Spartina* was overgrowing native *Spartina.* In single species stands we constructed a 1 m x 1 m exclosure cage of plastic mesh (4 cm x 4 cm openings) attached by electrical cable ties to polyvinyl chloride (PVC) posts. At border areas with both native and hybrid *Spartina,* we established exclosure cages 1 m x 4 m with the long axis of the exclosure perpendicular to the hybrid-native border. The center of the exclosure (at 2 m) was positioned to be over the approximate border edge at the time of construction. Adjacent control areas for both single species exclosures and border exclosures were established within 2 m of the exclosure cages. The control areas were delineated with PVC posts at the corners. We estimated the linear distance in spread for control and exclosure treatments by measuring the distance (to nearest 0.1 m) from the hybrid border at the start of the experiment to the leading edge of the spread after two years. We tested differences with Analysis of Variance (ANOVA) using log transformed values for distance and treatment (exclosure vs. control) as fixed factors using SAS version 9.1 (SAS Institute, Cary, North Carolina).

Grazing Trials with Intact Clones

In order to determine the extent that observed grazing was the result of preference for native *Spartina* rather than availability or other factors, we conducted grazing trials during February-March 2003 using a small group (six) of captive western Canada geese maintained by the Wildlife Department at Humboldt State University (HSU), Arcata, California. This group of geese had been maintained together for several years on processed diets. The experiment involved using intact segments (0.5 m x 0.5 m x 0.5 m) of two clones of either hybrid or native *Spartina* offered in brown plastic bins. Each plant segment had been excavated from field sites in San Francisco Bay (hybrid plants from Elsie Roemer Bird Sanctuary, City of Alameda and *S. foliosa* from Bothin Marsh, City of Mill Valley) and driven to the Humboldt aviary the next day. All stems and leaves were counted for each clone prior to the start of the experiment. At the beginning of the experiment, the tubs with the clone segments were established in a two by two array approximately 2 m apart. The geese were then allowed to enter the aviary and encounter the clones. We made focal observations on each clone at one minute intervals noting the number of geese grazing on each. After two hours, we collected the clones and measured the number of stems and leaves that had been grazed by geese. The entire experiment was repeated three times over the following three weeks. We estimated the intensity of grazing by counting the percentage of leaves and stems that had been grazed during the trial for both hybrid and native clones. We tested differences in grazing with ANOVA using arc-sin tranformed percentage of leaves or stems with treatment as a fixed factor (as above).

Grazing Trials with Clipped Stems

To determine whether the preferences of experimental geese were determined by differences in the physical properties of the plants versus differences in plant chemistry, we used the same group of geese during experiments from February-March 2004. We collected stems of both native and hybrid *Spartina* at the same sites used for collecting the clones the previous year. We cut the stems at the base of the plant and wrapped them in large plastic bags for shipment. Stems were kept refrigerated from the time of clipping and sent to the aviary at HSU within 24 hours and were used immediately thereafter in feeding trials. Geese showed the same interest in the clipped stems as they did in the intact clones, therefore we assume that the condition of the stems and leaves were similar to that in the previous experiment.

In this second experiment, we offered the geese a choice of cut stems of either hybrid *Spartina* or native *S. foliosa* using the same bins the geese are normally offered their pelleted feed. We arranged three bins of each of the two plant types in a 2 x 3 array with each bin approximately 2 m from the others. After setting up the feeding array, the geese were allowed to graze for at least one hour after which all the plants remaining in the bins were collected. Also, plants that had been removed from the bins and discarded were also collected and stored separately. We repeated this experiment two times over the following eight weeks. We estimated the intensity of grazing by weighing the dry weight of the plants in each bin remaining at the end of the experiment as well as the discarded plants adjacent to that bin. We tested differences in grazing with ANOVA using log-transformed weights for either bins or discards with treatment as a fixed factor (as above).

RESULTS

Grazing Intensity in San Francisco Bay

Our data from several sites in San Francisco Bay show intense grazing by western Canada geese on native *S. foliosa.* Our most extensive data from Robert's Landing (Fig. 1) show greater than 90% loss of aboveground biomass with nearly all stems grazed to within a few centimeters of the ground on all three transects (Fig. 1). By contrast, geese virtually ignored hybrid *Spartina* with no measurable grazing on any plants along the transect. Similarly intense grazing was measured at Oro Loma (greater than 85% of stems grazed) and Pt. Isabel (greater than 95% of stems grazed).

Field Grazing Exclosures

Hybrid *Spartina* grew laterally into the native *Spartina* zones in nearly all of the experimental areas independent of treatment. However, the rate of lateral spread of aboveground plant biomass was significantly greater ($p<0.005$) in areas where geese had been excluded from grazing. We assume that the lateral spread of aboveground biomass is a reasonable proxy for the presence of belowground biomass even if a tradeoff exists in allocation to above- versus belowground biomass. Although there may have been changes in the allocation from aboveground to belowground growth as the result of grazing, our results clearly show that the rate of growth of hybrid into areas occupied by the native was 25% greater when the aboveground portion of the native plants was subject to grazing (Fig. 2).

Grazing Trials with Intact Clones

The results of the first set of feeding trials showed that the captive geese strongly preferred native *Spartina* clones over hybrid clones. The results in Fig. 3 showed that the percentage of leaves remaining was nearly 10 times greater ($p<0.0001$) and the percentage of stems remaining was nearly 5 times greater ($p<0.01$) for hybrid clones compared to native clones. Results showed measurable consumption of hybrid plants, particularly the leaves. However, this was not the result of a reduced preference for natives over hybrids, rather it was a somewhat unavoidable artifact of the experiment. As geese grazed down the native plants and most of the leaves were consumed, they began to switch over to consuming some of the hybrid leaves, rather than consume the tougher stems of the natives. Leaves were generally preferred over stems of either type. Geese began to graze the hybrid as availability of the native ran low, and data from our focal observations clearly showed that consumption of hybrid clones occurred only after the native had been consumed.

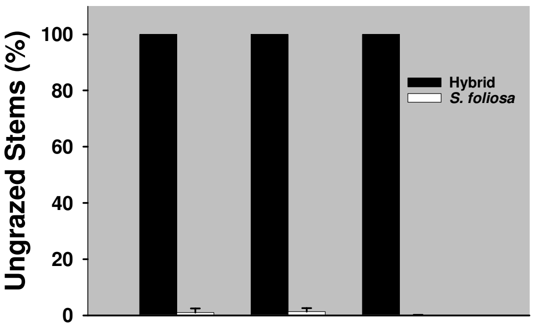

Fig. 1. Results of field grazing estimates for Robert's Landing showing mean (± 1 s.e.) of the percentage of ungrazed hybrid stems (black bars) and native stems (white bars at bottom) for each of three transects.

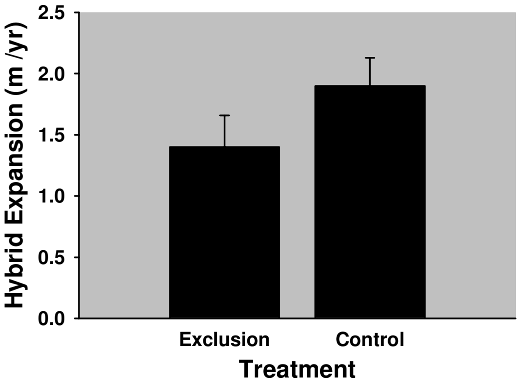

Fig. 2. The results of experimental exclusion of geese for two years from border areas where hybrid *Spartina* was overgrowing native *Spartina* at the Robert's Landing site. Bars represent mean (± 1 s.e.) of the lateral spread of hybrid into the native area (n=6).

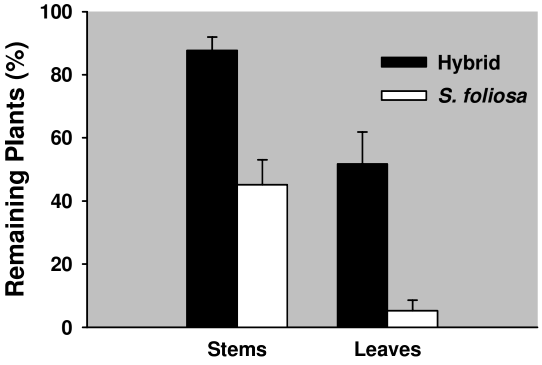

Fig. 3. Results of feeding preference experiments showing means (+ 1 s.e.) of remaining stems and leaves of hybrid (black bars) and native *Spartina* (white bars) after being grazed by captive geese.

Grazing Trials with Clipped Stems

The results of the second set of feeding trials with clipped stems produced very different results than the trials involving intact clones. The captive geese showed no significant preference for clipped stems of native *Spartina* compared with stems of hybrid *Spartina*. Geese grazing was vigorous overall and geese spent similar amounts of time grazing as they did in the trials with the clones ($p>0.05$). However, neither the amount of biomass consumed by grazing geese nor the amount discarded was significantly different for hybrid stems relative to native stems ($p>0.25$ for all).

DISCUSSION

Our findings from the field surveys suggest that grazing by western Canada geese on native *S. foliosa* is widespread throughout the central portion of San Francisco Bay. Data from at least five sites showed a large reduction in the aboveground biomass of native *Spartina*. By contrast, adjacent areas where hybrid *Spartina* was present showed little sign of grazing. Geese appeared to ignore hybrid *Spartina* entirely when it was present in our surveys. There is the possibility that geese may consume a higher proportion of hybrid genotypes that are morphologically more similar to native *S. foliosa*, however, we have not yet seen evidence for this.

Our results from the goose exclusion studies indicate that lateral spread of the hybrid into areas occupied by native *S. foliosa* is more rapid in areas where goose grazing has been excluded for two years. The rate of lateral spread is 25% greater than in adjacent control areas where goose grazing continued. This 25% increase in lateral spread may have significant consequences for the overall rate of invasion of hybrid *Spartina* in San Francisco Bay. Although the rate of sexual reproduction of hybrid *Spartina* is overwhelming native reproduction due to higher seed set and pollen swamping, plant clonal growth is also important for maintaining its presence in the bay. The degree to which hybrid plants can overgrow and outcompete native plants will have a significant effect on the speed that hybrid *Spartina* may be able to drive *S. foliosa* locally extinct in central San Francisco Bay (Ayres et al. 2004).

The eventual replacement of native with hybrid may also have some negative consequences for the nesting geese. Native *Spartina* is not likely to be the only food source for nesting geese in an urbanized estuary like San Francisco Bay, where lawns and golf courses offer easy forage. However, the high levels of grazing on native Spartina at our study sites suggest that this is still an important source of forage for these birds. Other sources of forage are not likely as proximate to nesting sites as native *Spartina* and, therefore may be costly in terms of foraging time and time spent away from the nest. This ultimately may affect nesting success. Unfortunately, there are no data with which to test these possibilities.

The results from our experiments with captive geese strongly support our observations in the field. The data from the first set of aviary trials show a strong ability of geese to discriminate between native *S. foliosa* and hybrid *Spartina* in side-by-side choice trials. This supports the idea that geese are also discriminating among these same plant types in the field. It is possible that geese may also discriminate among different hybrids, possibly preferring hybrids that more closely resemble the native *S. folioa.* However, we cannot determine that from the current study.

Our data from the second set of aviary trials is consistent with the idea that geese are able to distinguish hybrid from native *Spartina* based on physical characteristics rather than chemical characteristics. In contrast to the first trials (plants upright and intact), results from these trials (cut stems laid sideways in trays) showed that geese exhibited no ability to distinguish hybrid from native.

Physical characteristics of intact plants may allow geese to assess palatability of potential forage by tugging on the plant and gauging its resistance (Lang and Black 2001). Physical differences between native and hybrid *Spartina* are immediately obvious to any human investigator as hybrid stems and leaves are much thicker, tougher and more fibrous. Results from the second experiment, designed to eliminate physical differences experienced by geese tugging on leaves and stems, showed that geese failed to discriminate between native and hybrid *Spartina*. The general conclusion from studies of secondary defensive plant compounds is that if by removing physical differences, there is no subsequent preference shown by the herbivore, then plant chemistry can generally be ruled out as important. If geese preference in the first experiment was based on differences in defensive chemistry between native *S. foliosa* and hybrid *Spartina*, they would presumably still be able to use those cues in the second trial. We also measured differences in the amount of material that geese discarded to explore the idea that geese might discard a greater proportion of the hybrid if defensive chemistry was a cue. However, we measured no significant differences in discarded material between native and hybrid *Spartina*.

Our results parallel other studies of factors influencing goose grazing. Earlier work by Buchsbaum et al. (1981) with eastern Canada geese (*Branta canadensis canadensis*) suggested that geese can detect defensive compounds such as ferulic acid in lawn grass. Their results suggest that phenolics at relatively high concentrations could be important in grazing preferences. Other studies have generally found less support for the role of phenolics influencing goose grazing. A study with greater snow geese (*Anser caerulescens atlantica*) tested the influence of water, fiber, phenolic and protein content among grass species and found that only water content was important (Gauthier and Bedard 1991). Another study using barnacle geese (*Branta leucopsis*) found that feeding preferences of geese were best correlated with water and nitrogen content, although the relationship with water was stronger (Owen et al. 1977). Coleman and Boag (1987) found that for western Canada geese non-structural carbohydrate and not fiber or protein content was the best predictor of preferences among forage species. Structural compounds such as cellulose have also been found to be poorly digested by geese relative to proteins and soluble carbohydrates (Buchsbaum et al. 1986). The conclusions from our study are generally consistent with these other studies that found little evidence for a strong role for defensive chemicals such as phenolics in determining the observed preferences and stronger influences of structural compounds, nitrogen and water content. Interestingly, native *S. foliosa* has a lower carbon to nitrogen (C:N) ratio (more protein, less cellulose) relative to hybrid *Spartina* (Tyler et al. 2007), and also has a less coarse physical structure and presumably higher water content.

In summary, our results suggest that Canada geese may be influencing the rate of spread of hybrid *Spartina* in the central portion of San Francisco Bay. The eventual loss of native *S. foliosa* due to the hybrid invasion, at least in some portion of San Francisco Bay, could also have negative consequences for foraging by removing a potentially important food source. The intense and highly selective grazing on native *Spartina* and almost total avoidance of the hybrid results from a strong preference for the native that is apparently based on structural rather than chemical differences.

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IMPACT OF INVASIVE HYBRID CORDGRASS (*SPARTINA ALTERNIFLORA* X *FOLIOSA*) ON SONG SPARROW AND MARSH WREN POPULATIONS IN SAN FRANCISCO BAY SALT MARSHES

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Exotic hybrid cordgrass, *Spartina alterniflora* x *foliosa,* is altering the vegetative structure and composition of the San Francisco Bay tidal marsh ecosystem, which has multiple impacts on the Alameda song sparrow (*Melospiza melodia pusillula*), a resident passerine species that is a California Species of Special Concern. These sparrows are affected not only by the altered habitat, but also by the occupation of this habitat by a potential competitor, the marsh wren (*Cistothorus palustris*). To assess the impact of the *S. alterniflora* invasion on song sparrow and marsh wren populations we: 1) located song sparrow nests to observe nesting habitat preferences and nest success and 2) used focal observations of color-banded birds to assess the vegetation composition of each territory and the amount of territory overlap between the two species. Our findings suggest that the changes in salt marsh habitat associated with the invasion of cordgrass hybrids may favor marsh wrens over song sparrows and could eventually result in a decrease in salt marsh song sparrow populations.

Keywords: *Spartina alterniflora*, song sparrow, marsh wren, nest success, territory, competition, San Francisco Bay

INTRODUCTION

Native San Francisco Bay tidal salt marshes are characterized by broad expanses of open tidal mudflats, a narrow mid-marsh zone where *Spartina foliosa* occurs, and a high-marsh zone composed mainly of low-growing *Sarcocornia* spp. (formerly *Salicornia* spp.) with narrow areas of *Grindelia* that line the meandering tidal channels. *Spartina alterniflora*, a cordgrass native to the Atlantic and Gulf coasts of North America, was introduced to San Francisco Bay in the early 1970s (Ayres et al. 2004). The exotic cordgrass subsequently hybridized with the native cordgrass, *S. foliosa*, and these hybrids have spread and now cover more than 600 hectares (ha) of tidal flat and tidal marsh habitat (Zaremba et al., this volume).

The tall, dense exotic *Spartina* (*S. alterniflora* and/or the hybrid *S. alterniflora x foliosa*) can grow further down the tidal gradient than any native tidal marsh plant species and so is able to colonize open tidal flats. This exotic cordgrass can also grow much further up the tidal gradient than native *S. foliosa* and thus displaces other native plant species in the mid- to high-marsh zones as well (Ayres et al. 1999; Nordby pers. obs.).

The profound changes in habitat structure and composition that accompany the exotic *Spartina* invasion (Callaway and Josselyn 1992) will likely have the greatest impact on species, such as birds, that are wholly dependent on the tidal salt marsh system. The Alameda song sparrow

Fig. 1. Alameda song sparrow (*Melospiza melodia pusillula*). Photo by Jen McBroom.

Fig. 2. Marsh wren (*Cistothorus palustris*). Photo by Jen McBroom.

(*Melospiza melodia pusillula*) (Fig. 1), a California Species of Special Concern, resides entirely within salt marshes in South San Francisco Bay. In a native marsh, this sparrow is the main resident passerine species and occupies territories and nests in *Sarcocornia* and *Grindelia* near tidal channels. Another passerine species, the marsh wren (*Cistothorus palustris*) (Fig. 2), that normally occurs in fresh or brackish water marshes on the Pacific Coast, has started to occupy the newly available exotic *Spartina* habitat (Nordby and Cohen, pers. obs.). Marsh wrens are very aggressive and will defend their territories against other birds, even other species, by breaking the eggs in nests that are close to their own territories (Picman 1977).

To assess the impact of the exotic *Spartina* invasion on song sparrow and marsh wren populations in San Francisco Bay we 1) studied sparrow nesting habitat preferences and nest success and also looked for evidence of the destruction of song sparrow eggs by marsh wrens, and 2) assessed the vegetation composition of sparrow and wren territories as well as the amount of territory overlap between the two species.

SONG SPARROW NEST SUCCESS

During the 2002 and 2003 breeding seasons we followed and observed the fates of 351 nests in 45+ territories across three study sites (Newark, San Leandro and Alameda). Once the fate of a nest had been determined (failed due to predation, failed due to tidal flooding, failed for other reasons, or successful), we recorded the location and vegetation composition of the nest site.

We found that song sparrows did use exotic *Spartina* as nesting habitat, but these nests were much more likely to fail due to tidal flooding than nests placed in native vegetation. As a result, our model of daily nest survival (a generalized linear modeling approach using Akaike's Information Criterion) revealed that overall nesting success was estimated to be 30% lower in areas of exotic *Spartina* than in areas of native vegetation. We also found strong evidence of marsh wren destruction of sparrrow eggs, particularly in areas of high marsh wren density (Nordby et al. 2009).

SONG SPARROW AND MARSH WREN TERRITORIES

During the 2003 breeding season (from March to August), we conducted two to four focal observations on each color-banded male to map the territory boundaries for the 32 song sparrows and 16 marsh wrens in the two *Spartina*-invaded study sites (San Leandro and Alameda). Observers used binoculars, a compass, and a range-finder to map bird locations during each one-hour observation period. Observation locations were marked using a GPS unit, bird locations were then calculated and these data were added to an ArcView GIS database. The points were used to construct 100% minimum convex polygons of each territory. Using color-infrared aerial photographs, we identified areas of invasive *Spartina* throughout the sites. In ArcView, we combined the layer of *Spartina* vegetation with the layers of marsh wren and song sparrow territory polygons. We then determined the percentage of each territory that was composed of *Spartina* habitat, as well as the amount of overlap between the two species.

Although song sparrows did include some exotic *Spartina* habitat in their territories, all but one song sparrow territory included some areas of native salt marsh habitat. The one territory that was determined from aerial photographs to be entirely covered by *Spartina*, actually had a large portion of native pickleweed vegetation underlying the *Spartina* stand. In contrast, marsh wren territories were more highly correlated with exotic *Spartina* habitat and many territories were exclusively composed of the exotic cordgrass. We also found that there was little overlap between the territories of the two species (Nordby et al., in prep).

DISCUSSION

These results suggest that the changes in salt marsh habitat associated with the invasion of exotic *Spartina* may favor marsh wrens over song sparrows. While song sparrows are occupying and nesting in the exotic *Spartina*, those that do so may be at a disadvantage. It is possible that song sparrows are being drawn to nesting sites in exotic *Spartina* that are inappropriate because they are too low in elevation relative to the tides. This increase in nest failure due to flooding coupled with an apparent increase in interference competition from marsh wrens may serve to negatively impact salt marsh song sparrow populations in San Francisco Bay.

However, we do not yet know whether exotic *Spartina* is acting as an 'ecological trap' for song sparrows, where overall reproductive success is reduced. It is also not yet known whether marsh wrens are excluding song sparrows from the *Spartina* habitat or if song sparrows are selecting against those areas for other reasons (e.g., nesting habitat or food resources are limited).

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CHAPTER FOUR

Spartina Control and Management

TAKING ADVANTAGE OF *SPARTINA*'S SPATIAL PATTERN FOR EFFICIENT CONTROL

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The invasion of open mudflats by *Spartina alterniflora* takes on a distinctive spatial pattern. This pattern of spread offers opportunity for strategic placement of control efforts. *Spartina* seedlings establish on open mud and then spread vegetatively to form expanding circular patches, which dot the mudflats and eventually coalesce into a contiguous monospecific meadow. The invasion typically begins in the upper tide zone and then moves down the tidal gradient. Using a spatially explicit model, I simulated the spread of *S. alterniflora* and compared various strategies for control in a situation where only a fraction of the total infestation could be controlled each year. A strategy of killing outlying patches first and then attacking the dense meadows (moving up the tidal gradient) led to eradication in up to 44% less time and effort than a strategy of targeting the dense meadows first and outlying patches second (moving down the tidal gradient). In the control of contiguous meadows located adjacent to the shoreline, the best strategy was to approach one end of the infestation, moving across the meadow to the other end. Suppression of seeds was not an effective control strategy by itself. In general, effective control strategies were those that first eliminate the plant in areas where current or future vegetative growth is greatest. Field application of these results for *S. alterniflora* and similar invasive plants could greatly reduce the costs of control work and improve the likelihood of local or complete eradication.

Keywords: *Spartina alterniflora*, Willapa Bay, spatial pattern, control strategy, control efficiency

INTRODUCTION

When resources for control work are limited, often only a fraction of a weed invasion can be controlled in any given year. Under these conditions, the spatial pattern of a weed invasion can provide an opportunity for strategic placement of control efforts to achieve their greatest effect.

The invasion of mudflats by *Spartina* spp. (cordgrasses) takes on a characteristic pattern. Seedlings establish in open mud and then spread vegetatively to form expanding circular patches. These initially dot the mudflats and can eventually coalesce into a contiguous meadow. The invasion typically begins in the upper intertidal zone and then moves down the tidal gradient. A distinct boundary is formed by the higher native marsh, which *Spartina* rarely invades. This study uses a simulation to compare control approaches for this pattern of spread. A more detailed version of it appears Grevstead 2005.

METHODS

A stochastic grid-based model was developed using Matlab® software to simulate the spread of *Spartina alterniflora* on a mudflat and to compare different control strategies. The simulations used a grid dimension of 120 by 120 cells where each cell was 1 square meter (m^2). One axis of the model space follows a tidal elevation gradient, with the upper edge representing the native marsh boundary and the bottom edge representing the lower extent of *S. alterniflora* growth. Cells are considered either empty or occupied by *S. alterniflora*. They become occupied through vegetative spread from neighboring cells or by establishment of seedlings dispersed from an occupied cell. Parameter estimates were obtained from field data for *S. alterniflora* in Willapa Bay. Assumptions and parameters of the model were as follows:

- Vegetative spread = 0.77 m radial increase per year (measured from aerial photos)
- Seeds disperse from occupied sites according to a Gaussian distribution ($\alpha = 50$)
- Seedling recruitment declines linearly with tidal elevation (based on Feist 1999)
- Seedling recruitment produces a 17% increase in area per year (based on Murphy 2003)
- Once established, clonal patches do not die until treated

Outlying patches first vs. meadows first

The time and total effort needed to eradicate a population was compared for a meadows-first vs. an outliers-first treatment strategy. In each case a fixed amount of *Spartina* was removed each year starting with a 20-year-old invasion (Fig.1). Paired trials were replicated 10 times for each of three levels of yearly effort.

Approach direction for meadows

For a case where there are no outliers but only an oblong meadow adjacent to the native marsh boundary, three strategies were compared: (1) Approaching from the lower tide zone (mudflat) and moving toward the upper tide zone

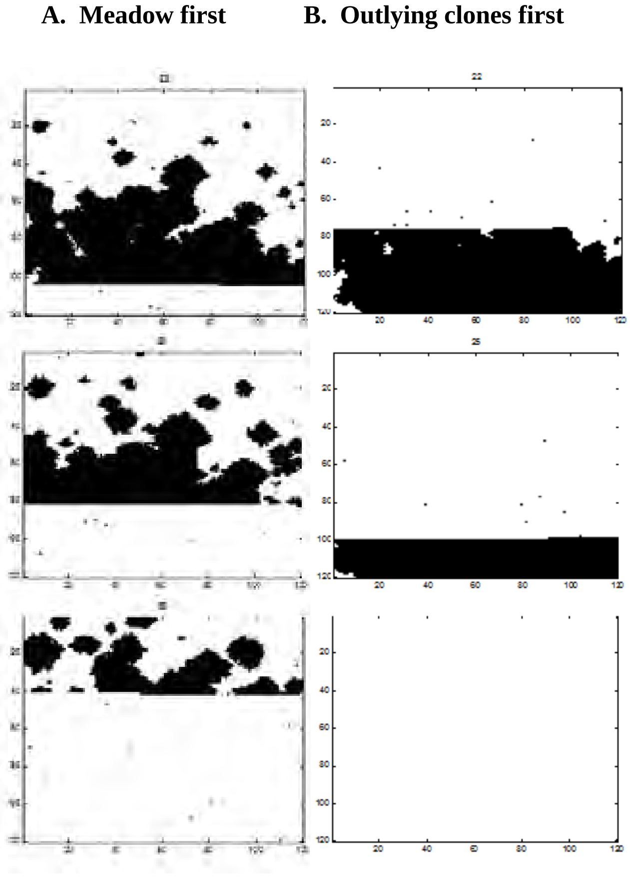

Fig. 1. Meadow-first (left) and outlier-first (right) approaches to controlling *Spartina*. Shown is the remaining *Spartina* (black areas) at times 2, 5, and 10 years. Removal of 500 occupied squares each year began on year 20.

(native marsh), (2) approaching from the upper tide zone and moving toward the lower tide zone, and (3) approaching from one end of the meadow and moving parallel to shore to the other end. Each year a fixed amount of *Spartina* was removed.

Is seed suppression effective?

Seed suppression, through mowing or low concentration herbicide spray, can be carried out at a much lower cost than completely killing the plants. To test the effectiveness of seed suppression, three start conditions were used (a 15-year-old invasion, a 20-year-old invasion, and a 15-year-old meadow without outliers). Seeds were suppressed at three levels: 0%, 50%, or 100%. In each case, seeds were suppressed every year, while established plants were allowed to spread vegetatively.

RESULTS

The outliers-first strategy achieved eradication substantially sooner and with less total effort than the meadow first strategy. The difference was especially great when the available yearly effort was low (Fig. 2).

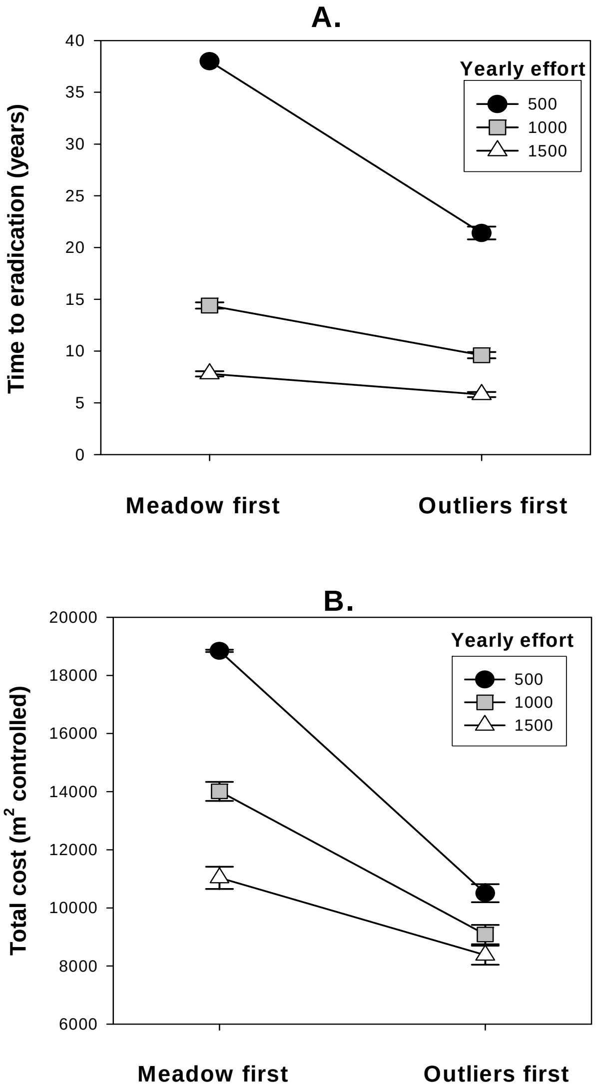

Fig. 2. Comparison of the amount of time (A) and amount of effort (B) needed to eradicate a *Spartina* population using a meadow-first or outliers-first approach. Plots show mean and standard error of 10 replicate trials for each strategy and yearly effort combination.

For oblong meadows, *Spartina* was eliminated fastest, and with the least total effort, when control work was applied first to one end of the meadow and then moved across the meadow in subsequent years. Slightly less effective was the approach of moving from lower to upper intertidal zone. The least effective strategy was to control from the upper tide zone, because this opened up an additional growing edge. Again, the largest difference in strategy effectiveness was found when the yearly control effort was low.

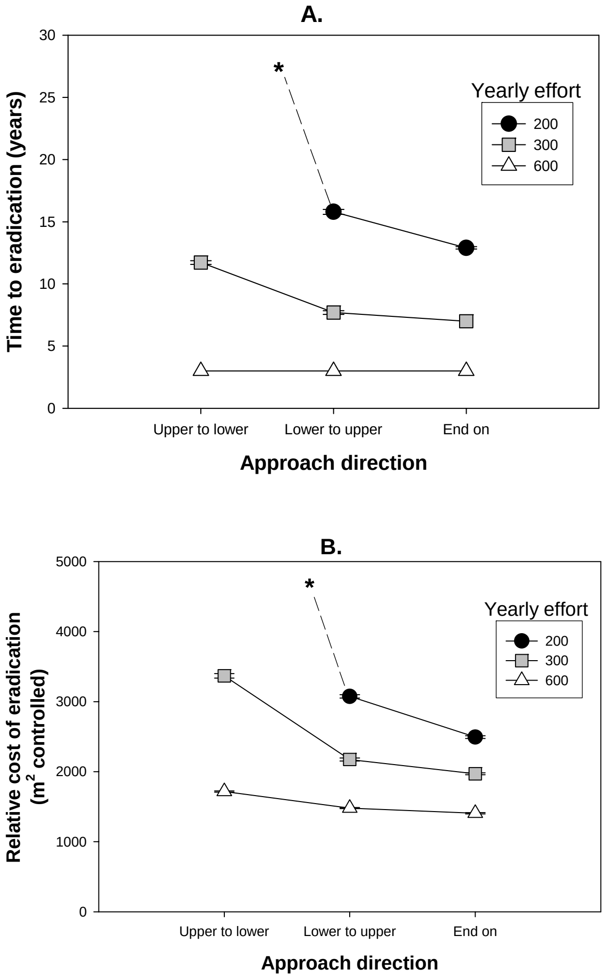

Fig. 3. Comparison of the amount of time (A) and amount of effort (B) needed to eradicate a *Spartina* population in the case of an oblong meadow adjacent to the native marsh using three approach directions. Plots show mean and standard error of 10 replicate trials for each strategy and yearly effort combination.

When the mudflat was already dotted with patches of *Spartina*, seed suppression only slightly reduced the rate of invasion and it took many years to see an effect. Seed suppression was somewhat more effective in the meadow-only case. However, in all cases, the area occupied by *Spartina* continued to increase.

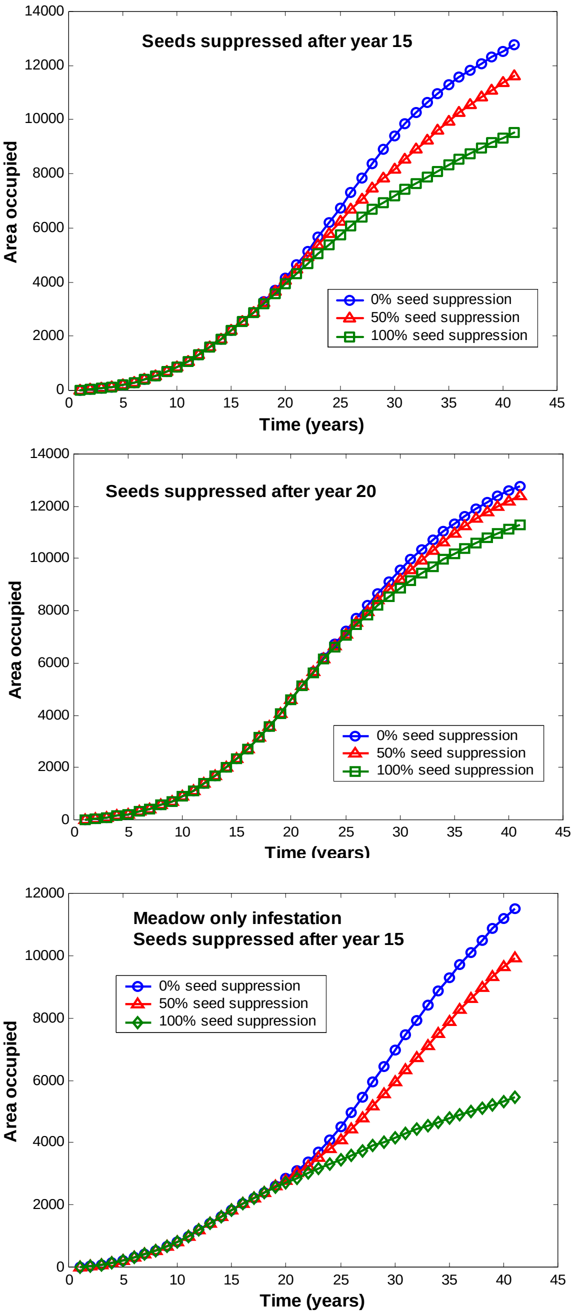

Fig. 4. Growth of simulated *S. alterniflora* populations for which seeds were suppressed at levels of 0%, 50% and 100% every year for a 20 year period. Seed suppression treatment was applied to the invading population after year 15 in (A) and after year 20 in (B). In (C) seed suppression was applied to a 15-year-old meadow surrounded by an uninfested mudflat.

CONCLUSIONS

In a situation where only a fraction of the invasion can be controlled each year, strategic placement of control treatments can greatly reduce the time and effort needed to eradicate *Spartina*. Control is more effective if it first targets outlying clones in the lower tide zone and then moves up the tidal gradient to target the larger meadow. These results are consistent with expectations based on the more generalized study by Moody and Mack (1988); they also agree with a model of *Spartina* control by Taylor et al. (this volume). The outliers-first approach eliminates *Spartina* in the areas where vegetative spread is greatest. In addition, it avoids opening up a new growing edge in the upper intertidal zone and also avoids opening up the habitat that is most favorable for seedlings.

For the control of solid meadows without outlying patches (i.e. after outliers have been controlled), treatment should begin with either the lower edge or, slightly better, one end of the meadow. Again, this more rapidly cuts down the amount of the growing edge and avoids opening a new edge in the upper zone.

An assumption behind these results is that the cost of control per unit area is similar in different parts of the invasion. This may or may not be true depending on what tools are available to the control crew. It is anticipated that land managers will consider the results of this study in combination with their own costs of control for different sections of the *S. alterniflora* invasion.

Seed suppression alone is not likely to be effective, especially when the invasion is already widespread. This is because the established plants will continue to spread vegetatively. Seed suppression may be warranted if used in combination with control of vegetative spread and when it can be done completely and economically.

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SHOREBIRD USE OF *SPARTINA*-AFFECTED TIDELANDS – CAN WE ACHIEVE FUNCTIONAL HABITAT POST-CONTROL?

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One of the major threats of invasive *Spartina* is the loss of shorebird foraging habitat. The Audubon Society lists the invasion of Willapa Bay (WB) by *Spartina* as the second most critical threat to shorebird habitat in the nation. Studies were conducted on how large-scale mechanical and chemical control efforts affect shorebird and waterfowl usage of *Spartina* meadows in WB. Food abundance and accessibility, shorebird, waterfowl and bird of prey density, and bird behaviour were evaluated on treated meadows and compared to untreated meadows and bare mudflats. Based on long-term point counts and remote video monitoring, there was no bird usage (of any species) in *Spartina* meadows. Highest food abundance and accessibility was found in mudflats. Waterfowl and birds of prey preferred herbicide-treated sites over the tilled and mudflat sites. Shorebirds preferred mudflats followed by tilling over that of herbicide-treated sites. Bird behaviour (feeding or resting) was variable and dependent on species, time of year and treatment. Although tilling appears to be initially effective in expediting restoration for shorebirds, it is too costly to implement on a large scale. The most significant long-term concern for shorebird usage is the *Spartina*-induced increase in tidal elevations on these meadows (>35 cm). Less than 20% of the gain in elevation was attributable to sediment accretion; the rest was root biomass. Due to the change in bathymetry, once *Spartina* was controlled at these sites, native salt marsh plants (*Salicornia*, *Triglochin* and *Spergula*) immediately invaded more than 400 meters out into what were previously intertidal mudflats. This potentially permanent large scale conversion of mudflat to salt marsh will have profound implications for shorebird habitat. Some potential remedies will be suggested.

Keywords: restoration, Dunlin, Western Sandpiper, birds of prey, native salt marsh, Willapa Bay, *Spartina*

INTRODUCTION

Spartina has colonized and eliminated much of the upper portion of the wide expansive intertidal mudflats of Willapa Bay. Species most threatened by *Spartina* are likely to be the thirty species of shorebirds that rely upon Willapa Bay's 47,000 acres of tideland for food and shelter during annual migrations to and from the Arctic. Much of the most-preferred shorebird habitat of Willapa Bay, sheltered upper tidal mudflats in the southern bay, has been displaced by *Spartina*. Peak winter and spring shorebird usage in sections of the bay has declined over 60 percent in the past decade as *Spartina* meadows have replaced the tidal mudflats (Jaques 2002). Census studies on shorebird abundance in Willapa Bay in 1991-1995, prior to the major increase in *Spartina* growth, found that 44 percent of the total bird usage was within two areas, the Bear River/Lewis Unit – South Willapa Bay region and the Willapa River area (Buchanan and Evenson 1997). These two areas have become almost contiguous *Spartina* meadows. Because of the loss of habitat caused by *Spartina*, the Audubon Society has listed Willapa Bay as the second most endangered shorebird habitat in the United States (Audubon 2004).

The ongoing chemical and mechanical control effort is the first step in recovering that habitat. The ultimate goal of a control effort should not be limited to control, but also needs to consider restoration of the affected habitat for maximal ecological value. Little information exists to date on how the numerous chemical and mechanical control methods being used to manage *Spartina* have expedited habitat restoration. The long-term ecological impact of invasive *Spartina* on shorebirds in England has been recently reviewed by Lacambra et al. 2004. They conclude that a return of shorebirds to English estuaries following *Spartina* removal is not axiomatic. In Washington, where there have been long-term control efforts by various agencies, use of the affected tideflats by shorebirds and waterfowl increases dramatically within several years of removal of invasive *Spartina* from mudflats (Patten and O'Casey 2007). The objective of this study was to assess the likelihood and the limiting factors involved in achieving functional habitat at *Spartina*-affected mudflats after control.

METHODS

Site information:

Direct and indirect assessments were made of shorebird, waterfowl and birds of prey usage of *Spartina* meadows (treated and untreated) in comparison to bare mudflats. These assessments were made for five sites: bare mudflat,

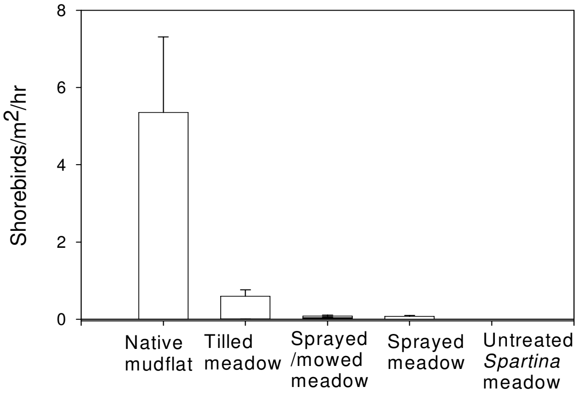

Fig. 1. A summary of the comparative use of *Spartina* affected tideflats by shorebirds during the winter/spring migration in 2003 based on foraging flux density data from remote sensing cameras as a funciton of *Spartina* control method. Bars = Std. Err.

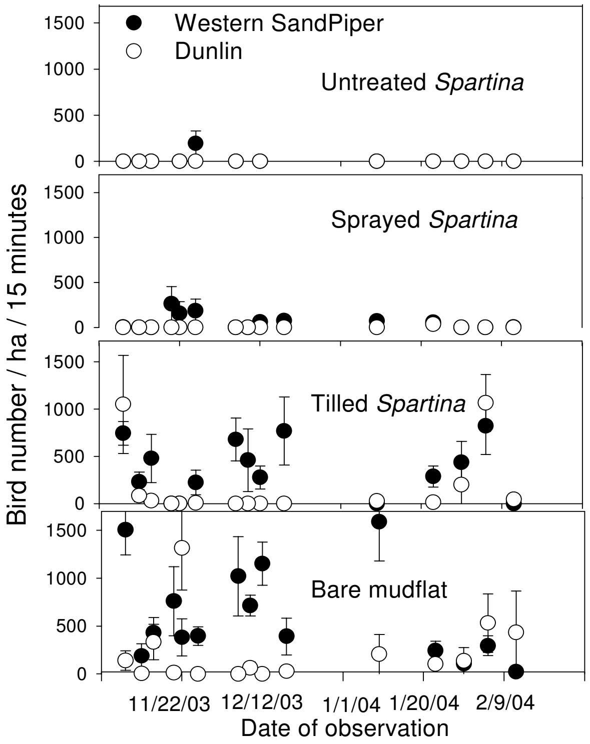

Fig. 2. Visual counts of the major shorebird species in Willapa Bay in Winter 03/04 as a funciton of the *Spartina* control method.

tilled *Spartina* meadow, sprayed *Spartina* meadow, spray-mowed *Spartina* meadow and an untreated *Spartina* meadow. Data collected included beak probe density, footprint density, fecal dropping density, visual counts during peak migration in spring 2003 and winter of 2003/2004, and remote monitoring with video cameras in winter/spring 2003. The study site was on Willapa National Wildlife Refuge property at the south end of Willapa Bay. The *Spartina* infestation was 10 to 14 years old and covered over 1000 hectares (ha). Treatment sites were adjacent to each other and large enough to be considered ecologically significant units (>60 ha). This part of the bay supported an abundant bird population prior to infestation by *Spartina* (Jacques 2002). Although the sites had similar bathymetry prior to *Spartina* infestation, their current elevations were measured to be greater than 35 centimeters (cm) above the adjacent mudflats. The bare mudflat site is and has been *Spartina*-free. The tilled site has been treated since 2000/2001 with mowing, tilling, and spraying for cleanup. It has been relatively free of *Spartina* since 2002. The sprayed and spray-mowed sites were treated with 2 gallons/acre of glyphosate (Rodeo®) in summer of 2002 and had follow-up spraying in summer of 2003. The spray-mowed site was mowed to a level of approximately 14 cm during the spring of 2003 to remove dead stubble and encourage bird usage. The untreated *Spartina* area is a large meadow comprising more than 200 ha at the southwest end of the bay.

Shorebird data

Beak probe density, footprint density, and fecal dropping density (#/0.25 m^2) data were collected on May 13, 2003, using five replications per habitat per location, with five subsample counts per replication. For each replication, comparative habitats (treatments) were located within 20 feet of each other. Remote monitoring of sites was done using video cameras in winter/spring 2003. A Mitsubishi Time Lapse Security Recorder, Model #HS-1280U, was used to record the black and white image from a Super Circuits PC23C camera with a 12-mm 1/3" CS TV lens. Power was provided using three 12-volt deep cycle marine batteries and a 16-watt solar pane with a DC to AC, 12-volt, 150-watt inverter. Cameras were mounted in weatherproof camera housing on 7-m poles 133 m from the native marsh. The camera focal area for each site varied slightly, ranging from approximately 90 to 180 m^2. Total bird usage (shorebird and waterfowl) from each tape was recorded every 30 seconds and the data was converted to mean daily flux densities (#/m^2/hour). For shorebirds, daily flux densities were based only on time periods during the day when the tideflats were exposed. The total number of days of complete data collection, from February 18, 2003 to May 14, 2003, ranged from 20 to 40 depending on the site. Visual observation of bird usage in the winter of 2003/04 was done using a single observer. Three plots (one hectare each) per site were observed for 10 minute intervals using a spotting scope. Observations were timed to coincide with peak usage at each site, just prior to tidal submergence or after tidal withdraw. Observation frequency was at least once a week. Bird species and behavior were noted.

Soil and plant data

Intact cores to the bottom of the root system (80+ cm) were collected by digging a 1-m wide and deep trench.

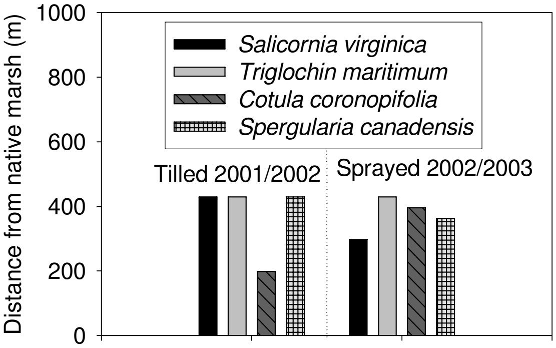

Fig. 3. The mean maximum distance from the shoreline that salt marsh species were located after *Spartina* was controlled. Data were collected June 2004.

Standard soil science methodology was used to determine porosity, bulk density, and the core sample composition. By washing the trench wall it was possible to identify all growing point meristems and record their points of origin (depth). The change in depth from the first occurring meristem to the current growing points over the 8-to-10-year period this meadow had been growing was assumed to be a change in tidal elevation resulting from *Spartina*-induced accretion. Data on vascular plant density (#/m^2) by species were collected in June 2004 from multiple transects from the native marsh line out to 500 m through each treatment site.

RESULTS

Shorebird foraging

Based on visual and remote observation data during the time course of this study, none of the *Spartina* control methods resulted in shorebird usage comparable to the bare mudflats (Figures 1 & 2). Flux density of shorebirds during winter and spring of 2003 was higher in the bare mudflat than in the tilled areas based on remote sensing (Figure 1), but comparable between the sites based on visual observations (Figure 2). Flux densities of shorebirds on the tilled site were higher than the sprayed or spray-mowed site (Figures 1 & 2). During 480 hours of video recordings, no shorebirds were ever observed at the *Spartina* meadow site. Shorebirds (Western sandpipers) were only noted once over 14 visual observations in the *Spartina* meadow, and they floated in on a *Spartina* wrack. From a behavioral perspective, it appeared that the tilled site had the lowest percentage of skittish feeding and the bare mudflat the lowest percentage of resting (data not shown).

Table 1. Shorebird usage of *Spartina* habitat at Porter Point based on footprint, fecal dropping and beak print densities as a function of accessibility.*

General site location	*Shorebird access descriptions*	*Density (#/0.25m^2)***		
		*Footprints**	*Fecal droppings*	*Beakprints*
Sprayed meadow, 80 m from native marsh; stubble 25-50cm high	Live *Spartina* canopy	0	0	0.8±0.4
	Dead stubble	50±50	0.9±0.4	29±4
	Exposed mudflat	134±11	1.8±0.3	68±10
Edge of sprayed meadow adjacent to bare mudflat, 80 m from native marsh; stubble 25 to 50 cm high	Live *Spartina* canopy	0.2±0.1	0	3±2
	Dead stubble	8±2	6±0.3	13±4
	Exposed mudflat	35±5	0.8±0.2	47±5
Tilled strips in between sprayed *Spartina*, 1000' from native marsh; stubble was mowed at 25 cm height	Dead stubble	87±12	0.5±0.2	25±4
	Exposed mudflat	130±19	1.3±0.3	76±9

*Data collected 5-13-03; 5 replications per habitat per location, with 5 subsample counts per replication. For each replication comparative habitats (treatments) were located with 20 feet of each other.

** mean ± standard error

Based on short-term comparisons in shorebird footprints, fecal droppings and beak probe densities, there were major differences in shorebird microsite habitat preferences (Table 1). All types of dead *Spartina* stubble or live *Spartina* stems drastically interfered with shorebird foraging. For three locations within Porter Point, there was almost no evidence of any shorebird usage where there was live *Spartina* growing. The bare mud and dead stubble locations usually displayed high counts of beak and foot prints and fecal droppings. Bare mud usually had twice as much shorebird usage as dead stubble.

Soil and plant data

Spartina meadows rapidly began a transition to native middle to upper salt marsh as soon as the *Spartina* was killed (Figure 3). Within two years of treatment, four salt marsh plant species extended 400 m out from their native marsh habitat. At this particular site, the transition from mudflat to *Spartina* meadow to salt marsh has all occurred with ten years and represents a permanent loss of hundreds of hectares of prime shorebird habitat. In an analysis of soil parameters at this meadow (data not shown), we have found the dead *Spartina* root mat extends down to 35 cm, with the bulk of the soil volume being comprised of organic matter and pore space. Only 15% of the elevation rise could be accounted for by sediment accretion.

DISCUSSION

Restoring mudflats back to their original form and function will be extremely difficult. Even with tilling and several years of follow-up chemical control, as well as natural restoration processes occurring over several years, *Spartina*-affected mudflats are far from having shorebird usage comparable to what normally occurred on a bare tidal mudflat. This may be especially true for low tidal energy sites in the southern half of Willapa Bay, where there is traditionally high shorebird usage. At these sites, the landscape-scale changes in bathymetry via *Spartina*-induced accretion and root mass accumulation make it unlikely that any restoration effort would be able to bring back the original bathymetry. This is especially true since native marsh is already succeeding in these areas. Once these sites have transitioned to stable salt marshes, there will be little likelihood that they could ever become functional mudflats again. To prevent irreversible loss of prime shorebird habitat, it is therefore absolutely essential to eradicate all existing *Spartina* in these critical sites as quickly as possible.

Can we realistically achieve restoration of functional shorebird habitat at *Spartina*-affected tidelands post-control? If the site has undergone major elevation changes, it is likely that it will become a stable salt marsh, and achieving shorebird habitat over the long term will be problematic. If the site has not undergone major *Spartina*-induced elevation changes, then habitat restoration is feasible. Restoration may be expedited with a process that breaks up root masses and removes stubble and traces of *Spartina* canopy, such as tilling. This process is not inexpensive. Tilling of large *Spartina* meadows is cost-prohibitive, requires very specialized equipment and is very slow (<1 ha/day). Tilling several small 2-3 ha restoration units throughout treated meadows might be a more cost-effective approach to restoring shorebird utilization of sites. It is not clear, however, how much of this tilling effect on shorebirds can be strictly attributed to the actual physical effects of tilling (breaking up the root mass) versus the creation of an open flat smooth surface that is more shorebird friendly. If the latter is the case, then waiting for natural processes to remove residual stubble to create an open surface would be sufficient.

From our data and that of others, it is still unclear what are the most critical factors driving shorebird usage of tideflats post-*Spartina* control: prey density, prey accessibility, predator avoidance behavior, or other variables. Research on changes in prey density post-*Spartina* control has been inconsistent (Lacambra et al. 2004). We found slightly higher benthic infauna on tilled vs. herbicide-treated *Spartina*-affected mudflats, but both were orders of magnitude less than on adjacent unaffected mudflats (data not shown). Based on our data, ease of access to prey is certainly a very significant factor. Removal of live canopy, dead stubble, or thick root mat immediately improves shorebird usage of a site. The presence of stubble and canopy is also likely to affect predator avoidance behavior. Our observational data (not shown) indicated birds of prey exclusively utilized tilled and herbicide-treated *Spartina* meadows rather than open mudflats to hunt shorebirds, even though the latter had orders of magnitude higher shorebird density.

Data from Great Britain on *S. anglica* indicate that shorebird usage of *Spartina*-affected tideflats in English estuaries has taken decades to occur once the *Spartina* has been eradicated or naturally died off (Lacambra et al. 2004). Unless there is a change in sea level or a major subduction event, the prospect of shorebird utilization of the thousands of hectares of *Spartina*-affected mudflats in Willapa Bay could take a similar or even longer time period.

ACKNOWLEDGEMENT:

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WHERE DO WE GO FROM HERE? ALTERNATIVE CONTROL AND RESTORATION TRAJECTORIES FOR A MARINE GRASS (*SPARTINA ANGLICA*) INVADER IN DIFFERENT HABITAT TYPES

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Little is known about the effects of removing invasive species and subsequent consequences for community restoration. Invasive species removal can have positive effects for some communities but may cause unexpected changes that lead the system to an alternative state. The consequences of invasive species removal are likely to be context-dependent with restoration occurring readily under some situations but not others. English cordgrass, *Spartina anglica*, has invaded large areas of protected shoreline in the Puget Sound, Washington State, USA, and is the target of intensive removal efforts. It invades and modifies a variety of habitat types, from unvegetated mudflats and cobble beaches to established low and high salinity native marshes. It binds sediment around its dense root system and changes biogeochemical processes, all of which can have significant consequences for shorebirds, infauna, and commercial aquaculture. Cordgrass invasion, modification, and removal varies among the different habitat types but post-removal colonization predictably results in colonization of native vascular plants. Although these plants are the dominant species in salt marsh communities, they are uncommon in mudflat and cobble beach communities, and thus do not represent a restored post-removal state. Instead, the legacy effects of cordgrass produce alternative outcomes. We hypothesize, based on the interaction between recruitment, physical disturbance (water movement) and sediment accretion, that cobble beach and high salinity marshes will be restored but that mudflats and low salinity marshes will retain the legacy of the invasion for the long term.

Keywords: English cordgrass, *Spartina anglica*, habitat modification, salt marsh, mudflat, cobble beach, restoration, alternative states, Puget Sound, Washington.

INTRODUCTION

In recent years there has been a strong focus on the impacts of invasive species both at community and ecosystem levels (Parker et al. 1999; Ruiz et al. 1999; Grosholz 2002). Some of these invasive species, known as foundation, dominant, or ecosystem engineering species (Jones et al. 1994; Power et al. 1996; Bruno and Bertness 2001; Crooks 2002), can transform communities, resulting in both positive and negative effects for native as well as nonindigenous species. These species can have a large influence on community structure relative to their biomass; they alter ecological processes in multiple ways and often create positive feedbacks that benefit their continued expansion and impact.

Much less is known about the consequences of removing invasive species, especially dominant or foundation species (Hobbs and Humphries 1995; Myers et al. 2000; Zavalata et al. 2001, Hacker and Dethier 2009). Invasive species removal can have positive effects for some communities, with restoration occurring soon after removal (Fig. 1A; Myers et al. 2000). However, in many cases, the results have been mixed, with unexpected and widespread impacts on natural communities (Zavalata et al. 2001; D'Antonio and Meyerson 2002). Communities may not simply return to their former state in a straightforward reversal of the invasion process but, instead, could be so modified by the invasion that they are not easily restored (Fig. 1B; Hobbs and Humphries 1995; D'Antonio and Meyerson 2002). These modifications are likely to vary in degree, depending on characteristics of the invader, the invaded community, and the time since invasion, but could prevent full recovery after the invader is removed. Just how the 'legacy' of the invasion influences post-removal community structure is poorly understood; yet such understanding is critical to be able to confidently predict whether management goals, originally intended to restore the integrity of highly invaded communities, will be met by removal alone or whether additional measures may be required. Given the numerous removal programs underway, development of testable theories that predict post-removal community dynamics are needed to better understand the benefits and risks of removing invaders that have large modifying effects (Hacker and Dethier 2009).

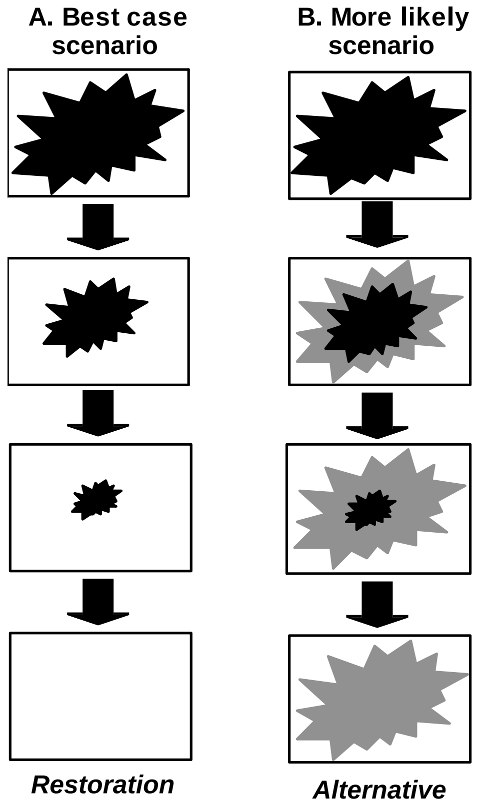

Fig. 1. Stages of invasive species removal (arrow) under A) the best case scenario for which the invader modification is lost and habitat is restored, and B) the more likely scenario for which the modification remains and community follows an alternative trajectory. From Hacker and Dethier 2009

In this paper, we explore the possible consequences of removing the invasive English cordgrass, *Spartina anglica*, which has invaded the shoreline of Puget Sound in Washington State, USA. *Spartina anglica* colonizes communities with different species assemblages and physical conditions, and thus produces variable degrees of invader modification. Our goal is to use these patterns of differential invasion along with a simple conceptual model to make predictions about post-removal community structure. Based on this analysis, we predict that some communities will be readily restored while others will follow alternative states. These states will depend on how the modifications and physical disturbance interact to influence both species recruitment and community maintenance through time.

STUDY SYSTEM

Spartina anglica was first introduced to Puget Sound from England in 1961. It did not become a management priority until the late 1990s when it had spread to a total of 3,300 hectares (ha) of intertidal habitat at 77 sites (Hacker et al. 2001). Cordgrass is a strong ecosystem modifier that accretes sediment around its dense root system and changes sediment biogeochemistry, which can have important community-wide consequences (Thompson 1991; Daehler and Strong 1996). Species affected by the invasion include native and commercial invertebrates (infauna and epifauna such as clams and oysters; Zipperer 1996; O'Connell 2002), plants (Hacker and Dethier 2006), and birds (Goss-Custard and Moser 1988; Triplet et al. 2002).

Cordgrass grows in a range of communities in Puget Sound from mudflats and cobble beaches, which are normally devoid of vascular plants, to low and high salinity marshes, where native vascular plants are the main biological component (Hacker et al. 2001). Our research shows that cordgrass invasion, removal, and post-removal patterns vary dramatically between these communities (Hacker et al. 2001; Reeder and Hacker 2004; Dethier and Hacker 2005; Hacker and Dethier 2006), thus suggesting that its removal will result in different post-removal community structure depending on habitat.

Habitat dependent invasion and modification by cordgrass

The abundance and distribution of English cordgrass varies between four habitat types within Puget Sound (Hacker et al. 2001). Low salinity marsh and mudflat sites have much larger infestations than cobble beach and high salinity marsh sites. These differences are driven mostly by variability in physical conditions across the four habitat types although biological interactions play a small role (Dethier and Hacker 2005). In a seed addition experiment (Dethier and Hacker 2005), we found that mudflat and low salinity marshes had the greatest cordgrass germination, survival, and growth of all the habitats. In mudflat communities, which are naturally devoid of vascular plants, seed germination was high and surviving seedlings grew quickly. This growth pattern eventually results in the coalescence of individual plants and widespread growth across all the intertidal elevations. Low salinity marshes, on the other hand, are dominated by a diverse native vascular plant assemblage. Cordgrass had high seedling germination, survival and growth; this pattern eventually results in large swards that outcompete native plants and form dense monocultures. In high salinity marsh habitats, cordgrass grows mainly in sparsely vegetated low intertidal stream channel areas. Seed additions show high germination and survival in low elevation areas without plant neighbors but not higher in the intertidal zone, pointing to the importance of high salinity and native plant interactions in restricting cordgrass distribution. Finally, cordgrass is rare in cobble beach habitats despite the lack of native vegetation. Here shifting cobble sediments contribute to the low germination, survival, and growth of the plant.

Cordgrass generally modifies its habitat by accreting sediment around its large root system, forming an elevated root mat and changing sediment biogeochemistry (Thompson 1991; Maricle and Lee 2002; Hacker and

Dethier 2006). In addition, it can provide substantial aboveground structure not normally present in some habitats. These structural and biogeochemical modifications have direct and indirect effects on other plant and animal species as mentioned earlier. In our study system, English cordgrass differentially modifies four habitat types, resulting in variable, community-wide consequences (Hacker and Dethier 2006). For example, we know that the accumulation of sediment by cordgrass differs between the four habitats, with mudflats experiencing the greatest sediment accretion (~30 cm) and low and high salinity marshes, the least (~10-15 cm) (Hacker and Dethier 2006). Cordgrass growing in cobble beaches accretes much less sediment (~20 cm) than mudflats. A likely explanation for this difference is wave energy; water movement is lower in mudflats but higher in cobble beaches.

In addition to accreting sediment, thus changing the structural characteristics of the substrate, cordgrass causes a number of chemical transformations. We found changes in sediment water content, redox potential (a proxy for oxygen content), and salinity in invaded versus native areas depending on community type (Hacker and Dethier 2006). For example, cordgrass caused a decline in sediment water content in mudflats and high salinity marshes suggesting that the elevated root mat had less tidal inundation, better drainage, and/or more water uptake by cordgrass. In cobble beaches, the opposite was seen; root mat sediments had higher water content than the unmodified cobble sediments. In low salinity marshes, there was no change in sediment water content with the presence of cordgrass compared to native vascular plants. Cordgrass generally increases oxygen content in the sediments of all the communities. This may be related, in part, to better drainage seen in some of the communities but is more likely due to oxygen leakage from root aerenchyma (Maricle and Lee 2002). Finally, surface salinities were generally lower in sediments with cordgrass although they did not change in low salinity sites with native plant cover. These results suggest that cordgrass shades the sediment surface thus decreasing water evaporation and salt accumulation compared to unvegetated areas although this hypothesis was untested. We have not investigated cordgrass effects on nutrient cycling but it is clear that cordgrass is a major carbon source unlike any other in these communities and likely modifies microbial and macrofaunal resource use.

English cordgrass modifications have significant consequences for community structure. We compared native vascular plant and algal abundance in areas with and without cordgrass in all communities (Hacker and Dethier 2006). In this study, we found that in mudflats, cobble beaches, and high salinity marshes, cordgrass invasion caused an increase in native vascular plant cover and decline in algal cover. By elevating sediments, increasing oxygen content, and decreasing salinity, cordgrass clearly provides a more suitable habitat for native vascular salt marsh plants but a less suitable habitat for algae, which require greater tidal inundation to avoid desiccation. In low salinity marshes, native vascular plants declined precipitously, presumably a consequence of cordgrass competitive dominance. We also compared marine invertebrates in mud and cobble sediments versus adjacent cordgrass patches. Cobble areas without cordgrass had oligochaetes, bivalves, and a variety of crustaceans; cobble with cordgrass had less infauna because of dense root mat formation. Uninvaded mudflats often had abundant clams and a variety of polychaete worms, while those with cordgrass had fewer infauna but more epifaunal crustaceans such as amphipods (presumably because of the three–dimensional structure provided by the vegetation). Invertebrate studies (Zipperer 1996; O'Connell 2002) in areas invaded by *Spartina alterniflora* in Willapa Bay, WA, similarly found that certain taxa are excluded by cordgrass (esp. polychaetes and bivalves) while others are increased (esp. dipteran larvae and spiders). Although we have not quantitatively measured possible changes in epifaunal communities, we have observed a general increase in higher marsh epifauna such as grasshoppers, spiders, snails, mice, and marsh wrens within cordgrass meadows and a decline in shorebirds, some species of snails, mussels, and oysters.

Cordgrass removal and consequences for post-removal restoration

Removal of English cordgrass, involving mowing and herbicide applications, began in 1997 and has caused a modest 10-20% decline as of 2002 (Hacker et al. 2001; Dethier and Hacker 2004). Although local eradication has occurred at some sites with minimal treatment repetition, most sites have required repeated removal spanning multiple years to achieve eradication. To investigate the factors hindering removal success, we conducted a multiple site study that linked removal data with ecological factors and removal methodologies. We found that removal success depended on removal regime and community type (Dethier and Hacker 2004). The bulk of cordgrass decline was due to consistent, multi-year removal (once per year) in certain communities. For example, cobble beaches, high salinity marshes, and mudflats showed the most promising response to consistent removal even when years of removal effort were similar to low salinity marshes. When removal was intermittent (≥ 2 years missed), low salinity marshes and mudflats were the least responsive to removal.

The pattern of regrowth with removal regime points to the resiliency of cordgrass. If photosynthesis is not continually interrupted to slowly kill the plant, removal success is compromised due to its ability to regrow. In a set of manipulative experiments, we found that biomass gains under intermittent removal greatly outweigh losses accrued under consistent removal both because of the highly productive nature of the species, the benefits of competitive release, and the habitat modifications that facilitate further growth (Reeder and Hacker 2004).

Preliminary study of post–removal community structure suggests that the response largely depends on how modifications created by the invader interact with habitat type (Reeder and Hacker 2004). In this study, all sites exhibited similar community responses following cordgrass removal. Native vascular plants increased with consistent removal in all communities, but declined under intermittent removal. In addition, the plant assemblages in the two salt marsh communities were different after cordgrass removal than their native counterparts. As a result, post-removal community structure and habitat restoration is more complex than simply removing cordgrass and anticipating a reversal of the invasion process.

IMPLICATIONS AND FUTURE DIRECTIONS

In this system, we found that removing invasive *S. anglica* resulted in an increase in native vascular plants irrespective of habitat type (Reeder and Hacker 2004). Although these plants are the dominant species in salt marsh communities, they are uncommon in mudflat and cobble beach communities, and thus do not represent a restored post-removal state. Instead, the legacy effects of the cordgrass infestation produced alternative short term outcomes, which may or may not continue for some time. Legacy effects include elevated, stabilized sediments and altered biogeochemical processes that make conditions better for vascular plants and poorer for macroalgae and infauna. We have observed a similar pattern of vascular plant colonization on relic cordgrass root mats both in the U.S. (Willapa Bay, Washington) and in other countries (Baie de Somme, France and Invercargill, New Zealand) suggesting that the effect is widespread (personal observation). Interestingly, in the Baie de Somme, native vegetation that colonizes cordgrass removal areas, in particular the salt marsh plants *Salicornia* and *Atriplex*, are harvested and sold as a specialty food. However, in most cases, long-term vascular plant colonization, especially in mudflat habitats, is likely to prolong the main management issues surrounding invasive cordgrass (i.e. reducing habitat for shorebirds, infauna, and commercially important shellfish).

Given the potential that removal of cordgrass can result in alternative community structure trajectories, we developed a conceptual model that helps predict the possible outcomes of cordgrass removal (Hacker and Dethier 2009). It is based on alternative stable state theory which explains the observation that different species assemblages can occur in the same general locality at different times (or different localities at the same time) because historical events or contingencies play an important role in creating community structure (Lewinton 1969; Sutherland 1974; Petraitis and Latham 1999). We suggest that it can provide a useful framework for identifying the processes important to post-removal community structure by identifying the factors that could lead a community toward or away from a restored state.

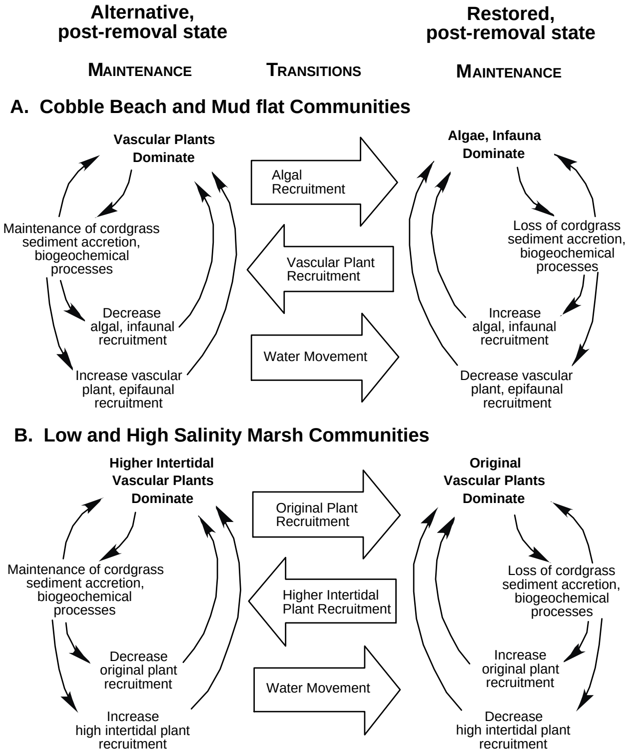

Fig. 2. Depiction of the hypothesized processes controlling alternative vs. restored community structure for either A) cobble beach and mudflat habitats (original communities lack native vascular plants) or B) high and low salinity marshes (original communities dominated by native vascular plants). Modified from Hacker and Dethier (2009).

In our model (Hacker and Dethier 2009 based on Petraitis and Latham 1999) there are two community states: 1) a restored state defined as the replacement of the lost community assemblage, and its function, after the invader is removed, and 2) an alternative state defined as one in which a new species assemblage colonizes and persists; it could also include reinvasion by the original invading species. There are transitional processes that include disturbance and stress, recruitment of species, and biological interactions. In addition, there are positive feedback processes in which the existing species assemblage acts to reinforce and maintain its current structure and function.

Applying these ideas to post-removal community structure, we can hypothesize what may happen to the four habitats when cordgrass is removed (Hacker and Dethier 2009). We predict that cobble beaches will assume a restored state due to the interaction of both transition and maintenance processes as outlined in Fig. 2. If we assume that vascular plant recruitment occurs in cobble beaches, but plant density is low due to high water movement, then an alternative state can only be produced if vascular plants can maintain elevated sediments and altered biogeochemical processes (Fig. 2A, left side). Active water movement and the scouring action of shifting cobbles and gravel should hamper this process. Ultimately, we predict that an alternative state will not be maintained due to the transitional process of water movement increasing sediment erosion around plant roots and decreasing subsequent vascular plant and infaunal recruitment. As a result, negative feedback processes in the maintenance component of the alternative state will shift cobble beach community structure to a restored state (Fig. 2A, right side). Algal recruitment will increase as sediment erosion occurs and cobbles re-emerge, pushing the community into a positive feedback loop that includes loss of sediment, decreased native vascular plant recruitment, and continued increases in algal and infaunal recruitment (Fig. 2A, right side).

We hypothesize that mudflat habitats, because they experience lower wave action, will have increased vascular plant recruitment, and allow for the maintenance of cordgrass sediment accretion and biogeochemical processes (Fig. 2A, left side). We expect that a positive feedback loop generated by slower water movement and the presence of vascular plants will maintain sediment characteristics created by cordgrass, decrease algal and infaunal recruitment, and continue to increase native vascular plant recruitment (Fig. 2A, left side).

Finally, we suggest that high and low salinity marshes previously invaded by cordgrass will experience recruitment of higher intertidal vascular plant assemblages, rather than the original assemblage, due to the increased tidal elevation produced by cordgrass-accreted sediments. We expect that these plants will be good at maintaining sediment depth and biogeochemical processes originally created by cordgrass. Their presence will be maintained via a positive feedback loop that increases their own continued recruitment while decreasing that of the lower intertidal plant community present before the invasion (Fig. 2B, left side). However, if water movement is sufficient to erode sediments or if plant recruitment is low or delayed, marsh communities may shift into a restored state (Fig. 2B, right side).

Testing these hypotheses will require both large and small-scale experiments in areas where cordgrass has been removed. Ultimately, this research will provide natural area managers with better ways of predicting and evaluating the consequences of post-removal impact across different habitat types. It may be that active removal of native vascular plant assemblages will be necessary in habitats such as mudflats where restoration could be continually hindered by the recruitment and positive feedbacks produced by these plants. Although invasive species removal and restoration is a critical component to the management of many natural areas, most research is site or species-specific. The proposed research will help establish the importance of a general theory on the contextual dependence of invasive species removal that can be applied to communities with varying species assemblages, physical conditions, and degrees of invasion.

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SPARTINA CONTROL STRATEGY AND EXPERIENCE IN THE SAN FRANCISCO ESTUARY

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In 2004, the San Francisco Estuary Invasive *Spartina* Project (ISP) initiated its first year of a region-wide, coordinated *Spartina* control program. The strategies for selection of the treatment locations and the coordination of the projects were developed jointly by the ISP and its partners, which include a number of local, regional, state, and federal agencies and non-profit organizations. Funding for the work was contributed by many of the partners, by the ISP's host agency (California State Coastal Conservancy), by grants from a state-federal consortium, and other sources. The control plans were developed by the ISP, and implemented by the partners. Approximately 176 hectares (ha) (435 acres [ac]) of non-native *Spartina* (*Spartina alterniflora* and hybrids, *S. densiflora*, and *S. patens*) were treated in 2004 using a variety of methods including covering, digging, and treatment with aquatic herbicide. The ISP facilitates acquisition of permits, grants, and contracts to implement the control work. Successful control of *Spartina* in the San Francisco Estuary is complicated by several factors, including an extremely short treatment season (September 1 to mid-October) to avoid disturbing endangered California clapper rails during their breeding season, and the greater-than-exponential spread of the *S. alterniflora* hybrid swarm. At the end of the 2004 control season, approximately 627 ha (1,550 ac) of non-native *Spartina* remained untreated in the San Francisco Estuary. In order to address this rapidly-expanding infestation, the ISP seeks to have control plans in place for all non-native *Spartina* in the estuary by the end of 2005, even though treatment may not be implemented on all sites until the following years to minimize impact on endangered species. One of the tools that the ISP hopes will help assure a successful control program is the aquatic herbicide imazapyr, which should be registered for use in California by the summer of 2005. The high efficacy and suitability of this herbicide for estuarine use bodes well for control efforts around San Francisco Bay. Building upon the structure and partnerships developed during the 2004 *Spartina* control season, the ISP believes it is possible to set in place coordinated, sustainable, estuary-wide management and control of non-native *Spartina*.

Keywords: *Spartina*, cordgrass, hybrids, hybrid swarm, imazapyr, glyphosate, estuary, California clapper rail

The spread of non-native *Spartina* in the San Francisco Estuary has been a topic of concern in the region since the early 1970s, following the initial introduction of several species: *S. densiflora* (Chilean cordgrass), *S. anglica* (English cordgrass), and *S. patens* (salt meadow cordgrass), but especially *S. alterniflora* (smooth cordgrass). By the early 1990s, ecologists and land managers in the estuary recognized the increasing impacts of the invasion as marshland within their respective jurisdictions began to support ever-expanding populations of non-native *Spartina* species. Around this time, a hybridization event between the non-native *S. alterniflora* and the native *S. foliosa* (Pacific cordgrass) produced the first individual clones of a vigorous hybrid swarm that swiftly outpaced its alien parent species in its ability to colonize a wide range of habitats in the San Francisco Estuary.

In 2000, the California State Coastal Conservancy (Conservancy) initiated the Invasive *Spartina* Project (ISP) to stave off the invasion of non-native cordgrass and its potential impacts. The ISP was intended to be a regionally-coordinated effort of federal, state and local agencies, private landowners, and other interested parties, with the ultimate goal of arresting and reversing the spread of non-native *Spartina* in the estuary. It was not until the late summer of 2004 that all of the required permitting and planning had been completed sufficient to initiate the first season of *Spartina* control work estuary-wide.

The process to produce an Environmental Impact Statement and Environmental Impact Report (FEIS/R), satisfying both Federal and State requirements, began at the inception of the ISP in 2000. In October of 2003, following the conclusion of the public comment period on the draft document and preparation of comment responses, the Final EIS/R was completed by the Conservancy and the U.S. Fish and Wildlife Service (California Coastal Conservancy and USFWS 2003). The FEIS/R was then used by the FWS to produce a Programmatic Biological Opinion (PBO) (USFWS 2004(a)) for ISP's Control Program, which outlined the requirements for creating detailed individual Biological Opinions (BOs) for each proposed *Spartina* treatment site. Issuance of site-specific BOs depended on the development of site-specific *Spartina* control plans by the ISP and its partners.

SITE-SPECIFIC *SPARTINA* CONTROL PLANS

Concurrent with, and subsequent to the completion of the FEIS/R and PBO, individual site-specific *Spartina* control plans (SSPs) (California Coastal Conservancy 2004(b)) for 2004 were drafted by the ISP and its partners for 16 sites scattered throughout the estuary. These sites were chosen based on a variety of characteristics, including existing partner involvement, infestation age and composition, endangered species issues, access issues, adjacent land uses and other criteria. The 16 sites chosen for *Spartina* control in 2004 included 45 sub-areas (areas further delineated for ease of treatment) encompassing an estimated 181 net ha (447 net ac) of *Spartina*, within just over 6,070 ha (15,000 ac) of estuary marshland. Each of the SSPs included:

- Scope of Work
- Work Program defining the schedule of work
- Budget
- Impact Identification Matrix
- Impact Mitigation Matrix
- Spill Prevention Protocols
- Drift Reduction Protocols
- Marsh Safety Recommendations
- Site Maps
- Site Photographs

As part of the requirements of the California Environmental Quality Act (CEQA), each SSP contained an Impact Identification Matrix and an Impact Mitigation Matrix. The Impact Identification Matrix evaluated the suite of *Spartina* control methods proposed for each site for potential impacts to environmental resources. Included in the matrix were impacts related to geomorphology and hydrology, water quality, biological resources, air quality, noise, human health and safety, visual resources, and cumulative impacts.

Once the impacts associated with *Spartina* control work were identified for a given site, an Impact Mitigation Matrix was prepared. Each site's matrix explicitly referenced those mitigation measures defined in the FEIS/R that were to be implemented in the field. Impact Mitigation Matrices were designed to require verification signatures next to each mitigation measure. Both the implementing agency representative and a representative of the ISP were required to verify that all mitigation measures were implemented.

PERMITTING AND GRANTS

The completion of the SSPs enabled FWS review of the documents for issuance of site-specific BOs which analyzed the effect of the proposed work on the suite of endangered species within the scope of the project (USFWS 2004(b)). Individual site-specific Environmental Assessments (EAs) were also prepared at this time to determine the effect of the proposed work on the greater environment beyond endangered species issues (USFWS 2004(c)). The results of these analyses provided the basis for a Finding of No Significant Impact (FONSI), which declared that the proposed *Spartina* control work would not produce "significant impact" on the estuary's natural resources (USFWS 2004(d)). Additionally, a Record of Decision (ROD) was published in the Federal Register announcing the completion of the FEIS/R (USFWS 2004(e)).

Soon after its inception, the ISP worked to identify partner agencies and groups that would be willing to join in the effort to control non-native *Spartina* in the estuary. Many of the partners involved in developing SSPs for their jurisdictions lacked the funds to initiate control work on their own, so outside funding for control operations were necessary for work to proceed. In some cases, funding was not the constraining factor for the partner, but instead, clearing permitting hurdles, logistics or knowledge of control methodologies presented the greatest challenge. In each case, the ISP provided the necessary assistance to implement the *Spartina* control plan. Where funding was the limiting factor, grants from the Conservancy were provided to enable work to proceed. The terms of these grant agreements were negotiated during late 2003 and the first half of 2004. Ultimately the Conservancy and the CalFed Bay Delta Authority issued a total of $350,000 in grants for *Spartina* control work in the estuary for the 2004 control season. Official ISP partners for this season included U.S. Fish and Wildlife Service, East Bay Regional Parks District, Alameda County Flood Control District, California Department of Transportation, Santa Clara Valley Water District, City of Palo Alto, Friends of Corte Madera Creek Watershed, Tiburon Audubon Society, Marin Conservation Corps, and California Department of Parks and Recreation. Inclusion of any organization or agency as an official ISP partner required that partner's governing body officially adopt the ISP's FEIS/R, develop an SSP in coordination with the ISP, and adhere to any mitigations defined in the Impact Mitigation Matrix developed for the site within their jurisdiction.

For each type of control method proposed for each site, certain permits needed to be acquired before work could begin. In the case of herbicide applications, all treatments were required to comply with the Environmental Protection Agency's National Pollutant Discharge Elimination System (NPDES), administered by the California Water Quality Control Board's (CWQCB) San Francisco Bay regional office. However, at the outset of 2004, the CWQCB had yet to develop an NPDES permit to cover the discharge of aquatic pesticides, a novel application of this portion of the Clean Water Act that had, until recently, been reserved for the regulation of effluent discharge. As a consequence, herbicide-based control operations throughout the state were on hold until the CWQCB revised its permitting procedures to address aquatic herbicides. The revisions were adopted in June of 2004, enabling ISP partners to issue Notices of Intent (NOIs) to be covered under the Statewide General NPDES Permit for Aquatic Weed Control.

The NPDES permit also requires developing an Aquatic Pesticide Application Plan (APAP) identifying the procedures for applying herbicides, the chemicals to be used, the species targeted for treatment, a description of the waterbody system, and other related information. Within the APAP is a Water Quality Monitoring Plan (WQMP), which defines the protocols for water quality sampling of treatment areas where herbicide is used. While the ISP developed the APAP on behalf of the ISP partners, they contracted the services of the San Francisco Estuary Institute (SFEI) to further develop and implement the WQMP for the work proposed by the ISP and its partners, and to report on its findings following the 2004 treatment season (California Coastal Conservancy 2004(c)).

In addition, access permits from regional and local agencies were necessary for treatment on many of the 16 sites slated for *Spartina* control and the ISP coordinated their procurement.

TREATMENT METHODS

A variety of methods can be used to treat non-native *Spartina* populations in the estuary. However, very rarely is any particular treatment method appropriate for all situations. In the FEIS/R, the ISP proposed a suite of control methods and approaches based on the cumulative expertise of *Spartina* control operations worldwide, especially the work being done to control *S. alterniflora* in Willapa Bay, Washington State, USA. In 2004, the ISP tested several possible control techniques to determine the relative benefits of each.

Chemical Control

Glyphosate herbicide (Aquamaster® or Rodeo®) was used in most of the area of *Spartina* infestation that was chemically treated in 2004. The application of herbicides comprised the largest part of the Control Program for a host of reasons, not least of which was the need to control large acreage at low cost to stay ahead of the rapid expansion rate of the invader. Moreover, this method has a relatively low impact on the treated marshlands in contrast to other treatment options. Drawbacks to herbicide use include the potential for herbicide or fuel spills, negative public perception, timing restrictions, and potential water quality impacts. Glyphosate herbicide was applied via tracked amphibious vehicles (Hydrotraxx or Argo) fitted with spray apparatus, truck-mounted spray equipment, backpack sprayers, shallow-bottomed boats with outboard motors and broadcast aerial applications via helicopter with a 30-foot boom fitted with a nozzle array.

Manual Control

For those sites not yet fully established—where there were very small, pioneering infestations to be treated—manual control techniques were used. These methods were often incorporated into public outreach efforts, involving volunteer groups that worked on marshland restoration projects. For smaller sites, digging the plants resulted in good control, but swiftly exhausted worker enthusiasm and resulted in heavy impacts to the marsh. Digging plants in infestations much larger than 25 square meters is prohibitively expensive and has the potential to leave lasting damage to the treated marsh in the form of deep holes or trenches. In such cases, tarping or covering of the plants was used to good effect. Heavy synthetic tarps were staked in place over trampled stands of non-native *Spartina* in several locations in the estuary, as well as at Point Reyes National Seashore on the outer coast north of the Golden Gate. Tarps were left in place for at least eight months and required regular monitoring to assure that the covering remained in place during tidal fluctuation, especially storms. Removal of the covering following successful control of the *Spartina* was often difficult because of the heavy accumulation of mud on top of the tarps, requiring laborious excavations to unearth the covering. Drawbacks to covering include the high cost of installation, maintenance and removal and the attendant damage to the marsh, as well as the negative environmental impacts of petrochemical production associated with the manufacture of the heavy synthetic fabric used in this treatment method. Two of the sub-areas identified for *Spartina* control in 2004 used small-scale mowing of the infestation. Gas-powered hand mowers fitted with a tri-blade were able to make quick work of the standing material which was either left in place in the marsh (early season, pre-seed set) or removed from the marsh and disposed of at a landfill (late season, post seed-set). Mowing in this way serves to control seed and pollen spread and may slow the vegetative expansion of the *Spartina* clone by usurping root reserves. Mowing alone does not kill the plants, however. Unless coupled with other control methods, mowing would need to be implemented several times each year in perpetuity and yet would still allow some vegetative expansion.

Other methods of *Spartina* control tried elsewhere were not attempted in the 2004 control season for various reasons. Macerating or tilling was not used because of the concerns of land managers that the high amount of urban refuse present in estuary marshes (tires, shopping carts, blocks of concrete, pieces of metal, wood debris embedded with bolts or nails) and natural wrack (driftwood logs), would result in high maintenance costs or operator injury. The extremely soft nature of most of the estuary mud where control would take place was also taken into consideration, as the machinery involved in these methods is heavy and very likely to get stuck in place. Crushing the plants, where machinery is used

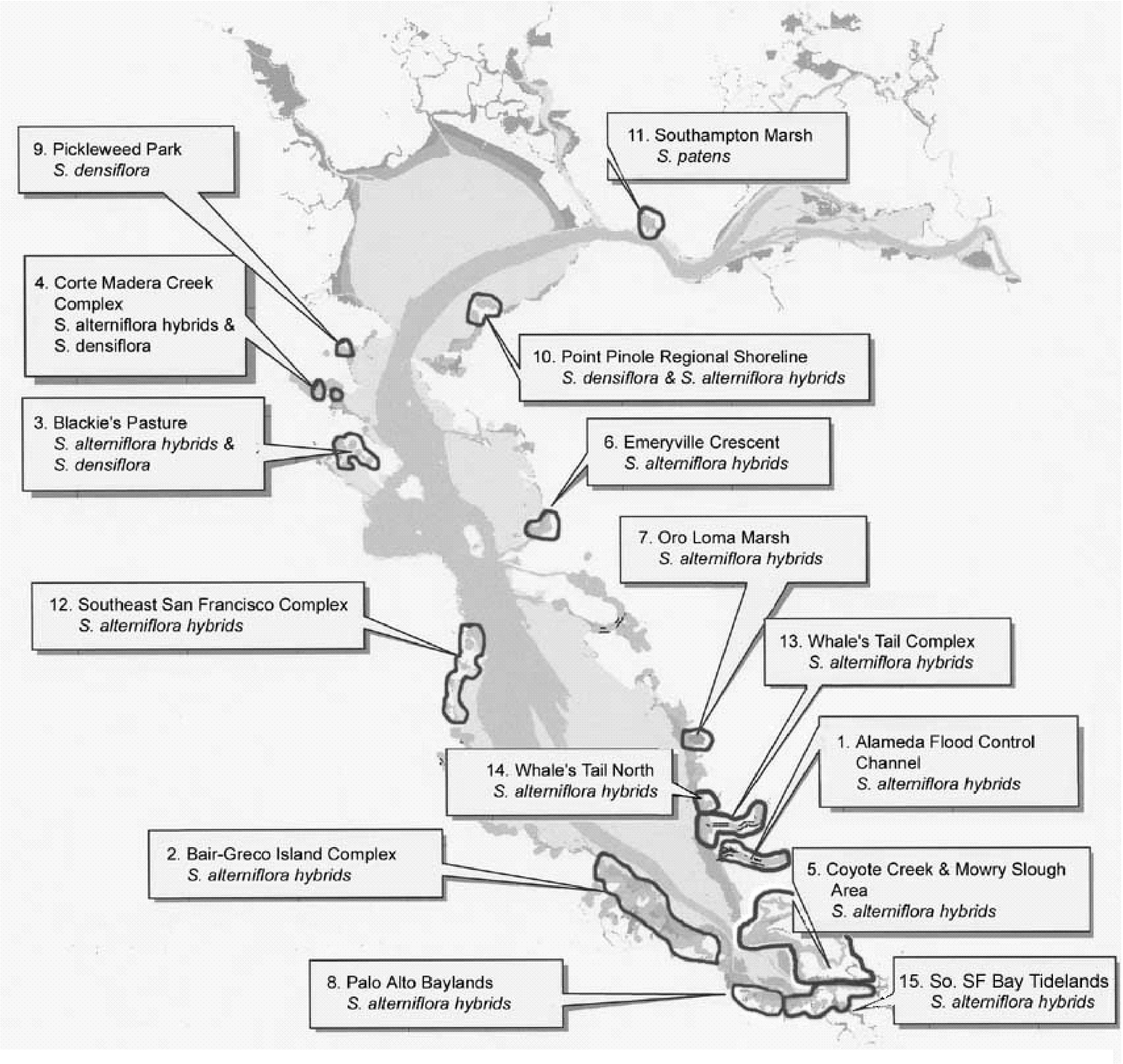

Fig. 1. 2004 Spartina treatment sites in the San Francisco Estuary, CA.

to press the plants into the mud, was tried on a very small scale, but the visual impacts of such a treatment method as well as potential impacts to endangered species made this option unsuitable for large-scale use.

TIMING

Spartina control work in the estuary is limited by more than just the daunting amount of paperwork necessary to initiate treatment. The estuary is a highly dynamic system, and the areas typically infested with non-native *Spartina* bear the brunt of much of this dynamism. Occupying many habitats, including the bayfront edge as well as more sheltered marshlands, non-native *Spartina* infestations are subject to daily tidal inundation, storms, wrack deposits, urban runoff and pollution, and sediment deposition. Treatment methods selected for these areas must account for all of these factors. Since large-scale treatment in 2004 relied heavily on herbicide use, the treatment parameters of glyphosate further dictated the available treatment window in the estuary.

Glyphosate herbicide has specific requirements that must be satisfied in order to achieve high treatment efficacies. For *Spartina* control these are: long dry times (12-24 hours) where the plant is neither inundated at high tide nor rained on, low amounts of silt and salt on the treated plants, and complete coverage of the treated plant ("spray-to-wet"). Where *Spartina* grows in the San Francisco Estuary, these requirements can be difficult to meet. Tidal fluctuations preclude most low and mid-elevation sites from adequate dry time for glyphosate most of the year. Siltation is a constant problem in the highly turbid waters of the estuary, often resulting in much of the herbicide binding to the accumulated silt on the plant's leaves rather than entering the plant's tissues and translocating properly. Finally, complete coverage of the targeted *Spartina* can be difficult to achieve over large stands or in areas difficult to access. Given all of these constraints, very few days are available in a given growing season (late May through mid-October) with the convergence of conditions necessary for efficacious treatment using this herbicide.

The San Francisco Estuary is also the habitat of the Federal and State endangered California clapper rail (*Rallus longirostris obsoletus*), whose breeding season extends from February 1 through August 31 of each year. Many of the marshes where larger populations of clapper rail breed have been invaded by non-native *Spartina*, and represent the majority of the infestation in the estuary. No *Spartina* control work can occur during the clapper rail breeding season in these marshes. Therefore, the larger infestations of non-native *Spartina* may only be treated between September 1 and roughly the end of October every year. During this time, tidal windows of opportunity that allow for the required dry time for glyphosate are limited. Finally, in most sites in the estuary, late-morning or early-afternoon winds develop that exceed spray drift reduction criteria for herbicide application (10 mph sustained winds). As a result, in the 2004 treatment season, *Spartina* control work within clapper rail occupied marsh was restricted to roughly 6-10 days where all necessary conditions were met.

2004 TREATMENT

At the outset of the 2004 treatment season, the SSPs called for treatment of 45 individual sub-areas within the selected 16 sites. The total area targeted was 181 ha (447 ac), which roughly coincided with the initial estimates of population size determined in 2001, just after the ISP's inception and initial inventory mapping of the non-native *Spartina* in the estuary. Figure 1 shows the locations of the various control sites within the estuary.

As of December 2004, 176 ha (435 ac) of targeted non-native *Spartina* had been treated, with roughly 0.8 ha (2 ac) slated for manual control work in January 2005. Herbicide treatment of several sub-areas with large infestations was cancelled as a result of heavy October rains, which prevented vehicle travel on bay-mud levees. Planned use of an amphibious excavator (Aquamog) was also postponed because of rain.

RATE OF SPREAD

In the fall of 2004 the ISP Inventory Monitoring Program produced a Monitoring Report based on selected sites surveyed during 2003 (California Coastal Conservancy 2004(a)). This report analyzed the change in area of non-native *Spartina* at 28 monitoring sites stratified across the estuary by subregion (latitude), site type and marsh type. Based on sample surveys, the report extrapolated an average 244% increase in area between 2001 and 2003 from all non-native *Spartina* species in the estuary. *Spartina alterniflora* hybrids were found to be spreading at the fastest rate, by as much as 317% over that time period. In 2001, an estuary-wide inventory mapped approximately 195 ha (470 acres) of non-native *Spartina,* with hybrids comprising all but five of these hectares. Applying the 317% rate of increase results in an estimate of as much as 793 ha (1960 acres) of *S. alterniflora* hybrids in the estuary in 2003.

Based on these numbers, 2004 treatment efforts resulted in some 20% of the estuary's *Spartina* population receiving treatment during the year, leaving 80% untreated. If the untreated area doubles by the 2005 treatment season, there could be as much as 1,294 ha (3,200 ac) of non-native *Spartina* requiring treatment during that year. This number becomes even larger if efficacy rates (40-80%) of the various treatment methods are factored into the calculation.

OUTLOOK FOR 2005 AND BEYOND

Given the "supra-exponential" expansion of the *Spartina* hybrid swarm in the estuary, the ISP has determined that only an aggressive, comprehensive strategy aimed at treating all of the *Spartina* during the 2005 control season has a realistic chance of eradicating the invader from the estuary. Building upon the partnerships and experience developed during the 2004 treatment season, the ISP will aim to put in place each of the four main components of *Spartina* treatment for each site or sub-area in the estuary. The components, broadly defined are:

- *Partner identification and buy-in.* Identification of the specific agency, landowner or land manager for the estimated 130 sub-areas requiring treatment during 2005 can be difficult given overlapping jurisdictional boundaries, property lines that bisect *Spartina* infestations, and other issues. Much of this work for public property has been accomplished during the 2004 treatment season; however, extensive public outreach will be necessary for the large number of individual private property owners whose properties are infested with non-native *Spartina*.
- *Development of Site-Specific Treatment Plans.* For each sub-area or site a site-specific treatment plan will be produced following the model of the 2004 SSPs, and incorporating elements of the FWS BO and EA. These plans will be developed in close

coordination with the ISP partners responsible for the infested area.

- *Procurement of Funding*. Identifying a funding source for the proposed work on the individual site can take many forms. In addition to grants awarded by the Conservancy, the ISP will seek grants from other sources and coordinate volunteer activities appropriate for each site based on the Site-Specific Plans.
- *Obtain Necessary Permits for Work*. In close coordination with the regulatory agencies whose purview encompasses the areas slated for *Spartina* treatment, the ISP will aid partners in obtaining the necessary permits for work on their site.

In 2005 treatment work will continue, and expand where appropriate, on the sites treated in 2004. As time is of the essence in treating *Spartina*, several enhanced control techniques will be employed to the fullest extent possible to maximize the treatment area in 2005.

Chief among these is the use of the herbicide imazapyr (Habitat®), which is well suited to the challenges of *Spartina* control in an estuarine environment. Application requirements for imazapyr herbicide are not as challenging as those for glyphosate; required dry times are shorter and plants need not be completely covered with the chemical for high efficacy (less chemical used). These qualities allow treatment of larger areas of *Spartina* in a given time period while also providing wider tidal windows for treatment.

The incorporation of imazapyr herbicide into the Control Program also enables greater use of aerial (helicopter) treatments. An estimated 42% of the total area slated for treatment may be suitable for aerial treatment. Some of this area is difficult to access on the ground; for these sites, replacing ground-based treatment with aerial applications will save time and money, and enable personnel to target less demanding sites.

Beyond the 2005 *Spartina* control ceason, the ISP anticipates establishing the initial stages of a land-manager-based *Spartina* monitoring and control program, as a pilot project for eventual dissolution of the ISP. The 2006 and 2007 *Spartina* control seasons will require the ISP to be in full effect coordinating estuary-wide eradication efforts, but later, and once the infestation has been reduced to more manageable levels, individual land managers will gradually need to assume responsibility for keeping low-level remnant infestations of non-native, invasive *Spartina* under control. In this way, the ISP seeks to render itself unnecessary following the 2010 *Spartina* treatment season.

CONCLUSIONS

The infestation of non-native *Spartina* in the San Francisco Estuary is spreading at a supra-exponential rate in the absence of widespread treatment. However, control efforts undertaken during the 2004 treatment season indicate that control and eventual eradication of non-native *Spartina* in the estuary is possible given continued funding, expanded partner involvement, and political will. New tools, including the use of imazapyr herbicide, are expected to greatly assist in achieving this goal.

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A COMPREHENSIVE LOOK AT THE MANAGEMENT OF *SPARTINA* IN WASHINGTON STATE

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Washington State has been fighting the spread of invasive *Spartina* since the early 1990's. Until recently the progress in Puget Sound was slow or, in the case of Willapa Bay, non-existent. However, with appropriate funding, increased community, agency and legislative support, improved tools, and better cooperation between the entities involved, the eradication in Puget Sound is progressing at a rapid pace, and the tide is finally being turned in Willapa Bay.

This paper discusses the challenges that have led to the current success in Washington State from an on-the-ground management prospective. Elements contributing to that success range from choosing the correct control tools to the importance of cultivating community support and cooperation necessary for a successful program, regardless of the size of the infestation that is being treated.

The lessons conveyed in this paper are intended to help others confronting infestations of non-native *Spartina* and other invasive species to make the most of their available resources. Not repeating the same learning processes and avoiding the mistakes that have already been made can save resource managers and field coordinators precious time and money and help to build a successful program to address invasive species problems.

Keywords: Invasive *Spartina, Spartina alterniflora*, *Spartina anglica*, *Spartina patens*, *Spartina densiflora,* Willapa Bay, Puget Sound, Hood Canal, Integrated Pest Management

INTRODUCTION

Spartina species introduced to Washington State have proven to be aggressive noxious weeds that severely disrupt the ecosystems of native saltwater estuaries. They outcompete native vegetation and convert mudflats into monotypic *Spartina* meadows. This is of great concern because estuaries serve as an important rearing area for numerous fish species, important breeding, migration and wintering grounds for many migratory birds, waterfowl and other wildlife and provide a critical economic resource for many communities dependent on commercial fishing, mariculture, shipping and tourism (Patten and Stenvall 2002). By 2002, in large areas of Willapa Bay, the area with the largest cordgrass infestation in the state, *Spartina alterniflora* had reduced the available foraging time for shorebirds by 50% of winter daylight hours because of the reduction in mudflat acreage (Jaques 2002).

In Washington, federal, state and local goverments, tribal entities, non-profit organizations, business interests, local universities and private citizens are working together to combat the invasion. This effort is becoming more successful each year with reduction of invasive *Spartina* statewide over the past two years. This report outlines the different components that have been instrumental in leading the *Spartina* eradication effort towards success, including program support, planning and coordination, adaptive management, and the evolution of the various tools used for eradication efforts.

EXTENT OF INFESTATION

Washington State has one of the largest invasions of non-native *Spartina* species on the entire U.S. west coast (Patten and Stenvall 2002). The invasion is comprised of four species, *Spartina alterniflora, Spartina anglica, Spartina patens* and *Spartina densiflora*, spread throughout four main waterbodies, Willapa Bay, Grays Harbor, Puget Sound and Hood Canal. Willapa Bay is known to contain only *S. alterniflora*; Grays Harbor contains both *S. alterniflora* and *S. densiflora*. Hood Canal is known to contain *S. alterniflora, S. anglica* and *S. patens*, while Puget Sound has infestations of *S. alterniflora, S. anglica* and *S. densiflora.*

The current size of the infestation in Washington is approximately 3,035 solid hectares (ha) (7,500 acres [ac]), affecting more than 7,300 ha (18,000 ac) of the intertidal marine environment (Murphy 2004). In Puget Sound and Hood Canal there are 95 sites with current or historical infestations (Fig. 1). The infestations cover approximately 260 solid ha (645 ac). The infestation in Willapa Bay covers more than 2,800 solid ha (7,000 ac) and encompases almost the entire shoreline of the bay (Fig. 2).

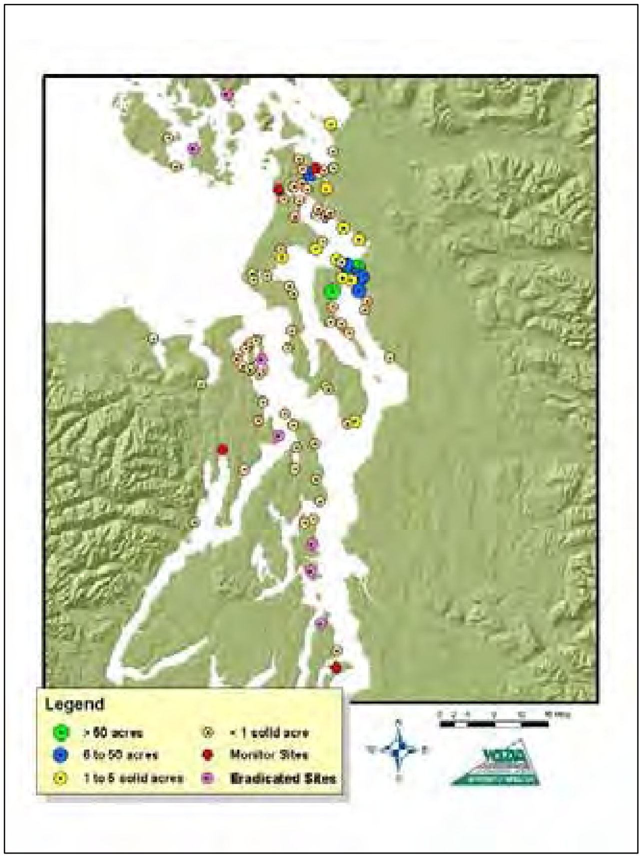

Fig. 1. Map showing *Spartina* invasion sites found within Puget Sound in Washington State, USA.

Fig. 2. Map showing *Spartina* invasion sites found within Willapa Bay in Washington State, USA. Infestation data provided by Washington State Department of Natural Resources.

PROGRAM SUPPORT

Support for the *Spartina* eradication program in Washington is vital for success and can be categorized into three types: community, agency, and legislative.

Community support for the program has come from private landowners, most of whom own property directly affected by *Spartina* or situated adjacent to infestations, but also includes local industries, sportsmen, tribal entities with land impacted by *Spartina* invasions, environmental groups, and local universities. At the outset of the eradication program in the early 1990s the biggest advocates for the program were those who depended on, managed, or owned property in the intertidal environment impacted by *Spartina.* During the early stages of the infestation, community support played a critical role in bringing the problem to the attention of both the goverment agencies who were in a position to acknowledge and act on the problem and local lawmakers who were in a position to mandate and fund appropriate actions.

Support for the program also has come from the various federal, state and local government agencies that must deal with the *Spartina* invasion either as part of their land management responsibilities or through their various legal mandates. These agencies include the U.S. Fish and Wildlife Service, which manages Willapa National Wildlife Refuge in Willapa Bay, as well as the Washington State Departments of Fish and Wildlife, Natural Resources, Ecology and State Parks. County noxious weed control boards are also involved with and support the *Spartina* eradication program as a part of their legislative mandates. Finally, the Washington State Department of Agriculture is mandated by law to lead the statewide eradication effort.

In the beginning stages of the eradication program, the early 1990s, some higher-level individuals within key agencies were reluctant to recognize *Spartina* as a threat. However, after field personnel familiar with the problem educated them on the pertinent issues, and with the passage of state legislation, these agencies became fully supportive of the effort. The most important step in this education process was the continued efforts by the field personnel and biologists working on the *Spartina* problem in educating their professional peers, making them aware of *Spartina's* threat to the larger ecosystem and viability of the estuaries.

Another aspect of agency support is interagency cooperation. The ability of each agency to work closely with each other, share equipment, resources and personnel has also been a very important part of the eradication program's success. The various partners in Washington have signed off on a Memorandum of Understanding (MOU) that helps to foster this cooperative process.

Legislative support has also been extremely important in helping to motivate the governmental agencies to make *Spartina* eradication a priority. Most importantly, strong political support from both local and federal politicians has ensured the program of adequate funding. Such support must be ongoing to ensure that adequate funding remains available.

PLANNING AND COORDINATION

The Washington State Department of Agriculture (WSDA) has been identified by law, RCW 17.26 (Revised Code of Washington), as the lead agency of the *Spartina* eradication program. Lead agency responsibilities include ensuring that the proper permits are in place, producing yearly progress reports for the Washington State Legislature, maintaining eradication program records, monitoring water quality in relation to herbicide applications, providing public educational information and developing management plans in cooperation with the other entities. Having one agency in the state manage these responsibilities has allowed the other entities to prioritize on-the-ground eradication efforts. However, no legal authority was given to WSDA requiring partners to follow specific WSDA plans or directives. This led to beneficial innovation in control methods as various partners tried control tools which they believed aided in eradication and built friendly competition between the agencies. However, the absence of lead-agency authority also sometimes resulted in unilateral decisions and a lack of budgetary transparency.

Spartina eradication efforts in Washington are planned cooperatively. In Puget Sound, the North Puget Sound *Spartina* Eradication Task Force, made up of representatives from local and state agencies, tribal entities, and non-profit organizations meets throughout the year to develop management plans and discuss the successes and failures of different approaches. In Willapa Bay, a technical group made up of field coordinators and biologists develops yearly management plans and brings them before an advisory committee for review and critique. While there has not always been agreement on all the specifics contained in the management plans, there has always been consensus on the overall goal of eradicating *Spartina*. With this common ground, the partners continue to return to the planning table.

ADAPTIVE MANAGEMENT

RCW 17.15.020 directs state agencies in Washington to implement integrated pest management (IPM) practices when carrying out the agency's or institution's duties related to pest control. IPM effectively extends the operating season and allows techniques to be applied when and where they work best (DOI 1996).

Adaptive management is a key element of a successful IPM program. It allows management policies and practices to continually improve by learning from the interim outcomes of the eradication program. http://www.for.gov.bc.ca/hfp/amhome/Admin/index.htm Washington's *Spartina* eradication program contains five adaptive management components: pre-treatment monitoring, implementing eradication practices on the proper scale, post-treatment monitoring and evaluation, adjusting future treatment based on evaluation, and controlling in a consistent manner.

Pre-treatment monitoring sets a baseline for comparison of post-treatment monitoring results. During the spring of 2002, the partners involved in control work developed a comprehensive monitoring plan that included protocols for pre-treatment monitoring. This ensured an accurate baseline of *Spartina* stem counts.

The next component of adaptive management is implementing eradication practices on an appropriate scale. It is important to choose the proper tools to use and set a goal of treating the entire site. For example, using backpack sprayers to completely treat a 200-hectare meadow would not be appropriate, whereas using a helicopter application would be time- and cost-efficient. Post-treatment monitoring helped determine that using the proper tool(s) for retreatment of a large *Spartina* infestation takes a minimum of three years. Ensuring that the entire site receives treatment is often dependent on funding, so matching appropriate funding to the infestation size is essential. Through field experience and research, it has become clear that consistency of treatment is as important as the method chosen for control. Early failures in Washington, in part, resulted from not treating the entire site, leaving viable plants to re-seed and spread back into the treated portion of the site.

Once eradication practices are implemented, post-treatment monitoring is conducted. The monitoring plan developed in 2002 ensured that enough samples were collected to allow for accurate comparison with the pre-treatment sampling. Through this comparison, managers and field coordinators were able to evaluate the effectiveness of the eradication practices employed.

Adjusting the treatments based on the completed evaluation becomes the next step in the process. For instance, crushing *Spartina* was found to be ineffective when the substrate was too firm and, as a result, the site in question was sprayed the next season. With adequate pre- and post-treatment monitoring and evaluation, managers and field coordinators get a good picture of the success or failure

Fig. 3. Field crew member mowing *Spartina anglica* with hand-held brushcutters.

Fig. 4. Field crew member treating *Spartina patens* with backpack herbicide sprayer.

of a treatment method. This allows for the adjustment of treatment methods for better control.

The most imporant component of adaptive management in *Spartina* eradication is consistent control from year to year. Research has shown that invasive *Spartina* is highly resilient and requires multiple years of consistent treatment to cause substantial declines. In some instances inconsistent control, specifically, when two treatment seasons were followed by a year without treatment, resulted in a 100% to 500% increase in cover and number of tillers (Reeder and Hacker 2004). This underscores the importance of consistently treating a chosen site year after year to achieve eventual reduction and eradication.

EVOLUTION OF TOOLS

Over the more than ten years of invasive *Spartina* eradication efforts in Washington State, the treatment tools have evolved and become more effective. In the beginning, crews treated infestations of all sizes with small hand held brushcutters (Fig. 3) and backpack herbicide sprayers (Fig. 4). While these tools are effective on small infestations and are still used to this day, they are simply too slow for effectively treating anything greater than a few hectares. The eradication program in Washington now employs helicopters and large, tracked amphibious machines to treat large meadows, and airboats with high pressure herbicide spray sytems to treat scattered clones and re-growth. The helicopters, applying herbicides at low carrier volumes, 97l liters per hectare (l/ha) (10 gallons per acre [10 gpa]), can treat more than 160 ha (400 ac) per day. While slower than the helicopters, the tracked machines with broadcast application systems are still able to treat upwards of 16 ha (40 ac) per day. Airboats may only be able to treat 3.2 ha (8 ac) per day at most, but they are able to manuever in the estuaries and on mudflats, allowing for rapid transport between scattered infestations and produce virtually no environmental footprint.

Having the ability to treat large infestations quickly and effectively has increased the success of the eradication program in Washington .

CONCLUSION

Over the past two years, the eradication program in Washington State has become more efficient and effective. In 2003, a total of 2,700 solid ha (6,695 ac) out of approximately 3,400 solid ha (8,500 ac) were treated (Murphy 2003). These treatments resulted in the first overall reduction in the statewide infestation. In 2004, a total of 2,500 solid ha (6,200 ac) out of an estimated 3,000 solid ha (7,500 ac) were treated statewide (Murphy 2004). Managers are confident that the 2004 treatments will also result in a

Fig. 5. Helicopter herbicide application, Willapa Bay, Washington, USA

Fig. 6. Tracked amphibious machine with ground broadcast herbicide application equipment, Willapa Bay, Washington, USA.

Fig. 7. Airboat with high-pressure spray equipment, Willapa Bay, Washington, USA.

continued overall decline of Washington's *Spartina* infestation.

Many things have contributed to the current success of the program. Consistent control made possible with adequate funding led to effective treatment. Washington's management plans called for a multi-year commitment to treatment sites, controlling a site until eradication has been achieved. Permitting consolidation also contributed to the program's success. WSDA is granted an National Pollutant Discharge Elimination System (NPDES) permit that allows for the chemical treatment of *Spartina*. As the permit holder, WSDA extends coverage to other entities to treat *Spartina* infestations, while WSDA retains the responsibily for monitoring and reporting. This allows the other entities to focus their resources towards on-the-ground control, rather than on manuevering through the bureaucratic permitting process.

Finally, through a long and painful learning curve, managers and field coordinators have discovered what works best to control *Spartina* infestations within a complicated, and sometimes controversial, environmental, social and political context. Garnering public support, having legislation in place to provide incentive and funding, working collaboratively with other agencies, having access to a wide breadth of tools, and knowing where and how to best use those tools have all contributed to the current success of the program. While there is still much progress to be made, the partners involved are confident that their eradication goal will be achieved. The effort in Washington will continue searching for better tools and equipment to increase efficacy and efficiency, while minimizing costs.

2010 UPDATE

At the time of this article's publication, the cooperative *Spartina* eradication effort in Washington State has continued to yield great success in effectively reducing the infestation statewide. During 2009 the partners involved in the eradication effort treated approximately 110 solid acres of *Spartina*. WSDA now estimates that, as a result of the 2009 effort, the infestation statewide has been reduced to only 40 solid acres (Phillips pers. com. 2010). This represents a 99.5% overall reduction from the historic high of 9,260 solid acres estimated in 2003.

This continued success provides evidence that the management effort, originally detailed in this article in 2004, was and continues to be an effective approach. Since the 2004 International Invasive *Spartina* Conference, the effort in Washington has continued to seek improvements in the tools and approaches used to eradicate *Spartina*. Imazapyr, first aerially applied to 2000 solid acres during the 2004 season (Murphy 2005), proved to be a huge success and a big reason for the success of the overall Washington State effort. Another obvious reason for this success was the continued program support and funding.

As major reductions were made in the overall size of the infestation, the remaining *Spartina* was scattered throughout a large area. This required the partners to modify the on-the-ground approach. The effort went from focusing on large-scale aerial and ground broadcast applications, to small-scale, targeted treatments of scattered infestations. This highlights the need to constantly re-evaluate the program to ensure that the most effective and efficient tools and approaches are used to continue the success of the cooperative *Spartina* eradication effort.

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WHY DID IT TAKE SO LONG TO TURN THINGS AROUND IN WILLAPA BAY? THE HUMAN SIDE OF THE *SPARTINA* INVASION

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Oyster growers first noticed clones of exotic cordgrass growing in Willapa Bay in the 1950s and brought their concerns about the alien grass to the attention of the staff of the Willapa Refuge. At that time, the infestation involved no more than a handful of clones. *Spartina* now infests over 15,000 acres of mudflats in Willapa Bay, seriously degrading one of the most ecologically productive and otherwise healthy estuarine areas along the entire Pacific Coast. The human story that parallels the expansion of *Spartina* reveals more than just the inevitable physical, logistical, and biological challenges of responding to an aggressive invasive species. Many of our environmental organizations were unprepared for this problem and reacted in ways that made the problem far worse. Our government structures were also not well designed to meet the challenges of a highly damaging but very natural process. This paper will present the views of one person who has been both an observer from the sidelines and an involved participant in the Willapa Bay *Spartina* program for the past 13 years. Since 1995, Miranda Wecker has served as leader of the marine research program of the University of Washington's Olympic Natural Resources Center. In that capacity, she supervised research on the feasibility of biological control of *Spartina* as well as spatial analysis of the weed's spread. She also collaborated with local leaders in the development and implementation of a long-term strategy for eradication based on the comparison of alternative control options using geographic information system (GIS) tools. It is important to acknowledge errors that have been made and draw lessons from them in order to assure that the same mistakes are not repeated when we face the next invasion.

Keywords: management lessons, long-term strategy

INTRODUCTION

My task is to summarize the key lessons that I have learned during the more than 15 years that we have been fighting the spread of *Spartina* in Willapa Bay. My aim is not to insult or embarrass anyone. My hope is that an honest appraisal of our problems will allow us to make improvements. I also hope our counterparts in San Francisco Bay will find it helpful to hear my opinions about the lessons we have learned.

What is at stake, it seems to me, is something much more than winning the battle against *Spartina*. I believe that the mismanagement of the *Spartina* control effort in Willapa Bay has damaged local attitudes towards government and its abilities to solve societal problems. During the past ten years, Willapa residents have watched *Spartina* continue to spread at an alarming rate despite the expenditure of millions of dollars of public funds. The ongoing failure to beat the *Spartina* invasion has added another chapter to a long history of rural skepticism towards government programs. Poor performance creates a double bind. If government agencies perform poorly, vocal criticism puts funding at risk. Without such criticism, poor performance is allowed to continue unquestioned. Without reform, the program would exhaust the public will and funds available to deal with the problem and fail anyway.

In the following sections, I will present my thoughts regarding what we have learned in fighting *Spartina* in Willapa Bay. These comments are intended to be subjective personal conclusions based my experiences in the human dimensions of the *Spartina* battle. For the battle was not just about deploying the physical resources to kill 20,000 acres of an invasive weed. Success required assembly of logistical, legal, financial, and political resources. Success depended on organizing an array of people and institutions to act in a concerted and effective manner.

LESSON 1: NEVER TOO MANY MAPS, BUT THEY CAN BE TOO EXPENSIVE

A biological invasion is an inherently spatial problem. Before you can formulate a sensible management plan, you need a good idea of the scale of the problem. You need to know where the enemy plants are. If your aim is to assist and facilitate management, you have to be concerned about the costs and the time delays associated with the various mapping options. Rarely does a weed control program have surplus funding. To see that most of the money goes toward eradication activities in the field, you must avoid overly expensive and labor intensive methods of mapping. GIS analytic services are often very expensive. Most agencies have geographic information system (GIS) expertise, but they are assigned to the highest priority projects for the agency. The *Spartina* infestation was not considered to be sufficiently important to justify dedication of internal GIS services by any of Washington's state agencies. Fortunately, precision is not critical. You really don't need maps and analysis worthy of scientific publications. For that reason, I believe that the use of standard-accuracy Global Positioning System (GPS) technology is an acceptable way to map for management purposes. We found that a basic survey of Willapa Bay could be done in seven-day period by delineating the outer edge of the infestation using a GPS unit. GPS surveys are inherently ground-truthed and geo-referenced. The data from a GPS survey can be easily exported and displayed as a GIS layer. Our total cost was less than $2,000. Infrared aerial photography and its interpretation, by comparison, was so expensive that agencies could only afford to have it done every third year. Image processing and interpretation required years of work. Katy Zaremba presented information earlier in this *Spartina* conference indicating that aerial photography misses a great many plants. While aerial photography may have academic value, I believe that we have learned that it is not the optimal way to serve day-to-day management needs.

In terms of quantity, however, I would say that it would be difficult to have too many maps. During the first five years of the Willapa *Spartina* program, we had too few. It is best to have surveys done at the beginning as well as at the end of each season. I would recommend that you have GIS and GPS services at your beck and call. You'll need maps to respond quickly to new challenges as they arise.

For example, in 2002, Dr. Kim Patten of Washington State University concluded that herbicide applications would only deliver optimal kill rates if a minimum of 12 hours of dry time followed treatment. Our challenge was to determine whether *Spartina* in the lowest elevations of Willapa Bay were exposed for at least 12 hours during any of the tides expected in the upcoming season. Using the new LiDAR bathymetry layer generated through the University of California at Davis and the NOAA Coastal Services Center project, Teresa Alcock and Keven Bennett of the University of Washington's Olympic Natural Resources Center (ONRC) formulated a method for presenting spatially-explicit tidal predictions. Another example of a spatial information challenge was the identification of owners of infested parcels. In many areas of Willapa Bay, the pattern of ownership is extremely complex with hundreds of plots shaped like jigsaw pieces. Before treatment could take place, landowners had to be contacted and permission forms had to be completed. ONRC staff generated maps with ownerships and *Spartina* coverage displayed.

In addition to its use as a monitoring and planning tool, GIS also played an important role in communications and public relations. An animated movie clip we generated graphically displayed how the *Spartina* infestation would expand throughout Willapa Bay. This short movie proved to be one of the most effective educational tools we created to communicate the risks. In addition, GIS played an invaluable role in generating concrete images to help facilitate discussion of alternative strategies. Because we had a GIS staff at the ready, we were able to rapidly react to the emerging information needs as they arose.

LESSON 2: TEACH THE PUBLIC WELL

In Willapa Bay, it was obvious from the beginning that broad-based public support for the control program was necessary. Roughly half the infestation occurred on private lands. The full costs of control far exceeded the local ability to pay, so help from the state and federal government was needed. The only practical option was a concerted and highly visible effort by public agencies. So it was obvious that the *Spartina* control program could not succeed without the consent, understanding and authentic support of the citizenry. Controversy would also have complicated the ability of lawmakers to secure the millions of dollars of funding needed to sustain the eradication effort to its conclusion. The public relations challenge was successfully met by focussing time and energy on educating and involving local residents; this kept the *Spartina* issue at the top of the priorities list. We also provided numerous opportunities for citizens to track control program progress and comment on its evolution. Because for years the S*partina* control effort was not making progress, many of our public meetings provided the opportunity for expressions of harsh criticism. In response, agency personnel became excessively defensive.

To overcome the climate of pessimism that surrounded the *Spartina* issue, we resorted to some rather unconventional approaches to public education. We understood that the standard environmental jargon and tactics that work well in receptive urban populations would only trigger sarcasm and hostile reactions in our area. One new way in which we delivered detailed information to audiences was through educational placemats. We designed placemats presenting key information along with beautiful images and distributed them free of charge to local coffee shops around Pacific County. Customers in restaurants proved to be very willing to read whatever was available as they waited for their orders. Copies of the placemats were also carried away and shared with others. Another successful device was our series of "science for the people" meetings in which research scientists presented progress updates on their work to the public. One of the more entertaining of those evenings occurred when Dr. Donald Strong of the University of California at Davis engaged in a lively debate over the feasibility of biological control with a senior official of the Washington State Department of Agriculture. Citizens learned about the uncertainties common to research and the ways in which enterprising scientists respond to them. They were able to draw their own conclusions after hearing firsthand the various sides of a debate.

LESSON 3: TIME IS OF THE ESSENCE

When the Willapa Bay *Spartina* invasion became evident as a serious threat, the outside world was not ready to see it as such. In the late 1980s, local civic leaders became convinced that the S*partina* invasion was fast becoming an ecological disaster. Unfortunately, the majority of environmental professionals across the country had not yet recognized the extent of the threat posed by invasive species. Within the ranks of the many public agencies with jurisdiction, professional staff held varying views. Conflicts over the acceptability of the impacts tied to control treatments made a prompt response to the spread of S*partina* impossible.

Responses were delayed because of the availability of legal mechanisms for individuals who held contrarian views to block agency action despite support for the program by the vast majority of the public. In Willapa Bay, such challenges deflected energies and added to the costs of the management effort. Contrarian activists made repeated efforts to derail the *Spartina* control program in the name of environmental concern. Their “monkeywrenching” tactics were ultimately defeated by the active participation of the Washington Chapter of the Nature Conservancy and Audubon Society Washington. It is vital to enlist the support and involvement of credible professional environmental organizations in order to prevent others from framing the issue in their own terms. During that early period of delay, an aggressive control program would have solved the problem *with the least costs and the least ecological impacts*. Instead, the invasion was allowed to accelerate.

LESSON 4: SOMEBODY HAS TO BE IN CHARGE

In Willapa Bay, three state agencies and one federal agency are directly engaged in field operations. They are independent of one another, have separate lands, and pursue different missions. In 1995, the Washington Legislature enacted RCW 17.26—a statute calling for coordination of *Spartina* control efforts and strong leadership under a single lead agency. The Legislature designated the Washington State Department of Agriculture (WSDA) as the lead agency, but also directed that each agency take responsibility for *Spartina* control on their own lands. WSDA has interpreted its lead role as comprised of two major functions: it convenes interagency meetings and drafts the annual report to the Legislature. WSDA declined to interpret its assignment expansively, in other words to include all mission critical leadership functions.

Without a single agency in charge, it was impossible for the agencies to develop and implement a comprehensive and coherent long-term strategy with an appropriate allocation of assignments and responsibilities. Without a single agency in charge, individual agencies were free to pursue their individual near-term interests rather than subordinate them to the interests of the overall program. No one agency was in a position to demand of others a timetable for success or to assess the level of funding needed for overall success. No one agency was held accountable for the success or failure of the program. No one agency was motivated to demand adherence to an overall vision and coordinating scheme. As a result, the control effort in Willapa Bay drifted down a path that was unnecessarily expensive and slow. Each agency was free to point to the inadequacies of the program without responsibility for the whole. From presentations during this conference, it is obvious that the California Coastal Conservancy’s Invasive *Spartina* Project has very competent, hard-working staff. They have generated a number of impressive documents that will go a long way toward gaining cooperation through persuasion. Our experience tells us that coordination will require more than persuasion. Legal and coercive authority will probably be needed.

LESSON 5: SELL THE NEED FOR SCIENCE

Looking back, I now think that one of our biggest errors was that we did not argue strenuously enough for a serious monitoring and research effort in the early years. We never persuaded our political leadership of the importance of monitoring and research designed to inform the management effort. From the outset, we should have more adequately countered the strong antipathy towards spending dedicated funds on research. Public hostility towards research was understandable: substantial sums had been spent exploring the non-target impacts of control tools that had been approved for years and used without problems in a wide variety of settings. Some of the scientists involved in early research were seen as biased. They were more concerned with the short-term and minor effects of control tools than with the long-term ecological devastation wrought by the invasion itself. In at least one case, a scientist was politically affiliated with groups opposed to the use of herbicides. This biased him to propose a research plan designed to ignore the longterm implications of the S*partina* invasion while highlighting the very transient benefits of S*partina* in creating edge habitat. Other scientists participating in the project objected to the inherent bias and forced a redesign of the workplan. In the end, because the results were not to the liking of the politically motivated scientist, he refused to publish the results. This intentional blurring of science and politics undermined trust in good science. It also led the political leadership to restrict the use of funds. Agencies were forbidden from using funds for research, even studies that would directly improve the efficiency and effectiveness of the control program. Rather than officially-sanctioned studies, evaluations of the costs and benefits of control techniques were done using grant funds cobbled together. Control technique trials were not conducted to provide reliable information on the efficacy of the tools. Alternative strategies could not be compared. Good consistent records were not kept. No agency could compare their work with others. The control program was conducted year after year without a good understanding of how well the tools and the application strategies actually worked.

The lack of objective quantitative information harmed the personal relations among both the allied agencies and local stakeholders as well. More and more frequently, interagency meetings provided the occasion for major squabbles over whether particular treatment techniques were sufficiently effective to continue their use. Without definitive studies, the subjectivity inherent in our arguments could not be overcome. The tension and distrust generated by these irresolvable debates eventually led factions to stop talking.

If I had it to do over, I would have spent much more time selling our politicians and the public on the need for good rigorous science and monitoring. We should have tried to learn how to kill *Spartina* effectively and affordably at the very start. A rigorous comparison of the tools should have been conducted to determine the conditions for maximum efficiency of each. We should also have gotten an earlier start on the feasibility of biological control, and started looking for alternative chemicals soon after hearing from our friends in Australia and New Zealand that glyphosate does not work well on *Spartina*.

LESSON 6: THINK AHEAD AND SHARE YOUR VISION

My experience has convinced me that long-term planning is very necessary despite the discomfort and humility you may feel in the face of many seemingly critical unknowns. By taking a decent stab at planning, you communicate that you are serious about solving the problem in a logical and efficient manner. If all the facts are not available, assumptions will have to do for the time being. The concept of adaptive management is nothing more than the explicit promise to update your plan as you learn from experience. You are likely to attract the money you need only if you have a compelling and well-defined plan of action. You are also likely to draw important allies if your vision is clear and convincing. If you are asking citizens to accept the use of chemical applications as a major part of the program, you had better have an endpoint in mind and be prepared to explain why it will take you that long to get there. You need a flexible timetable. You need graphic images showing how you can succeed with the resources you have at hand. Then you need to take your vision for success on the road and sell it to the key stakeholders.

LESSON 7: CONSOLIDATED SYSTEMATIC PROGRESS

Over the years, it became more and more apparent that we would make no progress unless we could eliminate large contiguous blocks of *Spartina* in a systematic fashion. Rather than dispersing the control effort here and there around the Bay, we needed to consolidate our efforts. To limit re-infestation from nearby untreated areas, we adopted a strategy of working in consolidated blocks. Theoretical weed management models advise that all outliers should be treated first in order to minimize the overall long-term costs. Logistical realities in Willapa Bay make the "outliers first" strategy untenable. Few tides are sufficiently low to allow time for effective treatment of all outliers before other parts of the infestation are tackled. Most of the outliers were also on private lands, while an agency's first priorities are their own lands. Chemical applications of glyphosate required dry times rarely available where the outliers occur. The time required and costs of moving heavy equipment dictate that crews limit hopping, skipping, and backtracking as much as possible. It is also far easier to keep track of where you have left off if the progression of treatments is simple and methodical. Based on these practical considerations, our crews have adopted a strategy of sweeping *Spartina* from the eastern side of the Bay first and then finishing up on the west side. The federal crews are moving from south to north, while the state crews in general are operating from north to south.

For many years, most parties were driven by narrow self-interest. Under those conditions, it was impossible to create a coherent and effective strategy. The lack of any—even a flawed—long-term vision allowed agencies to pursue their own near-term interests without political consequences. No one had the authority or permission to impose the discipline of working together to treat some lands first, others later, and some at the very end. Without such a multi-year commitment, no one had confidence the program would last long enough to serve their own interests. The brevity of budget periods constrained long-term thinking. With federal budgets set on an annual basis and state budgets set every two years, there were no absolute guarantees that the funding would be maintained to the very end. Still, with a well thought-out plan and the support of all parties, you can draw together enough political will to have a good shot at sustaining the program over the required number of years.

LESSON 8: LOSE THE THIN SKIN

I have to conclude with some cautionary words about the emotional toll that comes with difficult challenges like controlling a widespread invasive weed. Weed eradication work in the field is tough and dirty business. People get hurt, equipment breaks down, and tempers fray. In Willapa Bay, the social and political dynamics among agency staff and stakeholders were as challenging as the physical tests. Natural resource professionals should remember to keep perspective and understand why the affected stakeholders get very angry at times. In the Willapa Bay area, many people rely on the resources of the Bay for their livelihood. Their jobs and lifestyle are in jeopardy. Even if not economically tied to the Bay, many others feel very deep affection for their surroundings. Large amounts of public money were being expended, yet, year after year, S*partina* spread and no progress was evident. It is no wonder that meetings often became contentious. In my view, agency staff often reacted as if the criticisms of the program were personal affronts. Rather than acknowledging the reasons for frustration and the importance of re-evaluation in light of the slow progress, they decided to reduce their exposure to local stakeholders. The heightened sensitivity to criticism displayed by state agency staff involved in the Willapa S*partina* program became an obstacle that prevented serious evaluation and program improvement. If the agencies had taken an attitude that welcomed criticism as a means to seek program improvements, the agencies would have gone a long way to enlist public support and confidence. Because many of the agency staff asked to manage this complicated program were relatively young and inexperienced, it is understandable that their public relations skills were inadequate. Less understandable was the disinterest displayed by their supervisors in requiring conduct that would improve relations with local constituents. It appeared that the distrust of government decision-making common among citizens was met by its mirror image within agencies: a distaste felt by agency employees for public involvement in decision-making.

For better or worse, natural resource issues do belong to everyone. Professionals who choose this line of work should accept that the people most affected by the success or failure of their work will want to have a voice in decision-making. An attitude of intolerance towards criticism will only invite more criticism. When the results visible on the ground are extremely unsatisfactory, little else counts for much. Soul searching and healthy criticism are helpful after all. The mission to succeed should outweigh all petty resentments. In the end, there is no choice but to work together.

DISCOVERY AND MANAGEMENT OF *SPARTINA ANGLICA* IN THE FRASER RIVER ESTUARY, BRITISH COLUMBIA, CANADA

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Spartina anglica (English cordgrass) was discovered in August 2003, during intercauseway marsh surveys of Roberts Bank for a proposed Vancouver Port Authority (VPA) container terminal expansion. This was a new species discovery for the estuary and the province of British Columbia, and a control program was immediately initiated by VPA. The control program involved collecting preliminary data on the *S. anglica* infestation in September (measuring stalk density and height, conducting GPS surveys, GIS mapping) followed by manual removal in October. *Spartina* clones, measuring 3–4.5 meter (m) diameter, had an average stalk density of approximately 800 stalks per square meter (m^2) and average height of 1 m, but most of the growth was in the form of seedlings and small tussocks <0.3 m diameter. Manual removal included digging up plants, washing to remove excess sediment, bagging, and disposal by incineration at the Greater Vancouver Regional District (GVRD) incinerator. A subsequent survey of the outer Fraser River estuary conducted in November using the Canadian Coast Guard hovercraft located a 4-kilometer (km) patchy infestation in Boundary Bay. In 2004, a multi-agency committee was assembled, including Fisheries and Oceans Canada, Canadian Wildlife Service, Ducks Unlimited Canada (DUC), VPA, Ministry of Water, Land and Air Protection, Corporation of Delta, GVRD, and several NGO's to conduct a removal effort. Manual and mechanical methods, including using a specially equipped excavator for deep *in situ* burial, were used to remove *S. anglica*. The program also included an outreach and education component targetting naturalist groups and government agencies. Program costs were about $15,000 for 2003 and $50,000 for 2004, with substantial in-kind contributions from many agencies and volunteers. The work to date has removed all existing *S. anglica* from Roberts Bank and substantially reduced it in Boundary Bay.

Keywords: *Spartina anglica*, English cordgrass, invasive species, Fraser River estuary, Roberts Bank, Boundary Bay, mudflat, salt marsh

INTRODUCTION

On August 21, 2003, G. Williams discovered *Spartina anglica* (English cordgrass) on the mudflats at Roberts Bank in the Fraser River estuary while conducting intertidal salt marsh surveys for the Vancouver Port Authority (VPA) Deltaport Expansion Project. VPA was immediatey notified and a management program was initiated to assess and control the *Spartina* infestation. The main focus of the program was to contain the spread of *S. anglica* using manual and mechanical methods rather than using chemical herbicides.

The Fraser River estuary, the largest estuary on the Pacific coast of Canada, is situated in the southwest corner of the province of British Columbia, between the City of Vancouver and the international border separating Canada and the United States of America (Fig. 1). The delta includes three large tidal areas: Sturgeon Banks, north of the Fraser River Main Arm, comprising 5,150 hectares (ha) (12,700 acres[ac]), Roberts Bank, 5,500 ha (13,590 ac), and Boundary Bay, 11,000 ha (27,180 ac) to the south.

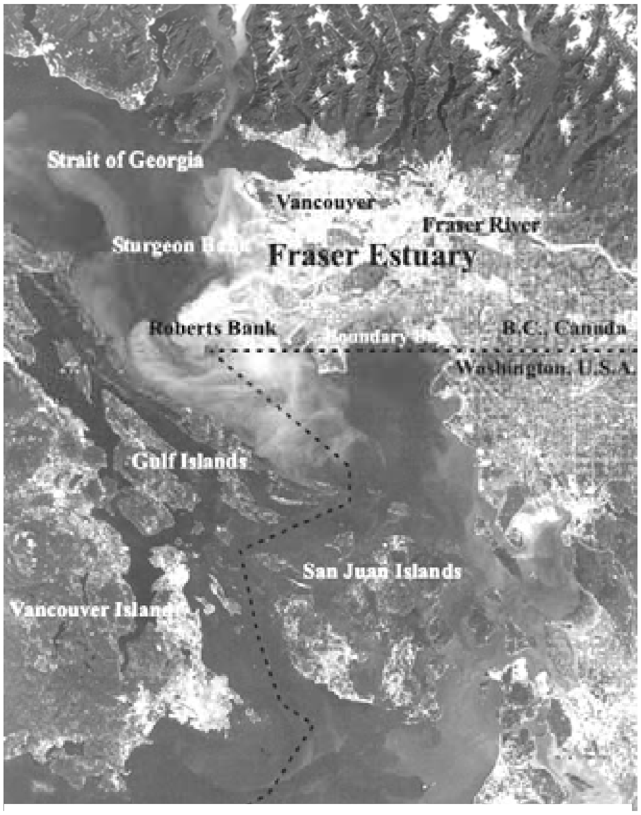

Fig.1. Location of Fraser River estuary.

The large tidal flats provide vast mudflat, eelgrass, and brackish and salt marsh habitats for birds, fish and wildlife (Adams and Williams 2004; Harrison and Dunn 2004; Schaefer 2004). Over one million migratory birds use the outer delta for critical feeding and staging areas annually (Butler and Campbell 1987; Important Bird Area (IBA) Site Summary, BC017, www.ibscanada.com; Pacific Estuary Conservation Program, www.ramsar.org). Numerous fish and invertebrate species utilize the flats, including Pacific salmon and Dungeness crab that support important commercial, recreational, and First Nations fisheries (Hoos and Packmann 1974; Levings 2004). Much of the area is protected within provincial Wildlife Management Areas, is recognized as a nationally Important Bird Area, and exceeds the criteria for Ramsar and Western Hemisphere Shorebird Network sites (Important Bird Area, IBA, Site Summary, BC017, www.ibacanada.com). The 586-hectare Alaskan National Wildlife Area on Westham Island, adjacent to Roberts Bank, is a designated Ramsar site.

Fraser River estuary is also a hub for domestic and international shipping with three port authorities: VPA's container and bulk terminals at Roberts Bank, Fraser Port's coastal and deep sea terminals on the Main Arm, and Port North Fraser's coastal shipping and log booming facilities on the North Arm. The concentrated port activity includes a constant movement of barges and ships through the estuary.

S. anglica is of particular concern in the Fraser estuary because of the known ecological impacts, including conversion of mudflats to monoculture *Spartina* marshes (Gray et al. 1991; Huckle et al. 2000), displacement of waterbirds (Goss-Cutard and Moser 1988; Nairn 1986), fish (Sullivan 2001 cited in Anon. 2002; Dethier and Hacker 2004), and invertebrates (Hedge and Kriwoken 2000; Jackson et al. 1985), accretion of sediment (Ranwell 1964; Gray 1991; Dethier and Hacker 2004), disruption of tidal drainage patterns (Gray et al. 1991), and impacts on native intertidal marsh, eelgrass beds, and unvegetated habitats (Hedge and Kriwoken 2000; Hacker et al. 2001). Experience in Washington State and elsewhere has shown that a quick response is required to avoid costly *Spartina* eradication programs. For example, $1.5 million was spent in 2003 to treat 6,000 ac of solid *Spartina* (70% of the infestation) in Willapa Bay and 694 solid ac (90% of the infestation) in Puget Sound (Murphy 2003). This paper summarizes the work undertaken in 2003 and 2004 in Roberts Bank and Boundary Bay and provides suggestions for further work to effectively control *S. anglica* in the Fraser River estuary.

METHODS AND PROGRAM PLANNING

2003 VPA Spartina Control Program for Roberts Bank

Following discovery of *S. anglica* in Roberts Bank in August, GL Williams & Associates Ltd. was retained by VPA to establish a control program. Since the infestation was limited in coverage and total area, the objective was to manually remove the plants as quickly as possible without the use of herbicide. The VPA control program consisted of confirming identification of the Roberts Bank *Spartina*, researching scientific literature to guide management options, conducting field GPS/GIS mapping and data collection, and undertaking plant removal.

Plant Identification

Plant specimens were collected, digital images obtained, and a web search conducted for information about *S. anglica*. The field identification manual prepared by the California Coastal Conservancy (www.spartina.org) was especially useful for preliminary identification and providing descriptions of the five *Spartina* species found along the Pacific Coast. Several wetland specialists in British Columbia and Washington State were contacted for appropriate keys and information. The indentification of the Roberts Bank specimens were confirmed using the *Spartina* key developed by Barkworth (2003). Specimens were also submitted to the Univeristy of British Columbia herbarium.

Fresh *S. anglica* specimens from Roberts Bank and Boundary Bay were shipped to Dr. Debra Ayres, University of California at Davis, for DNA analysis (Ayres and Strong

2001). DNA analysis was undertaken to confirm the identification and determine if the origin of the *S. anglica* could be established. Finally, specimens from B.C. were compared with samples of *S. anglica* and *S. alterniflora* from Padilla Bay during a meeting with S. Riggs at the Padilla Bay Estuarine Research Reserve in Mount Vernon, Washington.

Field Surveys and Mapping

In late September, *S. anglica* plants were located using a handheld Garmin XL GPS, and a GIS map was produced using Fraser River estuary orthophotos. To prevent the release of seeds, above-ground leaves and all inflorescences were cut and collected in plastic bags for disposal. Density and height were measured for a small subsample (n=4) within the largest clone.

Spartina Removal

On October 24, 2003, the first Fraser *Spartina* Busters were assembled on Roberts Bank The group was comprised of 21 volunteer representatives from VPA, federal, and provincial agencies, Ducks Unlimited Canada (DUC), Langely Environmental Partners Society, and Tsawwassen First Nations (TFN). The removal was conducted over one low-tidal cycle and involved digging up plants with shovels, washing the roots and rhizomes in pools to remove sediment, placing the washed plants in industrial-strength plastic bags, and collecting them in a shore-based disposal bin for incineration at the GVRD incinerator. All above-ground material was removed except for three clones measuring 3 m or greater in diameter.

Hovercraft Survey of the Outer Fraser Estuary

The Canadian Coast Guard hovercraft, operated by Fisheries and Oceans Canada (FOC), was used to survey the mudflat-marsh interface on Sturgeon Bank, Roberts Bank and Boundary Bay during low tide on November 19. Observers included the author (GW) and biologists from FOC and VPA. Environment Canada, GVRD, and FOC provided funding for the survey. GPS locations of *S. anglica* were recorded and the distribution mapped.

Outreach and Education

To increase the awareness of the ecological impacts of *S. anglica* to the Fraser River estuary, a PowerPoint presentation was shown to several local naturalist groups. Articles were published in the Vancouver Natural History Society newsletter (Williams 2004) and Botanical Electronic News (Williams 2004a). Interviews were also conducted and articles published in local newspapers. Canadian Broadcasting Corporation (CBC) radio ran a short feature on *S. anglica* during a newscast. VPA is also considering establishing an environmental stewardship program with TFN that would include the monitoring and removal of *Spartina* in Roberts Bank.

2004 Spartina anglica Control Program

Coordination of Management

Following the confirmation that *S. anglica* was not restricted to Roberts Bank, the invasive species coordinator for FOC, Pat Lim, was contacted and a committee established to deal with the *S. anglica* threat. On December 4, 2003, representatives of the agenices involved in the Roberts Bank control program met with invited *Spartina* eradication specialists from Washington State to develop an action plan for the control and removal of the *S. anglica* from Boundary Bay and Roberts Bank. Washington State representatives offered to assist in the control and removal program.

The action plan, developed over several meetings, consisted of using volunteers to manually dig out plants during low tides in June, with a follow-up removal in the fall. A preliminary budget was determined to cover hiring a volunteer coordinator, producing educational materials, and supporting a field operation. Matching funds were negotiated with partner agencies and organizations, and considerable in-kind support was obtained.

Mapping S. anglica Distribution

On May 31 and June 4, 2004, FOC mapped the eastern area of Boundary Bay between 104th and 112th Streets in Delta using handheld GPS units (Garmin eTrex Vista and GPS 76 Marine Navigator) with an accuracy of ± 5 m.. *Spartina* was classified according to one of four classes: seedling, clone <0.3 m diameter, >0.3 m to <1 m diameter, and >1 m diameter. A GIS map was produced using a recent orthophoto base map. The map was useful in indicating the amount of *Spartina* present and the allotment of resources for removal.

New growth of *S. anglica* was mapped in Roberts Bank in June and October using a Garmin 60 C handheld unit and a GIS map produced by DUC. On November 24, a hovercraft survey was conducted in Boundary Bay to map new and existing *S. anglica*. The GPS locations were added to the GIS map produced by DUC.

A survey of selected sites in Burrard Inlet (e.g. Maplewood, Port Moody mudflats, and Spanish Banks) was conducted at low tide on November 21, 2004. No *S. anglica* was observed at the Burrard Inlet sites.

Outreach and Education

On June 5, World Oceans Day was organized at Blackie Spit Park and a *S. anglica* poster and materials were put on display. The event also provided an opportunity to recruit volunteers for the *S. anglica* removal in Boundary Bay scheduled for June 17–19. FOC produced a fact sheet for distribution at the event and other forums.

PowerPoint presentations were made to several NGO's, including naturalist groups and environmental training

Fig. 2. *Spartina anglica* on Roberts Bank in August 2003.

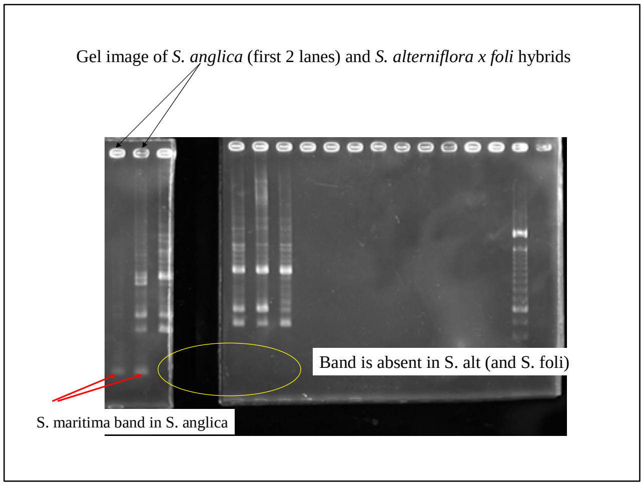

Fig. 3. DNA analysis gel image of Roberts Bank *S. anglica* (left samples) showing *S. maritima* bands and absence of *S. alterniflora* (courtesy of D. Ayres, University of California, Davis).

programs. Ducks Unlimited Canada and the Corporation of Delta issued several press releases. Delta established a web site on *Spartina anglica* as part of their outreach program. An oral presentation describing the Fraser *Spartina* Busters program was also made at the 3rd International Invasive *Spartina* Conference in San Francisco, November 8-10, 2004.

Volunteer Recruitment and Management

Volunteer organizations and individuals were contacted by email providing information on the meeting time and place, as well as mapping and field needs for the Boundary Bay *S. anglica* removal. A meeting room at the Delta Air Park was made available to the organizers for volunteer sign up, supply and equipment staging, and brief orientation. Laminated *S. anglica* identification cards were given to all volunteers to assist in accurate identification during removals.

Several volunteer removal efforts were held from June and October in Boundary Bay and Roberts Bank. Eighty-eight volunteers participated in the June 17–19 removal. A fall follow-up was held October 13–15 supported by 19 volunteers.

Volunteers received a brief instruction on manual removal methods in the meeting room during the June removal, with field demonstrations conducted in subsequent removals.

Manual and Mechanical Removal Methods

The manual removal methods were similar to the Roberts Bank program. However, an all-terrain vehicle, provided by Delta, was used to collect and transport bags to the dike. Filled bags were piled at collection points on top of the dike and subsequently loaded by backhoe into a truck and taken to the GVRD incinerator, where they were weighed prior to incineration.

For larger clones (i.e., >1 m diameter), an amphibious swamp excavator operated by Concord Excavating and Contracting Ltd. was used to bury the plants in deep holes excavated in the mudflat. The machine ran on large tracked pontoons and left a light footprint on the ground (< 1 psi). Excavated *S. anglica* was buried in holes about 3–5 m deep and backfilled with excavated material, which was graded to the surrounding substrate (referred to earlier as *in situ* burial). At some locations, GPS was used to record the location for future monitoring. The excavator was also used to remove several large clones in the western section of Boundary Bay in June.

Two removal efforts were also conducted at the Roberts Bank site. On August 5, the swamp excavator was used to complete *in situ* burial of the three large clones. A volunteer removal of seedings occurred on September 28, involving eight person-days of effort.

RESULTS

Spartina Identification

S. anglica was quite easily identified based on habitat and plant characteristics. Most plants colonized mudflat elevations well below native salt marsh. The oblique blade and vertical spikelet arrrangements were distinguishing characteristics in the field (Fig. 2).

DNA analysis showed the distinctive *S. maritima* bands, lacking in *S. alterniflora* (Fig. 3). However, due to the limited genetic variation in *S. anglica*, it was not possible to determine the source of the Fraser River estuary specimens (D. Ayres, pers. comm.).

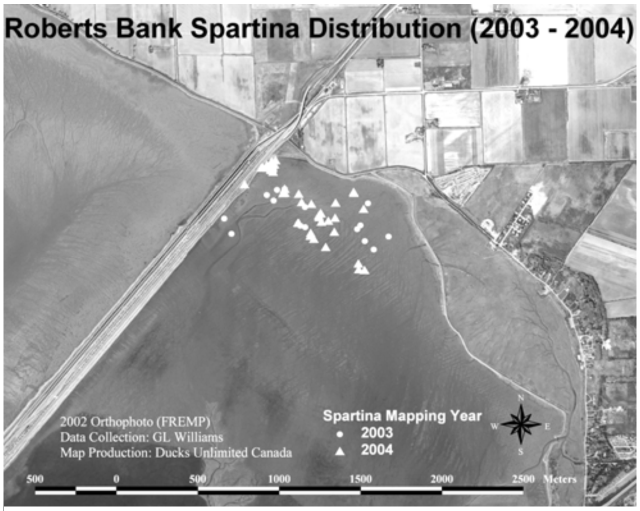

Fig. 4. Comparison of *S. anglica* distribution on Roberts Bank in 2003 and 2004.

S. anglica Distribution on Roberts Bank and Boundary Bay

In 2003, *S. anglica* was present on Roberts Bank in three 3-m diameter clones and approximately 50 scattered seedlings and tussocks up to 1 m in diameter. The total mudflat area affected was 60 ha (148 ac), but the actual total area covered by *S. anglica* was only about 0.1 ha (0.2 ac).

The average plant density and height in the main clone (n=4) was 217 ± 42 shoots/0.25 m^2 and 108 ± 6 cm, respectively). Individual seedlings were much smaller, ranging from 0.1–0.3 m (4–12 in.) in height.

In 2004, new seedling growth was found on Roberts Bank, despite previous clearing in 2003 (Fig. 4). Seedling distribution, mapped in June and October, showed that the distribution of the plants continued to expand through the summer. Above-average temperatures experienced in the summer of 2004 appeared to increase the spread of *S. anglica.*

In 2003, *S. anglica* in Boundary Bay was concentrated in two nodes near Beach Grove, in the west and between 96th and 112th Streets in the central portion (Fig.5). Most of the observed *S. anglica* was present as seedlings or small tussocks, with occasional clones ranging from 1–3m diameter. Total area impacted was about 135 ha (330 ac), but total coverage was estimated at 1% or 1.35 ha (3 ac).

Similar to Roberts Bank, Boundary Bay seedling abundance increased in 2004, and new areas were documented in Surrey and Mud Bay in the eastern section of Boundary Bay. The most densely infested area was located west of 112th Street, outside the FOC mapped area. The composite map of *Spartina anglica* locations is shown in Fig. 6.

Fig 5. November 2003 GPS locations of main *S. anglica* infestations recorded during hovercraft survey.

Spartina Removal

In 2003, the one-day removal effort (total of 21 person-days) was successful in removing all *S. anglica* plants from Roberts Bank, except for those in the three 3-meter diameter clones. Roberts Bank sediments were quite soft which made walking and digging more difficult. Manual removal of the dense growth in the clones was slow, and only half of one of the 3-m diameter clone was removed by six volunteers working a full day (Fig. 7). The sediments were washed from the plants to reduce the weight of collection bags. In Boundary Bay, the sediments were firmer (i.e. sand) which facilitated removal. The use of an all-terrain vehicle to remove the bags greatly reduced the labour. The 140 person-days of removal effort in 2004 was not sufficient to remove all the *S. anglica*.

The excavator and *in situ* burial used in 2004 proved to

Fig. 6. Distribution of *S. anglica* on Roberts Bank and Boundary Bay.

be a more efficient removal method for larger clones. It is unlikely that *S. anglica* will grow from a depth of 2–3 m.

Total plant material disposed of in 2004 was about 8,000 kilograms (kg) and it is estimated that approximately five times that amount was removed using *in situ* burial. The total weight included plants and some sediment.

Hovercraft Survey of the Outer Fraser Estuary

A hovercraft provides an efficient survey method for covering large areas of tidal flats, especially where mud substrates make walking difficult. Seedlings are easily observed from either inside or from the foredeck. The on-board digital charts and GPS provide accurate locating. The craft can travel either rapidly or at a very low speed, and is capable of stopping if there is a need to obtain plant specimens.

Outreach and Education

The outreach component of the VPA Roberts Bank program included PowerPoint presentations, local newspaper and radio, and printed/electronic publications. The PowerPoint presentations were well received and often led to invitations from other groups. The newspaper articles provided broad local coverage, which appeared to reach a larger audience than radio. The Vancouver Natural History Society (VNHS) newsletter article led to a request to prepare a more detailed article for the VNHS journal, Discovery. The electronic publication in Botantical Electronic News (BEN), being available on the web, led to communications and exchange of information with scientists in France and Oregon.

As well as increasing awareness of the *S. anglica* threat, the outreach was effective in recruiting volunteers. During conversations with removal volunteers, many said they were motivated to provide assistance after seeing the PowerPoint presentation or attending local World Oceans Day events where information about the problem was presented.

Program Costs

The 2003 VPA *S. anglica* management program was developed and coordinated by the author (GW), and volunteers conducted the actual removal. The total budget was approximately $15,000, covering consultant fees, disposal trucking, incineration, and supplies (e.g. plastic bags, gloves, etc.). The hovercraft survey cost about $1500, including reporting and mapping. There were also in-kind contributions of hovercraft fuel (provided by Fisheries and Oceans Canada) and staff salaries.

The 2004 program costs were over $50,000 plus a matching amount in-kind from many agencies involved in the *Spartina* removal program.

DISCUSSION AND RECOMMENDATIONS

The management efforts in 2003 and 2004 were useful in developing strategeies to effectively control *S. anglica* in the Fraser estuary. *Spartina. anglica* was fully controlled at Roberts Bank by the end of October 2004, although continued management will be required to address new recuitment from existing seedbank and new invasions. The distribution of *S. anglica* in Boundary Bay has been greatly reduced, but there is still a substantial amount remaining east of 112th St., and scattered new seedlings were observed throughout Boundary Bay in November 2004. The Fraser estuary program shows that control is possible without the use of herbicides, provided that the infestation is caught early and aggressive removal programs implemented (Daehler and Strong 1996; Anon 2002).

Fig. 7. Fraser *Spartina* Busters on Roberts Bank in October 2003.

Manual effort was effective in removing *S. anglica* in the Fraser estuary, but *in situ* burial is more effective for large clones exceeding 1 m. Digging and bagging plants is efficient for seedlings and tussocks, but the use of all-terrain vehicles for collection and transport to shore for disposal is required for large scale programs. Firmer substrates permit the use of mechanized equipment and are generally easier to work on than muddy sediments.

The Fraser estuary *Spartina* programs depended on volunteer manpower, but dedicated work crews are recommended for Boundary Bay. Boundary Bay will require a greater effort than 107 person-days of removal to reach the status of Roberts Bank. However, much of the *S. anglica* in Boundary Bay consists of seedlings, which can be easily removed. Denser coverage occurs in localized areas, such as the six, 3 m-diameter clones west of 104th Street or mixed within native marsh west of 112th Street.

Mapping was a critical component of the 2003 and 2004 programs. Handheld GPS units provide sufficient accuracy for mapping *S. anglica*, and classification of the growth as seedling, tussock (0.1–0.3 m), small clone (0.3–1m) or large clone (>1 m) provides sufficient detail for planning manual or mechanical removal. Seedlings and tussocks can be removed manually, while large clones will require mechanical, *in situ* burial. Mapping should be conducted in June when the plants have matured and are easily located, and removal efforts should be conducted during July. Flowering occurs from June to September, but may be as late as November, and most seed is set in September (Dethier and Hacker 2004).

Following the summer removals, FOC participation in the program was discontinued and Environment Canada assumed the chair of the steering committee. Senior agencies should make a higher commitment to *S. anglica* management and provide sufficient manpower and financial support to bring the infestation under control. Without sufficient follow-up *S. anglica* will spread and make control and removal much more difficult and expensive in the future.

Training is an important component of *S. anglica* removal. Some volunteers had difficulty distinguishing it from native salt marsh species. Although complete removal of above-ground plants and below-ground roots and rhizomes was stressed during site demonstrations, some removals were incomplete. These concerns can be minimized by providing better training and by implementing inspections by qualified supervisors during removal efforts.

During both 2003 and 2004 there were substantial in-kind contributions from many particpating agencies. For example, FOC covered the hovercraft fuel costs; Delta provided the all-terrain vehicle, bag collection, and dike access; FOC provided GPS and GIS support, a *Spartina* fact sheet, and meeting rooms; MWLAP arranged for the excavator; and DUC arranged for portable toilets in the field, GIS mapping, and press releases.

Increased surveillance is required, which benefits from better outreach. Providing presentations to interested parties is useful for increasing awareness and building support for the program. Attempts were made to enlist the help of birders from the VNHS, but there was no active follow-up to make it more effective. A *Spartina* contact telephone number should established. Material should be prepared to assist in the identification of *S. anglica* and other species (e.g. *S. alterniflora* and *S. densiflora*). Outreach should also be extended to Vancouver Island and the Gulf Islands, where suitable conditions exist for establishment.

POST PRESENTATION UPDATE:

To manage *Spartina* infestations in British Columbia, the BC *Spartina* Working Group (BCSWG) was formed, led by Ducks Unlimited Canada. The BCSWG has prepared annual reports and commissioned the BC *Spartina* Response Plan (Dresen et al. 2010). *Spartina* mapping and related information pertaining to the BCSWG efforts are provided on the Community Mapping Network website www.Spartina.ca.

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IMPLEMENTING A STRATEGY FOR MANAGEMENT OF RICE GRASS, *SPARTINA ANGLICA*, IN TASMANIA, AUSTRALIA

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INTRODUCTION

Spartina anglica, commonly referred to in Australia as rice grass, was intentionally introduced to Tasmania in the 1930s because of its potential value for coastal engineering and agriculture. Many decades later, however, the vast majority of the benefits provided by rice grass have been overshadowed by the ecological, social and economic costs of its continued spread (Hedge and Kriwoken 2000; Kriwoken and Hedge 2000). In 1996 the Rice Grass Advisory Group (RGAG) was established to provide advice to the Tasmanian Government on future management options for rice grass infestations. The RGAG facilitated the formation of a partnership between the Australian Government, Tasmanian Government, Tasmanian Fishing Industry Council and the broader community to fund the development of the Strategy for the Management of Rice Grass (*Spartina anglica*) in Tasmania, Australia (DPIWE 2002).

The Management Strategy set out a range of broad objectives and associated tasks to reduce the area of rice grass infestations in Tasmania and identified the Department of Primary Industries, Water and Environment (DPIWE) as lead management agency. It also identified area-based objectives for each of Tasmania's infested regions and pointed to Fusilade® (active constituent: 212 grams per liter (g/L) fluazifop-P present as butyl ester) as the only cost-effective and practicable technique for controlling and eradicating infestations. It recommended the establishment of a small, multi-disciplinary team to strategically reduce rice grass infestations in collaboration with affected industries and communities.

In 1997 DPIWE sought funds to implement the Management Strategy. After considerable lobbying by the RGAG, the Natural Heritage Trust Fisheries Action Program provided US $690,000 in funding, including US $487,000 of in-kind support. The DPIWE was charged with the responsibility to administer the project and a steering committee was established to guide its development.

This paper focuses on the implementation of the Management Strategy during the period from 1998 to 2002. It initially identifies the priority risks to program success and links these to key management tasks, including the development of area-based management plans, environmental monitoring programs and targeted research. The tools and approaches used to develop and maintain stakeholder support, environmental monitoring programs and reduce rice grass infestations are also identified and discussed. Lessons learned and current challenges for rice grass management in Tasmania are summarized.

IDENTIFICATION OF KEY RISKS TO THE MANAGEMENT PROGRAM

Reducing rice grass infestations presents a vast range of challenges to managers, including complexities associated with identification of suitable control techniques, logistics of field-based operations, and stakeholder support for control programs (Kriwoken and Hedge 2000; Hedge et al. 2003). Understanding and effectively addressing these challenges was recognized by the steering committee as being critical to program success. Table 1 lists the priority risks to program success and links these to key management responses.

An essential approach to understanding and effectively addressing priority program risks involved learning from past and current experiences of rice grass management in other locations. Gathering published information on the ecology and management of rice grass provided considerable information about rice grass ecology, biology, ecological impacts and overviews of various approaches to control programs in other regions, including Australia, New Zealand and the United States. Such information was useful for understanding the range of control techniques and strategies used to manage rice grass. Extensive collaboration and communications with experienced rice grass management teams in Victoria, Australia and Washington State, USA provided particularly useful insights into these risks and challenges in the field.

KEY ELEMENTS OF THE MANAGEMENT PROGRAM

Engaging and working with stakeholders

A high level of stakeholder engagement was critical to the success of the management program. The Rice Grass Advisory Group, established in 1996, provided an important mechanism to bring together relevant stakeholder groups so that their concerns, perspectives and ideas could be shared and understood. The Management Strategy recognized this group as the central integrating and advisory body for rice grass management in Tasmania.

The Management Strategy required area-based Management Plans to be developed, in close collaboration with local stakeholders, to meet the recommended management objectives for each infested area. Three main

Table 1: Priority risks to the Tasmanian Rice Grass Management Program and summary of key management responses.

Priority Risks to Program Success	*Management Responses*
Lack of key stakeholder support for management program	• Develop and review area-based Management Plans with stakeholders • Establish regional ‘champions’ and establish appropriate mechanisms for ongoing communication
Efficacy of control techniques is less than 90%	• Include experienced weed management persons in management team • Identify key variables and establish system to monitor performance or team members and conduct annual review of control technique efficacy
Actual and perceived toxicological impact of herbicide on the environment	• Commission risk assessment on toxicological risks of herbicide to the environment • Develop targeted monitoring program
Actual and perceived impact of rice grass removal to environment	• Provide scholarships/funding to encourage student research on the impact of herbicide to non-target organisms
Actual and perceived impact of herbicide on survival growth and marketing of commercial Pacific oysters, *Crassostrea gigas*	• Commission risk assessment that considers toxicological effects of herbicide on juvenile and adult *C. gigas* • Conduct field and laboratory-based research on effects of herbicide on *C. gigas*
Lack of funds to continue implementation of management program	• Demonstrate commitment to program objectives, wise use of funds and accountability • Seek key opportunities for media releases
Exponential increases in area of rice grass infestations	• Identify priority areas for control • Develop realistic seasonal goals for priority control areas and develop systematic approach to reduction of infestations

mechanisms were used to engage stakeholders in developing these plans: 1) identifying and involving champions, 2) convening regional meetings, and 3) holding targeted stakeholder meetings. Trusted community members who were well-informed on coastal natural resource management issues assisted with outreach to the local communities where grass infestations occurred, and identified the range of local stakeholder interests to be considered in developing these plans. Champions for the intertidal oyster industry were also identified and involved to ensure that their interests, concerns and potential contributions were clearly recognized/understood.

In collaboration with these champions, regional meetings were organized to seek community and industry, in particular the oyster industry, support for recommended management objectives, proposed control techniques and field-based operations. Stakeholder contributions during these meetings were very useful for identifying coastal access points, preferred periods for control activities and priority areas for control. Regional meetings provided valuable input to the development of area-based management plans, including agreement on the roles and responsibilities of relevant stakeholders.

In some cases regional meetings were followed up with more targeted stakeholder meetings to better understand specific stakeholder concerns about the potential ecological effects of various control techniques. These meetings often required substantial preparation and time but were important for generating broad stakeholder support for the program.

Media releases and a quarterly newsletter kept stakeholders informed of rice grass management progress. Media opportunities were sought to highlight the achievement of key management program milestones.

Environmental monitoring and targeted research.

During the mid 1990s the Department of Natural Resources, Victoria, Australia, conducted a range of studies that investigated the efficacy of control techniques and their ecological impacts. The studies collectively pointed to the highly selective post-emergent herbicide Fusilade as the preferred means of controlling rice grass infestations. Similar studies by the DPIWE in Tasmania also pointed to Fusilade as an environmentally responsible, safe, and cost-effective technique. However, Fusilade is not licensed for use in the coastal environment. In 1998 the DPIWE used these research findings to support an application for an off-label permit to use Fusilade to control rice grass in Tasmania, which was later approved that year by the National Registration Authority for Agricultural and Veterinary Chemicals.

Initially, DPIWE voluntarily restricted its use of Fusilade to the very small infestations (e.g., Derwent River and St Helens sites) pending the completion of an independent environmental risk assessment on the use of

Fusilade to control rice grass (Davies 1999). This assessment found that although the available information was limited, the use of Fusilade to control rice grass represents a low environmental risk. The report also contained a range of recommendations that DPIWE then used to refine its management program (e.g. initially limiting spray events to 0.5 hectares per day as a precautionary approach in view of limited available information), design an environmental monitoring program, and target future research.

The risk assessment recommended that further studies be conducted to better understand the potential effects of Fusilade on *Crassostrea gigas*. Laboratory-based research was conducted, in collaboration with the Pacific oyster aquaculture industry, to investigate the potential effects of Fusilade on survival and development of *C. gigas* (Hedge et al. 1999). The investigation also included a laboratory/field component that examined ingestion and depuration of the Fusilade active ingredient (fluazifop-P-butyl) and its primary degradation product (flauzifop-P) in *C. gigas* when exposed to expected field concentrations. The findings of the investigation provided further evidence that Fusilade presented a low environmental risk and was also an important factor in securing the support of the Pacific oyster aquaculture industry for the management program.

In 1999, an environmental monitoring program was designed to include the following components:

- Water quality monitoring to assess fluazifop-P butyl and fluazifop-P concentrations at a variety of locations in each estuary during and following initial control operations;
- Shellfish sampling to monitor ingestions of fluazifop-P butyl in *C. gigas*;
- Small-scale, comprehensively designed biomonitoring program of benthic macroinvertebrates in areas sprayed; and
- Mapping the location and size of rice grass infestations following treatments with Fusilade.

Collectively, results from research on *C. gigas* and the environmental monitoring program provided additional assurance of environmental safety; consequently the management program increased the use of Fusilade to allow treatment of up to one hectare per day.

More recently, two research projects are underway to investigate the impact of rice grass removal on benthic communities in the Rubicon/Port Sorell estuary and the potential effects of the release of sediments resulting from large-scale rice grass removal in the Tamar River. These projects will provide useful information to decision makers when reviewing management objectives for the two remaining large infestations (i.e. Rubicon/Port Sorell and Tamar River).

Reduction of infestations

The Management Strategy provided clear direction for prioritizing the reduction of infestations. The general approach was to focus on the smallest infestations (i.e. Derwent River, St Helens, Little Swanport estuary and Bridport infestations) progressively building up to larger infestations (i.e. Circular Head, Rubicon/Port Sorell and Tamar River). A small, multi-disciplinary management team was used to reduce all targeted infestations to the point where the efforts of local communities and industry became important for locating remnant patches or isolated plants. Area-based Management Plans contained the specific details of how each infestation would be reduced (i.e. management objectives and responsibilities, control techniques and control period). This was an important planning tool that was used to synchronize the efforts of the DPIWE, local communities and industry.

The management team consisted of a core of three people, one management officer and two technical officers. A hovercraft and operator were contracted and the vessel fitted with a 50-liter spray tank, low spray equipment and 100-meter remote-controlled, retractable hose. Other equipment used included all terrain vehicles fitted with low-pressure spray equipment and backpack low-pressure spray units. The primary control technique was application of Fusilade (application rate of 1000 litres/hectare of 1% Fusilade solution mixed with freshwater and the surfactant BS1000 at 0.2% vol/vol to maximize efficiency). A review of field-work data sheets showed the management team typically achieved efficacies of 90-99%. In 2002, the DPIWE decided to engage commercial weed management contractors to assist with treatment in the Circular Head region. A comparison of costs and efficacies between the management team and commercial weed contractors is provided in Table 2.

Table 3 provides a summary of the infestations, their current management objectives and size in 2002 relative to 1997. The area of infestation at Derwent River, St Helens, Little Swanport estuary and Bridport have all been reduced by 99% and are on target for achieving the management objective. The Circular Head infestation has been reduced by approximately 50% and is also on target to achieve the management objective. The Rubicon/Port Sorell infestation, although having increased in total area, has been successfully confined to the upper half of the estuary. The Tamar River infestation, the largest in Tasmania, appears to have increased its area but is thought to be confined to the

Table 2: Comparison of costs and efficacies between the management team and commercial weed contractors for the application of Fusilade in the Circular Head region in 2002 (estimate of costs provided by DPIWE).

Control Crew	*Average area treated (ha./day)*	*Efficacy (%)*	*Cost US $/ha*
Management Team	0.5	~95	2,500
Contractors	1.0	>90	1,120

estuary. Management options and objectives for the Rubicon/Port Sorell and Tamar River may be revised if significant developments in large-scale control techniques are forthcoming.

CONCLUSIONS

Between 1998 and 2002 Tasmania's rice grass Management Strategy used the experiences of rice grass management programs in other regions, particularly those in Victoria, Australia, and Washington State, USA, to identify priority program risks and to develop its approach to the reduction of infestations. The management program placed a high degree of importance on stakeholder participation and engagement at all stages of the management program. Targeted research and environmental monitoring programs have also been strategically used in developing and progressively scaling up the management program from small-scale to medium-scale control efforts.

The Management Strategy and area-based Management Plans set specific objectives for each of Tasmania's infested regions. In light of the progress made toward these objectives the implementation of the Management Strategy between 1998 and 2002 was successful. This achievement was acknowledged in 2001 when the Rice Grass Advisory Group was awarded the Australian Water Association Environment Award for its contribution to the restoration of aquatic habitat. Factors that significantly contributed to program success include:

- Development of a management strategy with clear objectives and direction for management;
- Establishment of the Rice Grass Advisory Group involving key stakeholder interests and the appointment of the DPIWE as lead management agency;
- Effective engagement of relevant regional and industry stakeholders at all stages of the management process;
- Development of a management program that integrates targeted research and adequate environmental monitoring; and
- A method of rice grass control that can be demonstrated to be environmentally responsible, safe, practicable and cost-effective.

It is worth pointing out, however, that the battle against the spread of rice grass in Tasmania is far from over. There are some important challenges still to be faced including:

- A clear understanding of what is required to achieve eradication as opposed to just controlling rice grass, and ensuring that those more stringent requirements are met (e.g. local communities and industries will play an important role in eradication and their continued support and commitment is vital);
- Maintaining effective, practicable and cost-effective arrangements to contain large infestations in the long term;
- Procurement of sufficient ongoing funding to meet the expense of reducing rice grass infestations, particularly in the Circular Head region.

Table 3: Summary of all infestations in Tasmania, including size and current management objectives in 2003 relative to 1997.

Infestation	*Management Objective*	*Infestation area 1997 (ha)*	*Infestation area 2002 (ha)*
Derwent River*	eradicate	1	0.0001
Little Swanport*	eradicate	10	0.0003
St Helens*	eradicate	1	0.0001
Bridport*	eradicate	5	0.0002
Tamar River	contain to estuary	415	415
Rubicon/ Port Sorell	contain to upper half of estuary	109	141
Circular Head	eradicate	50	25

* Priority infestations treated in 1999, 2000 and 2002

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CONTROLLING INVASIVE *SPARTINA* SPP.: THE NEW ZEALAND SUCCESS STORY

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Spartina is being successfully controlled in the estuaries of southern New Zealand. With an estimated 800 hectares (ha) (2000 acres [ac]) of the New River estuary near Invercargill being affected by *Spartina* in 1988, this has now been reduced to less than 1 ha (2.5 ac). Within the next two years, we expect *Spartina* to have been eradicated in these estuaries. *Spartina* was originally planted in the Invercargill district in the 1930s to reclaim land in the estuary for the increased industrial activities in the area. It wasn't until the 1970s that *Spartina* was first acknowledged as a serious problem causing significant impacts on the estuarine values, including wading birds and shellfish habitats, and thus declared an invasive weed to the area. Various control techniques were trialled starting in the early 1970s producing some good results but were discontinued in the mid 1980s. In 1988 the newly formed Department of Conservation took over the responsibility for the control of *Spartina* and trials began to find a method to control its spread.

Trials with different chemicals show Haloxyfop (registered as Gallant^{nf}) to be the most successful herbicide with up to 95% kill on first application. Trials on application techniques have seen the combination of helicopter boom spraying of meadows and follow-up with an eight-wheeled amphibious-tracked vehicle (Argo), fitted with tracks and spray unit, as the most efficient methods of applying this chemical. These methods are now being used extensively around New Zealand.

The New Zealand Department of Conservation has been involved with the control of *Spartina* in Southland for the past 16 years and has had some significant successes in the fight against this invasive weed. This document summarizes the lead-up to the department's involvement, the trial work undertaken, the results achieved to the present and the plans for the future.

Planting of *Spartina* started in the New River Estuary during the early 1930s to help reclaim parts of the estuary for industrial development. The initial plantings were of the sterile hybrid *Spartina x townsendii*, but these plantings did not prove very successful. In 1947, plantings of the species *Spartina anglica* were undertaken. These plantings continued until 1954 and proved to be very successful. In the following decades, the area covered by *Spartina* continued to increase in an uncontrolled manner and spread to other estuary environments within Southland.

In the 1960s and early 1970s, concern was first raised on the increasing spread of the infestation and the effect it was having on wading bird habitat. In 1972, a committee was established to look at the *Spartina* issue, and with funding from the Invercargill City Council, trials were undertaken to try to control the spread of *Spartina*.

Early work using the herbicide Tandex (active ingredient karbutilate) by spray application proved successful, but later work was less so, possibly due to silt build-up on *Spartina* leaves reducing the herbicide's effectiveness. A method of soil injection was developed; although successful, it was very difficult, time-intensive work. Successes achieved during these early control applications were due mainly to the dedication, perseverance and plain hard work of a few key people.

Aerial trials with the herbicides Herbex and Phytazol A were undertaken, but the consistency of the results was disappointing. Due to the restructuring of local authorities within Southland in the mid 1980s, fewer resources were allocated to the *Spartina* control program. Infestations of *Spartina* again grew in an uncontrolled manner and so some of the earlier successes were lost.

By the end of the late 1980s, it was estimated that infestations of *Spartina* within New River Estuary affected some 800 hectares (ha), approximately 20% of the entire estuarine area. In 1987, the newly formed Department of Conservation took responsibility for the control of *Spartina* within Southland.

A new interagency partnership was developed between Invercargill City Council, Environment Southland (a public environmental monitoring organization), and the Department of Conservation. With this approach, efforts could again be focused on methods to control *Spartina*, not only in the New River Estuary but also in all Southland estuaries. This partnership continues to this day and is one of the reasons for the success of the program. As some of the previously used herbicides were now unavailable, this led to a new series of trials being undertaken with new herbicides and application methods.

One of the first herbicides tried was glyphosate. Whilst this herbicide proved fairly successful and was used for a number of years, its effectiveness was often reduced when silt and dirt covered *Spartina* leaves, although kill rates of 50% to 60% were still achieved. Further trials were undertaken using the herbicide Gallant? (Haloxyfop-R-methyl). This grass-selective herbicide was being used by the Department of Conservation at that time for the control of marram grass (*Ammophila arenaria*) on sand dune systems along the Southland coast. Gallant™ proved very effective at penetrating silt and dirt with at least 90% kill rates being achieved with a single application. This subsequently became the herbicide of choice and its use has continued to this day.

Whilst an effective herbicide was now available, early application methods left something to be desired, proving costly in time and resources. Initial work with a high-pressure spray gun and 200 meter (m) hose mounted on a truck was slow and difficult, needing a team of six people to haul a hose across mudflats. Trials using a hovercraft in 1995 proved successful in allowing quick access to scattered *Spartina* patches that previously had to be approached on foot. This machine was fitted with a small spray unit and proved very effective. However, this form of transport was not able to travel across the now vast *Spartina* meadows. So even though the hovercraft had made work easier, there were still shortcomings. Further trials were undertaken using an Argo, an all-wheel-drive amphibious vehicle, with extremely successful results. When fitted with a small spray unit, this vehicle allows one person to undertake control work across the vast majority of areas within the estuary environment. When fitted with tracks, this vehicle is capable of travelling over waist-deep mud. Whilst the Argo was an extremely efficient vehicle, an effective method of herbicide application to the vast *Spartina* meadows was still sought. Trials with a helicopter fitted with a boom and valsecol foaming nozzles, flying at a height of 1-2 m above the tops of the plant and at very slow speeds, proved very effective for this task.

As a result of our experience acquired during all of these trials, we now had the right tools in the fight against *Spartina*. These tools were:

- an herbicide that was extremely successful in the harsh estuarine environment,
- a helicopter that could attack the vast meadows, and
- the Argo to control smaller areas on mud and to follow up on the helicopter work.

The correct use of the right herbicide by the most appropriate method at the right time has had tremendous results, which we were able to replicate in all estuaries within Southland.

Sixteen years ago, the control and eradication of *Spartina* seemed an impossible task. However, previous lessons and the development of some of the early pioneering work has led to the elimination of Southland's *Spartina* infestation.

To achieve eradication of *Spartina,* it is crucial for an active surveillance program to be undertaken to continue to search for previously unrecorded infestations. At the present time, the *Spartina* meadows and patches have been eradicated from all estuaries within Southland—sparsely scattered individual plants are all that remain.

Where such plants are discovered, the appropriate control action needs to be taken at the appropriate time. The use of the Argo is now the mainstay of our treatment operations because it is time and resource-effective.

In addition to present eradication work, monitoring the effects of *Spartina* removal on the estuary will need to continue.

ACKNOWLEDGMENTS

This has been a brief overview of the work that has been undertaken over the past 30 years to enable estuaries within Southland to approach the eradication of non-native *Spartina.* This would not have been achievable without the continuing support and cooperation of our partner organisations: Environment Southland, Invercargill City Council, and Dow AgroSciences.

In addition, a number of individuals have given support, advice and assistance from the beginning of this program. My thanks go to all who have been a part of it.

COMPARISON OF CHEMICAL AND MECHANICAL CONTROL EFFORTS FOR INVASIVE *SPARTINA* IN WILLAPA BAY, WASHINGTON

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Willapa Bay, Washington, hosts the largest invasive *Spartina* population in North America. State and federal agencies have been conducting large-scale mechanical and chemical control efforts on the affected lands during the past decade with varying degrees of success. Assessments of the long-term efficacy, cost effectiveness and ecological risk of the various control tools are presented. No long-term control was achieved with multiple years of disking. Crushing *Spartina* was only successful when the plant was driven well below the surface sediment. This occurred on soft sediment and a thin root mat. Winter tilling provided good control, but spring and summer tilling only marginal control. In sites where seed stalks were tilled into the sediment during the winter, there was a solid stand of seedlings the following spring. *Spartina* control with glyphosate at the high hand-sprayed rates (5–8% v/v) averaged approximately 50%, with permanent control taking several years of re-treatment. Control from late season (September/October) hand-sprayed glyphosate was poor. Control with broadcast application of glyphosate (8.4 kilograms active ingredient per hectare (kg ai/ha)) was highly variable. Although brown-down was usually observed, there was no permanent control except under ideal conditions (clean leaves, clean spray water and several days of dry time), in which case up to 74% control was achieved. Based on same season observations, large-scale control appears promising using broadcast (aerial and ground) and hand-sprayed imazapyr. Most variability with imazapyr occurred when hand-sprayed applications failed to achieve good canopy coverage. Best control, lowest long-term cost and least ecological risk occurred with broadcast imazapyr application in June.

Keywords: glyphosate, imazapyr, tilling, crushing, modeling, eradication, *Spartina*

INTRODUCTION

Research to develop better control methods for invasive *Spartina* has been on-going on a global scale for many years (Frid et al. 1999; Garnett et al. 1992; Kilbride et al. 1995; Major et al. 2003; Patten 2002; Patten and Stenvall 2002; Pritchards 1995; Shaw et al. 1995). How well these various methods work when implemented by large-scale control programs has been poorly documented. In addition, there is little information about how successful these programs are in achieving their control or eradication goals.

Willapa Bay, Washington State, USA currently hosts one of the largest *Spartina alterniflora* infestations in the world. In 1993 the state legislators unanimously passed the Revised Code of Washington 17.26 which mandates that *Spartina* shall be eradicated. To accomplish this goal, there have been large-scale control efforts on *Spartina* in Willapa Bay since 1995. Those efforts have included an array of mechanical and chemical practices implemented by state, federal and private stakeholders. Treating *Spartina*, however, has not led to eradication. Control has not come close to keeping pace with the 15 to 20% annual rate of spread (Hedge et al. 2002), despite state and federal agency expenditures of $500,000-$1 million to treat thousands of acres of *Spartina* per year (WSDA 1999 to 2003). A successful eradication strategy requires that the specific control practices being utilized have adequate efficacy to accomplish the task. Unfortunately, according to a study by the Government Accounting Office (GAO 2002), most invasive species management programs fail to objectively assess, improve and realign their control strategies to realistically correspond to their eradication goals. The *Spartina* control effort in Willapa Bay is no exception to this failure of management strategies (Hedge et al. 2002).

The objectives of this study were to monitor and evaluate the most commonly used *Spartina* control efforts in Willapa Bay and to determine if the efficacy of those efforts is sufficient to allow state and federal agencies to accomplish their eradication goals.

MATERIALS AND METHODS

Major mechanical and chemical control efforts conducted by Washington State Department of Agriculture (WSDA), Washington State Department of Fish and Wildlife (WDFW), Washington State Department of Natural Resources (WDNR), Willapa National Wildlife Refuge (WNWR) and private tideland owners in Willapa Bay from 2001 to 2004 were identified and selected for evaluation.

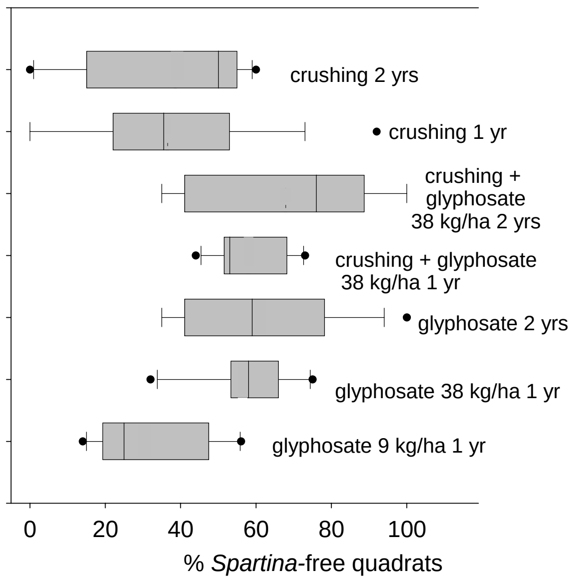

Fig. 1 Box whisker graphs of percent *Spartina*-free quadrats from different mechanical and chemical controls used in Willapa Bay from 2001 to 2003. Box whisker graphs only presented for treatments with five or more data points. (mean = dotted line, median = solid line, box = 25th and 75th percentiles, whiskers = 5th and 95th percentiles, data point = outliers).

Three mechanical control practices (tilling, crushing and disking) and four chemical control practices (glyphosate at two rates, crush and spray, and imazapyr) were assessed.

Tilling was done by the WNWR in the winter on large meadows that were previously mowed (2000/2001) or untreated (2004). Crushing was done by WSDA, WDNR or WDFW using tracked amphibious vehicles. Disking was done with a tandem disk. Crushing timing and frequency varied by site while disking was done only in the winter. Sites usually received multiple crushing or disking events per year. Imazapyr and the low rate of glyphosate (9 kilograms per hectare (kg/ha)) were applied by boom using approximately 500 liters per hectare (l/ha) spray volume. The high glyphosate rate was applied by hand using high pressure spray guns. A 5 to 8% v/v of product was applied with a spray volume of about 1,500 l/ha. The estimated glyphosate rate for hand applications averaged 38 kg/ha. Spraying of crushed sites occurred once *Spartina* had re-grown enough to provide adequate canopy coverage. Treatment sites ranged from 0.25 ha to 500 ha in size with the majority larger than 10 ha.

Attempts were made to assure that the treatment sites selected for monitoring were relatively free of confounding factors from previous control efforts at the sites, and that each site had a discrete treatment that was identifiable. Monitoring was done along multiple transects in each treatment site. *Spartina* stem and seedling density counts from 0.25m^2 quadrats were taken along transects. The number of quadrats and type of transect varied depending on the size and shape of each treatment area, with a range of 40 to 2,600 quadrats per site. Methods are fully detailed elsewhere (Patten 2003, 2004). Stem density data were classified as follows:

1) excellent *Spartina* control – 0 stems/0.25m^2;
2) good to moderate control – 1 to 5 stems/0.25m^2;
3) fair to poor control – 6 to 20 stems/0.25m^2, and
4) poor to no control – ≥21 stems/0.25m^2. Mean stem density before treatment was typically >50 stems/0.25m^2. Pooled and disaggregated data for each site were analyzed for stem density and overall stem density frequency distribution. Means and standard error values are presented.

The percent of *Spartina*-free quadrats was used as a conservative method to assess treatment efficacy. This value indicates a treatment's ability to prevent vegetative re-colonization and to minimize the cost of re-treatment. For example, a thinned-out *Spartina* canopy with a mean density of 5 stems/0.25m^2 could represent a greater than 90% decrease in stem density, but would cost almost the same to treat as a solid canopy; without re-treatment this would become a solid infestation again within a year. For this paper, the percent of *Spartina*-free quadrats was also assumed to approximate the extinction coefficient or the percent control. Years to achieve eradication for a given treatment, assuming minor re-infestation, was calculated as years = log 0.01/log (1-extinction coefficient). A discrete time, logistic growth model, with growth rate and outside seedling input parameters, was used to predict the amount of *Spartina* meadow remaining after four years of a given treatment.

Parametric comparisons of treatment efficacy were not feasible because treatments lacked true replication. That is, even sites with the same general treatment were treated by different agencies under vastly different conditions. Therefore to make inferences about overall treatment effectiveness from a management perspective, treatment variation is presented in box whisker format (mean, median, 5th, 25th, 75th and 95th percentiles, and outliers) for all treatments where there were more than five data sets.

For comparative purposes, box whisker graphs of efficacy on *Spartina* across selected herbicides are shown; this research was conducted by the author between 1998 and 2003 (Patten 2002; Patten & Stenvall 2002). Percent control data were pooled across each experimental unit for glyphosate at 18 kg/ha rate and imazapyr at 1.7 kg/ha.

RESULTS

Individual and pooled results are shown in Tables 1 and 2, and Fig. 1. Control with the broadcast rate of glyphosate (9 kg/ha) was highly variable (14% to 56%) and averaged half the efficacy achieved at the much higher hand-sprayed rates (38 kg/ha) (Table 1 and Fig. 1). There was less

Table 1. Summary of chemical control efficacy for *Spartina* in Willapa Bay from 1999 to 2003.

Control method	***Year***	***General location in Willapa Bay***	***Stem density*** ***(#/0.25m^2)*** ***±std. error of mean.***	***Percent stem density frequency***			
				0	***1 to 5***	***6 to 20***	***>20***
Glyphosate 9 kg/ha	2003	SE	21.0±3.0	20	13	22	44
Glyphosate 9 kg/ha	2003	S	27.0±1.5	14	13	19	54
Glyphosate 9 kg/ha	2003	SE	7.0±1.0	25	27	35	13
Glyphosate 9 kg/ha	2002	S	16.8±2.8	25	15	25	35
Glyphosate 9 kg/ha	2002	S	7.5±2.0	56	16	15	15
Glyphosate 9 kg/ha	2002	S	13.0±1.3	19	15	31	35
Glyphosate 9 kg/ha	2002	S	1.6±0.2	55	23	15	7
Glyphosate 9 kg/ha	2002/03	S	6.0±0.7	59	16	16	9
Glyphosate 38 kg/ha	2002	NE	7.0±1.0	75	7	5	18
Glyphosate 38 kg/ha	2002	Mid Peninsula	1.7±0.3	60	30	10	0
Glyphosate 38 kg/ha	2002	N. Peninsula	2.3±1.2	57	37	3	3
Glyphosate 38 kg/ha	2002	N. Peninsula	5.0±1.8	53	27	12	8
Glyphosate 38 kg/ha	2002	SE	2.7±1.1	74	14	7	5
Glyphosate 38 kg/ha	2003	N	5.0±1.0	32	13	42	13
Glyphosate 38 kg/ha	2003	Mid Peninsula	16.0±4.0	35	10	20	35
Glyphosate 38 kg/ha	2003	Mid Peninsula	5.0±1.0	58	12	18	12
Glyphosate 38 kg/ha	2003	SE	0.5 ±0.5	68	32	0	0
Glyphosate 38 kg/ha	2002	SE	3.4±1.1	54	28	10	8
Glyphosate 38 kg/ha	2002	N. Peninsula	6.8±2.1	60	20	15	0
Glyphosate 38 kg/ha	2002/03	N	9.0±2.0	35	18	33	14
Glyphosate 38 kg/ha	1999/02/03	N. Peninsula	1.3±0.5	83	10	8	0
Glyphosate 38 kg/ha	2000/02	S. Peninsula	5.2±0.7	63	7	18	12
Crushing+glyphosate 9 kg/ha	2003	E	8.0±2.0	47	13	25	15
Crushing+glyphosate 9 kg/ha	2003	E	2.0±3.0	8	8	43	41
Crushing+glyphosate 9 kg/ha	2002	SE	3.1±1.2	44	46	7	2
Crushing+glyphosate 9 kg/ha	2002	SE	1.5±0.5	71	19	10	0
Crushing+glyphosate 9 kg/ha	2002	SE	1.6±0.5	53	40	7	0
Crushing+glyphosate 9 kg/ha	2002	SE	1.9±0.6	60	20	15	0
Crushing+glyphosate 9 kg/ha	2002	N. Peninsula	6.8±1.7	53	40	7	0
Crushing+glyphosate 9 kg/ha	2002	SE	2.8±1.0	73	15	15	7
Crushing+glyphosate 9 kg/ha	2002/03	SE	0.5±0.5	85	15	10	0
Crushing+glyphosate 9 kg/ha	2002/03	SE	2.0±2.0	100	0	0	0
Crushing+glyphosate 9 kg/ha	2002/03	SE	6.0±1.0	51	18	22	9
Crushing+glyphosate 9 kg/ha	2002/03	N. Peninsula	8.0±1.0	35	15	40	10
Crushing+glyphosate 9 kg/ha	2002/03	SE	1.0±0.2	76	8	14	2
Crushing+glyphosate 9 kg/ha	2002/03	SE	5.0±1.0	43	15	37	5
Imazapyr 1.7 kg/ha	2003	E	1.0±0.7	82	15	0	3

variability in control at the high application rate. Glyphosate applied at the same site in consecutive years did not provide any marked improvement in overall control over a single year of treatment (Fig. 1). Crushing followed by the application of the high glyphosate rate did not provide any better control than glyphosate alone (Table 1 and Fig. 1). Similarly, multiple years of this treatment did not improve control over that of a single year of treatment. Control with imazapyr was the highest achieved by any treatment (82%). Although these results were obtained from a very limited data set, they are very similar to those obtained over seven years of small plot research (Fig. 2). In contrast, the control level found at monitoring sites where resource agencies used glyphosate was considerably less than that obtained from research trials.

Winter tilling provided the highest level of uniform control across all sites of any mechanical effort (77%). Control achieved with crushing was highly variable (ranging from 0 to 92% *Spartina*-free quadrats), depending on type of sediment and location (Table 2). Highest control (92%) occurred on one site where the crushing pushed the crown of the plant well below the surface of the sediment. No control was recorded at several sites with firm sediment. Average control was achieved from crushing on soft and firm sediment at 52% and 26%, respectively (Table 2). Crushing the same site repeatedly over consecutive years did not provide any marked improvement in control over a single year of treatment (Fig. 1). Control with disking was comparable to control from a good crushing event (Table 2). Control results from multiple years of crushing or disking sites have a level of uncertainty because only a few sites were available for comparison.

Seedling density data were also collected at all sites (data not shown). With two exceptions, density was low (<4 seedling/m^2) and no particular treatment effects were noted. The exceptions were a winter-tilled site and the imazapyr spray site where densities exceeded 100/m^2. Tilling effectively planted seeds on site, while the imazapyr site was in the middle of a 100-ha untreated *Spartina* meadow.

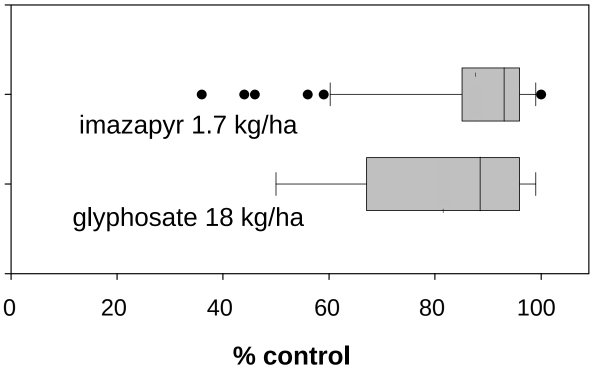

Fig. 2. Box whisker graph of pooled research results from 1997 to 2003 for imazapyr (n=46) and glyphosate (n=8).

An estimation of the time it will take to achieve near eradication (99%) using different control methods is shown in Table 3. These values underestimate the real time required to accomplish the task since re-infestation from new seedlings and continued vegetative spread are not taken into account. Only winter tilling and imazapyr provide any level of certainty that the target goal of near eradication could be accomplished in less than four years. Low rates of

Table 2. Summary of mechanical control efficacy for *Spartina* in Willapa Bay from 2001 to 2004

Control method	***Year***	***General location in Willapa Bay***	***Stem density (#/0.25m^2) ±std. error mean.***	***% stem density frequency***			
				0	***1 to 5***	***6 to 20***	***>20***
Winter tilling – silt	2004	SE	0.4± 0.1	75	25	0	0
Winter tilling – silt	2001	S	1.5±0.3	77	13	8	2
Winter tilling – silt	2001/02	S	3.2±0.6	67	19	10	4
Crushing –sand	2002	N. Peninsula	6.7±1.0	34	31	6	1
Crushing – sand	2002	N. Peninsula	4.0±0.6	47	29	21	4
Crushing – sand	2002, 2003	N. Peninsula	5.0±2.0	55	27	8	10
Crushing- sand	2002, 2003	N. Peninsula	3.0±1.0	60	15	22	3
Crushing- sand	2001, 2002, 2003	N. Peninsula	32.0±3.0	5	3	25	67
Crushing- soft silt	2002, 2003	NE	37.0± 2.0	0	0	5	95
Crushing- soft silt	2002, 2003	NE	7.0± 2.0	45	8	33	5
Crushing - soft silt	2001, 2002, 2003	N	3.0±1.0	50	33	13	3
Crushing - soft silt	2002, 2003	N	5.0±1.0	55	17	20	8
Crushing- firm silt	2003	N	10.0±2.0	54	20	20	6
Crushing -firm silt	2001	SE	46.0±3.0	0	0	0	100
Crushing- firm silt	2001	SE	25.0±3.0	22	16	29	33
Crushing- firm silt	2001	N. Peninsula	41.0±7.0	0	4	17	80
Crushing- soft silt	2001	SE	6.1± 0.6	37	30	28	6
Crushing- soft silt	2002	SE	5.8± 1.0	26	31	38	5
Crushing- soft silt	2001	SE	0.2± 0.1	92	8	0	0
Crushing- soft silt	2001	SE	4.0± 0.5	53	15	30	2
Disking + crushing	2001, 2002	SE	4.0± 0.7	71	19	10	0
Disking- soft silt	2001	N. Peninsula	3.8±1.2	53	30	10	7

glyphosate and crushing on firm surfaces extend the time period well beyond 10 years. If a more realistic and conservative approach to modeling is used that includes growth and seedling inputs, then only the use of imazapyr approaches 100% control after four years of treatment.

Treatment cost per hectare is only a minor component of the total cost of a control effort. Efficacy governs the majority of the total cost. For example, treating 100 hectares of *Spartina* with imazapyr or glyphosate at 38 kg/ha, would cost approximately $60,000/ha and $120,000/ha, respectively at current chemical prices. These values change dramatically when the modeling results shown in Table 1 are factored in. To control 99% of *Spartina* using imazapyr, a total of 130 ha would need to be treated over four years at a cost of approximately $78,000/ha, whereas to achieve that control with glyphosate, a total of 208 ha would need to be treated over seven years at a cost of approximately $250,000/ha.

DISCUSSION

The objectives of this study were to evaluate the *Spartina* control efforts in Willapa Bay and to determine if the efficacy of those efforts was sufficient to accomplish eradication. Developing inferences about treatment efficacy based on monitoring data from a few sites is problematic. Unlike research plots, these treatment sites lack replication and complete records on all the environmental and site conditions, and in general lack application precision. Traditional “in-house” monitoring also lacks objectivity. These concerns were minimized within this study by pooling data collected by an impartial party (WSU) across as many similar treatment sites and times as possible and using a very conservative estimate of control (percent *Spartina*-free quadrats). The tremendous variability across sites found in this monitoring effort is not atypical of that found in other *Spartina* control efforts (Patten 2002). Due to the lack of background data, however, the variations in this study are difficult to account for. Detailed data collection on the variables that affect *Spartina* control efficacy, such as herbicide dry time, tidal conditions, canopy quality (dirtiness, height, intactness/health, growth stage, and clonal age), spray water quality, and sediment type (Patten 2002), are usually not collected by field crews doing the control work.

Several observations on specific treatments should be noted. Tilling, although a superlative method for mechanically controlling *Spartina*, is costly, requiring an expensive ($250,000) amphibious tiller and is slow (~0.25 ha/hr). It has a limited window during the winter when it works, and unless it is preceded by summer mowing, it results in massive seedling density of more than 200 seedlings/m^2. Crushing is relatively less expensive (~$40,000 to $80,000 for the equipment) and is faster (1-2 ha/hr) than tilling, but it requires multiple crushing events per year. The greatest success in crushing appears to be limited to sites with certain sediment characteristics, such as areas with young *Spartina* on soft sediment. Crushing well-established *Spartina* meadows with a thick root mat or *Spartina* on sand provides marginal control. Disking is also relatively inexpensive and comparable to crushing, but was problematic in terms of sectioning and uprooting large mats of *Spartina*, which re-established in deeper tidal zones. The broadcast rate of glyphosate, although very inexpensive, only provided control under ideal conditions (>48 hour dry time and a clean, intact canopy). These conditions were rare in the field. The hand-sprayed rate of glyphosate (with or without previous crushing) provided fairly consistent control, but accurate rates and cost analysis are difficult to assess. Tank mixes ranged from 5 to 8% v/v of product with spray volumes from 1,000 to 3,000 l/ha spray volume. Application from airboats is limited to a few hectares per day. Variability in efficacy from hand applications largely reflected plants that were missed or only partially covered. Although large-site monitoring data is lacking, the broadcast

Table 3. Years to achieve 99% *Spartina*-free tideland using repeated annual treatment of different control practices.

Treatment	***Extinction coefficient ±std. error mean ****	***Calculated time to reach 99% Spartina-free tideflat** (years)***	***Amount of 100 ha meadow Spartina remaining at the start of year 5, after 4 years of treatment and total amount of area treated during that time******	
			ha remaining	***total ha treated***
Winter tilling	0.73±0.03	3.6	1	145
Crushing on soft sediment	0.52±0.14	6.3	11	211
Crushing on sand or firm silt sediment	0.26±0.09	15.4	73	360
Glyphosate 9 kg/ha	0.31±0.06	12.5	52	323
Glyphosate 38 kg/ha	0.57±0.04	5.4	7	192
Crushing + glyphosate 38 kg/ha	0.59±0.04	5.3	6	185
Imazapyr 1.7 kg/ha	0.82	2.7	0.3	127

* Extinction coefficient equals the mean % *Spartina*-free quadrats for the given treatment.

** Assumes that treatment is repeated annually, that the equation log 0.01/log (1- extinction coefficient) approximates the system’s response, and that there are no inputs from spread and seedlings.

*** Discrete time, logistic growth model, with growth rate parameter 0.15, carrying capacity 100 ha and additional seedling input from outside sources equal to 0.1% of carrying capacity per year. Developed by Dr. Caz Taylor, UC Davis.

application of imazapyr conducted in 2004 was relatively inexpensive (~$600/ha), fast (>100 ha/day), and fairly efficacious.

Repeated control measures conducted year after year on the same site should result in an overall reduction of *Spartina* at the site. This overall reduction in *Spartina* occupation over time (two years) was not evident from the pooled monitoring data for either chemical or mechanical control. These results are not inconsistent with those found in Puget Sound (Hacker et al. 2001). They report that sites required consistent control for four years to obtain an 86% decrease in *Spartina*. Most of the sites monitored in this study were also large (>10 ha) and, according to Hacker et al. (2001), less likely to show decline in *Spartina* density over time than small sites. Examples of this spatial scale effect were evident in this study. For three discrete sites that were smaller than five hectares and sprayed for two consecutive years, the percentage of *Spartina*-free quadrats went from 73% to 85%, 60% to 90% and 60% to 95%. Evidently on large sites, re-growth and seedling re-infestation compensated for any additional control achieved in subsequent years. This concurs with results from the discrete time, logistic growth model. When parameters for growth rate are set at 0.15, seedling input at 0.01, and control rates at 50%, 10 ha of *Spartina* within a 100-ha mudflat would still have 50% of *Spartina* left in the beginning of the third year. On the other hand, when a control rate of 73% is used and the parameter for outside seedling input is reduced to 0.005, the site is projected to be 81% *Spartina*-free in the beginning of the third year (this is similar to the 85% found at one of our monitoring sites).

Three major conclusions can be reached from this monitoring study. First, real world control data are highly variable between sites and years, and tend to show results that are less effective than what would be expected under ideal conditions. Efficacy can vary by at least 20% from the expected level. Second, with the exceptions of tilling and imazapyr, none of the treatments provided efficacy anywhere near the level of that which would be required to achieve eradication in a reasonable time frame. Based on the political landscape, it is not prudent to expect funding for a large-scale control effort to go beyond a six to eight-year time period. Thus, unless the average control rate for a given method can be expected to be greater than 75%, that method has minimal practical value. The tenfold expansion of *Spartina* in Willapa Bay during the last decade of control efforts testifies to this maxim. Third, even if the control rate is 75%, as long as there is significant seedling input from outside sources, eradication can not be achieved. The control efforts should be mounted over a scale large enough to minimize threats from new seedlings. Similarly, unless the sites continue to be treated year after year, no appreciable gains toward eradication can be achieved.

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FRAGMENT PROPAGULES OF *SPARTINA ALTERNIFLORA* AND POTENTIAL EASTERN PACIFIC DISPERSAL

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Commonly used mechanical control methods for *Spartina alterniflora* involve varying levels of disturbance to rhizomes and roots. We examined the viability of rhizome fragments and their potential role in dispersal. Production of rhizome fragments by rototilling in Willapa Bay, Washington, USA was studied. The top 10 centimeters (cm) of the sediment contained an average of 310 fragments per square meter (m^2). Median rhizome length was 3.7 cm. Eighty-seven percent of the rhizome fragments had at least one vegetative shoot attached. Survivorship of *S. alterniflora* rhizome fragments from Willapa Bay and San Francisco Bay populations was investigated using a three-way factorial design. Treatments included two fragment sizes, approximating those found in Willapa Bay, immersed in either freshwater, 15 parts per thousand (ppt) saltwater, or 35 ppt saltwater for 3, 8 or 15 days. Fragments were then individually planted and grown in greenhouse ponds for four months. Rhizome survivorship was low (8.6% or less) in all 35 ppt treatments. Survivorship was 37.3% and 87.5% in 15 ppt and freshwater treatments, respectively. Large rhizomes had higher survivorship than small rhizomes at all salinities, and the length of time the rhizome fragments were immersed prior to planting had a variable effect on survivorship. Results suggest rototilling for control of *Spartina* may spread the infestation within an estuary but is unlikely to result in spread to other estuaries by ocean transport. Thus, tilling should be used with caution in estuaries with small, isolated populations of *Spartina*.

Although ocean transport of rhizome fragments appears to be a small risk, ocean transport of wrack and viable *S. alterniflora* seed is likely. A drift card study was begun in late September 2004 to better understand potential dispersal from invaded west coast estuaries. Monthly releases of cards from Humboldt and San Francisco bays in California, as well as Willapa Bay, Washington will aid identification of wrack deposition sites. Data from the first two months of this year-long study indicate that long distance dispersal of up to 270 kilometers (km) over a four-week period can occur.

Keywords: *Spartina alterniflora*, rhizome fragment, propagule dispersal, drift card simulation

INTRODUCTION

Effective invasive plant management considers potential vectors of propagules as well as how to minimize propagule production. In the case of *Spartina alterniflora*, early detection and treatment efficacy are high priorities for many stakeholders wanting to preserve historic habitat, indigenous species, and other beneficial uses of mudflats and native salt marshes in the Pacific Northwest. Within the core infestation sites, thousands of hectares have already been colonized including an estimated 790 net hectares (ha) (1,960 acres [ac]) in San Francisco Bay, California (Zaremba and McGowan 2004) and 3,200 net ha (8,000 ac) in Willapa Bay, Washington. Additionally, thousands of hectares in 31 Pacific estuaries are at risk for future colonization by one or more invasive *Spartina* spp. (Daehler & Strong 1996; Pfauth et al. 2003). In Oregon alone, approximately 13,622 ha (33,660 ac) of intertidal mudflats and aquatic beds are vulnerable to invasion (Pfauth et al. 2003). Understanding both the potential risks and the efficacy of any control method is critical to refining management choices and early detection efforts.

Efficacy and cost data have been evaluated for a wide array of chemical and mechanical treatments, (Patten 2002; Hedge et al. 2003; Pfauth et al. 2003); however the risk assessments of non-target effects have focused more narrowly on chemical controls. Mechanical treatments such as rototilling, disking, crushing, pulverizing and digging have been problematic due their slow pace, variable efficacy, and high cost per area treated (Patten 2002). Yet rototilling and disking are still used in some situations to facilitate the decomposition of below-ground biomass after successful chemical treatment, which allows for more rapid restoration to usable shorebird habitat (Patten & Stenvall 2002); these mechanical methods have also been employed where landowners oppose chemical treatment options.

Cordgrasses are capable of reproducing by vegetative fragments (Landin 1990; Stiller & Denton 1995; Daehler & Strong 1996; Sayce et al. 1997; Patten & Stenvall 2002). Disturbances to *Spartina's* extensive below-ground structure, such as those caused by rototilling, could potentially produce rhizome fragments. While invasive cordgrasses are known for the resiliency of their rhizomes (Reeder & Hacker 2004; Patten 2003), the viability of mechanically produced rhizome fragments and their role in dispersal has not been closely examined (i.e., evidence is anecdotal) (Randall & Milne unpublished; Pfauth et al. 2003).

Research has focused on the sexual reproductive capacity of *Spartina* (Broome et al. 1974; Sayce 1988; Daehler 1996; Plyer & Proseus 1996; Sayce & Dumbauld 1997; Daehler 1999; Davis et al. 2004), rather than asexual production, since this is considered to be the primary source of new clones (Stiller and Denton 1995; Sayce et al. 1997). Seed as well as rhizome fragments could disperse S*partina* locally or across long distances if carried by tides and ocean currents (Daehler and Strong 1996; Stenvall and Patten 2002, Pfauth et al. 2003). Repeated reports of *Spartina* fragments washing ashore near Ft. Stevens (near Astoria, Oregon) suggest transport of wrack from nearby Willapa Bay, Washington (Grevstad and Graves pers. comm.; Howard et al. unpublished report 2004). Huiskes et al. (1995) collected seeds of *S. anglica* in floating and standing nets in a tidal salt marsh in the Netherlands. Eighty-eight percent of the seeds collected were captured in floating nets, indicating that tidal transport of seed was primarily on the water surface rather than along the sediment. In an earlier study in the same location, Koutsaal et al. (1987) released dyed sunflower seeds on outgoing and incoming tides to track tidal movement of seeds in the salt marsh. Seeds were found as far as 45 km away within one week of release. The final location of seeds was determined by the wind velocity and direction as well as by tidal currents.

Oregon's *Spartina* Response Plan (Pfauth et al. 2003) was developed to prevent the introduction and spread of any *Spartina* species in Oregon. Areas requiring further research were identified , including an investigation of the likelihood of the ability of *Spartina* fragments to resprout and an examination of potential transport of propagules via ocean currents.

Preliminary results from three studies addressing these research needs are presented here. First, a field study was performed to assess the production of *Spartina* fragments by rototillng. Secondly, a greenhouse experiment examined the ability of rhizome fragments to resprout. Thirdly, preliminary data from a propagule dispersal study are presented.

MATERIALS AND METHODS

Field Study of Rototilling Effects

Samples were collected on January 16, 2004 along the south shore of the Naselle River, which flows into the southeastern end of Willapa Bay, Washington. Staff of Willapa National Wildlife Refuge (NWR) was mechanically treating the site between high tides with a rototilling attachment towed by a Wilco amphibious vehicle. The Wilco operator made single passes within a solid meadow of *Spartina alterniflora* and tilled to a depth of approximately 15 cm. Immediately following rototilling, three quadrats (0.25 m^2) were randomly chosen approximately 30 m apart and excavated to a depth of 10 cm. Excavated material was rinsed clean and all fragments were measured for culm length, number of culms, and rhizome diameter and length. Fragments were divided into two rhizome sizes, small and large, by the median value for rhizome length. The mean value of each of these rhizome class sizes was then rounded to the nearest half centimeter and used as the experimental rhizome sizes for the greenhouse study of fragment viability.

Greenhouse Study

A 2x3x3 factorial design was used to evaluate survivorship of *S. alterniflora* fragments. Factors were initial rhizome size (large or small), immersion duration (3, 8 or 15 days), and salinity (freshwater, 15 ppt, or 35 ppt). Samples from two populations were compared. Rhizome fragments from San Francisco were collected on March 26, 2004 from the shoreline of Elsie Roemer Bird Sanctuary on Alameda Island (San Francisco Bay, California). Two to three samples were dug from each of ten clones. Samples from Willapa Bay were collected on April 5, 2004 from four riverbank locations (two along the Naselle River, one on the Niawiakum River, and one on the Palix River). Seven to ten samples were dug from each location. Sampling locations had not been subjected to any previous chemical or mechanical treatment.

Samples were returned to Portland State University and rinsed clean of all mud and organic matter within two days of field collection. Within 20 minutes of rinsing, fragments were cut to fit one of two rhizome class sizes (large ~7.5 cm or small ~2.5 cm). Fragments were then placed in open plastic tubs containing water at 0 ppt, 15 ppt or 35 ppt (Instant Ocean® aquarium salts). Tubs were maintained under ambient greenhouse conditions. Salinity concentrations were monitored daily and adjusted as needed. After floating for a period of 3, 8 or 15 days (referred to as immersion duration), each fragment was measured to determine rhizome length, rhizome diameter, number of attached culms and culm length. The 8-day immersion duration was eliminated from the San Francisco treatment design due to limited plant material. Fragments were then individually potted in six-inch diameter pots with a sterile potting medium and each pot placed into a wet bed (1.83 m x 2.44 m wood-framed beds lined with three layers of 6 mm clear plastic) containing either 0 ppt, 15 ppt or 35 ppt saline water to a depth of 10 cm. A total of six wet beds were created (two at each salinity level) with three utilized for the San Francisco fragments and three for the Willapa Bay fragments. Salinity of the wet beds was monitored every one to three days and adjusted with saline or fresh water as needed.

Table 1: *S. alterniflora* rhizome fragment metrics following single-pass, winter rototilling effects in Willapa Bay.

Measure	**Mean ± SD**
Number of fragments per 0.25 m^2 x 0.1m deep	77.7 ± 13.7
Percentage with ≥ 1 culm	87.8 ± 3.86
Rhizome length (cm)	4.96 ± 3.38
Rhizome diameter (cm)	0.58 ± 0.29
Culm length (cm)	5.37 ± 3.58
Culms per fragment	1.31 ± 0.89

A total of 234 fragments, all having at least one culm, were potted from the San Francisco samples. A total of 353 fragments having at least one culm were potted from the Willapa Bay samples. An additional 116 fragments with no culms attached were created from these samples. The purpose of these fragments was to test if survival was dependent upon the presence of at least one culm as suggested by Randall and Milne (unpublished). Small and large rhizome fragments were immersed in the saline baths (0 ppt, 15 ppt or 35 ppt) for either 4 or 16 days. The immersion duration treatments for these culmless fragments were extended by 1 day to allow adequate time for planting of the culm treatment groups. They were then individually potted in the same sterile potting medium and placed in the wet beds.

Pots were randomly positioned within the wet beds. All

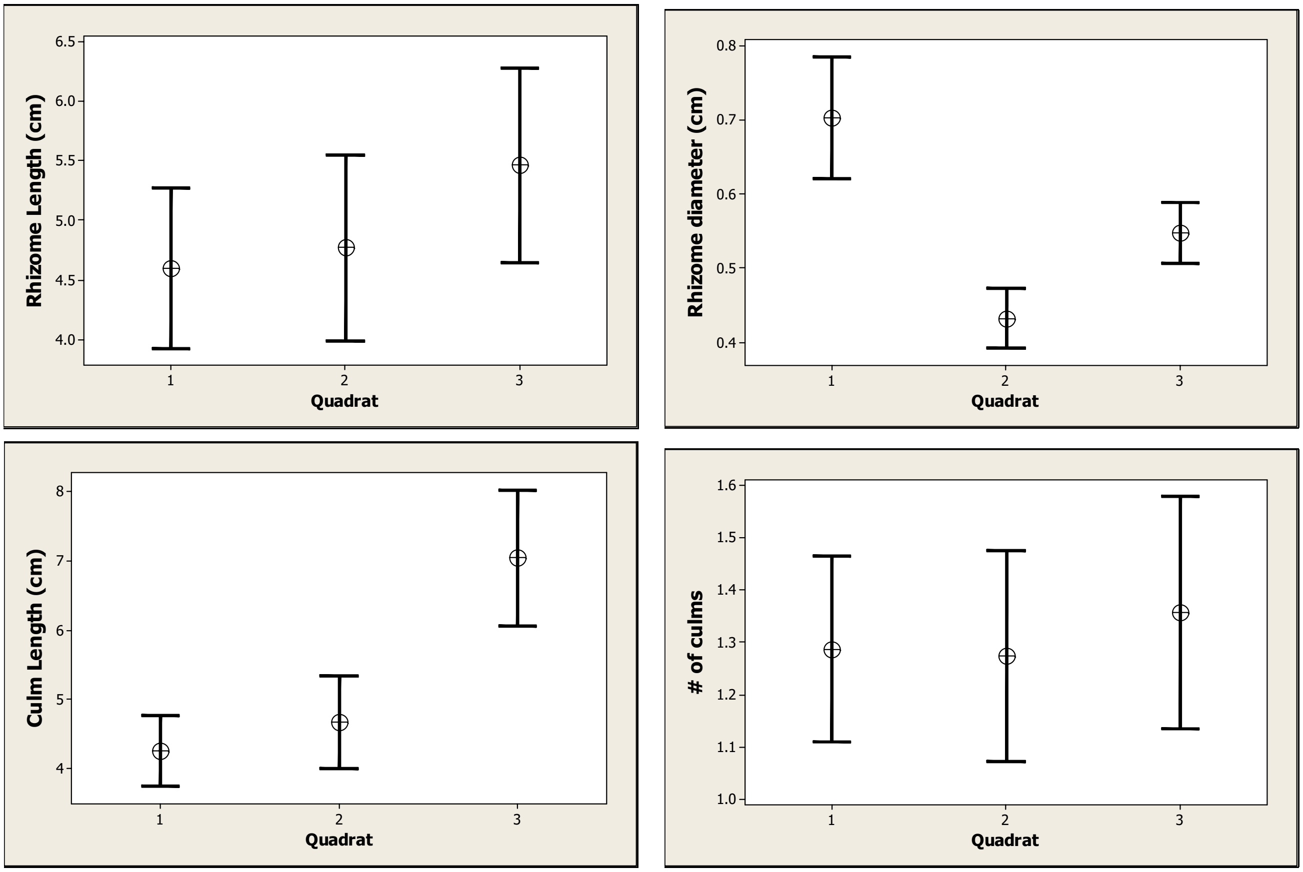

Fig. 1: Interval plots by quadrat (with 95% C.I.) of rhizome length, rhizome diameter, culm length and number of culms per rhizome fragment found immediately following single pass, winter rototilling in Willapa Bay, WA.

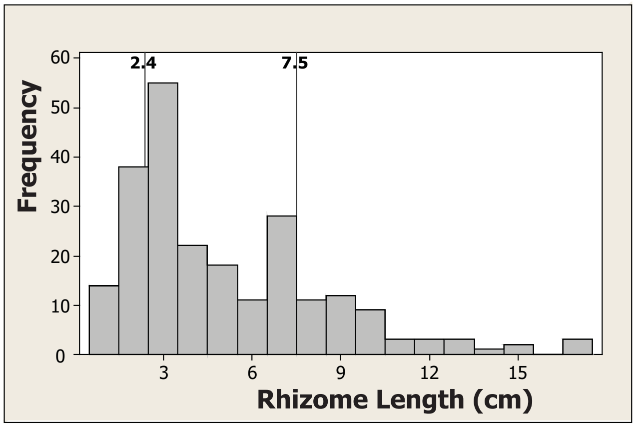

Fig. 2 Rhizome sizes found immediately following single-pass winter rototilling in Willapa Bay, WA.

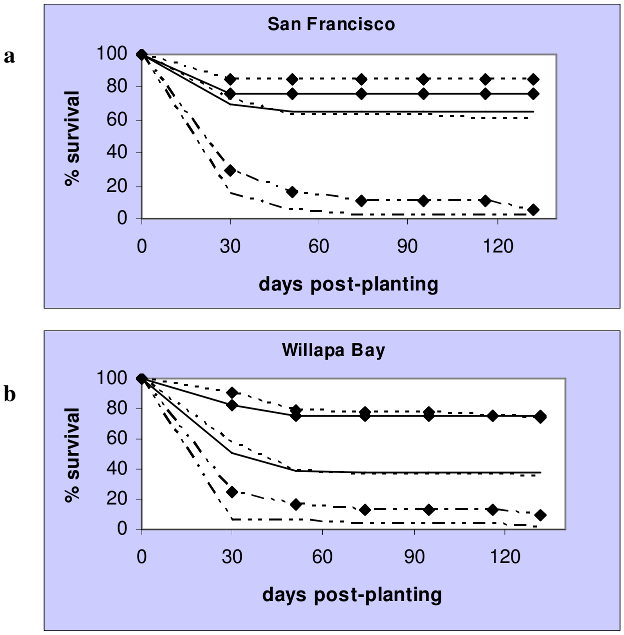

Fig. 3. Percent *S. alterniflora* fragment survival over time for (a) San Francisco and (b) Willapa Bay plants. Treatment groups are delineated by salinity (- - - - - for 0 ppt, ——— for 15 ppt, and — - - — for 35 ppt), and rhizome size (plain line = small, ◆= large).

plants were exposed to ambient light and temperature conditions for 132 days after planting. Survival, culm length and the number of culms were recorded at 30, 51, 74, 95, 116 and 132 days after planting. Survival was defined as the presence of at least one green culm. Roots, rhizomes, culms and inflorescences were separated 132 days after planting and their fresh weight (fw) recorded. Dry weight (dw) was obtained after drying the roots, culms and inflorescences to a constant weight in the greenhouse and oven drying the rhizomes at 65-70°C for 48 hours.

Propagule Dispersal Study

Monthly releases of buoyant, biodegradable wooden drift cards began in September 2004 from the mouths of Willapa Bay in Washington and Humboldt and San Francisco bays in California. A total of 600 cards are released each month—200 each per bay. Releases occurred within two hours after high tide to ensure an outgoing current. Each batch of cards was printed with a unique code denoting the location, month and year of the release as well as reporting instructions and contact information. Velocity estimates were made under the assumption that the recovery date was the same as date the card washed ashore and that the card followed a straight line of travel.

RESULTS

Field Study of Rototilling Effects

The mean fragment density was 310 (± 54.8)/m^2 within 10 cm of the surface. Of these, 87.7% had at least one culm attached (Table 1). No plant material other than *S. alterniflora* was present in any of the plots.

One-way Analysis of Variance (ANOVA) (α=0.05) comparing quadrats were performed on rhizome length and culm length (log(x+1) transformed) and rhizome diameter ((x+1)$^{1/2}$ transformed) and number of culms per fragment. The quadrats did not vary significantly in rhizome length (p=0.240) or the number of culms per rhizome fragment (p=0.322). There were significant differences between quadrats with regard to culm length (p<0.0001) and rhizome diameter (p<0.0001) (Fig. 1).

Rhizomes shorter than the median length (3.7 cm) had a mean of 2.4 cm (± 0.73 Standard Deviation (SD)). Those larger than the median length had a mean of 7.5 cm (± 2.98 SD) (Fig. 2). These two mean rhizome sizes, rounded to the nearest half centimeter, were used to define the small and large rhizome classes used for the greenhouse study.

Greenhouse Survival Study

No new culms were produced by any of the Willapa Bay (WB) rhizome fragments that were planted without culms. Since survival was defined as the presence of at least one green stem, 100 percent of these fragments were defined as dead within the first 30 days of the experiment. Subsequent observations of these fragments showed no signs of culm production. Prior to planting, the position of the fragment within the immersion tubs was observed. While all of the fragments having attached culms remained floating after 15 days, only a few of the fragments with no attached culms remained floating after 16 days.

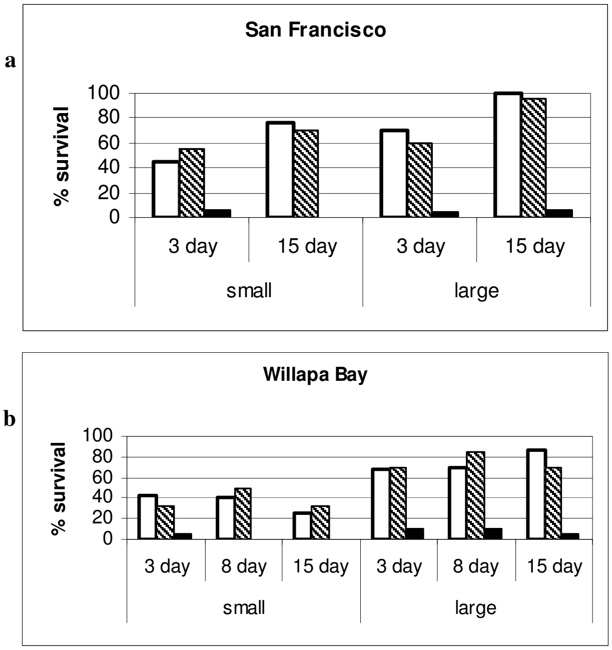

Fig. 4: Percent fragment survival 132 days after planting of *S. alterniflora* from a) San Francisco and b) Willapa Bay. White = 0 ppt, striped = 15 ppt, black = 35 ppt. All fragments represented here were planted with at least one attached culm.

Table 2: Summary data for two months of drift card releases from three S. alterniflora infested bays.

Release Date	**Willapa**	**Humboldt**	**San Francisco**
September 2004			
Recovery Rate	57.5%	21.5%	30.5%
Quantity of recovered cards			
North	79	10	12
South	34	31	19
Inside bay	2	2	30
Max distance traveled (km)			
North	95	75	45
South	35	65	20
October 2004			
Recovery Rate	29.5%	0.5%	24.0%
Quantity of recovered cards			
North	56	1	6
South	3	0	41
Inside bay	0	0	1
Max distance traveled (km)			
North	223	6	75
South	35	na	30

The proportion of surviving fragments per treatment group stabilized by the end of the growing period (Fig. 3). For San Francisco (SF) plants, survival 132 days after planting ranged from 45-100% in the freshwater treatments, 55-90.0% in 15 ppt water and 0 to 5.56% in 35 ppt water. For WB plants, survival ranged from 26.3-81.8% in the freshwater treatments, 27.8-88.9% in 15 ppt water and 0-11.1% in 35 ppt water.

For nearly all SF groups, rhizome fragments immersed for 3 days prior to planting showed lower rates of survival than those immersed for 15 days (Figure 4). The same pattern emerged with the WB 15 ppt fragments where the three-day immersion groups showed much lower survival than the eight-day immersion groups. Large rhizome fragments consistently had higher viability than small rhizome fragments. For SF plants, 80.3% of large fragments survived compared to only 62.5% of the small fragments. For WB plants, the difference was more pronounced with 74.8% of large and 36.2% of small fragments surviving. SF and WB populations were compared using a two-tailed test of two proportions; there were significant differences between the proportions surviving in both freshwater (72.5% vs. 55.5%, respectively, $p=0.012$, $\alpha=0.05$) and 15 ppt water (70.1% vs. 56.0%, $p=0.043$, $\alpha=0.05$). In both of these comparisons, SF fragments had higher survivorship. There was no notable difference between survival rates for the two locations (3.9% vs. 5.1%) in the high salinity treatment ($p=0.691$, $\alpha=0.05$).

PROPAGULE DISPERSAL STUDY

Drift card return rates have been over 20% for five of the six releases performed as of November 11, 2004 (Table 2). Cards have consistently been found both to the north and south of each release location. In four of the six releases performed to date, cards have been found inside the estuaries. The majority of cards are staying within 25 km of the release locations, although a few have traveled longer distances.

In Willapa Bay, over 69% of the September release cards and 95% of the October release cards were found to the north of the bay. Maximum northward velocities for September and October releases were 6.9 and 11.2 centimeters per second (cm/s) respectively, while maximum southward velocities reached 6.7 and 3.4 cm/s.

In Humboldt Bay, approximately 72% of the September cards were carried south. Maximum velocity for September release was approximately 3.4 cm/s both to the north and south. Only one card, found after six days approximately six km north of the Humboldt Bay entrance, was recovered from Humboldt's October release.

The highest number of cards found within an estuary occurred in the case of the San Francisco September release when thirty cards were recovered on the eastern edge of the bay, mainly near the Berkeley and Albany shoreline. Observed winds at the time of that release were from the west at approximately 7.7 – 10.3 m/s. Recoveries of San Francisco cards not blown back into the bay after the September release showed 61% were carried south. Maximum velocities for this release were approximately 2.4 cm/s to both the north and south. Eighty-seven percent of October cards were found to the south. Maximum velocities were 5.4 cm/s to the north and 6.9 cm/s to the south.

DISCUSSION

Rototilling appears to have fairly uniform cutting action on solid meadows; it produces rhizome fragments of consistent size and with similar numbers of attached culms. A high percentage (87.8%) of the *S. alterniflora* fragments produced by rototilling in Willapa Bay had at least one attached culm. The observed difference in culm length and rhizome diameter between quadrats was likely a result of variations in the age of coalesced clones, rather than variable tearing action by the tilling blades. Assuming uniformity in the production of rhizome fragments (approximately 312 fragments/m^2 within the top 10 cm) we could make a conservative estimate that 0.5% of fragments might be loosened by wave or tidal action, becoming suspended in the water column. Based on those assumptions, as many as 15,600 fragments might be distributed in the open water for every hectare rototilled (~6,300 per acre).

Viability of rhizomes after floating at the water surface seems to be primarily dependent on the presence of at least one culm. Randall and Milne (unpublished) found that rhizome fragments 2.5 to 15 cm long with no attached culms had 100% mortality regardless of position on the mud-flat substrate or beneath it at various depths. The presence of attached culms should allow the fragment to respire and thereby increase its chance of survival. In fact, repeated mowing to remove vegetative shoots caused a reduction of oxygen to the root system and was initially utilized as a control method for *Spartina* (Ebasco Environmental 1993; Hedge et al. 1997). Of the 116 rhizomes planted without attached culms during the greenhouse study, none survived. Compared to fragments planted with culms, this finding seems to support the theory that at least one vegetative shoot is needed for survival of vegetative propagules.

For fragments having culms, salinity and initial rhizome size determined survival. The 35 ppt treatment resulted in notably poorer survival rates compared to the lower salinity treatments. Differences in 0 ppt and 15 ppt treatments appeared largely due to initial rhizome size. Larger rhizomes would logically have higher chances of survival since they would be likely to have more nodes, a greater number of established roots and more non-structural carbohydrates to fuel new growth.

The length of immersion was a less important determinant of survival and establishment or rhizome fragments than salinity or rhizome size. Longer immersion durations may increase viability, although this effect was not consistent. Three to four months of wet, cool conditions may help break seed dormancy and increase germination rates by leaching a germination inhibitor. Similarly, the conditions that fragments are exposed to while floating in open water may retard growth of pathogens, encourage shoot production or elongation or otherwise increase chances of survival.

Repeated monitoring of treated sites in Willapa Bay has shown that mechanical treatments such as rototilling and disking have higher efficacy during the period of December through February (Patten & Stenvall 2002). All of the plants used for this study were collected four to five weeks after the normal rototilling period in Willapa Bay. Increased culm length, as well as higher air, soil and water temperatures at the time of collection, may have increased survival rates. Additionally, the vigorous action of rototilling produces more ragged edges and somewhat damaged culms than were reproduced in the greenhouse. This might also elevate rates of survival shown here.

Preliminary results from the first two months of the drift card study suggest propagule deposition from infested estuaries lessens with increased distance. Flow over the coastal shelf is predominantly poleward in the winter and early spring, with mean current velocities of 20 cm/s. Summertime flow is typically southward with mean velocities of 10 cm/s. Dispersal patterns seen from these preliminary findings may be due to a seasonal transition period between these predominant currents. Recovery patterns may also reflect wind forcing and local eddies from the mouths of the release estuaries. Frequent recoveries in beaches along Long Beach peninsula, where *Spartina* wrack is commonly found in the fall, suggests that the cards simulate wrack dispersal with some accuracy.

CONCLUSIONS

The estimate of 15,600 fragments per hectare may seem inconsequential when compared to estimates of seed germination rates for *S. alterniflora* which range from nine to nineteen million seeds per hectare (3.7 million to 7.7 million seeds per acre) (Callaway 1990; Daehler and Strong 1994). However, understanding the risks associated with all control methods is necessary when site-specific treatment decisions are made. Rototilling or other mechanical disturbance that produces fragments larger than 2.5 cm should be used with caution in areas with fresh to moderately brackish (mesohaline) waters. Mechanical disturbance following some other treatment method, such as herbicide application, may pose less of a risk of starting new clones. If the infestation is isolated and/or the population is not setting seed, caution may be warranted in using rototilling or similar mechanical treatments since it could produce viable propagules in areas with few other loci of dispersal.

A greater understanding of the dispersal patterns from infested bays should help to identify the risk of seed or vegetative propagule transport. This data, combined with known characteristics of susceptible habitat, will help identify natural deposition sites of invasive *Spartina* spp.

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COVERING THE *SPARTINA* THREAT: AN ALTERNATIVE CONTROL METHOD FOR NON-NATIVE *SPARTINA PATENS* IN A WEST COAST SALT MARSH

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On Oregon's central coast, the Nature Conservancy's Cox Island Preserve harbors the introduced salt marsh grass *Spartina patens* (saltmeadow cordgrass), the only known infestation in the state. The 187-acre preserve lies in the Siuslaw River, seven miles inland from the Pacific Coast. *Spartina patens* is native to the east coast of the United States, found from Newfoundland to Texas. It was probably introduced to the west coast in the early 1900s. *Spartina. patens* invades mid-marsh communities at elevations ranging from 1.83 to 2.05 meters (m) above mean low water (Frenkel and Boss 1988). Initially, it spreads primarily by rhizomes and forms circular, monotypic stands which crowd out native plants and eliminate wildlife habitat. These *Spartina* patches accumulate sediment and litter at a faster rate than the surrounding marsh vegetation, thus altering the natural succession of the site. To restore Cox Island and prevent *S. patens* from spreading to other estuaries, we field-tested methods that have shown promise at controlling *S. patens* elsewhere. Covering with heavy-duty landscaping fabric anchored by spikes was the most effective control method of those we tried. Leaving the fabric on for two years kills the *Spartina*. After removal, native salt-marsh vegetation re-colonizes on its own. To date, 0.81 hectares (ha) (2 acres [ac]) have been restored and another 1.62 ha (4 ac) are now covered. This methodology may not be feasible for large-scale control efforts on well-established infestations, but it is a very viable option for control efforts at the most effective time to eliminate invasive non-native species, during the early stages of an infestation.

Keywords: *Spartina patens*, non-chemical control, salt marsh management, geotextile covering

THE *SPARTINA* THREAT

The only known Oregon occurrence of the introduced salt marsh grass *Spartina patens* (saltmeadow cordgrass) occurs on the Nature Conservancy's 187-acre Cox Island Preserve located in the Siuslaw River. *Spartina patens* is native to the east coast of the United States, found from Newfoundland to Texas. It was probably introduced to the west coast around the turn of the 20th century.

Spartina. patens invades mid-marsh communities at elevations ranging from 1.83 to 2.05 meters (m) above mean low water (Frenkel and Boss 1988). Initially, it spreads primarily by rhizomes and forms circular, monotypic stands which crowd out native plants and eliminate wildlife habitat. These *Spartina* patches accumulate sediment and litter at a faster rate than the surrounding marsh vegetation, thus altering the natural succession of the site.

At Cox Island, *S. patens* apparently established in undisturbed vegetation before 1939. Since then, it spread to cover approximately 1.1 hectares (ha) (2.7 acres [ac]) by 1996. If uncontrolled, it could continue to spread until all available habitat is occupied (Frenkel and Boss 1988).

EARLY DETECTION PARTNERSHIP

Other invasive species of *Spartina* from the east coast have become established in Washington and California. For example, *S. alterniflora* has infested Willapa Bay in Washington where it is eliminating an important feeding area for migratory waterfowl by invading mudflats and forming a *Spartina* monoculture (Aberle 1993).

Studies indicate that 13 Oregon estuaries are at risk of invasion by non-native *Spartina* species (Daehler and Strong 1996). Seeds or other propagules could be dispersed to new areas by migratory waterfowl, dredging operations, shellfish harvesting or movement of materials between oyster-producing areas. Early detection is essential to prevent new infestations in Oregon (Pfauth et al. 2003).

The Nature Conservancy has partnered with the Oregon Department of Agriculture (ODA) and the Siuslaw Watershed Council to control *Spartina* on the Cox Island Preserve and to detect any new infestations in the state. In 2003, ODA developed a *Spartina* Response Plan to protect Oregon estuaries from *Spartina* invasions (Pfauth et al. 2003). Nature Conservancy and Watershed Council members have volunteered to survey salt marsh areas in two estuaries for invasive *Spartina* species. Two patches of *S. patens* were found in the Siuslaw Estuary on property adjacent to Cox Island and were controlled. No other *Spartina* was found.

RESTORING COX ISLAND

To restore Cox Island and prevent *S. patens* from spreading to other estuaries in Oregon, the Nature Conservancy field-tested several control methods from 1996 to 1998 that have shown promise at other sites, including covering with heavy-duty landscape fabric, repeated mowing, artificial inundation and removal by manual digging.

Results of these tests indicated that covering the *Spartina* with landscaping fabric (Mirafi 500 or Amoco 2002) anchored by gutter spikes pushed into the sediment was the most effective and least environmentally detrimental method, of those that were tried, for controlling this invasive species at Cox Island. Leaving the fabric on for two years effectively kills the *Spartina,* leaving behind bare ground. After removal of the fabric, native salt-marsh vegetation re-colonizes these bare patches on its own so active planting of native vegetation is not necessary.

The landscaping fabric we use (Amoco 2002) is a tough, woven black plastic fabric (geotextile) that resists UV light and holds up well in a saltwater environment. We have successfully used pieces of this fabric for six consecutive years (on three different patches for two years each) before it became too thin to block light. Since the fabric is woven, cut edges need to be folded under so the wind does not unravel the edges. This fabric is available from ACF West Inc. in Portland, Oregon (1-800-878-5115 or 503-771-5115). Its cost ranged from $315/roll (picked up) to $385/roll (locally delivered). These rolls contain 6,300 square feet ($ft^{2)}$ of fabric and measure 18 ft x 350 ft.

To install the fabric, we identify the spatial limits of the patch, mow the vegetation around the boundary of the patch so the fabric lays flat, cut a piece of fabric of sufficient size to extend well beyond the border of the patch, and pin in place with gutter spikes every two to three feet along the edges while pulling the fabric taut. The fabric should extend a minimum of two feet beyond the edge of patches to help prevent any rhizomes from growing out beyond the covering. We fold the edge of the fabric under and push a spike with a washer (to keep the head of the spike from going through the fabric) through the fabric and pound it into the substrate. If the ground is too soft in places to get a good grip with the gutter spikes, we use nine-inch nails instead. (These also tend to last longer in the saltwater environment and do not need washers.). We angle the spikes toward the center of the covering as we drive them in to help prevent the tidal currents and wind from pulling up on the edges. If additional anchoring is needed, for example where two pieces of fabric overlap, two spikes can be driven in at opposite angles in the same spot.

In the fall of 1998, we began efforts to control the small outlier patches of *Spartina* before they could turn into large patches. This strategy of beginning with small outliers has proven to be the most effective way to contain a non-native species invasion (Moody and Mack 1988). As of 2004, we have controlled all of the outliers and are making progress on the main infestation area. The *Spartina* in this area has grown from three patches in 1939 to a *Spartina* meadow today. We are covering the perimeters of these meadows to halt their spread and will work towards their interiors in successive years. Each year we mow flowering patches that have not yet been covered to prevent seed set. The patches are mowed from mid- to late-August with gas-powered string trimmers and are cut well above the ground surface to avoid tearing out rhizomes that might spread the infestation.

Overall, we have successfully restored about 0.81 ha (2 ac) of former *Spartina* patches to native salt marsh. At least another 1.62 ha (4 ac) are currently covered with fabric. We estimate that 0.43 ha (1.1 ac) of large *Spartina* patches remain uncontrolled on the island, which we hope to cover by the end of 2005. Controlling this invasive species on Cox Island will not only restore native salt marsh to the island, but will also help to prevent *Spartina's* spread to other parts of the Siuslaw Estuary and to other estuaries in Oregon and Washington.

I believe this weed control method shows promise for use on other invasive species as well (we are currently trying it on upland grasses and reed canarygrass [*Phalaris arundinacea*]). While it may not be practical for well established invasive species populations that cover more than 4-8 ha (~10-20 ac), it is a viable non-chemical control method for outlier patches and small pioneering infestations on a site.

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COMMUNITY *SPARTINA* EDUCATION AND STEWARDSHIP PROJECT

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People For Puget Sound's Community *Spartina* Education and Stewardship Project is a community-based *Spartina* control program targeted at small groups of private property owners and select public beaches. The goals of the program are to: 1) educate and mobilize shoreline property owners through stewardship to eradicate *Spartina* from their beaches, and 2) educate and involve all citizens in the Puget Sound-wide *Spartina* control effort through participation in large, organized community *Spartina*-removal dig events. This project includes a strong component of monitoring and long-term stewardship driven by property owners and partnering organizations/agencies.

Keywords: Community, stewardship, education

INTRODUCTION:

In Washington State, *Spartina* is an invasive, salt-tolerant weed that threatens our natural shoreline ecosystems. It aggressively displaces native habitat, and left untreated could cause irreversible damage to the nearshore environment of Puget Sound. State agencies and organizations involved in *Spartina* control agree that education of the public is key to the weed's eradication from Puget Sound. The goal of People For Puget Sound's Community *Spartina* Education and Stewardship Program, launched in 2004, is to enhance community education, involvement and stewardship in the Puget Sound by targeting areas of small *Spartina* infestations, generally under one acre, in sites with multiple private shoreline property owners.

Research indicates that if eradication of *Spartina* is to occur in an effective manner, individual infestations must be treated simultaneously and in a coordinated effort. In addition, the monitoring of sites post-eradication is necessary to ensure that re-invasion of *Spartina* does not occur. This education and stewardship project will act as an investment in the future of *Spartina*-free beaches in Puget Sound, by focusing on community involvement and highlighting the importance of stewardship. The project enhances our own current programs, other local stewardship programs, and builds the key link between the agencies committed to *Spartina* removal and the citizens who depend on the health of the sound.

The project works in close partnership with various regional, state, local and tribal agencies in Puget Sound to maximize the effectiveness of our efforts. Washington State Department of Agriculture (WSDA), Skagit and Island County Noxious Weed control boards, the Northwest Straits Commission, Washington State University "Beach Watchers" program, and tribal communities assist in building connections with the wider community and in staging large public dig events to raise awareness of the *Spartina* problem.

METHODS

Three to five priority shoreline communities are targeted for outreach each year of this project. The project manager works with the state agencies, especially WSDA, to prioritize sites for outreach, and works with other local partners to identify an interested neighbor in the target area to act as a community steward. The manager then assists the steward in hosting a '*Spartina* Social' with neighbors to educate them on *Spartina's* environmental impacts, control methods, eradication strategies, monitoring and stewardship, as well as roles community members can play in the project. Generally two to six additional neighbors are also interested in becoming stewards for the community following this initial meeting.

Community stewards are trained to conduct baseline surveys and monitoring at the site twice a year, and are equipped and supported in hopes that they will continue monitoring for up to 15+ years. We opted to use the protocol for photo-point monitoring and photo-plot monitoring outlined by Portland State University's Environmental Sciences and Resources Program Student Watershed Research Project (www.swrp.org). Photo-point monitoring involves taking landscape photos of the area of concern to monitor overall changes at the site each year, including the presence of *Spartina*. In addition, one or two photo plots were established in each site to capture an area of one square meter (1 m^2) of *Spartina* to monitor the percent coverage per year in those plots. Baseline monitoring was conducted in each community between August and October 2004. Community stewards will continue monitoring each year in late May and again post-control (September or October).

Community stewards were provided with all necessary monitoring materials. Each community received a photo-monitoring instruction manual, including monitoring guidelines, data entry sheets, photo marker tags, monitoring equipment, a copy of People For Puget Sound's Sound Stewardship native and invasive plant ID guide, and contact information for the Island County Noxious Weed Control Board. In addition to the manual, stewards received a 1 m^2

PVC photo plot, rebar stakes for marking photo points, and a compass for calculating directional bearings for photos. The communities provided their own digital cameras for the monitoring.

RESULTS AND DISCUSSION

People For Puget Sound's project manager established an initial steward in each community in July and August 2004 through our 2003 outreach efforts and with the assistance of the Washington State University Extension Beach Watchers Program. To date, the project has conducted outreach to the communities of Juniper Beach and Eagle Tree Estates on Camano Island, and Harrington Lagoon on Whidbey Island. A neighbor from a fourth community, Cavelero Country Club on Camano Island, attended another community's meeting and has been working with the project manager to get her community involved in the project.

The project manager met with the community stewards between August and October to conduct *Spartina* surveys of the neighborhood's shoreline, and training on monitoring techniques. Baseline visual and photographic surveys were conducted at this time, along with photo monitoring. The project manager also began training the stewards in native plant identification, focusing on plants commonly confused with *Spartina*.

People For Puget Sound and its partners also hosted three large, public *Spartina* dig events from June through August 2004. These events focused on manual removal in areas where chemical control is not desired. Volunteers were trained in manual removal techniques including digging and seedling removal with an emphasis on removing all rhizomatous roots to prevent regrowth.

The project manager worked with partners to educate the general public about *Spartina* through these community dig events. The 6th Annual Skagit Dig Days was held on June 19th at the Swinomish Casino lagoon. This event drew 38 volunteers for four hours of manual removal. Two additional dig events were held in Island County: Oak Harbor on Whidbey Island had 14 volunteers for three hours on August 14, and Iverson Spit on Camano Island drew 22 volunteers for four hours on August 28.

Monitoring surveys of approximately 20 hectares (48 acres) of area affected by *Spartina* were conducted between May 1 and October 31 This number was estimated from data in WSDA's 2003 *Spartina* Eradication Program Report to the Legislature, PUB 805-110 (available online at http://agr.wa.gov/PlantsInsects/Weeds/Spartina/default.htm). It is projected that this same area will be monitored one additional time during the course of this grant period, ending May 2005. The three dig events resulted in an estimated 0.6 hectares (1.5 acres) of *Spartina* removed from the nearshore. Outreach to the private communities was not intended to result in direct control of *Spartina*, since the Island County Noxious Weed Control Progrram is currently managing control efforts throughout the county. We expect that WSDA will report the results of acres treated at these sites in its 2004 report to the Legislature.

Thus far this project has trained over 70 citizens in proper identification and manual removal methods for invasive *Spartina* through the large community dig events. We have educated over 60 private shoreline property owners on *Spartina*'s environmental impacts, control methods, identification, and options for community-based eradication through community meetings and presentations. The project manager has trained 11 stewards from the three communities in proper monitoring techniques. The stewards have agreed to conduct photo monitoring of their community beaches twice a year, conduct visual monitoring throughout the year, report all monitoring activities to the project manager, provide copies of all monitoring photos, work with the project manager and Island County Noxious Weed Control Board to organize control efforts, and commit to long-term stewardship of their community beaches for hopefully 20 years or more.

We have received very positive feedback from the public for this project and we feel that this first year has been a great success. The project will continue to conduct outreach to three to five additional communities each year, while maintaining the necessary level of involvement in current communities' activities. The project manager is looking into expanding the project in upcoming years in several areas. There is a distinct need to conduct yearly GPS surveys of each community's infestation and to acquire GIS capabilities that would allow for the production of maps and other visuals.

The project manager is also investigating options for an online database that can be accessed by our staff, community stewards and neighbors, and our partnering agencies and organizations. The project manager will also be working with WSDA, NOAA, and Restore America's Estuaries to develop a *Spartina* stewardship manual and a regional *Spartina* information and identification flyer.

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BIOLOGICAL CONTROL OF SPARTINA

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Biological control using introduced natural enemies can be an effective approach to the long term control of widespread weeds. A biological control program against *Spartina* spp. is underway in Washington State, where more than 10,000 hectares (ha) of intertidal mudflat are affected by *Spartina alterniflora* and *Spartina anglica*. Releases of the planthopper *Prokelisia marginata* have been made into Willapa Bay each year since 2000 and into Puget Sound since 2003. Prior to introducing this insect, rigorous host specificity testing and a review by the Technical Advisory Group on Biological Control of Weeds confirmed that the risk to non-target plants was minute. Populations of the biocontrol agent were initially slow to establish and grow. However, early problems with high winter mortality have been remedied through a combination of improved release site selection and the use of cold-hardy east coast biotypes. At least two populations in Willapa Bay are well established and expanding. At a localized scale, we have measured 50 percent reductions of *Spartina* biomass and 90 percent reduction in viable seed set due to *P. marginata*. The full extent of the impact will only be known with time.

While the use of biological control in California may pose a risk to the closely related native *Spartina foliosa*, it would be an excellent option in other other parts of the world where *Spartina* has invaded and where there are no closely related native *Spartina* species. In addition to *P. marginata*, other candidate biocontrol agents from the Atlantic Coast are currently being investigated.

Keywords: Biological control, *Spartina alterniflora*, *Spartina anglica*, *Prokelisia marginata*, Willapa Bay, Puget Sound

INTRODUCTION

Classical biological control of a pest or weed involves the introduction of a natural enemy (biocontrol agent) from another geographic region. The goal is to establish a permanent population of the biocontrol agent that will provide long-term control. During the last century, close to 1,000 biological control introductions for weeds were made throughout the world (Julien and Griffiths 1998). Modern weed biocontrol projects in the United States proceed only after extensive testing of the natural enemy followed by a review by the federal Technical Advisory Group on Biological Control of Weeds to ensure that it will not harm other organisms. Weed biocontrol has proven to be a safe and often very effective method of long term control of widespread invasive plants (Cruttwell-McFadyen 1998).

Biological control has both advantages and disadvantages over traditional control. Unlike most chemical and mechanical approaches, biological control is highly specific to the target weed. Since biological control agents have been chosen for their host specificity, they will not harm other plant species intermixed with the weed. Biological control is economical over large areas. Once established, the biological control agent will reproduce, spread to new sites, and continue to damage the plant with little or no additional input. Biological controls have no toxic residues or health hazards as do some herbicides. When it is successful, biological control can provide a permanent solution to a weed problem, although it usually maintains a very low level of infestation rather than bringing about full eradication.

The disadvantages of biological control include the large amount of pre-release research that is required. Moreover, even with careful selection of host-specific agents, there will always be some small risk to non-target organisms from either direct or indirect interactions. Biological control is often slow in its action, taking several years and even up to a decade for an impact to be seen. Finally, the complexities of ecological interactions mean that the effectiveness of biological control is difficult to predict ahead of time. The probability of successful control increases when multiple biocontrol agents are used (Denoth et al. 2003).

A biological control program for *Spartina alterniflora* and *Spartina anglica* in Washington State was developed during the late 1990s. The biocontrol agent *Prokelisia marginata*, a sapsucking planthopper, was introduced beginning in 2000. In this paper, we provide background information about this biological control program. Then we present results of a comparison of the performance of four populations of *P. marginata* imported from different geographic locations. Finally, we present possible future directions for the biocontrol program including the screening of additional agents from *S. alterniflora*'s native range.

BACKGROUND ON *SPARTINA* BIOCONTROL IN WASHINGTON STATE

To date only one biological control agent has been introduced into Washington for control of *Spartina* spp. The delphacid planthopper, *Prokelisia marginata*, was introduced from California into Willapa Bay beginning in 2000 and into north Puget Sound in 2003. The introductions were made only after extensive testing demonstrated its high level of host specificity (Grevstad et al. 2003) and after ruling out the possibility that it could vector a disease (Davis et al. 2002). The project was reviewed and approved by the Technical Advisory Group on Biological Control of Weeds and permitted by the Washington State Department of Agriculture and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS).

P. marginata is native to the Atlantic and Gulf Coasts of North America and also occurs in San Francisco Bay, California. Genetic analyses (R. Denno and D. Hawthorne, University of Maryland, pers. comm.) indicate that *P. marginata* was probably introduced to California from the East Coast in recent decades. The absence of *Prokelisia* spp. in an early 1970's survey of insects on *Spartina foliosa* in San Francisco Bay also supports a recent introduction to the West Coast (Cameron 1972). *P. marginata* was selected as a promising biocontrol agent because of its narrow host range and its known potency against *S. alterniflora* and *S. anglica* (Daehler and Strong 1997; Wu et al. 1999). *P. marginata* adults and nymphs feed by sucking the sap from the plant, draining its energy supply. *Spartina* is also damaged by the scars that arise on the leaf surface where adult females insert eggs. If high enough densities of *P. marginata* are attained, feeding and oviposition scars cause the leaves to turn brown and eventually kill the plant.

The *Spartina* biocontrol program is unique in being the first classical biocontrol program to target a grass, although others are being considered (Tewksbury et al. 2002; Witt and McConnachie 2004). It is also the first application of classical weed biocontrol in a marine intertidal environment. This project differs from most classical biocontrol projects in that the targeted weed is invasive in the same country to which it is native (although a different region). The biocontrol agents are likewise transferred between states rather than between countries.

To the advantage of a biological control program, invasive *Spartina* in Washington appears to have lost resistance to herbivory since its introduction. In greenhouse experiments (Daehler and Strong 1997; Wu et al. 1999; Garcia-Rossi et al. 2003), plants from the invasive populations in Washington suffer much greater biomass reduction and mortality from *P. marginata* than plants from native locations. Herbivore exclusion and addition experiments in the field also demonstrate this difference in response (compare Daehler and Strong 1996 with Grevstad et al. 2003). The vulnerability of the Washington populations may be due to an evolved loss of resistance in the absence of herbivores (Garcia-Rossi et al. 2003). The mechanism of *Spartina* vulnerability is unknown, but it may be related to the structural breakdown of vascular cells as a result of piercing by the planthopper during feeding and oviposition (Wu et al. 1999). The possibility that the vulnerability is due to a disease vectored by the planthopper was ruled out by Davis et al. (2002).

Over the past few years, *P. marginata* has been released at 40 locations in Willapa Bay and Puget Sound. Results have been encouraging, but not without setbacks. Following release, *P. marginata* populations typically grow explosively during their first summer and cause visible damage to the plants by fall (Grevstad et al. 2003). Local densities in some sites have exceeded 50,000 insects per m^2. A 50% reduction in local biomass was measured in an early field cage experiment (Grevstad et al. 2003). A 90% reduction in field seed viability was found in localized areas where *P. marginata* density was greater than 30 per stem (Grevstad, unpublished data). However, low survival of nymphs over the winter has prevented these populations from building to the high densities over large areas required to have large-scale impacts on the *Spartina* population. In spite of the summer boom, *P. marginata* populations are typically much smaller the following spring than they were at the time of release. Some of these populations eventually build up densities, but many have gone extinct and others are dwindling or growing only slowly.

Determining the best geographic source of P. marginata

In selecting a geographic source of a biocontrol agent, it is important to consider the ways that herbivore populations may be locally adapted. Classical biological control programs often seek an agent source population from a location that has a climate similar to the region where it will be introduced. However, in the case of the *Spartina* biocontrol program, a close match to the Willapa Bay climate does not exist. The San Francisco Bay area has a similarly moderate climate but temperatures are approximately 5ºC warmer at all times of year. East Coast locations have more extreme seasonality and no location can match both winter and summer temperatures. A northeastern location such as Rhode Island has the best match during the summer months, but a mid-Atlantic location, such as Virginia, has the best match during the winter.

In addition to climate and host plant adaptations, seasonal adaptations affecting the phenology of the biocontrol agent are also likely to vary among potential agent source populations. Many insects use photoperiod as a cue for synchronizing life history events with seasonal change in environmental conditions. When an insect is moved from one geographic location to another, its phenology may not be synchronized to the new seasonal schedule. In Willapa Bay, the California population of *P. marginata* has been observed to emerge in late February, a time that may be too early for nymph survival in the cooler and longer winters of coastal Washington. Based on a match of the timing of arrival of warm temperatures, a Rhode Island source may be the best match. However, the possibility that late emergence could reduce the number of generations produced each year makes this outcome uncertain.

Another consideration in choosing a source is potential variation in ability to compensate for plant defenses. A population from a location farther south, such as Georgia, while poorly adapted climatically, could be better adapted for overcoming plant defenses to herbivory, which are known to be greater in southern *Spartina* plants (see Pennings et al. 2005 and Katz et al. 2005 in these proceedings). A "new association" between a southern herbivore and northern plant could make biocontrol more effective.

In the spring of 2004, after obtaining permits from USDA-APHIS and the Washington State Department of Agriculture, four populations *P. marginata* were introduced into Willapa Bay. The source populations were (1) Sausalito, San Francisco Bay, California, (2) Grayville, Rhode Island, (3) Quinby, Virginia, and (4) Jekyll Island, Georgia. All populations passed through a period of quarantine in a laboratory in Davis, California before being imported into Washington for rearing in a greenhouse.

At each of five sites in Willapa Bay, we set up four 4x4-meter (m) release plots located at similar tidal elevations within the same *Spartina* meadow, but separated by at least 100 m. Each plot was randomly assigned one of the four source populations. Five thousand insects of the assigned population were released into each plot by placing heavily infested *Spartina* stems clipped from five rearing plants uniformly throughout the plot. A 100 m distance between release plots ensures that populations will remain separate long enough to compare their phenology and performance. *P. marginata* were sampled in late September using an insect vacuum converted from a leaf blower. Eight uniformly spaced sample points 21-cm in diameter (corresponding to the vacuum tube's diameter) within each release area were vacuumed. Adults and nymphs were counted separately and adults were scored for wing form;

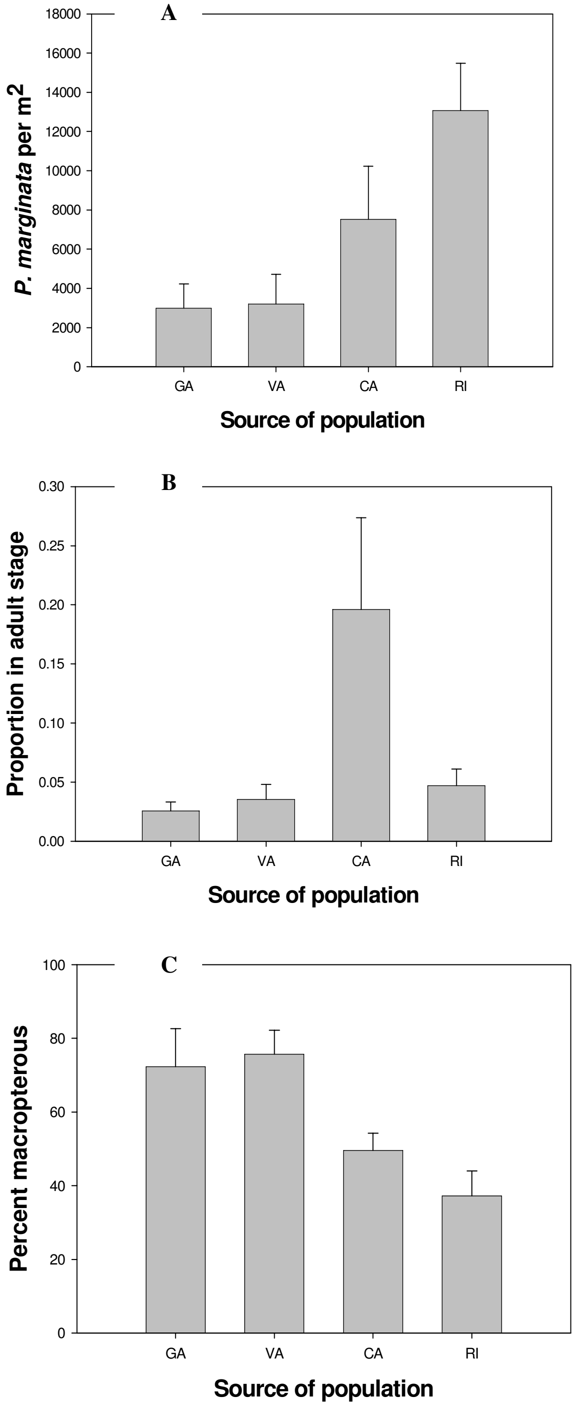

Fig. 1. (A) Densities of *Prokelisia marginata* measured at the end of September following introduction of 5,000 individuals from each of four geographic sources. (B) The proportion of each source population in the adult stage at the end of September. (C) The percentage of adults in each population that were macropterous (long-winged). Each bar represents the mean and standard error for five replicate populations from each source.

either brachypterous (rudimentary or abnormally small) or macropterous (long or large).

We found striking differences among the four populations in the densities attained by the end of summer (Fig. 1A; ANOVA F=9.227, P=0.002). The best performing geographic source, in terms of the densities of *P. marginata* obtained by end of summer, was Rhode Island, which is also the location with the best summer temperature match to Willapa Bay. In post-hoc tests, Rhode Island populations significantly differed from Georgia and Virginia, but did not significantly differ from California. California did not significantly differ from Georgia and Virginia.

The populations also differed in the proportion of the population that was in the adult stage at the end of September (Fig. 1B; ANOVA F=3.637, P=0.030). California populations had four to five times the proportion of adults found in the other populations. This may indicate differences in phenology although further data is needed.

Finally, we found differences among the four populations in the proportion of individuals that were macropterous (Fig. 4C; ANOVA F=8.71, P<0.001). Macroptery frequency in *P. marginata* is a plastic response that increases with unfavorable conditions (Denno et al. 1985). The low proportion of macropters in the Rhode Island populations could be an indication that the conditions in Willapa Bay are favorable for that ecotype. However, it could also reflect inherent genetic differences among populations.

So far, these results suggest that the Rhode Island population may be best suited to the Willapa environment, at least during the summer months. The timing of spring emergence, winter survival, and overall population growth from year to year will determine which population is most successful in the long term.

Potential future biocontrol agents

While we expect *P. marginata* to have an impact on *S. alterniflora* and *S. anglica* in at least some areas, it may not control these plants in all areas where they grow. In particular, the lowest tidal areas sustain high levels of disturbance during the winter and are unlikely to harbor dense populations of *P. marginata* because the insects will need to recolonize these sites each summer. The use of multiple biological control agent species can enhance biocontrol effectiveness by contributing to control in different habitats (Nowierski et al. 2002). It can also enhance control by imposing additive stress on the plants (Harris 1981) and by increasing the odds that at least one very effective agent will be established (Myers 1985). Denoth et al. (2003) found that the success rate of weed biocontrol programs increased from roughly 27% with one introduced agent to 50% with two agents, 70% with 3-4 agents, and it approached 100% for programs using five or more agents. Thus the addition of more agents would greatly enhance the likelihood of attaining long term biological control of *Spartina*.

Table 1. Natural enemies of *Spartina alterniflora* from the Atlantic Coast of North America. The list is a compilation from personal field surveys and the following literature: Davis and Gray (1966); Denno (1977); McCoy and Rey (1981); Montague et al. (1981); Vince et al. (1981); Stiling and Strong (1983); and Newton (1984).

Sap suckers	**_Prokelisia marginata_ (Homoptera: Delphacidae)
	*_Prokelisia dolas_ (Homoptera: Delphacidae)
	Delphacodes penadetecta (Homoptera: Delphacidae)
	*_Trigonotylus uhleri_ (Heteroptera: Miridae)
	*_Haliaspis spartinae_ (Homoptera: Coccidae)
	Haliaspis peninsularis (Homoptera: Coccidae)
	Sanctanus aesuarium (Homoptera: Cicadellidae)
	Draeculacephala portola (Homoptera: Cicadellidae)
	Ischnodemus badius (Heteroptera: Lygaeidae)
	Rhytidolomia senilus (Heteroptera: Pentatomidae)
Leaf chewers	_Orchelimum fulicinium (Orthoptera: Tettigoniidae)_
	Orchelimum fidicinium
Stem borers	_Chaetopsis aenea (Diptera: Otittidae)_
	Chaetopsis apicalis
	Calamomyia alterniflorae (Diptera: Cecidomyiidae)
	Mordellistena spendens (Coleoptera: Mordellidae)
	Languria taedata (Coleoptera: Languriidae)
	Donacaula sordidella Zincken (Lepidoptera: Pyralidae)
	Thrypticus violaceus (Diptera: Dolichopidae)
	Chilo demotellus Walker (Lepidoptera: Pyralidae)
Root borer	_Lissorhoptrus spp. (Coleoptera: Curculionidae)_
Seed feeders	_Oscinella carbonaria Loew (Diptera: Chloropidae)_
	Contarinia sp. near sorghicola (Diptera: Cecidomyiidae)
Leaf miner	_Hydrellia valida Loew (Diptera: Ephydridae)_
Snail	_Littoraria irrorata (Mesogastropoda: Littorinidae)_
Fungus	**_Claviceps purpurea (maritime variety) (Ascomycetes)_

* Present in California

** Present in California and Washington

We completed a literature review and field surveys along the Atlantic Coast in 2001 and 2002. These investigations revealed at least 22 insect species plus a fungus and a snail with apparent specialization on *S. alterniflora* (Table 1).

Two promising species are the picture-wing flies (Ottitidae) *Chaetopsis aenea* and *Chaetopsis apicalis*. The larvae of these flies develop inside young *S. alterniflora* shoots, feeding on meristem tissue and developing leaves. The result is death of the shoot tip and no flower production in nearly 100% of the stems that are infested. Both species occur from Florida to Maine. The potency of these flies lies in the fact that a single larva can kill a shoot. By comparison, it takes approximately 200 planthoppers to kill a shoot (Daehler and Strong 1997). In our surveys, we found several sites where the rate of shoot death due to this insect was greater than 50%.

Other promising candidates include the sapsucking insects, *Trigonotylus uhleri*, *Haliaspis spartinae,* and *Prokelisia dolas*. All three of these species have the advantage that they already occur in California, where the native *Spartina foliosa* occurs. Thus, introduction of these insects to Washington does not pose a risk to *S. foliosa* if they were to disperse from Washington to California. *T. uhleri* is a mirid bug that feeds primarily on the tips of the leaves. *H. spartinae* is a scale insect that occurs in low densities on the Atlantic Coast but can be found at very high densities in San Francisco Bay. *P. dolas* is a close relative of our current biocontrol agent. *P. dolas* has been shown to be more tolerant of low host quality than *P. marginata* (Denno et al. 2000), which suggests that it could be a potent biocontrol agent. However, this species has been shown to outcompete *P. marginata* (Denno et al. 2000). This could be detrimental to the overall biocontrol program because unlike *P. marginata*, *P. dolas* is unable to exploit the majority of *Spartina* occuring in mid to lower tidal elevations.

UPDATE ON BIOCONTROL PROGRAM

Much has happened with regard to control of *Spartina* in Willapa Bay and Puget Sound since this report was originally prepared in 2004. In particular, the state and federal agencies involved in the control program increased capacity and improved the effectiveness of the herbicide control treatments and eventually sprayed all *Spartina*-infested areas in Washington including biocontrol sites in an effort to eradicate the plant. As of 2007, prior to herbicide treatment of the last remaining biocontrol site, the *P. marginata* population was increasing and spreading in the north end of Willapa Bay with measured densities of more than 10,000 per m^2, sufficient to cause browning of the plants over a two-acre area. The full impact of biological control may never be known. If *P. marginata* is capable of persisting on the sparse shoots that remain, it may help suppress any reinvasion of *Spartina* if traditional control methods are discontinued.

CONCLUSIONS

The biological control agent, *Prokelisia marginata*, has a demonstrated capacity for reducing *Spartina* biomass and seed set in Washington. In the first few years of the biocontrol program, poor overwintering survival of nymphs kept populations from growing from year to year. With the introduction of *P. marginata* ecotypes from the East Coast, we are confident that we have provided the best opportunity for *P. marginata* to be successful. *P. marginata* from Rhode Island outperformed *P. marginata* from other locations in the first summer after release. Impacts to *Spartina* in the lab (Daehler and Strong 1997) and in field cages (Grevstad et al. 2003) have been clearly demonstrated. Impacts on a much larger scale were beginning to be seen in 2007 prior to herbicide treatment of biocontol sites.

The success of a biological control program for *Spartina* could be further improved with the screening and introduction of additional biocontrol agents. Several promising candidate agents have been selected from a diverse community of insects and other organisms that use *S. alterniflora* as a host in their native range. Biological control could be a valuable tool in other parts of the world where *Spartina* has invaded, especially where complete eradication is too expensive or not feasible. Even where traditional control programs are underway, biological control can contribute to *Spartina* reduction in an integrated weed management approach, or it can serve as a backup in the case that complete eradication is not achieved. Unfortunately, San Francisco Bay is not a good target location for biological control because of the risk to the native *S. foliosa.* However, it would be an excellent option in China, New Zealand, and Australia where there are no native *Spartina* species.

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ECOLOGICAL INVESTIGATIONS OF NATURAL ENEMIES FOR AN INTERSTATE BIOLOGICAL CONTROL PROGRAM AGAINST *SPARTINA* GRASSES

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Spartina alterniflora (smooth cordgrass) is a dominant member of salt marsh communities in its native range along the East Coast of the United States as well as an introduced invasive in several West Coast intertidal areas. In 2000, a biological control agent, *Prokelisia marginata*, was released in Willapa Bay, Washington, USA where *S. alterniflora* was rapidly spreading. Despite establishment of this agent, the invasive population was not brought under control. Although the natural enemies of *S. alterniflora* have been previously catalogued, little has been written regarding their ecology, with the exception of *P. marginata*. The purpose of this study was to provide additional information on the ecology of insect herbivores of *S. alterniflora* in Rhode Island in support of a larger effort to develop biological control agents for the West Coast.

An intensive survey of insect species found on *S. alterniflora* in Rhode Island was conducted to describe the composition of the insect herbivore community. Vacuum samples taken at both high and low marsh locations facilitated the characterization of species assemblages. Our results suggest that there is little variation in insect herbivore species richness among Rhode Island salt marshes. Damage due to *Chaetopsis* spp. is easily observed in the field, but the frequency of damaged stems varies greatly among sites. Additional research is needed to determine the factors responsible for this variation.

Keywords: *Spartina alterniflora*, *Chaetopsis*, biological control, natural enemies, invasive species

INTRODUCTION

Spartina grasses have become serious invasive pests in West Coast intertidal regions since their introduction in the late 1800s. Many Pacific intertidal areas are predominantly exposed mudflats at low tide, and *Spartina* is rapidly colonizing these areas. Its effects range from the reduction of valuable feeding habitat for migratory shorebirds to the possible collapse of some areas' shellfishing industries. Traditional control methods such as mowing, tilling, and herbicide application were not yet effective or economical at the writing of this article, and a long-term solution was still being sought. In 2000, a biological control agent, the planthopper *Prokelisia marginata* Van Duzee (Homoptera: Delphacidae), was released in Willapa Bay, Washington, to control *Spartina alterniflora* Loisel. (Grevstad et al. 2003), but the invasive population was not brought under control despite establishment of this control agent.

Past studies documenting the insect fauna in *Spartina* marshes (Davis and Gray 1966; Denno 1977; Vince et al. 1981; Newton 1984) have demonstrated the diversity of the associated insect communities, but relatively few have focused on New England marshes. The purpose of this study was to gather information on the ecology of insect herbivores of *S. alterniflora* in Rhode Island in support of a larger effort to develop biological control agents for release in Willapa Bay.

METHODS

Thirteen Rhode Island salt marshes (Fig. 1) were

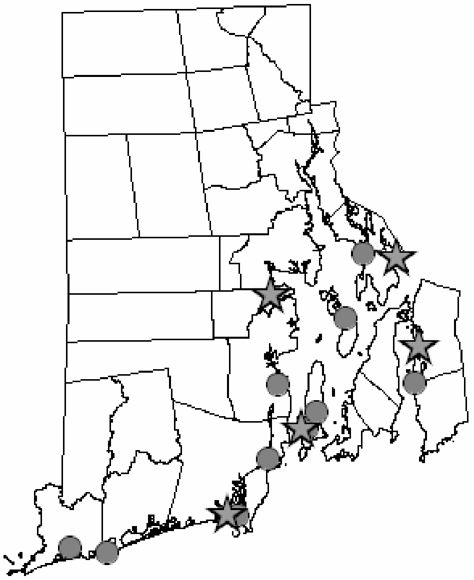

Fig. 1: Vacuum samples were collected at sites marked with a circle. Vacuum and stem samples were collected at sites marked with a star.

Table 1: Vacuum Survey Results: Number of salt marshes, of 13 sampled, inhabited by known *Spartina* herbivores by zone. Feeding Guilds: (LC) Leaf-chewer; (SS) Sap-sucker; (SB) Stem-borer.

Species	Feeding Guild	Zone	
		Low Marsh	High Marsh
Orchelimum spp.	LC	2	5
Prokelisia dolus	SS	3	13
Prokelisia marginata	SS	10	10
Trigonotylus uhleri	SS	11	10
Chaetopsis aenea	SB	11	11
Chaetopsis apicalis	SB	12	10

sampled once from mid July to late September using a Homelite® gas-powered leaf blower/vacuum with a fine mesh net fitted inside the intake tube. Samples were taken by walking along transects (approximately 30 meters (m) at both low and high marsh elevations) and sweeping the vacuum over *S. alterniflora* plants from base to tip. Samples were frozen prior to being sorted. Specimens of interest were then mounted and identified.

At a subset of five marshes, the frequency of damage due to specific species or feeding guild was measured. The total number of stems in a square meter quadrat was counted initially. Fifty stems chosen at random were harvested as well as every stem showing apical leaf damage. Four replicates were sampled at each site. Stem height and diameter were recorded before the stem was scored for external feeding damage and then dissected for stem-boring larvae. Data were analyzed using Microsoft Excel and Analyse-it® supplementary software.

RESULTS

Specimens collected in vacuum samples represented four genera of *Spartina* herbivores in three feeding guilds (Table 1; Fig. 2). Of the three guilds, sap-sucking and stem-boring species were collected at all but a few sites, while the sole leaf-chewing genus, *Orchelimum* Serville (Orthoptera: Tettigoniidae), was recovered rarely. *Prokelisia dolus* Wilson was also anomalous in that it was found much more frequently at high marsh than at low marsh. Additional species observed during stem dissections were not recovered at all in vacuum samples.

Harvested stem analysis revealed disparities in the infestation rates across species or feeding guild (Fig. 3).

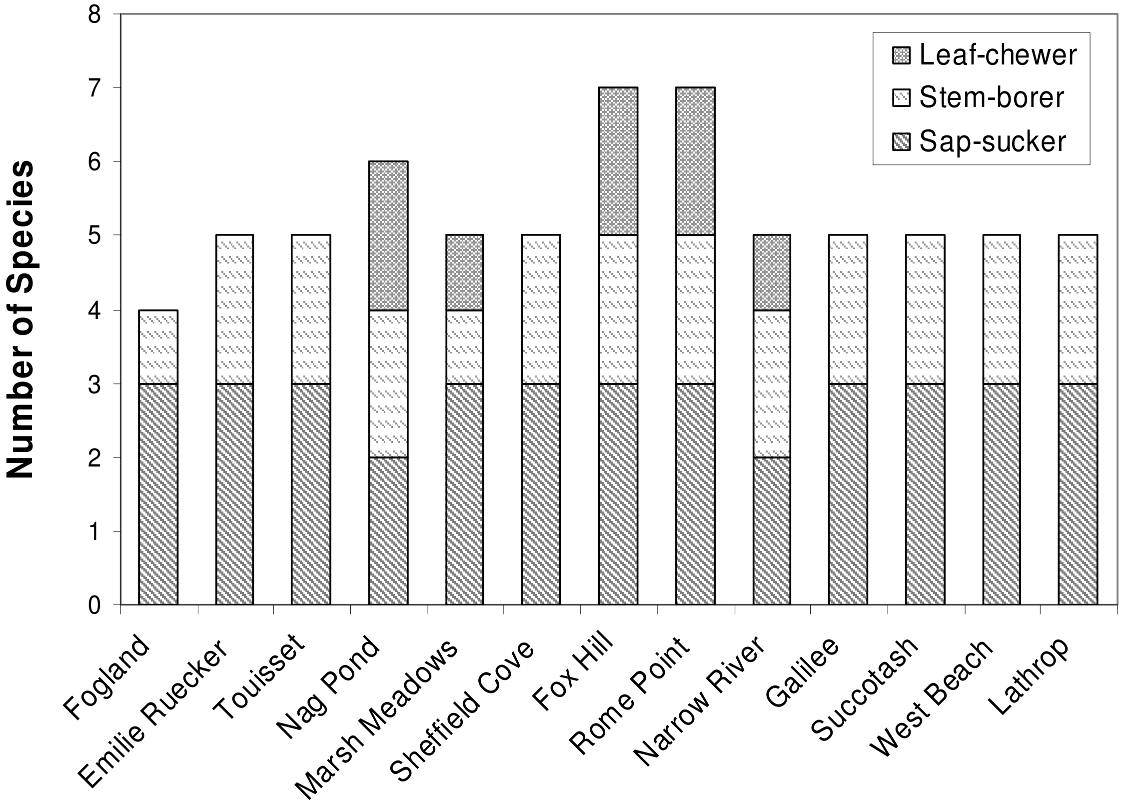

Fig. 2: Composition of *Spartina alterniflora* herbivore feeding guilds based on vacuum samples collected at thirteen marshes.

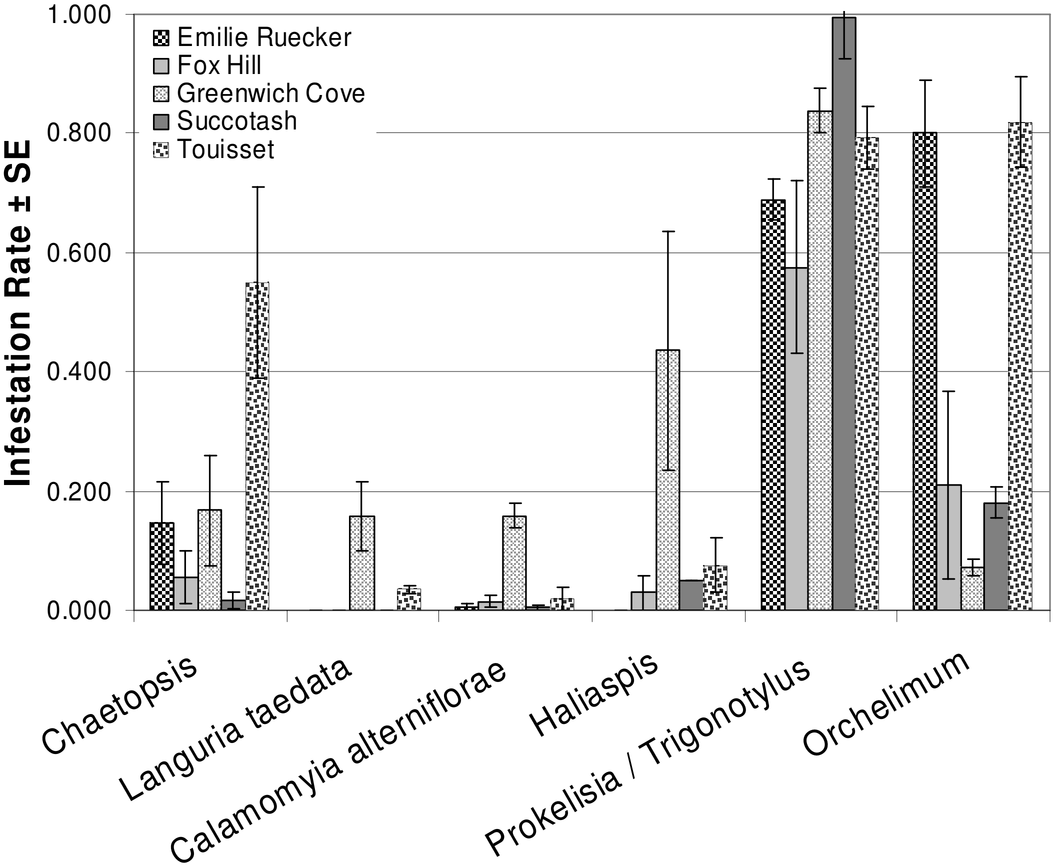

Fig. 3: Infestation rates of *Spartina* herbivores at five marshes. Feeding damage caused by *Prokelisia* spp. and that caused by *Trigonotylus uhleri* were not distinguishable.

There were significant differences among sites in the rates of infestation by *Chaetopsis* spp. Loew (Diptera: Otitidae) ($p = 0.0007$), *Languria taedata* LeConte (Coleoptera: Languriidae) ($p = 0.0025$), *Calamomyia alterniflorae* Gagne (Diptera: Cecidomyiidae) ($p < 0.0001$), *Haliaspis* spp. Takagi (Homoptera: Diaspididae) ($p < 0.05$), and of leaf-chewing damage ($p < 0.0001$). Infestation by *Chaetopsis* spp. was associated with an average stem height reduction of 24.8%, although these results were not significant.

DISCUSSION

The results of vacuum sampling suggest that there is little variation in *S. alterniflora* herbivore community composition among Rhode Island marshes. The apparent rarity of *Orchelimum* spp. and the absence of other species in vacuum samples was likely due to limitations of the sampling methods. Vacuum sampling used in this survey may have provided more opportunity for evasion than sweep netting used in previous studies (Davis and Gray 1966; Vince et al. 1981). Seasonal variation in community structure is also of importance as adult forms of many stem-boring species are only present in limited intervals (Newton 1984). To alleviate the inherent biases of this sampling method, surveys of community composition should include a wider array of sampling methods and increased frequency of sampling throughout the season.

Analysis of feeding damage to *S. alterniflora* stems provides sufficient evidence to warrant further investigation of some species as possible biological control agents. Although sap-sucking insects caused the highest frequency of damage, they did not cause the severity of damage associated with the infestation by *Chaetopsis aenea* Wiedemann and *C. apicalis* Johnson. The larvae of these flies destroy the shoot apical meristem of all infested stems, effectively stopping growth and preventing flowering. There exists, however, significant variation in the proportion of stems damaged by *Chaetopsis* spp. both among sites and within sites. This variation may be explained by differences in variables such as plant nutrition, plant resistance, tidal fluctuation, and water salinity. Newton (1984) demonstrated that salinity was strongly correlated to the infestation rates of several stem borers. Denno et al. (2000) investigated changes in intra-guild competition among sap-sucking species due to variations in plant resistance. Until the degree of niche overlap between *Chaetopsis* species is better understood, this sort of interaction ought to be considered possible.

Future studies should focus on gathering additional information on the potential effectiveness of *Chaetopsis* spp. as biological control agents. A major component of this research should seek to explain the variation in *Chaetopsis* infestation rates in an attempt to understand what factors are limiting the species where it is found at lower densities. It will be important to quantify the effect of *Chaetopsis* infestation on the productivity of *S. alterniflora* genets and to learn which ecological traits distinguish *C. aenea* from *C. apicalis*. Lab rearing techniques using a modified protocol from Allen & Foote (1992) currently in development at the University of Rhode Island should aid future work.

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POTENTIAL FOR SEDIMENT-APPLIED ACETIC ACID FOR CONTROL OF *SPARTINA ALTERNIFLORA*

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Note: This paper, presented at the Third International Conference on Invasive Spartina, was subsequently published in 2007. The citation and abstract of the published paper is provided below.

Citation

Anderson, L.W.J. 2007. Potential for Sediment-Applied Acetic Acid for Control of *Spartina alterniflora. J. Aquat. Plant Manage.* 45:100-105 [http://www.apms.org/japm/vol45/v45p100.pdf]

Abstract

Smooth cordgrass (*Spartina alterniflora*), a tall grass native to the east coast, has invaded Willapa Bay, Washington, and the San Francisco Bay, California. Management with glyphosate and imazapyr can be effective, but in the San Francisco populations, applications in several sites are confined to short periods in the fall in order to protect nesting habitats of Clapper rails (*Rallus longirostris*). Use of efficacious soil-active herbicides could mitigate this restriction. Acetic acid, a readily degraded natural product, has been shown to kill sediment-borne propagules of aquatic plants such as *Hydrilla verticillata* and *Stuckenia pectinatus.* Effects of acetic acid on sediment-free rhizomes of *S. alterniflora* were examined. Exposure of 0.1, 1.0, 1.5% vol/vol acetic acid for a few hours to several hours resulted in increased conductivity in distilled water compared to unexposed controls, indicating loss of cellular integrity and leakage of electrolytes. Regrowth from exposed rhizomes was significantly inhibited at higher (1.0% and 1.5%) concentrations applied for 2 or 4 hr. When rhizomes that had been directly exposed to 1.5% acetic acid were transferred to outdoor conditions in Albany, CA, both new shoot number and average plant height were reduced by over 90% at nine months post-treatment. The exposure to all concentrations of acetic acid for 4 hr also led to reduced frequency of inflorescence production, thus potentially diminishing the dispersal capacity of the treated plants. Field trials are needed to determine if judicious drenching of sediments with acetic acid (e.g., at low tide) may have utility as an alternative to foliar applied herbicides such as imazapyr and glyphosate.

Keywords: soil-active herbicide, electrolyte, pore-water, HPLC, seawater, smooth cordgrass, vinegar.