

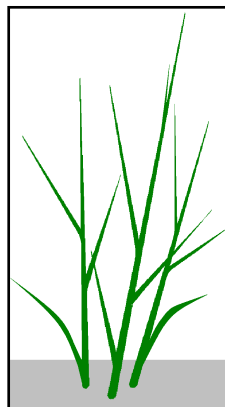


4th International Conference on Invasive *Spartina*

ICI-Spartina 2014

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PROCEEDINGS





4th International Conference on Invasive *Spartina*

Cordgrasses (*Spartina*, Poaceae) are perennial plants playing an important role in salt marsh sediment dynamics where they are considered as "ecosystem engineers". Multiple cases of species introduction outside their native range had important evolutionary and ecological consequences on several continents (hybridization with native species, speciation, biological invasion, shifts in species interaction...). Due to the rapid expansion of some *Spartina* species, their adaptative potential, their tolerance to various pollutants and their high biomass, they have been used in wetland restoration programs, shoreline stabilization, in phytoremediation and are also considered as a potential bio-energy source for biofuel production. However, the use of *Spartina* was not always beneficial when some of the introduced species became invasive in various regions, requiring their recurrent mechanical or chemical eradications. These different actions have mobilized large budgets and staff worldwide.

Beyond the *Spartina* biological system, this genus is an excellent model to address more general issues related to speciation, adaptation, invasion, stress tolerance (e.g. anoxia, salinity, pollution), biodiversity management, ...

After three International Conferences in the United States (Seattle, WA 1990; Olympia WA 1997; San Francisco CA 2004), this Conference crosses the ocean (like did several *Spartina* species) to be held for the first time in Europe. By bringing together international experts from complementary areas, this 4th International Conference on Invasive *Spartina* aims at promoting knowledge exchanges and discussion on the developments accomplished during the last decade in both fundamental biology (evolutionary history of the genus, impacts on ecosystems, evolution of the communities impacted

by the invasions) and applied research (management and control of the invasive populations). The contribution of the recent technological developments and advanced genomic approaches to the understanding and use of *Spartina* will be most particularly explored.

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SESSION 1: History of invasions,
distribution, species status

(chairman: A. Gray)

THE HISTORY AND CONSEQUENCES OF *SPARTINA* INVASIONS

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Abstract

Maritime *Spartina* are powerful ecosystem engineers that accrete sediment, define shorelines, create habitat, and generate prodigious primary productivity both where they are native and where they have been introduced. Invasive *Spartina* can overgrow resident marsh plants, compete vigorously with native species, accrete sediment to change hydrology, diminish biota, and confound human uses of estuaries. A mostly New World genus of the western Atlantic, 13 species of *Spartina* existed before the Columbian Exchange. Hybridization between native and human introductions of *Spartina* has created new species and hybrid swarms. Huge, expensive efforts have been mounted to kill some populations of invading *Spartina*. Sophisticated, long-term, and large-scale herbicide treatments have virtually eliminated this invader from Willapa Bay. In San Francisco Bay, CA an imbroglio continues to play out from the purposeful introduction of *S. alterniflora*, which hybridized with native California cordgrass. The hybrids spread rapidly into habitat of migratory shorebirds, which led to a large-scale herbicide campaign that was a success in saving shorebird habitat. However, the endangered California Ridgeway's rail, previously known as the California clapper rail, uses the tall hybrid *Spartina* as a surrogate for habitat that had been removed by dikes behind which cities and agriculture has been built around SF Bay. A twist of irony is that tall, dense *S. alterniflora* hybrids and even *S. alterniflora* itself could be used to protect the densely populated shoreline of the SF Bay from the early stages of sea level rise.

1. A Small Genus

Spartina comprises 13 original species, known to have existed before the Columbian Exchange (Strong and Ayres, 2013). New species and swarms have arisen from hybridization between native and human introductions of *Spartina* since travel between the New and Old Worlds accelerated at the end of the 15th Century. All but two of the original species are native to the Western Hemisphere. The exceptions are *S. maritima* native to Europe and *S. arundinacea* native to a few remote islands of the south Atlantic and Indian oceans. *S. alterniflora* and *S. patens* have the greatest native ranges. With interruptions by mangroves at low latitudes, the former occupies large fractions of low intertidal salt marsh from Newfoundland to Argentina, and the latter is a high marsh specialist that ranges from Newfoundland to Uruguay. *S. cynosuroides* is a giant plant that grows in oligohaline reaches of estuaries from the mid-Atlantic of North America through Texas. *S. bakeri* and *S. Spartinae* grow from high salt marsh into upland areas. The former occurs from South Carolina to Texas, and the latter occurs only on the Gulf coast. Native *S. densiflora* grows in the Atlantic of Brazil into the Pacific in Chile from mid intertidal into high salt marsh habitats. *S. foliosa* is an

intertidal specialist that ranges from San Francisco Bay to Central Baja California. *S. ciliata* and *S. argentinensis* grow on coastal dunes in Argentina. *S. pectinata* and *S. gracilis* are the only two species with continental distributions far from the sea. The former is a specialist of freshwater marshes with vast monospecific stands from central to northeastern North America, and the latter lives on inland salt pans and saline lakeshores of northwestern continental North America.

2. Ecosystem Engineers.

Powerful influences upon habitat and other species have earned marine *Spartina* species a central place in the ecology of salt marshes and in human affairs of shorelines of mid latitudes. At low latitudes, *Spartina* is usually overgrown by mangroves. *Spartina* species participate in vigorous reciprocal interactions between the biotic and physical environment and have been called "foundation species" (Pennings and Bertness, 2001). Their stiff tall stems reduce wave energy and current speed, and roots grow upward into the sediments precipitated from waters calmed by their presence. The consequence is stabilization and elevation of the marsh to match sea level. The equilibrium between

loss to erosion and gain of organic detritus and mineral sediment due to *Spartina's* ecological engineering has been shown to maintain shorelines for thousands of years (Redfield, 1972).

3. *Spartina* Invasions

Where maritime *Spartina* species are native, they are uniformly valued for providing habitat to wildlife, for defining and preserving a constant shoreline, and as a base to ecologically and economically valuable food webs. Where they have become invasive, the view of *Spartina* as a bane usually forms owing to the habitat transformations and threats to other animals and plants that these plants cause (Strong and Ayres, 2009).

Just as species introductions in general (Zenni and Nuñez, 2013), most *Spartina* introductions have failed. The longest list of known introductions are of the highly invasive hybrid *S. anglica*, which failed in 22 out of 44 purposeful introductions in the first half of the twentieth century (Ranwell, 1967); it also collapsed after spreading in China (Zuo et al., 2012), became extinct in the Falkland Islands after surviving for some time there (Orensanz et al., 2002), and failed to spread after introduction to San Francisco Bay (Hogle, 2011). Perhaps most notable, pertinent to the hypothesis of failure as a consequence of interspecific competition, no introduction of any *Spartina* species is known to have succeeded within the native ranges of 9 *Spartina* species on the east coasts of North America and South America (Strong and Ayres, 2013).

Most of the scores of recorded *Spartina* introductions have been purposeful. A few are reasonably inferred to have been incidental. Most known introductions failed, some persisted without much spread, and others have spread widely. *Spartina* spreads by seed that floats on the tide. *S. alterniflora*, *S. patens*, *S. densiflora*, and *S. anglica* have been most widely introduced and are the most consequential invaders. *S. densiflora* was the first known to have been introduced, to the Gulf of Cadiz, Spain from South America in the sixteenth century (Castillo et al., 2010). All of the known introductions to Pacific shores occurred in the twentieth century.

3.1. Early Invasions: Sources, Vehicles, and Propagules

The Columbian exchange across the Atlantic was but a prelude to the accelerating global exchange of species among all parts of the world that has followed in the five centuries since 1492 (Crosby,

2004). Inadvertent introduction by shipping was the most common vector of marine species introductions. *Spartina* was useful as ballast packing material. Perhaps from as early as the beginning of the sixteenth century, when Portuguese explorers first visited southern latitudes high enough for *Spartina*, this plant has been ferried around the world in the holds of sailing ships. Some was probably cast ashore without purpose from ships sailing from Atlantic America to other destinations. Similarly plausible is that *Spartina* was among the many species of New World plants brought home to Europe for cultivation in the centuries after Columbus' discovery. Transportation of some cordgrass in a manner that increased chances of surviving the voyage would have followed from the knowledge that *Spartina* is nutritious fodder and the fibrous leaves are fine thatching material. Another possibility is that it was feral New World *Spartina* thriving around the docks in Europe that was discovered and then propagated for animal feed and thatch there (Strong and Ayres, 2009).

Invasions derive from propagules that must arrive, survive, and then thrive in their new home. Most propagules introduced to new areas fail to make it through this sequence. *Spartina* ballast probably would have been composed of both kinds of potential propagules, plant fragments and seeds; more seeds would have been in ballast made up in late summer and early winter. Many more *Spartina* introductions and even locally surviving populations probably happened than are known. On the other hand, thousands of sailing voyages passed from shores with native *Spartina* to other parts of the world in the century or more between the first detection (from South America to Iberia) and next detections (of *S. alterniflora* from North America to France in 1803 and to England in 1816). This raises the possibility that transportation, or survival, and, or disposal of cordgrass was not very frequent. One intriguing possibility is that *Spartina* packing material was too valuable to throw away.

3.2. Another Vehicle

The next major introduction was of *S. alterniflora* to Willapa Bay WA at the end of the nineteenth century (Civille et al., 2005). Evidence suggests that the plant hopped the continent via the new transcontinental railroad in barrels of live oysters, iced, and wet, from New York Harbor and the Chesapeake Bay. More than 300 iced carloads each holding upwards of 100 barrels of live oyster seed and adults were imported from New York Harbor and Long Island Sound to

Willapa Bay WA between 1893 and the second decade of the twentieth century. Conditions in the barrels were as salubrious for *S. alterniflora* as for oysters, among which this plant grows. The seven distant areas of Willapa Bay, some 20 km from others, to which oystermen distributed the oysters taken from the trains, were where the earliest populations of *S. alterniflora* grew.

3.3. Spread by Seed after Introduction

While *Spartina* can be cloned readily from fragments of roots attached to a shoot, fragments are by far the least successful *Spartina* propagules in nature. Fragments of shoots and roots routinely break off in chunks of mud eroded from channel banks and marsh foreshores. However, the mud is denser than seawater, and these fragments sink in situ. While the odd live rhizome can be washed out from its muddy base and float away, there is little if any evidence of *Spartina* spread by plant fragments.

On the other hand, abundant evidence exists that *Spartina* invasions spread rapidly by seeds, which float while fragments sink (Strong and Ayres, 2009). Seed bearing wrack was probably how *S. anglica* spread similarly widely among English estuaries after its hybrid origin there. It floated to or was carried to the Netherlands and Ireland then probably spread by floating seed within both of these countries. *S. densiflora* spread by seed around the coastline of the Atlantic and Mediterranean coasts of Portugal and Spain after its introduction in the 16th c. It also spread widely by floating seed after its introduction to Humboldt Bay, CA early in the 20th c. *S. alterniflora* has spread by floating seed in the areas to which it has been introduced: Willapa Bay WA, San Francisco Bay CA, New Zealand, and South Africa. Floating seed has played a large role in dispersal of *S. alterniflora* to more than 19 degrees of latitude --- from 39 deg. N near North Korea to 20 deg N near Viet Nam --- since its introduction to China in 1979. China is the only place known where *Spartina* invades and threatens mangroves (Zhang et al., 2012). The hybrid swarm of *S. foliosa* x *S. alterniflora* spread rapidly and widely by floating seed around San Francisco Bay.

3.4. Introductions Accelerate

The dawn of the twentieth century saw the new hybrid *S. anglica* cross the English Channel, probably by seed, to Baie des Veys then spread southward, throughout Brittany from the colonization site in Normandy after its detection in 1906. *S. anglica* was

introduced to New Zealand and Tasmania early in the twentieth century, and to Australia in mid century and Puget Sound, WA a few decades later; *S. alterniflora* was introduced to New Zealand about this time. During the last half of the twentieth century four species of *Spartina* were introduced to San Francisco Bay part and parcel of marsh rehabilitation as this habitat segued from a trash dump to the waterfront view of expensive real estate. China, which has no native *Spartina* imported *S. anglica* in 1963, *S. cynosuroides* and *S. patens* in 1979; all three are either extinct or extremely rare now. In 1980, *S. alterniflora* was imported (Zuo et al., 2012), and it has spread over most of the country since its introduction. The most recent known introduction is of *S. alterniflora* to South Africa (Adams et al., 2012).

Spartina alterniflora is the most influential cordgrass in both native areas and where it has been introduced. It was introduced to San Francisco Bay in 1973 by the United States Army Corps of Engineers (Ayres et al., 1999). They planted seed from the Atlantic coast at one site in New Alameda Creek. Plants that grew from this seed hybridized with native *S. foliosa* to form a swarm that backcrossed to both parental species as well as among themselves. Seed of the hybrids floated to grow in many sites around San Francisco Bay. Hybrids were also disseminated in mitigation and marsh restoration projects to multiple parts of the Bay during the following two decades. The hybrid nature of these plants was discovered in 1997 (Daehler and Strong, 1997). The hybrids grew densely above and below the native *S. foliosa* with potential to negatively influence habitat, flood control, human uses of the shore, and other species.

4. Control

The most common motivation for introducing *Spartina* was to solidify mud and prevent erosion on the shores of estuaries. Following introduction, whether intentional, incidental, or of unknown cause, opinion about *Spartina* has almost always become negative as this plant changed the physical and biological nature of its new home (Strong and Ayres, 2009). Invading *Spartina* came to be seen as a threat to conservation of native species, fishing, mariculture, navigation, access to the shore, and to the aesthetic value of the landscape.

4.1. Herbicides were first used against *Spartina* in the 1950's in The United Kingdom, where *S. anglica* had become a bane. The attitude in New Zealand switched from enthusiastic in favor of *Spartina* to

extirpation over three decades from the 1950s to 1980. With vague rationalizations of possible value *S. anglica* was introduced to Australia during the first half of the twentieth century. Introductions ceased by 1962, and concerted control with herbicides has been practiced in recent decades (Strong and Ayres, 2013).

4.2. Willapa Bay WA was the site of one of the largest invasions of *Spartina*. It was followed by a huge sustained effort to eliminate the plant from this vast estuary. The cover of invasive *S. alterniflora* grew to ca. 27,000 ha of intertidal and supertidal habitat during the ca. 100 years of spread during the twentieth century. This invader was seen to threaten the wildlife of the Willapa Bay National Wildlife refuge, migratory shore birds, and a valuable century old oyster industry, as well as navigation, intertidal habitat, and the aesthetics of the estuary. In 2008 the herbicide glyphosate, which had been used with disappointing results for almost a decade, was replaced with imazapyr, which quickly gave favorable results (Patten, 2002). By 2011, only a few hectares total of scattered plants remained, and total extirpation was anticipated. The control effort had cost ca. \$30 million through 2011 (Strong and Ayres, 2013).

4.3. San Francisco Bay is the site of another expensive *Spartina* control program, motivated in particular by concern about the loss of open mud relied upon for foraging by migratory shorebirds. More than 900,000 of these birds rely upon the invertebrates in intertidal mud during their spring stopover in San Francisco Bay. A large program, The Invasive *Spartina* Project, was begun in 2003 to control the hybrid swarm with the same herbicides used in Willapa Bay WA. Large expanses of hybrid cordgrass, identified by correlation between morphology and molecular systematics, were sprayed first with glyphosate, then more recently with imazapyr. The primary conservation objective of protecting open mud for foraging by migratory shorebirds was a great success. However, by about 2008, concern mounted that the spraying program was harming the non-migratory and endangered California Ridgeway's rail, previously known as the California clapper rail (Overton et al., 2014). It is inferred in hindsight that the large stature and high density of the hybrid *Spartina* plants had created new habitat for the endangered California Ridgeway's rail, habitat that had been lost over the 20th century when more than 90% of saltmarshes of San Francisco Bay had been converted to agriculture and urban development. In 2010 the spraying program ceased

and replanting *S. foliosa* began in the areas where the hybrid had been killed. \$21 million was spent on the control program between 2000 and 2012.

5.0. Coda

The large maritime *Spartina* species, especially *S. alterniflora*, might afford protection to coastal areas from sea level rise. This potential depends upon open space for *Spartina* colonization in the high intertidal and supertidal. A second requirement is an abundance of sediments to build the upward marsh growth. Roads, levies, and other human built environments that would prevent upward marsh growth border many areas most in need of protection from sea level rise. As well, it is not clear that sediments sufficient for upward marsh growth exist in some estuaries. Ironically, areas where large *Spartina* is not native, such as San Francisco Bay, will experience much sea level rise in coming decades.

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THE HYBRID SPARTINA INVASION OF SAN FRANCISCO BAY

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Abstract:

Hybrids between *S. alterniflora*, introduced at least twice into San Francisco Bay, and the native cordgrass *S. foliosa* formed in the 1980s. Numerous studies investigated the genetics, morphology, physiology, reproduction, and spread of hybrids in San Francisco Bay. A hybrid swarm of genotypes arose through bi-directional introgressive hybridization. Self-fertility, absent in the two parental species and in early generation hybrids, arose in advanced generation hybrids. Hybrids transgressed the parental species for a broad range of traits and spread at an exceptional rate through the Bay. A mathematical model found that seedling recruitment drove hybrid *Spartina* spread, and studies showed that seedling recruitment was episodic in response to climatic events and dependant on safe-sites where seed was trapped and tidal waters were muted. These insights provide guidance to controlling hybrid *Spartina* spread in SF Bay, and perhaps other places where *Spartina* is spreading.

Keywords: introgression, speciation, *Spartina alterniflora*, *Spartina foliosa*

Hybridization is a recurrent theme in *Spartina* ranging from ancient hybridizations between *S. alterniflora* and *S. arundinacea*, or their ancestors, which resulted in *S. densiflora* (Fortune et al., 2008), to the modern hybridization-speciation event between *S. maritima* and *S. alterniflora* that led to the formation of *S. anglica* in the 1800s (Gray et al., 1991; Ayres and Strong, 2001). In contemporary times, the proclivity for *Spartina* to hybridize has resulted in abundant sterile hybrids between introduced *S. densiflora* and native *Spartina* species in Spain and California (Castillo et al., 2010; Ayres et al., 2008a), and the swarm of introgressing hybrids that occurred between native *S. foliosa* and introduced *S. alterniflora* in San Francisco Bay (SF Bay), California (Ayres et al., 1999). SF Bay supports large populations of the native *S. foliosa*, has been the recipient of introductions of *S. densiflora*, *S. alterniflora*, and *S. anglica*, and contains plants arising from interspecific hybridizations - in many respects SF Bay is the test garden for *Spartina* species and hybrids (Fig. 1). This paper will focus on hybrids between *S. alterniflora*, introduced into SF Bay in the 1970s, and the native cordgrass, *S. foliosa*.

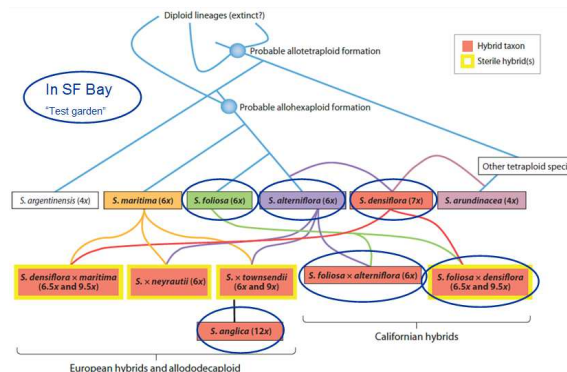


Fig. 1. Phylogeny of invasive *Spartina*; species and hybrids encircled are those found in SF Bay (From Strong and Ayres, 2013 (Redrawn and updated from Ainouche et al., 2009). Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae). Biol Inv 11: 1159–1173).

Spartina alterniflora is a dense, robust species, reaching 2m in height, and is able to grow onto intertidal mudflats where it accretes sediment, elevating the marsh plain and defining the shorelines where it is native on the US east and gulf coasts. In contrast, the native California cordgrass is a smaller (<1m tall), sparser species than its robust congener, less able to grow down the intertidal plane and

leaving vast areas of open mud in its native habitats along the Pacific coast of north America, south of Bodega Bay, California. The two species are closely related sister-species (Fig. 1), have the same chromosome number ($S. foliosa = S. alterniflora = 62$; Ayres et al., 2008a) and about the same genome sizes ($S. foliosa 2C = 4.47$, $S. alterniflora 2C = 4.36$); Ayres et al., 2008a). *Spartina alterniflora x foliosa* hybrids were first detected in SF Bay using molecular markers in 1997 (Daehler and Strong, 1997). Numerous studies since then have investigated the genetics, morphology, physiology, reproduction, and spread of hybrids in SF Bay. Hybrids had the same chromosome number (62) and genome size ($2C = 4.4$) as the parental species (Ayres et al., 2008a), both of which were

seed parents of hybrids (Anttila et al., 2000). The hybrid swarm contained diverse combinations of markers/alleles from each species (Ayres et al., 1999; Sloop et al., 2011) indicating introgressive hybridization was ongoing; F1 hybrids were rare (Strong and Ayres, 2013). Sloop et al. (2011) concluded that *S. alterniflora* was introduced from two genetically distinct source populations; one was from the mid-Atlantic US, while the other source population was unknown (Fig. 2). This same study found spatial genetic structure due to these two source populations, and local scale temporal and spatial genetic structure due to local production and dispersal of seedlings. In an earlier study they showed (Sloop et al., 2009) that self-fertility may have arose due to a reduction in inbreeding depression in later generation hybrids (but not in the parental species or early generation hybrids); up to 37% of seedlings recruiting onto mudflats in 2003 and 2004 were the product of self-pollination by nearby hybrid plants.

Spartina hybrids, like the parental species, reproduced both by vegetative expansion via rhizomes, and through the production of seed and pollen. Research showed that for a broad range of these traits hybrids transgressed the parental species (Zaremba, 2001; Ayres et al., 2004; Ayres et al., 2008b). Hybrids were transgressive for salinity tolerance as well; under greenhouse conditions hybrids exceeded the growth of both parental plants

by two to three times under conditions of high salinity, and by as much as six times under low salinity conditions (Lee et al., 2010). Time series data showed that the spread rate of the hybrid *Spartina* population was increasing between dates resulting in greater-than-exponential growth (Ayres et al., 2004). To determine which aspect of *Spartina* reproduction was driving this exceptional growth rate, Hall et al. (2006) developed a mathematical model.

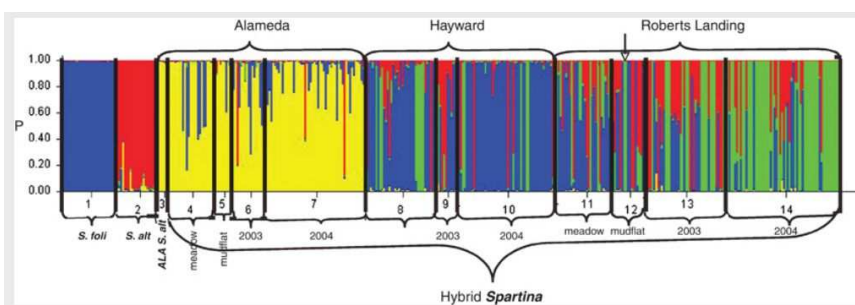


Fig. 2. STRUCTURE diagram of three populations of established hybrid cordgrass and their 2003 and 2004 seedling populations; the colors of each bar/genotype stands for the proportional microsatellite allelic contribution of *S. alterniflora*, *S. foliosa*, and a “new” green hybrid source. Seedling populations reflect similar genotypes to the established parental hybrid plants denoting local production and dispersal of seeds. The diagram also shows two source populations for *S. alterniflora* introductions in SF Bay; red color is from mid-Atlantic source populations found at Hayward and Robert’s Landing, yellow is from an unknown source at Alameda Island; blue is native *S. foliosa*.

Against a null model where all genotypes were inter- and self-fertile and equally fit, the model tested the effects of increased pollen production by hybrids, increased hybrid seedling recruitment, and increased vegetative expansion of hybrids. Increased pollen production did not affect spread, but the frequency of hybrids increased in the population at the expense of native and *S. alterniflora* genotypes. Increased vegetative growth contributed to spread rate, but increased seedling survival had the greatest effect by far on greater-than-exponential spread, leading to their conclusion that high recruitment is the key driver to hybrid invasion success (Hall et al., 2006) (Fig. 3).

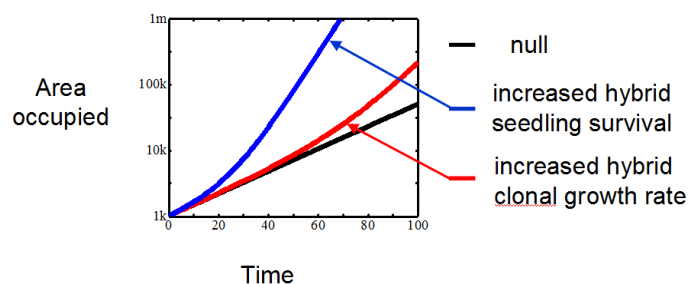


Fig. 3. Mathematical model outcome of hybrid *Spartina* spread through time portrayed on a semi-logarithmic plot showing the effects of increased clonal growth rate and increased hybrid seedling survival on spread rate compared to a null model where all genotypes are equal (diagonal line).

Seedling recruitment was a function of flower production and seed set, with some hybrids exceeding both parental species in these traits (Ayres et al., 2008b), and seed dispersal to a spatial and temporal “safe-sites”. Restoration sites – former salt ponds that were filled in and restored to tidal action – proved to be excellent sites to trap hybrid seed and increase survival of hybrid seedlings due to their muted tidal regime (Ayres and Strong, 2010) (Fig. 4). We also observed seedling recruitment on the lee side of established hybrids growing on mudflats and in the wrack deposits at the landward edge of marshes (Ayres and Sloop, pers. obs) – again seed was trapped in these micro-sites, and protected from tidal action during the vulnerable stages of seed germination and early seedling growth.

There was also a temporal component to seedling recruitment. California, along with much of the world, experiences climatic cycling due to the El Niño Southern Oscillation; during El Niño years California may receive greater rainfall than normal. During the strong El Niño event of 1997-98, in which 200% of normal rain fell in San Francisco

(<http://ggweather.com/ca2004rain.htm>), aerial photos showed extraordinary increase in cover by hybrid *Spartina* in a restored salt pond (Fig. 4). Our data showed a 3-fold increase in hybrid seed production during the 1998 El Niño compared to the 1999 La Niña (anti- El Niño) events (Ayres et al., 2008b). Sloop et al. (2009) documented 2.7- to 9- fold

increases in seedling occurrences on three mudflats between the moderate and weak El Niño events of 2002-03 and 2003-04, followed by an almost complete absence of seedlings during the 2004-05 La Niña event. While the mechanisms behind these episodes were not understood (increase in flowering intensity/seed production, and/or increase in seed germination/survivorship in response to higher rainfall/lower soil salinity), it is clear that there was a climatic “safe-site” component to *Spartina* seedling recruitment.

Seedling recruitment was key to *Spartina* spread, and seedling recruitment was safe-site-dependant and episodic in response to climatic events. These insights, as imperfect as they are, still provide guidance to controlling hybrid *Spartina* spread in SF Bay, and perhaps other places where *Spartina* is spreading (e.g. China). First, hybrid cordgrass should be eliminated to reduce hybrid seed production. Second, seedling safe-sites where seeds were trapped and tidal waters were muted – restored salt ponds, in the lee of established plants, and high in the marsh – should be reduced as much as possible. Finally, these safe-sites should be intensely monitored for seedling occurrence during and immediately following El Niño events.

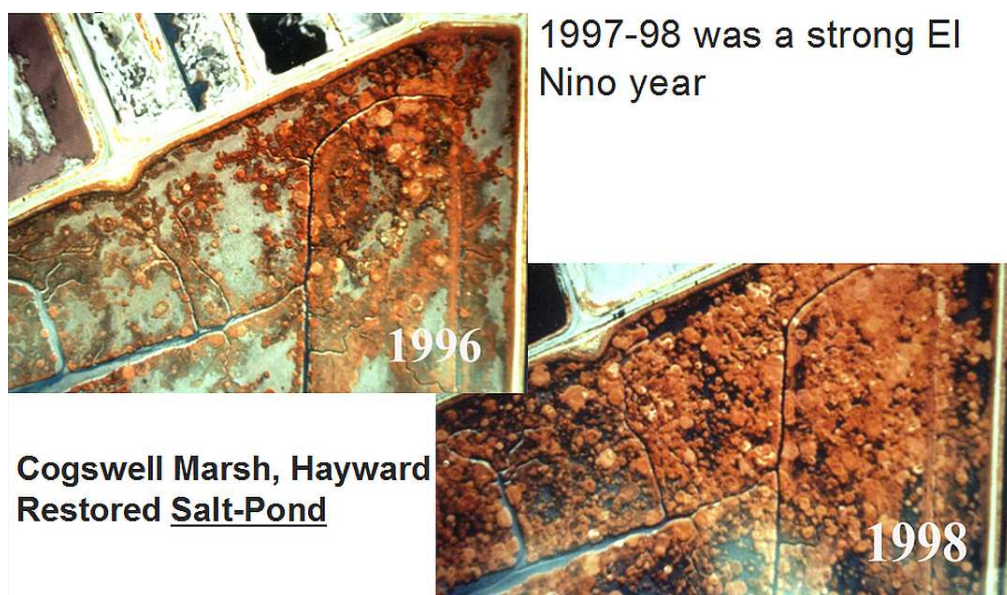


Fig. 4. Aerial photographs showing the same marsh, a restored salt pond, before and after the El Niño event of 1997-98. Pale grey is open mud, red is *Spartina* - many showing typical circular clonal growth.

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CHARACTERISTICS AND CONTROL OF *SPARTINA ALTERNIFLORA* ON THE SOUTHERN COAST OF AFRICA

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Abstract

Spartina alterniflora was first recorded in 2004 in the Great Brak Estuary, a system that closes to the sea along the southern coast of South Africa. This first record in Africa indicates the adaptive potential of this invasive grass. *Spartina alterniflora* spread from 2566 m² first measured in 2006 to a maximum area covered of 10 221 m² in 2011 despite being inundated for months at a time. Changes in sediment characteristics between 2009 and 2012 were measured in the dense monospecific stands. There was an increase in silt, sediment organic matter and a significant reduction in sediment redox potential at sites invaded by *S. alterniflora*. When the estuary closes the water level rises and *S. alterniflora* is flooded limiting opportunities for mechanical and chemical control. The glyphosate-based herbicide (Kilo Max) was approved in 2010 and application began in 2012. This chemical control was more effective in reducing the stands than mechanical removal and in November 2013 the area covered by *Spartina* had been reduced to 8 994 m². Spread of this invasive to the intertidal marshes in adjacent estuaries is a potential biodiversity threat although fortunately this population does not seem to produce viable seed. There is also the concern that hybridization with the resident *S. maritima* may occur. Important research and management questions remain i.e. how quickly will the natural marsh re-establish following eradication and how can we prevent movement of the grass to other estuarine salt marshes?

Keywords: sediment characteristics, redox potential, inundation, chemical control, salt marsh

Introduction

The suspect plant was identified as *Spartina alterniflora* using genetic analysis in 2007 (Adams et al., 2012). It was recognized by local home owners as something different and was sent away for identification as early as 2004. However it was only recognized as a potential invasive when we became involved and realized that it was a salt secreting grass. This was the first record of *S. alterniflora* in an African estuary and the first time it has been found in an estuary that closes to the sea. A sand bar forms at the mouth of the Great Brak Estuary during winter and it can remain closed to the sea for 3-8 months. It is not known how this invasive arrived at the site. The Great Brak Estuary is a disturbed system with surrounding development on both banks. It is a managed estuary with a dam a short distance from

the head of the estuary. Freshwater ($3 \times 10^6 \text{ m}^3$) is released annually from the dam in spring / summer to keep the mouth open to the sea and allow fish and invertebrate recruitment (e.g. mudprawn) and salt marsh germination. Recent studies have shown that the estuary is eutrophic with a tight cycling of nutrients between the sediment, macroalgae and submerged macrophytes (Human, 2014).

Objectives & Methods

The objective of the research has been to characterize the plant and sediment conditions and measure response to control methods. GIS mapping of the area covered by *S. alterniflora* was completed for 2006 and then for each year from 2008 to 2014. Sediment characteristics were measured in 2009, 2011 and 2012. The following parameters were measured

using standard methods (Adams et al., 2012) namely sediment particle size, redox potential, moisture content, organic content, pH and electrical conductivity. Measured plant characteristics were above and below ground biomass, plant height and stem density in 2009 and 2012. In 2011 only plant height and the sediment seed bank was measured while in 2013 (January, February, November) and April 2014 plant height and stem density were measured.

A water level recorder (K2H004) was installed under the national bridge over the Great Brak Estuary by the Department of Water Affairs. The bridge is located adjacent to the *Spartina* stands. The recorder measures the water level (m above msl) in the estuary every 10 minutes. From this the average daily water level was calculated. *S. alterniflora* occurs at an elevation range of 0.63–1.29 m above msl. Under these conditions the sediments are waterlogged and 50% of the aerial parts of the grass inundated. When the mouth is open water level can drop to 0.5 m msl and when it is closed water level can increase to 2 m msl (Figure 2).

Results & Discussion

Plant and sediment characteristics

The area covered by *S. alterniflora* has increased from 0.26 ha consisting of 12 stands to a maximum of 1.02 ha in 2011 (24 stands). Control measures in 2011 and 2014 have reduced the area occupied by the population (Figure 1).

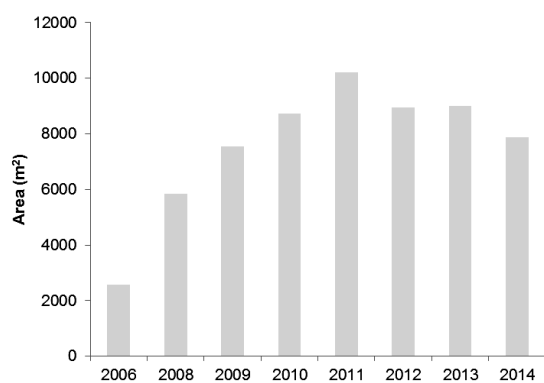


Figure 1. Area cover of *Spartina alterniflora* stands in the Great Brak Estuary over the last 8 years. Control measures took place in 2011 and 2013.

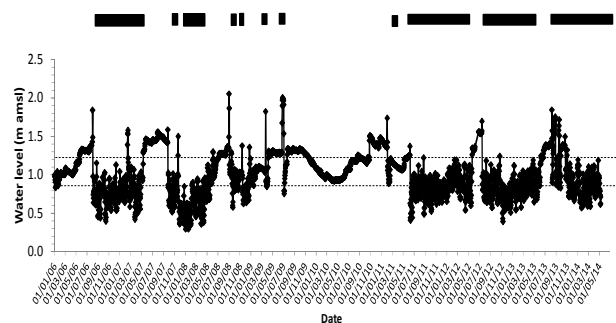


Figure 2. Average daily water level in the Great Brak Estuary. Dashed lines indicate the elevation range of *Spartina alterniflora* stands. Black bars indicate when the mouth of the estuary was open to the sea.

Figure 2 shows the water level in the estuary over the period of study. It is important to consider the water level prior to the sampling date as this would influence the measured plant and sediment characteristics. Before April 2009 stands were flooded for three months and eight months prior to March 2011. The mouth remained open with little flooding before sampling in April 2012. However there was little relationship between inundation time and area covered by *S. alterniflora*. There were some site specific differences as patches inundated during winter appeared to show greater signs of die-back. There was a decrease in plant height, stem density and biomass from 2009 to 2012 which indicated the response to control measures implemented in 2011 (Table 1).

Table 1. Plant characteristics of the invasive *Spartina alterniflora* in the Great Brak Estuary, South Africa from this study.

	2009	2011	2012
Plant height (m)	0.71 ± 0.03	0.96 ± 0.07	0.36 ± 0.06
Stem density (m ⁻²)	217 ± 34	-	101 ± 0.06
Above ground biomass (g m ⁻²)	933 ± 147	-	687 ± 96.9
Below ground biomass (g m ⁻²)	3120 ± 493	-	1842 ± 283

There are indications that the *Spartina alterniflora* invasion has influenced sediment conditions in the Great Brak Estuary. There was an increase in sediment organic matter and decrease in redox

potential (Table 2). Other conditions were clearly influenced by the state of the mouth. Open mouth conditions in 2012 were responsible for a higher sediment electrical conductivity measured at this time. When the mouth is open to the sea the estuary becomes tidal with more saline conditions.

Table 2. Sediment characteristics associated with invasive *Spartina* stands from this study in the Great Brak Estuary, South Africa.

n = 40	2009	2011	2012
Water content (%)	37.5 ± 1.7	14 ± 1.1	39.1 ± 1.4
Sediment electrical conductivity (mS.cm ⁻¹)	32.2 ± 0.9	31.2 ± 1.9	52.5 ± 1.6
Organic matter (%)	2.96 ± 0.16	2.47 ± 0.2	5.01 ± 0.23
Redox potential (mV)	-228 ± 20.2	-252 ± 26.4	-270 ± 29
% Sand	16 ± 0.02	40 ± 0.02	20 ± 0.02
% Silt	15 ± 0.01	13 ± 0.01	66 ± 0.02
% Clay	71 ± 0.01	48 ± 0.02	14 ± 0.02

History of control

There were delays in treatment of *Spartina alterniflora* which could be attributed to a number of factors. These were the short treatment season as a result of closed mouth conditions and inundation of the stands in winter. There were delays in getting a suitable herbicide approved by the Department of Agriculture. Implementation initially suffered from financial constraints and the transition of operational responsibility from one agency to another and there were administrative delays in putting contractors in the field. Once they were in the field treatment was confined to low tides, low wind speeds and no rain.

The Working for Water Invasive programme of the Department of Water Affairs first initiated control measures. A glyphosate-based herbicide (Kilo Max) was approved in 2010 (700 g kg⁻¹). In April / May 2011 two treatments were applied; namely the

chemical treatment using Kilo Max and mechanical removal by hand of the above and belowground biomass. In October / November 2011 there was a follow up treatment. No treatments were applied in 2012. Biomass and plant height showed a significant decrease at sites where there was chemical control. Mechanical removal actually stimulated plant growth as indicated by the increase in stem density (Figure 3).

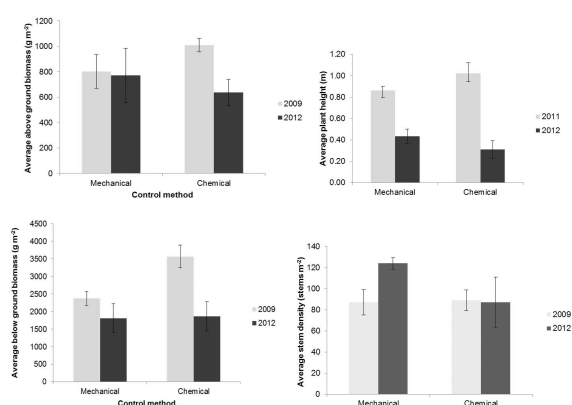


Figure 3. Effect of chemical and mechanical treatment on plant height and biomass of the invasive *Spartina alterniflora* in the Great Brak Estuary, South Africa.

No control took place in 2012 as administrative responsibility was transferred from the Department of Water Affairs to the Department of Environmental Affairs. The Invasive Species Programme of the South African National Biodiversity Institute is now responsible for the control, management and monitoring of this invasive plant. In 2013 plants were treated in February, April and November with glyphosate-based herbicide (Kilo Max). From November 2013 all plants were foliar sprayed with Kilo Max (10 kg ha⁻¹) with 0.5% imazapyr (100 g l⁻¹) added to the herbicide mix. Early in 2013, a portion of patch 9 was mowed, allowed to grow and foliar sprayed with Kilo Max 1 month later.

The data once again showed that chemical treatment was more effective than mechanical control (Table 3). For the chemically controlled sites stem density decreased by almost 50% with mostly (54%) dead stems found in November 2013. Recent measurements in April 2014 indicated that most plants were dead and those remaining had 5% or less of living stems.

Table 3. Effect of chemical and mechanical treatment of the invasive *Spartina alterniflora* in the Great Brak Estuary, South Africa.

	February 2013		November 2013	
	1 x chemical	1 x mow	2 x chemical Feb. & April	1 x mow & April chemical
Density (stems m ⁻²)	161 ± 12	157 ± 23	87 ± 11	179 ± 24
Live stems	85 %	60 %	46 %	58 %
Dead stems	15 %	40 %	54 %	42 %

Potential threats

In the Great Brak Estuary if left unchecked *S. alterniflora* has the potential to replace 42.9 ha or 41 % of the total estuary habitat. It would displace five different intertidal salt marsh species. It also has the potential to invade other estuaries in South Africa, especially those with extensive intertidal habitat and containing *S. maritima* (19 estuaries in total). There is also the concern that hybridization with the resident *S. maritima* may occur. In the past, *S. alterniflora* hybridized with *S. maritima* to form a sterile hybrid (Marchant, 1967) and as a result, the fertile *S. anglica* was derived (Stapf, 1913), which showed to be tolerant to wider environmental conditions than other *Spartina* species (Gray et al., 1991).

In conclusion this study has shown the adaptive potential of this invasive grass as it has been recorded for the first time in an African as well as a closed estuary. It has a wide environmental tolerance range although sediment and plant characteristics were similar to that reported elsewhere. The research has shown the urgent need for persistent follow up control and monitoring if this invasive is not to become a threat to the biodiversity of South African estuaries. Important research questions remain regarding whether the

population supports viable seed, how quickly will the natural marsh re-establish following eradication and how can we prevent movement of the grass to other estuarine salt marshes? Genetic analysis could be used to determine the method of spread and the relationship between eutrophication, algal mat decomposition and *S. alterniflora* expansion is important.

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NATIVE OR INTRODUCED?

THE STATUS OF *SPARTINA VERSICOLOR* FABRE

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Abstract:

Intercontinental introductions are widespread in genus *Spartina*, with important ecological and evolutionary consequences. Species status is then critical with regard to conservation biology, biodiversity assessment and management, especially for Mediterranean coastline ecosystems threatened by urbanization. *Spartina versicolor* was first described in southern France in 1849, then successively in various places on the European and North-African Mediterranean and Atlantic coasts. This species is considered either as European native or as an invasive species introduced from North America, according to its high morphological similarity to the Atlantic American species *Spartina patens*. We performed extensive sampling of *Spartina versicolor* in Europe and North Africa, from both natural populations and herbarium collections. These samples were compared to other European and American *Spartina* species (including *S. patens*). Genome size estimation and chromosome counts were reported for the first time and revealed that this species is tetraploid ($2n=40$). Various molecular markers were employed for genetic diversity and phylogenetic analyses. Our phylogenetic results group *S. versicolor* in a unique clade with the American species *S. bakeri* and *S. patens*. *Spartina versicolor* probably originated from *S. patens* due to their high molecular similarities. The low level of genetic diversity within *S. versicolor* suggests that the introduction of *S. versicolor* occurred from a narrow genetic pool of *S. patens* from North America.

Keywords: Poaceae, DNA, chromosomes, coastline, Mediterranean.

INTRODUCTION

Wetland habitats are among the most threatened in the Mediterranean. Assessment of their vulnerability needs to be based on robust knowledge of their composition, in terms of species diversity, population distinctness and genetic variability. In the western Mediterranean, humid depressions in dune habitats are colonized by *Spartina versicolor* Fabre, one of the rare European species of the *Spartina* genus which is mainly distributed on American

coastline salt marshes and inland salt grasslands. This species has a controversial taxonomic status. According to the Mediterranean flora, e.g. Tison et al. (2014) *S. versicolor* is a native Mediterranean and Atlantic plant. However Mobberley (1956), in his monography on *Spartina*, described it as synonymous of *S. patens*, assuming that the Mediterranean populations were introduced from the North-East American coast where *S. patens* is abundant in high marsh and dunes.

"The presence of this species in Europe is probably a result of introduction of rhizomes or seeds from North America by means of ship ballast. It is known from Southern France, Corsica and Italy." Mobberley 1956.

Since then, some authors indicated *S. patens* to be native on the American coasts and introduced in the Mediterranean (e.g. Silander and Antanovics, 1979), while in the European floras it was continuously described to be native (Tison et al. 2014). New World *Spartina* species received important attention from botanists who conducted comprehensive studies in the 1950s and 1960s (Mobberley, 1956; Marchant, 1967) whereas the Mediterranean *S. versicolor* remained poorly studied after its discovery (1839) by Fabre in France. Recent studies on saltmarshes along the Spanish Atlantic coast have underlined the presence of *S. patens* in Europe (Page et al., 2010) and renewed interest in the status of *S. versicolor* (Prieto et al., 2011). Worldwide invasions of *Spartina* are now well-documented (Strong and Ayres, 2013) and the Mediterranean could be one of the various places where American *Spartina* have settled after dispersal by ships during the 18th or 19th centuries.

In this paper, we report cytogenetical and comparative molecular analyses to clarify the native or introduced status of *S. versicolor*. Gaining insight into the status of this species will be of particular importance to better understand the biogeography and diversification of *Spartina*, as well as to determine the conservation priority level and management policy of *S. versicolor*.

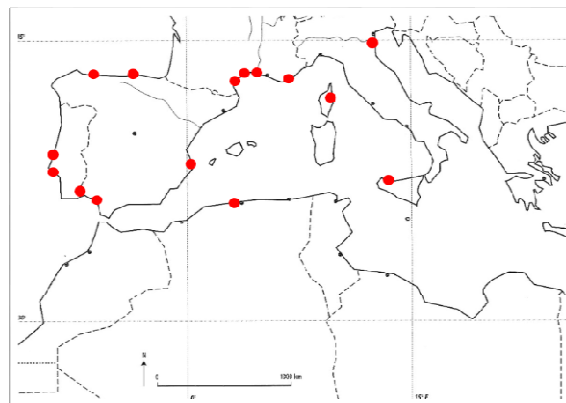
MATERIAL & METHODS:

Spartina versicolor sampled from several Mediterranean sites (n=47) and from herbarium collections (n=10) were compared to *S. patens* sampled from the east American coast (n=8). A particular effort was made to perform a sampling scheme representing as much as possible the species range (Fig.1a,b,c).

Chromosome counts were performed on mitotic chromosomes from different *S. versicolor* samples of two Mediterranean populations in France (Vieux Salins and Saint-Louis du Rhone).

DNA sequence comparisons among samples were based on the Internal transcribed Spacers (*ITS*) of nuclear ribosomal genes (rDNA) and chloroplast DNA intergenic spacers or introns (*ndhC-trnV*, *petA-psbJ*, *trnL-trnF* and *trnT-trnL* regions). Sequence data were analyzed to report for nucleotide variation

differentiating *S. versicolor* from *S. patens* and to assess the phylogenetic position of both taxa in the *Spartina* genus.



(a)



(b)



(c)

Figure 1: Sampling map of *S. versicolor* (a), *S. patens* (b) and structure of *S. versicolor* in Vieux Salins (Hyères, France) (c).

Genotyping was performed for 60 samples (52 *S. versicolor* and 8 *S. patens*) using 9 microsatellite (Simple Sequence Repeat SSR) loci designed from BAC-end sequencing in *Spartina maritima* (Ferreira et al., 2013). Out of these nine markers, seven SSR were useful and accounted for 37 alleles in total. Matching genotypes, bearing evidence for identical clones were searched using the Genalex 6.51 software (Peakall and Smouse, 2006). A discriminant analysis was conducted on the correlation of presence/absence of alleles in order to distinguish genetic groups in *S. versicolor* or *S. patens* (DAPC analysis, *adegenet* R package, Jombart, 2008). Allele frequencies within these groups were used to compute Nei distances (Nei, 1972) and to build a neighbor joining network to explore relationships between groups.

RESULTS & DISCUSSION

Chromosome counts revealed that, in both analyzed populations, *S. versicolor* individuals have $2n=40$ chromosomes, indicating that this taxon is a tetraploid species.

Ribosomal DNA *ITS* (457 nucleotides) and cpDNA (2268 nucleotides) sequencing revealed a total absence of differences between *S. versicolor* and most of *S. patens*. The only variation was a private substitution within the cpDNA sequence for one accession of *S. patens* from Cheesapeake state park. *Spartina versicolor* and *Spartina patens* are closely related to *S. bakeri* having few specific differences (1 substitution for rDNA *ITS* and 1 for cpDNA). Then the three species are positioned as a sister clade to either *S. pectinata* (cpDNA) or the clade of *S. densiflora* and *S. arundinacea* (rDNA *ITS*, Fig. 2).

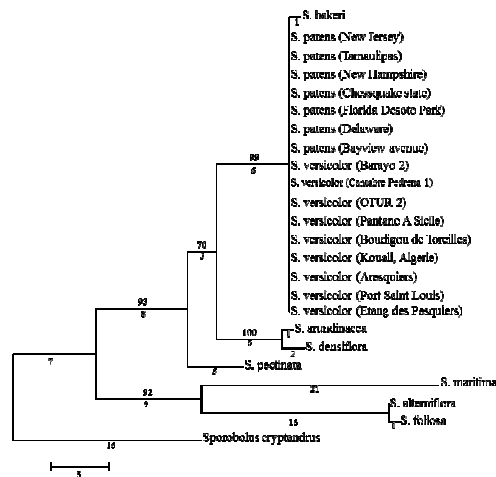


Figure 2: Maximum parsimony tree obtained using rDNA *ITS* sequences (457 bp) from *Spartina* species, especially from *S. patens* and *S. versicolor* individuals. The bootstrap percentages (1000 replicates) and the number of change are indicated above and below the branches, respectively.

Most SSR genotypes were heterozygous with mostly 3 or 4 alleles per locus. Within *S. versicolor*, search for matching genotypes revealed 35 genotypes among 52 samples, 31 being unique and 4 being repeated between 4 and 11 times. The 35 genotypes of *S. versicolor* are very similar (the number of matching genotypes even increased to 41 if accounting one possible allele error of genotyping). Higher allelic diversity was encountered in *S. patens* than in *S. versicolor*, i.e. for 6 samples *S. patens* has 30 alleles against 25 for *S. versicolor*.

The discriminant analysis based on microsatellite data groups European samples from France (natural populations and herbarium samples), Italy, Corse and North Spain). A second group encompasses individuals from the southern Iberic peninsula (south-Spain and Portugal) and Algeria, whereas 3 genetic groups could be distinguished in the American samples of *S. patens*: (i) Mexico-Florida-Delaware (ii) New Jersey and (ii) New-Jersey Hampshire). The *S. patens* group being the most similar to *S. versicolor* is observed all along the eastern Atlantic coast from Mexico to Delaware. However, according to the limited sampling size in the native region of *S. patens*, this result needs to be confirmed by further sampling and phylogeographic analyses.

Our results indicate that *S. versicolor* cannot be differentiated genetically from *S. patens*. *Spartina versicolor* appears to be a tetraploid species with 40 chromosomes, as found in *S. patens* (Mobberley, 1956). These species are morphologically similar, although several variants were described for *S. patens* (Mobberley, 1956). Our molecular analyses indicate that they have the same rDNA *ITS* and the same cpDNA, and although they can be differentiated on the basis of allelic frequency, their genotype differences are slight. These molecular analyses thus support the introduced origin of *S. versicolor* that has, as would be expected, a reduced diversity by comparison to the diversity present in its native American area. Multiple genotypes could have been introduced in the Mediterranean area considering the light genetic structure. This hypothesis could explain the lack of recombination of genotypes observed in *S. versicolor* in agreement with the observation that this taxon very rarely produces seeds (Tison et al., 2014, our personal observation). The ecology of *S. versicolor* could be nested within the ecological amplitude of *S. patens* ranging from high salt marshes, dunes to inner grasslands (Silander and Antonovics, 1979; Silander, 1984). Our data therefore support the phenotypic observations of Mobberley.

In conclusion, our study provides new insights into the worldwide spread of *Spartina* and on the origin of *S. versicolor*, which raises the question of its taxonomical status. Further sampling and molecular studies are needed for a comprehensive analysis of the genetic diversity of the highly variable *S. patens*, which appears to be another successful invasive *Spartina* species in the Old-World.

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ECOPHYSIOLOGICAL RESPONSE OF NATIVE AND INVASIVE *SPARTINA* SPECIES TO EXTREME TEMPERATURE EVENTS IN MEDITERRANEAN MARSHES

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Abstract

The recent on-going environmental changes tend to modify the climatic habitats available for colonization within ecosystems. Salt marshes are no exception to this. In these environments species distribution is mostly modulated by marsh elevation, which is a key factor affecting the sediment flooding, redox potential, pH and salinity. Nevertheless air temperature affects all the marsh indiscriminately. In recent years, high and low temperature events have become more frequent and intense in the Mediterranean area. Although these events only last for 2-4 days the species resistance and resilience during these periods shape their ability to maintain their biomass and therefore their colonized area. *Spartina patens* is an invasive species already spread along several Mediterranean countries, like France and Spain. On the other hand, *Spartina maritima* is an endemic species present in all estuarine systems along the Iberian coast. Recently there has been verified an increased invasion of *S. patens* in the Portuguese marshes. Considering this fact it becomes important to evaluate the impact of extreme climate events, such as heat and cold waves, on the ecophysiological fitness of these two species as an important factor in their spread across the Mediterranean marshes. These events can both enhance the maintenance of *S. maritima* and/or its drawback and can possibly favour a capable invader like *S. patens*. To investigate this mesocosmos trials were performed simulating realistic heat and cold wave events and the species ecophysiological performance was evaluated, by assessment of their photosynthetic efficiencies (chl fluorescence). *S. patens* appears to have a higher tolerance to both events without evidencing showing disturbances in its biophysical energy production mechanisms, while *S. maritima* suffered a more drastic decrease of its photosynthetic ability.

Introduction

Wetlands have great ecological value, providing a wide range of ecosystem services that have been evaluated as some of the most valuable services for humanity (Constanza et al., 1997; Teal and Howes, 2000), like nutrient cycling, primary production, habitat for wildlife, and shoreline stabilizers, and immobilization of pollutants. The recent IPCC WG2 5th Assessment Report (IPCC, 2014), points out to not only an increase in global air temperature, driven from CO₂ increase, but also to an increase in the frequency and intensity of heat waves, particularly in Southern Europe with mostly adverse implications

for health, agriculture, forestry, energy production and use, transport, tourism, labour productivity, and the built environment. On the other hand, cold waves seem to be tending to decrease in frequency but not in intensity (IPCC, 2014).

Halophytes are typically adapted to stressful environments, subjected to high levels of abiotic stress like flooding (Duarte et al., 2014a), high salinity (Duarte et al., 2013a) and pollution (Duarte et al., 2013b), showing morphological and physiological adaptations that allows them to adapt and survive in such harsh environments. The *Spartina* genus is one of the more successful halophytes, being present in a

wide range of latitudes across the globe. Latitudinal population differentiation of *Spartina* species has already been described (Seliskar et al., 2002; Lessmann et al., 1997; Daehler et al., 1999; Otero et al., 2000; Proffitt et al., 2005; Álvarez et al., 2010). The members of the *Spartina* genus have C₄ photosynthesis using phosphoenolpyruvate carboxylase (PEPC) to concentrate CO₂ at higher amounts than a C₃ organism (Hatch, 1992; Álvarez et al., 2010). Although this metabolic advantage PEPC is controlled by light-dependent phosphorylation (Vidal et al., 1996), making it very dependent on the surrounding climatic environment. Differences are likely to be found since leaves have high sensitivity to environmental pressures, such as climatic variations, contaminants and grazing (Heide, 2005; Stephenson et al. 2006). In addition, the activation of PEPC by its specific kinase is poor in low light (Bailey et al., 2007) and at low temperatures (Lara et al., 2001), both of which are abiotic factors that change with latitude.

Alongside climate change, the invasion of natural communities by non-indigenous species (NIS) constitutes one of the most serious threats to biodiversity (Heywood, 1989). While invasion potential of riparian plant communities, patterns and causes of NIS invasion throughout the river corridors have been widely studied (Planty-Tabacchi et al., 1996, 2001), the invasiveness of NIS aquatic plant species in wetlands has received far less attention. One of these NIS is the American *Spartina patens* (Ait.) Muhl. (Poaceae) traditionally reported in the Western-European coasts as *Spartina patens* (Fabre) and native from these coasts (Fabre, 1849; Tutin, 1980; Van der Maarel and Van der Maarel-Verluys, 1996). It is probable that this plant was introduced first to various ports around the Mediterranean Sea, since it was used as packing material in crates, allowing it to eventually establish and spread along the Mediterranean coasts (SanLeón et al., 1999). Its strongly rhizomatous character puts at risk the presence and survival of native species in the Mediterranean marshes (Daehler and Strong, 1996). Recently it has been widely found in Galicia and nowadays in several estuaries and costal lagoons from the west coast of Portugal. Simultaneously and as an important part of the Portuguese salt marsh flora, *S. maritima* is present in the same marshes and locations as *S. patens*. *Spartina maritima* (Curtis) Fernald, has a very wide distribution in the northern hemisphere native to the coasts of western and northern Europe and western Africa, with also a disjunct population on the Atlantic coasts of Namibia

and South Africa (Marchant and Goodman, 1969). This indicates a new problem. The fast colonization occurring in Mediterranean marshes by *S. patens* can lead to a loss in the area of indigenous *S. maritima*, due to competition for resources.

Recently the Mediterranean countries have been affected by a higher frequency of heat and cold waves (Niu et al., 2014). Extreme thermal events are defined by the World Meteorological Organization (WMO) as an extreme thermal event when the daily maximum (or minimum) temperature by 5 °C during more than five consecutive days, using as reference the period comprised between 1961–1990. Recently in 2003 and 2005 a severe heat and cold wave affected southern Europe respectively. Based on this, the present study aims to understand the biochemical and photochemical processes underlying *S. maritima* and *S. patens* response to extreme temperature events, in order to understand the ecophysiological fitness of both species under adverse thermal environments.

Material and Methods

For this propose plants from both species were collected in a Tagus estuary salt marsh and brought back to the laboratory. Plants placed in pots with a mixture of sand and perlite (1:1) and irrigated with ¼ Hoagland solution and placed in a FitoScope 130 RGBIR chamber (Photon System Instruments, Czech Republic) configured to simulate field conditions (Maximum PAR 250 μmol photons m⁻² s⁻¹, 16/8 h day/night rhythm; day temperature 20 ± 0.5 °C, night temperature 18 ± 0.5 °C). After 2 weeks of acclimation plants were separated into 3 groups (N=5) and subjected to different thermal treatments (day/night): control (20/18 °C), cold wave (9/5 °C) and heat wave (42/38 °C). Heat and cold waves simulation were performed according to the records of air temperature for the Tagus estuary from the 2003 heat wave and the 2005 cold wave (www.snirh.pt). All experiments lasted for 3 days after which plants were sampled. Pulse Amplitude Modulated (PAM) chlorophyll fluorescence measurements were performed using a FluoroPen FP100 (Photo System Instruments, Czech Republic), on 30 min dark-adapted leaves. Rapid light curves (RLC) measurements, in dark-adapted leaves, were attained using the pre-programed LC1 protocol of the FluoroPen (Duarte et al., 2014a). Each ΦPSII measurement was used to calculate the electron transport rate (ETR) through photosystem II according to Genty et al., 1989. Rapid light curves

(RLC) were generated from the calculated ETRs and the irradiances applied during the rapid light curve steps. Each RLC was fitted to a double exponential decay function in order to quantify the characteristic parameters, alpha and ETRmax (Platt et al., 1980; Marshall et al., 2000). The onset of light saturation (Ek) was calculated as the ratio between ETRmax and α . The polyphasic rise in fluorescence (known as OJIP test or Kautsky curves) transient depicts the rate of reduction kinetics of various components of PS II. When dark-adapted leaf is illuminated with the saturating light intensity of 3500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ it exhibits a polyphasic OJIP curve. Each letter reflects distinct inflection in the induction curve. The level O represents all the open reaction centres at the onset of illumination with no reduction of QA (fluorescence intensity lasts for 10 ms). The rise of transient from O to J indicates the net photochemical reduction of QA (the stable primary electron acceptor of PS II) to QA⁻ (lasts for 2 ms). The phase from J to I was due to all reduced states of closed RCs such as QA⁻ QB⁻, QA QB2⁻ and QA⁻ QB H2 (lasts for 2–30 ms). The level P (300 ms) coincides with maximum concentration of QA⁻ QB2 with plastoquinol pool maximally reduced. The phase P also reflects a balance between light incident at the PS II side and the rate of utilization of the chemical (potential) energy and the rate of heat dissipation (Zhu et al., 2005).

Results and Discussion

Under normal and extreme heat conditions, *S. patens* presents a comparatively higher photosynthetic efficiency (α). By contrast during cold wave events the native *S. maritima* is far more efficient. This is not only true on an overall basis, but also if the different light levels is considered. This can be related to the ecology of both species. *Spartina maritima* ecological distribution, ranges between Mediterranean and North Atlantic habitats, prone to prolonged cold during the winter (Duarte et al., 2013a), providing this specie with an ecological adaptation to this condition. On the other hand *S. patens*, is native from warmer climates and thus is highly adapted to high temperatures (SanLeón et al., 1999).

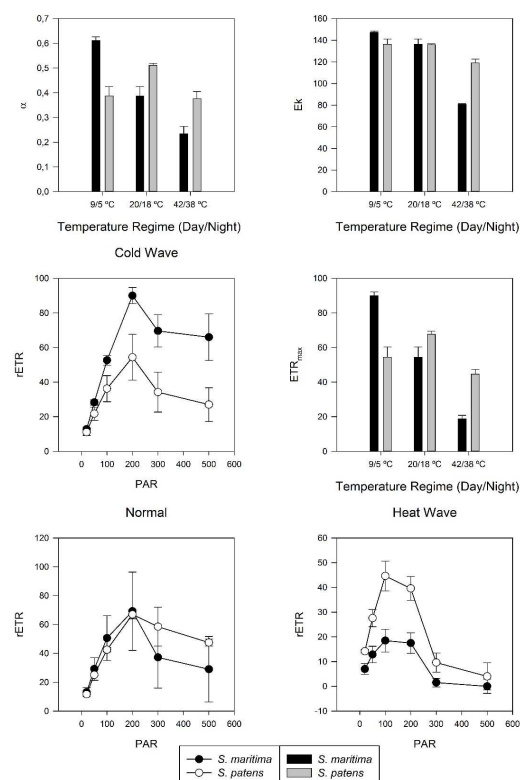


Figure 1. Rapid light curves and derived parameters in *S. maritima* and *S. patens* individuals exposed to the three experimental thermal regimes (average \pm standard error).

Going deeper into the photobiology of both species, these differences are enlightened. The Kautsky of both species (Fig. 2) revealed that during cold waves the electronic processes between both photosystems are very similar, and thus are not specie-dependent. On the other hand during heat waves the interspecific differences are exacerbated, showing a significant reduction of the fluorescence signal in *S. maritima* leaves exposed to extreme heat. Furthermore, these signals can be translated into energetic fluxes unveiling where in the energy transduction pathway is affected (Fig. 3).

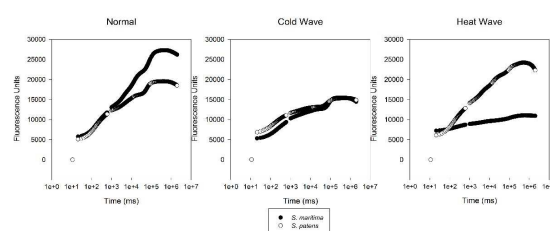


Figure 2. Kautsky curves in the leaves of *S. maritima* and *S. patens* individuals exposed to the three experimental thermal regimes.

The phenomological examination (Fig. 3), reveals that in both species there was no thermal effect on the energy absorption capacity (ABS/CS). Crossing this information with the number of reaction centres available for energy reception (RC/CS), it is possible to observe that *S. maritima* had this value reduced under extreme heat, while *S. patens* showed an increase in the reaction centre density on a cross-section basis.

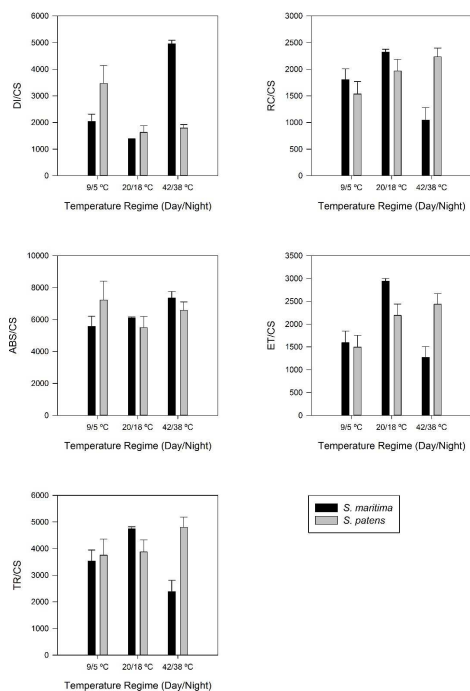


Figure 3. Phenomological energetic fluxes in the leaves of *S. maritima* and *S. patens* individuals exposed to the three experimental thermal regimes (average \pm standard error).

Nevertheless, this decrease verified in *S. maritima*, the energy absorption capacity was maintained mainly due to a higher net rate of reaction centre (M_0) closure (Fig. 4), improving light harvesting capacity. The same could be observed in which concerns the efficiency with which an electron can move from the reduced intersystem electron acceptors to PS I end electron acceptors (δR_0). At this level *S. maritima* shows an increase in $\square R_0$ under the exposure to both extreme thermal treatments, while *S. patens* only had these parameters increased under extreme cold treatment. Previous studies, showed that heat-treated leaves exhibited increased δR_0 indicative of lower damage at the PS I level comparatively to PS II (Chen and Cheng, 2009). Considering the appearance of the so-called K-phase anomalies in the OJIP curves (W_K). *S. maritima* showed an increase in the K-phase amplitude, typically indicative of thermal stress, contrarily to

what was observed in *S. patens*. This increase in K-phase amplitude in *S. maritima* individuals exposed to heat stress, is in accordance with the low values of trapped energy flux (Fig. 3, TR/CS) associated with a decrease in the quinone pool size available for reduction (Fig. 4). In summary, two differences arise between the species electronic energy processing. While *S. maritima* responded with a decrease in the photosynthetic efficiency during heat waves due to an inefficient trapping mechanism of the absorbed energy, *S. patens* responded more negatively to extreme cold with a reduction of the electronic transport chain (ETC). Nevertheless, all these interconnected electronic processes will reduce the maximum yield of primary photochemistry and consequently the probability of an absorbed photon move an electron into the ETC. This creates a new problem with an inevitable increase of excessive energy inside the chloroplasts that needs to be dissipated. In fact this was observed by the increase in the quantum yield of the non-photochemical reactions and in the dissipated energy flux itself. Also the performance index (PI), usually used to assess the overall electronic energy flux, showed a decrease due to the lack of efficiency while trapping and processing the absorbed energy. This was already found for other halophytic species under several abiotic stresses (Duarte et al., 2013; 2014a; 2014b).

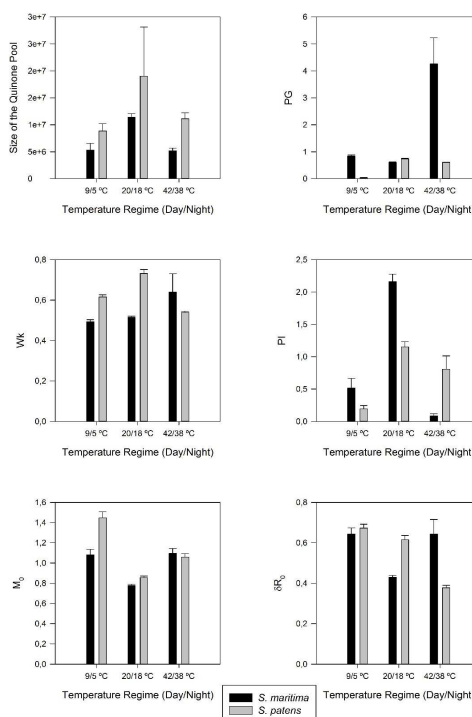


Figure 4. Transient fluoresce parameters in the leaves of *S. maritima* and *S. patens* individuals exposed to the three experimental thermal regimes (average \pm standard error).

Conclusions

It is clear that there is much to be learned about how extreme thermal events affect plants. At an ecological perspective, the most evident differences among the indigenous *S. maritima* and the invasive *S. patens* is their tolerance to heat and cold. *Spartina maritima* appears to suffer more damage in its photochemical apparatus during extreme heat events, while *S. patens* presented higher stress signs during extreme cold treatment. As stated by the IPCC report, the frequency and duration of extreme cold events tend to decrease in the Mediterranean region compared to the occurrence of heat waves, leading to an ecophysiological disadvantage of *S. maritima*, facilitating the expansion of the invasive *S. patens*.

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SESSION 2: Evolutionary genomics, genetics,
epigenetics

(chairman: M. Ainouche)

THE CHALLENGES OF GENOMICS IN POLYPLOID *SPARTINA* SPECIES: HOW TO DEAL WITH HYBRID GENOMES AND HIGH REDUNDANCY?

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Abstract

Hybridization and genome duplication (polyploidy) are particularly well illustrated in genus *Spartina*, resulting in complex genomes which exhibit several duplication events, including those dating back to the Poaceae family. The most complex genome (namely the invasive allo-dodecaploid *Spartina anglica*) arose recently in Europe c.a. 150 years ago, by genome duplication of the homoploid hybrid *S. x townsendii* resulting from an interspecific cross between the introduced *S. alterniflora* ($2n=6x=62$) and the European native *S. maritima* ($2n=6x=60$). This well described system represents one of the rare known examples of recent allopolyploid speciation that gave rise to a highly successful new species.

For more than a decade, genomic approaches have underscored the prevalence of genome redundancy in eukaryotes, resulting from both the accumulation of repeated sequences (e.g. Transposable Elements) and polyploidy. In the perspective of understanding the role of reticulate evolution and whole genome duplications in the phenotypic plasticity and adaptation of the recently formed *Spartina anglica*, the high ploidy levels of this species (dodecaploid) and its parents (hexaploids) makes genetic and genomic studies particularly challenging. We are using massive parallel sequencing technologies to explore the genomes and transcriptomes of these species, and we have developed bioinformatic approaches and tools for detecting the different putative orthologous copies originating from the parents (duplicated homoeologs) in *S. anglica*.

Keywords: polyploidy, evolution, genomics, transcriptome, bioinformatics

1 - Introduction: what are the challenges of *Spartina* genomics?

Spartina is a polyploid genus of perennial plants belonging to the Chloridoideae sub-family of Poaceae that is poorly investigated in a genomics point of view. It is composed of about 13-15 species (Mobberley, 1956), some of them having experienced several interspecific hybridizations, or even are currently crossing with each other (Strong and Ayres, 2013). It has evolved in two main lineages, tetraploid and hexaploid. Interspecific hybridization within and between these two lineages has resulted in the formation of introgressant hybrids or new invasive allopolyploid species (Ainouche et al., 2012).

Several rounds of polyploidy followed by genome fractionation all along plant genome evolution have shaped the *Spartina* genomes: it has been recently established that Spermatophytes have a polyploid origin (Jiao et al., 2011), as well as Angiosperms and Poaceae, adding many levels of ancient duplications. We have recently assembled and annotated the chloroplast genome of *Spartina maritima*, and one of the outcome of this work (poster presented by M. Rousseau-Gueutin, S. Bellot et al., p. 165) is the estimation of the divergence of *Spartina* species compared to other Poaceae, allowing us to estimate the divergence of the tetraploid-hexaploid lineages to about 9 MY, and between *S. alterniflora* and *S. maritima* to 3 MYA. One last Whole Genome Duplication event happened recently, about 150 years ago, leading to the new invasive allopolyploid *S. anglica*.

If the basic number of chromosomes is $x=10$ (Marchant, 1968), many chromosomes can be encountered due to the high ploidy level of *Spartina* species (at least $2n=4x$: tetraploid), up to 124 in the allododecaploid *S. anglica*. Because of this huge number of chromosomes and lack of agronomic interest (until now *S. pectinata* may be a "biomass" candidate), no genetic or physical map is available yet.

Reticulate evolution, high duplication levels, and huge chromosome numbers make genomics particularly challenging in *Spartina*, but new sequencing technologies are making some studies possible; we seized then this opportunity for assembling reference transcriptomes of five *Spartina* taxa, and initiated a survey of duplicated genes to analyse the repetitive component of the *S. maritima* genome.

2 - Development of genomic resources for naturally occurring and introduced species in Europe

Using *S. anglica* as a model of gene and genome evolution our research topics are mainly focused on the consequences of allopolyploid speciation on the transcriptome, and the epigenome that might explain *Spartina* adaptation, or even the evolution of new functions. To answer these questions, we combine traditional molecular markers and Next-Gen Sequencing technologies for building *Spartina* reference transcriptomes, and genomic sequences.

To study the evolution of the invasive *S. anglica*, both parental species (the introduced *S. alterniflora*, sampled from Brittany and the European native *S. maritima*) and their F1 sterile hybrids *S. x townsendii*, and *S. x neyrautii* are concomitantly studied for testing the null hypothesis of genome additivity and to parse the effects of hybridizations from genome doubling in *S. anglica*.

In the last decade, our laboratory members have demonstrated using PCR-derived markers a genome stasis after the hybridization and genome doubling while the methylated regions sampled were altered mainly after hybridization (Baumel et al., 2002; Yannic et al., 2004; Salmon et al., 2005; Parisod et al., 2009), and the transcriptome of both the hybrid and *S. anglica* was not additive (Chelaifa et al., 2010). Because these previous studies were using anonymous molecular markers or a rice heterologous microarray, we started a project of transcriptome sequencing of *Spartina* taxa; this was published for the two hexaploid species, *S. maritima* and *S. alterniflora* (Ferreira de Carvalho et al., 2013b).

2.1 Reference transcriptome assemblies for *S. maritima* and *S. alterniflora*

We sampled *S. alterniflora* and *S. maritima* populations in Brittany, produced cDNA libraries from several individuals, for two separate organs (leaf and root), and have normalized a cDNA library for *S. maritima* from samples collected in the field. Half a million reads were produced for each pooled library, using Roche-454 technology.

2.2 Assemblies and Functional annotations

De novo assemblies were performed using the Roche software, Genome Assembler (Newbler v. 2.5.2). After cleaning vectors and low quality sequences, different parameters were tested to identify the most

accurate. We decided to use a *de novo* assembly with a minimum percentage of 90% of identity and minimum length coverage of 100 bp. Each dataset was assembled separately first, then pooled per tissue and per species – and functional annotations were done using homology searches against various databases and GO Annotations for both species.

About 38 000 contigs were assembled from leaves and roots when considering both species. We were able to annotate 99% of these contigs using a combination of various grass databases, which resulted in about 17 000 annotated genes (which represents about 50% of the genes annotated in the related *Sorghum* genome).

2.3 Outcomes and Perspectives

The Functional annotation allowed the identification of ecologically relevant genes in *Spartina*. For instance, we have now identified a set of genes involved in salt tolerance and stress responses, in developmental processes, or genes that were found affected by hybridization and polyploidization in previous differential expression studies. This reference transcriptome is also useful for specific gene expression analyses and for validation of transcriptomics studies.

These also allowed us to design a *Spartina* custom array based on the *Spartina* annotated cDNA contigs: this array is now used in collaboration with the C. Richards lab from the University of South Florida to analyse gene expression in *S. alterniflora* populations, following the oil spill which occurred in the Gulf of Mexico in 2010. Mariano Alvarez presented first results during the Conference (p. 61).

We have also presented a second generation of *Spartina* transcriptomes using combined datasets from Roche-454 sequences and Illumina Hi-seq sequences (Boutte et al., *in prep*) for *S. maritima* (~19,000 contigs), *S. alterniflora* (~17,500 contigs), *S. x townsendii* (~20,000 contigs), *S. x neyrautii* (~20,500 contigs), and *S. anglica* (~18,600 contigs), increasing the number of differentially annotated contigs to 28,485 (versus 17,000 with the first generation of *S. maritima* and *S. alterniflora*).

3 – Genomic resources

We are focusing our production of genomic resources on the European native hexaploid species *S. maritima*. A BAC library was produced (70, 000 120 kb-long clones) in collaboration with the Genoscope (CEA Evry, France) and the CNRGV (Castanet-Tolosan, France) covering about 2x of its genome. BAC-end sequences (BES) were sequenced at the Genoscope (~40 000 BES) and were used for Comparative Genomics and the estimation of the repetitive fraction of the genome (Ferreira de Carvalho et al., 2013a). Next-Generation sequences were also produced, including 400 Mb of Roche-454 sequences, and 5 Illumina Hi-Seq lanes totalling 160 Gb. From the 1.6 billion Illumina genomic reads for the European *S. maritima* species many contigs were assembled using Velvet (Zerbino and Birney, 2008) and custom scripts and scaffolding using paired BES is on-going. These first contigs of the *S. maritima* genome are currently under investigation for gene and repetitive fraction annotations, as well as for copy number estimations based on sequencing depth.

From Roche-454 pyrosequencing data, and using the Repeat Explorer clustering and Transposable Elements Annotation tool (Novák et al., 2010), we estimated the proportion of repetitive elements to a quarter of the *S. maritima* genome.

4 – Duplicate gene evolution

If we consider a diploid ancestor of *Spartina* species, including some ancient duplications – called “paralogs” – resulting from the paleopolyploid origin of grasses, tetraploid *Spartina* species present two sets of homeologous chromosomes, and hexaploids three sets, that have diverged for 3 to 9 MY (Figure 1). *S. anglica* has six homeologous genome sets. In order to describe the fate of duplicated copies in such highly repeated genomes, i.e. their retention or loss, and their expression regulation, we first need to develop analytic tools to detect duplicated genes. We are currently using three main methods to detect and discriminate old duplications (paralogs or paleologs) from duplications resulting from 9 MY, 3MY and 150 years old duplications (homeologs resulting from tetra- hexa- and dodecaploidy in *Spartina*, respectively).

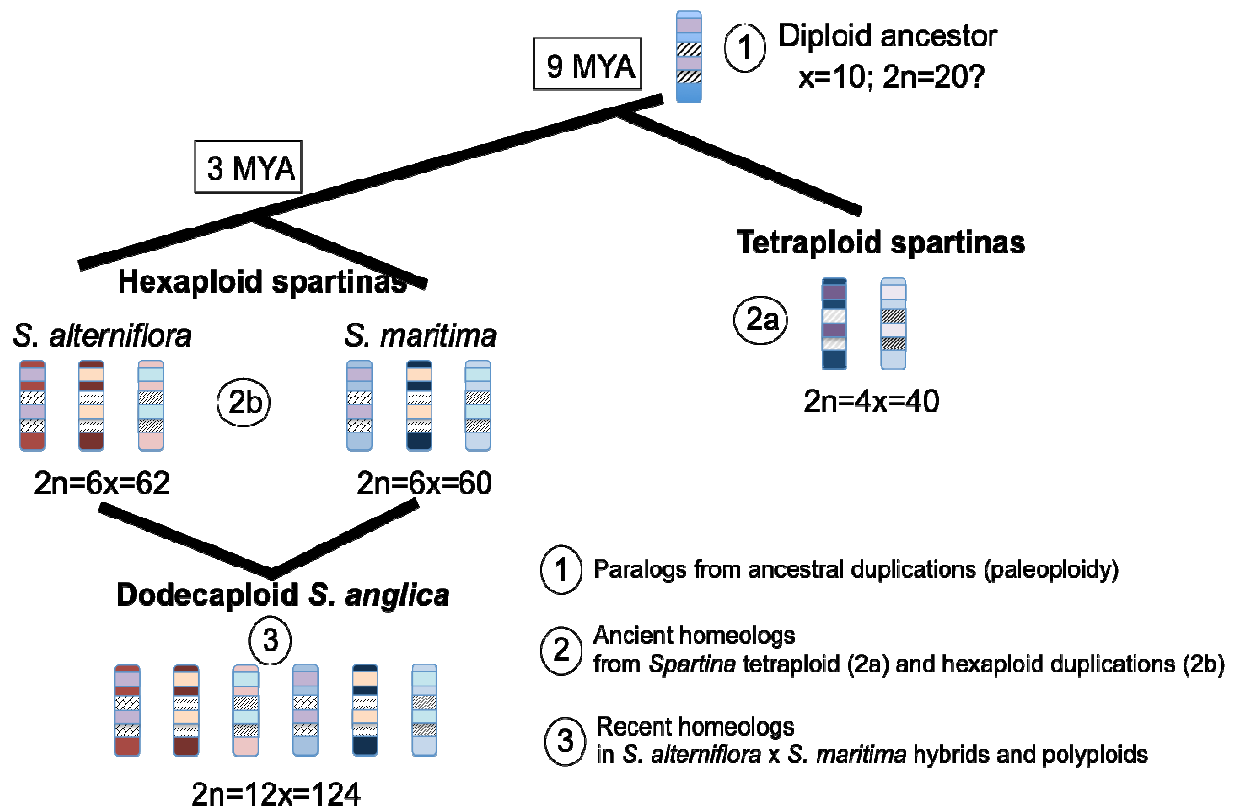


Figure 1: The origin of duplications in *Spartina* genomes

The first method relies on the phylogenetic construction of duplicated copies of low copy number genes. This method corresponds to the traditional cloning/sequencing of low copy number regions, such as what was done for the *waxy* gene as presented in the previous ICI-Spartina Conference, which allowed the demonstration that some duplicates were retained in hexaploid species, while some others were lost (Fortune et al., 2007).

The sequencing of BAC homologous regions for two regions of interest (the Adh1 and CAD regions, results presented as a poster during the Conference by Carine Charron et al.) is also a powerful method for homeologous region identification and analysis. After selecting putative homeologous BACs of these two regions of interest in *S. maritima*, we detected two haplotypes per region corresponding to two homeologous regions mainly diverging by retroelement insertions. These regions display either a high gene collinearity compared to other Poaceae (Adh1 region), or some rearrangements in the case of the CAD gene (Charron et al. *in prep.*)

The last method we are using is inspired by the work developed for cotton polyploid species (Udall, 2006; Flagel et al., 2009; Salmon et al., 2010; 2012) and uses *in silico* SNP/indels detection from parental/hybrid/polyploid sequences alignments well suited for NGS data analyses. In *Spartina*, as no diploid species is available, parental and hybrid haplotypes need first to be detected, to make possible the assignment of a hybrid haplotype to one parental haplotype. This was used for detecting paralogous rDNA copies in *S. maritima*, where 18 SNPs and 8 haplotypes were detected and validated by cloning and Sanger re-sequencing on five IGS polymorphic sub-regions from a 10 to 20x coverage. From pooled transcriptomic datasets, we applied this method and were able to assign to hybrid haplotypes their parental haplotype (Boutte et al., 2016).

5 - Perspectives

The genomic datasets as well as the appropriate analytic tools we are developing now allow a shift from a single-gene scale to a whole genome scale to study *Spartina* species evolution and adaptation. These datasets allowed us (1) to assemble reference transcriptomes for the five taxa (2 hexaploid parents,

2 independently formed F1 hybrids and the allododecaploid); (2) to assemble genomic contigs for the European hexaploid *S. maritima* and estimate the repetitive fraction of its genome; and (3) to detect the duplicated copies within parental hexaploids and to assign their parental origin in the homoploid hybrids and allododecaploid. It is now possible to explore at the whole-genome level in *Spartina* the evolutionary fate of duplicated copies (loss, pseudogenization, concerted evolution, differential mutation accumulations, sub-or neofunctionalization of gene expression) and to analyze the evolutionary forces (e.g. putative release of selective pressures) acting on redundant genes involved in different metabolic pathways.

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This report includes also the work from our laboratory presented as posters at the International Conference on Invasive *Spartina* 2014 in Rennes, France:

- Homoeolog gene detection and phylogenetic reconstruction in allopolyploids using Next-Generation Sequencing by Julien BOUTTE *et al.*
- BAC sequencing analyses in the hexaploid *Spartina maritima* (Poaceae): homoeolog divergence and microsynteny in the grass family by Carine CHARRON *et al.*
- The complete chloroplast genome of *Spartina maritima*: insights from comparative analyses in the Poaceae family and molecular dating between polyploid lineages by Mathieu ROUSSEAU-GUEUTIN *et al.*

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CHARACTERIZATION OF THE TRANSCRIPTOME AND GENOME OF *SPARTINA PECTINATA*

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Abstract:

With the goal of improving the understanding the biology of key traits of *Spartina pectinata* we have developed a transcriptome of its key vegetative tissues; leaves, roots and rhizome buds under different environmental stresses using over 1.2 billion Illumina reads. The analysis of this data set has allowed us to identify tissue specific transcripts as well as to increase our understanding of the adaptation of *Spartina pectinata* to its growing environment. The assembly of the transcriptome has also allowed us to identify over 5,000 SSR markers that could be of interest in genetic and breeding programs. To take advantage of comparative genomics with other grasses we have also developed a 5x coverage BAC library of 4X clone with an insert size of ~123kb. Using a pooling approach we have sequenced 2304 random clones using Illumina technology. These sequences were assembled in ~12000 contigs. We used the transcriptome reads to annotate the BAC contigs discovering over 7000 full length genes including introns.

Keywords: *Spartina pectinata*, genome, transcriptome

**ESTIMATION OF PLOIDY LEVELS AND rDNA GENOTYPES IN TWO MIXED
POPULATIONS OF RECENTLY FORMED *SPARTINA* HYBRIDS
IN SOUTHERN ENGLAND**

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Abstract

In Southampton Water, recent interspecific hybridisation between the hexaploids *Spartina alterniflora* ($2n=6x=62$) and *S. maritima* ($2n=6x=60$) gave rise to the homoploid hybrid (*S. × townsendii*, $2n=6x=62$). Subsequent chromosome duplication resulted in formation of the invasive allododecaploid species *S. anglica* ($2n=12x=120-124$) that spread worldwide. To address the question of dynamics of mixed ploidy populations involving these plants, we analysed two populations in Southern England, one of which was the presumed place of origin of the homoploid hybrid and the derived allopolyploid (Hythe). We genotyped forty individuals using rDNA markers and Southern blot hybridisation. We found that the homoploid hybrids dominate the population at Hythe (82% of the individuals analysed) and that hybrids are also detected on Hayling Island (66% of the analysed plants). These results indicate that the homoploid hybrid has not been outcompeted by the allopolyploid. Parental *S. alterniflora* was recorded at low frequency (~12%) in Hythe while *S. maritima* was not found in either population. Compared with the situation six years ago there was little or no change in the frequency of any particular cytotype in Hythe. We observed significant morphological plasticity of *S. × townsendii*, often masking its hybrid origin and underlining the necessity of genotyping by homeolog analysis. The occurrence of *S. × townsendii* at a location beyond its likely origin indicates its dispersal ability by vegetative means or some homoploid hybrid fertility.

Keywords: *Spartina*, allopolyploidy population dynamics, rDNA, biodiversity

Introduction

Interspecific hybridization between the hexaploid *Spartina alterniflora* (♀, $2n=6x=62$, genomic composition AA) from North America and the

native British hexaploid *S. maritima* (♂, $2n=6x=60$, genomic composition MM) produced the largely infertile homoploid hybrid *S. × townsendii* ($2n=6x=62$, genomic composition AM), first identified in 1880 at Hythe, Southampton, UK

(Groves and Groves, 1880). The derived allopolyploid *Spartina anglica* C.E. Hubbard dodecaploid ($2n=12x=120-122$, genomic composition AAMM) is thought to have formed by spontaneous duplication of chromosome sets soon after the hybridisation event (Marchant, 1963; Hubbard, 1968). The allopolyploid species is fertile, vigorous and has colonized salt marshes worldwide (Strong and Ayres, 2013).

The original population of *S. × townsendii*, as well as individuals of the derived allopolyploid *S. anglica*, exhibit additivity of multilocus parental molecular markers (Raybould et al. 1991; Ayres and Strong, 2001; Baumel et al., 2001). In addition, the subgenome integrity seems to have been maintained without bursts of retroelement activity (Baumel et al., 2002; Parisod et al., 2009) despite such activity frequently being generated by allopolyploid formation in other species (reviewed in Parisod et al., 2010). Instead, it has been suggested that such a lack of genomic diversity resulted from a genetic bottleneck at the time of formation (Ainouche et al., 2004). Indeed, the genetic uniformity observed in extant individuals suggests a single origin or multiple origins involving similar genotypes (Baumel et al., 2001; Baumel et al., 2003). In fact, where hybridisation occurs, there is little genetic variation amongst the parental species.

The hybridisation zone in Southampton Water, UK has been intensively studied since *S. × townsendii* was first discovered at the beginning of the last century. Using classical cytogenetics, Marchant discovered populations of mixed cytotypes (hexaploids, nonaploids and dodecaploids) at Hythe (Marchant, 1963, 1968a). Charman (1990) proposed that the expansion of *S. anglica* had been concomitant with the reduction of the homoploid hybrid and parental species across the entire southern coast of Britain. More recently, Renny-Byfield et al. (2010), taking a flow cytometry approach, showed that the Hythe population was composed mainly of homoploid hybrids (94%), co-occurring with a low proportion of *S. anglica* plants (6%). In contrast, the nearby population at Eling Marchwood consisted of *S. anglica* (36.4%), nonaploids ($2n=90$; 27.2%) and *S. alterniflora* (36.4%). Some plants that are probably derived directly from the original *S. alterniflora* colonisation event are still extant in Southampton Water. In contrast, the native species *S. maritima*, is now rare, whereas it was abundant before the arrival of *S. alterniflora*. Thus, the occurrence of parental *S. alterniflora* and *S.*

maritima is now restricted to a few populations. Nevertheless, the population dynamics may be complex since *S. anglica* is known to locally contract in population size and even die-out (Goodman and Williams, 1961; Gray and Pearson, 1984).

Here, we studied the ploidy dynamics in mixed populations of this recently formed *Spartina* homoploid hybrid and allopolyploid and addressed the question of their possible genetic diversification. Population-level studies are often complicated by difficulties in species and cytotype identification due to morphological similarities and phenotype plasticity (Thompson et al., 1991). To overcome these problems, we applied flow cytometry and rDNA markers on samples collected from two populations (at Hythe and Hayling Island) in Southern England during 2012 and 2013 and determined the variation in ploidy levels. The evolutionary dynamics of ploidy were assessed through a comparison of current and previous analysis.

Material and Methods

Plant material

The sampling included place of origin (Hythe, Southampton Water) and Hayling Island located ca. 40 km to the east. The later location was investigated as it was considered as one of the places where remnant *S. maritima* populations still occur (Raybould et al., 2000). For GPS coordinates, see Table 1. *S. maritima* and *S. alterniflora* were also collected in French localities (Brittany). Fresh leaf material (~1 g) was collected and stored in RNA later solution until use. At each locality we collected plant material from 4-6 clones. The sample labels (A-E) roughly reflected the distance from the sea, with "A" being the most distal. For Southern blots, DNA was extracted from leaf samples stored in RNAlater solution using the Plant DNA extraction kit (Qiagen, Germany). For flow cytometry, fresh leaf tissues were used.

Southern blot hybridisation

Southern blotting followed the protocol described by Kovarik et al. (2005) using rDNA probes labelled with [α -³²P]dCTP (Izotop, Hungary) in a random-primed reaction (DekaPrime kit, Fermentas, Lithuania). The hybridisation signals were visualized by Phosphor imaging (Typhoon, GE healthcare, USA) and signals were quantified using ImageQuant software. The probe was a 220 bp-long

26S rRNA gene fragment from tobacco (Lim et al., 2000).

Flow cytometry

Flow cytometry was used to screen for ploidy level using methods described in Hanson et al. (2005) and a Partec CyFlowSL flow cytometer. Parsley (*Petroselinum crispum*) was used as a calibration standard for assessing ploidy levels. Nuclei were isolated by chopping the leaf tissue with a razor in the GPB buffer of Loureiro et al. (2007) supplemented with 3% PVP.

Results

Identification of hybrids by Southern blot hybridisation

We used Southern blot hybridisation to genotype the samples. Genomic DNAs were digested with *Bst*NI and hybridised with the 26S rDNA probe. *Bst*NI had one conserved recognition site in the 26S coding region and a second, more variable site in the IGS region (Fig. 1a). An example of Southern blot analysis of representative samples is shown in Fig. 1b. In *S. alterniflora* (A-genome), a 1.8 kb band was visualized after probe hybridisation (right panel). In contrast, digests of *S. maritima* (M-genome) DNA generated a single 2.3 kb band. In hybrid and allopolyploid digests, the probe hybridised to both parental bands. In most cases, the band ratio was close to 50% while there were few notable exceptions from the rule. For example, the hexaploid 15B individual from Hayling Island had markedly reduced the A-genome hybridisation fragment.

Distribution of ploidy levels

Table 1 summarizes the ploidy levels identified for the samples collected in 2012 and 2013, together with data for 2007 taken from Renny-Byfield et al. (2010). Combining the data for 2012 and 2013 shows that the population at Hythe was quite homogenous with prevalent hexaploids corresponding to the *S. × townsendii* homoploid hybrid (82% of individuals analysed). Only 3 individuals (12%) were assigned as *S. alterniflora*. In 2012 one individual was found to be dodecaploid, whereas no dodecaploids were identified among the 2013 collection.

The Hayling Island salt marsh was also visited twice. Both samplings in 2012 and 2013 resulted in near equal numbers of dodecaploids and hexaploids (Table 1). Neither *S. alterniflora* nor *S. maritima* was found on Hayling Island based on rDNA

genotyping though some plants phenotypically resembled *S. maritima*.

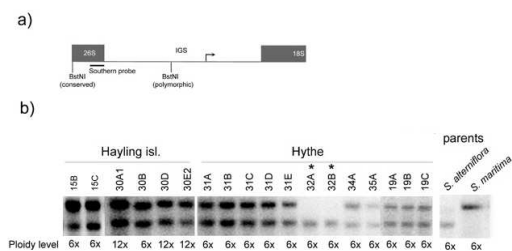


Figure 1. Example of Southern blot hybridisation analysis of homeologous rDNAs. (a) Diagram representing part of the rDNA unit showing the position of the diagnostic restriction *Bst*NI sites. The region of probe hybridisation is indicated. (b) Southern blot analysis of selected DNA samples from Hythe and Hayling Island. Parental DNAs (*S. alterniflora* and *S. maritima* sampled in France). The hybridisation profiles were mapped to ploidy levels (given below the blots) determined by flow cytometry. Note two individuals (asterisks) from Hythe lacked M-genome bands indicating *S. alterniflora*. Note, 19A-C plants contained both parental bands despite strong *S. alterniflora* morphology.

Discussion

Evolution of mixed ploidy populations

Interspecific hybridisation is generally thought to generate “genomic stress” arising from incompatibilities between parental genomes and chromosome pairing problems during meiosis. In many cases, allopolyploidy is thought to restore fertility by enabling chromosome pairing. Consequently, newly formed allopolyploids may be expected to competitively replace parental hybrids. Indeed, Charman (1990) argued that the expansion of *S. anglica* had been concomitant with the reduction of the homoploid hybrid and parental species across the entire southern coast of Britain. Surprisingly, we repeatedly found no evidence of homoploid loss.

At Hythe, the place of the original hybridisation event, we found only a few *S. anglica* plants, the majority (82%) of the individuals analysed in 2012 and 2013 were homoploid hybrids. A similar cytotype frequency was previously recorded at the same place in 2007 indicating that the population is stable (Table 1). In Hythe, we identified one nonaploid ($2n=90$) individual in 2013 while these were not recorded in 2012 (this study) and 2007 (Renny-Byfield et al., 2010). However, plants with nonaploid karyotypes were reported in this locality in the sixties (Marchant, 1967) and more recently,

Table 1 : Distribution of ploidy levels in mixed populations of *Spartina* hybrids and allopolyploids

Locality	Year	N	Hybrids			Parents		Source of data
			6x	12x	9x	<i>S. alterniflora</i>	<i>S. maritima</i>	
Hythe 50°51'39.9"N 01°23'11.9"W	2012	13	11 (85%)	1 (7.5%)	0	1 (7.5%)	0	This work
	2013	15	12 (80%)	0	1 (7%)	2 (13%)	0	This work
	2012 + 2013 combined	28	23 (82%)	1 (3%)	1 (3%)	3 (12%)	0	
	2007	34	32 (94%)	2 (6%)	0	0	0	Renny-Byfield et al. 2010
	2012 + 2013 + 2007 combined	62	55 (89%)	3 (5%)	1 (1%)	3 (5%)	0	
Hayling Island 50°49'55.9"N 00°58'29.8"W	2012	7	6 (86%)	1 (14%)	0	0	0	This work
	2013	5	2 (40%)	3 (60%)	0	0	0	This work
	2012 + 2013 combined	12	8 (66%)	4 (34%)	0	0	0	

they were located in the nearby Eling Marchwood (Renny-Byfield et al., 2010). In the latter population, the genomic composition of a nonaploid individual was assessed by genomic in situ hybridization (GISH) and shown to be AAM. This suggests that nonaploids may be scattered throughout the whole Southampton Water region at low frequency.

It will be interesting to cytogenetically characterise further individuals by GISH in order to see if reciprocal genotypes also occur. Despite the ploidy homogeneity, phenotypically, the Hythe population was not uniform. Most homoploid hybrids (87%) took their phenotype from the *S. maritima* parent (as seen in *S. anglica*) while four individuals (13%) resembled *S. alterniflora* according to morphological features (Marchant, 1967). This can be explained by known morphological plasticity of hybrid genotypes (Raybould et al., 2000). However, we cannot exclude the possibility that the parental species have hybridised more than once in Southampton Water, and that these ancient clones persist today. It is, perhaps, noteworthy that an independent hybrid between *S. alterniflora* and *S. maritima* has been formed in the South of France that is morphologically similar to *S. alterniflora* (Jovet, 1941).

It may be surprising why the highly invasive and fertile *S. anglica* dodecaploid has not outcompeted the homoploid hybrid despite the long time of their co-existence and the fact that *S. anglica* has spread worldwide. We are left with several hypotheses:

- (i) There may be recurrent and ongoing hybridisation between progenitor species leading to the continuous generation of F1 hybrids. However, this is unlikely since the last record of *S. maritima* at this area is dated to around 1900 (Marchant, 1967, 1968b). At that time, both parental species co-existed for about 20 years. Certainly, in Hythe, we never found *S. maritima* during our collecting trips in 2007, 2012 and 2013 (Table 1).
- (ii) There was more *S. anglica* soon after the time of species origin than is seen today. If so, then there must have been a massive extinction of *S. anglica* at the Hythe locality. Such “die-outs” have been occasionally documented in recently formed allopolyploid populations (Abbott and Lowe, 2004), including *S. anglica* (Goodman and Williams, 1961; Gray and Pearson, 1984). However, it remains unexplained why such a decline did not also affect *S. × townsendii*. Subtle morphological differences exist between *S. × townsendii* and *S. anglica*. For example, at Hythe, tiller counts are higher in *S. × townsendii* than in *S. anglica* (Marchant, 1964). Furthermore, stomatal density is greater and their size smaller in *S. × townsendii* than in *S. anglica*. Perhaps, *S. × townsendii* physiology is better adapted to the lower tide conditions Hythe than *S. anglica*.

- (iii) Expression incompatibilities may be less pronounced in *S. × townsendii* than in *S. anglica*. In this context, at the genomic level, more expression and methylation changes (compared to parents) have been documented in *S. × townsendii* than in *S. anglica* (Salmon et al., 2005; Chelaifa et al., 2010). Furthermore, nucleolar dominance (silencing of one rDNA homeolog) is strong in *S. × townsendii* but appeared to be labile in *S. anglica* (Huska et al., unpublished results). Perhaps, robust epigenetic silencing in *S. × townsendii* contributes to hybrid vigour and its long-term survival.

Introduction of *S. × townsendii* to distal locations

A mixed ploidy population at Hayling Island located about 40 km east of Hythe has been identified for the first time. In this population hexaploid and dodecaploid individuals are encountered. Hexaploid plants exhibit morphological similarities with *S. maritima*, but rDNA patterns suggest a hybrid origin with additive “maritima” and “alterniflora” diagnostic markers, as in *S. × townsendii*. Previously, abundant populations of *S. × townsendii* were identified in Poole Harbour, located west from Hythe (Hubbard and Stebbing, 1968) suggesting that *S. × townsendii* is not limited to a single population but occurs in at least three locations in the area. Currently, it is difficult to make quantitative estimates over the proportions of *S. × townsendii* and *S. anglica* because of the relatively small sample size. Clearly, more extensive screening is needed. How can the presence of *S. × townsendii* in these additional localities be best explained? One possibility is that the Hayling Island population may have an independent origin. Although multiple origins seem to be frequent attributes of many successful allopolyploids (Soltis and Soltis, 2009), we consider this possibility unlikely since there is no evidence that *S. alterniflora* was introduced to Hayling Island. Previous reports have indicated that, in England, its occurrence is restricted to Southampton Bay (Charman, 1990). In addition, while the second parent, *S. maritima*, has been reported on Hayling Island in the past (Raybould et al., 2000), the only hexaploid plants encountered during our sampling in 2007, 2012 and 2013 exhibited hybrid nuclear rDNA patterns. An alternative explanation for the occurrence of *S. × townsendii* at these additional localities is that they have originated from Hythe. Indeed, vegetative fragments (tillers), which have a high regenerative capability, could have been introduced to these locations by sea currents or human activity. One further possibility is that *S. × townsendii* may actually be partially fertile and produce seeds which can be dispersed. In this context, despite irregular chromosome pairing, rare bivalents have been noted in their pollen mother cells (Marchant, 1977).

The usefulness of combined ploidy and rDNA marker analysis in identification of hybrids

Flow cytometry analysis of *Spartina* provides information about the ploidy levels but it cannot distinguish homoploid hybrids from their parents. On the other hand, molecular analysis of rDNA homeologs distinguishes between hybrids and parents while it cannot assess ploidy. Thus both methods are clearly complementary, efficiently determining plant identity in mixed populations. Often, plants first identified as parental species according to morphology have subsequently turned out to be hybrids following the genotyping. The morphological plasticity of the homoploid hybrid remains unexplained, but we cannot exclude multiple origins at the time of species hybridisation.

Conclusions

There is no evidence for the somatic age-related decline of the homoploid hybrid *S. × townesendii* in Southern England since its origin more than 100 years ago. Instead, the hybrid seems to be a highly successful competitor of the invasive *S. anglica* dodecaploid, at some localities at least. Long-term survival of homoploid hybrids together with large effective population size establishes an opportunity for further evolution.

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GLOBAL METHYLATION OF DNA AMONG *SPARTINA ALTERNIFLORA* CLONES DIFFERING IN AGE AT NORTH INLET, SC.

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Abstract:

“Brown marsh” or “sudden marsh dieback” is an ecosystem condition resulting in the loss of thousands of hectares of *Spartina alterniflora*, most notably along the coastline of Louisiana. In 2000, approximately 8,000 hectares of *S. alterniflora* died back following a 100-year drought, which prompted Louisiana Governor Foster to declare a state of emergency. Other cases have been documented at North Inlet, SC as well as various other marshes along the southeastern United States. Currently, there is much dispute regarding the cause of “sudden marsh dieback” but environmental stress is one of the acknowledged constants across sites. We hypothesize an alternative mechanism in which methylation of the genome increases with plant age, reducing stress tolerance. DNA methylation occurs on the cytosine nucleotide and is an epigenetic modification that is crucial for stable gene regulation along with silencing of harmful transposable and repetitive elements (TE and RE respectively) in the plant genome. This modification is initiated and maintained through different pathways and methyltransferase families, but only one family of methyltransferases can propagate and maintain DNA methylation through a process denoted as *de novo* style methylation. We will present data addressing the level of global methylation and differences among *Spartina alterniflora* clones of variable age at North Inlet, (South Carolina, USA).

Keywords: Brown marsh, Methylation, *Spartina*, Dieback, Epigenetics

DNA methylation on the cytosine nucleotide (m⁵C) is an epigenetic modification that is crucial for stable gene regulation and silencing of transposable elements along with other repetitive elements in the plant genome (Henderson and Jacobsen, 2007). Previous studies have demonstrated that m⁵C are not added to template strands as respected nucleotides but rather they are enzymatic modifications of ordinary cytosines (Vanyushin and Ashapkin, 2011). The sequence context of this epigenetic modification has also been illustrated to differ between plants and animals predominantly in that the later preferentially chooses to methylate cytosines only in the CG context, while the former commonly occurs in all sequence contexts: CG, CHG, and CHH, where H can be A, T, or C (Henderson and Jacobsen, 2007). In plants, DNA methylation is initiated through a *de novo* style pathway via RNA Directed DNA Methylation (RdDM) and maintained through progressive generations through maintenance methylation pathways. This process is essentially a two-fold procedure consisting of laying initial methyl marks on the cytosine nucleotide targeted as well as maintaining those marks during the process of replication and postreplication (Vanyushin and Ashapkin, 2011). First observed by Wassenaar (2005), this process of RdDM is propagated by small

interfering RNAs (siRNAs) and involves a conglomerate of mechanisms including the well known RNA interface machinery (RNAi), a methyltransferase capable of perpetuating *de novo* style methylation named DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), and two specific RNA polymerases, PolIV and PolV, working in tandem with chromatin remodeling factors (Matzke et al., 2009; Huettel et al., 2007; Pikaard et al., 2008). Matzke et al. (2009) further expanded on the RdDM pathway by identifying specific methyltransferase families and the sequence context to which they act upon. The symmetric CG sequence is maintained predominantly by a subfamily of transferases, Dnmt1, known as METHYLTRANSFERASE 1 (MET1) in plants while the CHG (H being any nucleotide other than C or G) sequence is maintained by MET1 and a transferase unique to plants CHROMOMETHYLASE 3 (CMT3) (Matzke et al., 2009). The asymmetrical sequence is both established and maintained by the Dnmt3 homolog mentioned earlier, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), the only methyltransferase capable of perpetuating *de novo* style methylation of both symmetrical and asymmetrical methylation in plants (Matzke et al., 2009). The degree of specificity of DNA methylation is not restricted to the nucleotide or sequence context

but also depends upon the accessibility and structural organization of the chromatin, hence why chromatin-remodeling factors are an essential part in RdDM (Vanyushin and Ashapkin, 2011).

DNA methylation has been mostly characterized as a developmental process for eukaryotic organisms, particularly showing dynamic changes in methylation status during embryogenesis or gametogenesis in plants. For both male and female gametogenesis, a genome wide loss of methylation occurs in the tricellular pollen grains and ovule respectively (Slotkin et al., 2009; Huh et al., 2008). The pollen grains consist of two sperms cells and one vegetative nucleus; the later undergoing hypomethylation leading to reactivation of many previously silenced transposons (Slotkin et al. 2009). Genetic information is only being passed on from the sperm cells, meaning that their genetic integrity must be kept in tact unlike the vegetative nucleus. Recent studies conducted by Slotkin et al. (2009) have depicted an interesting theory as to the purpose of the reactivation of transposons within the vegetative nucleus. Sequencing of the sperm cells has revealed high levels of siRNAs corresponding to silenced transposons, these particular siRNAs are thought to be products of the vegetative cell that travel to the sperm cell and reinforce the RdDM already occurring. By undergoing hypomethylation, the vegetative cell is affectively allowing potentially harmful TEs to be expressed, which triggers the production of siRNAs theoretically initiating RdDM in the sperms cells thus ensuring a deeper level of genetic security (Law and Jacobsen, 2010). Similarly, the female gametophyte DNA undergoes hypomethylation at discrete loci in the endosperm while the embryo remains predominantly methylated (Mosher et al., 2009; Hsieh et al., 2009). It is important to note that the 21-nt siRNAs produced from the vegetative cell and endosperm act in *trans* on their subsequent targets, being the sperm cells and embryo respectively (Anges et al., 2013).

Dynamic changes in methylation status also occur during cell proliferation and differentiation within meristematic regions. During proliferation, multitudes of cells are produced with heavily decreased methylation counts while the process of cell differentiation reestablishes lost methylation marks in addition to new ones generating specific cell types (Testillano et al., 2013). Agnes et al. (2013) illustrated that active movement of methylation groups aid in restricting vascular propagation of *Pto* DC3000, *Pseudomonas syringae* pv *tomato*, in *Arabidopsis* making this defense mechanism active in regions where cell division is still occurring and maintenance methylation is ongoing, silencing targets of RdDM. Once the plant has detected a microbe, a MAMP-triggered response is activated (microbe associated

molecular pathway) that inactivates transcriptional gene silencing (TGS) by downregulating key components of RdDM while derepressing a subset of immune-response genes in *cis*, namely RESISTANCE METHYLATED GENE 1 (RMG1) (Anges et al., 2013). Much like with the pollen grains, an accumulation of siRNAs acting in a *trans* manner was noted during MAMP-triggered release of TGS. Agnes et al. (2013) proposes that the release of TGS through both PAMP (pathogen associated molecular pathway) and MAMP-triggered responses leads to the production of TE-based substrates for DICER LIKE (DCL) proteins that initiate the biogenesis of 24-nt siRNAs. These *trans*-acting siRNAs then work on the regulators of the defense genes in the cells surrounding the areas of TE activation, ultimately silencing them. Once the regulators of the defense genes are turned off, the expression of genes like RMG1 form an immune cell layer around the vasculature, preventing the propagation of *Pto* DC3000 from xylem cells to neighboring mesophyll cells (Anges et al., 2013). These findings agree with Slotkin et al. (2009) and the proposal of enhanced sperm cell genetic integrity. By undergoing a MAMP-triggered repression of TGS, the pollen grain is ensuring that the sperm cell will have a constitutively expressed antimicrobial immune response, which will limit the transmission of pathogens from the pollen grain (Mink, 1993).

DNA methylation can be mitotically inherited and local changes in methylation status have the possibility to be transgenerational for apomictic clones, increasing the probability of establishing and maintaining epigenetic changes due to environmental stresses in apomictic vegetative clones. Here, a differentiation must be established between apogamy and vegetative apomixis the former still undergoing a portion of sexual reproduction, either through selfing or spores, while the later relies on a reduced internment sexual stage and prolonged expansion by vegetative means (Klekowski, 2003). Reproducing through a strictly vegetative process should allow for the transmission of local and global changes in DNA methylation status, perhaps even histone or polycomb variants. The gametic reprogramming responsible for resetting the epigenetic status of both pollen and the embryo is sidestepped and genetically identical plants are propagated through a network of underground stems known as rhizomes. As previously stated, biotic and abiotic stress can influence the methylation status of DNA facilitating an adaptive response that has the potential to last generations. When considering a vegetative clone, it can be thought of as either a new plant, or perhaps more accurately, the branching of an existing plant that is genetically identical to the parental strand. Adaptive responses established in the parental clone can be passed onto the vegetative

progeny mitotically, perhaps in a much more successful manner.

Spartina alterniflora is a vegetative apomictically reproducing species growing predominantly across East coast marshes. Expansion of this species occurs primarily through the propagation of vegetative clones from underground rhizomes, though brief stages of sexual reproduction can occur to establish new colonies. Since this species is chiefly a clonal species, the mitotic heritability component and proposed memory capacity seen within progeny of Dandelion clones shown in Verhoeven et al. (2010) can also be assumed to be present in *S. alterniflora* along with the mitotic heritability displayed through gametogenesis. Currently there is an ecosystem condition occurring on the eastern coast of the United States dubbed “brown marsh” or “sudden marsh dieback” resulting in the rapid browning and death of thousands of hectares of *Spartina alterniflora*, most notably along the coastline of Louisiana. This phenomenon gained notoriety in the summer of 2000 when 8000 hectares of *S. alterniflora* suddenly died in coastal Louisiana prompting Louisiana Governor Foster to declare a state of emergency. Diebacks can occur throughout the range of *Spartina*, including a well-studied site in North Inlet, South Carolina. Instances of brown marsh prior to the 2000 event are also known, including one in the Charleston Harbor in 1985 coincident with the redirection of the Cooper River and a drought. In both cases, 1985 and 2000, a drought and increase in salinity was observed within the afflicted areas. Presently there is much dispute regarding the cause brown marsh, but environmental stress is one the acknowledged constants across sites. We hypothesize an alternative mechanism in which methylation of the genome increases with plant age, reducing stress tolerance.

Methylation may accumulate within a marsh stand in a clock-like fashion, with each passing year increasing the scale of genomic dysregulation. In addition, methylation may also be accumulating in response to environmental stresses themselves. In both cases, the accumulation of methylation will be synchronized across large sections of a stand since individual stems experience essentially the same stresses as their neighbors. We are proposing a generalized mechanism of increasing stress susceptibility with stand age in which intrinsic properties of a marsh stand breakdown gradually over time. The quality of the stand degrades, but the degradation has no outward effects other than increasing the susceptibility to environmental stressors. Eventually, a tipping point is triggered by the combination of the degraded marsh state and an environmental stress resulting in a brown marsh event. Preliminary sampling using an ELISA-like format for fluorescence based quantification of DNA methylation was taken from North Inlet, SC and has shown global

methylation for *S. alterniflora* clones to range from ~2-6% (Fig 1). Various sections of the marsh were sampled including a sight in which a dieback event occurred in 2002. The dieback event took place within an isolated population, 60 Bass or SB, but has since recolonized; samples were taken from the center of dieback (SBDB), the periphery (SBDP), and the outer most regions where no dieback event was recorded (SBND). Inland short form populations from Crab Haul Creek were also sampled (CHCA, CHCB), along with three open channel populations Debidue Creek (D), Old Man Creek (OMC) and South Town (ST), and a population of seedlings found at the South Town location (STS). Significant differences were seen between the South Town seedlings and the adult populations as well as the inland Crab Haul Creek populations. At the CHC location,

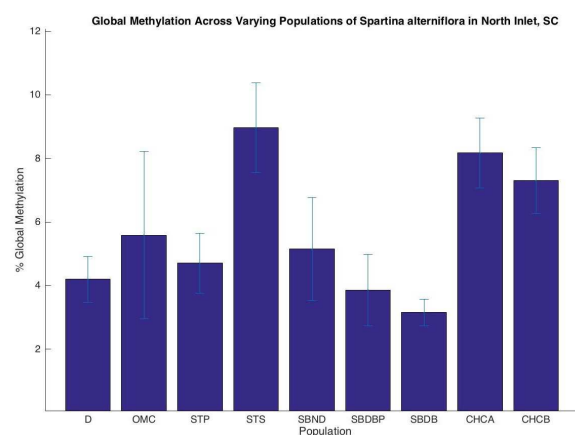


Figure 1. A field survey of various populations across North Inlets, SC measuring global methylation. Significant differences were seen comparing the South Town Seedlings (STS) and Crab Haul Creek (CHCA, CHCB) populations to the remaining 6 populations tested. Each of the sites are geographically isolated and cover a range of salinities. CHCA and CHCB were further analyzed looking at methylation coverage within clearly identified genets. Samples were taken at 1-meter intervals both horizontally and vertically across the genet to give a range of coverage of old and young plants. p-value: 3.38×10^{-6} for the global methylation study across various populations.

separate genets were identified and sampled to test the hypothesis that global methylation rates increase based on location or “age” within the genet. Preliminary findings have shown that no significant differences can be seen in global methylation percentages based upon location within the genet structure, though the percentages varied. Figure 2 is an image of each of the study sites from Google Earth. In addition to the field survey, a greenhouse experiment is currently being conducted to test the hypothesis that methylation predicts the susceptibility to stress in addition to investigating the effects of drought caused by increasing salinity. We chose to

use 3 test groups based on the populations that showed significant differences in methylation status from the field survey; the South Town seedlings, inland and creek bank populations. Each of these will be grown under greenhouse conditions in a 30 ppt saline solution in a Hoaglands nutrient media. The

plants will be subjected to a sequence of 5 ppt step increases in salinity, with a week between each step. Each week, nondestructive biomass measurements will be taken along with rates of photosynthesis and leaf respiration.



Figure 2. Google earth image of North Inlet, SC. The 4 studies sites throughout the marsh are identified with a yellow pin. Seedlings were found at the South Town Creek location while a dieback event has been documented at the 60 Bass Creek location in 2002.

In conclusion, significant differences in global methylation can be seen in the South Town seedling as well as the Crab Haul Creek samples when compared to other members of the adult populations. Global methylation rates among adult members were not significantly different and the clonal studies from Crab Haul Creek (CHCA and CHCB) showed no definitive difference among clones in methylation. When considering methylation and its influence throughout the genome, it is important to note that plants have a unique ability to undergo gene-body methylation in which large sections of genes will become hypermethylated without compromising the efficiency of the gene in question. Bearing that in mind, increases in global methylation might not be the answer to the brown marsh ecosystem condition but rather the strategic placement of methyl marks on prominent defensive genes resulting in a decreased or diminished response to both biotic and abiotic stressors.

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**MOLECULAR RESPONSE TO CHALLENGING ENVIRONMENTAL CONDITIONS
IN *SPARTINA ALTERNIFLORA*: AN INTEGRATED APPROACH**

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Abstract

Native populations of the invasive grass *Spartina alterniflora* provide a unique opportunity to explore mechanisms of phenotypic response to a range of stressors, including persistent environmental gradients and periodic disturbance events, that may inform our understanding of the global *S. alterniflora* invasion. Here, we use Amplified Fragment Length Polymorphism (AFLP), methylation sensitive-AFLP (MS-AFLP), and microarrays to examine genetic, epigenetic, and transcriptional response to natural and anthropogenic stressors in native populations of *S. alterniflora*. Preliminary data from these experiments suggests that epigenetic variation is at least partially structured by predictable natural gradients in environmental factors, whereas response to the severe disturbance of the *Deepwater Horizon* oil spill changed the genetic make-up of populations and had a strong effect on gene expression. These findings suggest that the ability of *S. alterniflora* to persist across a range of conditions may be due to a combination of epigenetic and genetic molecular mechanisms. Invasive species often display high phenotypic variation and are successful in a wide range of habitats. Our results indicate that both epigenetic and genetic molecular mechanisms may provide insight into the regulation of phenotype in response to the novel stressors present in an invaded habitat.

Keywords: Epigenetics, gene expression, oil spill, native salt marsh

Introduction

Salt marsh habitats are dynamic and heterogeneous environments, and we have a rich history of ecological study of native salt marsh systems (reviewed in Pennings and Bertness, 2001). Native salt marshes are important to coastal ecosystems in part, because they provide critical services such as erosion mitigation and

sediment filtering (Pennings and Bertness, 2001). The strong nutrient and salinity gradients present within the salt marsh create unique community patterns. For instance, tidal and rainfall influences generate salinity gradients across the salt marsh, which affect the types of plants present along the gradient (Bertness and Ellison, 1987; Pennings and Bertness, 2001). Salt marshes also experience frequent, but unpredictable,

anthropogenic impacts including land development, oil spills, and the effects of climate change (RamanaRao et al., 2012; Silliman et al., 2012; McCall and Pennings, 2012). Because of these stressors and impacts, salt marsh plants like *Spartina alterniflora* must orchestrate a variety of processes at several biological scales to cope in its natural habitat and modulate phenotype.

While there are several possible molecular mechanisms with which a population could respond to environmental stimuli, four major ones are: selection of genetic variants, differential expression of genes, modification of proteins, and modification of epigenetic marks (Nicotra et al., 2010). In particular, epigenetic mechanisms may allow for rapid modification of phenotype in response to immediate and acute stressors. There are several epigenetic mechanisms that can alter gene expression (e.g. chromatin remodeling, histone modifications, small interfering RNAs), but DNA methylation of cytosines is most commonly studied (Schrey et al., 2013). In many eukaryotes, DNA methylation occurs at the fifth carbon of a cytosine—as 5-methylcytosine—located within one of several DNA sequence motifs: CpG, CpCpG, CpHpH, CpHpG (where H is any nucleotide except guanine) (Rapp and Wendel, 2005; Zilberman and Henikoff, 2007; Zhang et al., 2008; Laird, 2010). Additionally, stress-induced changes in methylation may persist after the stress is relieved (Verhoeven et al., 2010; Richards et al., 2012a). In ecology and evolution, the ability to rapidly alter phenotype is of great interest, and DNA methylation may be an important source of variation and a means by which *S. alterniflora* modulates phenotype in response to various environmental stressors (Salmon et al., 2005; Ainouche et al., 2009).

The most immediate consequences of genetic and epigenetic change in a population is the differential expression of genes. Transcriptome analyses have shown that mRNA expression is generally positively correlated with protein expression, and changes in gene expression may alter the expression of phenotype (Rees et al., 2011). Because gene expression affects phenotype, it may play an important role in ecological processes such as adaptive divergence and phenotypic plasticity (reviewed in Alvarez *et al. in press*). Previous ecological transcriptomic studies have identified numerous gene networks that are responsive to environmental stimuli (e.g. Richards *et al.* 2012b, Richards *et al.* unpublished) and have been used to clarify the effects of molecular-level regulation on phenotype. In addition, gene expression may capture the effects of functional genetic and epigenetic variation, making it an important tool for assessing stress response.

Our laboratory group is currently investigating natural populations of *S. alterniflora* at the molecular level by examining genetic, epigenetic, and gene expression patterns in response to complex environmental stressors. In three studies, we assessed the response of *S. alterniflora* to both the persistent, complex environmental stress gradient and the unpredictable, but severe, disturbance of the *Deepwater Horizon* oil spill. We hypothesized that *S. alterniflora* responds to these stressors through a combination of genetic differentiation and epigenetic regulation, which results in differential gene expression. Together these differences likely contribute to variation in phenotype and fitness.

Response to Persistent Stressors

In native populations of *S. alterniflora* from Sapelo Island, Georgia, USA, we detected a broad range of variation in eight morphological and fitness proxy traits that were correlated to salinity, waterlogging and organic matter content of the soil (Richards et al., 2005). Ecologists long assumed that populations of *S. alterniflora* were comprised of a few large clones that were tolerant to the range of conditions across the salt marsh; however, using allozyme markers we revealed that native populations of *S. alterniflora* have high genetic diversity (Richards et al., 2004). While there was high genetic diversity, there was no association of genotypes to microhabitats along the gradient (Richards et al., 2004). Thus, *S. alterniflora* may respond to microhabitat by adjusting phenotypes through non-genetic mechanisms (i.e. phenotypic plasticity), which may be modulated by molecular epigenetic mechanisms (Nicotra et al., 2010).

To better understand the relative contributions of the mechanisms that underlie response to persistent stress, we used AFLP and MS-AFLP markers to assess genetic and epigenetic population structure across the salt marsh gradient (Foust et al., 2015). We sampled 20 individuals from each of three habitats (corresponding to low, medium, and high salinity) across five natural populations of *S. alterniflora* on Sapelo Island in May 2011 (Foust et al., in review). Instead, we found weak genetic population structure exhibiting a pattern of isolation by distance as determined by Bayesian clustering performed with STRUCTURE v.2.3.4 (Figure 1). This pattern may represent higher similarities between northern (i.e. Apex and Cabretta) and southern (i.e. Hunt Camp, Lighthouse, and Marsh Landing) sites, or they could indicate a continuous gradient ranging from the northern to the southern portions of the island. We will explore these trends with more detailed analyses.

Response to Disturbance

In April 2010, the *Deepwater Horizon* oil spill dramatically impacted salt marsh populations across the Gulf of Mexico. As the dominant plant on the leading edge of the salt marsh, many *S. alterniflora* populations experienced heavy deposits of weathered crude oil that caused extensive mortality (Lin and Mendelsohn, 2012; Silliman et al., 2012). Despite large dieback, populations of *S. alterniflora* rebounded quickly and showed resilience to this stress (Lin and Mendelsohn, 2012). Although tragic, this event presented a unique opportunity to study the effects of sporadic, severe stressors on the ecology and evolution of *S. alterniflora*. In August 2010, we collected samples of leaf tissue from 10 individuals in three paired, contaminated, and uncontaminated populations of *S. alterniflora* along the Gulf of Mexico. We examined genetic and epigenetic variation within and among the populations using AFLP and MS-AFLP, and we used GENALEX (Peakall and Smouse, 2006, 2012) to perform a hierarchical AMOVA. We found significant genetic differentiation between the contaminated and uncontaminated populations, suggesting that the oil spill may have selected for particular genotypes (Robertson et al., unpublished). However, we did not find a correlation between DNA methylation and oiled vs. non-oiled sites (Table 1). Although our other work suggests that epigenetic variation may play an important role in coping with persistent environmental stressors in the salt marsh (Foust et al., in review), we were unable to identify a clear role for DNA methylation in this catastrophic disturbance (Robertson et al., unpublished). In this case, because we sampled several months after the oil spill, we may have missed a critical period during which epigenetic response to the oil spill could be detected. Additionally, we found high amounts of epigenetic variation within populations (Table 1), which may have hindered our ability to detect epigenetic variation in response to oil contamination. However, the strong genetic differentiation among oil contaminated and uncontaminated sites suggests the severe stress of the oil spill and the accompanying mortality may have overwhelmed the ability of certain genotypes to respond to the stress, selecting for individuals with particular alleles instead.

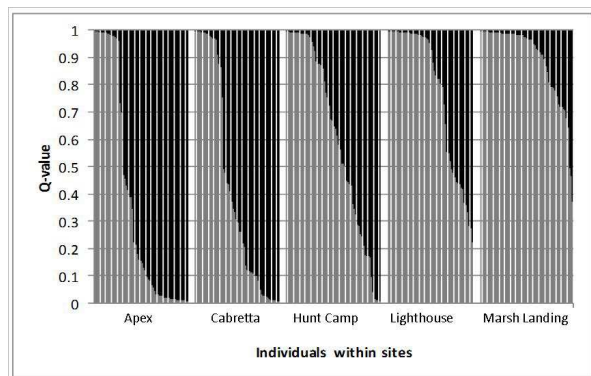


Figure 1. Bayesian assignment testing results for *Spartina alterniflora* assigned to two clusters. Each bar represents an individual plant. The fraction of the individual's genotype attributed to each of the clusters (Q-value) is indicated by color (Cluster 1=black, Cluster 2=grey). This pattern suggests weak genetic differentiation exhibiting isolation by distance across the island.

Our results suggest that epigenetically-mediated differentiation may play a more important role in the response to local environmental challenges among habitats than genetic differentiation does. We detected a significant correlation between epigenetic variation and micro-habitat when we controlled for the correlation between genetic and epigenetic variation (Foust et al., in review). This relationship is even more telling given that there was no significant genetic structure within sites among micro-habitats. We also found MS-AFLP loci that were significantly differentiated in at least one habitat based on locus-by-locus AMOVA (Figure 2).

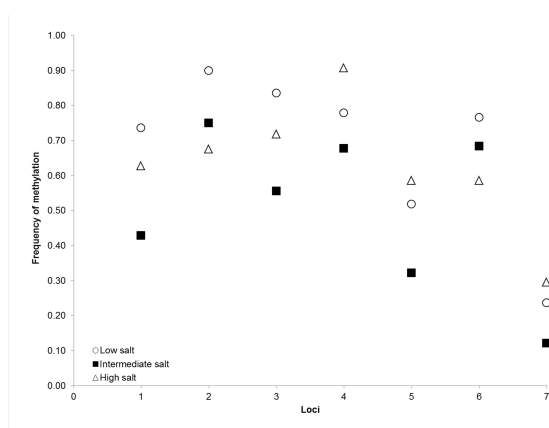


Figure 2. MS-AFLP loci from *Spartina alterniflora* individuals sampled among three habitat types (low/medium/high salt) across five sites on Sapelo Island, GA that had significant differences in frequency of DNA methylation among the habitat types (low salt, intermediate salt and high salt).

Table 1. Results of hierarchical AMOVA, among all populations, among populations within site type, and between site types (oiled and non-oiled) for genetic (AFLP) and epigenetic (MS-AFLP) loci. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

	Φ statistics	
	Genetic	Epigenetic
Within populations	$\Phi_{PT} = 0.215$ ***	$\Phi_{PT} = 0.071$ ***
Among populations	$\Phi_{PR} = 0.168$ ***	$\Phi_{PR} = 0.055$ *
Among site type	$\Phi_{RT} = 0.056$ *	$\Phi_{RT} = 0.017$ NS

In addition to genetic and epigenetic analyses, we surveyed gene expression variation in the populations of *S. alterniflora* that were affected by the *Deepwater Horizon* oil spill. We used a microarray, specifically developed for *S. alterniflora*, to perform a genome-wide expression survey on the samples previously mentioned. After normalization and ANOVA, our preliminary results show widespread differential gene expression in response to oil contamination, which may modulate the ability of *S. alterniflora* to respond to this stressor (Alvarez et al., unpublished). Through our expression analysis, we identified 3622 genes responsive to oil stress (FDR < 0.05). To further understand the functional implications of this transcriptional variation, we looked for enrichment of Gene Ontology categories, and visualized the categories as gene networks (Katari et al., 2010). In particular, we focused on gene categories that represented stress responses, such as "Response to Salt Stress" and "Response to Cadmium Ion" (Figure 3). Cadmium is particularly important in this context because it is one of the plant toxins present in petroleum. Using these networks, we identified highly connected genes, such as AtRNR1 and AtOST1, which may have been regulators of other genes in their network. In addition to their connectivity, these gene targets may also play an important role in cellular function. AtRNR1, for instance, is a ribonucleotide reductase involved in the production of dNTP for DNA replication and repair. AtOST1 is a protein kinase activated by osmotic stress. Mutations in AtOST1 disrupted stomatal closure and opening in *Arabidopsis thaliana*, affecting organismal phenotype beyond cellular phenotype (Katari et al., 2010). Like other traits, gene expression is heritable, and may contribute to divergence between populations that is suggested by our AFLP results (Alvarez et al., 2015). However, the long-term stability and impact of differential transcription on oil-impacted *S. alterniflora* populations is still unclear, and further study is required to separate the transcriptional differences that are due to genetic structure and those caused by regulatory changes.

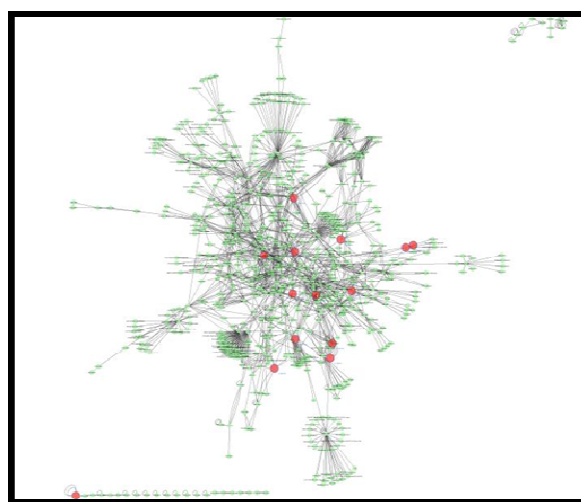


Figure 3. All genes in the gene ontology category: "Response to Cadmium Ion". Green points represent genes known to respond to cadmium ion in *A. thaliana*. Red points show genes in the network that responded experimentally.

Future Directions and Conclusions

In healthy native marshes, *S. alterniflora* displays phenotypes that are correlated to the environmental gradient within the salt marsh, including reduced above-ground biomass in response to increasing salinity (Pennings & Bertness, 2001; Richards et al., 2005). *Spartina alterniflora* is tolerant to salinity and temperature stress in controlled conditions (Baisakh and Subudhi, 2009; Subudhi and Baisakh, 2011) and shows resilience to oil stress from the *Deepwater Horizon* oil spill across populations (Lin and Mendelssohn, 2012; Silliman et al., 2012). Gene targets, identified from genetic, epigenetic, and transcriptional data, may be used to correlate alternative phenotypic responses to molecular processes.

Further research integrating different levels of biological organization will be necessary to understand response to stimuli in this important non-model species and specifically link molecular level changes to phenotype and fitness (Alvarez et al., 2015). Although we studied native populations of *S.*

alterniflora, our findings provide critical insight into the mechanisms by which short-term and long-term environmental challenges alter population structure. These mechanisms may be the same molecular pathways exploited by *S. alterniflora* during invasions, when populations are faced with novel stressors. The gene targets provided from genetic, epigenetic, and transcriptional assays may help provide further insight on invasive *S. alterniflora* populations by providing diagnostic markers for the ability to respond to various types of stressors. Once a target is identified and its effects are confirmed, it may be used to assess the potential for native *S. alterniflora* populations to become invasive, or the potential for invasive *S. alterniflora* populations to spread.

We intend to use the aforementioned design to continue our exploration of stress response in *S. alterniflora*. Gene targets, identified from our *Deepwater Horizon* oil spill expression survey, will be further analyzed in a controlled study using tDNA knockout genotypes of the grass *Brachypodium distachyon*. We will measure changes in biomass and photosynthetic rate in replicates of these lines that are exposed to crude oil to isolate the impact of differential expression of the target genes on phenotype. This design will allow us to isolate the effects of genetically-induced transcriptional variation on phenotype, potentially providing a link between molecular mechanisms and organismal fitness. We also intend to collect rhizomes from the same pairs of populations in Louisiana for further controlled greenhouse studies. These studies will allow for exploring long-term response to the oil spill through controlled application of oil.

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SESSION 3: Ecology and ecosystem effects of
Spartina

(chairman: S. Pennings)

MARSH EQUILIBRIUM THEORY

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Abstract:

Marsh equilibrium theory explains how the vegetated marsh surface tracks rising sea level and maintains relative marsh elevation within the vertical range of tolerance of the vegetation. Smooth cordgrass, *Spartina alterniflora*, has a wide vertical range of tolerance, and this is important for the stability of coastal wetlands where this species dominates. The equilibrium elevation and primary productivity are functions of the rate of sea-level rise. Marsh elevation increases with increasing primary production due to the trapping of sediment by vegetation and to the growth of biovolume via belowground production. For marshes located high in the tidal frame, primary production responds positively to rising seas; subsequently, sediment trapping and elevation increase. But the equilibrium elevation decreases as the rate of sea-level rise increases, moving the marsh toward a tipping point. The resilience of a marsh to an increasing rate of sea-level rise is proportional to the vertical distribution of vegetation within the tidal frame and the skewness. Resilience is greatest when the biomass distribution is positively skewed with respect to elevation, or when the optimum elevation for maximum biomass production is lower in the tidal frame. Selection for varieties of marsh vegetation with those characteristics should increase the chances of intertidal wetlands surviving in place.

Keywords: model, sea level, simulation, salt marsh, *Spartina*, accretion ratio

Introduction

Salt marshes that developed during times of low sea-level rise have maintained elevations in sync with sea level for several thousand years (Redfield, 1972). However, sea-level rise (SLR) is currently accelerating (Church and White, 2006; Kemp et al., 2011) and is expected to reach levels (Rhamstorf et al., 2012) that threaten to submerge our existing coastal wetlands by the end of the century (Kirwan et al., 2010). The resilience of these ecosystems is dependent on their ability to maintain elevation relative to sea level within a suitable vertical tidal range that lies approximately between mean high water (MHW) and mean sea level (MSL) (McKee and Patrick, 1988).

Research conducted in marshes at North Inlet, SC has shown that marsh productivity has a positive effect on the rate of vertical accretion of the marsh surface (Morris et al., 2002), and as shown at North Inlet and Plum Island, MA, the relative elevation of the sediment surface exerts an important control over the productivity of the salt marsh plant community (Morris et al., 2013). This results in stabilizing feedback at low rates of SLR that maintains relative marsh elevation in the upper part of the tidal frame, but as SLR accelerates this feedback could switch to a destabilizing form of control that results in the rapid submergence and loss of marshes.

This manuscript updates and summarizes the history of marsh accretion at North Inlet, applies the original

Marsh Equilibrium Model (MEM) to those data, and explores theoretically the characteristics of marsh vegetation that influence these feedbacks between vegetation and vertical accretion. The original MEM (Morris et al., 2002) assumes, like the model of Krone (1985), that vertical accretion dz/dt is proportional to the depth (D) of the marsh surface below MHW, which affects inundation time and the opportunity for mineral sedimentation. Marsh vegetation also contributes to vertical accretion by aiding in the trapping of mineral sediment and by producing biovolume. Biovolume is created largely from production of roots and rhizomes, a process that has the capacity to build peat. The effects of depth and biomass on vertical accretion can be expressed simply as

$$dZ/dt = (q + kB)D, \text{ for } 0 < D < T \quad (1)$$

where T is tide range, depth D (cm) is positive for marsh elevations lower than MHW. B is aboveground biomass density (g/cm^2). The parameters q and k account for mineral deposition in the absence of plants and for the effect of biomass on vertical accretion, respectively. Parameter k accounts for trapping of mineral sediment as well as creation of biovolume.

Biomass B is a function of the relative elevation of the marsh surface or, alternatively, its depth D below MHW. I will refer to this as the biomass profile:

$$B = aD + bD^2 + c \quad (2)$$

At the low end of the biomass profile (high D), marsh vegetation succumbs to hypoxia, and at the upper end (low D) the vegetation suffers from osmotic stress from drought and salinity.

Of course relative elevation is not the only determinant of primary production. Wieski and Pennings (2013) have shown that salinity, altered by variation in river discharge, is a major determinant of primary production in the Altamaha river estuary on the coast of Georgia, USA. Fresh water flow into North Inlet estuary is insignificant (see below), but rainfall there has a significant effect on primary production (Morris and Haskin, 1990). Consequently, the biomass profile (Eq. 2) is variable, depending on a number of factors. One can think of Eq. 2 as belonging to a family of curves or, alternatively, a slice through a three-dimensional surface in which time is the third dimension.

Taking the approach that Eq. 2 represent the average biomass profile, and making the substitution for B in Eq. 1 yields the following:

$$dZ/dt = [q + k(aD + bD^2 + c)]D, \text{ for } D > 0 \quad (3)$$

When in equilibrium with sea level, $dZ/dt = r$ where r is the rate of SLR. Thus, as rate r increases, then D will increase (Eq. 3). In fact, D can increase beyond the limits of the vegetation. I will show below how the equilibrium depth changes with different biomass profiles and for accelerating SLR.

Methods

Marsh Elevation

Elevation change was measured with a nine-pin, Sedimentation-Erosion-Table (SET), which is a portable, mechanical device designed to attach to a stable, benchmark pipe driven into the ground. The experimental design and details were discussed earlier (Morris et al., 2002). Three SET benchmark pipes were installed during May 1996, and sampled approximately monthly beginning in June 1996 to the present. Each benchmark pipe was established in the center of a paired plot, with a control plot on one side and a fertilized plot on the other. Elevations were measured at six different compass positions around each benchmark pipe, three positions each in the control and fertilized plots of each pair. Change in elevation was determined by comparing repeated monthly measures of SET pin lengths relative to the means of marsh elevations, established subsequently by real-time kinematic (RTK) receiver and back-calculated to time-zero.

Measuring NPP with marsh organs

End-of-season (EOS) standing biomass has been measured from annual harvests taken from three bioassay "marsh organ" experiments that have been running at North Inlet since 2002. Each marsh organ has

six to eight rows of 6 inch diameter PVC pipe. Each row consisted of 6 replicate pipes. The organs were oriented to face south to avoid self-shading and were placed at an elevation that spans the upper half of the tidal frame in a location that is protected from waves and strong currents. Pipe heights ranged from 0.46 m to about 1.5 m. The pipes were filled with sediment collected from a nearby creek and planted each spring with sprigs of *S. alterniflora* collected nearby (Morris, 2007; Morris et al., 2013).

Statistics

Parameters q and k in Eq. 1 were estimated using a least-squares non-linear regression procedure (Proc Model, SAS 9.4) with a choice of the dynamic model solution option. A dynamic solution refers to a numerical integration of a differential equation (Eq. 4) obtained by using only solved values for the lagged value, marsh elevation (Z). The procedure finds the parameter values that minimize the sum of squares of errors: $\sum (Z_{\text{obs}} - Z_{\text{pred}})^2$. The time step was 1 yr. The dependent variable marsh elevation (Z) was taken from the end-of-year SET measurements. Independent variables in the model were EOS standing biomass, annual mean high water level (MHW) computed from monthly means, and the initial marsh elevation. EOS biomass was calculated from a parabola fitted to marsh organ harvest data (Morris et al., 2013) and depth of the top of the pipe below MHW. Depth of the marsh surface was computed as annual MHW minus the marsh elevation.

Simulations

Eqs. 1 and 2 were solved numerically using current initial conditions at North Inlet for marsh elevation and the current rate of sea-level rise. I assumed two alternative sea-level rise scenarios: a constant rate of sea-level rise equivalent to the 20th century rate of 3.4 mm/yr and an accelerating rate that reaches 80 cm above the current MSL in 100 yr and computed as $MSL(t) = MSL(0) + c_1t + c_2t^2$ (NRC, 1987). Constants c_1 and c_2 are uniquely dependent upon the assumed end point (80 cm), elapsed time (100 yr), and current rate (0.34 cm/yr).

Results

Marsh elevations at the North Inlet Goat Island site have been monitored for 18 years (Fig. 1). Over that period of time control sites have gained an average of 0.1 cm/yr (estimated by linear regression), which is less than the rate of sea-level rise. The 20th century rate of sea-level rise, measured at the NOAA Charleston station 8665530 was 3.4 mm per year (Morris et al., 1990). The slope of the predicted (fit of the MEM) accretion rate in control plots was 0.15 cm/yr.

The greatest changes in marsh elevation have been observed in plots that were fertilized during the first 9 years (Fig. 1). During the treatment period, fertilized plots gained elevation at a rate of 0.4 ± 0.06 cm/yr (by linear regression). Since the fertilizer treatment was halted in 2005, elevations have dropped nearly 2 cm from 2005 to 2014. At the post-treatment accretion rate of -0.18 ± 0.05 cm/yr, the elevations of the post-fertilized and control plots should intersect in the year 2020.

A fit of the model (Eq. 3) to the combined data from the fertilized and control sites gave a coefficient of determination of 0.89 (Fig. 1). With these data there is no way of parsing the plant contribution to accretion between sediment trapping and biovolume growth, because biovolume was not measured, but the plant contribution ($k=0.0735$ g⁻¹ yr⁻¹) in general is highly significant ($p<0.0001$). The mineral sedimentation rate independent of the plant contribution ($q=1.76 \times 10^{-3}$ yr⁻¹) was less important to the fit of the model ($p=0.11$). The coefficient values derived from this regression were used in the following simulations.

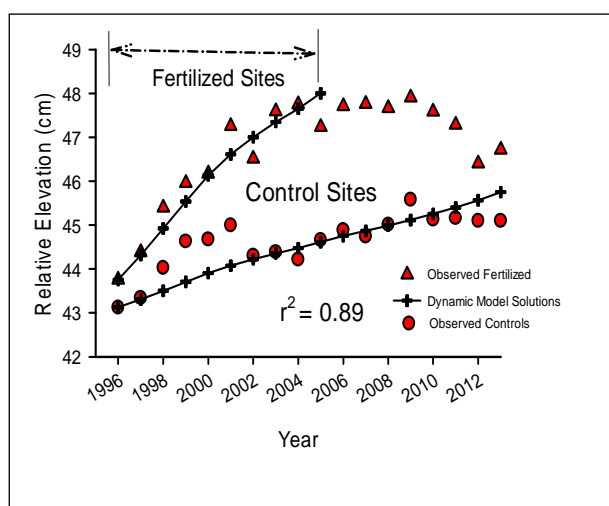


Figure 1. Time series of mean marsh surface elevations (NAVD 88) in plots that were fertilized with N and P from 1996 through 2005 and in control plots. The solid lines with crosses are from the best fit of the model (numerically integrated Eq. 3) to the data.

Using a biomass distribution typical of North Inlet marshes (Fig. 2A), the marsh remains in equilibrium with sea level with little or no loss in relative elevation when sea level rise was linear and constant at 0.34 cm/yr (Fig. 2B). However, when sea level was assumed to rise 80 cm in 100 years, accelerating to that level by the end of the century, marsh elevation decreased to nearly 0 cm relative to sea level (Fig. 2C). A marsh could still exist at that elevation, but would be approaching the lower limit of the vegetation (Fig. 2A). Biomass and accretion rate would be declining.

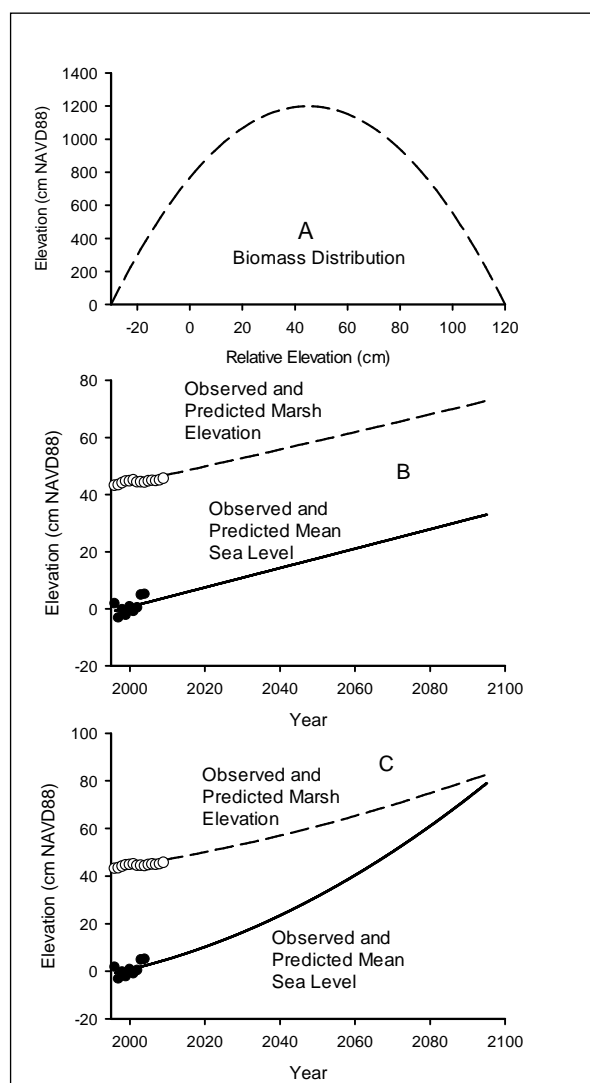


Figure 2. A) A biomass profile typical of North Inlet and used in simulations resulting in outputs shown in 2B and 2C. B) Predicted marsh elevation (---) and sea level (—) rising at constant rate 0.34 cm/yr. C) Predicted marsh elevation (---) and sea level (—) rising at an accelerating rate to a level 80 cm above present in a century. Observed marsh elevations (o) and sea level (●) are also shown.

From simulations that explore the consequences of the size and shape of the biomass profile, all using the 80 cm sea level scenario, it was shown that the marsh did not survive when its biomass profile was distributed over a range of 10 to 80 cm (Fig. 3A and 3C). Reducing the breadth of the biomass profile (Fig. 3A vs Fig. 2A) increased the relative decline in elevation (reduced the accretion rate) (Fig. 3C vs Fig. 2C) and resulted in marsh extinction by the year 2084. The combination of a limited vertical range (10 to 80 cm) and a reduced maximum biomass (600 g/m²) (Fig. 3B) resulted in an even lower vertical accretion rate and faster extinction time (Fig. 3D).

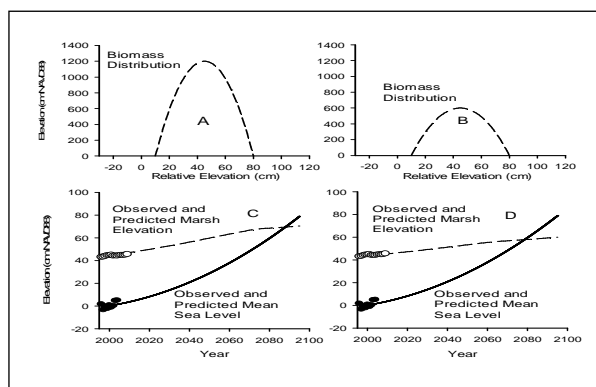


Figure 3. (A-B) Different biomass profiles used in simulations C-D. The maximum of profile A is typical of North Inlet, but is compressed. This profile generated the output in C. Profile B is also compressed and has a maximum biomass that is 50% of profile A. This resulted in the output shown in 3D.

Biomass profiles are not necessarily symmetrical as depicted in Figs. 2 and 3. Simulations were run to explore the consequences of varying degrees of asymmetry in the biomass profile (Fig. 4). The results are expressed as an accretion ratio, defined as the total vertical accretion of the marsh in 100 years (cm/century) divided by the sea level rise, which in these simulations was 80 cm. Thus, an accretion ratio of 0.5 would represent a marsh that accretes vertically only half as much as sea level has risen in 100 yr.

The consequences of varying the minimum and optimum elevations of the biomass profile, while holding the maximum elevation constant at 120 cm showed that accretion ratio was generally increased by reducing both the optimum and minimum elevations. Reducing either the optimum or minimum increased the accretion ratio, while reducing both had the greatest effect (Fig. 4). For minimum elevations ranging from -30 to 30 cm, the accretion ratio varied from 0.46 to 0.19 for optimum elevations that were 30 to 10 cm higher than the minimum, respectively. This also is a case of a right-skewed biomass profile having the greatest positive effect on accretion ratio.

Discussion

Marshes are stable and resilient against relatively low rates of sea-level rise. As the rate of sea-level rise increases, relative marsh elevation decreases, which raises both biomass and accretion rate. The rise in accretion rate reestablishes the equilibrium. The equilibrium depth of the marsh surface below MHW is directly proportional to the rate of sea-level rise, and the depth will slowly increase until it passes over the optimum level. Beyond that point, further increase in rate of sea-level rise depresses biomass and accretion, and the feedback switches from negative to positive.

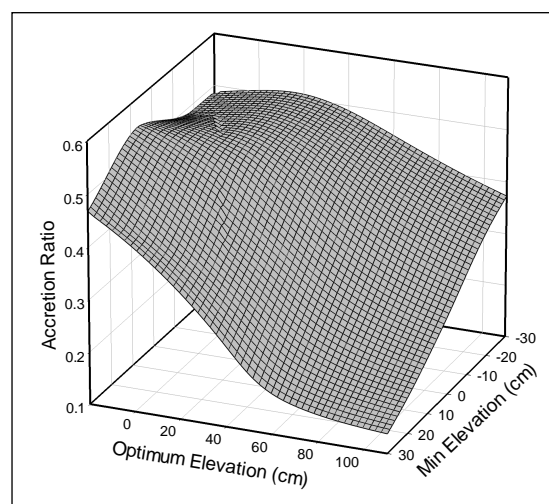


Figure 4. The forecasted vertical accretion ratio (see text) as a function of the optimum and minimum elevations of the biomass profile. The response surface is generated from simulations that assume a century of rising sea level, accelerating to a level 80 cm above present, a maximum biomass at the optimum elevation of 1200 g/m², and a maximum elevation of 120 cm.

An updated version of the original marsh equilibrium model (MEM) suggests that existing marshes in North Inlet estuary will survive an 80 cm rise in sea level by the end of the century. However, the elevation of the marshes at that time should be approaching mean sea level (Fig. 2), which is perilously close to the lower limit of the vegetation. Moreover, that elevation is suboptimal for the vegetation, which implies that further increase the rate of sea-level rise will decrease biomass and, consequently, sedimentation rate, which moves the marsh closer to extinction. It should be noted that with acceleration to the 80 cm level, the rate of sea-level rise at the end of the century will be close to 1.4 cm/yr, not the linear and constant rate of 0.8 cm/yr. It will be the acceleration in rate near the end of century that drowns massive areas of coastal marshland.

The model statistics suggest that the vegetation is relatively more important to vertical sediment accretion in North Inlet salt marshes than is the purely physical process of sedimentation in the absence of vegetation. This is a good generality for conditions at this site; there is no alternative explanation for the rapid rise in elevation in the fertilized plots, and we think that the increased biovolume in fertilized plots is important (see Wigand et al., 2015). This can be illustrated by a simple calculation. The depth of the marsh surface in SET plots was approximately 30 cm below MHW (i.e. $D = 30$ cm). The sedimentation rate in the absence of vegetation at that depth is (q/D) or 0.05 cm/yr. The vegetation should account for DkB of accretion or 0.1 cm/yr, while in the fertilized plots with 3.8 times the biomass of the controls,

DkB accounted for 0.5 cm/yr of accretion. The tidal creeks in North Inlet have about 20 mg/l of suspended sediment, which is on the low end of the spectrum (Kirwan et al., 2010), and the relative importance of vegetation needs to be tested in systems with high sediment loads.

The model makes several interesting generalities about the influence of the biomass profile on vertical accretion. An obvious finding is that biomass matters. While North Inlet with maximum biomass of about 1200 g/m² and wide vertical range survives an 80 cm rise in sea level (Fig. 2), vegetation having a narrower vertical range and equivalent maximum biomass does not (Fig. 3). I have shown that maximum biomass, independent of the vertical range, also makes a difference (Fig. 3D and 3E).

The skewness of the distribution is also important. The accretion ratio (the ratio of vertical accretion to total sea-level rise) is most sensitive to both the position of the optimum elevation within the vertical biomass range and the minimum elevation (Fig. 4 and Fig. 5). Having a lower optimum and a lower minimum extends the life of a marsh. Somewhat surprising, the accretion ratio was insensitive to the maximum elevation of marsh vegetation (Fig. 4). The explanation for this is simply that the equilibrium marsh elevation at North Inlet, at the prevailing rate of sea-level rise and higher, is already considerably lower than the maximum elevation. Based on these results I would recommend that marsh restorations be planted with varieties of marsh vegetation that generate maximum biovolume and thrive at the lowest possible elevations.

Acknowledgement

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SPARTINA ALTERNIFLORA: THREAT OR SAVIOR FOR COASTAL SALT MARSHES?

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Abstract:

Coastal salt marshes are subject to a variety of disturbances that threaten their sustainability. Often such disturbances, for example tropical cyclones, drought, oil spills, cause plant mortality and subsequent habitat deterioration. Natural and human-induced disturbances threaten the sustainability of coastal wetlands globally, especially in an environment of accelerating sea-level rise. Thus, an understanding the role of coastal vegetation in ameliorating the impacts of disturbances and buffering the habitat from the effects of sea-level rise is paramount. We investigated the capacity of *Spartina alterniflora* to maintain an intertidal position, via the accumulation of inorganic and organic materials, after disturbance and in an environment of accelerated relative sea-level rise. To determine the importance of *S. alterniflora* in these processes, we measured elevation change and surface accretion over a four-year period in recently disturbed and unvegetated marshes. Disturbance resulted from a drought-induced marsh dieback. We compared soil and vegetation responses in paired planted and unplanted plots with adjacent reference plots that had not experienced dieback. All treatments (unvegetated, planted, and reference) were replicated six times. The disturbed areas at the beginning of the study were 6 to 10 cm lower in elevation than the reference marshes due to post-disturbance soil compaction; consequently, mean water levels were 6 to 10 cm higher in these areas versus the reference sites. Surface accretion rates at 2.3 mm/yr were lowest in plots without *S. alterniflora*, but increased in the presence of this species to 16.4 mm/yr in the reference marsh and 26.1 mm/yr in the *Spartina*-planted plots. Rates of elevation change were also bolstered by the presence of *S. alterniflora*. The unplanted areas decreased in elevation by 9.4 mm/yr; while the *Spartina*-planted areas increased in elevation by 13.3 mm/yr, and the reference marshes increased by 3.5 mm/yr. These results highlight the importance of *Spartina alterniflora* in the accretionary processes that maintain marsh surface elevation within the intertidal zone and its importance in accelerating marsh recovery after disturbance in an environment of rising sea levels.

Keywords: disturbance, elevation change, *Spartina alterniflora*, accretion, SET

LAND LOSS RATES INFLUENCED BY POPULATION GENETICS

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Abstract:

S. alterniflora is a well-known ecosystem engineer, able to shape the physical structure of its surroundings. Where invasive, the species can rapidly accumulate sediment and organic material, initiating positive feedbacks that alter hydrology and convert intertidal mudflats into dense meadows of tall grass. Introductions to San Francisco Bay in North America and the Yangtze River Delta in China provide notorious examples of such transformations. In its native range, these characteristics are essential to the persistence of coastal marshes that are undergoing rapid subsidence and threatened by rising sea levels. Recently, studies using transplantation experiments have begun to document *S. alterniflora* ecotypes that can regulate ecosystem function in different ways regardless of local environment, indicating ecosystem-level consequences of intraspecific genetic variation. The utility of management efforts working either to control harmful ecosystem engineering in the case of invasions, or to promote beneficial engineering activities by restoring natives, may depend on the underlying genetic identities determining relevant functional traits for a population.

To examine the influence of *S. alterniflora* genotypic identity on biogeophysical processes related to erosion, field transplantation studies and controlled greenhouse experiments were conducted. Plant traits, soil properties, accretion/subsidence, and rates of land loss were compared across lines of wildtype and cultivated clones. We found significant differences in rates of erosion between genotypes. Differences in erosion corresponded to variation in soil properties including shear strength, soil organics, and subsidence. These properties in turn were correlated with differences in plant traits such as belowground biomass and root tensile strength. This genetic and phenotypic variation in *S. alterniflora* establishes the functional basis of an extended phenotype in coastal salt marshes. Our results demonstrate the importance of genetic variation to salt marsh functioning, elucidating the relationship between evolutionary and ecological processes in these systems, and helping to inform marsh restoration and management activities.

Keywords: ecosystem-engineer, genetics, erosion, restoration, extended-phenotype

LATITUDINAL TRENDS IN VEGETATIVE GROWTH AND SEXUAL REPRODUCTION OF THE INVASIVE *SPARTINA ALTERNIFLORA* IN CHINA

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Abstract:

Understanding performance of plant species across the latitudinal gradient will help to know the capacity of plant species to adapt environmental conditions, and to predict species potential distribution range, particularly for those invasive plant species with wide-ranging distributions.

Spartina alterniflora, native to the Atlantic and Gulf Coasts of the United States, was introduced into China in 1979 and now has spread over 19 degrees of latitude along west Pacific Coast. However, few previous studies on performance of *Spartina alterniflora* along its whole latitudinal distribution range.

We studied the patterns of the relative trends in vegetative growth and sexual reproduction of *Spartina alterniflora* along their present latitudinal range in China. In the field our 2 years end of growing season's results indicated a significant latitudinal gradient in plant height, shoot density, and sexual reproduction, which with increasing individual reproductive reproduction, decreasing population density from low to high latitude, whereas plant height, reproductive reproduction of population were of bell-shape curve in relation to latitude. Growth of those corresponding populations in field common garden showed that only individual seed setting and proportion of flowing culm maintaining the same patterns. These results suggested that alien invasive *Spartina alterniflora* were of adaptive phenotypic plasticity along latitude in China, which significantly response to mean annual temperature and total annual precipitation along latitudinal locations. Besides geographic variation, we also find a significant relationship between vegetative growth and sexual reproduction of *Spartina alterniflora*, which showed that populations with medium plant size have higher reproductive investment.

With tall plant height and high reproductive production, *Spartina alterniflora* populations perform well in mid-latitude locations. With smallest plant size and lowest reproductive production, *Spartina alterniflora* populations perform poorest in lowest latitude location. High temperature and high precipitation may inhibit equator-ward distribution of the temperate plant species of *Spartina alterniflora*.

Keywords: *Spartina alterniflora*, latitudinal gradient, phenotypic plasticity, reproductive investment

CLIMATE DRIVERS OF *SPARTINA ALTERNIFLORA* PRODUCTION IN SALT MARSHES IN GEORGIA, USA

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Keywords: climate period, precipitation, primary production, river discharge, sea level

The spread of invasive *Spartina alterniflora* in its introduced range is likely mediated by abiotic conditions. At the same time, within the native range of *S. alterniflora*, tidal wetlands are threatened by global changes related not only to sea level rise but also to altered weather patterns. To predict invasion success in the introduced range, and the consequences of global changes in the native range, it is necessary to understand how temporally varying abiotic conditions drive *S. alterniflora* performance. A limited number of studies have addressed this issue, with contradictory results.

As reported in *Ecosystems* (Więski and Pennings, 2014), we conducted annual surveys of *S. alterniflora* biomass in 2000–2011, in tidal marshes at nine sites in and around the Altamaha river estuary on the coast of Georgia, USA (Figure 1). Non-destructive measurements of each shoot (height and flowering status) were taken at the end of the growing season (October) of each year in permanent plots in the creekbank and midmarsh zones. These data were used to estimate end-of-year biomass using allometric equations, with biomass serving as a proxy for annual net primary production (ANPP). Plots suffering from obvious disturbances were omitted from the analysis. We evaluated five potential drivers (river discharge, sea level, local precipitation, average temperature and maximum temperature) as predictors of *S. alterniflora* ANPP, using multiple regression and multilevel modeling. We also explored whether there were critical climate periods—short periods of time when the plants were highly sensitive to abiotic variation.



Figure 1. Salt marsh in Georgia, USA, with *Spartina alterniflora* dominating the creekbank and midmarsh zones.

River discharge was the most important driver of *S. alterniflora* ANPP, especially in creekbank vegetation. Increased river discharge reduces water column salinity, and this was most likely the proximate driver of increased production. In the midmarsh zone, the patterns were less distinct. River discharge was again the best predictor, but maximum temperature had a similar predictive ability. Poor growth of *S. alterniflora* in years with high maximum temperatures is consistent with physiological data showing that temperatures in GA often exceed the thermal optimum for *S. alterniflora*. ANPP of *S. alterniflora* in both marsh zones was also related to sea level. Sea level was found to be correlated with *S. alterniflora* production in previous studies. High sea levels cause increased flooding of the marsh platform, and this likely affects ANPP by diluting porewater, thereby reducing porewater salinities.

In contrast to results from terrestrial grasslands, we found no consistent evidence for a sharply delimited critical period for any climate driver in the tidal marsh, which indicates that plant growth was responsive to abiotic drivers at any time during the growing season. Results were broadly consistent across multiple sites

within the geographic region. Our results differ from previous analyses of production in *S. alterniflora* marshes, which either identified oceanic drivers of *S. alterniflora* production or were unable to identify any drivers, likely because the low-latitude sites we studied were hotter and more affected by river discharge than those in previous studies.

Our study was limited by a modest number of replicate years (12), and by the fact that the full historical (62 years) range of abiotic variation did not occur in the 12 study years. For example, none of the 12 study years had sea levels or temperatures that fell within the lower range of the historical distributions for these variables. We are continuing to monitor plant productivity at these sites, which will allow a future analysis with more annual replication. In addition, with collaborators we are exploring the use of historical satellite imagery to quantify plant biomass over the past three decades, thereby extending our analysis further into the past.

Finally, we plan to conduct comparisons with other dominant plant species in Georgia tidal marshes to see if they respond similarly to abiotic variation.

Our findings indicate that, in its native range, *S. alterniflora* may benefit in coming decades from moderate increases in river discharge, but suffer from rising temperatures. Alterations to river discharge caused by climate change or anthropogenic use will also affect *S. alterniflora* productivity. In the introduced range, *S. alterniflora* is likely to be most invasive in brackish marshes, and in regions with a moderate climate.

Acknowledgements

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THE POSSIBLE ROLE OF *FUSARIUM PALUSTRE* IN *PHRAGMITES AUSTRALIS* DIEBACK IN CHINESE SALT MARSHES TRANSFERRED BY INVASIVE *SPARTINA ALTERNIFLORA*

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Abstract:

A dieback of *Phragmites australis* has occurred in the Dongtan wetland on Chongming Island off Shanghai, China where *Spartina alterniflora* has invaded. *Fusarium palustre*, a slightly virulent endophytic fungus, found on *S. alterniflora* in North America, was readily isolated from monocultures of *S. alterniflora* and from mixtures composed of the invading *S. alterniflora* and the declining *P. australis*. The fungus was not recovered from healthy monocultures of *P. australis*. Pathogenicity tests showed that *F. palustre* could induce foliar dieback symptoms on *P. australis* and was re-isolated from the plant. Partial sequences of the TEF- α gene were amplified from 15 Chinese isolates of *F. palustre* and shown to cluster with known sequences from USA isolates of *F. palustre* and may have been introduced on *S. alterniflora* from North America. Our findings highlight how the destabilization of native ecosystems by exotic plants can be driven by pathogens that lower obstacles for colonization.

Keywords: fungus, disease

Introduction

The salt marshes on Chongming Island, Shanghai, China were historically populated by native sedges (e.g., *Scirpus* spp. and *Carex* spp.) and reed (*Phragmites australis* (Xu and Zhao, 2005)). In 2001, ramets of smooth cordgrass (*Spartina alterniflora*) were introduced from Yancheng in Jiangsu Province. These transplants were produced from seeds and ramets of *Spartina* obtained in 1979 from marshes in the US states North Carolina, Georgia, and Florida (An et al., 2007; Li et al., 2009). Since then, *Spartina* has spread and disrupted local plant species, driving *Scirpus mariqueter* close to extinction (Chen et al., 2004), reducing native biodiversity (Li et al., 2009) and altering nutrient cycling (Liao et al., 2008). In 2008, native stands of *P. australis* experienced dieback, but only when proximal to *S. alterniflora*.

In the USA, the opposite is occurring, where a dieback of *S. alterniflora* occurs and the invasive *P. australis* continues to make inroads into marsh environments (Elmer et al., 2013). Studies in the USA found that diebacks of native *S. alterniflora* were associated with

high densities of a slightly virulent endophytic pathogenic fungus called *Fusarium palustre* (Elmer and Marra, 2011; Elmer et al., 2012). In China, the close proximity of the invading *S. alterniflora* to the declining *P. australis* led to the hypothesis that *S. alterniflora* may carry and transfer some deleterious agents. Our objectives were to sample the *S. alterniflora* and *Phragmites* communities and determine if *F. palustre* was present and whether or not it was associated with the dieback on *P. australis*. We also sought to determine whether or not *F. palustre* was pathogenic on *P. australis*.

Methods

In the spring and summer of 2012, plant samples were removed from four marsh communities in the Dongtan wetland on Chongming Island in the Yangtze River estuary. Four communities were sampled: (1) *P. australis* monoculture, (2) *S. alterniflora* monoculture, (3) both species coexisting in healthy stands, or (4) where *Spartina* was healthy and *P. australis* was dying. Other *P. australis* monocultures along the east coast of

China where *S. alterniflora* had not yet arrived were also sampled to serve as controls. Three to five plants were sampled at each site. Tissue was surface-disinfested and placed on peptone PCNB agar. Single conidia from the *Fusarium* colonies were sub-cultured to carnation leaf agar and identified morphologically as either belonging to the *Fusarium equiseti-incarnatum* species complex or as, putatively, *F. palustre*. DNA was extracted from 15 of the putative *F. palustre* isolates and 6 other nonpathogenic isolates that belonged to the *F. equiseti-incarnatum* species complex that were also recovered. Partial sequences of the translation elongation factor (*tef*) gene were amplified, aligned, and compared to known sequences of *F. palustre* in GenBank, as previously described (Elmer and Marra, 2011).

Isolates of the putative *F. palustre* isolates were stab-inoculated into healthy *Phragmites* stems along with sterile agar controls. After sterilizing the inoculation site with 75% ethanol-soaked cotton, an agar plug (1 cm × 0.5 cm) with mycelia (treatments) or without mycelia (negative controls) was then stabbed into the leaf. The inoculation was then immediately wrapped in moist sterilized cotton and sealed with Parafilm. Inoculations were watered regularly with tap water to maintain humidity. Symptoms of infection (i.e., red or brown spots or stripes on stem; dark brown spots on sheath) were observed every three days for one month after inoculation.

Results and Discussion

Fusarium palustre was readily isolated from monocultures of *S. alterniflora* and from mixtures composed of the invading *S. alterniflora* and the declining *P. australis* in the Dongtan wetland (Figure 1). The fungus was not recovered from healthy *P. australis*. Partial sequences of the TEF- α gene amplified from 15 Chinese isolates clustered with known sequences from USA isolates of *F. palustre* (Figure 2), confirming the species designation of these isolates as *F. palustre*.

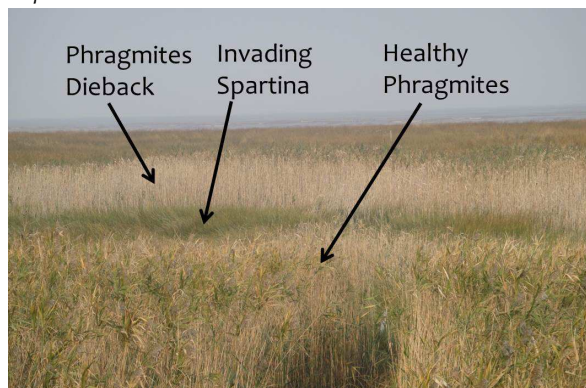


Figure 1. Symptoms of *Phragmites australis* dieback on Dongtan wetland on Chongming Island off Shanghai, China where *Spartina alterniflora* has invaded.

Pathogenicity tests and the fulfillment of Koch's Postulates showed that *F. palustre* could induce foliar dieback symptoms on native Chinese *P. australis*. Interestingly, strains of *P. australis* in the USA appeared to be immune to *F. palustre* (data not shown).

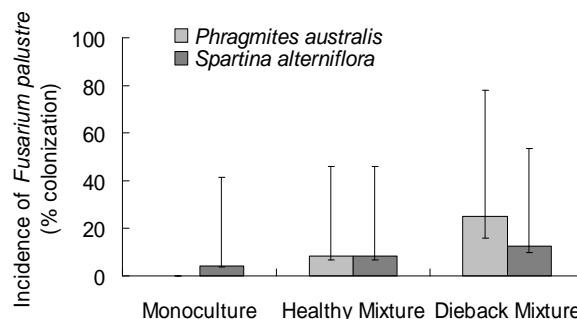


Figure 2. Incidence of *Fusarium palustre* on *Spartina alterniflora* and *Phragmites australis* in dieback sites on Chongming Island, Shanghai, China.

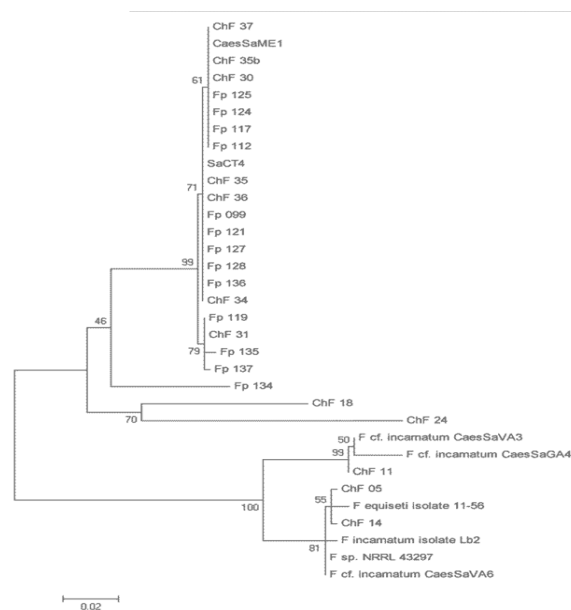


Figure 3. Phylogenetic relationships among isolates of *Fusarium palustre* and *F. incarnatum* from *Spartina* and *Phragmites* from salt marshes in China and from the Northeastern and Mid-Atlantic States. The maximum likelihood (ML) tree is based on a combined analysis of partial gene sequences from translation-elongation factor 1-a. Chinese isolates have the CHF prefix while all other isolates are from marshes in the Northeastern and Mid-Atlantic States. Isolates with the Fp prefix are confirmed *Fusarium palustre* from the United States.

It is tempting to suggest our findings support the hypotheses that *F. palustre* was first introduced to China via introductions of *S. alterniflora* from North America, and that *P. australis* infections may be a result of their proximity to *S. alterniflora*. However,

more genetic testing from a larger bank of isolates from China and North America are needed. These findings highlight how the destabilization of native ecosystems by exotic plants may be driven by pathogens that lower obstacles for colonization.

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EFFECTS OF THE INVASIVE *SPARTINA ALTERNIFLORA* ON SILICA RETENTION IN TIDAL MARSHES: A CASE STUDY IN THE BAY OF BREST

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Abstract:

Silicon is the second most important element composition the terrestrial crust. Through weathering, the main inputs of Si to oceans come from lands and are delivered by rivers. Dissolved silica (dSi), is bioavailable for number of aquatic, marine and terrestrial organisms as diverse as terrestrial plants, micro algae, fungi and bacteria. Plants accumulate biogenic silica (bSiO₂) as phytoliths, which represents an important component of the riverine Si input along the land-ocean continuum. The role of macrophytes and their phytoliths in the Si cycle remains unclear. Anthropogenic activities have an important impact on riverine nutrient inputs. Eutrophication, dam constructions, and invasive species that can significantly modify the retention of Si in estuaries. This study focuses on *Spartina alterniflora*, a tidal marsh plant from the East coast of North America that accumulates bSiO₂, and is considered as an invasive species in the Bay of Brest. In this study we investigate the effect of this invasive plant on the retention of Si in tidal marshes. We investigate temporal and spatial variability and studied the effect of salinity and duration of immersion on the concentration of bSiO₂ and dSi measured in sediments of an invaded and non-invaded marsh. In addition, we realized a dissolution experiment of *Spartina* litter and of marsh sediment in order to estimate the dissolution constant (k_{diss}). In the second part, we calculate Si budgets in a marsh invaded by *S. alterniflora* and in a non-invaded marsh from previously obtained results. This work showed a possible seasonal trend in dSi concentrations increasing from spring to fall. The bSiO₂ and the dSi annual mean concentrations in sediment increase with decreasing salinities along the estuarine gradient. We also showed that bSiO₂ dissolution rate increases with salinity and that the bSiO₂ in *Spartina* litter dissolve 5 times faster than in marsh sediments. Comparing the budgets of the invaded and the non-invaded marsh, we observed that the retention of Si is two times higher in the invaded marsh (8 %) than in the non-invaded marsh (4%). Burial increase by 50 % in the invaded marsh. These differences are explained by the fact that *S. alterniflora* is a bioengineer species and increase sediment deposition. The impact of the invasive *S. alterniflora* on the retention of bSiO₂ seems to be more due to physical than biological processes as uptake of the invasive plant itself represent only 0.1% of the total Si input.

Keywords: *Spartina alterniflora*, invasive species, silica budget, estuaries, intertidal sediments

SEED DYNAMICS IN TIDAL SALT MARSHES: IDENTIFYING KEY FACTORS GOVERNING SEEDLING ESTABLISHMENT OF *SPARTINA* SPP.

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Abstract:

Seeds often play a critical role in the colonization of mudflats by *Spartina* species, especially in the spreading of invasive *Spartina* populations. Identifying key factors determining whether seed can arrive/survive at the right place and at the right time is critical for the understanding the demographic process of *Spartina* species, and may hold the key for effective and sustainable management and control strategies. We conducted a case study on seed dynamics of *Spartina anglica* in tidal salt marshes in the Westerschelde, The Netherlands, through a series of field surveys and manipulative experiments. Results showed that seed arrival at the mudflat is distance dependent and controlled by tidal cycles and dispersal phenology. Once arrived, seed persistence in the seed bank at the mudflat is determined by vertical position of seeds and sediment disturbance depth. Furthermore, flume and mesocosm experiments showed that invertebrate ecosystem engineers and tidal currents can act in synergy in seed burial at the mudflat. Our study indicated that the existence of source population in vicinity and shallow seed burial are essential for generating a viable annual soil seed bank at the tidal mudflats, highlighting the importance of hydro-morphodynamic processes and bio-physical interactions involved in seed dynamics on the tidal flat, for the colonization potential of *Spartina* species.

Keywords: *Spartina*, seed, colonization, burial

**SPARTINA ANGLICA IN THE MONT SAINT MICHEL' BAY:
A GREEN PERIL REVISITED**

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"Prestigious Site, beautiful, the bay of Mont Saint-Michel is also the largest area of salt marshes of the French coast"
(Géhu 1979)

Abstract:

Spartina anglica was introduced in France in 1906, in the Bay "des Veys" (Normandy, France) where it has since invaded most of the salt marshes located near the estuaries of four rivers that flow into the bay. Hundred years later, it covered 200 hectares of the 325 hectares of total salt marsh area, but was only dominant on 54 ha. This species was first recorded around 1925 in the Mont-Saint-Michel bay. Four years later, its expansion is cause for concern, so that the Municipal Council of Mont-Saint-Michel qualifies this phenomenon as the "green peril". Currently, this non-native invasive species, which only occurs in lower parts of salt marshes, does not form dense monospecific stands as it occurs in the Bay "des Veys". This is not the case of a native species, characteristic of the high part of salt marshes, namely the sea couch grass *Elymus athericus*. Located at the foot of dikes 30 years ago, this clonal species (as *S. anglica*) has progressively spread along the natural drainage channels of the salt marsh before gradually invading intermediate (formerly dominated by the shrub *Atriplex portulacoides*) and low parts (covered with *Puccinellia maritima*) of the marsh. Between 2002 and 2007, its rate of spread was about 100 hectare.yr⁻¹. Functionally, this native invader leads notably to a decrease in the outwelling of organic matter and to significant changes in terms of services provided by an ecosystem whose interaction with the marine environment decreases as the invasion continues. These modified salt marshes were presented during the *Spartina* Conference field trip.

Keywords: Invasion, *Spartina anglica*, *Elymus athericus*

For researchers who are interested in *Spartina* and particularly in the spread capacity of *Spartina anglica*, the bay of Veys and the Mont Saint-Michel's bay are two mythical places. This new species, resulting from hybridization in England between

the American *Spartina alterniflora* and the European *Spartina maritima*, was observed in France for the first time in bay of Veys (Cotentin) in 1906 (Corbière, 1906). From there, it was first recorded in the bay of the Mont Saint-Michel in 1926 (Jacquet,

1949) and concomitantly invaded other bays and estuaries of North Channel and the North Sea, among which the Seine Bay and several estuaries (Somme Bay) (Rauss et al, 2008). Except in the Gironde estuary (where it was recorded since 1935), it spread more recently in Brittany and on the Atlantic coast (Arcachon Bay in 1980, Fig.1).

Its high expansion rate in the Bay of Mont-Saint-Michel since 1930 the Mont Saint Michel municipality, considering this expansion as a "green peril". Analysis of the changes in the saltmarsh vegetation of the bay indicated that we were far from an invasion threatening native species. But, and this is the paradox, this monitoring every five years since 1983, showed that a native species *Elymus athericus*, present in the early 1980s in small population along some creeks and dike to the sea in areas with *Festuca rubra* dominant, invades now the entire saltmarsh. Its spread-rate reached 100hectaresper year through the years 2002 to 2008.



Fig. 1: Progression of *Spartina anglica* along the French coast from the Bay of veys since its introduction in 1906 (Rauss, 2003, from Jacquet, 1949; Guénégo and Levasseur, 1988; Baumel et al., 2001; Verger, 2005)

It is important to stress out that there is a big difference between American saltmarshes where *Spartina* spp. are flooded 2 times daily by tides while in European saltmarshes, the entire surface is covered only some hours per year by very high tides (over 110). Their lower part is only submerged by the tide (near 80). This explains why in French bays, *S. anglica* is mostly located in pioneer areas, often with *Salicornia* spp, or in low marsh with *Puccinellia maritima*.

Current bays were formed in the last 7500 years during the Flandrian transgression. The sediment accumulation in the bay generated a gradual elevation and when the topographic level permits, a salt-tolerant vegetation colonizes this area. In Western Europe, based on the frequency of flooding by tides and salinity variation, we can distinguish a high marsh zone with *E. athericus* and *F. rubra*, a middle marsh with *Atriplex portulacoides*, a low marsh with *P. maritima* and a pioneer zone with *Salicornia* (Lefeuvre, 2010). In the Mont-Saint-Michel's bay *Spartina anglica* colonized this pioneer zone, with subsequent extension to part of the low marsh. It never forms monospecific area but currently is distributed either as isolated individual or little patches, which contrasts with its distribution in the Bay of Veys. These two bays differ considerably:

-The Mont-Saint-Michel's bay with a total area of 500 km² has only over 4,000 acres of salt marsh, sometimes extending up to 3 km wide. This bay was used as a the model by a European program on saltmarsh functioning along the western European coasts, specifically designed to evaluate the Odum out welling hypothesis ("*saltmarshes are the richness of the sea*") and to assess ecosystems services (Lefeuvre and Dame, 1994; Lefeuvre et al., 2004).

- Bay of Veys has a total area of 72 km², and its salt-marshes extend on 325 acres, more often not larger than 300 m. Primary production and functionality of this salt-marsh were analyzed in Rauss (2003) PhD thesis. Follow-ups are now conducted with new European projects and the French Coastal Conservancy ("Conservatoire du littoral") to answer management issues of these estuarine areas (climate change, managed realignment, and others). In the bay of Veys, *Elymus athericus* remains localized in the highest part of the saltmarsh and is not spreading as monospecific zone. *Atriplex portulacoides* forms a monospecific zone and it is replaced on the slight lower level by low marsh

species *Puccinellia maritima*, *Spartina anglica* and *Aster tripolium*. *Spartina anglica* marshes gradually become monospecific in the last 100 meters, which is characteristic of the Bay of Veys (Rauss, 2003; Rauss et al., 2008).

The almost total absence of high marsh in the Bay of Veys can be explained by the reduced area of the bay, a low tide magnitude (about 7 meters; tide range in the Bay of Mont-Saint-Michel is one of the largest in the world, up to 14m) and younger saltmarsh as the last land reclamation took place between 1957 and 1972. It is also possible that the lack of spread of *Elymus athericus* is linked to a better quality of fresh water arriving on salt marshes. The rivers flowing into the bay of Veys have been crossing through important wetlands.

In the Mont Saint-Michel's bay, *Spartina anglica* remains restricted to low marshes and does not threaten populations of the autochthonous *Puccinellia maritima*, which are in contrast competing with a rapidly expanding native species characteristic of high part of saltmarshes, namely the sea couch grass *Elymus athericus*. Located at the foot of dikes 30 years ago, this species has progressively spread along the natural drainage channels of the saltmarsh before gradually invading the middle marshes dominated by a low shrub *Halimione portulacoides* and a part of the low marshes where dominated by the native species *Puccinellia maritima* (Valéry and al., 2009). Between 2002 and 2007, its spread rate was about 100 ha.yr⁻¹. Now it covers over 45% of the marshes. This type of invasion by a native species is not unique to the Bay of Mont-Saint-Michel; it was also reported in the Netherlands, where two assumptions were made by Dutch colleagues: one, the most common, considers that the progress of *Elymus* is linked to significant deposition of nitrogen compounds in the atmosphere. The other hypothesis supposes that the sediment deposit in salt marshes resulting in elevation of the substrate on low and middle marsh, and limiting seawater arrivals would cause the extension of *Elymus athericus* populations. Although sedimentary deposits are important in the Bay of Mont-Saint-Michel (one million m³ per year in the estuarine bay) and result in a gain of 15 acres of new saltmarshes/year on average, this assumption is hardly acceptable in this bay as the species which is now colonizing all low and middle marshes is still subject to severe flooding in comparison to high marsh (Valéry, 2006). The prevailing assumption is now based on the nitrate level increase in these salt

marshes. The river waters of the bay, including those of the Couesnon river, increased from less than 10 mg/l. 40 years ago, to sometimes more than 50 mg/l. today. This coincided with a significant increase in nitrate levels in the water creeks along which grow populations of *Elymus athericus* at first. Furthermore, it has been shown experimentally that *Elymus athericus* grown in enriched nitrate conditions, exhibits increasing osmoprotectant compounds such as glycine betaine, which would enhance the plant tolerance to salinity (Lepout et al., 2006). This suggests that this species could occupy saltmarsh parts subjected to flooding such as pioneer zone and middle marsh. Research has to be done (1) to show how the freshwater rivers loaded with nitrates may deploy on saltmarshes at high tide, (2) to explore the nitrate levels in the roots of spreading *Elymus*, (3) the effect on individual growth and size.

That's the paradox of the Mont-Saint-Michel's bay, where after having given concerns regarding the risk of *Spartina anglica* spread, is currently threatened by the demographic explosion of a native species. This autochthonous species is uncontrollable and could undermine the ecosystem services provided by saltmarshes such as outwelling or sheep grazing (Valéry and al., 2009).

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Visit to salt-marshes in the Bay of Mont Saint-Michel

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CHANGES IN TUSSOCK ARCHITECTURE OF INVASIVE *SPARTINA DENSIFLORA* (POACEAE) ALONG THE PACIFIC COAST OF NORTH AMERICA

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Abstract:

It is important to link functional trait-based responses of invaders to environmental changes to improve our understanding of mechanisms that promote invasiveness. The invasion of alien *Spartina densiflora* Brongn. along the Pacific coast of North America provides a natural model system for studying the response of invasive populations to contrasted environments. We hypothesized populations of *Spartina densiflora* from this region would show different responses to the environment based mainly on phenotypic plasticity, since the species has been shown to have low genetic diversity. To probe this hypothesis, we compared architecture traits of tussocks from *S. densiflora* populations along the Pacific Coast of North America growing in contrasted habitats and we grew plants from the studied field populations in a common garden experiment. Our results suggest plants from these populations are able to adjust key functional tussock traits across a wide latitudinal gradient in response to widely variable climate (specifically, air temperature) and sedimentary conditions.

Keywords: biomass, climate change, phenotypic plasticity, salt marshes, *Spartina*

Introduction

Plant species that successfully invade novel habitats beyond their native range are thought to have broad ecological amplitudes and/or physiological tolerances to environmental change. Phenotypic acclimation of individual plants and genetic differentiation by natural selection within invasive populations are two potential mechanisms that may confer fitness advantages and allow plants to cope with environmental variation (Drenovsky et al., 2012).

It is important to link functional trait-based responses of invaders to environmental changes to improve our understanding of mechanisms that promote invasiveness, predict future invasions, forecast changes in the distribution of alien species, and manage those at hand (Kirwan et al., 2009; Drenovsky

et al., 2012). Clonality as a plant growth strategy has been a successful adaptation contributing to clonal plants being the dominant vegetation in many ecosystems and has been implicated as a significant factor contributing to invasiveness (Martina and von Ende, 2013).

Phenotypic variation within species is widespread among salt marsh plants and the invasion of alien *Spartina densiflora* Brongn. across a wide latitudinal gradient from California (USA) to British Columbia (Canada) provides a natural model system for an integrated study of the potential mechanisms underlying the response of invasive populations to substantial variation in climate and other environmental variables. *Spartina densiflora* is a halophyte native to southeastern South America (Brazil, Argentina) with a low detectable genetic

variation (Ayres et al., 2008) that has invaded the Pacific coast of North America from San Francisco Bay, California (ca. 37°56' N) north to Vancouver Island, British Columbia (ca. 49°20' N) (Bortolus, 2006; Saarela, 2012). We hypothesized populations of *Spartina densiflora* from a geographic gradient along the Pacific coast of North America would show different responses to the environment based mainly on phenotypic plasticity, rather than on adaptive differentiation. To probe this hypothesis, we compared tussock traits from populations along the Pacific Coast of North America growing in contrasted habitats and we collected and grew *S. densiflora* plants from the each of the studied field sites in a common garden experiment to test whether inter-population differences recorded in the field were due to genetic differentiation or phenotypic plasticity (Castillo et al., 2005a).

Materials and methods

Study Sites

Our field study was carried out in summer of 2010 at five coastal marshes invaded by *Spartina densiflora* along the Pacific Coast of North America: The southernmost population was at Corte Madera Creek (37°56'33" N, 122°30'55" W) in San Francisco Bay Estuary, the second at Vance Marsh (40°49'53" N, 124°10'17" W) in the Humboldt Bay Estuary, the third population was near the mouth of the Mad River Estuary (40°56'10" N, 124°7'48" W), the fourth population was at Grays Harbor Estuary (46°57' N, 124°8'08" W) and the northernmost population was at Baynes Sound, Vancouver Island in British Columbia (49°33" N, 124°52'09" W) (see Castillo et al. (2014) for a description of sampled marshes).

Characterization of *Spartina densiflora* habitats

Latitude was recorded for every location using handheld GPS technology. Meteorological conditions were characterized using mean daily temperature (°C) for the last week before sampling reported in local climatological stations. In addition, we examined mean daily global horizontal insolation (W h m⁻²) for the sampling month, day-length (h) for the sampling day, and the number of annual growing degree days for all sites. Growing degree days measure the number of days that daily temperature exceeds a threshold temperature necessary for significant plant growth. We used 10°C as a threshold for *S. densiflora* growth, as it has been applied previously for *Spartina alterniflora* Loisel. (Kirwan et al., 2009). Mean tidal level and range (m) for each location were obtained from Internet websites of NOAA Tide Predictions and Fisheries and Ocean Canada. *Spartina densiflora*

populations were visited during low tides and redox potential (Eh), electrical conductivity and pH, organic matter concentration by loss on ignition method, nitrogen concentration and texture of the sediments (as percent of gravel, sand, silt and clay) were recorded (n = 3-10).

Tussock traits

Ten adult clumps of *Spartina densiflora* with between 10 and 80 live shoots (ca. 2-3 years old) were individually marked in every studied population. The following traits were recorded for the marked tussocks in the field (n = 10 tussocks): (1) live, spiked and dead shoot density; and (2) height and diameter of live shoots (5 randomly selected shoots per tussock).

Ten entire tussocks similar in size to those marked were collected in the field per population, and total nonstructural carbohydrate (TNC) in rhizome (mg g⁻¹ DW) and biomass of root, rhizome, live and dead shoot, and live and dead leaves of live shoots (in dry weight, DW) were recorded in the laboratory. Oven-dried and ground rhizomes were analyzed for total nonstructural carbohydrates (TNC) using a modified procedure by Swank et al. (1982). The dry weight of the different organs per plant (n = 10 tussocks per population) was obtained after drying samples in a forced-air oven at 80 °C for 48 h, and total standing above-ground and below-ground biomass (AGB and BGB, respectively) and AGB:BGB ratio were calculated. In addition, leaf area index (LAI) was recorded (n = 10 tussocks) (Carrión-Tacuri et al., 2011).

Common Garden Experiment

Every marked tussock of *Spartina densiflora* used in the field study was collected and rhizomes were separated and grown for 27 months in plastic pots (20 cm diameter X 18 cm height) filled with perlite substrate in the greenhouse facilities of the University of Seville (37°21'42" N, 5°59'15" W). The pot bases were kept permanently flooded with fresh water, and watered with 20% strength modified Hoagland's nutrient solution. After 27 months of growth, every tussock trait recorded in the field, except below-ground biomass, was recorded again for the same plants acclimated to common greenhouse conditions.

Data analyses

Statistical analyses were carried out using SPSS 12.0 (SPSS Inc., Chicago, USA). Tussock traits were compared between *S. densiflora* populations by one-way analysis of variance (ANOVA) and Kruskal-Wallis H-test. Principal Component Analysis (PCA) was performed to reduce the number of abiotic and

trait variables to extract independent PCA factors with eigenvalues > 1 . Multiple linear regression analyses were used to characterise the relationships between PCA factors of the abiotic environment with PCA factors of plant tussock traits. Simple linear regression analyses were used to characterise the relationships between abiotic variables and plant traits.

Results and discussion

Four factors were obtained for the abiotic environment in the field from the PCA, explaining 86.0% of the variance. The first factor (PC1-A) was positively correlated with latitude, day length, mean tidal level and with coarse sediments (with higher sand and gravel percentages, and lower clay percentage), and negatively with the number of growing degree days. PC2-A was positively correlated with mean tidal range and negatively with more anoxic and acidic sediments (lower Eh and pH, respectively) exposed to less mean daily isolation. PC3-A was positively correlated with saltier sediments (higher conductivities) and mean daily temperatures, and PC4-A negatively with the silt percentage.

Five factors were obtained for the tussock traits in the field from the PCA, explaining 77.7% of the variance. The first factor (PC1-C) was positively correlated with AGB (marked by live leaf and live shoot biomass, and varying between $1972 \pm 298 \text{ g m}^{-2}$ for Mad River and $6866 \pm 622 \text{ g m}^{-2}$ for Vancouver Island), LAI (varying between 0.36 ± 0.04 for Grays Harbor and 2.49 ± 0.31 for Vancouver Island) and root biomass (varying between $512 \pm 81 \text{ g m}^{-2}$ for Mad River and $1228 \pm 127 \text{ g m}^{-2}$ for Vancouver Island). PC2-C was positively correlated with rhizome biomass, dead shoot biomass, BGB (varying between $1617 \pm 255 \text{ g m}^{-2}$ for Grays Harbor and $3678 \pm 946 \text{ g m}^{-2}$ for Humboldt Bay) and AGB:BGB ratio. PC3-C was positively correlated with live shoot diameter (varying between $3.1 \pm 0.1 \text{ mm}$ for Grays Harbor and Humboldt Bay and 3.4 ± 0.1 for Vancouver Island) and height (varying between $29.4 \pm 2.0 \text{ cm}$ for Grays Harbor and $46.4 \pm 1.9 \text{ cm}$ for Vancouver Island), and PC4-L positively with rhizome TNC and spiked shoot density.

PC1-C correlated positively with PC1-A and PC3-A. Thus, tussocks accumulating more roots, more AGB and with higher LAI were growing on coarser sediments at higher latitudes, influencing mainly through higher air temperatures (Fig. 1). Our results are in accordance with previous studies reporting higher root development to explore more soil volume looking for nutrients in coarse-textured and poor sediments than in fine-textured sediments (e.g. Wang et al., 2014). In addition, root biomass increased with

air temperature and rhizome biomass with the number of growing degree days. Roots are organs with a high turnover in grasses, which development would be very sensible to short-term environmental changes such as an increase in temperature and sediment aeration, while rhizomes are more stable structures that would be influenced mainly by long-term environmental conditions such as an increase in the duration of the growing season with latitude. Root biomass of opportunistic invasive species, such as *S. densiflora*, can increase with temperature due to increasing soil nutrient availability through accelerating microbial activity (Thakur et al., 2014).

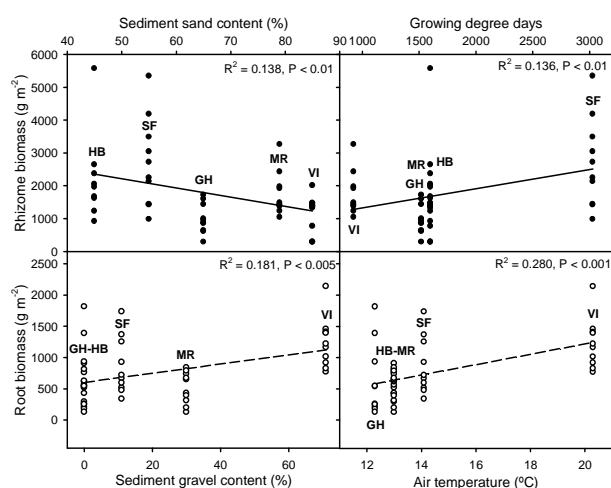


Figure 1. Relationships between rhizome biomass and sediment sand content and growing days, and between root biomass and sediment gravel content and mean daily air temperature during last week before sampling for tussocks from five invasive populations of *Spartina densiflora* along the Pacific Coast of North America. Populations: SF, San Francisco Bay Estuary; HB, Humboldt Bay Estuary; MR, Mad River Estuary; GH, Grays Harbor Estuary; VI, Baynes Sound, Vancouver Island.

AGB and LAI were related mainly with mean daily air temperature. Curiously, the highest values of AGB and LAI were recorded for Vancouver Island, the most northern location presenting warmer temperatures during summer than the other three locations to the south along the Pacific Coast of the United States. *S. densiflora* AGB in its native range decreases with latitude and salinity increases at higher latitude due to more xeric climates (Montemayor et al., 2014), while in our study the population at the highest latitude showed the highest AGB and salinity had not a significant relationship with AGB since humid climates dominated at higher latitudes and no hypersaline conditions were recorded. *S. densiflora* is a halophyte with a high tolerance to salinity that is limited by hypersaline conditions (Castillo et al., 2005b).

PC2-C decreased with PC1-A and increased with PC4-A, reflecting that the clumps with higher BGB (marked by higher rhizome biomass) were growing in saltier and more fine-textured sediments at higher latitudes (Fig. 1). PC3-C decreased with PC2-A, reflecting that live shoot height and diameter increased in more oxygenated sediments exposed at lower mean tidal ranges and more mean daily isolation, and PC4-C correlated positively with PC2-A and slightly with PC3-A ($P < 0.05$), showing that rhizomes with higher TNC and higher spiked shoot densities were recorded at more anoxic and saltier sediments exposed to higher air temperatures and insulations. Previous studies have described *S. densiflora* as a halophyte whose distribution, physiology and biomass are limited by anoxia (Castillo et al., 2000, Castillo et al., 2008), in contrast to halophytes that are well-adapted to low marsh conditions, such as *Spartina maritima* (Curtis) Fernald that increased its shoot height when growing on anoxic sediments (Castillo et al. 2005a). The decreases of rhizome TNC at lower salinities could reflect the depletion of rhizome reserves due to growth stimulation under brackish conditions. In this sense, *S. densiflora* has been described as a highly competitive species in estuaries subjected to marked freshwater discharge (Costa et al., 2003). On the other hand, spiked shoot density increased with air temperature, reflecting that flowering was more advanced at warmer locations on the beginning of the summer when our sampling was carried out and/or that plants flowered more at warmer locations.

Each tussock trait showed significant inter-population differences in the field (ANOVA or Kruskal-Wallis H-test, $P < 0.05$) and every population converged in almost all architectural traits when growing in the common environment (ANOVA or Kruskal-Wallis H-test, $P > 0.05$) suggesting a relatively low level of genetic variation for architectural traits of tussocks in invasive *S. densiflora* populations in this study region, which may be related with the process of genetic drift experienced by those genotypes introduced sequentially from one estuary to another along the coast. Although environmental conditions appear largely to determine tussock traits in *S. densiflora*, a genetic component influencing these traits due to local adaptation cannot be excluded, since slight significant interpopulation differences in live and spiked shoot density after more than 2 yr in a common environment were recorded. Furthermore, our common garden experiment may not necessarily have provided conditions for different genotypes to express distinct trait phenotypes (Thompson, 1991).

The recorded high phenotypic plasticity for traits of tussocks adds to the same behavior reported in relation to leaf traits for the same *S. densiflora* invasive populations along the Pacific coast of North America

(Castillo et al., 2014). In addition, Abbas et al. (2012) reported also high physiological plasticity for *S. densiflora* seedlings in response to flooding. The considerable phenotypic plasticity of *S. densiflora* is in agreement with that found in other *Spartina* species (Elsey-Quirk et al., 2011) and it may be related with its ancestral hybrid origin (Fortune et al., 2008). The high degree of phenotypic plasticity showed by *S. densiflora* would allow it to adjust important functional traits in response to variable environmental conditions, resulting in the invasion of very contrasted habitats. These results are in agreement with those recorded in Southwest Iberian Peninsula, where *S. densiflora* has invaded very different estuarine habitats (Nieva et al., 2001), and a variety of phenotypes are recognized (Castillo et al., 2003; Castillo et al. 2008).

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INTRASPECIFIC AND PHENOTYPIC VARIATION IN SALINITY RESPONSES OF INVASIVE *SPARTINA DENSIFLORA* FROM PACIFIC ESTUARIES OF NORTH AMERICA

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Abstract:

Salinity and tidal inundation induce physiological stress in vascular plant species and influence their distribution and productivity in estuarine wetlands. Plants in these wetlands are subjected to climate change and magnified physiological stresses as these key abiotic processes increase with sea level rise. Understanding the potential of invasive plants to respond to predicted salinity increases will elucidate their potential niche breadth. To examine potential phenotypic plasticity and mechanisms underlying responses to variation in salinity stress in *Spartina densiflora*, we collected rhizomes from four invasive populations occurring from California (USA) to Vancouver Island (Canada) on the Pacific Coast of North America. In a common garden experiment, we measured plant traits associated with growth and allocation, photosynthesis, leaf pigments, and leaf chemistry and calculated plasticity indices across imposed salinity treatments. Leaf chemistry, leaf pigments, leaf morphology and physiology all expressed strong plastic responses to salinity, and population was also an important factor for several measured plant traits. Growth and allocation measures were less plastic, with the exception of total leaf area. Salinity-induced changes in leaf chemistry and physiology may compensate to support invasive plant growth with rising estuarine salinity.

Keywords: *Spartina*, invasion ecology, phenotypic plasticity, salinity stress, tidal wetlands

INTRODUCTION

Invasive plant species often colonize and spread in environments quite different from their native range. Spatially and temporally dynamic salinity and tidal inundation regimes interact to control soil water potential, and induce biochemical and physiological responses in vascular plant species, greatly influencing their growth, biological interactions, distribution and productivity (Wilson et al., 1996; Howard and Mendelssohn, 1999). Invasive plant species in estuaries also face rising sea level, increases in aqueous salinity regimes, and changes to other influential abiotic processes affected by global climate change that together can be expected to dramatically change plant community and ecosystem processes. Interspecific variation in salinity acclimation among dominant species in these communities is an important driver of both landscape-scale vegetation zonation and small-

scale vegetation pattern in salt marshes (Bertness, 1991; Shumway and Bertness, 1994).

As environments change, some introduced species can rapidly evolve by adapting via genetic differentiation in response to local selection pressures (Leger and Rice, 2003; Maron et al., 2004), genetic drift or mutation (Dlugosch and Parker, 2008) or by epigenetic processes (Richards et al., 2012). Rapid evolution of invasive plants may be facilitated by genetic variation and/or novel genotypes that result from intra- or interspecific hybridization (Strong and Ayres, 2013). Phenotypic plasticity may be a contributing adaptive mechanism that can convey fitness benefits to individual plants and promote invasiveness by increasing niche breadth in spatially heterogeneous environments (Richards et al., 2006; Caño et al., 2008; Drenovsky et al., 2012). Improved understanding of plant traits of dominant species that affect variation in their responses to changes in salinity is needed given their cascading

influences on critical ecological processes (Hester et al., 2001). Our objective was to evaluate whether growth and foliar traits of *Spartina densiflora* populations from four invaded estuaries expressed differences in phenotypic plasticity relative to experimentally imposed salinity gradients and to determine if responses differed among populations.

METHODS

Study area and focal species – We collected *Spartina densiflora* Brong (Poaceae) plants in summer 2010 from four estuaries distributed latitudinally along the Pacific west coast of North America, including: 1) San Francisco Estuary (SF), California (37°56'33"N, 122°30'55"W); 2) Humboldt Bay Estuary (HB), California (40°49'53"N, 124°10'17"W); 3) Mad River Estuary (MR), California (40°56'10" N, 124°7'48"W) Baynes Sound (VI, Vancouver Island), British Columbia (49°33'N, 124°52'09"W). Salinity regimes at the collection sites ranged from oligohaline to near marine conditions (Castillo et al., 2014).

Propagation techniques and experimental design – Rhizomes were transplanted into pots with drainage holes (15 cm d x 17.5 cm h) and filled with expanded perlite. Potted rhizomes were placed in 180-liter aquatic mesocosms filled with fresh water to 10 cm below substrate surface, and grown for five months in a glasshouse at Davis, California. Following tussock propagation, experimental plants with up to 41 live stems per tussock were exposed to experimental treatments. Salinity treatments were created using 20% modified Hoagland's solution and Hoagland's solution plus sea salts (Instant Ocean®, Aquarium Systems Inc., Mentor, Ohio USA) to achieve desired concentrations, and solutions were refreshed weekly. Experimental plants were subjected to freshwater (0.5 ppt) and brackish (15 ppt) treatments in mesocosms for two weeks. One brackish mesocosm was then increased to marine salinity (35 ppt) level for an additional two weeks of conditioning. After salinity conditioning, plants were randomized into the experimental design using fifteen 84-liter plastic mesocosms (0.53-m l X 0.53-m w X 0.30-m d). Treatments were randomly assigned within a nested, full factorial design. Five potted tussocks (replicates) from each of the four population sites were randomly assigned to one of three aqueous salinity treatments for a total of 60 experimental plants. Replicates from each of four sites were randomly nested within each of the 15 mesocosms. Water level in each mesocosm was maintained at 10 cm below substrate surface throughout the experiment. The experimental treatments were imposed for 30 days (March 8 – April

6, 2011) in a glasshouse with controlled temperature between 21-25 °C and a 15 h daily photoperiod.

Plant growth, foliar response and leaf traits – The following plant traits were measured: shoot and root biomass, root mass ratio, leaf morphology, leaf area, leaf elongation rate, leaf gas exchange, stomatal conductance, leaf water potential, fluorescence parameters, photosynthetic pigment concentrations, free proline accumulation, glycinebetaine concentration, leaf tissue total N, leaf Na concentration, and salt exudation rates. Shoot biomass (SM; g) and root biomass (included all subsurface biomass, RM; g) were measured for each tussock after samples were oven-dried at 70 °C for 48 h, and root mass ratio (RMR) was calculated as root + rhizome biomass divided by shoot biomass. Each tussock was photographed and total leaf area (LA; m²) was calculated using WinFOLIA (Regent Instruments Inc., Saint-Foy, Quebec, Canada). Leaf mass (LM) was determined from 0.5 cm diameter drilled samples from five random flag leaf samples per plant. Specific leaf area (SLA; m² g⁻¹) was then calculated as LA/LM. Apical leaf elongation (growth) rate and adaxial leaf rolling were measured and calculated per Castillo et al. (2014).

Photosynthetic traits. Measurements of integrated net photosynthesis rate (A_{sat}), stomatal conductance (g_s), and light- and dark-adapted fluorescence over the same leaf area were carried out on days 21-26. Measurements were made on the second-youngest leaf of each of five tillers chosen at random (each from a different tussock) from each treatment using a LI-COR 6400 portable infrared CO₂ analyzer (LI-COR Biosciences, Lincoln, NE, USA) in differential mode and in an open circuit coupled to a pulse-amplitude modulated (PAM) fluorometer. Photosynthetic photon flux density inside the chamber was maintained at 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$, with the LI-6400 LCF actinic light source (90% red, 10% blue), 400 $\mu\text{mol mol}^{-1}$ CO₂ concentration, and flow rate set to 400 $\mu\text{mol s}^{-1}$. Light-adapted measures were conducted on sunny days within 2 h of solar noon. Before measurement, leaves were rinsed with deionized water to remove salts that had accumulated on the leaf surfaces and allowed to dry. Each individual gas exchange measurement consisted of three values logged at 10 s intervals with stable conditions inside the chamber. Then the measured leaf sections were marked and dark-adapted fluorescence measures were taken on the same leaf sections at night (pre-dawn, 0330-0515 h), avoiding illumination of the leaves while taking measurements. The marked leaf sections were harvested to determine leaf area. Simultaneous measurement of photosynthesis and stomatal conductance allowed us to calculate water use efficiency (carbon fixed/carbon lost) of experimental plants (WUE; mmol CO₂ mol

H₂O⁻¹). Leaf water potential (Ψ_{leaf}) was measured with a Scholander pressure bomb within 2 h of solar noon on days 23-27 on fully expanded adult leaves exposed to direct sunlight from each of 5 tillers chosen at random (each from a different tussock) from every treatment.

Leaf Chemistry, Compatible Solutes and Pigments— Approximately 2 g of fresh tissue were collected from young fully mature leaves and frozen for analysis of proline and photosynthetic pigments. Leaf, stem, and root tissues were collected separately and dried at 70 °C and weighed. Dry leaf tissue was ground to pass through a No. 40 mesh screen for nitrogen (N), sodium (Na), and glycinebetaine analysis. Total leaf N concentration was determined using a CHNS/O analyzer (Perkin Elmer 2400, Waltham, MA, USA). Leaf Na was measured with a sodium electrode on dry-ashed samples that were dissolved in 1M HCl. Proline concentration in leaf samples was determined (*sensu* Bates et al., 1973), and glycinebetaine was estimated as quaternary ammonium compounds (*sensu* Grieve and Grattan, 1983). Photosynthetic pigments were extracted in 80% aqueous acetone and chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (C_{x+c}) concentrations were determined with a spectrophotometer. Leaf sodium exudation rates were measured (*sensu* Christman et al., 2009).

Plasticity Assessment— Phenotypic plasticity indices were calculated following Molina-Montenegro and Naya (2012) for each population and plant trait to evaluate differences in the expression of phenotypic traits. The phenotypic plasticity index for each trait and locality was quantified as the percentage of change in absolute high and low mean trait values, irrespective of salinity treatment; that is, $P = [(X_{\text{max}} - X_{\text{min}}) / X_{\text{max}}] * 100$, where *P* is plasticity, X_{max} is the highest mean value for a given trait and X_{min} is the lowest mean value for a given trait. Means represent 5 replicated measurements from the experiment.

Statistical analyses — Measured response variables were classified into five functional trait groups for use as dependent variables in the analyses: 1) growth and allocation, 2) leaf traits, 3) photosynthetic traits, 4) Leaf N and pigment concentrations, and 5) leaf Na and osmotic solute concentrations. Dependent variables were tested for homogeneity of variances and transformations were performed on SLA, Na exudation rate, leaf Na, and leaf proline, and Ψ_{leaf} to meet assumptions for parametric analyses. To protect analyses from type I error, we used the ‘protected analysis of variance (ANOVA)’ protocol (Scheiner 2001). We used Pillai’s Trace to evaluate significance in MANOVAs. All multivariate and univariate GLMs tested the same factors: population site (VI, HB, MR, and SF), salinity treatment (0.5 ppt, 15 ppt, and 35 ppt),

population by salinity interaction, and mesocosm nested in salinity treatment, in which mesocosm was considered a random factor. Post-hoc Tukey tests with Bonferroni-corrected α -levels were performed for each trait when salinity treatment or population site were significant in the univariate ANOVA model. Analyses were performed using SAS V. 9.2 for Windows (SAS Institute Inc., Cary, NC USA).

RESULTS AND DISCUSSION

Trait Responses— Salinity had a significant effect on plant growth and biomass allocation traits ($F_{6,70}=3.88$, $p=0.0021$), leaf traits ($F_{8,68}=7.66$, $p=0.0001$), instantaneous photosynthetic traits ($F_{12,64}=8.93$, $p=0.0001$), leaf nitrogen and pigments ($F_{8,68}=6.69$, $p=0.0001$), and leaf sodium and compatible solutes ($F_{8,68}=37.53$, $p=0.0001$). Univariate GLM analyses for each of the measured trait groups indicated population site was a significant factor for all growth and biomass allocation traits, including shoot mass (SM; $F_{3,59}=20.71$, $p=0.0001$), root mass (RM; $F_{3,59}=18.94$, $p=0.0001$), and root mass ratio (RMR; $F_{3,59}=3.63$, $p=0.0218$). SM and RM were highest in plants collected at VI as compared to all other sites; SM of VI plants was 3.25 times higher and RM, 2.6 times higher than the growth measured in plants from MR. Plants in the salinity treatments had higher RMR, although RMR in plants from HB decreased in the highest salinity treatment ($F_{2,59}=3.35$, $p=0.0001$).

While leaf traits as a composite response group responded significantly to salinity treatments, LA was only moderately influenced ($F_{3,59}=2.61$, $p=0.0876$). On the other hand, population site strongly influenced total LA production (LA; $F_{3,59}=16.99$, $p=0.0001$), where LA was 2.3 times higher in plants from the BC population site. Specific leaf area (SLA) was affected by both salinity treatment ($F_{2,59}=4.77$, $p=0.0145$) and population site ($F_{3,59}=6.91$, $p=0.0009$). Plants collected at MR had slightly higher SLA on average, but all plants in the highest salinity treatments (30 ppt) had up to 25% lower SLA than plants exposed to 0.5 or 15 ppt salinity treatments. Leaf elongation rate did not respond to salinity treatment, nor population site, but population site was important in determining the degree of adaxial leaf rolling ($F_{3,59}=4.07$, $p=0.0138$), and leaf rolling strongly responded to salinity ($F_{3,59}=103.08$, $p=0.0001$).

Both the rate of CO₂ assimilation per unit leaf area (A_{sat}) and stomatal conductance (g_s) suggest the process of photosynthesis was influenced by population site (A_{sat} : $F_{3,59}=3.50$, $p=0.0250$; g_s : $F_{3,59}=2.93$, $p=0.0467$), and g_s was additionally influenced by salinity treatment ($F_{2,59}=5.61$, $p=0.0076$). A_{sat} and g_s were highest in plants collected in VI, and g_s was

highest in the 15 ppt salinity treatment. Leaf water potential responded strongly to salinity treatment ($F_{2,59}=52.06$, $p=0.0001$) and was increasingly negative with increased salinity treatments. Variation in WUE was high in the fresh water treatments, and although WUE decreased for all plants with increasing salinity level, individual variation was high and a salinity effect was not detected at this level of replication.

Salinity treatment had a significant effect on light-adapted quantum efficiency of PSII ($F_{2,59}=14.20$, $p=0.0001$). The quantum efficiency was 20% higher for plants in the 15 ppt salinity treatment compared to the 35 ppt salinity treatment. The effect of mesocosm on potential efficiency was not significant ($F_{12,59}=1.95$, $p=0.0608$).

Salinity treatment was a strong driver of leaf N and associated pigments, although the direction of the effect was not consistent for the four traits in this response group. Leaf N was 20% lower in the freshwater treatment than the 35 ppt salinity treatment ($F_{2,59}=5.84$, $p=0.0001$). Chlorophyll *a* ($F_{2,59}=7.13$, $p=0.0025$), Chlorophyll *b* ($F_{2,59}=13.34$, $p=0.0001$), and carotenoids ($F_{2,59}=6.94$, $p=0.0028$) all responded significantly to salinity. All plants from VI, MR, and SF generally followed a trend of decreasing chlorophyll pigments with increasing salinity (Chl *a*: $F_{3,59}=3.75$, $p=0.0192$; Chl *b*: $F_{3,59}=4.03$, $p=0.0001$). However, plants from HB had the highest chlorophyll concentrations in the 15 ppt salinity treatment. Population site was a marginally significant factor for the amount of carotenoids ($F_{3,59}=2.83$, $p=0.0521$), following the same pattern as described for the amount of chlorophyll.

Unsurprisingly, salinity was a major determinant of leaf Na and related osmotic solutes ($F_{8,68}=37.53$, $p=0.0001$). The significant effect of salinity was consistent, and followed increasing levels of salinity treatment for sodium exudation rate ($F_{2,59}=151.55$, $p=0.0001$), and leaf Na ($F_{2,59}=461.26$, $p=0.001$), proline ($F_{2,59}=173.33$, $p=0.0001$), and glycinebetaine ($F_{2,59}=137.07$, $p=0.0001$) concentrations. Post-hoc tests showed that sodium exudation was statistically the same for the 15 ppt and 35 ppt salinity treatments.

Trait Plasticity – There were no clear differences in overall degree of plasticity among population sites. However, all individual plant traits and each plant trait group exhibited some degree of plasticity in response to salinity, varying from weak to strong expression (Fig. 1). Individual traits from within growth and allocation, leaf trait, and gas exchange trait groups were relatively less plastic, and consistent across all traits in a trait group (Fig. 1). However, reaction norms suggest relatively large differences in percent plasticity among fluorescence traits, in which

dark-adapted measures of PSII potential efficiency were much less plastic than the three other measures. Similarly, leaf N was substantially less plastic than Chlorophyll *a* and *b* and carotenoids. Leaf sodium concentrations, osmotic solute concentrations, and other foliar stress indicator measurements of *Spartina densiflora* reveal strong, plastic responses of these traits in response to imposed salinity regimes (Fig. 1).

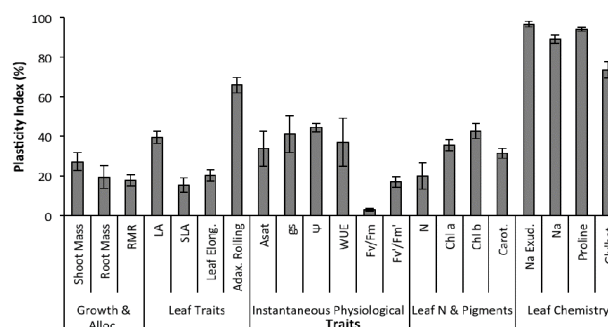


Figure 1. Percent Plasticity Index (PPI) for traits responding to salinity treatment, calculated using minimum and maximum mean trait values, organized into trait response groups.

Our experiment suggests the *Spartina densiflora* populations invading the Pacific estuaries of North America are phenotypically plastic in response to salinity. Acclimation to higher salt concentrations occurs through a suite of interacting morphological, physiological and salt-induced leaf chemistry responses to maintain growth. Across sampled populations and traits, plasticity responses to the experimentally imposed salinity gradient did not vary, but at the level of individual traits, some degree of local adaptation was observed between populations. This was largely due to more significant growth VI plants relative to all other populations. *S. densiflora* growth can be highly responsive to local heterogeneity, as observed in an Argentine estuary (Di Bella et al., 2014). In Spain, invasive populations of *S. densiflora* tolerate high salinity, but expansion and colonization rates of invasive populations are highest in brackish tidal wetlands influenced by freshwater outflow (Castillo et al., 2005).

Acclimation of *S. densiflora* to increasing salinity suggests the invasive cordgrass will continue to maintain growth, persist and spread as estuarine salinity rises, though we expect plant performance will vary depending on the interaction of salinity with inundation levels and other changing environmental factors. Understanding potential niche breadth via plasticity or adaptation may help improve assessments of invasion risk and management strategies in this time of rapid climate change.

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BIOPHYSICAL AND BIOCHEMICAL MECHANISMS UNDERLYING THE DROUGHT-TOLERANT *SPARTINA PATENS* TO POLYETHYLENE GLYCOL MEDIATED WATER STRESS

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Abstract

Along with the recent increasing temperature climate scenarios observed all around the globe, drought events are also becoming more frequent and severe. Although at first site, salt marshes are typically areas where drought would not be one of the most evident scenarios to consider, this is not true to all marshes. Some wetland areas and more precisely the upper marsh areas where the elevation is considerable, the tidal influence can become much reduced, being only felt during spring tides, occurring only sporadically during few days per year. In Portugal, the invasive species *Spartina patens* typically inhabits the higher marshes although recently it has been spreading towards lower elevations. Its typical sediment habitat is mainly composed by sands, with a reduced water retention potential. All these factors can impose to this species some periods of drought that will inevitably affect its physiological performance, shaping its biomass production and therefore its invasive potential. To evaluate this species performance in moderate and severe drought events, *S. patens* individuals were subjected to nutritive solutions containing 10% and 20% polyethylene glycol (PEG) respectively. Surprisingly, *S. patens* presented an elevated tolerance to moderate stress and even in severe drought conditions showed only minor disturbances on its biophysical energetic processes indicating low impacts on its biomass production mechanisms. This physiological fitness makes this species drought-tolerant facilitating the expansion of its range of influence under adverse conditions.

Introduction

On a global basis, drought, in conjunction with coincident high temperature and radiation, poses the most important environmental constraints to plant survival and to crop productivity (Turkan et al., 2005). *Spartina patens* is a non-indigenous species (NIS) that is widely expanding in the Western European marshes. In saline environments, halophytes frequently experience salt and drought stresses, especially for the species that colonize the upper marsh, where the tidal irrigation is not frequent. Considering the new predictions in the IPCC report (IPCC, 2014) pointing out an increase in the number of warm days and nights and of desertified areas, especially in the Mediterranean, becomes important to understand how this drought increase will affect the expansion of this invasive cordgrass.

Polyethylene glycol (PEG) compounds have been used to simulate water stress effect in plants (Murillo-Amador et al.,

2002). Drought stress results in stomatal closure, which limits CO₂ fixation and reduces NADP⁺ regeneration by the Calvin Cycle (Satoh and Murata, 1998). These adverse conditions increase the rate of reactive oxygen species (ROS), by enhanced leakage of electrons to molecular oxygen. These cytotoxic ROS can destroy normal metabolism through oxidative damage to lipids, proteins and nucleic acids (Fridovich, 1986; Rabinowitch and Fridovich, 1983). Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant mechanisms. These mechanisms include α -carotens, α -tocopherol, ascorbate, glutathione and enzymes including SOD, POX, APOX, CAT and GR (Halliwell, 1987; Assada, 1992). There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to environmental stress (Acar et al., 2001).

Considering this, in the present study the ecophysiological feedback of *S. patens* towards moderate and severe PEG-induced drought is examined, using a biophysical and biochemical approach, in order to understand how this specie will cope with future drought periods.

Material and Methods

For this propose plants from both species were collected in a Tagus estuary salt marsh and brought back to the laboratory. Plants placed in pots with a mixture of sand and perlite (1:1) and irrigated with $\frac{1}{4}$ Hoagland solution and placed in a FitoScope 130 RGBIR chamber (Photon System Instruments, Czech Republic) configured to simulate field conditions (Maximum PAR 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 16/8 h day/night rhythm; day temperature 20 ± 0.5 °C, night temperature 18 ± 0.5 °C). After 2 weeks of acclimation plants were separated into 3 groups (N=5) and subjected to PEG5000 concentrations: control (0% PEG), 10% and 20%. All experiments lasted for 7 days after which plants were sampled. Pulse Amplitude Modulated (PAM) chlorophyll fluorescence measurements were performed using a FluoroPen FP100 (Photo System Instruments, Czech Republic), on 30 min dark-adapted leaves. Rapid light curves (RLC) measurements, in dark-adapted leaves, were attained using the pre-programed LC1 protocol of the FluoroPen (Duarte *et al.*, 2014a). Each ΦPSII measurement was used to calculate the electron transport rate (ETR) through photosystem II according to Genty *et al.*, 1989. Rapid light curves (RLC) were generated from the calculated ETRs and the irradiances applied during the rapid light curve steps. Each RLC was fitted to a double exponential decay function in order to quantify the characteristic parameters, alpha and ETRmax (Platt *et al.*, 1980; Marshall *et al.*, 2000). The onset of light saturation (E_k) was calculated as the ratio between ETRmax and α . The polyphasic rise in fluorescence (known as OJIP test or Kaustsky curves) transient depicts the rate of reduction kinetics of various components of PS II. When dark-adapted leaf is illuminated with the saturating light intensity of $3500 \mu\text{mol m}^{-2} \text{s}^{-1}$ it exhibits a polyphasic OJIP curve. Each letter reflects distinct inflection in the induction curve. The level O represents all the open reaction centres at the onset of illumination with no reduction of QA (fluorescence intensity lasts for 10 ms). The rise of transient from O to J indicates the net photochemical reduction of QA (the stable primary electron acceptor of PS II) to QA⁻ (lasts for 2 ms). The phase from J to I was due to all reduced states of closed RCs such as QA⁻ QB⁻, QA QB⁻ and QA⁻ QB H₂ (lasts for 2–30 ms). The level P (300 ms) coincides with maximum concentration of QA⁻ QB₂ with plastoquinol pool maximally reduced. The phase P also reflects a balance between light incident at the PS II side and the rate of utilization of the chemical (potential) energy and the rate of heat dissipation (Zhu *et al.*, 2005). For anthocyanins quantification, leaves were grinded in 99 % methanol acidified at 1% with HCl. To ensure complete disaggregation of the leaf material, samples were subjected to a cold ultrasound bath during 2 min. Extraction occurred at -20 °C

during 24 h in the dark. After extraction samples were centrifuged at 4000 rpm during 15 min at 4 °C and the absorbance of the extract was read at 530 nm (Sepúlveda-Jiménez *et al.* 2004). Anti-oxidant enzymatic activities were analysed according to standard methods for Catalase (Teranishi *et al.* 1974), Ascorbate Peroxidase (Tiryakioglu *et al.*, 2006), Guaiacol Peroxidase (Bergmeyer, 1974) and Superoxide Dismutase (Marklund, 1974).

Results and Discussion

The first evident change in the *S. patens* individuals exposed to PEG-induced drought was an increase of the leaves water content (Fig. 1), more accentuated under severe drought stress.

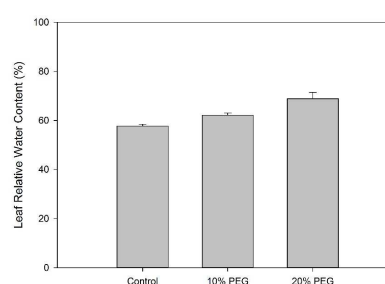


Figure 1. Leaf relative water content (RWC, %) in *S. patens* individuals exposed to different PEG-induced drought levels (average \pm standard error).

On the other hand, the photosynthetic efficiency (\square) of the individuals decreased with the increased drought level (Fig. 2). This was mostly due to a decrease in the electron transport rates (rETR) at different light intensities. This was more evident at higher light levels, mostly due to the incapacity of a stress cell to deal with excessive light as it was already observed in other halophytes subjected to stress (Duarte *et al.*, 2013). Nevertheless the maximum ETR was not affected by the drought exposure. This points out to incapacity of the stressed individuals to absorb and trap light energy. In fact, this can be confirmed while analyzing the phenomological energy fluxes (Fig. 3). Both levels of PEG-induced drought lead to a decrease in the number of reaction centers available for light harvesting (RC/CS) leading to a concomitant decrease of the absorbed (ABS/CS) and trapped (TR/CS) energy fluxes. Alongside there was also a decrease in the transported energy flux, as well as of the dissipated energy flux contrarily to most of the photosynthetic systems subjected to stress (Duarte *et al.*, 2013; Duarte *et al.*, 2014a; Duarte *et al.*, 2014b). This lack of efficient means to dissipate the excessive energy leads to the accumulations of excessive redox potential inside the cells and consequent activation of enzymatic counteractive measures to detoxify the cells from dangerous ROS.

This is evident in while observing the anti-oxidant enzymatic activities of the individuals subjected to PEG-induced drought, especially in the plants exposed to severe drought.

All enzymes showed a concomitant increase in their activities, stimulated by the increase in the production of ROS (Fig. 4).

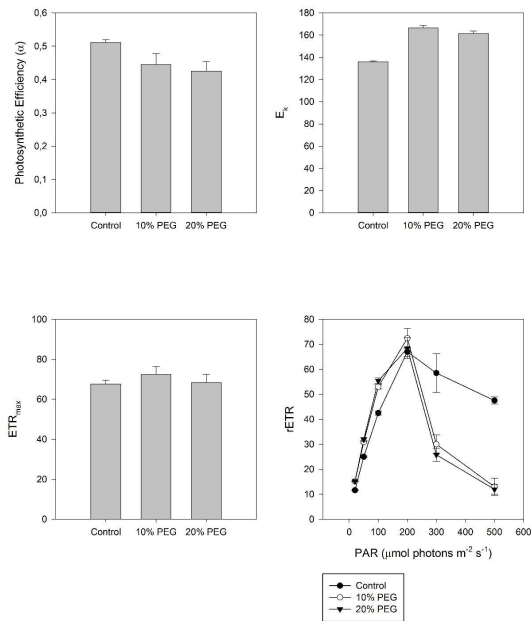


Figure 2. Photosynthetic efficiency (α), ETR_{max} and $rETR$ in leaves of *S. patens* individuals exposed to different PEG-induced drought levels (average \pm standard error).

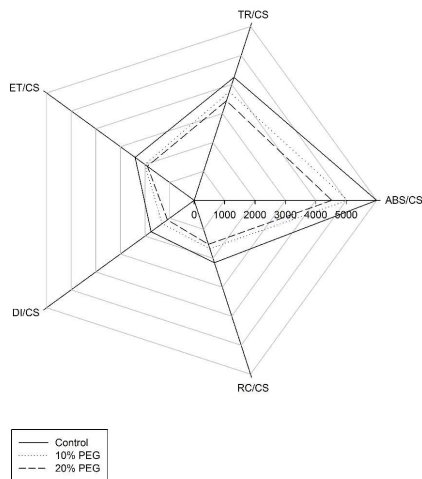


Figure 3. Phenomological energy fluxes in leaves of *S. patens* individuals exposed to different PEG-induced drought levels (average).

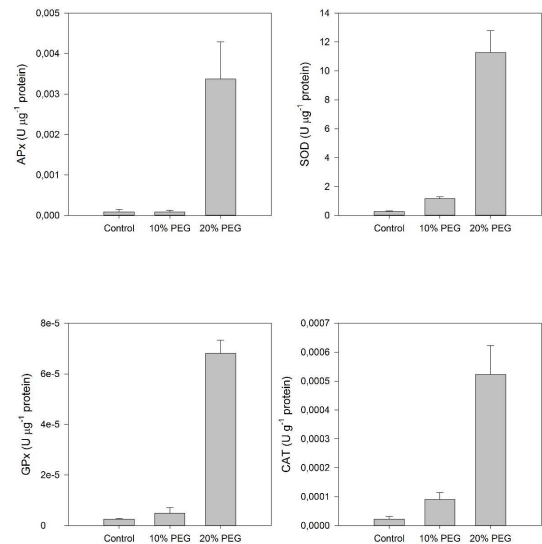


Fig. 4. Anti-oxidant enzymatic activities of catalase (CAT), ascorbate peroxidase (APx), guaiacol peroxidase (GPx) and superoxide dismutase (SOD) in leaves of *S. patens* individuals exposed to different PEG-induced drought levels (average \pm standard error).

Another typical feature characteristic of plants under drought is the production of anthocyanins. These pigments have as main function to dissipate the light excessive energy the reaches the leaves (Duarte et al., 2013). Regarding the total anthocyanin content of the leaves of the individuals exposed to severe drought, there could be observed a marked increase in the concentration of these photoprotective pigments (Fig. 5). Overlooking the UV-visible spectra of the acidic pigment extract of the stressed individuals, an anthocyanidin could be identified, cyanidin. This pigment is a precursor of anthocyanins which a formed by anthocyanidin glucosidation (Tulio et al., 2008). This production of anthocyanins is due to the diversion of metabolites from the primary metabolism to secondary metabolism under stress conditions (Fowler, 1986; Tholalalabavi et al., 1994). Anthocyanins have the potential to mitigate photo-oxidative injury in leaves, both by shielding chloroplasts from excess high-energy quanta, and by scavenging reactive oxygen species (Wang et al., 1997; Manetas, 2006), offering an effective and versatile protection to leaves without significantly compromising photosynthesis (Neill and Gould, 2003), that in the present study seem to cope with the also increased anti-oxidant enzymatic activity.

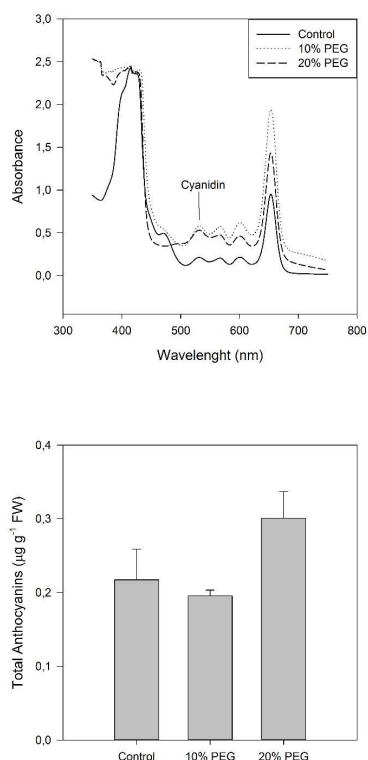


Fig. 5. UV-visible spectra of the acidic extract and total anthocyanin content of the leaves of *S. patens* individuals exposed to different PEG-induced drought levels (average \pm standard error).

Conclusions

Although it is widely accepted that salt marshes are wetlands, the halophytes that inhabit in these ecosystems can undergo drought periods, especially in the upper marsh areas. These plants are typically characterized by being able to colonize and adapt to their natural adverse environments, presenting a very efficient cellular machinery to overcome abiotic stresses. The invasion of the upper marsh areas by the NIS *Spartina patens* has been accelerating recently. Due to the absence of a frequent daily tidal flooding in this area of the marshes, this species can be exposed to moderate and drought stress. Nevertheless, it was found that this species can cope very well with this adverse conditions, decreasing the absorption of light energy in order to prevent the accumulation of redox potential inside the chloroplasts. Alongside with a high anthocyanin production, an efficient battery of anti-oxidant enzymes is also activated during severe drought conditions protecting the cells. The IPCC report states that the occurrence of extreme heat events tend to increase leading to longer drought periods in the Mediterranean region. Although with a slight reduction in its primary photochemistry, this species is apparently prepared to this scenario and thus will probably prosper under severe drought conditions competing with less adapted halophyte species.

Acknowledgments

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SESSION 4: Management, control, restoration
(chairman: D. Ayres)

10 YEARS AFTER: PROGRESS IN THE SAN FRANCISCO BAY SINCE THE 3RD
INTERNATIONAL CONFERENCE ON INVASIVE *SPARTINA*

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Abstract:

A decade ago, the Third International Conference on Invasive *Spartina* was convened in San Francisco, California (USA) to provide a forum for the latest *Spartina* research, and an opportunity to discuss new developments in *Spartina* science with marsh 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 science and management through increased exposure to one another. The San Francisco Estuary Invasive *Spartina* Project (ISP), initiated by state and federal agencies four years previous and a main driver behind the conference, was eager for information and guidance from scientists, and from managers with experience controlling invasive *Spartina*. We wanted to know: how big is our *Spartina* problem, and what are our chances of being able to control it? In addition to dozens of presentations, we provided participants a first-hand look at the San Francisco invasion through helicopter and mud-level field trips, after which we convened an expert panel and posed a list of questions. Overwhelmingly, the panel agreed that control was both possible and needed to conserve and protect important physiological and biological functions of the tidal marsh. The management experts stressed that action should be taken quickly and aggressively, particularly because of the accelerated rate of spread of the *S. alterniflora* × *foliosa* hybrids. With that affirmation and with partnerships, permits, and funding nearly in place, the ISP quickly ramped up an aggressive regional program to eradicate introduced *Spartina* from the estuary. Ten years later, the ISP and its partners have reduced the area of invasive *Spartina* from more than 800 net acres to 33, and achieved the first, second, or third year of zero-detection at 11+ previously-invaded marshes. Half of the remaining acreage is in 11 other marshes where treatment was suspended by a federal agency in 2011 because of concerns about the endangered California clapper rail. In response, the ISP began implementing a major tidal marsh revegetation program to improve habitat for this bird so that treatment can eventually resume. The ISP operates over an area of 50,000 acres of tidal marsh and mudflat providing regional planning and coordination, *Spartina* monitoring and treatment, clapper rail monitoring, and marsh revegetation, at a cost of \$24 million since 2000.

Keywords: Weed control, tidal marsh, conservation, *Spartina* hybrids, management

MANAGEMENT OF INVASIVE *SPARTINA* IN SAN FRANCISCO BAY, USA: ATTAINING AN ERADICATION TRAJECTORY WITHIN 50,000 ACRES OF URBAN ESTUARY

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Abstract:

The California Coastal Conservancy's Invasive *Spartina* Project (ISP) leads a regionally-coordinated effort to eradicate non-native cordgrass from the San Francisco Estuary. ISP developed partnerships from a wide array of stakeholders and contractors including local, state and federal agencies with diverse missions. This coalition has reduced the infestation by 96%, from 805 net acres down to 33, through the use of integrated landscape-scale monitoring and treatment.

The primary targets were hybrids between introduced *Spartina alterniflora* and the native *Spartina foliosa*. Over decades of backcrossing, a hybrid swarm had formed diverse morphologies and phenologies capable of exploiting all intertidal niches from naturally-unvegetated mudflats and channels up to high marsh. As the vast monocultures were diminished by imazapyr applications, treatment platforms transitioned from helicopters and amphibious vehicles to airboats and backpack sprayers. Detecting and treating hybrid targets scattered throughout the Estuary, while ensuring *S. foliosa* preservation and sensitivity in endangered species habitat, necessitated a comprehensive system of ISP biologist oversight.

While the infestation of *Spartina densiflora* never reached the scale or geographic scope of *S. alterniflora* × *foliosa*, eradication brought its own suite of challenges. Acquiring access to hundreds of residential properties took years of outreach and education from Friends of Corte Madera Creek. An adaptive Integrated Vegetation Management strategy was developed to overcome imazapyr's highly-variable efficacy on *S. densiflora*, as well as the persistence of the above-ground biomass. By 2013, only 64m² of *S. densiflora* remains; the eradication trajectory is now maintained by manual removal to exhaust the seed bank.

Keywords: *Spartina alterniflora*, hybrids, *S. densiflora*, imazapyr

The California State Coastal Conservancy (Conservancy) and U.S. Fish and Wildlife Service (USFWS) began the San Francisco Estuary Invasive *Spartina* Project (ISP) in 2000 in response to the growing threat posed by non-native cordgrass to the functions and values of the San Francisco Estuary (Estuary) tidal ecosystem. The majority of the cordgrass invasion throughout the Estuary was the product of hybridization between native *Spartina foliosa* (Pacific cordgrass) and *Spartina alterniflora* (smooth cordgrass) that had been introduced by the U.S. Army Corps of Engineers circa 1975 as an experiment in dredge spoils stabilization (Faber, 2000).

The genetic similarity of *S. alterniflora* to *S. foliosa* allowed multiple hybridization and backcrossing events that produced the 'hybrid swarm' that has posed the most widespread threat to the Estuary (Daehler and

Strong, 1997). This introgression has produced many morphologies and phenologies, but *Spartina alterniflora* × *foliosa* (hereinafter referred to as 'hybrid *Spartina*') is normally characterized by: (1) greater height (allowing for establishment at lower tidal elevation with greater frequency and duration of inundation, dominating mudflats and clogging tidal channels); (2) culm density (increasing competition and exclusion of native plants, allowing rapid sediment accretion altering marsh hydrology); and (3) greater flower size and fertility (allowing for pollen swamping of adjacent native cordgrass, as well as greater individual seed production) [Rhymer and Simberloff, 1996].

Extant tidal marshes and mudflats around the Estuary were undergoing rapid transformation by the hybrid *Spartina* invasion, and newly-breached restoration sites

were particularly vulnerable to domination due to their low biotic resistance in the absence of vegetative cover, and their position behind partial levees with reduced wave energy allowing for more rapid establishment. Hybrid *Spartina* can significantly alter the hydrology and development of the plant community, pushing restoration off a native marsh development trajectory. This invasion threatens the South Bay Salt Pond Restoration Project, attempting to restore native wetland functioning to 15,100 acres of salt evaporator ponds acquired from Cargill in 2003. As these systems mature, if the intended trajectory can be maintained, they promise to provide much-needed support to many species that have been seriously impacted by the loss of 85-90% of the historic wetlands around the Estuary.

Imazapyr Herbicide

In 2004, after several years of research and environmental documentation, including a Programmatic Environmental Impact Statement and Report (California Coastal Conservancy and USFWS, 2003), various treatment methods were evaluated including the use of imazapyr herbicide under an Experimental Use Permit from the California Department of Pesticide Regulation. Several land managers had attempted to control the invasive cordgrass over the years with little success. Imazapyr enabled ISP partners to implement much more effective treatment than they had achieved with glyphosate, the only other herbicide approved by the U.S. Environmental Protection Agency (EPA) for use in sensitive estuarine ecosystems. It is well documented that glyphosate adsorbs to sediment, inactivating its herbicidal properties. The turbidity of the Estuary allows for the accumulation of depositional material on the *Spartina*, as well as salt crystals that the halophyte exudes. Glyphosate produced highly variable results on *Spartina* (Patten, 2010), likely due to this adsorption, inhibiting uptake and translocation down into the rhizomes.

Imazapyr is a systemic herbicide that works by inhibiting the enzyme acetolactate synthase (ALS) needed for the biosynthesis of three branched-chain aliphatic amino acids. Animals do not produce these but rather acquire them by consuming plants, thereby presenting low toxicity in the absence of a relevant pathway of activity. EPA considers imazapyr 'practically non-toxic' to wildlife (mammals, birds, fish, amphibians, aquatic invertebrates, etc.). Leson & Associates (2005) synthesized two decades of available literature on the herbicide, examining the toxicity to a wide variety of organisms and the ecologically-relevant doses and exposure pathways, concluding that using imazapyr to eradicate non-native *Spartina* presents very low risk to the Estuary.

Imazapyr is short-lived in the aquatic environment, primarily broken down by photolysis (half-life of 2.5-5.3 days) (Shaner and O'Connor, 1991). In *Spartina* treatment field studies, Patten (2003) found that imazapyr concentrations in estuarine water quickly dropped below detectable levels (mean of 40 hours), and disappeared from mudflat sediment within an average of 400 hours. This dissipation is consistent with ISP data from implementation of their Water Quality Monitoring Plan for National Pollutant Discharge Elimination System (NPDES) compliance (Kerr, 2014). One week post-treatment sampling results over the four-year period 2007-2010 showed a mean 95.8% reduction in the imazapyr concentration regardless of the concentration previously measured at treatment (Kerr, 2011).

ISP Treatment Program Development

In addition to the secure, multi-year funding that the Conservancy provided, ISP brought the regional coordination that was essential to the success of landscape-scale aquatic vegetation management involving the tidal areas of nine counties. ISP biologists mapped 805 net acres of non-native cordgrass when Estuary-wide treatment began in 2005, more than 90% of which was hybrid *Spartina*. To gain control over the rapidly-invading hybrid swarm, helicopter applications utilized over the large monocultures. Any native *S. foliosa* at these sites had been extirpated by the hybrid swarm, so there was no need to be selective with cordgrass removal, making broadcast methods appropriate. By 2008, these applications helped to reduce the Estuary-wide infestation down to 273 net acres.

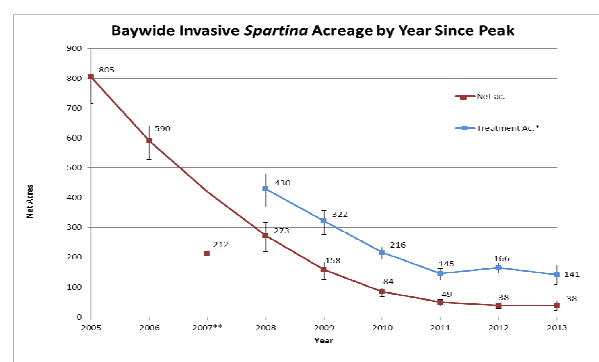


Figure 1. Bay-wide trend of invasive *Spartina* from 2005-2013 by net cover and treatment cover.

As the large meadows and mudflat infestations came under control, treatment methods began to shift to greater use of amphibious tracked vehicles (e.g. ARGOs). ARGOs became invaluable as the second phase of the eradication at many of the aerial treatment monocultures, and they were also utilized at some extensive infestations that were too close to residential development to allow for helicopter operations.

ISP found through a 2008 pilot project that many of the Estuary's mudflat and channel substrates could be driven by airboat at low tide with no water present. This allowed for treatment on a receding tide which was necessary to provide sufficient dry time for the imazapyr, an essential factor that can make the difference between 100% efficacy and 0%. Experience has shown that at least four hours of dry time over the majority of the plant's height is required for effective treatment with imazapyr.

There are a suite of factors that confine *Spartina* treatment to relatively narrow temporal windows of opportunity each year. Applications to a perennial such as *Spartina* are best conducted later in the phenology of the plant, but before senescence, when the downward direction of carbohydrate flow into the rhizomes for energy storage assists with translocation. Since the primary target of ISP treatment is part of a highly-fertile hybrid swarm, some intermediate forms are hard to discern from neighboring native cordgrass, necessitating that biologists wait to conduct inventory until later in the maturation of the plants to allow for the most thorough detection. In addition, the Federally-endangered California Ridgway's rail (*Rallus obsoletus* ssp. *obsoletus*, hereinafter 'rail') is present in many of the marshes of the Estuary, and USFWS had placed entry restrictions on treatment until the end of the bird's breeding season.

ISP greatly expanded the use of airboat as a treatment platform in 2009, utilizing Aquatic Environments Inc. to treat Don Edwards San Francisco Bay National Wildlife Refuge where many small to moderate infestations were scattered over thousands of acres of the best remaining tidal marshes in the Estuary. Applications can be conducted directly from airboat using the spray apparatus, best for mudflat clones or shoreline infestations. This has proven especially valuable in recently-breached restoration marshes, where new clones may be scattered across hundreds of acres of open mud just beginning to develop vegetative cover. Applicators can haul out up to 100m of hose from the airboat to reach larger stands on the marshplain or in remote channels. This treatment platform is also used to deploy and resupply backpack sprayers to tackle smaller, scattered infestations, or those beyond the reach of the hose.

Treatment Surveys

By 2009, the changing face of the non-native *Spartina* infestations around the Estuary required further adaptation of the Integrated Vegetation Management (IVM) strategy. Large infestations were becoming sparser, making thorough treatment challenging. Detecting a variety of hybrid forms at sites with abundant native *S. foliosa* required a high level of botanical expertise and site-specific experience. In addition, access during rail breeding season required strict oversight by certified biologists. Just as ISP was grappling with these new realities, a study from Willapa Bay, Washington, USA (Patten and Milne, unpublished data) added empirical evidence of the challenges to achieving eradication with unsupervised applicators. Eradicating non-native *Spartina* requires that there are no new introductions to a site, that all the plants in a given site are found each season (made more challenging by the hybridization), and that herbicide applications within the site are 100% successful. However, they found that approximately 63% of the target *Spartina* plants are detected (in the absence of a native cordgrass and highly-fertile hybrid swarm). Combined with an average efficacy of 75% from the imazapyr application, this results in only a 47% kill each year with a single inventory and application.

The combination of these factors led ISP to institute Treatment Surveys and acquire additional staff as biological chaperones. Inventory is first conducted by experienced ISP biologists, particularly where native cordgrass presence may confuse the issue. Non-native *Spartina* is mapped using GPS dataloggers (Trimble™ Yuma 2) and uploaded to ISP's Geographic Information System (GIS) geodatabase. Samples of morphologically indistinct plants are submitted to a lab at University of California Los Angeles (UCLA) for genetic testing. Inventory data and genetic results are loaded onto GPS units for treatment, and ISP biologists navigate back to the previously-mapped targets, updating each feature as it is successfully treated. This oversight allows for *S. foliosa* preservation through biologist identification of hybrid targets, improves the efficiency of the treatment crews and reduces disturbance to the denizens of the tidal marsh, while enhancing efficacy by ensuring thorough coverage of each target.

Benefits to the Estuary from ISP Treatment

In addition to preserving tidal marsh and protecting it from further degradation by this biological pollutant, a driving force behind the eradication of non-native *Spartina* was to restore the displaced native tidal marsh plant communities and thereby protect the fauna that depend on them for habitat. Perennial pickleweed (*Sarcocornia pacifica*) is the dominant native plant of the mid-marsh in the Estuary, yet it had been virtually

extirpated from many sites by late-stage infestations of hybrid *Spartina* growing in tall, dense meadows. Largely unimpacted by imazapyr, it experienced a strong competitive release from the tall, dense invader. Pickleweed is the preferred habitat and primary food source for the Federally-endangered salt marsh harvest mouse (*Reithrodontomys raviventris*), and treatment by ISP partners restored hundreds of acres of this critical habitat component.

In 2011, Point Blue Conservation Science revisited 183 plots where ISP biologists had collected vegetation data in 2005 prior to the initiation of *Spartina* treatment. The resulting Habitat Assessment Monitoring (HAM) report (Elrod et al., 2013) found that 15 of 24 sites had an increase in perennial pickleweed, with the greatest increase of 47.4% at Bunker Marsh that had received both aerial broadcast and ground-based treatment over the years. Passive revegetation for some of the key tidal marsh plants has been rapid in the wake of hybrid *Spartina* removal. This recuperation through native plant establishment also lends support to the assertion that imazapyr is short-lived in the estuarine environment, not presenting residual impacts to seedling establishment.

Another impetus for the eradication of non-native *Spartina* was to reclaim the naturally-unvegetated mudflats from the invader and preserve tens of thousands of acres from conversion to hybrid *Spartina* meadows with very low biodiversity. Just as Estuary-wide treatment was commencing, University of California Davis research (Neira et al., 2006, Levin et al., 2006, Brusati and Grosholz, 2006) showed that the domination of mudflats by hybrid *Spartina* caused a food web “dead end”. Hybrid *Spartina* produced so much belowground structure that it functionally excluded invertebrates. This essential invertebrate biomass that represents the foundation for the members of the greater food web (birds, fishes, crabs, etc.) declined by >70%. In addition, the remaining invertebrates shifted from surface feeders available to shorebirds and other consumers to inaccessible belowground feeders. The HAM report included analysis of the changes at two sites, Elsie Roemer and San Bruno, which had been converted from open mudflat to hybrid *Spartina* monocultures by the invader. Successful treatment at Elsie Roemer resulted in a 48.2% increase in open mud, while the San Bruno marshes had a 72% reduction in vegetative cover. These late-stage infestations had accreted substantial sediment and raised the elevation to an appropriate level for mid-marsh perennial pickleweed to now thrive, while the lower elevation portions could convert back to bare mudflat.

ISP's *Spartina densiflora* Treatment Program

While the infestation of *Spartina densiflora* never reached the scale or geographic scope of *S. alterniflora* × *foliosa*, its eradication has posed unique challenges and has required a significant investment of time and labor. As with much of ISP's work around the Estuary, the presence of endangered species has played a pivotal role in determining the IVM strategy. In the case of *S. densiflora*, the presence of rails at many of the infestation sites initially pushed the treatment work into sub-optimal timing, and also affected the methods that were chosen. For the first four years (2004-2007), entry to the marshes where rails had been confirmed by winter call counts was prohibited until their breeding season ended on September 1. Unfortunately, this timing was in conflict with the phenology of *S. densiflora* and would not allow for successful eradication. By late July, much of the *S. densiflora* around the Estuary had added to the seed bank and potentially dispersed propagules to new marshes. The plants had also begun to senesce by September, greatly reducing uptake and translocation. In addition, because of the presence of rails, mowing had not even been fully evaluated as a potential treatment in order to preserve refugia for the birds in the form of the residual marcescent necromass. Unlike the other species of *Spartina* in the Estuary, *S. densiflora* does not lose all of its above-ground biomass in senescence to subsequently regenerate from the perennial roots in spring.

By 2008, USFWS permitted ISP to enter rail sites by May to conduct inventory and treatment on *S. densiflora*. However, a new challenge had come to light; it had become evident that imazapyr had highly-variable efficacy on this cordgrass species and could not be relied upon in isolation to complete the eradication. Established stands of *S. densiflora* one year post-treatment can display a yellow-grey, half-dead appearance and are not healthy enough to translocate another herbicide application. Since this necromass is very persistent, it inhibits future treatment for a year or more and doesn't allow for an accurate assessment of the true status of each plant. Digging can be very effective on this species, a discretely-rooted bunchgrass; since it does not spread asexually by rhizome in the Estuary, the infestation is not exacerbated by digging. However, on a large scale it can be very damaging to channel banks and the marsh plain, especially when a meadow of *S. densiflora* has developed over the course of decades.

From 2008-2010, a new IVM strategy was developed that would minimize the impacts to the marsh and disturbance to rails while accomplishing the eradication in an efficient and cost-effective manner. In early winter after rail breeding season had ended, plants were mowed to slightly below the surface of the substrate

with gas-powered brush cutters equipped with tri-blades. This removed partially-dead above-ground biomass allowing for the best assessment of current plant status, and added further stress towards starving the plants' reserves.

By spring, the mowed plants that were still alive had regrown and seedlings had emerged. Large-scale digging and hauling is not permitted during this time, the height of rail breeding season. Instead, an imazapyr application was performed in early June that proved effective at arresting plant development and stopping seed production, even if it did not always produce mortality. In early winter the sites were evaluated; any remaining high-density stands were mowed again to reduce impacts to the marsh plain, while scattered individual plants were dug and disposed off-site. Each year less and less mowing was required until the dwindling infestation only required spot digging.

By 2012, mowing was discontinued at the last site, the original introduction site that had once contained the most extensive meadows. That site still continued to receive a June imazapyr application to thwart seed production, with digging performed in the winter. All other *S. densiflora* sites around the Estuary receive twice-annual survey and manual removal by ISP biologists, once in the winter when the senescent pickleweed allows for better detection and once in late spring when the flower stalks rise up above the surrounding vegetation. By 2014, only 64m² of *S. densiflora* was detected throughout the Estuary, a 99.7% reduction, leaving the remaining task of exhausting the seed bank. ISP experience suggests 3-5 year seed viability for this species.

A great deal of the Estuary falls under the category "highly-urbanized". Areas with the highest concentration of *S. densiflora* were in the Corte Madera Creek watershed in eastern Marin County where most of the shoreline is developed, with literally hundreds of individual residential, commercial and municipal landowners and tenants. Sandy Guldman, President of Friends of Corte Madera Creek Watershed, conquered the challenge of parcel research and developed a database to store the interactive history for each, facilitating regular notifications required for ISP inventory and treatment year to year. Long-time resident of the community who remained very active with environmental and other political issues, Sandy helped facilitate ISP's presence in the watershed by certifying the legitimacy of the intentions behind the eradication. Yet with so many people involved, it was not surprising to find some landowners against the removal, even without the use of herbicide that can be contentious in the absence of an accurate understanding of the risks. Landowner concerns ranged from property rights to the aesthetics of a mature *S. densiflora* hedge

lining their shoreline, to one landowner who valued the "debris fence" that kept all manner of litter from washing up on the tide. Unfortunately, *S. densiflora* is not a sterile landscape plant, and maintaining it on one property affects neighboring properties and allows it to disperse on the tides to infest new marshes. Counties have the authority to require the control of plants listed by the State of California as noxious weeds. In the end, the final holdouts allowed for the removal of *S. densiflora* after being contacted by Marin County Deputy Agricultural Commissioner Stefan Parnay, and none of the cases required enforcement action.

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INNOVATIVE MONITORING STRATEGIES TO SUPPORT INVASIVE *SPARTINA* CONTROL EFFORTS

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Abstract

The California Coastal Conservancy's Invasive *Spartina* Project (ISP) has been using GPS and GIS to map hybridizing invasive *Spartina* populations across 20,000 hectares of potential habitat within the San Francisco Estuary (California, USA) since 2001. As weed populations decline and we approach the goal of eradication, monitoring becomes more difficult, and the need for thorough, accurate and precise data to inform management and treatment becomes increasingly important. Such data is even more critical when the target of eradication is a hybrid requiring genetic testing for identification of some individuals. The ISP has developed several novel methods for the efficient, accurate capture of a full suite of mapping data, including presence data, area surveyed, and patch-level treatment tracking. These innovative monitoring strategies are proving critical in these final stages of eradication at many sites, when treatment of every instance of the target plant is required to reach our goals.

Keywords: *Spartina*, Weed Mapping, GIS, Monitoring

Background

Wildland invasive weed control efforts often include a weed mapping component. Various organizations around the world have developed guidelines to help facilitate and standardize weed mapping efforts. We reviewed the California Weed Mapping Handbook, the published North American Weed Mapping Association standards and Australian Environmental Weed Mapping Standards, and the results of a web search to identify various European weed mapping methods. Our review identified five major components that are the basis of all invasive weed mapping standards: who found the plant; when, where, and what was found; and how much of it was found. The added components of where the observer looked (that is, recording of spatially accurate absence data, in addition to presence data), and if and how the plant was treated during any control efforts, were mentioned as optional for data collection.

Within our project, we have found tracking these two optional components –spatially explicit absence data and patch-level treatment information – to be critical to our project's success. Although their collection is time-consuming, the additional information obtained by tracking these parameters allows us to ensure the thoroughness of our survey and treatment activities.

Such thoroughness is critical to meeting the goal of not just control but eradication of invasive *Spartina* from the San Francisco Bay.

This paper reviews the weed mapping workflow of San Francisco Estuary Invasive *Spartina* Project (ISP) and discusses some of the creative strategies we have developed to efficiently implement the above-mentioned major and additional weed mapping standard components via the use of commercial Geographic Positioning System (GPS) and Geographic Information System (GIS) software.

Workflow

The ISP *Spartina* Monitoring Program involves two components: Inventory and Treatment Monitoring.

Inventory monitoring begins with the extraction of past years' data for viewing on a GPS-enabled electronic device, such as a tablet PC or handheld GPS unit running Windows Mobile. We extract site-specific data from our ArcMap GIS software for viewing and editing in our ArcPad GPS mapping software. ArcPad's ability to display background imagery, digitized water features, and attribute-rich *Spartina* data from past

years while allowing collection of tracklogs and new features make this software ideal for assisting biologists with navigating the marsh, revisiting old patches of invasive *Spartina*, and ensuring complete coverage of the area to be surveyed for new patches. Current-year features are mapped as new layers viewed over past years' data. Field-collected GPS data are checked into a GIS database for viewing in ArcMap. This data is then used to plan for treatment, which can take place anywhere from the next day to several months later.

The ISP also collects *Spartina* samples for genetic testing, to help with the identification of hybrid *S. alterniflora x foliosa*. Genetic samples collected during inventory monitoring are shipped to a genetics lab for microsatellite testing. Results from the lab are linked to their associated GIS features, and the species identifications of these features are then updated accordingly.

Although the ISP has coordinated treatment efforts since 2004, treatment monitoring efforts did not begin until 2009, when ISP biologists began using GPS units to guide treatment crews to previously-mapped, current-year *Spartina* patches that were mapped during inventory monitoring using GPS units. Prior to 2009, treatment crews were responsible for finding and treating invasive *Spartina* patches on their own based only on paper maps generated after inventory mapping. Treatment was not monitored on a patch-level basis, and patches were sometimes missed as a result.

At the end of the year we summarize our collected data for analysis and reporting purposes.

Weed Mapping Components

Who/When

Using our GPS units and ArcPad software, we record the standard information of "who" and "when" for each feature recorded. All treated features have two sets of surveyor and date fields: one set that records the name of the surveyor and the date of the initial capture of the feature during inventory monitoring, and one set that records the surveyor's name and date of treatment monitoring. The ISP currently has about 20 field biologists on staff who work to monitor and record the location and treatment of invasive *Spartina* throughout our inventory and treatment sites. We inventory for *Spartina densiflora* in the winter and early spring, and we map and treat all other types of invasive *Spartina* between June and December each year. Staff record approximately 35,000 features annually. To help with efficiency and accuracy of data for this quantity of data collection, we employ ArcPad customization scripts to automate the recording of the surveyor name and date for each feature.

Where Looked

The "where" component of our mapping data includes collection of weed locations (also known as "presence data") and the careful tracking of area surveyed (sometimes referred to as "absence data"). Our review of standard mapping protocols revealed that some programs consider it optional to record the extent of the area surveyed, however with so many people to coordinate and such a large area to cover, the ISP requires excellent strategies to keep track of where we inventory each year to ensure complete coverage. We use what we call "inventory boundaries" to track where we inventory for invasive *Spartina* each year, and we use ArcPad's tracklog capabilities to ensure that we cover all of the ground we intend to survey.

Our inventory boundaries encompass over 20,000 hectares (50,000 acres) of marsh, mudflat, riprap, flood control channels, and even roadside ditches that experience enough tidal inundation to be possible *Spartina* habitat. These boundaries were initially derived from a variety of GIS sources in 2006, and have been refined by our field staff every year to include all areas assessed for potential invasive *Spartina* habitat. These boundaries are used by our field staff as a guide for where to look, so that we are sure to fully survey every location with suitable *Spartina* habitat every year. We attempt to maintain a high degree of spatial accuracy (+/- 5 meters) in our inventory boundary layers so that they will be as useful as possible in the field, guiding biologists to small side-channels and other areas that could otherwise be easily overlooked during monitoring. We track our season's inventory progress in our GIS by using these inventory boundaries. We also track areas that we have checked for possible habitat and determined are not potential *Spartina* habitat due to lack of tidal action or too much freshwater.

Navigation in the field is also assisted via use of a marsh channels layer and aerial imagery as background layers within our ArcPad maps.

In large areas without a lot of physical landmarks, division of labor (deciding who will map where within a marsh) can be difficult. We use GIS tools to create what we term "tracking polygons" in the office that help break up the marsh into navigable sections for field staff. We use aerial imagery and the channels layer to try to make sure that each colored section delineates a reasonable area for a single person to navigate, and does not require crossing of major channels to complete inventory of that section. The creation and editing of tracking polygons in the office is relatively time-consuming, but the time savings in the field make the effort to create these background features worthwhile.

We use ArcPad's tracklog capabilities to visualize and record the path of each biologist in the field. Tracklogs are converted to tracklines in the office, and these are used by managers to assess whether monitoring efforts were completed within the designated inventory boundaries. This information allows us to confirm that surveys were complete, thus boosting our confidence in the thoroughness of our presence data and the accuracy of our reported absence data (that is, confirming that we did indeed fully survey those areas where no invasive *Spartina* was detected).

Where Found

All patches of invasive *Spartina* are mapped annually. We record new data each year, rather than updating previous-year features, since patch geometries change over time as weed patches coalesce as they expand, or dissolve into multiple patches due to treatment effects. Our "minimum mapping unit" can be as small as a single stem of *Spartina* mapped as a point with a radius of 5 cm. Our maximum patch size can be a contiguous meadow of *Spartina* mapped as a large polygon. We use points, lines and polygons, as appropriate, to map our findings. Field staff are trained to choose which type of feature geometry to use for which patch based on balancing the efficiency of data collection with the usefulness of the feature to guide treatment. Point features are the most efficient to record, but polygon features can be much more informative when trying to delineate the exact outline of a patch of invasive *Spartina* that is growing within a meadow of native *Spartina*, for example. Line features are especially useful for mapping linear features, such as patches along channel edges.

What: Species Identification

The recording of what species or hybrid was found is often the most difficult part of our data collection process, since field identification of hybrid *Spartina* can be very difficult. So in addition to our field identification, we record our level of confidence in that identification, and the reasons for our field identification. We also take samples for microsatellite testing to help distinguish native *Spartina foliosa* from *S. alterniflora* and/or *S. alterniflora x foliosa*.

When mapping *Spartina*, staff are required to record their species/hybrid identification, the basis of their field identification, and their level of confidence in this identification for each new feature recorded. Field identification can be based on plant morphology, lab results, past years' data, location, or simply the fact that the plant appears to have been treated with herbicide. For features recorded with a low or moderate confidence, staff are instructed to enter ancillary information to document what characteristics influenced their decision-making. Common

characteristics that influence species identifications in the field include culm color, phenological timing, plant density or morphological heterogeneity within the patch, and the width, height or length of culms, inflorescences and/or leaves. Checkboxes associated with the most common characteristics are available on our electronic data forms for efficient data collection, and a comments field is available for typing in additional information. This patch-specific descriptive information is helpful to staff returning to the patch for later treatment or mapping in subsequent years, as it helps to guide their field identification and related decision-making regarding the need for treatment.

Annual genetic samples help us to inform and validate our identification of native and hybrid *Spartina*. We link lab results from all genetic samples to the corresponding GIS features that are recorded during sampling efforts, so that lab results can inform future treatment actions and mapping efforts.

The majority of our genetic samples are collected during our mapping efforts in an effort to help inform difficult patch-level species identifications within sites where native and hybrid *Spartina* co-exist. Such samples are often collected from plants growing within salt pannes or adjacent to previously treated patches, as such microhabitats and/or herbicide treatment effects can stunt hybrid *Spartina* growth, leading to shorter stunted plants that are difficult to distinguish from native *Spartina*. Because of the turnaround time for receipt of genetic results, samples collected during mid-season monitoring efforts often cannot inform current-year treatment efforts. To address this constraint, we use early-season sampling at some of our more difficult sites in an effort to inform our current-year monitoring and treatment efforts. Since plants are extremely difficult to identify during the early growth stages, especially prior to stem elongation, early season samples are not collected solely based on their current-year morphologies, but rather are collected from patches that were mapped with lower or moderate confidence the previous year. Results are then used to inform current-year mapping efforts several months later, when the sampled plants are exhibiting more distinguishable characteristics in their later growth stages.

Validation samples are those taken from plants field-identified by staff as *S. foliosa* or *S. alterniflora x foliosa* with very high confidence. Many native *Spartina* validation samples have been processed in recent years in support of *S. foliosa* collection and revegetation efforts, however these come from only a few locations in the Estuary from which we collect *S. foliosa* for propagation and outplanting. Hybrid and native *Spartina* validation samples were collected to inform

genetic analysis during the first year of microsatellite testing (2010) and again in 2014 onward.

Mapped patches of *Spartina* and genetic results are color-coded by species and by year within ArcPad and ArcMap, with past years' data displayed in pastel colors and current-year data displayed using a bright color scheme. Past years' data are also displayed as smaller features than current-year features.

What: Patch-Level Treatment Effort

During our treatment monitoring we navigate to previously-mapped, current-year features and update these at time of treatment. When updating features, we once again record our field identification and confidence level, if it has changed, and we record information about how the patch was treated, such as the method used (herbicide or digging) and any notes about the treatment itself. Again, as an aid to efficiency, commonly used treatment descriptions, such as if the plant had started to senesce or if the tide was coming in, both of which might affect the efficacy of the treatment, are listed on the data form and can be recorded by checkbox. Treatment notes can be typed to record any additional information about treatment.

As patches are treated, the features in ArcPad change color to indicate when treatment is complete (blue) or to highlight when a patch of invasive *Spartina* was not treated (yellow). This allows us to easily track our progress during a treatment survey and ensure that all mapped patches are treated.

Efforts are made to make our interactive field maps in ArcPad as useful as possible by displaying helpful layers, such as previous years' mapped *Spartina* locations and channel layers, but also attempting to keep the map as simple and uncluttered as possible so that the information is comprehensible. However, the maps are still relatively complex and take time and training to use. Figure 1 shows an example of an ArcPad map prepared for a second day of treatment monitoring at a large marsh site. This map contains non-editable background layers of inventory boundaries, channels, colorful tracking polygons and past year's data in pastel, with editable, current-year data in bright colors on top. This map also contains line and polygon centroids (symbolized as black X marks), which can be helpful in the field to make sure no mapped patches get overlooked during treatment due to screen display issues. Some current-year *Spartina* features are labelled with notes that were recorded during inventory monitoring specifically to inform treatment of these individual features.



Figure 1: ArcPad interactive map prepared for treatment monitoring.

How Much

Every GPS feature (point, line or polygon) mapped has a patch size associated with it. This is recorded as a radius for points and as a width for lines. At the end of the year, points and lines are converted to polygons for data summary and analysis purposes.

We record two cover class values for each *Spartina* feature. The first, relative net cover of invasive *Spartina* in the patch, is a standard weed mapping component and a typical measurement used in botanical surveys. The second, termed "treatment cover", is defined as the percent of the feature area that will require treatment. This "treatment cover" concept was developed by the ISP in 2008 to allow us to accurately plan for treatment efforts.

When invasive *Spartina* is thick and lush, net and treatment cover are virtually identical, but as patches thin out over time due to weakening by treatment, for example, net cover can become significantly lower than treatment cover. By taking both measurements, we can track change in vegetative cover over time and also prepare properly for treatment efforts.

We multiply feature area by net cover to derive net area, and by treatment area to derive what we term "treatment area", at the patch level. These data can then be summarized for planning and reporting purposes.

Summary

The ISP *Spartina* Monitoring Program has successfully met the complex mapping and monitoring needs of the project by using GIS and GPS software to record all five standard weed mapping components (Who, What, When, Where Found and How Much) as well as two additional components (Where Looked and How Treated) that are critical to meeting our project's goal of weed eradication.

We have customized our ArcPad data entry forms to increase the efficiency and accuracy of our data collection of the five standard weed mapping

components, which we have expanded to include thorough methods of recording species identifications and with ancillary information about these identifications. We developed the unique concept of “treatment area” to supplement our net area estimates. This measurement has proven useful in assessing the size and extent of the infestation in terms of treatment effort required.

We have developed innovative strategies for tracking area surveyed, which include GIS preparation of “inventory boundaries” and “tracking polygons” to

identify areas where surveys are needed, and the use of automated tracklogs to record actual area surveyed during field work.

Our treatment survey methods of guiding treatment crews to pre-mapped features and recording patch-level treatment information has allowed us to make sure that we are treating all plants detected at a site, which is essential if we are to meet our goal of eradicating invasive *Spartina* from the San Francisco Estuary.

SPARTINA IN THE SAN FRANCISCO BAY: POPULATION GENETICS IN A MANAGEMENT CONTEXT

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Abstract:

Spartina foliosa is native to the San Francisco, California, Estuary. *Spartina alterniflora* was introduced to the estuary in the 1970s and subsequently hybridized with *S. foliosa*. Backcrossing created an invasive hybrid swarm, outcompeting *S. foliosa* within its range and spreading into higher and lower intertidal zones, drastically altering ecological communities. *S. foliosa* acreage has declined, and the species is locally displaced near the epicenter of the invasion. The San Francisco Estuary Invasive *Spartina* Project (ISP) has reduced the net acreage of non-native *Spartina* by 95%, but threats posed by hybrid expansion persist. Since some hybrid plants appear morphologically similar to native *S. foliosa*, the ISP has used a variety of genetic tools to aid in identification, initiating the use of microsatellite DNA markers in 2010. We have accumulated geo-referenced microsatellite genotypes of over 3,500 samples from all regions of the bay and some locations outside the bay. Using population genetic analyses, we can address important management issues that are not easily accessible without genetic data. Here, we test for population genetic structure (i) among samples near the site of the original introduction of *S. alterniflora*, (ii) among new, hybrid recruits at multiple locations in the southern portion of the bay, and (iii) among hybrids from throughout the bay. The ISP is the curator of a large, robust database of *Spartina* genetics. Exploiting the information we already have, we can learn more about invasion dynamics in order to inform management priorities and future sampling strategies.

Keywords: *Spartina foliosa*, *Spartina alterniflora*, San Francisco Estuary, Population Genetics, GIS

Discussions about managing invasive species generally boil down to a set of fundamental questions: "What is it?" "Where is it?" "How does it spread?" and "Can it be controlled or eradicated?" The San Francisco Bay in California, USA, is a large, urban estuary and contains several important commercial ports, and has been the recipient of many biological introductions and invasions. One invasion involves several species of marsh cordgrass in the genus, *Spartina*. *Spartina foliosa* is native to the estuary, its range extending from Baja California, Mexico, to Bodega Bay, northern California, USA (Spicher and Josselyn, 1985). *Spartina densiflora*, *S. patens*, and *S. anglica* have been introduced in multiple locations, with important but geographically limited effects. *Spartina alterniflora*, on the other hand, was introduced by the U.S. Army Corps of Engineers in the 1970s as part of a program of salt marsh restoration at New Alameda Creek (Fremont, California, see Figure 3), then hybridized with native *S. foliosa* (Daehler and Strong, 1997). *Spartina alterniflora*, and probably hybrids, were also

planted by the USACE in the 1970s for erosion control on the Alameda Island shoreline (US Army Corps of Engineers, 1978). Morphology-based field observations identify the presence of *S. foliosa* and *S. alterniflora* x *foliosa* hybrids in all major regions of the bay, although individual marshes may be composed of only *S. foliosa*, only hybrids, or a mixture of both. Hybrids outcompete *S. foliosa* within its tidal range, as well as colonizing higher tidal zones up to the high tide line, and lower zones, converting mudflats to *Spartina* marshes (Callaway and Josselyn, 1992).

The San Francisco Estuary Invasive *Spartina* Project (ISP) (www.spartina.org) was established in 2000 by the California State Coastal Conservancy, with United States Fish and Wildlife Service and more than 50 regional partners, to respond to the threats posed by invasive *Spartina* to tidal marshes and the species that depend on them in the San Francisco Bay. The ISP has an effective treatment program that has reduced the net area of the infestation by

over 95% from its peak of approximately 325 hectares (805 acres) in 2005 (Rohmer et al., 2014), but restoring tidal exchange at new restoration sites threatens to allow for hybrid expansion into these vulnerable systems. As progress has been made in removing the most easily identifiable plants, those hybrids that are less readily identified by morphology have become a greater proportion of remaining plants. Genetic sampling provides an additional tool for species identification. In 2010, the ISP made a transition from using RAPD genetic markers to using 13 to 15 polymorphic microsatellite markers (Blum et al., 2004; Sloop et al., 2005).

Here we present microsatellite-based analyses that provide additional information regarding population structure, and rates and dynamics of hybrid dispersal. A drift card study (D. Kerr, unpublished data) suggests that *Spartina* propagules could potentially be dispersed widely throughout the bay, and even beyond the bay up to 100 km from point of release, every generation. On the other hand, prior work (Sloop et al., 2011) and an ISP study (Thornton et al., 2013) suggest that most recruits come from nearby sources. The population genetic predictions of these competing scenarios differ. Frequent long-distance dispersal would have the effect of homogenizing populations, preventing the persistence of population genetic differences. Localized recruitment favors the establishment of population structure that could be detected by microsatellites. Persistent population structure just north of the area of the original introduction of *S. alterniflora* was detected by Sloop et al. (2011), with evidence supporting another *S. foliosa* hybridization event with a second and distinct *S. alterniflora* introduction further to the north, at Alameda Island (Figure 2). An improved understanding of the invasion dynamics will inform decisions regarding strategies for eradication of these plant pests. In these analyses, we test for population genetic structure among samples near the documented site of introduction of *S. alterniflora*, among new, hybrid recruits at multiple locations in the southern portion of the bay, and among hybrids from throughout the bay.

Methods

Spartina samples were collected from nearly every tidal marsh in the San Francisco Bay and Estuary from 2010 through 2013 (Figure 1). Sampling was primarily motivated by the need for genetic information to help identify individual plants as *S. foliosa* or *S. alterniflora* × *foliosa* hybrid, and was therefore not carried out under randomized sampling except under certain circumstances.

Analyses focused on the area near the initial introduction(s) of *S. alterniflora* were performed on 98 samples collected from 2010–2013 that were identified as hybrid using the lab techniques described below, receiving a %Hyb score of 0.6 or above. For the study of new recruits, samples were collected in 2013 to determine the composition of young plants in sites with various types of likely seed input: native, hybrid, or a mix. Genetic structure of newly recruited hybrids was analyzed using 122 of those samples that received a %Hyb score of 0.2 or above. Baywide analyses were carried out on the full 2010 – 2013 dataset with %Hyb score of 0.6 or above. All analyses were limited to samples with at least seven successfully amplified loci.

Samples were stored at 4°C for up to a week, rinsed with water, and shipped to Genomic Advisors, Inc. (La Mirada, CA, USA) for DNA extraction, using a combination of maceration and Qiagen DNeasy Plant Extraction columns (Qiagen, Inc.). Extracted DNA was shipped to the Genotyping and Sequencing Core at the University of California, Los Angeles, David Geffen School of Medicine, where microsatellite genotyping took place. Samples were genotyped using 13 to 15 of the following primers: SPAR2, SPAR8, SPAR9, SPAR10, SPAR16, SPAR17, SPAR20, SPAR21, SPAR22, SPAR23, SPAR26, SPAR27, SPAR28, SPAR31, and SPAR32 (Blum et al., 2004; Sloop et al., 2005). PCR was carried out on 96-well plates using a Qiagen Taq PCR Master Mix Kit and fluorescently labeled forward primers. PCR products were pooled into three different panel mixtures and run through an Applied Biosystems 3730 DNA Analyzer. Raw data was analyzed in GeneMapper (Applied Biosystems). STRUCTURE (Pritchard et al., 2000) was used to compute the probability of the genotypic data given K genetic clusters, recording credible regions, assuming admixture among populations, with allele frequencies correlated, with a burn-in length of 10,000 iterations followed by 100,000 iterations of the model. The post hoc method of Evanno et al. (2005) was used to determine the number of genetic clusters K detected in the data by STRUCTURE.

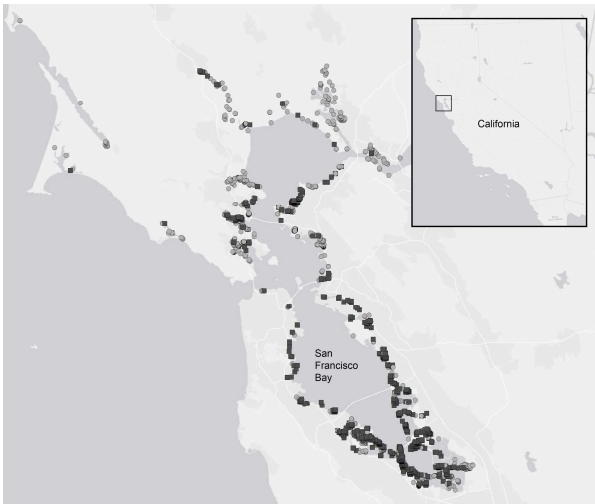


Figure 1: Map of San Francisco Estuary and surrounding coast, showing locations of all samples of *S. foliosa* (light grey dots) and *S. alterniflora* and hybrids (dark grey squares) used in this study.

For all samples, we estimated the presence of two genetic clusters ($K = 2$), corresponding to *S. foliosa* in one cluster and other species or hybrids in the other cluster. We then defined samples as *S. foliosa* or hybrid based on the predicted percentage of ancestry in the hybrid cluster (%Hyb). Samples with estimated %Hyb < 0.10 were deemed to be *S. foliosa*. Samples with %Hyb > 0.20 were deemed hybrid, while samples with %Hyb between $0.10 - 0.20$ were called “uncertain” and not used in further analyses. These genetically based species definitions were used to determine which samples to include in subsequent population genetic analyses of hybrids. Baywide population genetic analyses on hybrids were more stringently limited to those 817 samples with %Hyb > 0.6 and successful amplification at seven or more loci.

A subset of 98 samples from 2010 – 2013 was selected to test for population structure in the area near the initial introduction(s) of *S. alterniflora*. Samples were selected from the Alameda/Bay Farm Island/San Leandro Creek complex, Dogbone Marsh, Citation Marsh/San Leandro Shoreline complex, Oro Loma Marsh, and Cogswell Marsh, and H.A.R.D. Marsh (Figure 2). The criteria for inclusion were that samples successfully amplified at a minimum of seven loci and received a %Hyb score of 0.6 or higher in STRUCTURE, a conservative cut-off to minimize potential effects of *S. foliosa* influencing population structure.

New recruits, defined as young, newly-detected plants or clonal patches not seen in previous years, were collected using randomly-generated collection points in 2013 from ten locations in the South Bay

(Figure 3). Out of 166 samples collected, 122 were identified as hybrid using a %Hyb criteria of 0.2 or above in STRUCTURE, with at least seven successfully amplified loci, and were used in subsequent analyses.

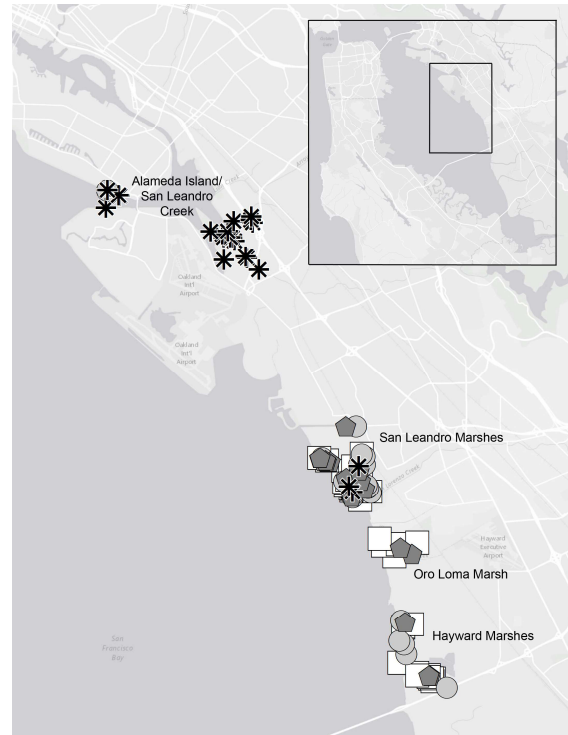


Figure 2: Map of hybrid sampling near the location of the original introduction of *S. alterniflora*. Different symbols represent the four different genetic clusters identified in STRUCTURE.

Pairwise F_{ST} calculations were carried out in Arlequin v3.11 (Excoffier et al., 2005). Genotypes, field- and laboratory-determined species identifications, and sampling location for all samples were joined and mapped using Geographic Information System software ArcMap 10.x (Esri, Redlands, CA, USA).



Figure 3: Sampling map of hybrids less than approximately one year old sampled from ten locations. The site of the original introduction of *S. alterniflora* by the U.S. Army Corps of Engineers is marked with a star.

Results

From 2010–2013, over 3,500 samples were collected from tidal marshes throughout the San Francisco Bay and analyzed at 13–15 microsatellite loci (Figure 1). Two genetic clusters were detected by log-likelihood and delta- K scores among all samples collected baywide over the four year period, corresponding to a *S. foliosa* group and a *S. alterniflora* or *alterniflora* × *foliosa* group. The estimates of the percentage of hybrid ancestry in the samples were distributed continuously from less than 0.1% to 99.9%.

We analyzed a subset of hybrid samples from marshes near the location of the original documented introduction of *S. alterniflora* by the USACE in the 1970s. Four genetic clusters were revealed among this geographically limited subset, with one cluster dominating on Alameda Island, an area relatively isolated from the other three by a narrow passage between two islands, and known to have been planted with *S. alterniflora*, and probably hybrids, by the USACE. The other three genetic clusters were mixed geographically among more closely adjoining areas along the San Leandro and Hayward shorelines (Figure 4).

An analysis of hybrid plants approximately one-year-old or less at ten locations gave strongest support for only one or possibly two genetic

clusters. The Evanno (2005) method cannot discern between one or two clusters, but log-likelihood scores increased sharply from -5002 for $K = 1$ to -4577 for $K = 2$. F_{ST} values detected significant genetic differentiation in most pairwise comparisons between sampling locations, but that differentiation was weak in some cases, showing evidence for some immigration among sites, including among sites on opposite sides of the South Bay (data not shown). STRUCTURE analysis of all hybrid plants across all locations over the entire four-year study period showed no evidence of population structure, with the hypothesis of a single genetic cluster receiving the greatest support, again with the caveat that the method cannot discern $K = 1$ from $K = 2$. The log likelihood of the data immediately began leveling off after $K = 1$, but does not completely rule out the possibility of the existence of two weakly differentiated genetic clusters of hybrids in the bay.

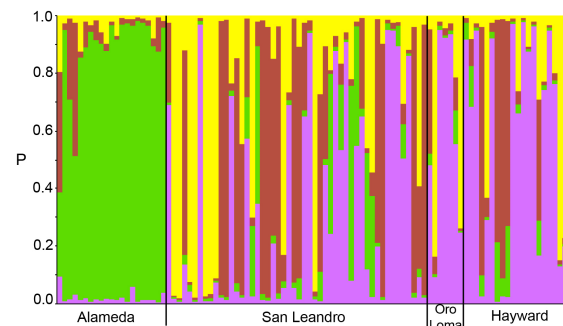


Figure 4: Bar plot of STRUCTURE-based estimates of genetic ancestry for four locations along the San Leandro shoreline. Each individual is represented as a column, with estimated proportion (P) of membership in each of four genetic clusters shown on the y-axis as a colored segment of the column.

Discussion

Thornton et al. (2013) found that the species or hybrid identity of new recruits is likely to match that of nearby potential source populations, suggesting that localized recruitment plays a prominent role in the colonization of marshes by *Spartina*. Sloop et al. (2011) also emphasized the importance of local recruitment. However, the lack of genetic structure among baywide hybrids supports the hypothesis that longer distance dispersal plays an important, homogenizing role. A blend of the two dispersal modes, in which most recruitment is local but is punctuated by migration over longer distances, could allow for short-lived, highly localized genetic structure as new

populations are founded by one or few individuals, but eventually this structure would be erased through the arrival of new immigrants.

In this context, our results reveal a puzzling pattern of localized population structure among hybrids near where *S. alterniflora* was introduced, but that pattern disappears as the scale of analysis encompasses more of the San Francisco Bay. This is in contrast to the lack of structure over larger areas of the bay, and to typical patterns seen in many other systems in which genetic differentiation increases with distance between samples. One explanation is that the original infestation and hybridization has persisted relatively free from any influx of immigrants in the subsequent decades, possibly due to space limitations, so that we can still detect remnants of population structure that was present among the original plantings and hybrids. If each of these original subpopulations contributes relatively equally to the colonization of the rest of the bay, no structure would be detected at that larger scale. Occasional long-distance dispersal among sites, as little as one migrant per generation per population, may continue to prevent the establishment of isolation by distance. However, in order to retain the structure we detected, the original populations would have to be relatively resistant to any influx of migrants from elsewhere in the bay. An alternative hypothesis is that one or more recent, unreported introductions, from a source other than that used for the original introduction, have taken place in the same area, and there has not been enough time for the homogenizing effects of dispersal to erase its genetic signature. The pronounced clustering among Alameda Island samples, found in this study and previously (Sloop et al., 2011), provides the strongest support for this hypothesis.

The goal of the Invasive *Spartina* Project is ultimately to eradicate *S. alterniflora* and its hybrids from the San Francisco Estuary. We have made great strides toward this goal, having reduced net acreage by over 95 % over the last ten years. However, the work has become increasingly difficult as the most distinctively hybrid phenotypes are eradicated. Many of the remaining hybrids are difficult to tell from *S. foliosa* using field characteristics alone. The incorporation of genetic tools into the identification process has helped greatly. The final push to eradicate the hybrid will likely become increasingly difficult, and genetic methods will likely play an even more important role. Few plants are truly "cryptic," however, in that most hybrids do display telltale phenotypes, but sometimes those phenotypes appear later in the season than when monitoring occurs, take multiple

seasons to manifest, or may be location dependent and are therefore less reliable for use in identifying new colonizers. Improvements in genetic methods, such as are now available with genome-wide scans, should be explored for their ability to more accurately find the genotypic space between native *S. foliosa* and invasive hybrids.

Acknowledgment

We are grateful to the California State Coastal Conservancy for the establishment and funding of the San Francisco Estuary Invasive *Spartina* Project, and to the large network of entities that have contributed to its execution.

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HOW DO RESTORATION SITE CHARACTERISTICS, PLANT CAGING, AND PARENTAL SOURCE AFFECT NATIVE PACIFIC CORDGRASS (*SPARTINA FOLIOSA*) ESTABLISHMENT?

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Abstract:

A key programmatic goal of the San Francisco Estuary Invasive *Spartina* Project's restoration program is to reestablish native Pacific cordgrass (*Spartina foliosa*). Pacific cordgrass forms the foundation of native low marsh structure in the San Francisco Estuary and provides critical habitat to the endangered California clapper rail (*Rallus longirostris obsoletus*). Pacific cordgrass was displaced by invasive hybrid *Spartina* (*S. alterniflora* x *S. foliosa*), and restoration efforts target areas in which invasive *Spartina* removal has been successful. Five large scale experiments conducted from 2010-2013 have tested how restoration site characteristics, plant caging, and parental source of *S. foliosa* transplants have effected establishment rates of native cordgrass. Throughout all experiments, outplanting location (including geographic location, substrate, and elevation) and caging were strong predictors of planting success. Establishment rate of native cordgrass was highest on uniform mudflats and wide channel banks (62%) with lower establishment rates occurring in 2nd order channels and bayfront habitat (15%). Cage effects varied by marsh, with the strongest cage effects occurring at sites with nesting Canada goose (7% survivorship in uncaged plots, 78% survivorship in caged plots). In a separate experiment, parental source was a strong predictor of planting establishment. Plants were collected from eight different marshes, grown in identical nursery conditions, and genetically tested using microsatellites. After 10 months, source populations differed in terms of culm height and density. Following nursery growth, 300 plants from each donor source were outplanted into two marshes and monitored quarterly. Sources varied significantly in terms of survivorship, flower production, and culm density. Field performance was not predictable from nursery bed performance. Successful restoration of native cordgrass requires understanding site specific conditions including marsh hydrology, elevation, substrate, and herbivore presence. Continuing research will determine long term effects of transplant source.

Keywords: *Spartina foliosa*, restoration, native, revegetation

A HIGHLY EFFICIENT PLANT REGENERATION SYSTEM THROUGH MULTIPLE SHOOT DIFFERENTIATION OF CORDGRASS (*SPARTINA PECTINATA*) USING BASAL MERISTEMS FROM GERMINATED MATURE SEEDS

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Abstract:

A successful *in vitro* regeneration system is presented for cordgrass based on basal meristems excised from *in vitro* germinated mature seeds. Germination was carried out in Murashige and Skoog (MS) medium or in the medium used for the development of meristematic clumps tissue (MCT) composed by MS-based medium containing 30 g/l maltose and a combination of 3 mg/l Thidiazuron (TDZ) and 2 mg/l Picloram. Explants were cultured in the dark for 2 weeks before exposure to light or directly in the light. Within 8–12 weeks, the proliferation into clumps with rapid and continuously forming adventitious buds containing clusters of meristemoids was obtained. The percentage of basal meristems that produced clumps of multiple buds was 0–72% depending on the light. The worst results were observed when basal meristems were cultured in dark. Lowering the levels of growth regulators, 1 mg/l TDZ and 0.1 mg/L Picloran, resulted in differentiation of multiple elongated shoots, which rooted and developed into morphologically normal plants, when they were cultured on hormone free MS medium. Subcultures every four weeks in the MS medium resulted in a continuous process of elongation and rooting of these sectors without any loss of morphogenic potential. The average number of shoots per responding basal meristem was 6–18. Normal and rooted explants were transferred to 250 ml pots containing a mixture of peat and perlite (2:1). Plants were acclimated in a growth chamber, under a 16/8 h light/dark photoperiod and 25° C of temperature, and 100% of plants survived to the acclimatization.

Keywords: Basal meristems, Cordgrass, Meristematic clumps tissue, Picloram, Shoot morphogenesis, Thidiazuron

Introduction

Prairie cordgrass (*Spartina pectinata* Link.) is a tall, rhizomatous, perennial, warm-season species found predominantly in marshes, wet meadows, potholes, and drainage ways throughout Canada to 60°N latitude and throughout the continental United States (Hitchcock, 1950; Mobberley, 1956; Stubbendieck et al., 1982). It is recognized for tolerance to salinity and valued for wetland revegetation, streambank stabilization, wildlife habitat, and forage. It is adapted to soils that are too wet and not sufficiently aerated for big bluestem (*Andropogon gerardii* Vitman) and switchgrass (*Panicum virgatum* L.), grows more rapidly than other tall grass prairie dominants, and is conspicuously taller than big bluestem and switchgrass where their distributions overlap (Weaver, 1954).

Evaluations of prairie cordgrass at 52°N latitude in Europe (Potter et al., 1995), in southwestern Quebec (Madakadze et al., 1998), and in eastern South Dakota (Boe & Lee, 2007) have indicated its high potential for biomass production, relative to switchgrass and other warm-season grasses, in short-season areas.

Increasing interest in the production of biofuels has warranted research in the production and genetic manipulation of high biomass crops such as switchgrass. With this increased interest, it is necessary to develop higher throughput transformation systems that are enabled by an efficient and reliable tissue culture system for target tissue production and plant regeneration.

In vitro plant regeneration of *Spartina* species from different explants has been reported. Li et al. (1995) analyzed the *in vitro* behavior of different explants of *S. patens* and regenerated plants from caryopses-derived

callus. Other authors reported the *in vitro* plant regeneration of *S. cynosuroides* and *S. alterniflora* from immature inflorescences (Li and Gallagher, 1996) and Wang et al. (2003) regenerated plants of *S. alterniflora* using mature inflorescences. Recently, Bueno et al. (2012) developed a simple and efficient protocol for *in vitro* plant regeneration of *S. argentinensis*. To our knowledge, plant regeneration of *S. pectinata* has not been achieved yet.

The aim of this work was to determine the *in vitro* regeneration requirements for prairie cordgrass through multiple shoot differentiation using basal meristems from germinated mature seeds.

Materials and Methods

Plant material and germination of seeds. Mature seeds of cordgrass were surface sterilized and germination was carried out on hormone-free MS basal medium (Murashige and Skoog, 1962) or in the medium used for the development of meristematic clumps tissue (MCT), composed by MS-based medium containing 30 g/l maltose and a combination of 3 mg/l Thidiazuron (TDZ) and 2 mg/l Picloram. Explants were cultured in the dark for 2 weeks before exposure to light, under a 16/8 h light/dark photoperiod, or directly in the light.

Differentiation of multiple bud clumps. For the isolation of meristems, roots and leaves were cut off from 2 to 4-week-old seedlings leaving 0.5–1 cm meristematic basal shoot portion. The remaining plant portion was cultured on fresh MCT-medium in the dark or under a 16/8 h light/dark photoperiod.

Development, elongation and rooting of shoots. Clumps, with multiple buds, were cultured in the medium for the development and elongation of shoots (SE), composed by MS-based medium, 30 g/l maltose and a combination of 1 mg/l TDZ and 0.1 mg/l Picloram. Elongated shoots were cultured on hormone free MS medium. Explants were cultured under a 16/8 h light/dark photoperiod.

Acclimatization. Normal and rooted explants were transferred to 250 ml pots containing a mixture of peat and perlite (2:1).

Results and discussion

Germination of seeds. Mature embryos started to germinate few days after culturing. Germination was higher in the light than in the dark. The germination rate of cordgrass mature seeds was of 23% in the dark compared to 33% when cultured under a 16/8 h photoperiod (Fig. 1A).

No differences were observed when seeds were germinated in MS or MCT, with a germination rate of 34% on both media (Fig. 1B).

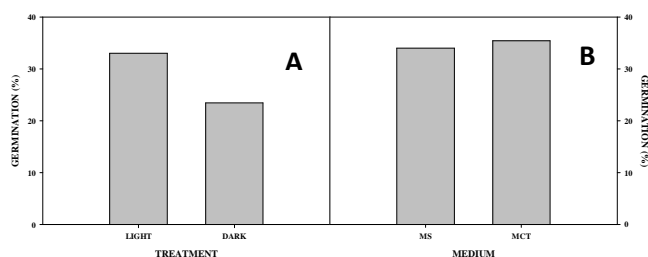


Figure 1. Effect of the presence or absence of light (A) or of different culture media (B) on the germination percentage of cordgrass mature seeds.

Differentiation of multiple bud clumps. After isolation and culture of mature embryos (Fig. 2A) the proliferation into clumps with rapid and continuously forming adventitious buds containing clusters of meristemoids, without callus, was obtained (Fig. 2B). The percentage of basal meristems that produced clumps of multiple buds was 0–60% depending on the presence of light. The smallest percentage was obtained when basal meristems were cultured in the dark (Fig. 3). While the survival of meristem cultured in the dark was 31%, almost 60% of meristem survived when they were cultured in the light. Sharma et al. (2007) observed that in few explants the internodal portion of a nodal shoot segment of wheat and barley did not respond and turned yellow or brown. In our study, the necrosis of cordgrass meristems cultured in dark was very high.

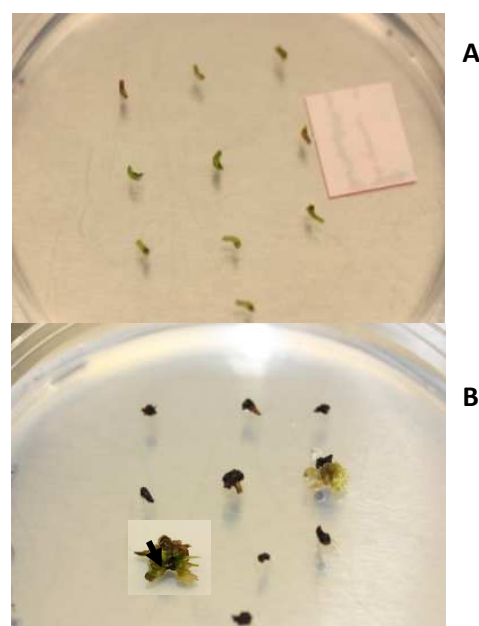


Figure 2. Isolation and culture of mature embryos (A). Necrosis of mature embryos (n) and differentiation of multiple bud clump (m) (B).

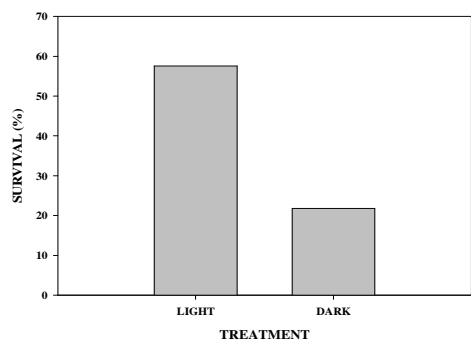


Figure 3. Effect of the presence or absence of light on the survival rate of cordgrass mature embryos.

Development, elongation and rooting shoots. The culture of clumps in SE medium, with lower levels of TDZ and picloram, resulted in differentiation of multiple elongated shoots (Fig. 4A), which rooted and developed into morphological normal plants, when cultured on hormone free MS medium (Fig. 4B). Subcultures every four weeks in the MS medium resulted in a continuous process of elongation and rooting of these sectors without any loss of morphogenic potential. The average number of shoots per responding basal meristem was 6–100, depending on the treatment. Currently, some clumps continue producing shoots in the hormone free MS medium. These results are in agreement with Sharma et al. (2007) in wheat and barley; these authors observed that within 8–10 weeks upon further subcultures, the proliferation into callus with rapid and continuously forming adventitious buds containing clusters of meristemoids was obtained and lowering the levels of growth regulators resulted in redifferentiation of shoots and developed into normal plants. Explants of wheat and barley were transferred to basal medium with indole-3-butyric acid for rooting (Sharma et al., 2007), however, in our study, auxins were not necessary and explants of cordgrass rooted in a hormone free MS medium.

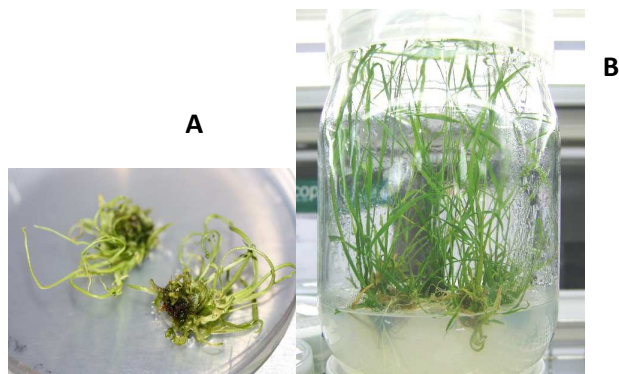


Figure 4. Development, elongation and rooting of shoots from multiple bud clumps in SE medium (A). Elongation and rooting of shoots in MS medium (B).

Acclimatization and growing in the greenhouse. Normal and rooted explants were transferred to pots containing a mixture of peat and perlite (2:1). Plants were acclimated in a growth chamber (Fig. 5A). All plants survived to the acclimatization and were growing in the greenhouse (Fig. 5B).

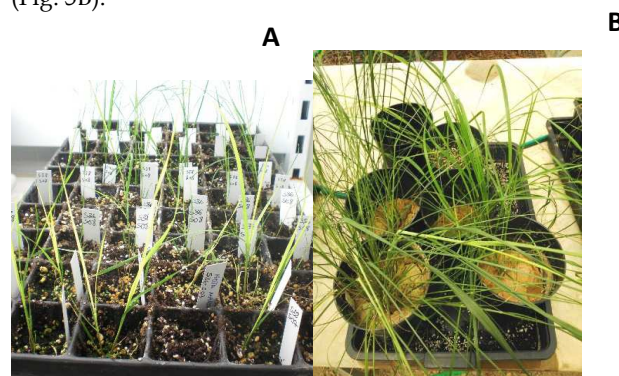


Figure 5. Acclimatization of normal plants of cordgrass on the growth chamber (A). Growing of cordgrass plants on the greenhouse (B).

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SAN FRANCISCO BAY *SPARTINA* CONTROL: SINGLE SPECIES VS. ECOSYSTEM MANAGEMENT IN A NOVEL ECOSYSTEM

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Abstract :

The San Francisco Bay Estuary in California, USA is a highly modified ecosystem. Centuries of human manipulation have fundamentally altered the physical and biological structure of this globally important hub of commerce and culture. Hydrologic diversions, shoreline development, species introductions, and tidal marsh reclamation for agriculture have changed the system beyond any historical baseline, creating a novel assemblage of species and interactions. In the late 1990's, introduced cordgrass (*Spartina alterniflora*), and hybridized forms with native *S. foliosa*, posed a significant biological threat to both historic and restored tidal marshes, becoming the subject of a regional eradication program. The eradication program is styled as an ecosystem-based management effort, given the ability of invasive *Spartina* to readily spread throughout the estuary, and act as an ecosystem engineer, transforming tidal marsh communities. An endangered native bird species, Ridgeway's Rail (*Rallus obsoletus*), preferentially inhabits stands of *S. alterniflora* hybrids. Ridgeway's Rail use invasive *Spartina* for nesting substrate, cover, and refuge during high tide and storm events in the absence of mature stands of native tidal marsh plant species. Under United States federal law, endangered species populations and habitats are subject to strict protections against disturbance. As a result, invasive species management and endangered species preservation have become unlikely adversaries within this novel ecosystem. Complete eradication of invasive *Spartina* now hinges on innovative habitat restoration strategies and renewed engagement from regional partners after almost a decade and a half of *Spartina* control work. The history and context of this management challenge are discussed along with prospects for resolution.

Introduction

Invasive *Spartina* (cordgrass) in the San Francisco Bay Estuary (SF Bay) of California in the United States has been the subject of over a decade of control work, and represents one of the largest invasive species control efforts currently underway in the world. The stated goal of the San Francisco Estuary Invasive *Spartina* Project (ISP) is to arrest the spread of and control non-native *Spartina* in SF Bay. To date, the ISP has been extremely successful in controlling the four species and two hybrids of non-native *Spartina* present (*S. alterniflora*, *S. densiflora*, *S. patens*, *S. anglica*, *S. alterniflora* x *foliosa*, *S. alterniflora* x *densiflora*), with some 95% of the historic maximum extent removed from SF Bay, at a cost approaching \$30M US (Rohmer et al., 2014). However, recent developments regarding the effect of control efforts on an endangered bird species, Ridgeway's rail (*Rallus obsoletus*, formerly the California clapper rail, *R. longirostris obsoletus*) have stalled control efforts. The US

Fish and Wildlife Service (USFWS) has precluded further invasive *Spartina* treatment on 11 marshes spread around SF Bay until such time as the current population of Ridgeway's rail has increased by an average of 80 individuals over 3 years. The initial impetus for the ISP was an ecosystem-based approach to the regional impacts associated with an invasive species (invasive *Spartina*); the current management strategy imposed on the program follows a single-species management paradigm under the auspices of the Endangered Species Act (ESA). The net effect of suspended comprehensive control efforts is the potential for re-invasion of previously controlled marshes, further development of cryptic hybrid *Spartina* morphs, development of resistance to herbicide treatments, partner fatigue and mission inertia. In a climate of funding uncertainty, the potential for reversion to a large-scale infestation of the SF Bay ecosystem is significant and imperils eradication efforts. This conflict in conservation goals - endangered species protection (single species management) vs.

invasive species eradication (ecosystem management) - has policy implications for ongoing restoration in SF Bay, as well as for *Spartina* control efforts, conservation programmes and restoration strategies globally.

The SF Bay watershed

The San Francisco Bay Estuary is the recipient of over 40% of the total river flow of the State of California, draining some 190,000 km² of the state. It is bordered to the east by the Sacramento-San Joaquin Delta, the only inland delta system in the world, and to the west by the Pacific Ocean through the narrow cleft in the Coast Range at the Golden Gate (Conomos et al., 1985). The entirety of the watershed that drains this portion of California from the Sierra, Cascade, Klamath and Coast Range mountains to the Pacific Ocean has been dramatically modified by human activity over the last ~150 years, affecting almost all aspects of the Estuary's ecology. For example, over 18,100 km² of the Great Central Valley alone has been converted to agricultural uses in what was historically a complex riparian network of permanent and seasonal channels, wetlands and other habitats. In the 1850's, the upper portions of the watershed were subjected to hydraulic mining operations which destroyed whole mountainsides in pursuit of gold. The sediment and pollution generated by this scouring was deposited along riparian corridors downstream all the way to SF Bay, causing severe disruption of channel hydraulics, marsh accretion rates and watershed habitat condition. Further, during this time settlers began importing invasive species, modifying channel hydrology directly with large and small damming projects, urban and industrial development, fill and reclamation of wetlands, and overharvesting of native commercial fish, shellfish and other species (Nichols et al., 1986).

As a result, only an estimated 8% of the historic extent of tidal marsh habitat remained by the late 20th century (Service, 2013). This limited habitat was often fragmented, bordered on the landward side by hardscaping in the form of dikes, levees, or development, and subject to pollution and general neglect. Further, SF Bay had an estimated 250 introduced species, predominantly in the infaunal and epifaunal soft-bottom benthos, fouling, brackish-water zooplankton and freshwater fish communities, but also in the vascular plant assemblages of tidal marsh communities (Cohen and Carlton, 1998). Many of these conditions continue to this day, though restoration activities in SF Bay have increased the current extent of tidal marshes from historic lows.

Climate change

The SF Bay ecosystem has been in a state of slow flux for at least the last 3000 years as it transitioned from a dry riparian valley to a flooded bay during the current

interglacial period. The slow march of sea level rise from fringing coastal areas upstream to fill SF Bay allowed plant and animal communities time to adapt to changing local environmental conditions. Current global climate models predict significant and accelerated sea level rise and temperature changes for this area over the next 50-100 years. Due to the anthropogenic modifications of the SF Bay shoreline over the last 150+ years, the predicted impacts of these changes threaten to submerge most of the remaining tidal wetland habitat currently present in San Francisco Bay (Veloz et al., 2011; Ackerly et al., 2013; Grewell et al., 2013).

SF Bay as a novel ecosystem

Designation of an ecosystem as 'novel' has been promulgated by Hobbs and others with the general definition involving anthropogenic modifications to biotic, abiotic and social components of a system that are impractical if not impossible to reverse, and tend to self-organize without further human intervention (Hobbs et al. 2013a). Using this construct, the San Francisco Bay easily qualifies as a novel ecosystem. Prescriptions for management, restoration or reconciliation of such a system must necessarily include novel approaches to enhance ecosystem functionality and services commensurate with regional management goals (Rosenzweig, 2003; Hobbs et al., 2006; Hobbs et al., 2013b).

To this end, land managers, researchers, regulators and other interested partners have worked in SF Bay to develop a cohesive vision for the entirety of the Bay ecosystem that generally accepts the constraints of the current novel system, while working where possible to restore or enhance historic habitat function and value. Efforts were aimed to maximize management value of individual actions and coordinate habitat enhancement and restoration efforts Bay-wide. In 1999 the Baylands Ecosystem Habitat Goals were published, which were "intended to be a guide for restoring and improving the baylands and adjacent habitat of the San Francisco Estuary" (Monroe et al., 1999). This effort has proven a nexus for restoration work for the past two decades in the Bay, and an update to this influential project is currently being finalized for publication (Grenier, 2014). Additional regional plans have subsequently addressed specific portions of the bay ecosystem not directly covered by the initial project goals including subtidal habitats (Conservancy, 2010). Other work by USFWS has resulted in the production of a Recovery Plan for Tidal Marsh Ecosystems of Northern and Central California (Service, 2013).

Ecosystem-based management

What all of these strategies have in common is a general recognition of the SF Bay ecosystem as a whole unit, requiring ecosystem-based management approaches to

provide effective, sustainable stewardship. As a result of this consensus, one of the largest wetland restoration efforts in North America is currently underway in SF Bay. Begun in 2000, the South Bay Salt Ponds Restoration Project (SBSRP) is in the process of restoring 6,500 ha of tidal marsh habitat (Conservancy, 2014). This regional effort involves re-engineering a portion of SF Bay that was diked for industrial salt production in the late 1800's and into the early 1900's and returning various levels of tidal inundation to support a wide variety of habitats including mudflats, seasonal ponds, vegetated marsh plain, upland transition zones and others.

The general approach to restoration in these areas has been an 'if you build it, they will come' attitude wherein much of the proposed revegetation would be accomplished via propagules suspended in the water of twice-daily tidal flux. A significant impediment to this approach was the expanding infestation of non-native *Spartina* that had established in SF Bay at this time. Introduced into SF Bay in the 1970's by the US Army Corps of Engineers to stabilize marshes and levees, East Coast or smooth cordgrass (*Spartina alterniflora*) had, by the early 2000's spread throughout the Bay to a population estimated at 250 net hectares (Faber, 2000; Ayres et al., 2004a; Grijalva and Kerr, 2005; Strong and Ayres, 2013). One of the original introduction sites was in Fremont, CA, on the southeastern shoreline of the Bay, and correspondingly, almost all of the tidal marshes on the southern bay edge were infested at the inception of the SBSRP in 2000.

In response, the California Coastal Conservancy (Conservancy) and the USFWS created the ISP to "arrest the spread of and eventually control" non-native *Spartina* in SF Bay (Conservancy, 2003). Without a coordinated control effort, almost all restoration activities would be threatened by rapid non-native *Spartina* colonization, jeopardizing the suite of habitat restoration goals predicated on a heterogeneous native tidal marsh plant community. Additionally, remnant tidal marshes in SF Bay were threatened by the expanding non-native *Spartina* population. This was occurring by two main mechanisms. The first was via simple propagule spread from the introduced population. *S. alterniflora* was able to exploit relatively calm, temperate conditions in SF Bay and outcompete the native *S. foliosa* (Pacific cordgrass) and other native tidal marsh plant species. Second, *S. alterniflora* hybridized with the native *S. foliosa* producing a hybrid swarm with a wide array of phenotypic expression. Hybrid plants were capable of growing both higher and lower in the tidal prism, form more propagules (seeds) than either parent, and spread hybrid pollen into otherwise native stands of *S. foliosa*.

S. alterniflora and *S. alterniflora* x *foliosa* hybrids posed a threat on many levels, including increased sedimentation which would alter channel elevation and morphology as well as increase mudflat elevations. A complex system of open channels winding through vegetated marsh plains is a core requirement of many tidal marsh species, affording essential forage and shelter. The open mudflats of SF Bay are an important annual stopover for shorebirds migrating along the Pacific Flyway providing a significant annual caloric subsidy in the form of epifaunal and infaunal invertebrates (Warnock et al. 2002). Tidal mudflats converted to *Spartina*-vegetated plains would significantly degrade this important habitat (Levin et al., 2006; Brusati and Grosholz, 2009; Grosholz et al., 2009). Additional concerns related to increased flooding from channels clogged with thick invasive *Spartina* stands and attendant maintenance costs, mosquito-abatement costs in ponded water behind stands of *Spartina*, and loss of genetically intact native *S. foliosa* through unabated hybridization (Conservancy, 2003; Ayres et al., 2004b).

Ridgway's rail

The relative vigor of the hybrid plant posed a special concern to several endangered species that rely on heterogeneous native plant assemblages within the marsh plain. Chief among these were Ridgway's rail and the salt marsh harvest mouse (*Reithrodontomys raviventris*). Both species are listed as federally endangered (35 FR 16047). *R. obsoletus* inhabits coastal and tidal marshes and is almost entirely restricted to SF Bay. It is a generally secretive, diurnally active marsh bird that breeds from February through July of each year. Importantly, it forages along the edges of the vegetated marsh in sloughs and at the edges of mudflats, typically where native *S. foliosa* grows. It uses *Spartina* as cover, nesting substrate and nesting material (McBroom, 2013; Overton et al., 2014). An intricate slough network affords the rail foraging space for invertebrates as well as escape routes from predators. The harvest mouse inhabits mature stands of pickleweed (*Salicornia* spp.) within the marsh plain (Shellhammer et al., 2010). Both habitats were directly threatened by invasion by hybrid *Spartina* through homogenization of the tidal marsh habitats where the species were found.

The sum of the previous considerations generated bay-wide support for the ISP's control program and from 2004 through 2010 the program developed a coordinated network of regional partnerships. The result was a reduction of the invasive *Spartina* infestation by approximately 93% by the beginning of the 2011 treatment season (June-November) (Grijalva et al., 2008; Rohmer et al., 2014). Concurrently however, both the ISP's Clapper Rail Monitoring Program and other regional organizations including Point Blue Conservation Science (formerly PRBO), US Geological

Survey and others noticed a decline in *R. obsoletus* populations throughout SF Bay (McBroom, 2012, 2013). Observations of *R. obsoletus* in marshes showed that the birds were preferentially using tall, dense hybrid *Spartina* stands for nesting and high tide refugia. In the absence of other tall tidal marsh vegetation and the destruction of upper marsh edge transition zones, tall hybrid *Spartina* represented the only habitat available in many marshes for the rail during seasonal king tide events or storms. *Spartina* control work was removing these habitats within the marsh, and rail were exposed to increased predation and diminished locations for nesting. This decline followed a significant increase in the rail population that coincided with the initial expansion of the hybrid *Spartina* footprint. Superficially, Ridgway rail population dynamics were closely tracking the changing dynamics of the invasive *Spartina* population (McBroom, 2012, 2013).

Single species paradigms

There is a large literature that seeks to find ways to assess the relative importance of a given species to an ecosystem (Simberloff, 1998). Single-species conservation strategies are born of social, political, cultural and ecological considerations among others. The confluence of the Ridgway's rail and invasive *Spartina* issues in SF Bay necessitates some clarification of where Ridgway's rail fits within some of the constructs of single species conservation.

The case can be made that the overall direct contributions of *R. obsoletus* to the ecology of SF Bay tidal marshes, at least in the current, hybrid condition of the Bay, is limited. Typical estimations of relative ecological value include valuations using terms like *keystone* (a species whose impact on the environment is out of proportion to its abundance), *foundation* (species impacts are proportional to abundance, generally applicable to larger populations), and *ecosystem engineers* (species that change resource availability necessary for ecosystem function). Under these constructs, Ridgway's rail is a lesser member of a larger tidal marsh community. However, Ridgway's rail, by virtue of its conservation status, provides other ecological benefits. Helpful single-species constructs more appropriate for the SF Bay situation include *umbrella* (conservation of an individual species translates into conservation of habitat, benefitting other organisms within the ecosystem), *flagship* (charismatic species that provide the opportunity to capture public interest in conservation) and *indicator* (those species that provide insight into overall ecosystem health by extrapolation from the health of the species' population). Both the concepts of umbrella and indicator species are only partially apt for SF Bay. Some species may indeed benefit from habitats appropriate for the health of the rail population, However, in the current hybrid ecosystem, the best

situation in the short term for Ridgway's rail (continued invasive *Spartina* presence and even renewed expansion) represents a significant threat to many other organisms and features of the ecosystem, and may even negatively impact the rail itself in the future. The rail can be said to have value as a flagship species, however, attracting birders and the public at large to restoration activities designed to benefit the rail, political support and a tangible subject for education and engagement.

Endangered Species Act and a change in perspective

The Endangered Species Act of 1973 (ESA) is a seminal document in the history of conservation in the United States (1973). Numerous species have survived inevitable extinction through the robust regulatory framework of the ESA (Neel et al., 2012). The ESA is designed to protect both the focal rare species and the habitat upon which that species depends. At its core, however, it is a single-species focused document, developed when understanding of ecosystem-wide management strategies were in their infancy. As ecological understanding has expanded in the ensuing four decades, regulatory agencies have sought to address larger ecosystem management questions and to expand the ESA's purview within the existing framework whenever possible (Bernazzani et al., 2012).

In the case of SF Bay, declining numbers of Ridgway's rail triggered a change in perspective from the USFWS regarding the primacy of invasive *Spartina* control in managing the SF Bay ecosystem. The USFWS is a co-sponsor of the ISP along with the Conservancy, and together they jointly produced an Environmental Impact Report and Environment Impact Statement (EIR/EIS) in 2003 addressing both Federal and state level environmental impacts associated with *Spartina* control in SF Bay (Conservancy, 2003). Using the EIR/EIS as a project description, the USFWS issued a programmatic Biological Opinion and a Record of Decision, a requirement under the ESA to assess the impact of proposed projects on federally-listed endangered species within the project area (Service, 2004b). At that time, the EIR/EIS was explicitly developed with an ecosystem-based approach, identifying the preferred alternative as offering the "greatest overall (net) environmental benefits in the long-term, despite greater short-term impacts" compared with the other alternatives. Successive Biological Opinions in 2004, 2005 and 2008 (Service, 2004a, 2005, 2008) relied on yearly rail population estimates, written site-specific ISP *Spartina* control plans, and invasive *Spartina* monitoring data provided by the ISP.

In 2011 this ecosystem-based approach changed. As described above, increased Ridgway's rail population estimates associated with an expanding hybrid *Spartina* population were reversed as the hybrid *Spartina* was

removed from infested marshes. This decrease triggered a curtailment of control work on the remaining hybrid *Spartina* in SF Bay, and has continued through the 2014 *Spartina* control season. The new directives require implementation of habitat enhancement work in a number of marshes around SF Bay and no treatment at 11 marshes located throughout SF Bay until the Ridgway's rail population has increased by 80 individuals (3-year average) from 2010 population numbers (Service, 2011, 2012).

The USFWS also determined that "although bay-wide invasion of exotic *Spartina alterniflora* and its hybrids with the native *S. foliosa* may threaten [Ridgway's] rails in future decades, hybrid *Spartina* currently provides habitat for the rail and eradication of exotic hybrid *Spartina* is a current threat." (Service, 2013).

Habitat enhancements

In early 2011 the Conservancy and the ISP began implementing habitat enhancement efforts to develop strategies and projects aimed directly at providing habitat for Ridgway's rail in as short a time as possible. The ISP developed a planting palette utilizing tidal marsh species like *Grindelia stricta* that exhibit a tall and vigorous growth habit, along with other species to provide heterogeneity in marshes that were treated for non-native *Spartina*. Additionally, the US Geological Survey working with researchers at UC Davis installed floating islands in select marshes to test whether rail would use artificial structures as refuge during high tide events or as nesting sites in the absence of (or preferential to) stands of adjacent non-native *Spartina*. The Conservancy and a consulting firm (HT Harvey and Associates) developed 'earthen islands' or 'high tide refuge islands' that were constructed in select marshes around the bay to provide elevated substrate for subsequent revegetation efforts and potential high-tide refugia for rail. Additionally, extensive plantings of native *S. foliosa* in select marshes continues to be the subject of restoration research. Each of these efforts seeks to enhance existing habitat and provide additional resources for rail while restoration work in the SBSP marshes continues to develop. All parties anticipate that once the large marshes of the SBSP effort are mature (5-20 years) they will provide significant additional habitat for Ridgway's rail (Olofson Environmental, 2013).

This abrupt shift in perspectives from eradication-focused to restoration-focused was jarring to the ISP, the Conservancy, and the bay-wide partner network. While habitat conservation and restoration were always integral tenets of the eradication effort, regional land managers became unsure as to the ability of the ISP to complete control and eventual eradication of the invasive *Spartina* if significant stands of the plant were left untreated and thereby sources of reinvasion for

previously treated marshlands. Further, funding for control activities and management was becoming increasingly scarce. The prospect of an indefinite time horizon for eradication significantly impacted morale throughout the ISP's network of partners.

Current and future directions

The ISP has seen significant success in the control of non-native *Spartina* in SF Bay, and is experiencing increasing success in innovative habitat enhancement techniques aimed at the Ridgway's rail. However, many outstanding questions yet loom over the longer-term success of the ISP. Changes to SF Bay tidal elevations associated with sea level rise threaten to inundate most of the remaining and restored tidal marshes currently present. This would be catastrophic for the entire SF Bay ecosystem, and would very likely result in the extinction of the Ridgway's rail as well as a number of other species endemic to SF Bay. It has been suggested that the advent of a vigorous hybrid invader exhibiting diverse and robust phenotypes capable of trapping sediments and building up marsh elevations is a windfall solution to many of the threats posed by sea level rise in SF Bay. This perspective argues for limited invasive *Spartina* control, perhaps relegated to marshes that maintain the maximal amount of historic native tidal marsh diversity, while suggesting that new restoration areas may benefit from the presence of the invader, especially in the long term. This perspective fully embraces the novel ecosystem idea, assuming the threat from sea level rise sufficient to subsume other considerations.

In terms of eventual eradication of hybrid *Spartina* within SF Bay, what does this mean in practice? Typically, an invasive species is considered 'eradicated' if it has not been detected on a previously infested site for three consecutive years. This is especially true in the case of invasive *Spartina* which produces seed that degrades within a year. But with individual clones within the hybrid *Spartina* population often exhibiting morphologies similar to native *S. foliosa* stands (termed 'cryptic' by the ISP), there is little agreement on what 'eradication' means on the ground. Does eradication mean elimination of morphologically distinct 'bad actors' whose phenotypes lead to invasiveness (high seed or pollen production, rapid rhizomatous clonal expansion, tall dense vegetation, or some combination of these)? Does eradication mean elimination of all non-native *Spartina* genes from SF Bay? The former represents an approachable goal, even with observed lag times following colonization of new sites. The latter represents a supreme technical challenge and will require long-term dedicated funding, further research, and development of a sustainable management structure capable of overseeing the effort.

The ISP has long since completed control of invasive *Spartina* in SF Bay. However, until and unless the Ridgway's rail population in SF Bay increases sufficient to satisfy the requirements of the USFWS and the ESA, invasive *Spartina* eradication in the SF Bay will be on hold. It remains to be seen whether institutional support for the ISP will continue into the future sufficient to maintain the current status of control while large marshes remain untreated.

Single-species vs. ecosystem or community-wide considerations color this complicated picture with regulatory, ecological and cultural valuations that are difficult to disentangle and reconcile. *Spartina* control itself can be viewed as a single-species management effort, and therefore the actual debate is between the relative value of two single-species approaches. However, given the potential for ecosystem-wide transformation of SF Bay tidal marsh community structure as a consequence of uncontrolled invasive *Spartina*, control of this invader should be more properly be viewed in the an ecosystem-based management context. The ISP, Conservancy and the USFWS have implemented innovative projects in an attempt to work within the many constraints of the current SF Bay ecosystem, regulatory framework and projected future change to the ecosystem. As predicted from the outset of the ISP, the paradigm has shifted from a *Spartina* control-dominated restoration effort to a habitat enhancement and maintenance effort. The longevity of such an effort remains to be seen. The future success of the ISP will hinge on renewed buy-in from existing regional partnerships, cultivating new relationships, developing new leadership, and finding some measure of flexible resolution to apparently intractable ecological and political challenges.

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REVEGETATION FOLLOWING REMOVAL OF INVASIVE *SPARTINA ALTERNIFLORA X FOLIOSA* IN SAN FRANCISCO BAY TO ENHANCE HABITAT FOR ENDANGERED CALIFORNIA RIDGEWAY'S RAIL

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Abstract:

The California Clapper Rail (*Rallus longirostris obsoletus*) is an endangered bird endemic to tidal marshes of San Francisco Bay and the nearby coast. In many San Francisco Bay tidal marshes, invasive hybrid *Spartina alterniflora x foliosa* provides habitat for the California Clapper Rail, but, in many sites, the rapid expansion of hybrid *S. alterniflora x foliosa* across a wide elevation gradient is inferred to have displaced or inhibited recruitment of two native plants which are critical components of California Clapper Rail habitat: Pacific cordgrass (*Spartina foliosa*) and marsh gumplant (*Grindelia stricta*). Following successful control of hybrid *S. alterniflora x foliosa*, there has been rapid recruitment of some native plant species, such as pickleweed (*Sarcocornia pacifica*), but, as a result of the local species displacement, there has been little recruitment of *S. foliosa* or *G. stricta* to some sites. Therefore, the California Coastal Conservancy and U.S. Fish and Wildlife Service San Francisco Estuary Invasive *Spartina* Project (ISP) has established a revegetation program to rapidly enhance California Clapper Rail habitat through planting *S. foliosa* and *G. stricta*. During the first three years of this program, the ISP has planted over 200,000 seedlings at 25 sites, most of which have been impacted by invasive *S. alterniflora x foliosa*. Sites either contain or are near existing California Clapper Rail populations and have extensive areas for potential *S. foliosa* or *G. stricta* habitat enhancement. Native *Spartina foliosa* has been planted on channel banks and in mudflats to provide foraging cover and *G. stricta* has been planted along channels to provide nesting substrate and on berms to provide high tide refuge. Additionally, at some of these sites, earthen islands planted with *G. stricta* and other native species have been constructed to function as high tide refuge. To meet the goal of mean 40% survivorship for all plantings, the ISP revegetation program follows an adaptive management strategy, using data from previous years' plantings and the advice of technical advisory committees to improve the success of subsequent plantings. For example, adaptive management for *G. stricta* plantings, including an examination of factors such as salt hardening, elevation, pot size, caging, and the function of existing plants as indicator species, has helped to increase *G. stricta* survivorship from 31.6% in the first year to 54.6% in the second year.

Keywords: hybrid *Spartina*, *Spartina foliosa*, *Grindelia*

The California Ridgeway's Rail (*Rallus obsoletus*) is a secretive marsh bird with a range restricted to San Francisco Bay and Tomales Bay. An obligate salt marsh species, the California Ridgeway's Rail suffered significant population declines during the early to middle twentieth century due to the loss of approximately 85% of San Francisco Bay's salt marshes (Harvey 1988). The California Ridgeway's Rail utilizes Pacific cordgrass (*Spartina foliosa*) for cover from predators while foraging and uses gumplant (*Grindelia stricta*) for cover from predators during extreme high tide events and for nesting

substrate. The ecological relationship between the California Ridgeway's Rail and *G. stricta* is strong enough that *G. stricta* has sometimes been referred to as 'rail-bush' (DeGroot 1927). The majority of mortality of California Ridgeway's Rail occurs as the result of predation by both avian and mammalian predators during extreme high tide events, when the majority of vegetation is submerged and cover is highly reduced (Albertson 1995). The physical structure of the marsh is also important for survival of the California Ridgeway's Rail; narrow deep channels are used for movement through the marsh and areas of

vegetated higher elevation ground within the marsh may be used as refuge during extreme high tide events.

Smooth cordgrass (*Spartina alterniflora*), a dominant salt marsh species on the east coast of North America, was introduced to San Francisco Bay in 1977 as part of a restoration project conducted by the US Army Corps of Engineers. The introduced *S. alterniflora* soon hybridized with the native *S. foliosa*, resulting in offspring which grow to a larger size than either parent and which flourish in a wider elevation range than the native *S. foliosa*. This invasive hybrid threatened to extirpate the native *S. foliosa* from San Francisco Bay and to engineer alterations to the San Francisco Bay salt marsh ecosystem. Hybrid *S. alterniflora* x *foliosa* threatened to reduce foraging habitat for migratory shorebirds by colonizing mudflats and to eliminate habitat for native salt marsh obligate species such as the endangered salt marsh harvest mouse (*Reithrodontomys raviventris*) by displacing native salt marsh vegetation. There are concerns that, allowed to spread unchecked, hybrid *S. alterniflora* x *foliosa* may negatively impact the population of the California Ridgeway's Rail by engineering changes to the physical structure of salt marshes including loss of small channels. In the short term, however, hybrid *S. alterniflora* x *foliosa* may provide cover from predators and nesting substrate for the California Ridgeway's Rail.

The Coastal Conservancy's San Francisco Estuary Invasive *Spartina* Project (ISP) was initiated with the goal of eradicating invasive *Spartina* from San Francisco Estuary. Control of hybrid *S. alterniflora* x *foliosa* began in 2005 and by 2012 control efforts had reduced the net area of the San Francisco Estuary which is covered by hybrid *S. alterniflora* x *foliosa* from a height of 805 acres to 49 acres, a 94% reduction (Roemer et al 2014). Because hybrid *S. alterniflora* x *foliosa* completely displaced native vegetation, including both *S. foliosa* and *G. stricta*, in many sites, there has been a decrease in vegetation structure for California Ridgeway's Rail at some sites following successful control of hybrid *S. alterniflora* x *foliosa*. At the same time the South Bay Salt Ponds Project is opening many former salt ponds to tidal flow. These two factors, displacement of native plants by hybrid *S. alterniflora* x *foliosa* and the opening of former salt ponds to tidal influence has resulted in many areas of salt marsh or potential salt marsh which lack many of the key plant species of San Francisco Estuary salt marshes. Some plant species, such as pickleweed (*Sarcocornia pacifica*), naturally recruit to these sites very quickly but other species, including *S. foliosa* and *G. stricta*, are often slower to recruit and establish populations, particularly in areas of the bay with limited local seed source.

In the winter of 2011-2012, the ISP began major revegetation installation with the goal of enhancing habitat for the California Ridgeway's Rail. The primary species which the ISP has planted are the aforementioned

key components of California Ridgeway's Rail habitat, *S. foliosa* and *G. stricta*. In addition, the ISP has planted saltgrass, *Distichlis spicata*, which is frequently used as nest-building material by the California Ridgeway's Rail, at sites where *D. spicata* is absent or in very limited supply. The ISP has also enhanced habitat for California Ridgeway's Rail by altering the physical structure of marshes through construction of earthen islands, elevated and vegetated mounds intended to provide refuge and cover from predators for California Ridgeway's Rail during extreme high tide events. From the beginning of plant installation in the winter of 2011-2012 through the most recent plant installations in the winter of 2013-14, the ISP installed greater than 200,000 plants in San Francisco Estuary and plans to have installed greater than 500,000 plants by 2017. Plants are typically grown in a nursery setting prior to installation in our salt marsh revegetation sites. The ISP's revegetation program has followed an adaptive management strategy, testing a variety of revegetation methods throughout the course of our revegetation program and altering our methods as we gain information based on the results of these different revegetation methods.

Outcomes of planting *S. foliosa* are detailed in another portion of these Proceedings. Therefore, we will only discuss results of monitoring *G. stricta* revegetation methods here. During 2012-13, *G. stricta* plantings were planted in plots of ten plants either on existing elevated mounds or berms or along channels to allow the vegetation to complement the existing physical structure in providing ideal California Ridgeway's Rail habitat. Two of the methods of improving survivorship which we have tested in the *G. stricta* plantings are salt hardening and caging. Salt hardening is a process of preparing plants which were grown in freshwater nursery conditions for the salinity of the salt marshes that they will be planted into. In this process, over a period of several weeks, plants are watered with a solution containing gradually increasing levels of salinity. Caging is a method of deterring herbivores by installing cylindrical plastic mesh cages around each *G. stricta* plant during installation. Cages are left on for a period of under one year, then removed during monitoring. Both salt hardening and caging increase the labor and expense required for each plant, therefore it is vital that we achieve an understanding of their effect on *G. stricta* survivorship to make decisions regarding how to utilize these strategies. To evaluate the effects of these two revegetation methods on *G. stricta* survivorship, we installed plantings which integrated these methods at several sites during the winter of 2012-2013. These plantings were then monitored for survivorship during the fall of 2013. The ten plant plot was used as the basic unit of comparison in analysis of survivorship data.

We tested the effect of salt hardening on *G. stricta* survivorship at four sites: Bunker Marsh, Citation Marsh,

Cogswell B, and Greco Island. Within a section of each of these sites, we planted paired plots of salt hardened and non-salt hardened pots of *G. stricta*. Pairs of plots were located in close geographic proximity, typically on either side of a channel. Number of plots varied by site: Bunker Marsh had 19 plots of each treatment, Citation Marsh had 20, Cogswell B had 32, and Greco had 39. Conditions including elevation, soils, and existing plant community varied enough between each plot within a pair that it was determined that a pairwise comparison would be inappropriate. Because distribution of survivorship was non-normal at all sites and the two treatments had unequal variances at Citation Marsh and at Greco Island, a nonparametric one-sided Kolmogorov-Smirnov test was used to compare survivorship of the two treatments within each site. Salt hardened plots were found to have significantly higher survivorship at Bunker Marsh ($p=0.0344$), Citation Marsh ($p=0.0002$), and Greco Island ($p=0.0318$). At Cogswell B, however, no significant difference in survivorship between the two treatments was observed ($p=0.2597$) (Figure 1). Speculatively, the lack of effect of salt hardening on *G. stricta* survivorship at Cogswell B could be due to the fact the Cogswell was planted much earlier in the winter and received early winter rains shortly after planting which reduced the shock of planting into saline soil.

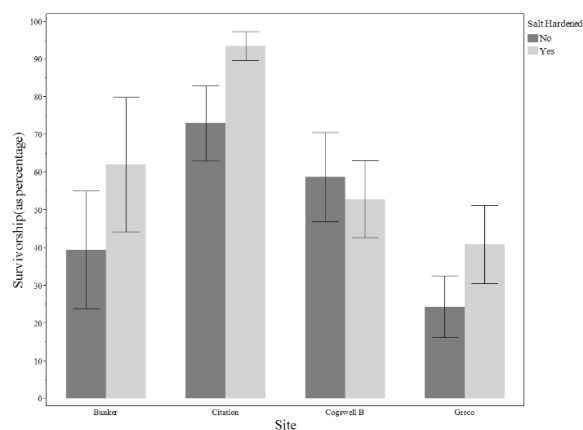


Figure 1: Effect of salt hardening on *G. stricta* survivorship by site

Caging was tested at four sites: Cogswell B, Mt Eden Creek, North Creek Marsh, and Oro Loma West. At these sites, some plots were caged and others were left without cages. At all sites, survivorship data was not normally distributed but Levene's tests showed no evidence of unequal variance between the caged and uncaged treatments, therefore a chi-square approximation Wilcoxon ranked sum test was used to examine differences between the two treatments within each site. At North Creek Marsh, caged plants had significantly higher survivorship than uncaged plants

($p<0.0001$). At Cogswell B, there was a slight trend toward greater survivorship in the caged plants, but this was not statistically significant ($p=0.0884$). However, at Mt. Eden Creek, there was a trend toward greater survivorship in the uncaged plants, though this also fell a little short of statistical significance ($p=0.0534$). At Oro Loma West, there was no difference in survivorship between caged plants and uncaged plants ($p=0.6700$) (Figure 2).

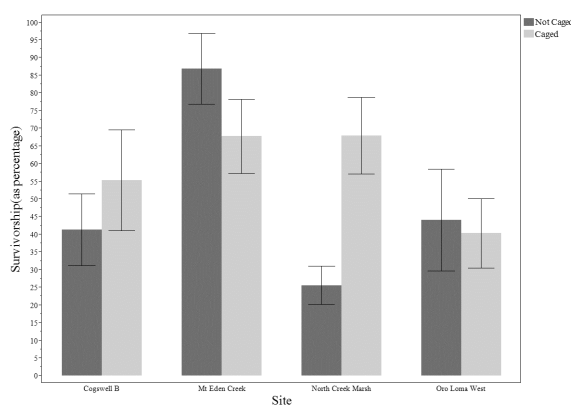


Figure 2: Effect of caging on *G. stricta* survivorship by site

Based on these results, salt hardening was found to be an effective means of increasing *G. stricta* survivorship at the majority of sites, but caging was found to have mixed results, sometimes increasing survivorship, sometimes decreasing survivorship, and often having no discernable effect. Because of what we have learned, salt hardening will continue to be used as a technique for increasing survivorship of *G. stricta* but caging will only be used in limited situations in which there is a particularly compelling reason to do so, such as extremely high herbivory pressure. The ISP's revegetation program will continue to pursue a program of adaptive management, refining our methods as we gather new information.

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POSTERS

HOMOEOLOG GENE DETECTION AND PHYLOGENETIC RECONSTRUCTION IN ALLOPOLYPOIDS USING NEXT-GENERATION SEQUENCING

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Abstract:

Next generation sequencing (NGS) technologies offer new opportunities to explore polyploid genomes and transcriptomes. However, transcriptome assembly and identification of duplicated (homoeologous) gene copies using different technologies (Roche-454 and Illumina) remain challenging, particularly when the actual (diploid) parental genomes are not available, and in the context of recurrent whole genome duplication events.

We developed strategies and bioinformatic tools to assemble several transcriptomes and to detect polymorphisms and haplotypes. This approach includes a de novo assembly process using long-reads and short-reads (Roche-454 and Illumina technology respectively), a hybrid assembly using custom script and a self-blast analysis. After transcriptome assembly, polymorphisms and haplotypes are detected using a homemade SNP detection tool (including removal of putative false-positive SNPs) and haplotype construction with appropriate parameters (number of SNPs to consider, SNP coverage and the read-depth). In order to detect the different copies within hybrid or allopolyploid species, the different consensus sequences and previously assembled haplotypes are aligned to identify homologous regions. From these alignments we detect the different SNPs and assign the parental origin of haplotypes within hybrid species using shared polymorphisms. The evolutionary origin of the detected haplotypes will be assessed using phylogenetic approaches.

We applied this pipeline on each transcriptome including Roche-454 and Illumina reads of 5 polyploid *Spartina* species (Poaceae, Chloridoideae) including the hexaploid parents *S. maritima* and *S. alterniflora*, their homoploid hybrids (*S. x townsendii* and *S. x neyrautii*) and the recently formed allododecaploid *S. anglica*. We were able to detect different SNPs and haplotypes within 286 937 contigs corresponding to 1,161,845 annotations obtained from the *Arabidopsis thaliana* and *Oryza sativa* annotated genomes. These results now allow us identifying duplicated copies at any different ploidy level in *Spartina* and to explore the fate of duplicate genes in these complex genomes.

Keywords: bioinformatics, duplication, homoeology, SNPs, sequence-assembly

A STUDY ON THE RANGE EXPANSION OF SALTMARSHES AND ITS UNDERLYING MECHANISM IN THE YANGTZE ESTUARY, CHINA

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Abstract:

Estuarine mouth bars and associated submerged delta features represent major locations of sedimentation resulting from the large amount of silt brought down the Yangtze River in eastern China. The range expansion of saltmarshes in the Yangtze Estuary depends on accretion of dynamic mudflats and influenced complex bio-physical interactions. Two typical patterns of saltmarsh advancing fronts at Chongming Dongtan wetlands are found: the *Spartina alterniflora*-mudflat (S-M front) and the *Spartina alterniflora* - *Scirpus mariqueter* - mudflat (S-S-M front). It is an ideal place to study the impact of bio-physical interactions on the range expansion of saltmarshes. In this study, both the range expansion in terms of seedling recruitment, establishment and formation of expansion pattern at these two advancing fronts and the accretion/erosion dynamics and hydrodynamic conditions used as a proxy for comparable hydrodynamic regimes were investigated during 2011 and 2012. The results showed that the hydrodynamic condition and sedimentation dynamics, especially during the critical stage of seedling establishment during April-June, were the most important factors responsible for the formation of these two typical expansion patterns. At the site with a regime of autumn/winter erosion and spring/summer accretion, the sediment accretion and seedling recruitment of *S. alterniflora* were higher, while the wave energy and current velocity were relatively gentle during the critical period of seedling establishment. At such site, the original pioneer species of *Scirpus mariqueter* was replaced by *Spartina alterniflora* and a S-M pattern of range expansion developed. In contrast, at the site with a relatively stable accretion regime and relatively strong wave energy and current velocity during the critical period of seedling establishment, the original pioneer species of *Scirpus mariqueter* remained within the advancing front and a S-S-M pattern of range expansion developed. The results from this study provide valuable insights into the bio-physical interactions on the formation of saltmarsh expansion patterns and the mechanisms underlying the range expansion of saltmarsh in the Yangtze Estuary, which could be important for the maintenance of coastal saltmarsh resource and biodiversity as well as the prediction of dynamics of coastal saltmarshes under the impact of climate change.

Keywords: Saltmarsh, *Spartina alterniflora*, Expansion pattern, Bio-physical interaction, Yangtze Estuary

BAC SEQUENCING ANALYSES IN THE HEXAPLOID *SPARTINA MARITIMA* (POACEAE): HOMOEOLOG DIVERGENCE AND MICROSYNTENY IN THE GRASS FAMILY

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Abstract:

Spartina maritima is a hexaploid species ($2n=6x=60$, $2C=3700$ Mb) native to the Atlantic European and African Atlantic coasts, and which hybridized with different *Spartina* species introduced from America, contributing to the formation of new hybrids and invasive allopolyploid species. We used a Bacterial Artificial Chromosome (BAC) library constructed in *S. maritima* in order to analyse homoeologous regions in this hexaploid genome and (2) to perform comparative analyses between *Spartina* (Chloridoideae) and representatives from other grass subfamilies.

Ten BAC clone sequences, including two target chromosomal regions were analysed. The first analysed region is homologous to that containing the Adh1 gene in *Sorghum* and *Saccharum*, and is known to be rearranged in some grass lineages. The second region includes a cinnamoyl alcohol dehydrogenase (CAD-6) gene, involved in the lignin biosynthetic pathway. For each of the analysed regions, we were able to distinguish two divergent homoeologous sequences within *S. maritima*, exhibiting differential retro-element insertions, and an average sequence identity of 96% in exons and 91% in introns. Perfect gene colinearity between homoeologues was observed for the Adh1 region whereas the CAD-6 region appeared more rearranged. These results shed new light into the origin of polyploidy in the hexaploid *Spartina* lineage. Local comparative analyses between *Spartina* and representatives of other grass subfamilies allowed examining synteny relationships and detecting structural rearrangements that occurred following divergence of the different grass lineages. We show that the translocation previously detected in the Panicoids (*Sorghum-Saccharum-Zea*) and containing the Adh1 gene is not shared with *Spartina*, suggesting that this event took place after divergence between Panicoideae and Chloridoideae sometimes 45-60 MYA.

Keywords: BAC sequencing, homoeolog divergence, microsynteny

BIOPHYSICAL AND BIOCHEMICAL MECHANISMS UNDERLYING THE DROUGHT-TOLERANT *SPARTINA VERSICOLOR* TO PEG MEDIATED WATER STRESS

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Abstract:

Along with the recent increasing temperature climate scenarios observed all around the globe, drought events are also becoming more frequent and severe. Although at first site, salt marshes are typically areas where drought would not be one of the most evident scenarios to consider, this is not true to all marshes. Some wetland areas and more precisely the upper marsh areas where the elevation is considerable, the tidal influence can become very reduced, being only felt during spring tides, occurring only sporadically during few days per year. *Spartina versicolor* typically inhabits the higher marshes although recently it has been spreading towards lower elevations. Its typical sediment habitat is mainly composed by sands, with reduced water retention potential. All the factors can impose to this species some periods of drought that will inevitably affect its physiological performance, shaping its biomass production and therefore its invasive potential. To evaluate this species performance in moderate and severe drought events, *S. versicolor* individuals were subjected to nutritive solutions containing 10% and 20% PEG respectively. Surprisingly, *S. versicolor* presents an elevated tolerance to moderate stress and even in severe drought conditions showed only minor disturbances on its biophysical energetic processes indicating low impacts on its biomass production mechanisms. This physiological fitness makes this species drought-tolerant whereas other neighbour species allowing to expand its range of influence under very adverse conditions.

Keywords: PAM, drought, OJIP, tolerance

A HIGHLY EFFICIENT PLANT REGENERATION SYSTEM THROUGH MULTIPLE SHOOT DIFFERENTIATION OF CORDGRASS (*SPARTINA PECTINATA*) USING BASAL MERISTEMS FROM GERMINATED MATURE SEEDS

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Abstract:

A successful in vitro regeneration system is presented for cordgrass based on basal meristems excised from in vitro germinated mature seeds. Germination was carried out in MS medium or in the medium used for the development of meristematic clumps tissue (MCT) composed by MS-based medium containing 30 g/l maltose and a combination of 3 mg/l Thidiazuron (TDZ) and 2 mg/l Picloram. Explants were cultured in the dark for 2 weeks before exposure to light or directly in the light. Within 8–12 weeks, the proliferation into clumps with rapid and continuously forming adventitious buds containing clusters of meristemoids was obtained. The percentage of basal meristems that produced clumps of multiple buds was 0–72% depending on the light. The worst results were observed when basal meristems were cultured in dark. Lowering the levels of growth regulators, 1 mg/l TDZ and 0.1 mg/L Picloran, resulted in differentiation of multiple elongated shoots, which rooted and developed into morphologically normal plants, when they were cultured on hormone free MS medium. Subcultures every four weeks in the MS medium resulted in a continuous process of elongation and rooting of these sectors without any loss of morphogenic potential. The average number of shoots per responding basal meristem was 6–18. Normal and rooted explants were transferred to 250 ml pots containing a mixture of peat and perlite (2:1). Plants were acclimated in a growth chamber and 100% of plants survived to the acclimatization.

Keywords: basal meristems, cordgrass, meristematic tissue, picloram, shoot morphogenesis

DEEP SEQUENCING OF PCR AMPLICONS REVEALS A DIFFERENTIAL MODE OF rDNA EVOLUTION IN *SPARTINA ALTERNIFLORA* AND *SPARTINA MARITIMA* POLYPLOIDS

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Abstract:

Introduction: Internal transcribed spacers (ITS) of ribosomal DNA (rDNA) units encoding the 18S-5.8S-26S genes are important genetic markers repeatedly used in many phylogenetic studies. Though hybrids and allopolyploids inherit alleles from their parents, in many cases, we observe altered gene ratios and even loss of rDNA homeologs due to poorly understood homogenization processes. The degree of homogenization is uneasy to tackle by classical cloning approaches due to high numbers of paralogs, often exceeding thousands of copies in plants. Here we explored the intragenomic rDNA variation in two hexaploid species, *S. alterniflora* and *S. maritima* that are believed to diverged ~3 myrs ago. Recent transcriptomic study revealed the presence of multiple protein coding homeologs in each genome likely to be inherited from the progenitors (de Carvalho *et al.*, Heredity 110, 181-193, 2013).

Methods: PCR products covering a part of the 18S gene and non-coding ITS1 sub-region have been amplified by emulsion PCR and sequenced on a 454 GS-FLX platform. We obtained >10000 reads representing about 4 x rDNA coverage. The reads were subjected to clustering, SNP analysis and quantitative analysis of variants.

Results: There were 32 (~12%) highly conserved polymorphic sites between consensus ITS1 sequences of *S. alterniflora* and *S. maritima* indicating rapid evolution. While *S. alterniflora* had almost homogeneous composition of both 18S and ITS1 sub-regions, two dominant ITS1 variants were found in *S. maritima* and significant polymorphisms were also discovered in the 18S coding region. Moreover, *S. alterniflora* major ITS1 ribotypes were found in *S. maritima* in a low copy (0.2%) while no *S. maritima* ribotypes were present in the *S. alterniflora* genome. Thus, the process of rDNA homogenization (and perhaps whole genome) is highly accelerated in *S. alterniflora* while it is relatively slow in *S. maritima*. Hypothesis on differential mode of rDNA evolution between the closely related species will be presented.

Keywords: NGS, rDNA, homogenization, phylogeny

ANALYSIS OF ACTIVE NUCLEOLUS ORGANIZING REGIONS IN POLYPLOID PRAIRIE CORDGRASS (*SPARTINA PECTINATA* LINK) BY SILVER STAINING

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Abstract:

Prairie cordgrass has recently gained attention as the future bioenergy feedstock on marginal lands which are not suitable for conventional food crops. This polyploid species is distributed broadly across the U.S. and Canada. More sufficient cytogenetic data are needed to investigate the cytogenetic evolution and phylogeny of prairie cordgrass polyploidy populations, as well as understand how a stable polyploidy species is formed. Silver staining is the cytogenetic method commonly used to study the number, size, and distribution of silver-nucleolar organizer regions (Ag-NORs) at metaphase and at interphase. Silver atoms deposit onto argyrophilic proteins associated with nucleolar organizer regions (NORs) which are involved in the transcriptional activity of ribosomal RNA genes and synthesis of ribosomes. The purpose of this study is to estimate the number of rDNA loci, their locations, and their activities in metaphase chromosomes and to determine the number, size, and distribution of nucleoli of interphase nuclei for different ploidy levels in prairie cordgrass populations. A variable number of AgNOR chromosomes were observed among six prairie cordgrass populations. The distribution of cells with different numbers of nucleoli per interphase, the percentage of interphase cells with heteromorphic nucleoli, total size of nucleoli per interphase, size of interphase nuclei, and proportion of nucleoli per nucleus are variable among the populations. These results can help to detect intraspecific NOR polymorphisms within polyploid populations and to understand the differences in growth and performance associated with the stabilization of polyploidy species.

Keywords: Ag-NORs, polyploidy, *pectinata*

***SPARTINA ALTERNIFLORA*: A SOURCE OF BIOACTIVE COMPOUNDS**

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Abstract:

Spartina alterniflora, an invasive halophyte growing in temperate salt marshes, develops effective adaptative responses, such as chemical adaptation, face to abiotic stresses, thus explaining in part its invasive nature. On another hand, biotechnological researches are currently focused on natural compounds showing promising bioactivities for specific application. In this context, within the framework of the MARMED project (European Union ERDF - Atlantic Area Program), our research activities are focused on the valorisation of *Spartina alterniflora* with a particular interest in polyphenols and their potential biological activities. Three different extracts were obtained by liquid/liquid extraction: i) a crude hydroalcoholic extract, and two semi-purified fractions ii) ethyl acetate fraction and iii) aqueous fraction with the aim to get rid of mainly lipids, proteins, sugars and to concentrate polyphenols. We assessed a variety of antioxidant activities by in vitro assays (DPPH, ORAC assays and reducing power), antibacterial and sunscreen activities ... for crude extract and fractions generated by our protocol. Moreover, chemical fingerprint of active fraction was determined by analytical tools (NMR and LC-MS analysis). The liquid-liquid process was effective increasing highly biological activities tested as antioxidant capacity for ethyl acetate fraction of *Spartina alterniflora*. The results of the biological activities together with the content of phenolic compounds were discussed in relation with chemical analysis.

This study highlights the high potential of an invasive species, *Spartina alterniflora*, which presents ecological concerns on salt marshes, as valuable source of natural compounds in therapeutic and cosmetic applications.

This work was co-financed with the support of the European Regional Development Fund (ERDF) - Atlantic Area Program, MARMED project nr. 2011-1/164 and with COMPETE project PEst-C/MAR/LA0015/2011.

Keywords: *Spartina alterniflora*, invasive, polyphenols, biological activities

ENDOGENOUS PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IMPROVE *SPARTINA DENSIFLORA* PHYTOREMEDIATION CAPACITY IN CONTAMINATED SALT MARSHES

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Abstract:

Spartina densiflora Brongn. is a cordgrass and a facultative halophyte that has demonstrated a high physiological and morphological versatility, which allows it to tolerate a wide range of salinity, tidal submergence and drainage. Furthermore, this species has shown to be useful for phytoremediation purposes, since it possesses a high capacity for accumulating heavy metals in its tissues, especially in its roots, as well as for phytostabilizing it in its rhizosphere. Recently, twenty-two different cultivable bacterial strains were isolated from the rhizosphere of *S. densiflora* grown in the Tinto river estuary (SW Spain). Seventy percent of the strains showed one or more plant growth-promoting (PGP) properties, including phosphate solubilisation and siderophores or indolacetic acid production, besides a high resistance towards copper. Despite strong evidence that PGPR influence overall plants performance, their detailed effects on growth, physiology and metal uptake of *S. densiflora* plants under metal stress have not yet been studied. Thus a glasshouse experiment was designed to investigate the effect of inoculation with a bacterial consortium integrated by *Pseudomonas composti* SDT3, *Aeromonas aquariorum* SDT13 and *Bacillus* sp. SDT14 on *S. densiflora* plants grown at two contrasting soils (polluted and non-polluted) for 50 days. Growth parameters together with total concentrations of arsenic, chromium, copper, iron, manganese, nickel, lead and zinc were determined in roots and leaves. Photosynthetic traits were followed by measurement of leaf gas exchange, efficiency of PSII biochemistry, total content of photosynthetic pigments and leaves water content. The results confirm that bacterial inoculation improved growth of *S. densiflora* at both soil conditions, and this growth enhanced was mainly reflected in greater length and diameter of roots. Improved growth was associated with the beneficial effect on *S. densiflora* photochemical apparatus, which seems to be related with a positive impact on chlorophyll concentration. Modification of the pigments concentrations was linked to an increment of leaves magnesium content. Moreover, this consortium was able to stimulate plants metal uptake in roots and leaves. On basis of these results, inoculation of *S. densiflora* with proposed bacterial consortium improves the potential of this species for metal phytoremediation purposes.

Keywords: *Spartina densiflora*, PGPR, metals, phytoremediation, estuary

SCOUTING CONTAMINATED ESTUARIES: HEAVY METAL RESISTANT AND PLANT GROWTH PROMOTING RHIZOBACTERIA TO ENHANCE METAL BIOACCUMULATION BY *SPARTINA MARITIMA*

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Abstract:

Spartina maritima is a C4 halophyte that is an important pioneer and ecosystem engineer in salt marshes of the Atlantic coast of southern Europe. In salt marshes of the joint estuary of the Tinto and Odiel Rivers (SW Spain), one of the most polluted areas by heavy metals in the world, this species grows in sediments with metal concentrations greater than toxicity thresholds for plants. On the other hand, plant growth-promoting rhizobacteria (PGPR) live in intimate association with plant roots, contributing to plant growth and physiology and playing a crucial beneficial role in plant responses to heavy metal stress, often facilitating metal phytoremediation. This contribution of the rhizomicrobial population to phytoremediation is usually referred to as rhizoremediation. Hence, phytostabilization of estuarine metal-polluted soils could be positively affected by the inoculation with indigenous PGPR. In this work, rhizospheric bacteria were isolated from *Spartina maritima* plants collected in Tinto salt marshes. The best-performing strains (*Bacillus methylotrophicus* SMT38, *Bacillus aryabhatai* SMT48, *Bacillus aryabhatai* SMT50 and *Bacillus licheniformis* SMT51) tolerated many heavy metals (Cu, Zn, Cd, As, Ni, Co, Hg and Pb) and also exhibited multiple PGP properties (nitrogen fixation, production of indole-3-acetic acid, siderophores and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, capacity to solubilize phosphates and biofilm formation). A preliminary experiment in gnotobiotic conditions was performed with *Medicago sativa* seedlings, which showed a higher main root elongation as well as an improved secondary root and root hair formation when they were inoculated with the selected bacteria. Actually, a glasshouse experiment is being developed to test the utility of this consortium to increase the growth and the phytostabilization capability of *S. maritima*. What is more, bacterial inoculation interaction with *S. maritima* metal tolerance will be analyzed through the measurements of leaf gas exchange, efficiency of PSII biochemistry and total content of photosynthetic pigments. This information will provide new insights into metals tolerance and biotechnological application of this important ecosystem engineer plant.

Keywords: *Spartina maritima*, estuary, heavy metal, phytoremediation, PGPR

VARIATION OF GROWTH AND PRIMARY PRODUCTION OF THE INVADING *SPARTINA ALTERNIFLORA* AND EFFECT ON THE MACROFAUNA IN TIDAL MARSHES IN AN EUROPEAN SITE: BREST, FRANCE

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Abstract:

The American *Spartina alterniflora* has largely colonized the tidal marshes of the Bay of Brest (France). In its new habitat, cordgrasses can be found from freshwater tidal marshes to salt marshes and from low to high marsh. In absence of competitors, the effects of wave action, salinity and immersion duration were studied on the density, growth and primary production of the population of *Spartina alterniflora*. We also studied the effect of the presence or the absence of the alien *Spartina* on the macrofauna.

In our study, *Spartina* aerial production is nearly four times higher in the freshwater tidal marsh compared to salt marshes. Along the tidal elevation gradient, the highest primary productions are found in the middle of the marsh. The primary production of *Spartina* exposed to wave activity are twice lower than in protected marshes but their densities are higher. The primary production in the Bay of Brest (1034 g DW m⁻² y⁻¹) is in the same range of plants growing in Virginia (US). We also show that the invasive *Spartina* induce three changes on the macrofauna. First, it increases the abundance of the epibenthic grazers represented by *Hyrobia ulvae*. Secondly, the endofauna's abundance, size and diversity, are reduced. And lastly, the trophic groups varied with the presence of the invasive plant.

These results permit to better understand how *S. alterniflora* can display such a large distribution outside its natural habitat along different salt marsh gradients and the impact of the plant on the salt marshes macrofauna in an European site.

Keywords: Salt marshes, invasive species, primary production, abiotic stress, macrofauna

THE COMPLETE CHLOROPLAST GENOME OF *SPARTINA MARITIMA*: INSIGHTS FROM COMPARATIVE ANALYSES IN THE *POACEAE* FAMILY AND MOLECULAR DATING BETWEEN POLYPLOID LINEAGES

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Abstract:

The polyploid *Spartina* genus (Poaceae, Chloridoideae) represents an excellent system for analyzing reticulate evolution and recurrent polyploid speciation. In an effort to better understand the evolutionary history of this genus, we reconstructed the complete chloroplast genome of the Euro-African hexaploid *S. maritima* (135,592 bp) using Roche-454 GS FLX pyrosequencing data. Due to its haploid nature and maternal inheritance, this genome is particularly useful for phylogenetic, phylogeographic and molecular dating studies in the contexts of hybridization and polyploidy that are particularly recurrent in genus *Spartina* (from tetraploid: $2n=4x=40$ to dodecaploid: $2n=12x=120-124$ in the currently living species). Moreover, this genome is the first representative of the Chloridoideae subfamily, a particularly poorly investigated lineage in grasses.

Comparative analyses of the *S. maritima* plastome with other *Poaceae* confirm the conserved structure and gene content of grass plastid genomes previously reported in other subfamilies. The multiple alignments of various *Poaceae* plastomes allowed the detection of 12 large indels (55 to 548 bp) most often flanked by direct repeats and that occurred in the Chloridoideae lineage since its divergence with other *Poaceae* subfamilies (≈ 50 million years ago). The *S. maritima* plastome appears poor in repetitive sequences and more than one third of these repeats is conserved among several *Poaceae* species, again highlighting the low plastome evolution in this family. Investigations on the evolutionary rate of all coding and non-coding sequences among the *Poaceae* plastomes allowed the identification of some fast-evolving regions that will be extremely useful for phylogenetic reconstruction at various taxonomic levels. Finally, phylogenetics and molecular dating based on 4 non-coding and 3 protein-coding plastid regions allowed to estimate the divergence time between different *Spartina* species of various ploidy levels and between the different *Poaceae* clades. Issues related to molecular dating using chloroplast sequences in polyploid species are discussed.

Keywords: *Spartina maritima*, chloroplast-genome, sequence-divergence, molecular-dating, *Poaceae*

EARLY DETECTION, RAPID RESPONSE AND SUSTAINED MONITORING OF *SPARTINA* IN THE STATE OF OREGON, U.S.A.

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Abstract:

Four non-native species and one hybrid of *Spartina* are known along the North America's West Coast. The alarming spread of these plants within estuaries of neighboring states during the 1980's and 1990's posed a persistent threat to Oregon's mudflats and saltmarshes, spurring the development of the Oregon *Spartina* Response Plan (ORSP). First written in 2003 and revised in 2007, the ORSP stresses early detection of new infestations, rapid response to any findings of *Spartina* and education to increase public awareness. Extensive surveys of all susceptible estuaries in the state are conducted on an ongoing basis with surveys conducted by ground, boat, and air at a targeted detection limit estimated between 0.2 to 0.4 ha. Small infestations of *S. alterniflora*, *S. patens*, and *S. densiflora* (all < 80 m²) have been found and treated in Coos Bay, the Siuslaw River, and/or the Columbia River. To better understand the threat from infestations in neighboring states, we conducted a year-long drift card study to examine potential ocean dispersal of *Spartina* in the nearshore coastal environment and to increase awareness at a regional scale. Implementing the ORSP over the last 10 years has reinforced the essential need for a responsible management agency; the importance of prolonged monitoring of all treated infestations; continued vigilance for all *Spartina* spp.; use of flexible survey methods; and the necessity for collaboration at a regional scale.

Keywords: invasive species, survey, estuary, management

USING GENETICS TO IDENTIFY NATIVE AND INVASIVE CORDGRASS RECRUIT COMPOSITION

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Abstract:

Establishing Pacific cordgrass, *Spartina foliosa*, is vital to the success of ongoing large scale tidal marsh restoration in the San Francisco Bay. However, since the 1970s, introduced *Spartina alterniflora* and its highly invasive hybrids with *S. foliosa* have established in many saltmarshes throughout the estuary. Established hybrid clones displace native vegetation, alter marsh hydrology, and change benthic food webs. Marshes newly restored to tidal action have been particularly susceptible to hybrid invasion due to lack of established vegetation. The Invasive *Spartina* Project was created in 2000 by the California State Coastal Conservancy to coordinate regional control and monitoring of invasive cordgrass. Prior to eradication efforts, restored marshes located near the introduction site in the central San Francisco Bay were typified by a dense monoculture of non-native *Spartina*. As distance from the invasion epicenter increased, higher rates of native recruitment were observed, especially in restoration marshes near established native clones. Our current project seeks to genotype *Spartina* seedlings in newly tidal areas in order to determine the influence of local populations of established *Spartina* clones on the genotypic identity of recruiting seedlings. In this ongoing study, we are sampling seedlings at four classes of sites: (i) untreated areas dominated by hybrids, (ii) previously treated areas that have been planted with nursery-grown *S. foliosa*, (iii) naturally occurring mixed stands of native and hybrid plants, and (iv) stands of naturally occurring *S. foliosa*. We sample populations in 12 distinct geographic locations in the central and southern portion of the San Francisco Bay using a suite of 16 diagnostic microsatellite markers to identify young clones and nearby established clones as hybrid or native. We test for similarity of genetic composition using a Bayesian approach in Structure, and with summary statistics in AMOVA. The results demonstrate the potential for active management of nearby *Spartina* hybrids to positively affect the long-term recruitment trajectory of invasive plants in San Francisco Bay wetlands.

Keywords: *Spartina*, seedlings, recruitment, restoration, genetics

CHLOROPLAST HAPLOTYPES IN PRAIRIE CORDGRASS (*SPARTINA PECTINATA* LINK)

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Abstract:

Prairie cordgrass is a C₄ perennial rhizomatous grass that is highly tolerant to abiotic stress such as drought, oversaturation, salinity, and extreme temperature. For these reasons, this species has been recommended as a future source of biomass used for bioenergy production on marginal lands. Prairie cordgrass is well known as a polyploid species consisting of tetra-, hexa-, and octoploidy. In the previous study, we have defined geographic distribution of cytotypes throughout the different regions of the U.S. and reported three distinct chloroplast DNA (cpDNA) haplotypes, which strongly associated with geographic distribution. An intraspecific chloroplast sequence variation within prairie cordgrass populations has been recently analyzed to assess the intrapopulation haplotype variation of prairie cordgrass over a natural distribution. The intraspecific cpDNA sequence variations were found within populations, which may not be associated with cytotype variations due to the lack of haplotype variations found in mixed cytotype populations. This result provides useful insights into population-level evolutionary processes and will aid in identifying useful indels or SNP for population identification.

Keywords: chloroplast, *pectinata*, diversity

HIGH SOIL SULPHUR PROMOTES INVASION OF EXOTIC *SPARTINA ALTERNIFLORA* INTO NATIVE *PHRAGMITES AUSTRALIS* MARSH

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Abstract:

Sulfur plays an important role in plant physiological process functioning as one of the macro-elements, but, high soil sulfur is toxic to most of the plants. Exotic *Spartina alterniflora*, a blacklist invasive species in China, actively accumulated and stored sulfur in their tissues and led to a high sulfide concentration in the invaded environment. *S. alterniflora* may be using high sulfur as a chemical tool in successful competition with the indigenous species, *Phragmites australis*, and lead to the sulfur-liked exotic species successfully invaded the indigenous habitats and replace the native species through increasing its competitive ability over the natives. As the supply of sulfur increases due to acid rain and rising sea levels, plant invasions will likely become more problematic around the world.

Keywords: biological invasion, chemical weapon

GENETIC DIVERSITY, ECOTYPE HYBRID AND MIXTURE OF INVASIVE *SPARTINA ALTERNIFLORA* LOISEL IN COASTAL CHINA

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Abstract:

Spartina alterniflora, a species native to the east coast of North America, is currently the focus of increasing management concern due to its rapid expansion in coastal China. To better understand the plant traits associated with the success of invasion, we examined the genetic variation and the possible existence and distribution of ecotype hybrids and ecotype mixtures of the species in China. We collected and analyzed 144 samples from seven populations throughout coastal China (21.6°-38.6°N; 109.7°-121.8°E) using amplified fragment length polymorphisms (AFLP) markers. The results indicated that the genetic diversity of *S. alterniflora* in China was low, with PPB = 23.24%, HE = 0.2979 and Hpop = 0.4427 at the population level, as well as PPB = 28.07%, HE = 0.3743 and Hsp = 0.5520 at the species level. The majority of the variation was found within rather than among populations, as estimated by Nei's genetic diversity (20.39%), Shannon's Index (19.80%) and AMOVA (23.56%). Results of assignment show that both ecotype hybrids and ecotype mixtures exist in coastal China, especially in southern populations. Therefore, the species' success in coastal China may be attributable largely to the coexistence of various ecotype hybrids and ecotype mixtures resulting from three independent introductions of the species' three ecotypes from their native ranges coupled with increasing human disturbances in the region.

Keywords: genetic diversity

LABILE AND RECALCITRANT SOIL CARBON AND NITROGEN POOLS IN TIDAL SALT MARSHES OF EASTERN CHINESE COAST ARE AFFECTED BY SHORT-TERM C4 PLANT *SPARTINA ALTERNIFLORA* INVASIONS

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Abstract:

In recent years, the effects of plant invasions on ecosystem processes and function particularly on soil carbon (C) and nitrogen (N) cycling have been increasingly recognized. However, terrestrial ecosystems carbon (C) and nitrogen (N) cycling as affected by plant invasions are not fully understood. In this study, we examined the impacts of 10 years *Spartina alterniflora* invasions on soil organic matter (SOM) pool, labile and recalcitrant pools by comparing with bare flat and native *Suaeda salsa* and *Phragmites australis* communities in tidal salt marshes of Eastern Chinese coast. Short-term *S. alterniflora* invasions significantly raised C and N concentrations in SOM pool, labile and recalcitrant pools compared to bare flat, *S. salsa* and *P. australis* soils. C stock in recalcitrant pool of *S. alterniflora* soil was significantly higher than that in labile pool. Meanwhile, *S. alterniflora* soil had higher recalcitrant index for C (RIC) relative to bare flat. The $\delta^{13}\text{C}$ abundance of *S. alterniflora* soil indicated that the proportion of *S. alterniflora*-derived C and decomposition rate of old C in labile pool were significantly higher than those in recalcitrant pool, but *S. alterniflora*-derived C stock of recalcitrant pool was much greater than that of labile pool. We also found that SOM pool and recalcitrant pool in *S. alterniflora* soil had significantly higher $\delta^{15}\text{N}$ levels compared with bare flat, indicating an increased N loss following *S. alterniflora* invasions. Our results suggested that short-term *S. alterniflora* invasions significantly enlarged soil organic C and N pools via altering SOM input and decomposition.

Keywords: plant invasions, soil fractionation

A STUDY ON THE SOURCES OF SULFUR TO *SPARTINA ALTERNIFLORA* IN COASTAL WETLANDS OF CHINA

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Abstract:

Recently, researchers has found the growth of *Spartina alterniflora* has a special demand on the content of sulfate. It is one of the rare terrestrial species which can release sulfur-contained greenhouse gas such as DMS. It is helpful and significant to discover the mechanisms and impacts of *S. alterniflora* invasion by studying on sulfur. Based on the fact that sulfur is quite important to the growth of *S. alterniflora*, we has finished a series of yield investigation and sampling at Jiangsu Yancheng Wetland National Nature Reserve in China, of course with laboratory experiment and stable isotopic technique test. Main results showed that, in the *S. alterniflora* marsh (SPM), the contribution of sediments to *S. alterniflora* is 69.59% while that of seawater is only 30.41%, which is significant less than that of sediment ($p < 0.05$). By contrast, the contribution of sediments to *S. alterniflora* is 94.79%, which is too much significant greater than that of the seawater (5.21%) in the *S. alterniflora* near the tidal ditch (SPM-TD). These results indicated that the sediment is the steady and reliable source of sulfur to *S. alterniflora* rather than the seawater in the coastal salt marsh during the growing season. Seawater is just a supplemental source, even though seawater contains unlimited sulfate ion.

Keywords: sulfur, *Spartina*, source, isotopic

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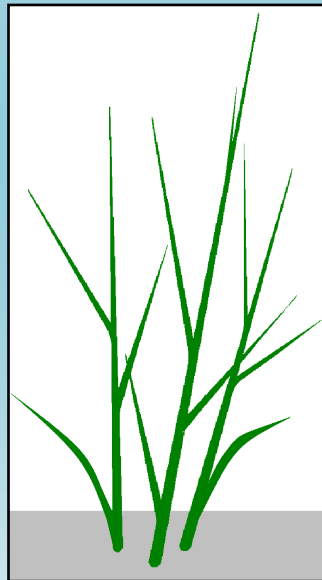
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