

Hydromulching in Tidally Influenced Wetlands:

Testing methods to alleviate seed wash-away and revegetate native plant communities

by

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ABSTRACT

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Estuaries are one of the most productive, and degraded, ecosystems on earth. Functioning estuaries provide habitat for 75% of the U.S. commercial fish catch, yet large-scale conversion of these wetlands to agricultural uses has resulted in estuarine habitat loss of up to 60% in some areas. With the advent of the National Estuary Program in 1987, local programs were developed to restore lost habitat functionality. Restored or created tidal wetland projects often include a revegetation facet to kick-start productivity and habitat development. Direct-seeding methods of revegetation have been the most cost-efficient, however seed wash-away has been a problem in establishing planned native plant communities in tidally influenced wetlands. This thesis tests a direct-seeding method augmented by the addition of a layer of hydromulch (a water and wood mulch slurry) in a set of recently created tidal channels on the Bayshore Preserve, in Shelton, Washington, U.S.A. We compared first season recruitment densities of one native salt-tolerant forb and four graminoid species under five different treatment conditions—broadcast seeded, two seeded treatments augmented by burlap or hydromulch, and two controls. The forb species, *A. patula*, was found to have statistically significant ($p < .0007$) higher stem densities in treatments which implemented a burlap fabric and hydromulch layer over the broadcast seed. The four graminoid species (*C. lyngbyei*, *C. obnupta*, *E. palustris*, & *S. americanus*) did not germinate in this experiment. While seeding *A. patula* into created tidal channels using this method shows promise, further research is needed to determine if it is feasible for other species and whether survivability in subsequent seasons compares with other wetland revegetation methods.

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Introduction

Estuaries—where the river meets the sea—have historically been viewed as wastelands, fertile ground for agricultural activities, or convenient locations for the logging industry to store and barge logs (Sedell, Leone, & Duval, 1991). They were essentially ignored as important habitats until the 1980s, when public awareness in the U.S. rose and the National Estuary Program was created by Congress in 1987.

Representing the lowest altitudinal point of a watershed, estuaries provide highly productive habitat for anadromous fish, shellfish, seabirds, and more. These ecosystems improve water quality by storing nutrients and pollutants that would otherwise immediately enter surface or groundwater (Wetzel, 1993). Finally, estuaries provide ecosystem services that benefit society—fisheries maintenance, coastal protection, erosion control, and water filtration (Barbier, et al., 2011). Estuary restoration aims to bring functionality back to these dynamic and important ecosystems.

The Bayshore Preserve, on Oakland Bay in Shelton, Washington, is an example of an ambitious restoration project in the midst of a region where much of the Puget Sound shoreline is industrially and privately developed, and therefore degraded. Bayshore, a golf course from 1930 to 2013, was purchased with grant money by Capitol Land Trust (CLT) in 2014. CLT recognized this property’s potential for ecological restoration, removed a dike that was installed in 1947, and restored tidal influence to a portion of the property that hadn’t been touched by saltwater for over 60 years.

The tidal flats—considered high quality habitat—were once prime shellfishing beds for the Squaxin Island Tribe, and are still used by people today. The shellfishing industry produces 40% of the nation’s Manila clams in Oakland Bay (Mason

Conservation District, 2004). Johns Creek, which runs through the property, hosts one of the largest summer chum salmon runs in Washington State (WDFW, 2017) and provides habitat for chinook, coho, bull trout, steelhead, and cutthroat salmon (Mason Conservation District, 2004). Even as a golf course, Bayshore provided excellent habitat for salmon, oysters, and clams. CLT is leading restoration efforts to further increase habitat quality and create a publicly open space for learning—promoting cultivation of sense of place—and provide access to nature near the city.

Estuarine habitat restoration is commonly approached from an experimental perspective, with many approaches being tested for viability in many types of estuarine environments (Zedler, 2001). When it comes to habitat creation, restoration sites are commonly evaluated on performance standards such as vegetation development and plant community make-up. Revegetation, when performed, has been applied using methods such as planting seedlings, cuttings, and sod plugs with high survivorship in the first growing season (Gilbert & Anderson, 1998; Sullivan, 2001). While planting propagules tends to result in higher initial survivorship overall (Keammerer, 2011; Mazer, Booth, & Ewing, 2001; Sullivan, 2001; Tiner, 2013), direct seeding is the simplest and least expensive method available to revegetate tidal marshes and wetlands (Hanslin & Eggin, 2005; Wright, 1992; Zedler, 2001).

Although direct seeding into tidal marshes is simple and inexpensive, it tends to result in low germination and plant establishment. Developing a method that keeps direct-sowing in tidal wetlands cost-effective and results in high rates of plant establishment would be beneficial for organizations, maximizing project budgets and restoration impacts.

While limited success has been observed from surface sowing methods (Broome, Seneca, & Woodhouse Jr., 1988; Sullivan, 2001), significantly higher germination of annual plants has been achieved when seeds were mixed into a mud/organic matter slurry and applied to the marsh surface (Sullivan, 2001). While this method seems promising, some seed is still washed away and, therefore, wasted. We think that a method of broadcasting dry seed onto the marsh substrate, then covering it with a “cap” of hydromulch will result in higher seed germination and plant establishment over the first growing season by providing a stabilizing effect against seed migration caused by tidal flow.

This thesis is focused on testing this novel method of revegetation in the created tidal channels. Hydroseeding, or hydraulic mulch seeding, is a planting method that uses a slurry of paper or wood mulch and seed. In this case, hydromulch will be used to augment prior broadcast seeded native salt-tolerant forb and graminoid establishment on the channel surfaces. Seeds will be sown onto the substrate, and a layer of hydromulch applied onto the pre-seeded soil. Influx and outflow of tides in the created channels at Bayshore Preserve is generally gentle and slow-moving, which should provide ample time for the mulch mixture to set before the first tidal inundation. As this method is monitored throughout the season for planted species’ germination and establishment success, conclusions can be drawn about whether larger-scale applications of the method are ecologically and financially feasible for wetland restoration projects.

The following chapters provide background on the Bayshore Preserve, the current science and trends in estuarine restoration, and the variables present in this experiment. In subsequent chapters, methods and materials for the experiment are outlined, results are

summarized and potential causal factors discussed, and conclusions and recommendations are shared.

Chapter 1: Bayshore Preserve Site History & Restoration Significance

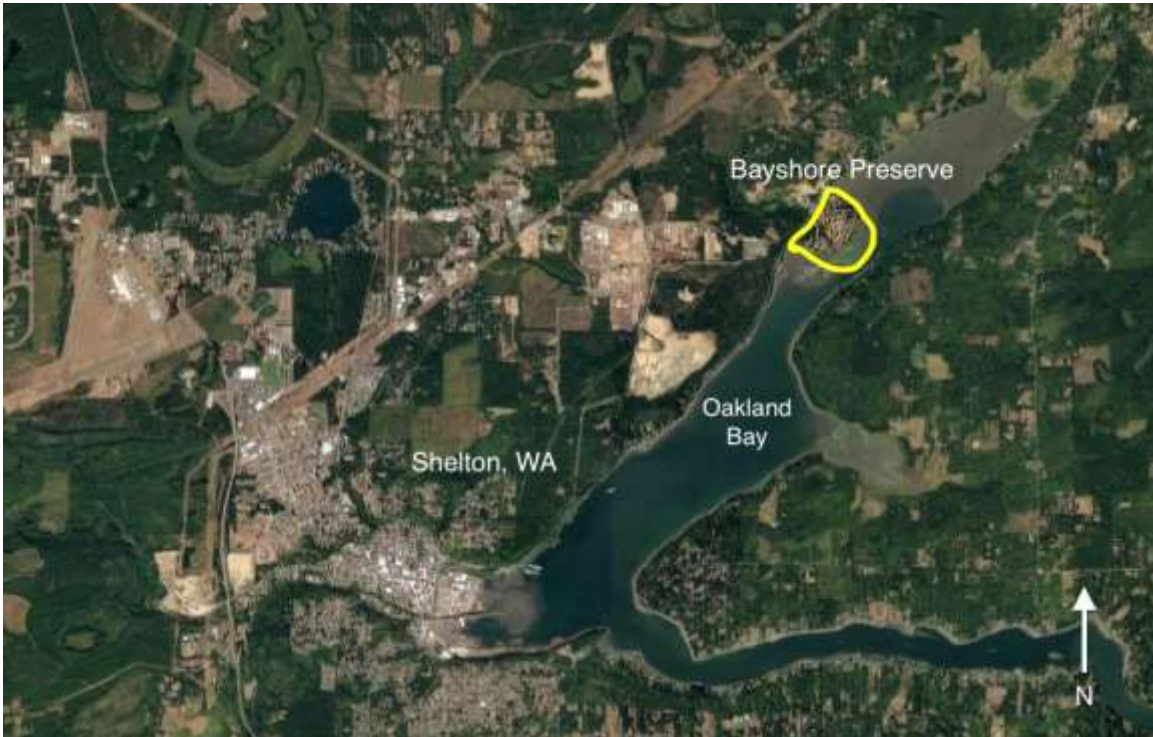


Figure 1.1 Location of Bayshore Preserve (formerly Bayshore Golf Course) northeast of Shelton, WA.

1.1 Site use history

The Bayshore Preserve was traditionally Squaxin land until the ratification of the Medicine Creek Treaty in 1856. Specifically, the Bayshore Peninsula was territory of the Sa-He-Wa-Mish of Big Skookum Inlet (now Hammersly Inlet). The Treaty's ratification resulted in the tribes losing thousands of acres of land to the Federal government, including this area. Coast Salish Tribal members' lives were—and still are—oriented toward the Puget Sound's inlets, which provided transportation, sources of fish, shellfish, and other marine resources. Puget Sound uplands provided plants and animals for food and materials. Watersheds were relied upon to support flourishing salmon runs that occurred each spring, summer, and fall. The Squaxin Tribe (People of the Water or

Saltwater People), depended primarily on the Puget Sound for their ways of life (Jolivette, Huber, Van Galder, Foster, & Henry, 2014).

Oakland Bay was home to the Squaxin, and there were several occupied longhouses present on the Bayshore Peninsula until they were demolished in 1867 (Hunn, 1993; Howard, 1949), after which time Native Americans used the area as a camp when hiking to and from Hood Canal. Undoubtedly, the peninsula was home to productive shellfish beds, and pre-contact shell midden was documented running 200 yards along the edge of the peninsula (Howard, 1949).

Historically, the land of Bayshore Preserve has been used by the Squaxin Island Tribe as a temporary living location during shellfish harvest times. It is notable that the largest Squaxin longhouse was present on the site—near the mouth of Johns Creek—next to one of the most productive natural oyster beds in the area. The Squaxin people are reliant on the waters of the Puget Sound for much of their food sources, and way of life. To American Indians, the land is not merely a resource to be used. It is a living entity with which every person has a living relationship. The cultural importance of restoring habitat to a high-quality state is immense—functional nearshore ecosystems provide all people food, and opportunity for deeper understanding of reasons to hold reverence for nature and its gifts.

The Willey family settled the land in 1866, and, with the logging of the entire Bayshore Peninsula, opened the Willey Mill in 1871 at the mouth of John's Creek. The mill was powered by water channeled from a dam built on John's Creek. By 1903 the mill had been abandoned (Deegan, 1959) and the Willeys developed the land into a 9-hole golf course and resort, completed in 1931 (Jolivette et al, 2014). The mill was

completely dismantled in 1947 when the new Shelton-Bayshore Golf and Country Club was built (Sideliner, 1947a). A soil dike was built along the entire southwest border of the property to prevent saltwater damage to the course (Sideliner, 1947b). The golf course was closed and abandoned in 2013, then purchased in 2014 by Capitol Land Trust (CLT) in response to the Oakland Bay Action Plan (Kenny, 2007).

Since purchasing the land, CLT has worked in partnership with the Squaxin Island Tribe, Taylor Shellfish, and Mason Conservation District to support the restoration and protection of these 325 acres of Oakland Bay nearshore habitat. To aid in reaching the long-term ecological goal for the property—maintaining ecological integrity of the shorelines, tidal wetlands, and riparian corridors of Johns Creek—CLT has removed the tidal dike to reconnect tidal processes to the land; removed most of the golf course infrastructure; installed a riparian buffer of native plants along Johns Creek; revegetated portions of the uplands; and removed all invasive plant populations from the property. Bayshore Preserve property is protected by a State of Washington deed of right to use the property for salmon recovery and conservation purposes in perpetuity. The U.S. Fish & Wildlife Service and Washington Department of Ecology hold a Restrictive Covenant for the property that additionally ensures its permanent dedication to conservation (Guthrie, 2014).

1.2 Capitol Land Trust Restoration Plan

Beside the major landscape alteration of dike removal and reconnection of tidal wetland function, CLT has focused on removing invasive species from the Bayshore Preserve property, and planting the golf course area with native forest and prairie species.

To improve water quality in Johns Creek, banks and buffer areas have been planted with native riparian tree and shrub species, and all groundwater usage from wells on the property has ceased (Capitol Land Trust, 2014).

During initial archeological surveying, evidence of fire was observed in soil horizons on-site. This implies that perhaps the area represents remnant prairie habitat—Native Americans traditionally managed prairies for control of unwanted species by burning. Because of this evidence, a 5-10 acre dry upland area of the Preserve will be revegetated and managed as prairie habitat. Native prairie plants will be introduced with a long-term goal of establishing viable populations of species including *Quercus garryana* (Garry oak) and the state endangered *Castilleja levisecta* (golden paintbrush). It is worth noting that although evidence of historical fire was detected on-site, active burning will not take place in the future—an initial application of herbicide will be used to prepare the area for revegetation (Capitol Land Trust, 2014).

1.3 Biodiversity of the Oakland Bay Area

Oakland Bay hosts a variety of fish, including five salmonid species: chinook salmon and steelhead trout (both federally listed as threatened), coho salmon (federal species of concern), chum salmon, and cutthroat trout. Hammersley Inlet hosts a stock of chum salmon (*Oncorhynchus keta*) that depends on the lower reaches of Johns Creek for its spawning grounds. Other documented fish species found in the bay include herring, sole, starry flounder, speckled sanddab and Pacific staghorn sculpin (Jolivette, Huber, Van Galder, Foster, & Henry, 2014).

The intertidal wetland habitat in and around the mouth of Johns Creek includes areas of continuously diluted saltwater and emergent vegetation that provide this critical

habitat for juvenile anadromous fishes. Intertidal salt marshes and mudflats provide high-quality habitat for salmonids, and nearby unconsolidated shorelines and sandy beaches provide currently functional habitat for a variety of shellfish (Guthrie, 2014; Capitol Land Trust, 2014). Beside industrially important shellfish species like manila clams, Pacific, and Kumamoto oysters, Oakland Bay supports populations of butter clams, native littleneck clams, horse clams, cockles, mussels, and other gastropods (Jolivette, Huber, Van Galder, Foster, & Henry, 2014).

Marine mammals are commonly observed, including harbor seals, sea lions, and elephant seals. The Southern Resident orca whale population occasionally visits Oakland Bay, and the city of Shelton has designated the bay as critical habitat for the species' recovery (Jolivette, Huber, Van Galder, Foster, & Henry, 2014). The dynamic estuary, home to these myriad species, also provides excellent habitat for hundreds of bird species. At least 70 species of birds use nearshore environments like Oakland Bay, including geese and swans, ducks and mergansers, loons, grebes, petrels, cormorants and more (Buchanan, 2006).

Oakland Bay is located within the western hemlock (*Tsuga heterophylla*) vegetation zone typical of the Puget Sound Basin. This zone is characterized by dense, tall evergreen forests with long-living trees that historically commanded shoreline landscapes. Dominant tree species in this zone include western hemlock, western red cedar (*Thuja plicata*), and Douglas' fir (*Pseudotsuga menziesii*). The understory generally consists of woody shrub species such as salal (*Gaultheria shallon*), Oregon grape (*Mahonia* spp.), salmonberry (*Rubus spectabilis*), huckleberry (*Vaccinium* spp.), and ferns such as sword fern and bracken fern (*Pteridium* spp.) (Kruckeberg, 1991).

Bayshore Preserve's intact marsh communities support halophytic (salt tolerant) plant species such as gumweed (*Grindelia integrifolia*), saltweed (*Atriplex* spp.), pickleweed (*Salicornia virginica*), saltgrass (*Distichlis spicata*) and others (Brennan, 2007). Much of the former golf course area is primarily vegetated with exotic grass species, with a few relic domestic fruit trees from the Willey homesteading days (Jolivette, Huber, Van Galder, Foster, & Henry, 2014).

CLT's restoration of the Preserve's riparian and upland habitats aim to enhance existing functionality with an ultimate goal of plantings becoming self-sustaining (i.e. requiring no maintenance interventions). This thesis study focuses on revegetating excavated tidal channels with those goals in mind. CLT did not plan to systematically seed the channels, but did have a high marsh seed mixture they intended to sow in tidal basins and near basin edges. This mix included the following species: meadow barley (*Hordeum brachyantherum*), tufted hairgrass (*Deschampsia caespitosa*), saltgrass (*Distichlis spicata*), slough sedge (*Carex obnupta*), Douglas aster (*Symphotrichum subspicatum*), Pacific silverweed (*Argentina egedii* ssp. *egedii*), and spear saltbush (*Atriplex patula*). While this project included only one of these species, it provided opportunity to compare methods for maximizing seed germination potential by attempting to create favorable seedbed conditions. If this experiment is successful, CLT will have a reproducible method and can continue to study efficiency of native seed application in wetlands, ultimately saving money and time while restoring critical salmon habitat.

Chapter 2: Literature Review

2.1 Tidal Wetland Restoration

Estuaries and connected tidal marshes are some of the most productive environments in the world (Tiner, 2013). Intact marshes undertake net primary production at rates between 2 to 4 kg above-ground dry matter per m² every year—vascular plants producing this matter contribute to the food web and provide energy for a wide range of organisms (Keefe, 1972). Salt marshes provide important ecological functions including shoreline erosion protection, wave and storm surge dampening, trapping water-borne sediments, nutrient cycling, and acting as nutrient sinks (Matthews & Minello, 1994). All of this contributes to health of the greater environment, and all organisms which rely upon it. This literature review will detail the importance and benefits of tidal saltmarsh restoration and review methods that have proved promising.

2.1.1 *The Importance of Tidal Saltmarsh Restoration*

Most restorations are undertaken because human intervention with the original environment caused degradation—whether this is urban development, dredging, draining and diking for agricultural uses, diversion of natural waterways and installation of dams or tidal gates to prevent flooding. Other impacts to estuarine systems can stem from pollutant discharge, agricultural run-off or accidental oil or gas spills. Effects can include alteration to soil and water chemistry, sedimentation rates, and changes in salinity levels (Broome, Seneca, & Woodhouse Jr., 1988). These effects combined alter the primary production in an estuary, which affects the quality of the food web upon which wildlife depend (NOAA, 2008).

The goal of estuarine restoration is to create a self-sustaining ecosystem that mimics the original habitat's structure and function. While it is impossible to totally recreate what was originally lost, restoration can aim to provide conditions that allow the site to become like the natural system through succession of flora and fauna (Broome, 1990; Gallego Fernandez & Novo, 2007) The foundation of tidal marsh restoration rests on restoring hydrologic connectivity to the site by simply removing barriers. This step—reintroducing natural tidal flow—allows natural flora and fauna to restore itself (Broome & Craft, 2000; Peck, et al., 1994). Seeding or transplanting dominant vegetation types into the restoration site can accelerate these processes (Sullivan, 2001; Zedler, 1992).

2.1.2 *Estuarine Wetland Restoration and Salmon*

Salmonids depend on estuarine habitats during key developmental stages of their life cycles. Chinook (*Oncorhynchus tshawytscha*) and chum salmon (*Oncorhynchus keta*) spawn in freshwater streams, depositing their eggs in gravelly eddies. Many juvenile salmon species use brackish waters of the estuary and nearshore environments to acclimatize to increased water salinity levels before migrating out to sea (Fresh, 2006). The ever-changing nature of estuaries provides an environment where species evolve and adapt to variable and extreme conditions.

Restoring estuarine wetlands is clearly beneficial for salmonid and other fish species in the Puget Sound. Reconnecting hydrology through tidal channels promotes fish and other marine organism usage of the wetland—increasing sediment, nutrient, and organic matter exchange between the marsh and the larger estuary (Minello, Zimmerman, & Medina, 1994).

Young restored estuaries support high numbers of juvenile salmon. Increased primary production in early stages of recovery supports larger invertebrate populations, and in turn support higher populations of juvenile chinook salmon (Gray et al. 2002). Assessments at the Nisqually River Delta show newly restored habitat compares well with undisturbed reference sites, providing juvenile chinook salmon similar foraging opportunities and potential for growth. With maturation of the restored sites, juvenile chinook salmon densities increased and diet composition displayed a trajectory toward reference conditions (David, et al., 2014).

Researchers have studied the effects of revegetation versus natural development of tidal wetland sites, and how these methods affect juvenile fish populations. Grey et al. were able to study structural *and* functional development of recovering marsh sites of different ages compared to adjacent relatively undisturbed, undiked reference sites. This gave researchers the opportunity to evaluate biotic and physical development of estuarine wetlands at different stages of recovery (establishing a trajectory toward reference conditions), and determine how and when dike removal timing impacts recovering juvenile salmon habitat. They found that the ecological functioning juvenile fish rely on does not necessarily result from the rapidly established vegetation, macrofaunal, and sedimentary structural attributes that occur in many restorations—it can be gained from simply allowing the restoration site to develop naturally after saltwater reintroduction. Planting vegetation to simulate later successional stages doesn't provide every structural attribute that would increase juvenile fish populations in an estuary (Cornu & Sadro 2002; Moy & Levin 1991), but does provide buffer benefits that can create healthier salmon habitat years down the road. For example, planting native vegetation in a newly

restored site can prevent invasion by aggressive exotic or native species that could negatively impact habitat (Broome & Craft, 2000). While revegetation provides quickly available habitat for fish, hydromorphic structural development and other factors impact fish recruitment as well. Tidal wetland revegetation in a restoration context is not fully understood, and it is worthy of deeper study from the perspective of proactively and adaptively creating self-sustaining salmon habitat.

From a human-centered perspective, functional estuaries provide ecosystem services beyond maintenance of fisheries. Porous soils of estuaries absorb water readily, providing a natural buffer against floods and storm surges. Marsh grass populations on tidal flats catch sediment and nutrients such as nitrogens from agricultural fertilizers, filtering water as it flows to the bay. Microorganisms that live in estuarine soils digest nutrients that enter through the greater watershed, buffering coastal waters against eutrophication. The many unseen processes occurring in an estuary build the foundation for a habitat that has become increasingly appreciated for its benefits to humankind. Whether it be for birding, salmon watching, shellfishing, or pure beauty, healthy estuaries are a lively environment to enjoy. Restoration of these dynamic ecological processes are critical to wildlife, humans, the Puget Sound, and the environment at large.

2.2 Tidal Marsh Revegetation Methods

2.2.1 *Halophytic plants*

Halophytic (salt-tolerant) plants are the logical choice when revegetating tidally influenced wetlands. Estuary soil salinities are naturally variable—salinities change depending upon soil characteristics, precipitation and seasonal variation, and where in the

wetland measurements are taken. Soil salinities can range from a few parts per thousand (ppt) to twice the concentration of seawater (35 ppt). Seeds and seedlings are generally more intolerant to salinity than mature plants (Broome, Seneca, & Woodhouse Jr., 1988), so plants are often grown in a greenhouse or harvested from other estuarine sites and transplanted (Zedler J. B., 2001).

Because elevation of marsh surfaces determines tidal inundation time, it is important to choose plant species with appropriate elevation requirements and salinity tolerances (Broome & Craft, 2000). Revegetation can be undertaken systematically by imitating climax plant communities of a similar reference site, or by experimentally planting species broadly across elevation zones of the wetland (Broome, Seneca, & Woodhouse Jr., 1988). This second method estimates species establishment ranges by observing plantings' survival at different elevations, and may be effective if no access to a reference site is available *and* organizations are willing to undertake experimentation.

2.2.2 Transplanting

Tidal wetlands are commonly revegetated by transplanting or plugging greenhouse grown stock in appropriate microhabitats to maximize plant community diversity and cover. To avoid more aggressive species dominating the restoration site, it is recommended to plant less aggressive or rarer species densely in their preferred microhabitat and less densely in other areas throughout their full elevation range. Leaving open spaces for natural recruitment of desirable species can be successful if the surrounding area supports plant communities which spread propagules, and soil does not become excessively saline (Sullivan, 2001).

2.2.3 Direct-seeding

Direct-seeding presents timing challenges. Many species need lowered salinity to exhibit optimal germination rates (Boyd, 1981; Dawe & White, 1986; Disraeli & Fonda, 1979; Ewing, 1982; Hutchinson, 1982; Hutchinson, 1988; Jefferson, 1976; Karafatzides, 1987; Macdonald, 1984; Mall, 1969; Moody 1978; Palmisano, 1971; Smith, Mudd, & Messmer, 1976; Smythe, 1987; Thom, 1981; Westley, 1962), so timing sowing after periods of rainfall or freshwater flooding provides lower soil salinity conditions favorable for germination (Kuhn & Zedler, 1997; Zedler, Nordby, & Kus, 1992). Seasonal low-salinity gaps exist in some regions during early spring months with higher precipitation rates, giving plants greater opportunity to successfully germinate and establish (Zedler, Nordby, & Kus, 1992) since seeds are usually more salt sensitive than mature plants (Broome, Seneca, & Woodhouse Jr., 1988).

Tidal influx and outflow present additional challenges when direct seeding a restoration site (Sullivan, 2001), however if there is protection from wave action seeding is more feasible (Broome & Craft, 2000). Storm-free periods are also of great help when attempting to establish marsh plant communities from seed (Broome, Seneca, & Woodhouse Jr., 1988). Since broadcast seeding alone often results in seed migration, several different methods have been used in attempts to keep seeds in place.

Atriplex patula has been tested to determine if broadcast or shallowly covering seeds with about one centimeter of soil would result in higher germination. The researchers found that sowing seeds onto compact soil and covering with soil resulted in the highest plant densities (Young, et al., 2011) Mulching mats have been anchored over

seeded areas (Zedler J. B., 2000), but details from this specific restoration are unknown. Similar to hydroseeding, one method mixes seeds with mud and organic matter to create a slurry which is “dropped” onto the marsh surface—this resulted in higher germination in annual species only, but most of the seed did remain within the mixture rather than being washed away (Sullivan, 2001). In the context of this project, the only drawback of hydroseeding is that since seeds are evenly mixed throughout the mulch slurry, much of it does not actually come into direct contact with the soil after application (NRCS, 2005).

2.2.4 Fertilization

Many restorations provide fertilization for new plantings—and while doing so can provide a “boost” to young plant communities, it is short lived. Especially in nutrient-poor sites, supplementing nitrogen and sometimes phosphorus through fertilization can determine whether restored plant communities are initially successful (Broome & Craft, 2000; Sullivan, 2001). Long-term, additions of N have not shown to increase aboveground vascular plant growth (Boyer & Zedler, 1998), and have actually been found to shift plant community dynamics in favor of nitrogen-competitive species (Boyer & Zedler, 1999). Sullivan (2001) recommends applying fertilizers conservatively only before transplanting and at the initial plant establishment period. This seems wise, especially when considering the sensitivity of estuaries to eutrophication.

2.3 Hydromulching in Restoration Projects

Hydromulching (a.k.a. hydroseeding—the application of seed in or with a water and mulch slurry) has been utilized in aspects of tidal wetland projects in Washington State. As part of project plans, hydroseeding has been used as a seeding method for

temporary slope stabilization during construction of setback dikes and storage pond side slopes. Grass seed mixes used in these projects were applied to provide soil stabilization and vegetated buffer between farm fields and created tidal channels, not directly onto restored estuary areas (Shannon & Wilson, Inc., 2014; Houghton & Ehlig, 2003).

Because projects were focused on creating or restoring estuarine habitat, it appears hydroseeded dike and buffer areas were not monitored, therefore effects of these seeding applications are unclear.

In another study of storm-water biofiltration swales, hydroseeding showed mixed success in establishment of six species of Pacific Northwest native grasses, but illuminated challenges faced when seeding in hydrologically dynamic environments. All seeded bioswales except two served as drainage for stormwater retention ponds, and one bioswale had a significantly steeper slope than the others. Due to storm-induced erosion, one swale was reseeded one week after initial seeding and another was hand reseeded due to poor establishment. These two swales showed little success in establishing native grass cover by hydroseeding because of persistent inundation and high flows which caused seed migration. One bioswale exhibited a strong germination response within two weeks, and continued to support multiple grass species one year after seeding with 98% mean vegetative cover (Mazer, Booth, & Ewing, 2001).

Hydroseeding was determined to be equally as effective, but no better than traditional broadcast seeding of Fremont cottonwood in a Colorado River Basin test restoration site. However, the researchers suggested hydroseeding might be preferred, when site location makes it feasible, because it requires less seed preparation (Grabau, Milczarek, Kapiscak, Raulston, Garnett, & Bunting, 2011).

The planting method has popularly been used for erosion control and bank stabilization, and has potential for terrestrial revegetation in areas where non-native invasive plants are a concern (e.g. wildfire sites, roadside construction). In Hawaii, where non-native invasions are of special concern, a native sedge (*Frimbristylis cymosa*, or Mau`u aki `aki) was tested in nursery beds for density and survival. Results indicated highest success with hydroseeded and handsowing combined with hydromulch cap methods. Researchers consequently concluded the both methods would be suitable for large-scale establishment of the species (DeFrank & Baldos, 2007).

The Natural Resource Conservation Service has suggested that a best method for using hydromulch in restoration projects is to apply seed first, and then perform hydromulching over the seed in a second operation. This gives the highest seed to soil contact ratio (NRCS, 2005). This information, however, is offered for restoration of terrestrial environments. As of this writing, literature has not been identified that explicitly deals with hydroseeding or hydromulching of wetland environments for the purpose of restoration with native species.

2.4 Species Selection

Species tested in this experiment are *Atriplex patula*, *Carex lyngbyei*, *Carex obnupta*, *Eleocharis palustris*, and *Schoenoplectus americanus*. The species are chosen because they can tolerate a range of soil salinities, periodic inundation, and are known to inhabit brackish marsh environments in the south Puget Sound region. Because soil salinity is expected to increase as excavated tidal channels are subjected to tidal influence, these species should be adaptable as soil salinity conditions change. See Table

1.1 for salinity tolerance ranges of each species. Following are descriptions of each species:

- *Atriplex patula*, commonly called spear saltbush or orache, is a fleshy, branched and leafy annual that grows up to 100 cm tall. It is often covered with a whitish, mealy substance which dissipates with age (Pojar & MacKinnon, 1994). A “morphologically variable” annual, it commonly occurs in saline intertidal marshes and less frequently in brackish marshes, occupying a wide range of elevations, substrates, and salinity conditions (Hutchinson, 1988).
- *Carex lyngbyei*, or Lyngbye’s sedge, is a singly growing to clumping sedge that spreads by rhizomes and stolons, growing from 20-100 cm tall. It is very common along the Washington coastline, colonizing tidal marshes and flats (Pojar & MacKinnon, 1994), and is a dominant plant of brackish marshes (Knudson & Woodhouse, 1982). Freshwater flushing is required to promote germination (Hutchinson & Smythe, 1986; Smythe, 1987), and though mature plants can tolerate a broad salinity range (Gordon, 1981) this species is absent in marshes where persistent soil salinities above 20 ppt exist for most of the growing season (Knudson & Woodhouse, 1982).
- *Carex obnupta*, or slough sedge, is a rhizomatous sedge typically common to freshwater marshes, swamps, bogs and stream-banks (Pojar & MacKinnon, 1994), but has also been found in “high salt/brackish marsh” habitats (Boule, Brunner, Malek, Weinmann, & Yoshino, n.d.). *C. obnupta* does not normally occur in the same habitats as *C. lyngbyei*, with the occasional exception of brackish sloughs and upper parts of tidal marshes (Pojar & MacKinnon, 1994).

- *Eleocharis palustris*, also called common spike-rush, is a rhizomatous perennial that grows singly or in clusters, from 10-100 cm tall. It thrives in wet ditches, brackish tidal marsh and shoreline habitats, and can tolerate constant inundation in shallow water (Pojar & MacKinnon, 1994). This species may have a wide range of salt tolerances that comprise several distinct populations, due to its taxonomical complexity (Hutchinson, 1988).
- *Schoenoplectus americanus* (synonym *Scirpus americanus*), commonly named three-square bulrush, is a rhizomatous perennial that grows singly or in small groups, with strongly triangular stems and stalkless, clustered flowers. It grows in brackish marshes and on shorelines, but prefers substrates that receive more freshwater influence than the generally finer, more saline substrate that dominates tidal marshes (Pojar & MacKinnon, 1994). It can be a dominant littoral species in low elevation and low salinity brackish marshes (Hutchinson, 1988), thriving in salinities between 5-10 ppt (Palmisano, 1971).

Species	Salinity tolerance range	Hutchinson (1988) Salinity Tolerance Rating—Max. Salinity in Field
<i>A. patula</i>	1-30 ppt (Boyd, 1981; Dawe & White, 1986; Hutchinson, 1982; Mall, 1969; Smith, Mudd, & Messmer, 1976; Westley, 1962; Hutchinson, 1988)	Very Tolerant— 0-45 ppt
<i>C. lyngbyei</i>	0-27 ppt (Boyd, 1981; Dawe & White, 1982; Dawe & White, 1986; Disraeli & Fonda, 1979; Ewing, 1982; Hutchinson, 1982; Jefferson, 1976; Macdonald, 1984; Smith et al., 1976; Smythe, 1987; Thom, 1981; Westley, 1962)	Tolerant— 0-20 ppt
<i>C. obnupta</i>	4-13 ppt (Macdonald, 1984)	Sensitive (estimate)— N/A
<i>E. palustris</i>	0-12 ppt (Dawe & White, 1982; Disraeli & Fonda, 1979; Ewing, 1982; Macdonald, 1984)	Moderately Tolerant— 0-12 ppt
<i>S. americanus</i>	0-17 ppt (Boyd, 1981; Disraeli & Fonda, 1979; Hutchinson, 1982; Karafatzides, 1987; Macdonald, 1984; Moody 1978; Smith et al., 1976; Westley, 1962)	Moderately Tolerant— 0-15 ppt

Table 1.1 Salinity (in parts per thousand) tolerance ranges of species chosen for this experiment.

2.5 Implications

Section 2.1 illustrates the importance of estuarine marsh restoration in the Puget Sound and how revegetation can act as a catalyst to providing new and available productive fish habitat. When revegetation is desired by restoration organizations, simple direct seeding has largely been abandoned in favor of planting plugs of halophytic species. While survivability is better when already established plugs are transplanted, this method is labor and cost-intensive. Additionally, often replanting of plugs is necessary to meet vegetation performance standards. Broadcast seeding and hydromulching as the initial seeding regime for an estuarine wetland may provide a cost-effective method to

revegetating a site, and can be augmented by planting plugs when needed. This study examines the viability of this seeding-hydromulch method using five halophytic plant species that naturally occur in tidal wetland environments.

Chapter 3: Methods and Materials

To prepare any site for restoration requires clear planning. Because every site is different, methods can be adapted to the site locality while adhering to the general principles of ecological restoration. In this experiment, we used recommended methods to calculate seed planting densities, prepare the seeding areas, measure elevations, collect and analyze soil samples, and test seeds for viability. We were not aware of an existing methodology for application of hydromulch in a wetland or estuarine environment at the time of this experiment's planning, so we used creative freedom and tested our own method of sowing.

This section will go over methods of seed preparation and experimental design, followed by sampling methods. Methods for a germination test that was performed to test seed viability are explained. Finally, the types of data analysis performed are introduced before results are reported.

3.1 Seed Preparation

Species for revegetation were chosen based on tolerance to saline conditions, inundation, and their classification as wetland plants. Calculations were made to determine a sowing rate of seeds per square foot, for each species, based on the literature's recommended rates of seeding densities per acre (Bishop & Bunter, 1999). We encountered variables such as unknown percentages of pure live seed (PLS), unknown chaff volume present with seed (purity), and unknown amount of tidal wash-away that would occur. Because of this, recommended seeding rates were inflated to

compensate for any losses these variables could potentiate. Additionally, we simply had enough seed to apply at higher rates than recommended by the literature.

Total area to be seeded was 387.5 ft² (36 m²). Eighteen plots were to be seeded, each measuring approximately 21.53 ft² (2 m²). To find the amount of seed needed for each individual plot, the total allocated weight of seed per species was divided by 18. Grams of seed needed per plot for each species were combined to make a seed mixture for each plot. Each identical seed mixture was pre-mixed with a 12oz scoop of sand before broadcast seeding. Table 2.1 shows seed weights allocated and sowing rates.

Weight ratios of each species within the seed mixture were determined based on what was known about each species performance in a brackish wetland environment, and the size of seeds. For example, *S. americanus* has large seeds and makes up over 40% of the seed mixture by weight, but the sowing rate is lower for this species.

Species	Avg. seeds per gram	Total grams allocated	Grams per plot	Seeds per ft ² sowing rate	% of seed mixture by weight
<i>Atriplex patula</i>	339 (Bishop & Bunter, 1999)	31.11	1.72	27.25	11.4
<i>Carex lyngbyei</i>	1,814 (Buenning, 2011)	60.76	3.37	311.45	22.3
<i>Carex obnupta</i>	1,203 (AOSA, 2007)	30.48	1.69	142.76	11.2
<i>Eleocharis palustris</i>	1,986 (Bishop & Bunter, 1999)	31.45	1.74	97.64	11.6
<i>Schoenoplectus americanus</i>	476 (Harwell, 2014)	118.1	6.55	109.51	43.4

Table 2.1 Experimental seed mixture species makeup and sowing rates per species. Note seed mixture percentage does not add up to 100.0% due to rounding.

3.2 Experimental Plot Design & Installation

Six sets of five side-by-side plots, measuring one meter high by two meters wide, were installed in three different tidal channels—two sets in each channel. The two sets were positioned as directly across from each other as possible, one on each side of the channel. Sets of plots were placed so each plot in a set was situated along an elevation gradient on the channel’s side wall, with no part of any plot on the channel floor. The tops of plots were positioned at the visible average high tide line (based on deposited debris).

A one by two (1x2) meter PVC frame was built to act as a guide when installing plots. The *inside* edges to corners of the frame measured one meter high by two meters wide. Holes were drilled one half meter in from both corners of the long (2 m) section of PVC—this served as a location to run twine through, which easily delineated the sample plot for monitoring.

For each set of plots, a piece of rebar was sunk into the ground at the location of the upper left corner of the plots, at the observed average high tide line. A meter tape was run ten meters from this corner, parallel to the high tide line, and rebar was sunk into the upper right corner of the plots. Making sure the meter tape was taut, rebar was sunk every two meters between the outer corners to mark the upper corners of each plot. The PVC frame was then laid over the top-edge corners and rebar was sunk into the lower corners to complete installation of each plot.. This installation method was used to create six sets of five side-by-side 1x2 meter plots—one set on each side of three separate channels.



Figure 2.1 Aerial photo of Bayshore Preserve showing channels and locations for each set of experimental plots.

3.2.1 *Plot Preparation*

To prepare for seeding treatments, each plot in every block was scarified to a depth of three inches using a bow-style metal garden rake. Any tidal deposited detritus in plots was measured for depth and area, and removed before treatments were applied. Nursery staples were used to secure polyethylene sheeting over control plots and plots adjacent to active treatment plots, to avoid contamination during seeding.



Figure 3.1 Affixing polyethylene sheeting to plots during seeding preparation.

3.3 Treatment Applications

Five different sowing treatments were applied:

- Unseeded control: no treatment
- Hydromulch only control: no seed
- Broadcast seeded + hydromulch (hereafter referred to as “seed + hydromulch”)
- Broadcast seeded with burlap cover
- Broadcast seeded only

Figures 4.1 through 4.3 show layout of experimental plots in each channel, and Tables 3.1 to 3.3 show associated treatments. Plots 1.1 and 2.1 were positioned across from each other in every channel, closest to the channel terminus. Plots 1.5 and 2.5 are closest to the bay in every channel.

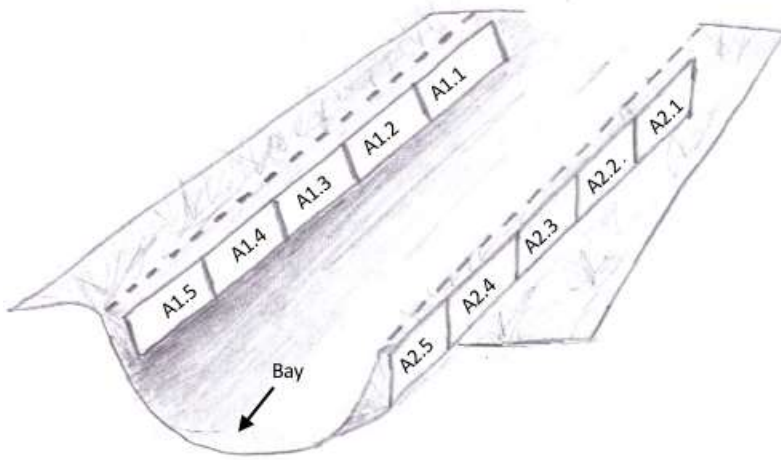


Figure 4.1 Plot layout in Channel A.

Plot	Treatment
A1.1	Hydromulch only control
A1.2	Seed + hydromulch
A1.3	Burlap
A1.4	Seed only
A1.5	Unseeded control
A2.1	Seed only
A2.2	Seed + hydromulch
A2.3	Hydromulch only control
A2.4	Unseeded control
A2.5	Burlap

Table 3.1 Treatments applied to each plot in Channel A.

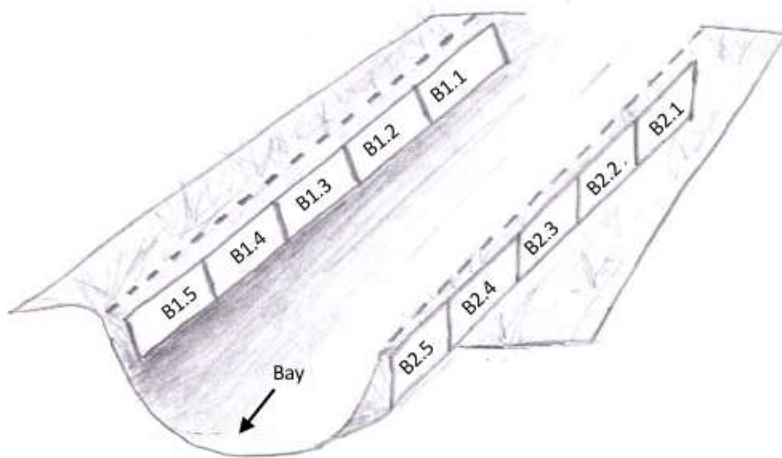


Figure 4.2 Plot layout in Channel B.

Plot	Treatment
B1.1	Seed + hydromulch
B1.2	Burlap
B1.3	Hydromulch only control
B1.4	Seed only
B1.5	Unseeded control
B2.1	Seed + hydromulch
B2.2	Hydromulch only control
B2.3	Seed only
B2.4	Unseeded control
B2.5	Burlap

Table 3.2 Treatments applied to each plot in Channel B.

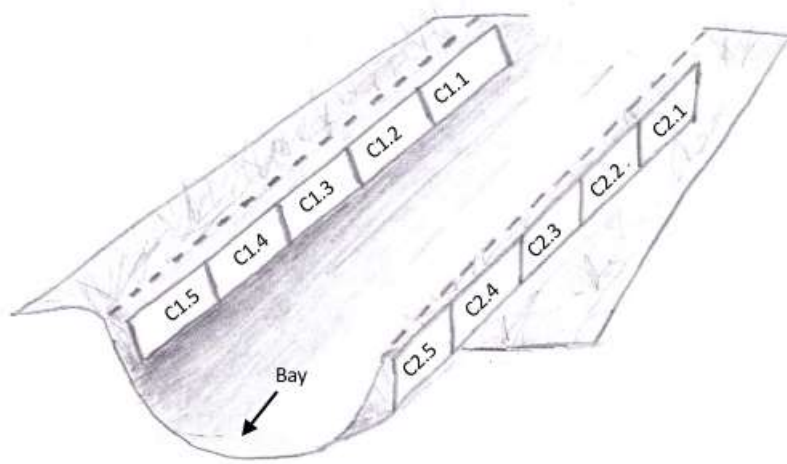


Figure 4.3 Plot layout in Channel C.

Plot	Treatment
C1.1	Burlap
C1.2	Hydromulch only control
C1.3	Seed only
C1.4	Seed + hydromulch
C1.5	Unseeded control
C2.1	Burlap
C2.2	Unseeded control
C2.3	Hydromulch only control
C2.4	Seed only
C2.5	Seed + hydromulch

Table 3.3 Treatments applied to each plot in Channel C.

3.3.1 Unseeded control treatment

There was one unseeded control plot in each set of plots. This control treatment was covered with polyethylene sheeting during the treatment application process.

3.3.2 Hydromulch only control treatment

One plot in each set received a hydromulch only control treatment. Adjacent plots to this treatment were covered securely by polyethylene sheeting to avoid contamination. Hydromulch was applied, by a contractor (Hoyt's Hydroseeding, Tahuya, WA), two inches deep to each hydromulch only treatment plot. The hydromulch product used was Rainier Fiber™ Premium Wood Fiber Mulch For Hydroseeding and Erosion Control. See Appendix A for details on hydromulch specifications and mixing instructions.

3.3.3 *Broadcast seed plus hydromulch treatment*

One plot in each set received a broadcast seeding plus hydromulch cap treatment. Plots adjacent to the area to be treated were covered using polyethylene sheeting and nursery staples. The treated plot was broadcast seeded using the prepared seed mixture, then covered by two inches (2”) of hydromulch by the contractor.

3.3.4 *Broadcast seed plus burlap cover treatment*

One plot in each set received a broadcast seeding plus burlap cover treatment. Adjacent plots were covered to avoid contamination. Prepared seed mixture was broadcast onto the treatment plot, and a 1x2 meter piece of burlap was tacked overtop the plot.

3.3.5 *Broadcast seed only treatment*

One plot in each set received a broadcast seeding treatment. Again, adjacent plots were covered to avoid contamination. Prepared seed mixture was broadcast onto the plot. No covering or mulch of any kind was used to secure seeds in this treatment, nor were seeds raked into the soil after application.

After all five treatments were applied, remaining polyethylene sheeting was removed, and treatments were checked 24 hours later.



Figure 5.1 Hydromulching in progress on March 9, 2016.



Figure 5.2 Examples of finished sets of seeded plots, showing all treatments applied on March 9, 2016.



Figure 5.3 Plots 24 hours after seeding on March 10, 2016. Note migration of hydromulch, especially in plot at left. Hand broadcast sowing and hydromulching was performed on March 9, with hydromulch applied between 9:00am and 10:00am (seed was applied prior, the same morning). Low tide occurred at 1:11pm at 2.58 feet (MLLW), and the next high tide occurred 6:22pm at 14.35 feet.

3.4 Plot Elevations

Elevations were measured at the midpoints of the top and bottom edges of each plot using the standard method of differential leveling. To prepare, several benchmark points near experimental channel edges were located using Google Maps and coordinates were recorded. These benchmark point coordinates were input into the USGS Elevation Point Query Service (NAD83) to retrieve point elevations in meters. A horizontal laser level on a tripod was used to measure vertical differences in elevation of each plot's top and bottom edge, relative to the elevation of the benchmark point used.

The laser level was affixed to the tripod and set up so its height was just above eye level, in a location where the line of sight allowed me to see the backsight and the foresights. The instrument was leveled, making sure the bubble was within the circle on

the tripod, and that the bubble was between the two lines on the laser level. The instrument was moved 90 degrees to both sides to check leveling.

Using a handheld GPS device with benchmark coordinates input, we navigated to and marked the benchmark elevation point. A reading was taken at the benchmark point to determine elevation difference between the height of the instrument and the known elevation—this is known as the backsight. The backsight rod reading value (BS) was added to the known elevation value at the benchmark point—this gave us the height of the instrument (HI) relative to the benchmark’s known elevation. From this point, we took rod readings at the midpoint of each top and bottom edge of each plot—these were the foresights (FS). To calculate elevation of each point, we subtracted the foresight reading from the height of the instrument (i.e. $HI - FS = \text{elev}$). To end the survey, we returned to the same benchmark point and took another reading to confirm the instrument height had not deviated outside an acceptable margin of error (0.03m) (University of Colorado Boulder, n.d.).

3.4.1 *Calculating tidal inundation*

To calculate how many tides completely inundated all experimental plots, converting elevations from the horizontal datum (NAD83), in which original data was collected using the USGS elevation benchmark points, into a vertical datum (NAVD88) was required. Vertical datums are used to measure heights of various points relative to a set zero elevation, and tides are often measured using Mean Lower Low Water (MLLW). MLLW is the average elevation of the daily lower low tide over a 19-year recording

period (also known as the National Tidal Datum Epoch), relative to a primary benchmark at the tidal station (NOAA, 2017).

Tide information was calculated based on the MLLW datum predictions for Barron Point, Little Skookum Inlet Entrance (NOAA Subordinate Station ID 9446742) located in Shelton, WA. Little Skookum station is referenced to Seattle (Station ID 9447130), so plot elevations were adjusted using NAVD88 referred to MLLW at this Seattle location to calculate the total number of high tides that submerged the plots between soil sample collection dates. See appendix Table A1 for a chart showing original elevations collected in NAD83 and converted elevations to relative datums.

3.5 Soil Sampling

3.5.1 Collection

Soil samples were collected on March 4, 2016 from each plot to test for soil salinity. In each plot, five six-inch deep scoops were collected from random locations and mixed together in a clean bucket to create a composite sample. This composite sample was screened through a large screen into a new clean bucket to remove rocks and debris. The rocks and debris were discarded back into the tidal channel, below and not in the plot the sample was taken from. Approximately one quarter pound of this soil was reserved and placed into a new, labeled 1-quart ziplock bag. Any remaining soil was returned to the sample holes in the plot. Before moving on to the next plot, buckets and the sieve were wiped with a towel until clean, rinsed with distilled water, and dried with a separate clean towel.

A second round of soil samples was collected on February 13-14, 2017 to measure salinity changes across control plots' elevation gradients. Composite samples were collected from three locations along the elevation gradient in the unseeded control plot in each block, 0.1, 0.5, and 0.9 meters from the top of each control plot. Three six-inch deep scoops were collected from each elevation within the plot, mixed together, and processed as above.

3.5.2 *Drying*

All first-round samples were air dried at room temperature by leaving ziplock bags open and periodically shaking the samples to redistribute the soil until completely dry. Second-round soil samples were dried in a drying oven, stirring periodically, at 90°F for 24-48 hours (or until completely dry) in paper bags.

3.5.3 *Electrical conductivity testing for salinity*

Each sample was subjected to soil electrical conductivity testing for soluble salts, using a Hanna HI 9813-6 Portable pH/EC/TDS/°C Meter. The meter was calibrated before each testing session and after every tenth sample to a known electrical conductivity standard using Hanna aqueous electrolyte calibration solution (HI 70031; 1413 $\mu\text{S}/\text{cm}$) and temperature. Before testing began, each sample was sieved again through a 2mm (U.S. #10) soil sieve and mixed well. A 1:5 extraction method was used—20 grams of soil were measured and mixed with 100mL of distilled water in a glass beaker. The solution was mixed well with a stainless steel lab spoon spatula for 30 seconds every five minutes, for 30 minutes. After the 30-minute mixing period, the solution was allowed to rest for 30 minutes so fine sediment could settle. This solution was strained through VWR Scientific 28213 Grade 617 (Fast) Qualitative filter paper into

a separate clean, dry beaker. The electrical conductivity in mS/cm (millisiemens per centimeter) of this filtrate was read with the Hanna meter and recorded. Each sample was retested in duplicate to determine sample variability.

3.6 Vegetation Establishment Monitoring

Experimental plots were monitored for vegetative germination and plant establishment at low tide, once per month beginning one month after seeding. Monitoring was conducted on the following dates: April 8, May 5, June 1, June 21, July 23, August 16, September 20-24, and October 20, 2016.

Qualitative observations were made at each visit regarding changes, patterns and effects of tide on each type of plot; thickness of new litter and debris deposits; estimated percent of hydromulch washed away; and development of plant communities in greater tidal channel areas. Quantitative measurements taken included first germination dates of observed species, density of each planted and naturally occurring species, and total vegetative cover in each plot.

3.6.1 *Sampling for density & percent cover*

Using the PVC frame constructed for plot installation, 1 m² sample areas were delineated by running twine through pre-drilled holes and laying the frame over the rebar plot corners. Species densities were measured by counting each live stem that occurred within the 1m² sample area—as a rule, stems were counted if they fell underneath the top edge of the frame or right-side twine, and omitted if they fell underneath the bottom edge of the frame or left-side twine. When estimating percent total vegetative cover, any and

all vegetative plant parts that fell within the sample area were counted, even if a plant's stem was itself outside of the sample area.



Figure 6.1 PVC frame and twine delineating 1m² sample area. Top: Plot A2.5 Bottom: Plot A2.1.

3.7 Germination Testing

Germination testing was performed on *Atriplex patula* to determine viability of seed. A first germination test failed due to equipment malfunction, so a second test was

performed to gain usable results. Due to time constraints, testing of *A. patula* seed was prioritized based on occurrence of two *Atriplex* species in the experimental plots (see Chapters 4 & 5).

Testing was performed in a controlled environment, under non-saline conditions. Fresh (deionized) water was used to maximize the likelihood of germination—based on measurements of environmental conditions on-site at the time of sowing, field conditions in which sowing took place revealed close to freshwater soil salinity concentrations.

Before germination testing occurred, *A. patula* seeds underwent a period of cold-moist stratification for 30 days (Baskin & Baskin, 2001). 100 seeds of *A. patula* were wrapped in cotton gauze, moistened with distilled water, and wrapped in a paper towel moistened with distilled water. This seed packet was put into a plastic bag and twist-tied shut. Seeds were stratified in a dedicated refrigerator at a temperature of 38°F.

Once the stratification period was complete, 100 *A. patula* seeds were separated into five sterilized petri dishes (20 seeds per dish) lined with one piece of filter paper (Double Rings 90mm) and 3mL of distilled water was added. Petri dishes were placed into the germination chamber (SG30 Controlled Environment Chamber), and started on the 12-hour dark cycle. Although Baskin & Baskin (2002) tested *A. patula* at a 5/25 °C alternating temperature cycle, this test used a 5/20 °C setting with 12 hours of dark at 5°C and 12 hours of grow lights (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPF) at 20°C. The decision to lower the upper temperature resulted from a desire to test multiple species at once in the interest of time.

During the germination testing period, each set of seeds was monitored for germination (emergence of a radicle—the embryonic root). As seeds sprouted, successful germinants were recorded for the day, and removed from the petri dish before returning samples to the germination chamber. Seeds were monitored every other day for a period of 35 days.

3.7 Data Analysis

Data was analyzed using JMP software. A one-way analysis of variance (ANOVA) was performed on vegetative recruitment data to compare treatment effects on planted species against two controls (unseeded control and unseeded hydromulch only control). A two-tailed t-test was run to compare the treatment means to each other in significant datasets.

Linear regression analysis was performed to reveal correlations between elevation and species recruitment and richness on select dates. Continuous variables were plotted against each other, and appropriateness of fit was checked by plotting residuals.

Chapter 4: Results

This chapter contains the vegetative survey analysis results, along with results of soil salinity and germination testing. First, section 4.1 introduces *Atriplex patula* as the sole successful species in this experiment and addresses reasons for plant identification confusion that occurred in this experiment. Section 4.2 highlights overall species occurrence in the tidal channels and discusses natural vegetative recruitment. In section 4.3, results showing *A. patula* recruiting with higher success in burlap and hydromulch treatments are explained in detail by delving into results from each monitoring date. Section 4.4 shows that naturally recruiting species did not show significant density differences between treatment types, but did show an unusual relationship between species richness and elevation. Increasing soil salinity and tidal inundation time are then discussed in section 4.5. Finally, section 4.6 reveals germination test results showed that four species tested were indeed viable, indicating their potential for germination in the field.

Graphs in this section reflect results analyzed from six replicates of each treatment. Oneway ANOVA graphs are set up as follows: x-axes are labeled with manipulative treatment types, and y-axes represent the mean live stem densities recorded for each treatment type. Linear regression graphs show species richness plotted against elevation per plot in which data was collected on the date of highest richness.

4.1 Planted Species Germination and Establishment to Maturity

Of five planted species, one was positively identified to have reached maturity within the experimental plots: *Atriplex patula* (Figure 7.1). This species was not

positively identified until June 2016—at the first two monitoring visits it was confused with a naturally occurring species, *Atriplex prostrata*. Both species were counted together—they were thought to be the same—and this is reflected in the data for the April through May 2016 monitoring period. By June, distinctive differences in leaf shape between the observed specimens prompted an in-depth identification effort, revealing that two species were present in the experimental plots. Beginning in June, both distinct *Atriplex* species are reflected in the data.¹

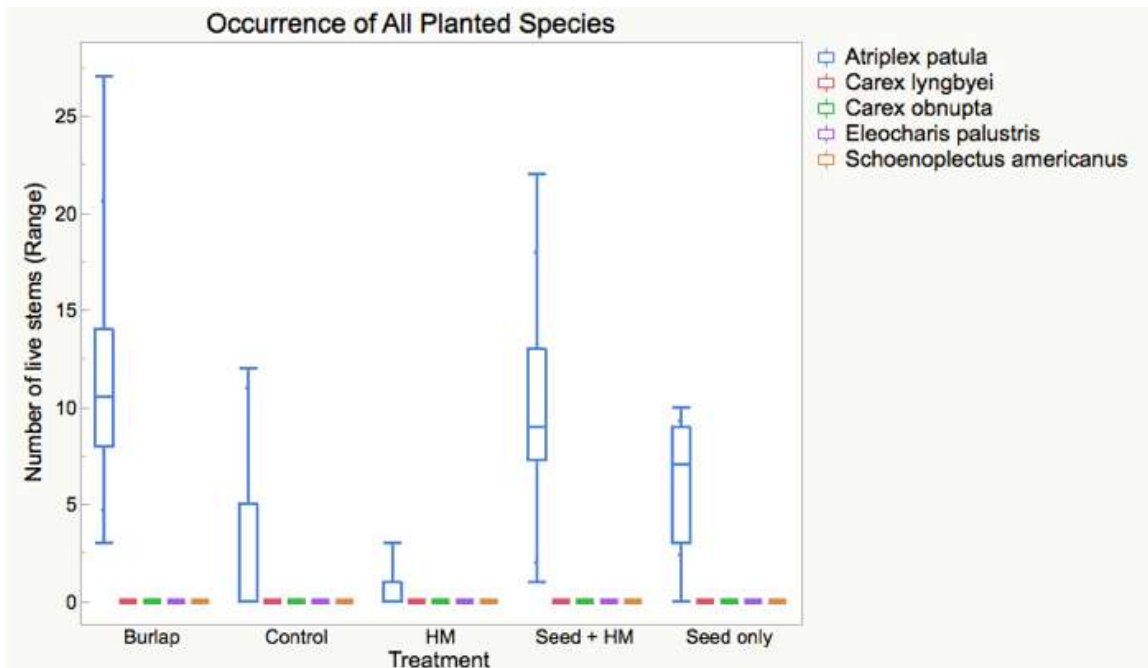


Figure 7.1 Recorded observations of each of five planted species within experimental plots.

¹ Since completion of the experiment, a potential misidentification of the naturally occurring species *Atriplex prostrata* has come to light. Based upon observations by Capitol Land Trust (CLT) ecologists, the plant referred to in this study may in fact be *Chenopodium album*—which is difficult to distinguish from *A. prostrata* to the naked eye. The plant in question was keyed to species *A. prostrata* in summer 2016, however specimens were beginning to senesce and few intact flowers remained. This presents a problem of ambiguous identification. Therefore, in the following sections, all mentions of *A. prostrata* could potentially be referring to *C. album*—final identification of which species is present on-site will be determined by CLT in 2017 when flowering specimens are present. This potential misidentification in no way affects the data analysis or results, as only the planted *Atriplex patula* was analyzed for treatment effects.

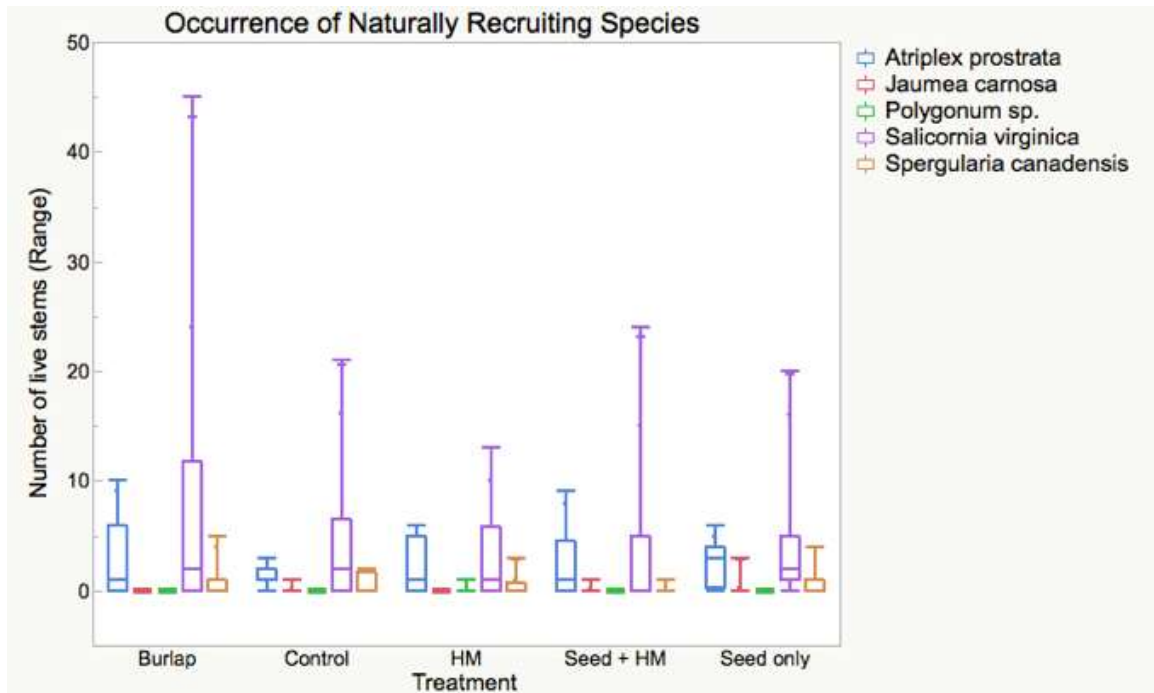


Figure 7.2 Recorded observations of naturally recruited (non-planted) species within experimental plots.

4.2 Naturally Occurring Diversity in Tidal Channels

Several naturally recruited native species colonized the experimental plots (Figure 7.2) and the tidal channel areas that were not part of the experimental plots. All tidal channel floors were abundantly vegetated by *Salicornia virginica* and *Spergularia canadensis*, while *Atriplex prostrata* was commonly observed. In tidal Channel A, *Jaumea carnosa* was less commonly observed, and *Atriplex patula* was rarely observed on the channel floor in addition to species mentioned above. All tidal channel walls supported *A. prostrata* (commonly observed), *A. patula* (common to less common), *S. virginica* and *Spergularia canadensis* (both common to less common).

Channel A also supported the greatest species diversity on channel walls, with common observations of *A. prostrata*, less common occurrences of *A. patula* (becoming more common approaching experimental plots), *Grindelia integrifolia*, *S. virginica*

(generally present on lower channel walls), and *Spergularia canadensis*, and rare occurrence of *J. carnosa*. Two unidentified species occurred on tidal channel walls. One appeared to be in the *Cyperaceae* or *Poaceae* families (species in these families exhibit very similar structure at young growth stages), and occupied upper elevation areas in some experimental plots (hereafter referred to as UNKN1; Figure 8.1). The other (UNKN2; Figure 8.2) occurred near channel edges (one specimen each in Channel A & B), having thick, fleshy leaves to eight inches long.



Figure 8.1 (Left) Species UNKN1 occurred in experimental plots. **Figure 8.2 (Right)** Species UNKN2 occurred outside of experimental plots on the edge of tidal channel.

4.3 *Atriplex patula* Treatment Responses

Results seem to indicate a pattern showing higher *A. patula* stem densities in treatments that were manipulated by adding seed. Beginning on June 1, 2016, we clearly see higher recruitment rates in the seed + hydromulch and burlap treatment groups than the control treatments. Plots which were dry broadcast seeded also exhibit a pattern of higher plant establishment than controls, but less so than more intensely manipulated plots.

Overall, the pattern seems to indicate that hydromulching or otherwise providing a stabilizing fabric over this species after broadcast seeding onto the soil results in higher rates of plant establishment. Higher numbers of planted *A. patula* in the first season after saltwater reintroduction adds value by providing more aboveground biomass to the system, increasing the ability of the channels to trap sediment, and a faster rate of habitat development benefitting benthic macroinvertebrates and other species.

Figure 9.1 shows an overview of mean live stem densities of *A. patula* on each sampling date for each treatment type. Results from each individual monitoring date are discussed in more detail below.

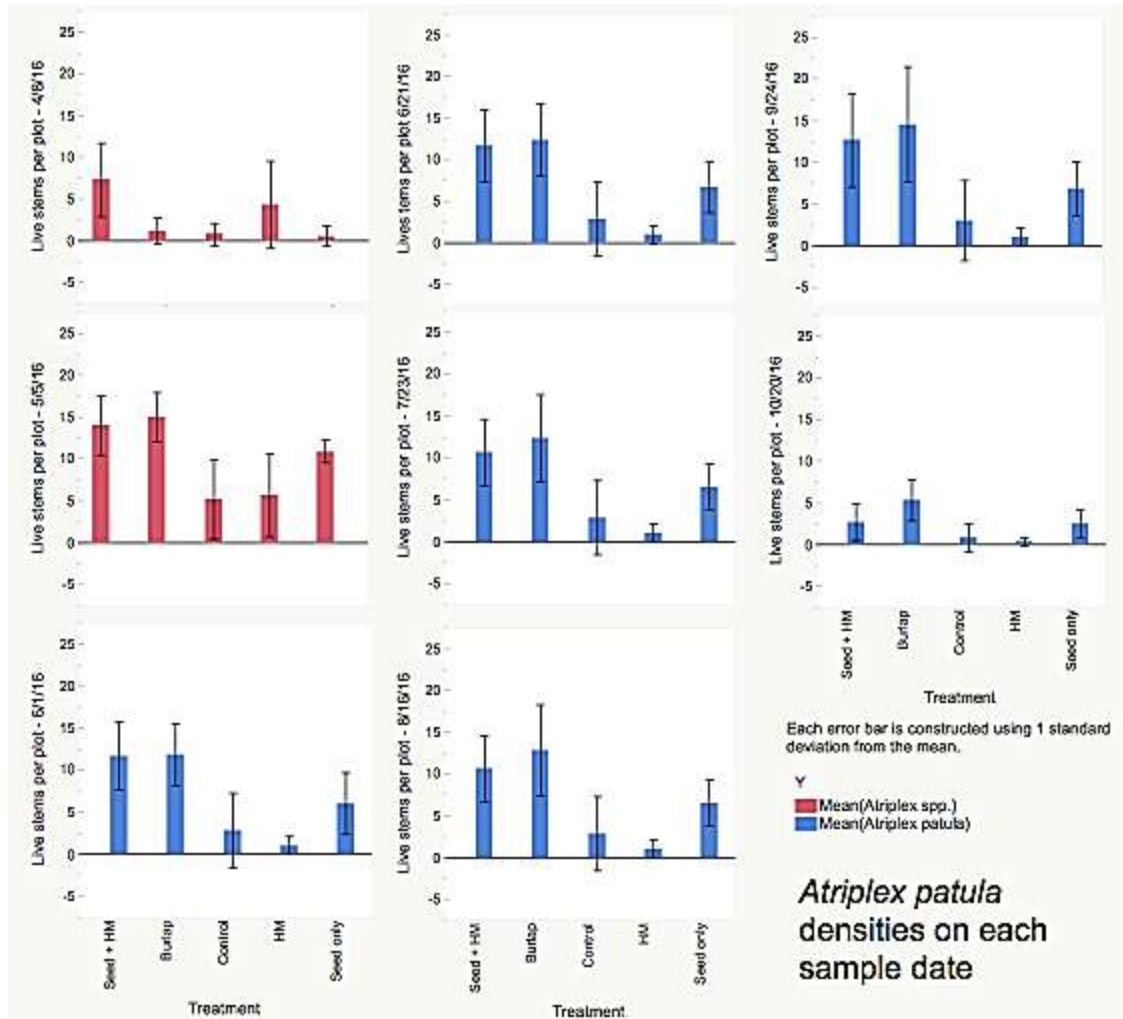


Figure 9.1 Mean *Atriplex* species densities shown for each monitoring date. Graphs in red show data gathered before *A. patula* was differentiated from *A. prostrata*, and therefore simply reflect mean densities of *Atriplex* spp. Note that over time, stem densities are clearly higher in plots that received the hydromulch-over-seed and burlap treatments.

4.3.1 April 8, 2016

On April 8, germinants of *Atriplex* spp. were observed in each treatment plot. Because germinants were so young—most had cotyledons, some had developed one set of true leaves—it was impossible to identify to species. Significant differences ($p=.0045$) in germination were observed between treatment groups on this date.

A two-tailed t-test showed that *Atriplex* spp. germinated in significantly greater numbers in the seed + HM plot. The seed + HM group had a sample mean (\bar{x}) of 7.33 germinants, significantly higher ($p=.0019$) than the unseeded control ($\bar{x}=1.873$). The hydromulch only control group (hereafter referred to as “HM”) exhibited a sample mean of 4.33 germinants per plot. The burlap and seed only treatment groups had much lower germinant counts, and were both significantly lower ($p=.0030$ and $.0012$, respectively) than the seed + HM group on this date. The burlap treatment resulted in $\bar{x}=1.67$ germinants. The seed only treatment resulted in $\bar{x}=0.50$ germinants. No significant differences were observed between the unseeded control and either the burlap ($p=.8604$) or the seed only ($p=.8604$) treatment groups.

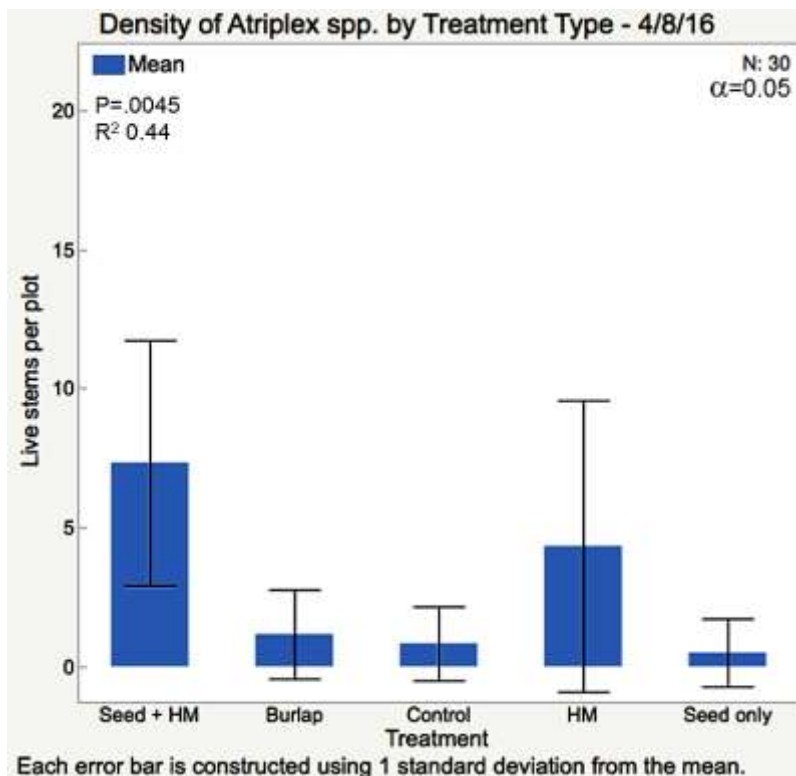


Figure 10.1 4/8/16 Establishment of *Atriplex* spp. in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.2 May 5, 2016

By this date, highly significant differences ($p < .0001$) are seen between treatment groups for *Atriplex* spp. At this point, *Atriplex* spp. were at a young stage of maturation, with most plants showing several sets of true leaves and no flowers. While noticeable differences in leaf shapes of *Atriplex* plants were observed, they weren't distinct enough to prompt investigation into the possible presence of two "varieties." Therefore, at this date, all *Atriplex* spp. were again counted and analyzed together.

When performing a two-tailed t-test, the sample means of all seeded treatments reflected significantly greater numbers of *Atriplex* spp. germination and establishment compared with the two controls. The sample mean of the burlap treatment ($\bar{x}=15.00$) is significantly higher than the unseeded ($p=.0001$, $\bar{x}=5.17$) and HM ($p=.0002$, $\bar{x}=5.67$) control groups. The seed + HM treatment also exhibited significantly higher germination and establishment of *Atriplex* spp. ($\bar{x}=14.00$) compared with the unseeded ($p=.0004$) and HM ($p=.0007$) controls. The seed only treatment exhibited a sample mean ($\bar{x}=10.83$) significantly higher than the unseeded ($p=.0143$) and HM ($p=.0241$) controls. There were no significant differences detected between the seed only and burlap ($p=.0641$) or seed + HM ($p=.1534$) groups. No significant differences were observed between the unseeded and HM control groups ($p=.8181$).

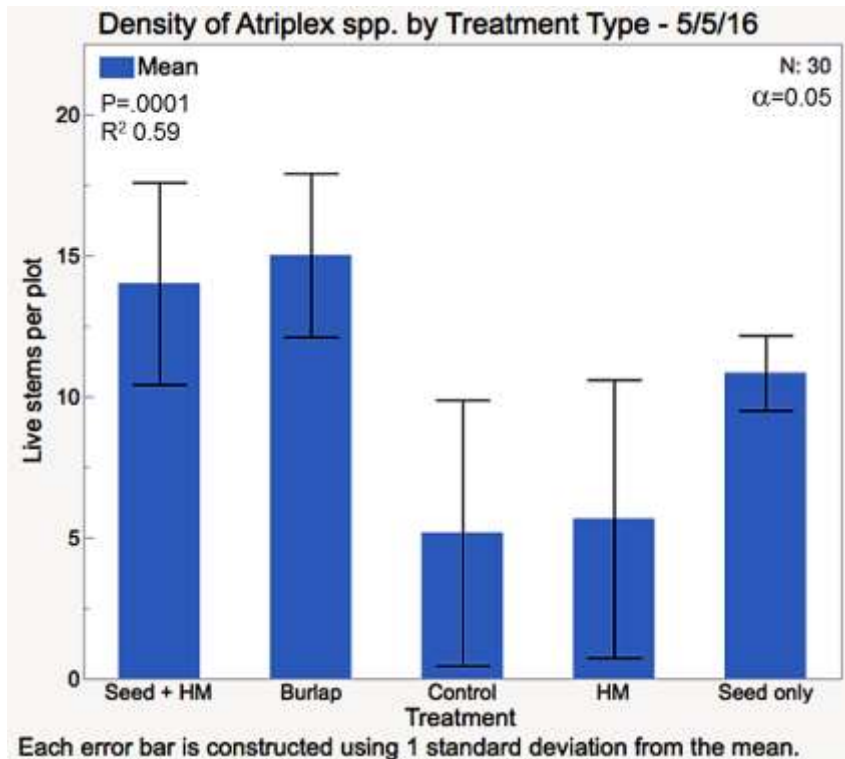


Figure 10.2 5/5/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.3 June 1, 2016

This is the first date in which data reflects positive identification of *Atriplex patula* and *Atriplex prostrata* as two separate species, both occurring in the experimental plots. For the first two data collection dates, this distinction was not confirmed and all *Atriplex* species within the sample area were counted together. On this date, a oneway analysis of variance (ANOVA) showed significant differences ($p < .0001$) in *A. patula* live stem densities between treatment groups.

A two-tailed t-test showed significantly higher live stem counts in all seeded treatments. The seed + HM group had a significantly higher number of established plants

(\bar{x} =11.67) than the unseeded (p =.0003, \bar{x} =2.83) and HM (p <.0001, \bar{x} =1.00) controls. The burlap treatment was also significantly more successful (\bar{x} =11.83) than unseeded (p =.0002) and HM (p <.0001) controls. Additionally, both the seed + HM (p =.0114) and burlap (p =.0095) treatments supported significantly higher densities of live stems than the seed only (\bar{x} =6.00) treatment. The seed only treatment had significantly higher stem densities compared to the HM control (p =.0237)—there was no observed difference when compared with the unseeded control (p =.1396).

Identical analysis of *A. prostrata* was performed to determine if any patterns between treatment groups existed, and because this species was unintentionally counted on the first two monitoring dates. Oneway analysis (p =.8938, R^2 =.04) and a two-tailed t-test (p -values between .3337 and .9137) of *A. prostrata* data revealed no significant differences between any of the treatment groups.

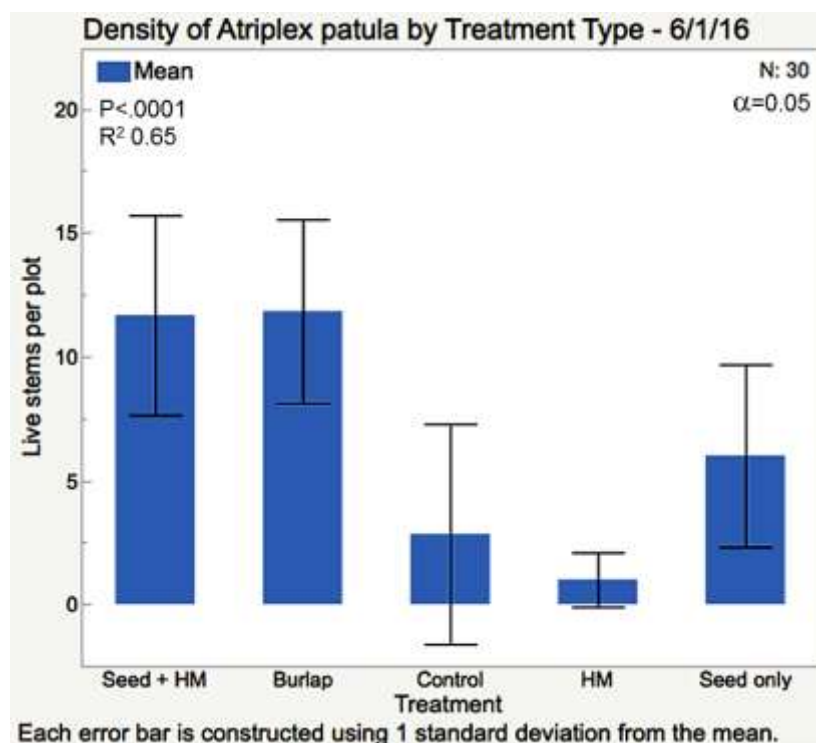


Figure 10.3 6/1/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.4 June 21, 2016

Oneway analysis revealed significant differences ($p < .0001$) between stem densities of *A. patula* across treatment groups. Two-tailed t-tests showed significant differences between the seed + HM treatment ($\bar{x} = 11.67$) compared with unseeded ($p = .0003$, $\bar{x} = 2.83$) and HM ($p < .0001$, $\bar{x} = 1.00$) controls. The burlap treatment recruited significantly higher live stem densities ($\bar{x} = 12.33$) than unseeded ($p = .0001$) and HM ($p < .0001$) controls. The seed only treatment group exhibited higher stem densities ($\bar{x} = 6.67$) than only the HM ($p = .0133$) control group—when compared with unseeded controls no significant difference was detected ($p = .0834$). The seed only treatment densities were additionally significantly lower than seed + HM ($p = .0268$) and burlap ($p = .0133$) treatments.

A. prostrata showed no significant differences between treatment groups ($p = .8490$, $R^2 = .05$).

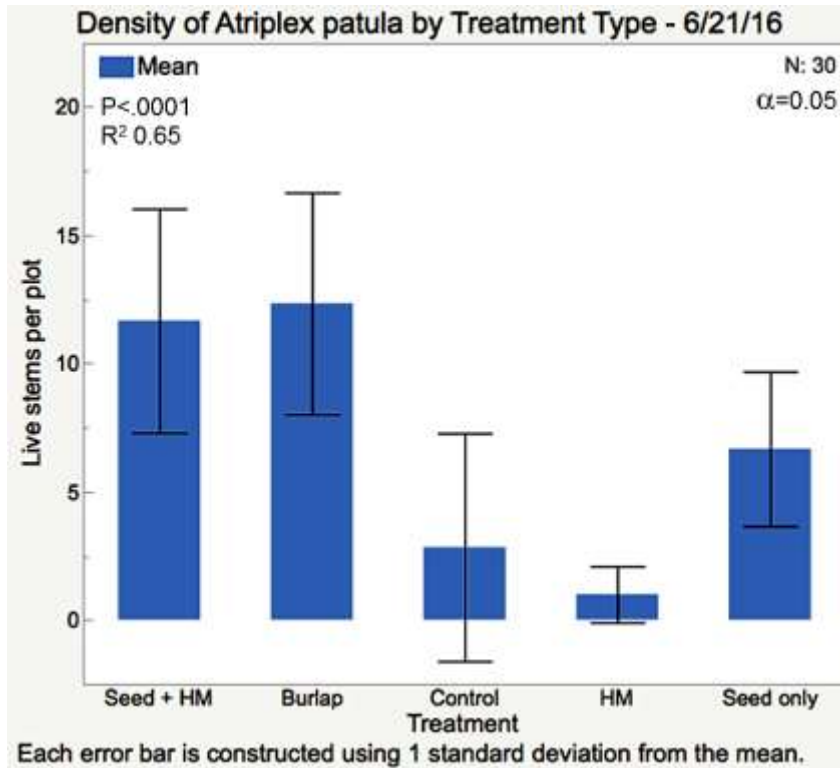


Figure 10.4 6/21/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.5 July 23 and August 16, 2016

On July 23 and August 16, 2016 monitoring dates, *A. patula* continues to show significant differences between treatments ($p < .0001$). On respective dates, the seed + HM treatments show significantly higher stem densities ($\bar{x} = 10.67$ on both dates) than unseeded ($p = .0002$ on 7/23/16, $p = .0017$ on 8/16/16) and HM ($p = .0002$ on 7/23/16, $p = .0002$ on 8/16/16) controls. Burlap treatments show significantly higher stem densities ($\bar{x} = 12.33$ on 7/23/16, $\bar{x} = 12.83$ on 8/16/16) than unseeded ($p = .0002$ on 7/23/16, $p = .0001$ on 8/16/16) and HM ($p < .0001$ on both dates) controls. Seed only treatment densities

(\bar{x} =6.50 on both dates) continue to only be significantly higher than HM controls (p=.0185 on 7/23/16, p=.0205 on 8/16/16).

A. prostrata continues to show no significant differences between treatment groups (p=.8761, R^2 =.05 on 7/23/16 and p=.8591, R^2 =.05 on 8/16/16).

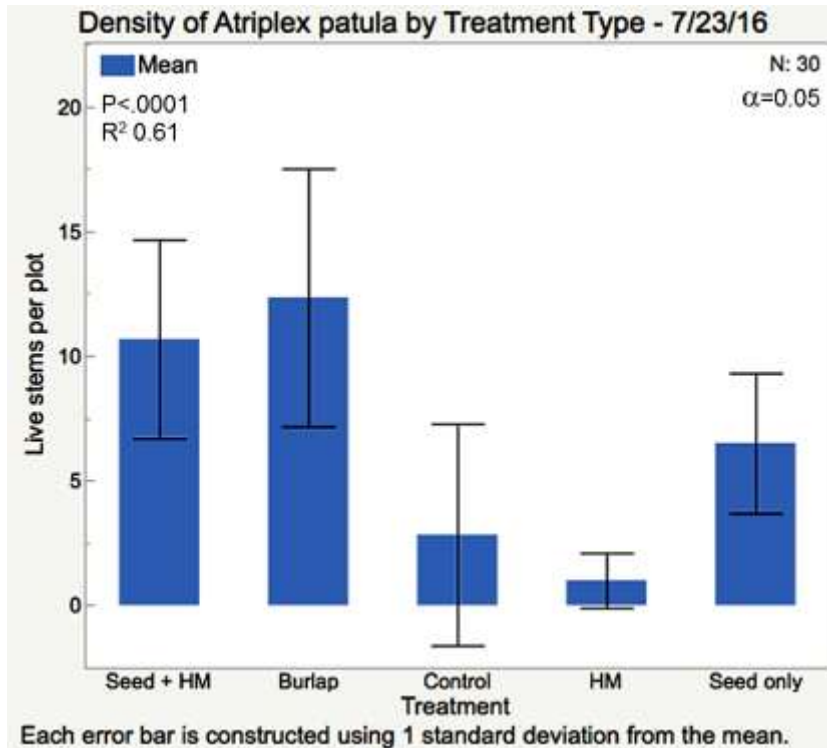


Figure 10.5 7/23/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

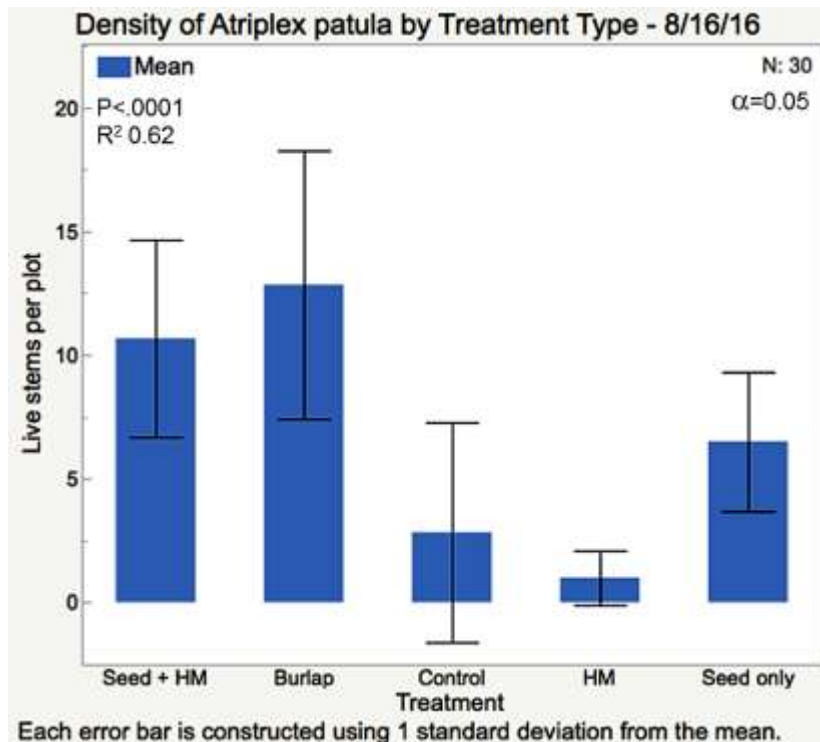


Figure 10.6 8/16/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.6 September 24, 2016

Again, ANOVA reveals significant differences in live stem densities of *A. patula* ($p=.0001$) between treatment groups. Two-tailed t-tests show greater seed + HM treatment stem densities ($\bar{x}=12.67$) than unseeded ($p=.0017$, $\bar{x}=3.00$) and HM ($p=.0003$, $\bar{x}=1.00$) controls. Burlap treatments also achieved significantly higher stem densities ($\bar{x}=14.50$) than unseeded ($p=.0003$) and HM ($p<.0001$) controls. Seed only plots continue to show lower stem densities ($\bar{x}=6.83$) than the previous two treatments ($p=.0437$ and $.0099$, respectively). Seed only treatments again are only higher than HM controls ($p=.0437$).

ANOVA reveals no significant differences in *A. prostrata* stem densities between treatments ($p=.8832$, $R^2=.04$).

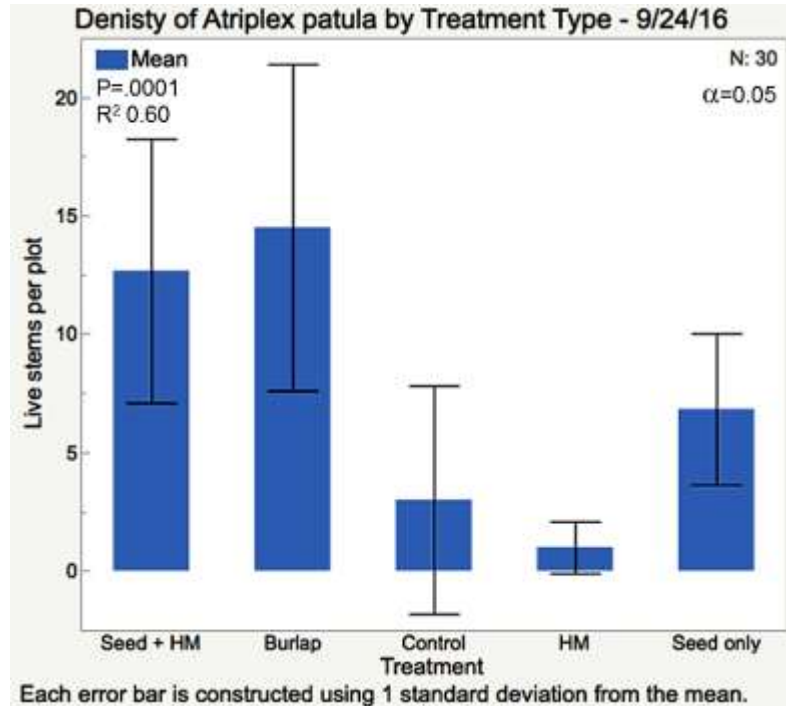


Figure 10.7 9/24/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments.

4.3.7 October 20, 2016

On this date most plants were almost fully senesced (dying, or entering dormancy). Plants were counted if they exhibited signs of life—dry, dead specimens were ignored. ANOVA showed significant differences between treatments ($p=.0007$). The burlap treatment contained significantly higher live stem densities ($\bar{x}=5.33$) than all other treatment types on this date. Two-tailed t-tests confirmed this when compared with unseeded controls ($p=.0003$, $\bar{x}=.83$), HM controls ($p<.0001$, $\bar{x}=.33$), seed + HM ($p=.0187$, $\bar{x}=2.67$), and seed only ($p=.0131$, $\bar{x}=2.50$) treatments. The seed + HM treatment still

exhibited higher live stem densities than HM controls ($p=.0372$), but was not significantly different from the unseeded controls ($p=.0962$) on this date.

A. prostrata again shows no significant differences between treatments ($p=.7359$, $R^2=.07$).

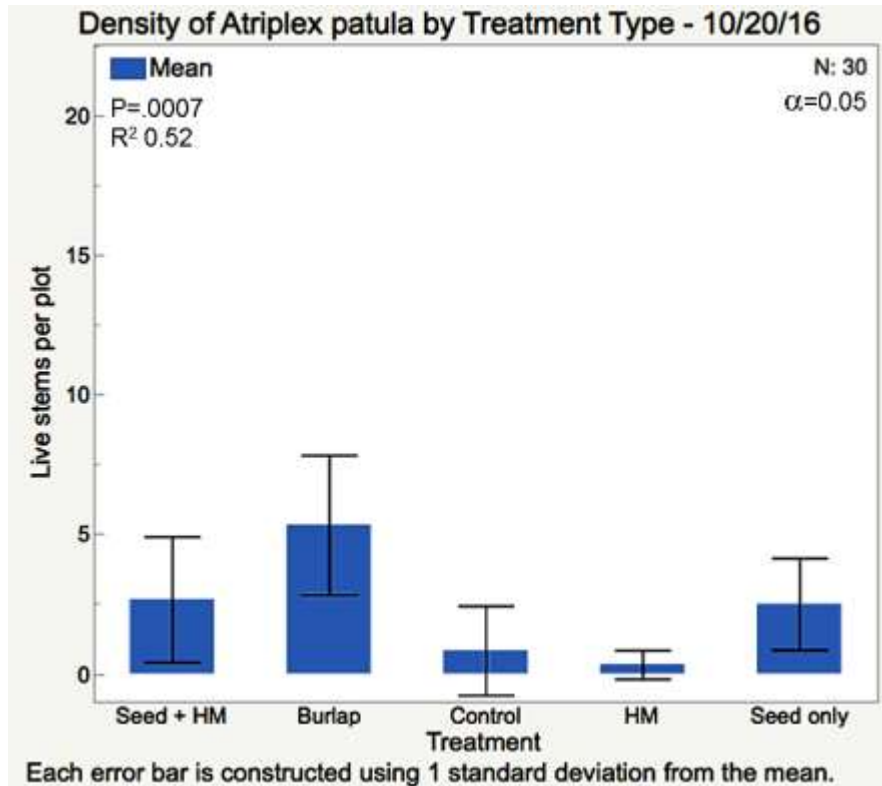


Figure 10.8 10/20/16 Establishment of *A. patula* in seed + hydromulch, burlap, and seed only treatment plots—compared with unseeded control and unseeded hydromulched control treatments. Lower densities reflect end of season plant senescence and die-off.

4.4 Natural Recruitment in Experimental Plots and Treatment Effects

4.4.1 Stem densities across treatments

Naturally recruited species colonized the experimental plots, and live stem counts were performed during data collection. Two species (*Salicornia virginica* and

Spergularia canadensis) occurred most commonly in the experimental plot area. A oneway ANOVA was performed to determine if stem densities were affected by the manipulative treatments. No significant differences were found between treatment groups for either species (Figure 11.1). In this experiment, it appears that applying hydromulch or burlap over the soil substrate did not affect natural recruitment of these two halophyte species. Potentially, this indicates that these treatments, when applied as part of a salt marsh restoration project, may not hinder natural recruitment of vascular plant species.

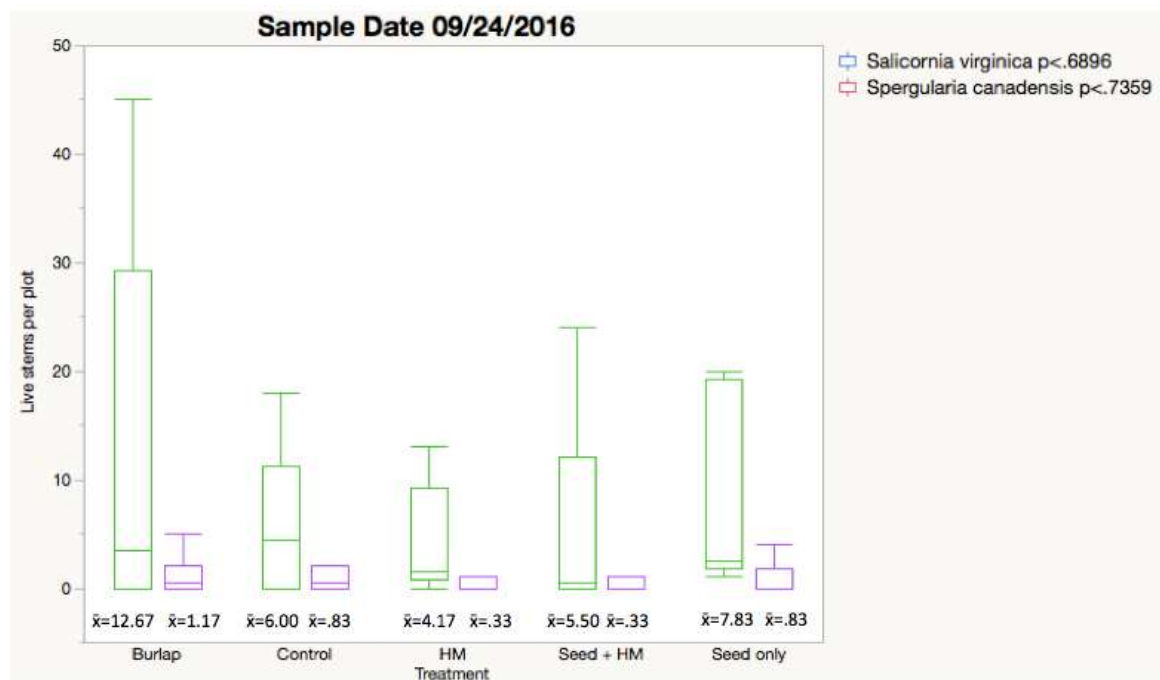


Figure 11.1 9/24/16 Establishment of the two most commonly occurring naturally recruited species: *S. virginica* (Rsquare=0.08) and *S. canadensis* (Rsquare=0.07) in each treatment group. Neither species appeared to have a relationship with any of the applied treatments.

4.4.2 Species richness and elevation

Species richness (i.e. how many different species occurred) data were collected for each treatment. Analysis was performed for data collected on September 24, 2016—species richness was highest on this date. A oneway ANOVA showed no significant

differences between number of species occurring in different treatment groups ($p=.9069$, $R^2=.03$). We can reason that species richness was not affected by the manipulative treatments in this experiment.

A regression analysis of species richness on the same date showed negative correlation with elevation (Figure 12.1)—higher richness occurred in plots with lower elevations. Naturally recruiting halophytic species could be occurring more commonly at lower elevations where higher soil salinities occur. Because this restoration site is in early stages of development, soil salinities will take some time to stabilize. Once that condition is reached, it will be interesting to examine how this relationship has changed.

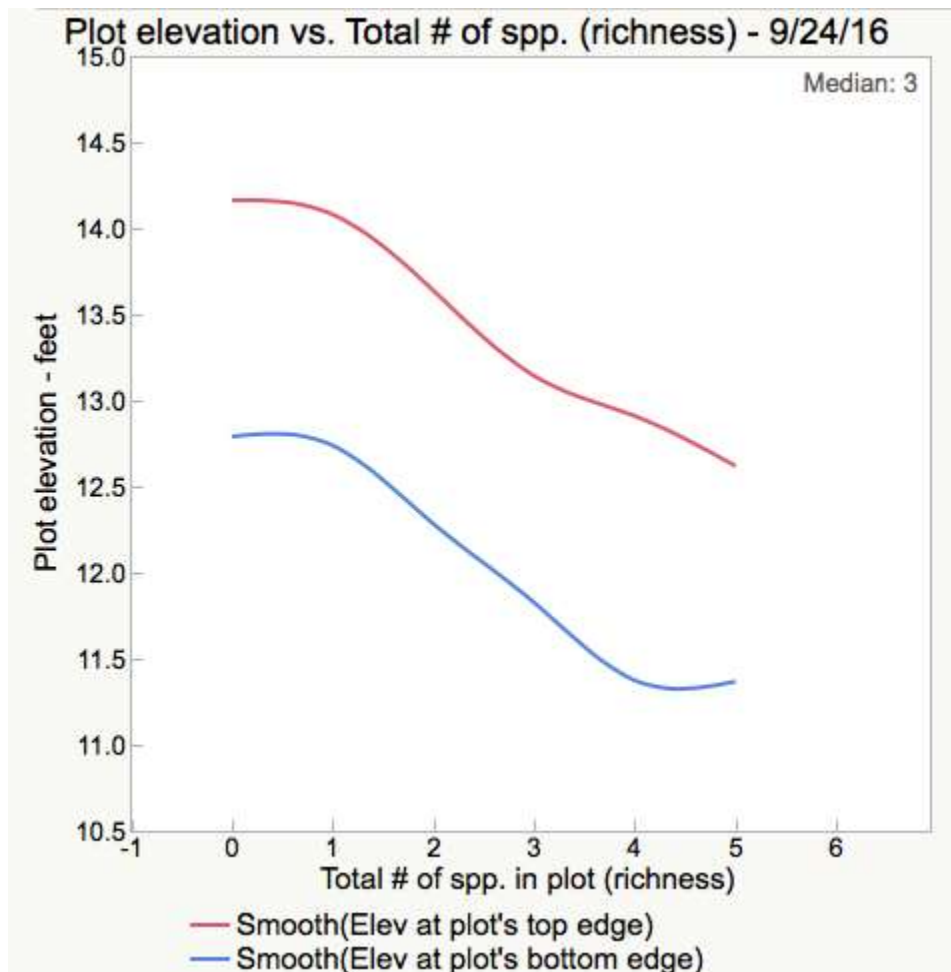


Figure 12.1 Species richness increased as elevation decreased ($R^2=0.44$, $p<.0001$), an unusual result. Significance was determined based on $p < 0.05$.

4.5 Soil Salinity

Soil salinity in all experimental plots was sampled on March 4, 2016 and fell within the freshwater range, below 0.5 ppt. In fact, all soil samples except for one—plot C1.3—tested below 0.1 ppt. A second sample was taken on February 14, 2017. These results showed an increase in soil salinity in the unseeded control plots—all samples fell between 0.5 and 0.75 ppt. See chart below (Figure 13.1) comparing soil salinity levels on the two sample dates.

Salt content is expressed in parts per thousand (ppt).

- 0.0 to 0.5 ppt = fresh water
- 0.6 to 30 ppt = brackish water
- >30 to 50 ppt = saline
- Seawater = 35 ppt
- Drinking water supply generally restricted to <0.5 ppt.
- Irrigation water must be less than 2 ppt or it will kill most crop plants.

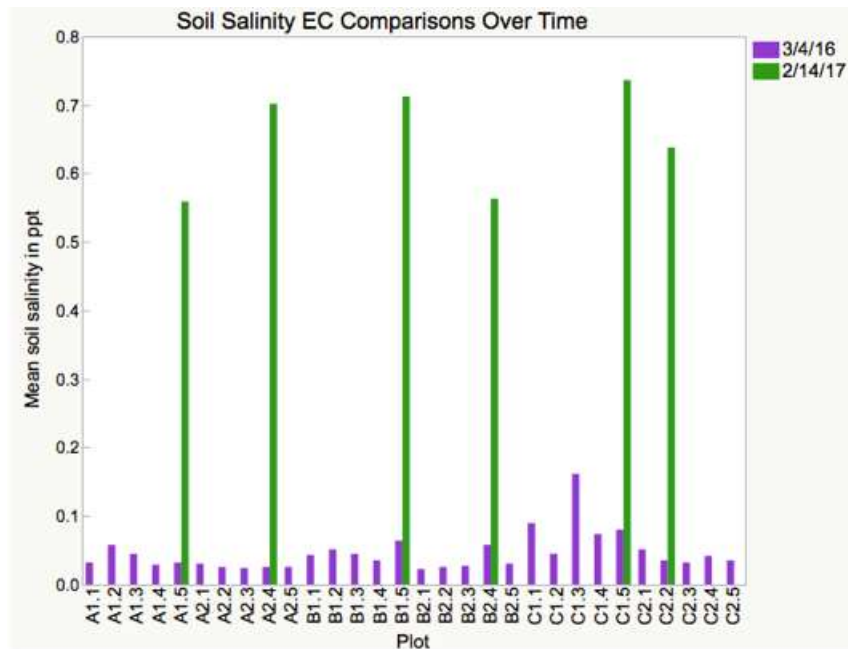


Figure 13.1 Soil salinity levels measured by electrical conductivity in parts per thousand (ppt). Samples in green were collected from the unseeded control plots.

4.5.1 Elevation and tidal inundation

After conversion to the Mean Lower Low Water (MLLW) datum, all plots fell between 8.48 and 12.12 feet in elevation. Using NOAA Tide Predictions charts from 2016 and 2017, each high tide above 12.1 feet was counted for Barron Point, Little Skookum Inlet Entrance tide station (Station ID 9446742) between the dates of 3/9/2016 and 2/14/2017. Between these two dates it was estimated that 530 high tide events completely submerged all experimental plots. As the new tidal channels were exposed to these tides, allowing seawater to absorb into the substrate, soil salinities rose (Figure 13.1). Soil salinities should be expected to continue rising over time until they stabilize. As soil becomes more saline, plant community compositions should shift toward vegetation types reflected in mature salt marshes.

4.6 Germination Test Results

A germination test of *A. patula* resulted in 83% of the seeds germinating quite rapidly—at 20°C germination began within 24 hours, peaked at four days, and ceased altogether after 11 days in the chamber. After 11 days, the temperature was raised to 25°C because *A. patula* germination was assumed to be nearly complete. At the conclusion of the germination test on 5/17/17, *Carex lyngbyei* and *Carex obnupta* showed low germination rates in freshwater conditions (3% and 8%, respectively), while *Schoenoplectus americanus* germinated at 41%.

Chapter 5: Discussion

5.1 Hydromulching onto Broadcast Seed—Success with *Atriplex patula*

Data from this experiment show a pattern potentially indicating a relationship between *Atriplex patula* plant establishment and the hydromulch treatment applied over broadcast seed. The oneway ANOVA for this species showed statistically significant differences of means between the six hydromulched samples and six unseeded control samples, for all monitoring dates. This may be promising for future restorations of tidal wetlands in which direct seeding of this species is desired. This species could be broadcast seeded mechanically or by hand during an ebb tide, and hydromulch applied directly after. The other four species did not yield results in this study. This could be due to several factors, which will be discussed in subsequent sections.

5.1.1 Identification difficulties of *Cyperaceae* species

The four species for which no data was collected were all in the *Cyperaceae* family. Species in this family are quite challenging to identify when very young, and can easily be confused with germinating grasses (*Poaceae* family). When graminoids present as germinants, it is very difficult to distinguish between key vegetative identification features such as stem shape and whether leaf sheaths are open or closed (Chase, Clark, & Pohl, 1996; Carex Working Group, 2014)—and of course floral features are lacking. Germinants were observed on several monitoring dates that may have been species belonging to either the *Cyperaceae* or *Poaceae* families. Because of their young age, identification to family was not determined.

While these plants in question appear on the first two monitoring dates, they had disappeared by June 2016, and did not reappear in experimental plots until August. It is speculated that unusually hot weather could have caused spring die-off of these germinants, drying and heating the soil beyond what the tender seedlings could handle. Perhaps a second, late emergence of germinants occurred that would account for the period with no observations. Temperatures reached the mid to high-eighties (°F) during a four-day period in mid-April, and in the first week of May temperatures rose to a record high of 97°F by 5/5/16. Germinants were noted on that date, but subsequently were absent until observed again starting on 8/16/16. Temperatures between May and August ranged generally between 60°F and 89°F, with three temperature events above 90°F for two or more days in a row during this time. Historical weather observations are based on readings from Sanderson Field in Shelton, WA (Weather History for KSHN, 2016).

5.1.2 Seed limitations

Quality of seeds and sourcing locations are critically important in restoration. Ideally, seed should be sourced as closely as geographically possible to the restoration site. This maintains local genetic diversity and is critical in avoiding propagation of genetic bottlenecks across the region. Seeds for this experiment were sourced from Inside Passage Seeds, a specialty native seed company focused on providing seeds from the Salish Sea bioregion and coastal areas ranging north and south. *A. patula*, *C. lyngbyei*, and *C. obnupta* were all collected within Jefferson County, Washington. *Eleocharis palustris* and *Schoenoplectus americanus* were collected in Benton and Lane Counties, Oregon, respectively.

Beyond seed quality, age and storage conditions can affect viability. Seed for this experiment were used within a month of receipt from Inside Passage Seeds, however seed used for the germination test had been stored for a year in cool, dark and dry conditions. Many species lose the ability to germinate as time passes or if exposed to high temperatures, but not all. Because four out of the five planted species were not observed during the first growing season at Bayshore Preserve, seeds were tested for viability in a germination chamber under controlled conditions. *E. palustris* was excluded from testing because it required temperatures above 27°C (Bartow, 2007) to germinate in a reasonable amount of time. *A. patula* seeds tested were from a more recently collected (2016) lot than seed used in the hydromulching experiment (collected 2015), and both lots were collected from the same location (Shomer, 2016).

5.1.3 *Controlled versus natural environments and seed germination*

While germination testing can show whether seeds are viable or not, it is important to note that the controlled environment of a germination chamber eliminates several factors present in natural environments. During this test, seeds received consistently adequate moisture throughout their germination phase and a non-variable light and temperature regime. Seeds were not subject to potential stressors like pests, pathogens, variable temperature and moisture inputs, tidal movement, or soil salinity fluctuations. All of these factors can impede germination in the field, and this test merely shows whether seeds of each species had the potential to germinate in the tidal channels.

The high rate (83%) of germination achieved for *A. patula* in controlled conditions reflects results in which Baskin & Baskin achieved rapid germination of the

species (2001). The seed lot used in our germination test was collected in 2016. The remaining lots of other species were collected in 2015. *S. americanus* achieved a 41% germination rate, while *C. lyngbyei* germinated at 3% and *C. obnupta* germinated at 8%. Because these three species were stored one year longer than *A. patula*, their viability could have been reduced. Additionally, factors such as heat exposure during transport may have reduced viability. Germination tests were conducted 12 months after the field experiment was installed, introducing a potential decrease in viability over this time.

While seed viability is generally expected to decrease over time, these seeds showed at least low levels of germination. These seeds were exposed to salinity stress—even though within their expected tolerance ranges—and other environmental factors immediately upon planting. As discussed, natural conditions make successful germination a gamble, and it seems the conditions on-site were less than perfect for all but *A. patula* seeds.

5.2 *Atriplex* spp. Treatment Responses

Though *A. patula* and *A. prostrata* were recorded as one species in early data collection², this mishap does begin to illuminate some interesting trends. The first monitoring date shows two plots supporting high numbers of *Atriplex* germinants: seed + hydromulch and the hydromulch only control groups. All the other treatments show very little germination on the first date (4/8/16). The second monitoring date (5/5/16) shows the hydromulch only control group supporting about the same amount of *Atriplex* spp. plants, but the numbers rise significantly for all the other treatments. On 6/1/16, when *A. patula* was positively identified and recorded separately from *A. prostrata*, we see the *A.*

² See footnote 1, section 4.1 for information regarding ambiguous identification of *A. prostrata*.

patula density in the seed + hydromulch and burlap treatments decrease somewhat, along with a notable decrease in the two control and seed only treatments. This general pattern persists throughout the data collection period for *A. patula*. Since we see the density numbers drop so drastically after *A. prostrata* was omitted, one could assume that the hydromulch only control plot supported more of this naturally recruiting species at the beginning of the season. Perhaps the hydromulch offered a more hospitable environment for seeds already present in the seed bank to germinate—potentially by retaining soil moisture during low tides (NRCS, 2003).

The *A. patula* density pattern reflected across the monitoring period shows the highest recruitment in the burlap rather than the seed + hydromulch treatment. Although the burlap treatment group did not show much germination at all on the first monitoring date, from May on it supported a higher density of plants than any other treatment. It is possible that the burlap itself may have slowed germination of *Atriplex*, blocking out light. It is likely, however, that small germinants were simply not observed in April because they were still underneath the fabric, and had emerged through by May. Overall, there was not a significant difference in the densities of *A. patula* that were supported between the burlap and seed + hydromulch treatments. Either way, both of the treatments did a good job of increasing the ability of *Atriplex patula* to establish, compared with the seed only treatments in the tidal channels. The USDA Natural Resource Conservation Service recommends mulch usage when seeding for most types of restoration projects, stating that they “reduce seed movement, mortality and predation, retain soil moisture and fertilizers, and reduce erosion (2003).” From the results attained in this experiment, it seems both types of “mulch” did a good job and it would be worth repeating the

experiment with other species, while calculating costs per square foot to determine the most cost-effective method.

5.3 Recommendations

Repeating this experiment with *A. patula* and other species is recommended. At Bayshore Preserve, second season (and beyond) data collection should determine survivability of *A. patula* past the first growing season to determine if this planting method augments longer-term development of the tidal wetland restoration—data should be compared with reference plots in areas of the tidal channels that did not receive these experimental treatments. An additional recommendation is identification of nursery grown stock of Inside Passage’s *A. patula* seed to positively ensure that data collected was indeed from planted seed (specimens are being grown at the time of this writing). In the future, if possible, it may be desirable to grow seed stock to maturation prior to experimentation to ensure positive species identification.

With future research of this method, it will be interesting to more closely study influential factors (precipitation, extreme tides, elevation, aspect, etc.) on hydromulch treatments more closely. For example, stratifying samples at different micro-elevations could provide finer resolution data regarding species recruitment and secondary effects of the hydromulch treatment on the soil substrate. It also seems wise to analyze chemical and physical effects of hydromulch on seawater to determine any potentially detrimental changes to water quality caused by this method. Monitoring soil development, benthic invertebrates, and performing fish counts in treated areas compared to reference sites would provide additional information related to functional development of revegetated

tidal wetlands, and aid in determining whether the methods are cost-effective into the future of the restoration site.

Conclusion

Estuaries are an essential part of earth's biosphere, providing unique habitat that once sustained fisheries, ecosystem services for humans such as flood mitigation, and critical habitat for wildlife. Estuary restoration efforts have increased since the establishment of the National Estuary Program, authorized by the Clean Water Act in 1987. The science of estuary restoration has evolved over the years, with many techniques developed and adaptively managed as scientists learn more about this dynamic ecosystem.

Revegetation techniques such as direct seeding have often produced less than ideal results, and producing plugs in a nursery and planting out can be costly. This thesis researched a novel method to amend direct seeding techniques—hydromulching on top of seeded areas to provide stabilization and buffer seed migration from tidal influence, with hopes to gain higher revegetation establishment than with direct seeding alone. Our hypothesis that hydromulch “seed anchoring” would increase germination and establishment for five native saltmarsh species was not fully substantiated. Only one of the planted species, *Atriplex patula*, was observed to have significantly greater establishment in this experiment. None of the planted *Cyperaceae* family species were positively identified as germinants or established plants during the course of this experiment.

Natural recruitment of several native saltmarsh species did occur in the first year post-dike removal time period in which this experiment took place. Prior estuary restoration researchers have often recommended allowing natural vegetation regeneration to occur instead of manipulating restoration sites, and long-term studies have shown

return of healthy ecosystem functioning without extra effort. However, if restoration biologists or organizations desire specific plant communities and have the resources to do some experimentation, this method could be worthy of continued research study. If found to be successful with certain plant species, hydromulching could provide a quick and easy revegetation method and allow organizations to stretch their project dollars to be as effective as possible.

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Station	Backsight (BS) meters	Height of Instrument (HI) meters	Foresight (FS) meters	Elevation in meters	Elev in feet	Remarks	Benchmark (BM) long, lat used in GPS	BM long, lat used in GPS	USGS National Map - Elevation Point Query Service coords	Adjusted BM elevation in NAVD88 (ft)	Difference	Adjusted elev - NAVD88 (ft)	Adjusted elev in MLLW (ft)
A	0.22			2.06	6.76	USGS BM CHA2 begin	47.24473, -123.04085	N47.24473, W123.04085		13.31	6.5514696		
		2.28							1/3 arc-second dataset	47.24774, -123.03905			
		2.28	0.39	1.89	6.20	A1.1 TOP					A1.1 TOP	12.75	10.41
		2.28	0.8	1.48	4.86	A1.1 BOTTOM					A1.1 BOTTOM	11.41	9.07
		2.28	0.48	1.8	5.91	A1.2 T					A1.2 T	12.46	10.12
		2.28	0.9	1.38	4.53	A1.2 B					A1.2 B	11.08	8.74
		2.28	0.55	1.73	5.68	A1.3 T					A1.3 T	12.23	9.89
		2.28	0.94	1.34	4.40	A1.3 B					A1.3 B	10.95	8.61
		2.28	0.57	1.71	5.61	A1.4 T					A1.4 T	12.16	9.82
		2.28	0.97	1.31	4.30	A1.4 B					A1.4 B	10.85	8.51
		2.28	0.52	1.76	5.77	A1.5 T					A1.5 T	12.33	9.99
		2.28	0.98	1.3	4.27	A1.5 B					A1.5 B	10.82	8.48
		2.28	0.35	1.93	6.33	A2.1 TOP					A2.1 TOP	12.88	10.54
		2.28	0.83	1.45	4.76	A2.1 BOTTOM					A2.1 BOTTOM	11.31	8.97
		2.28	0.45	1.83	6.00	A2.2 T					A2.2 T	12.56	10.22
		2.28	0.84	1.44	4.72	A2.2 B					A2.2 B	11.28	8.94
		2.28	0.36	1.92	6.30	A2.3 T					A2.3 T	12.85	10.51
		2.28	0.82	1.46	4.79	A2.3 B					A2.3 B	11.34	9.00
		2.28	0.39	1.89	6.20	A2.4 T					A2.4 T	12.75	10.41
		2.28	0.81	1.47	4.82	A2.4 B					A2.4 B	11.37	9.03
		2.28	0.36	1.92	6.30	A2.5 T					A2.5 T	12.85	10.51
		2.28	0.85	1.43	4.69	A2.5 B					A2.5 B	11.24	8.90
	0.25			2.03	6.66	USGS BM CHA2 end							
B	0.11			2.7	8.86	USGS BM CHB2 begin	47.24687, -123.03963	N47.24687, W123.03963					
		2.81											
		2.81	0.7	2.11	6.92	B1.1 TOP					B1.1 TOP	13.47	11.13
		2.81	1.24	1.57	5.15	B1.1 BOTTOM					B1.1 BOTTOM	11.70	9.36
		2.81	0.76	2.05	6.73	B1.2 T					B1.2 T	13.28	10.94
		2.81	1.18	1.63	5.35	B1.2 B					B1.2 B	11.90	9.56
		2.81	0.75	2.06	6.76	B1.3 T					B1.3 T	13.31	10.97
		2.81	1.15	1.66	5.45	B1.3 B					B1.3 B	12.00	9.66
		2.81	0.7	2.11	6.92	B1.4 T					B1.4 T	13.47	11.13
		2.81	1.14	1.67	5.48	B1.4 B					B1.4 B	12.03	9.69
		2.81	0.73	2.08	6.82	B1.5 T					B1.5 T	13.38	11.04
		2.81	1.14	1.67	5.48	B1.5 B					B1.5 B	12.03	9.69
		2.81	0.48	2.33	7.64	B2.1 TOP					B2.1 TOP	14.20	11.86
		2.81	0.98	1.83	6.00	B2.1 BOTTOM					B2.1 BOTTOM	12.56	10.22
		2.81	0.58	2.23	7.32	B2.2 T					B2.2 T	13.87	11.53
		2.81	1.02	1.79	5.87	B2.2 B					B2.2 B	12.42	10.08
		2.81	0.56	2.25	7.38	B2.3 T					B2.3 T	13.93	11.59
		2.81	1.01	1.8	5.91	B2.3 B					B2.3 B	12.46	10.12
		2.81	0.5	2.31	7.58	B2.4 T					B2.4 T	14.13	11.79
		2.81	0.92	1.89	6.20	B2.4 B					B2.4 B	12.75	10.41
		2.81	0.49	2.32	7.61	B2.5 T					B2.5 T	14.16	11.82
		2.81	0.94	1.87	6.14	B2.5 B					B2.5 B	12.69	10.35
	0.17			2.64	8.66	USGS BM CHB2 end							
C	0.17			2.62	8.60	USGS BM CHC1 begin	47.24774, -123.03905	N47.24774, W123.03905					
		2.79											
		2.79	0.72	2.07	6.79	C1.1 TOP					C1.1 TOP	13.34	11.00
		2.79	1.03	1.76	5.77	C1.1 BOTTOM					C1.1 BOTTOM	12.33	9.99
		2.79	0.95	1.84	6.04	C1.2 TOP					C1.2 TOP	12.59	10.25
		2.79	1.24	1.55	5.09	C1.2 B					C1.2 B	11.64	9.30
		2.79	1.04	1.75	5.74	C1.3 T					C1.3 T	12.29	9.95
		2.79	1.25	1.54	5.05	C1.3 B					C1.3 B	11.60	9.26
		2.79	0.89	1.9	6.23	C1.4 T					C1.4 T	12.79	10.45
		2.79	1.19	1.6	5.25	C1.4 B					C1.4 B	11.80	9.46
		2.79	0.83	1.96	6.43	C1.5 T					C1.5 T	12.98	10.64
		2.79	1.16	1.63	5.35	C1.5 B					C1.5 B	11.90	9.56
		2.79	0.59	2.2	7.22	C2.1 TOP					C2.1 TOP	13.77	11.43
		2.79	0.96	1.83	6.00	C2.1 BOTTOM					C2.1 BOTTOM	12.56	10.22
		2.79	0.53	2.26	7.41	C2.2 T					C2.2 T	13.97	11.63
		2.79	0.95	1.84	6.04	C2.2 B					C2.2 B	12.59	10.25
		2.79	0.51	2.28	7.48	C2.3 T					C2.3 T	14.03	11.69
		2.79	0.95	1.84	6.04	C2.3 B					C2.3 B	12.59	10.25
		2.79	0.38	2.41	7.91	C2.4 T					C2.4 T	14.46	12.12
		2.79	0.81	1.98	6.50	C2.4 B					C2.4 B	13.05	10.71
		2.79	0.5	2.29	7.51	C2.5 T					C2.5 T	14.06	11.72
		2.79	0.86	1.93	6.33	C2.5 B					C2.5 B	12.88	10.54
	0.16			2.63	8.63	USGS BM CHC1 end							

Table A 1: Differential leveling plot elevation data. Adjusted elevations to local tidal datum (MLLW) in right-most column.

Soil Samples collected by Allie Denzler, Josh Carter, Brendan Duffy on March 4 2016.													
Plot	g/100ml	Factor	mS/Cm Reading	Adjusted value	ppm	ppt	g/100ml	Factor	mS/Cm Reading	Adjusted value	ppm	ppt	Mean soil salinity (ppt)
A1.1	20	1	0.05	0.050	32	0.0320	20	1	0.05	0.050	32	0.0320	0.032
A1.2	20	1	0.09	0.090	57.6	0.0576	20	1	0.09	0.090	57.6	0.0576	0.058
A1.3	20	1	0.07	0.070	44.8	0.0448	20	1	0.07	0.070	44.8	0.0448	0.045
A1.4	20	1	0.04	0.040	25.6	0.0256	20	1	0.05	0.050	32	0.0320	0.029
A1.5	20	1	0.05	0.050	32	0.0320	20	1	0.05	0.050	32	0.0320	0.032
A2.1	20	1	0.045	0.045	28.8	0.0288	20	1	0.05	0.050	32	0.0320	0.030
A2.2	20	1	0.04	0.040	25.6	0.0256	20	1	0.04	0.040	25.6	0.0256	0.026
A2.3	20	1	0.04	0.040	25.6	0.0256	20	1	0.035	0.035	22.4	0.0224	0.024
A2.4	20	1	0.04	0.040	25.6	0.0256	20	1	0.04	0.040	25.6	0.0256	0.026
A2.5	20	1	0.04	0.040	25.6	0.0256	20	1	0.04	0.040	25.6	0.0256	0.026
B1.1	20	1	0.07	0.070	44.8	0.0448	20	1	0.065	0.065	41.6	0.0416	0.043
B1.2	20	1	0.09	0.090	57.6	0.0576	20	1	0.07	0.070	44.8	0.0448	0.051
B1.3	20	1	0.07	0.070	44.8	0.0448	20	1	0.07	0.070	44.8	0.0448	0.045
B1.4	20	1	0.05	0.050	32	0.0320	20	1	0.06	0.060	38.4	0.0384	0.035
B1.5	20	1	0.1	0.100	64	0.0640	20	1	0.1	0.100	64	0.0640	0.064
B2.1	20	1	0.04	0.040	25.6	0.0256	20	1	0.03	0.030	19.2	0.0192	0.022
B2.2	20	1	0.04	0.040	25.6	0.0256	20	1	0.04	0.040	25.6	0.0256	0.026
B2.3	20	1	0.045	0.045	28.8	0.0288	20	1	0.04	0.040	25.6	0.0256	0.027
B2.4	20	1	0.09	0.090	57.6	0.0576	20	1	0.09	0.090	57.6	0.0576	0.058
B2.5	20	1	0.045	0.045	28.8	0.0288	20	1	0.05	0.050	32	0.0320	0.030
C1.1	20	1	0.14	0.140	89.6	0.0896	20	1	0.14	0.140	89.6	0.0896	0.090
C1.2	20	1	0.07	0.070	44.8	0.0448	20	1	0.07	0.070	44.8	0.0448	0.045
C1.3	20	1	0.26	0.260	166.4	0.1664	20	1	0.245	0.245	156.8	0.1568	0.162
C1.4	20	1	0.12	0.120	76.8	0.0768	20	1	0.11	0.110	70.4	0.0704	0.074
C1.5	20	1	0.13	0.130	83.2	0.0832	20	1	0.12	0.120	76.8	0.0768	0.080
C2.1	20	1	0.08	0.080	51.2	0.0512	20	1	0.08	0.080	51.2	0.0512	0.051
C2.2	20	1	0.06	0.060	38.4	0.0384	20	1	0.05	0.050	32	0.0320	0.035
C2.3	20	1	0.06	0.060	38.4	0.0384	20	1	0.04	0.040	25.6	0.0256	0.032
C2.4	20	1	0.07	0.070	44.8	0.0448	20	1	0.06	0.060	38.4	0.0384	0.042
C2.5	20	1	0.06	0.060	38.4	0.0384	20	1	0.05	0.050	32	0.0320	0.035

Table A 2: Soil salinity electrical conductivity data for samples collected 3/4/16, run in duplicate.

Samples collected by Allie and Conrad on Feb 13-14, 2017							
Plot	g/100ml	Factor	mS/Cm Reading	Adjusted value	ppm	ppt	Avg ppt for entire plot
A1.5 U	20	1	0.75	0.750	480	0.4800	
A1.5 M	20	1	0.97	0.970	620.8	0.6208	0.5589
A1.5 L	20	1	0.9	0.900	576	0.5760	
A2.4 U	20	1	1.24	1.240	793.6	0.7936	
A2.4 M	20	1	1.35	1.350	864	0.8640	0.7019
A2.4 L	20	1	0.7	0.700	448	0.4480	
B1.5 U	20	1	0.77	0.770	492.8	0.4928	
B1.5 M	20	1	1.25	1.250	800	0.8000	0.7125
B1.5 L	20	1	1.32	1.320	844.8	0.8448	
B2.4 U	20	1	0.88	0.880	563.2	0.5632	
B2.4 M	20	1	0.82	0.820	524.8	0.5248	0.5632
B2.4 L	20	1	0.94	0.940	601.6	0.6016	
C1.5 U	20	1	1.31	1.310	838.4	0.8384	
C1.5 M	20	1	1.16	1.160	742.4	0.7424	0.7360
C1.5 L	20	1	0.98	0.980	627.2	0.6272	
C2.2 U	20	1	0.89	0.890	569.6	0.5696	
C2.2 M	20	1	1.06	1.060	678.4	0.6784	0.6379
C2.2 L	20	1	1.04	1.040	665.6	0.6656	

Table A 3: Soil salinity electrical conductivity data for samples collected 2/14/17.

Control Plot	Mean soil salinity ppt - 03/04/2016	Mean soil salinity ppt - 02/14/2017
A1.4	0.03	0.56
A2.4	0.03	0.70
B1.5	0.06	0.71
B2.4	0.06	0.56
C1.5	0.08	0.74
C2.2	0.04	0.64

Table A 4: Comparison of soil salinities in unseeded control plots on two dates.

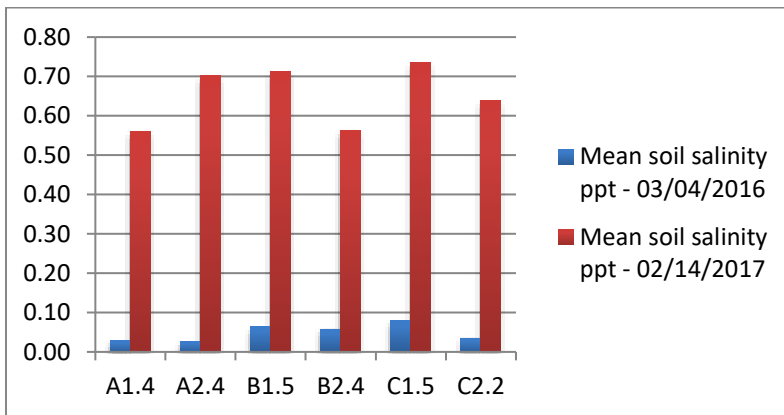


Figure A 1: Graphical comparison of soil salinities in unseeded control plots on two dates.

Appendix A

Materials

Easy Gardener brand 100% natural burlap fabric. Address: easy gardener products, inc., p.o. box 21025, Waco, tx 76702-1025

Rainier Fiber™ Premium Wood Fiber Mulch For Hydroseeding and Erosion Control. Net Wt. 50 lbs/22.7 kg. Manufactured by: Rainier Veneer Inc. P.O. Bos 1250 Graham, Wash. 98338. 253-846-0242. Date stamp on bag: 07/21-15 15:37.

SPECIFICATION	TEST METHOD	TEST RESULTS
Moisture content	ASTM D 644	12% ± 3%
Organic matter (minimum)	ASTM D 586	98%
Ash content (maximum)	ASTM D 586	2%
Water holding capacity (minimum)	ASTM D 7367	1300%
pH @ 3% fiber concentration	SW846 9045	4.5 ± 0.5
Color	Observed	Green

Mixing instructions

All Rainier products are smooth loading wood fiber mulches with load rates up to 25 bags per 3000 gallons of water or 50 lbs per 120 gallons of water. Loading rates may vary depending on the type of machine and its working capability.

Mixing instructions:

1) Mechanically agitated hydroseeders:

- A. Fill tank with water to bottom of agitator shaft.
- B. Start agitation
- C. Keep water running while adding fiber until proper amount is reached.
- D. Mix slurry approximately 5 minutes for Rainier Fiber. Mix for minimum of 8 minutes for +Tac, BFM, SMM and Supreme to fully activate the additives.

2) Before spraying:

Slow agitator speed down to approximately ¼ speed—just fast enough to keep the slurry in an active rolling mix. Do not run agitators fast or they will bear air in the slurry. Slowing down the agitators will help avoid cavitation problems.

Appendix B

Contact Information

- Bayshore Preserve
3800 WA-3
Shelton, WA 98584
- Capitol Land Trust
4405 7th Ave SE, Suite 306
Lacey, WA 98503
(360) 943-3012
info@capitolandtrust.org
- Inside Passage Seeds
P.O. Box 639
Port Townsend, WA 98368
1-800-361-9657
(360) 385-6114
forest@insidepassageseeds.com
- Hoyt's Hydroseeding
Tahuya, WA
360-204-3053
steve@hoytshydroseeding.com
- Mason Conservation District
450 W. Business Park Rd.
Shelton, WA 98584
(360) 427-9436
<https://www.masoncd.org/>