

HAWAIIAN COASTAL WETLANDS:
GERMINATION AND EARLY GROWTH OF FIVE NATIVE HAWAIIAN COASTAL
SPECIES AND THE INVASIVE *BATIS MARITIMA*

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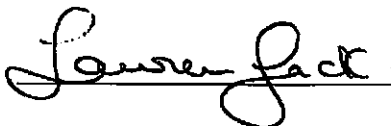
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Abstract

The effects of salinity and flooding on germination and early growth of five native Hawaiian coastal species was conducted to determine whether these native species would be appropriate for restoration at the Waiawa Unit of the Pearl Harbor National Wildlife Refuge. Soil salinity and soil moisture samples were investigated to determine if any gradients existed at the site. In addition, the effects of salinity and flooding on the germination and early growth of the invasive species, *Batis maritima*, was also investigated. The invasive species, *Batis maritima*, and the native species *Sesuvium portulacastrum* exhibited the highest salinity tolerances during germination and early growth, but all species were appropriate for replanting at the site. In addition, a competition experiment between *Batis maritima* and *Sesuvium portulacastrum* was conducted to determine if salinity or flooding affected the competitive ability of either species.

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Chapter 1. General Introduction to Hawaiian Wetlands and Proposed Research

Introduction

What is a Wetland?

Wetlands are a major feature of the landscape in almost all parts of the world (Mitsch and Gosslink 1993). One of the most widely accepted definitions by wetland scientists in the United States is presented in *Classification of Wetlands and Deepwater Habitats of the United States* (Cowardin and Carter 1979, Mitsch and Gosslink 1993).

“Wetlands are the lands transitional between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water...

Wetlands must have one or more of the following three attributes: (1) at least periodically, the land supports predominantly hydrophytes, (2) the substrate is predominantly undrained hydric soil, and (3) the substrate is non-soil and is saturated with water or covered by shallow water at some time during the growing season of each year.”

Wetland Change in the Continental United States

North America has always had an abundance and diversity of wetlands. However, the wetlands that exist now may only represent a fraction of those seen by pioneers as they advanced across the continent 200 years ago (Mitsch and Gosslink 1993). In the middle to late 20th century, few people were aware of the term wetlands; even fewer people were aware of the significance of wetlands to the physical, cultural, and biological environment. Wetlands were long regarded as mosquito-ridden wastelands providing

little or no benefit to our environment or economy. In the continental United States they were considered a hindrance to settlement, a nuisance that needed to be eliminated in order to develop agricultural land (Elliott 1981, Wallace 1985).

Farmers initially bypassed wetlands in favor of dry lands with a good supply of water and trees in the continental United States. Through legislation such as the Swampland Acts of 1849, 1850, 1860, and 1882, government subsidies were given for drainage of millions of acres of wetlands (Elliott 1981). Numerous agencies were involved in the conversion of wetlands to agricultural land. Between 1929 and 1974, the Army Corps of Engineers constructed flood control projects that affected nearly 5.5 million acres in the lower Mississippi alluvial plain (Interior October 1988). Federal assistance to drain wetlands made it economically feasible to expand agricultural production. As a result, over half (53%) of the wetlands in the lower 48 states were lost between the late 1700's and mid 1980's (Mitsch and Gosslink 1993).

Early Uses of Hawaiian Wetlands

Very little is known about Hawaiian intertidal wetlands that existed prior to human settlement, but these wetlands were modified beginning with the arrival of Polynesians 1500 or more years ago (Kirch 1982, Allen 1998). When Polynesians first arrived in Hawai'i, they settled along the coasts. As planters, they fire cleared the lowlands to build house sites and to grow their introduced crops, such as Kalo (*Colocasia esculenta*) (Eyre 2000).

Alterations of these coastal plant communities may have been massive, resulting in little remaining natural vegetation in this zone (Cuddihy and Stone 1990). Salt production, development of agricultural and aquacultural systems (construction and

maintenance of fishponds), and the use of native vegetation for crafts (such as niihau mats from makaloa sedge) were some of the early uses of wetlands. These agriculture and aquaculture systems were developed in areas that were originally low-lying uplands, natural wetlands, or shallow open water (Kikuchi 1976, Kirch 1982, Allen 1998). Wagner et al (1999) supports the notion that native Hawaiian wetland communities were disturbed by the ancient Hawaiians because nutrient rich flood plains, marshes, and lowland riparian areas were largely converted into fishponds or irrigated farmlands for cultivation of Kalo (*Colocasia esculenta*).

With the arrival of European and Asian immigrants, land use and land cover in Hawai'i began to change. Early uses of wetlands gradually gave way to those that persist today. Wetland-based agriculture and aquaculture declined, except for rice production to support the needs of Asian immigrants. Western values and population increase accelerated urban development, particularly along the coast where many wetlands were concentrated. Waikiki, Ala Moana, Salt Lake, Kuapa Pond, Kaelepulu Pond, and Keehi Lagoon are examples of wetland areas on O'ahu that supported Kalo, fishponds, and native water birds, but have since been replaced with urban land use (Elliott 1981).

Values of Wetlands

Wetland functions depend on the location, size, various ecosystem characteristics of the wetland, and also upon the nature and degree of human intervention (Gopal 1995). These low-lying areas have the capacity to detain and retain water. Capture of water from uplands delays downstream flow, thereby preventing lateral and downstream flooding as well as minimizing bank erosion (Gopal 1995). Coastal wetlands, especially lagoons and backwaters, also help in mitigating the effects of flooding by tides and minimizing storm

damage. In Hawai'i, mangrove swamps have developed in sediment-laden areas such as the southern coast of Molokai, Pearl Harbor, and Kaneohe Bay; these swamps can filter out excess nutrients and other pollutants before they enter the open ocean (Elliott 1981).

Wetlands are some of the most biologically productive natural ecosystems in the world. Beneficial effects of wetland primary productivity include the provision of food and shelter for fish, shellfish, other invertebrates, microorganisms, mammals, reptiles, and birds (EPA 2001). In Hawai'i, migratory and resident water birds find habitat in low elevation wetlands (Elliott 1981).

Types of Wetlands

Due to the abundance and diversity of wetlands, a number of common terms have been used over the years to describe different types of wetlands such as bog, swamp, vernal pool, etc. The classification scheme used in the United States, as part of the National Wetlands Inventory, is all-encompassing but also very complex. Generally, wetland ecosystems can be classified into two groups: coastal and inland wetlands (Gopal 1995).

The focus of this research project is on coastal wetlands, which are influenced by alternate floods and ebbs of tides. There are three types of coastal wetland ecosystems: (1) tidal salt marshes are found throughout the world, along protected coastline in which plant and animals have adapted to the stresses of salinity, periodic inundation, and extremes in temperature, (2) tidal freshwater marshes are inland of the tidal salt marshes; they still experience tidal effects, but lack the salinity stress, and (3) mangrove wetlands occur in subtropical and tropical regions of the world. Salt tolerant trees dominate mangrove wetlands, which occur in wide range of salinity levels and tidal influences

(Mitsch and Gosslink 1993). Hawaiian coastal wetlands may include mud flats, salt flats, mangrove swamps, pickleweed (*Batis maritima*) marshes, and fishponds, all occurring together with subtle gradations. (Elliott 1981).

Hawaiian (O‘ahu) Wetlands and Associated Vegetation

A variety of wetland types are found on the Hawaiian Islands; coastal wet communities (e.g. coastal wet herblands, coastal wet sedgeland, coastal wet shrublands, and coastal wet forests) occur on Laysan, French Frigate Shoals and all of the main Hawaiian Islands except Lana‘i and Kaho‘olawe (Wagner et al. 1999). The remaining natural vegetation is limited in coastal regions of Hawai‘i (Cuddihy and Stone 1990) and most of these remaining wetland areas are vulnerable to displacement by urbanization, resort development, and agriculture (Wagner et al. 1999). On O‘ahu, the most populated of the Hawaiian Islands, many coastal and inland wetlands have been destroyed due to urban development (Elliott 1981). Despite their importance in Hawai‘i, low elevation wetlands have received limited treatment in the literature.

It has been suggested that prior to the arrival of humans, intertidal wetlands in Hawai‘i had few species of vascular plants. The aquatic plant *Ruppia maritima* L. could be found in the lowest portions of the intertidal zone and herbaceous or small shrub species such as *Heliotropium curassavicum*, *Lycium sandwicense*, and *Sesuvium portulacastrum* were present at the upper end of the intertidal zone (Gagne and Cuddihy 1990), but the bulk of the intertidal zone may have been inhabited primarily by algae, microbes, bacteria, and viruses (Egler 1947).

Egler (1947) suggested that the following successional stages have occurred in many Hawaiian intertidal areas after Western discovery: (1) pre-Hawaiian and Hawaiian

communities of widgeon grass (*Ruppia maritima*) and various algae, (2) the introduction of pickleweed (*Batis maritima*) sometime prior to 1859 and subsequent development of pure *Batis maritima* meadows, (3) introduction and spread of red mangrove (*Rhizophora mangle*) onto the meadows, and (4) eventual replacement of *Batis maritima* meadows by mangroves (Allen 1998).

Paleobotanical studies can supplement written documentation and provide great insight on the prehistoric vegetation of Hawaiian lowlands. Palynological data indicate that plants such as Hala (*Pandanus tectorius*), Naupaka (*Scaevola sericea*) and Pa'ūohi'iaka (*Jacquemontia ovalifolia*), all currently existing in coastal and strand vegetation communities, were present in the mid-Holocene (Burney et al. 2001).

The prevalence of exotic species in Hawai'i's low elevation wetlands is evident when examining Elliot's 1981 plant species list for surveyed wetlands. Elliot's 1981 study revealed that ten plant species are more prevalent than others in low-elevation inland and coastal wetlands of Hawai'i, including: *Bacopa monnieri* (Water hyssop), *Batis maritima* (Pickleweed), *Brachiaria mutica* (California Grass), *Commelina diffusa* (Honohono), *Hibiscus tiliaceus* (Hau), *Ludwigia octovalvis* (Primrose willow), *Pluchea indica* (Indian pluchea), *Pluchea odorata* (Sour Bush), *Scirpus validus* (Great Bulrush), and *Rhizophora mangle* (American mangrove).

Batis maritima, *Brachiaria mutica*, *Commelina diffusa*, *Ludwigia octovalvis*, *Pluchea indica*, *Pluchea odorata*, and *Rhizophora mangle* are non-native species; *Bacopa monnieri*, *Hibiscus tiliaceus*, and *Scirpus validus* are native to Hawai'i, but none are endemic. Many of these exotic and indigenous species are considered weedy and aggressive in certain areas. Pickleweed and mangrove are the two most common coastal

wetland plants found in Hawai'i and are commonly associated with one another, with mangroves forming the seaward portion of the coastal wetlands and pickleweed occupying the more landward portion (Elliott 1981).

Mangroves, especially *Rhizophora mangle*, have become well established and currently occupy much of the intertidal habitat. They are having some beneficial contributions such as sediment retention and water quality improvement in Hawai'i (Drigot 2001). Nevertheless, negative impacts, such as the colonization of other alien plants and loss of important foraging and nesting habitat of endemic and endangered water bird species, have also occurred (Allen 1998).

European-introduced plant species, such as *Batis maritima*, became dominant in the intertidal zone simultaneous with the physical modification of wetlands (Egler 1947, Elliott 1981, Allen 1998). The principal community type, coastal wet herblands, is represented now by a single alien community, *Batis maritima*, occurring on Kaua'i, O'ahu, Moloka'i, and Maui. This species was first reported in the Hawaiian Islands in 1859 by Hillebrand (1888) and has since spread widely, likely displacing native communities such as *Sesuvium portulacastrum* herbland. The vegetation of coastal wet herblands is now dominated by alien species, with almost complete displacement of native species (Wagner et al. 1999).

Invasive Species: *Batis maritima*

Certain plants are viewed as undesirable because of their negative impact on wildlife and because they are aggressive competitors (Mitsch and Gosslink 1993). The U.S. Federal government definition regarding invasive species was provided in Executive Order 13112 signed by President William Clinton on February 3, 1999. An "invasive

species" is defined as an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health (<http://www.invasivespecies.org/resources/DefineIS.html>).

Invasive plants can be problematic in restoration projects because of their rapid growth and ability to take advantage of unused resources (Cronk and Fuller 1995). Nevertheless, "alien invaders were not statistically more likely to have higher growth rates, competitive ability, or fecundity than their native counterparts. The relative performance of invaders and co-occurring natives often depend on growing conditions"(Daehler 2003).

Batis maritima, also called 'akulikuli-kai, was accidentally introduced and was first collected on Sand Island in 1859; it is now abundant along coastal areas on all of the main Hawaiian Islands. It is native to tropical and subtropical America as well as the Galapagos islands and is commonly known as saltwort or pickleweed. It is highly salt tolerant and rapidly colonizes moist soil and shallow water such as mudflats, fishponds, salt marshes, the upper edge of tidal flats, and the edge of mangrove stands (Stemmermann 1981, Nelson 1996, Schlucker 2003).

These short but monotypic stands of pickleweed exclude shorebirds and water birds from foraging or nesting on the mudflats. Furthermore, excessive pickleweed provides cover for alien mammalian predators like rats, cats, and mongoose (Rauzon and Drigot 2002). Presently, a dense *Batis maritima* mat dominates the upper intertidal zone at the Waiawa unit, Pearl Harbor National Wildlife Refuge (NWR) and allows little space or light for any species to grow under it (personal observation).

Batis maritima is being controlled at various sites around Hawai'i, including Nu'upia ponds Marine Corps Base Hawai'i on O'ahu as well as at Kealia Ponds NWR on Maui. Monitoring of areas where mangroves have recently been removed (Nu'upia Ponds Wildlife Management Area) show that systematic pickleweed control has been crucial in maintaining open habitat for stilt feeding, loafing, and nesting. Mangrove removal at the Waiawa unit is intended to increase foraging habitat and this goal may be compromised by the spread of *Batis maritima*.

Wetland Restoration in Hawai'i

Alien species in Hawaiian coastal wetlands have received little attention in the past (Cuddihy and Stone 1990, Rauzon and Drigot 2002), but recently there has been an interest in controlling invasive species and restoring native vegetation to wetland sites (Brimacombe 2002). Many state, federal and non-profit agencies have begun to implement wetland restoration projects in Hawai'i, but rarely has information regarding restoration design and results been published. Encouraging results have been achieved in some areas; native plants have been found to spread and colonize in response to removal of non-native vegetation. Unfortunately, many of these areas are not being regularly monitored, so it is unknown whether these native species will persist. Presumably, control of non-native vegetation is essential to maintain these populations (Brimacombe 2002).

A handful of restoration efforts have been initiated on the main islands of Hawai'i. O'ahu and Maui have the highest number of restoration efforts including projects at Hamakua Marsh, Kawainui Marsh, Marine Corps Base Hawai'i (Kaneohe Bay), Pouhala on O'ahu, Kealia Pond, Kanaha Pond, Ahihi Kinau, Cape Hanamanioa,

and Ulupalakua Ranch on Maui. The majority of these efforts include removal of alien plant species including *Batis maritima*, *Brachiaria mutica*, *Pluchea indica*, *Rhizophora mangle*, and *Schinus terebinthifolius* (Brimacombe 2002) .

Some of the most commonly used restoration plants include: *Bolboschoenus maritimus*, *Cyperus laevigatus*, *Cyperus javanicus*, *Dodonaea viscosa*, *Gossypium tomentosum*, *Jacquemontia ovalifolia* , *Pandanus tectorius*, *Sida fallax*, *Sesuvium portulacastrum*, *Vitex rotundifolia*, and *Wikstroemia uva-ursi*, (Brimacombe 2002).

While these plants have been used in several restoration projects in the Hawaiian Islands, results have varied. Plants have flourished at some sites including the native riparian demonstration garden at the Youth Activities Center, Puha Gardens at the Marine Corps Training Area Bellows (SRGII 2001), and Kanaha Pond on Maui (Brimacombe 2002). Plants experienced rapid deterioration at other sites leading to death soon after replanting (e.g. the native riparian demonstration gardens at the Muliwai site on the Marine Corps Base on Mokapu Peninsula) (Brimacombe 2000).

United States Fish and Wildlife Service Mangrove Removal Project

“The Recolonization and Succession of Wetland Communities” is a Sea-Grant-funded project assessing the impact of mangrove removal on coastal Hawaiian ecosystems. The project’s objective is to remove four acres of introduced mangrove in the Waiawa unit, Pearl Harbor National Wildlife Refuge (NWR) in Middle Loch Pearl Harbor. To restore natural wetland habitat, both sediment flats and native Hawaiian plants will then be re-established in the coastal zone. To facilitate rapid re-establishment of native Hawaiian plant species and restoration of native habitat after mangrove removal, outplanting of native wetland species is desirable (Smith 2001).

The U.S. Fish and Wildlife Service (U.S. FWS) intends to restore native plants at the Waiawa unit. Unfortunately, there is a minimal amount of information about species selection, placement, planting time and optimal conditions for germination and early growth of many native Hawaiian coastal plant species.

The removal of fringe mangroves is supposed to enhance the coastal habitat for endangered Hawaiian Stilts and migratory birds, but it may initially promote the colonization of opportunistic invasive species (Smith 2001). Allen (1998) suggests that mangrove presence in the intertidal zone is a major factor limiting the potential for restoration of native wetland ecosystems, but other aggressive alien species such as *Batis maritima* would also need to be controlled.

Waiawa Unit

The Pearl Harbor NWR is managed by the U.S. FWS as a breeding area for endangered Hawaiian water birds and is located in the northwestern corner of the Pearl City Peninsula. The wildlife refuge is separated into two major diked pond areas and was previously connected to the Waiawa Springs complex (Appendix D). There is very little information about the Pearl Harbor NWR regarding potential soil salinity gradients, seasonal fluctuations of salinity, soil texture, moisture, or nutrient availability.

Vegetation along the Pearl Harbor shoreline was characterized by Hosaka (1937) as a *Batis-Bolboschoenus (Scirpus) maritimus* (pickleweed-bulrush) community. Photographs dating between 1900 and 1920, prior to the introduction of mangroves, provide evidence of other native plant species, not mentioned by Hosaka including *Schoenoplectus (Scirpus) lacustris* subsp. *validus* and *Sesuvium portulacastrum* (Englund and Preston 2000).

Plant Species Background

Bolboschoenus maritimus, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* were selected for germination and early growth studies, performed in the laboratory and greenhouse, in order to assess the potential of these species for restoration at the Waiawa unit of Pearl Harbor NWR, Waiawa. All species are indigenous to Hawai'i and occur in coastal wetland areas on the leeward side of O'ahu.

Previous research showed that *Bolboschoenus maritimus* does not germinate in salinities equal to or greater than 8.5 ppt and *Cyperus javanicus* does not germinate in salinities above 17.5 ppt (Brimacombe 2003). *Sesuvium portulacastrum* has been shown to have 75% germination in approximately 10 and 20 ppt and 20% in 34 ppt (Martinez and Valverde 1992). I am not aware of any research conducted on the remaining species salinity tolerances at the germination stage.

Sesuvium portulacastrum and *Batis maritima* are often seen growing in similar areas (personal observation); therefore *Sesuvium* was selected for inclusion in a competition experiment with *Batis*.

Jacquemontia ovalifolia ssp. *sandwicense* is sometimes known as oval-leaf clustervine, but is more commonly known in Hawai'i as Pā'ūohi'iaka. This species belongs to the Convolvulaceae family; it is herbaceous and often roots at the nodes. (Stemmermann 1981, Merlin 1986). *Jacquemontia ovalifolia* occurs in coastal habitats on a variety of substrates, particularly on the leeward sides of islands; it occurs on all of the main Hawaiian islands (Ni'ihau, Kaua'i, O'ahu, Moloka'i, Lāna'i, Maui, Kaho'olawe, and Hawai'i). This plant is fast growing and requires minimum water and maintenance

and its dense cover may be useful in shading out emergent invasive species (White 1999). From personal observation at Kalaeloa and Honouliuli NWR, *Jacquemontia ovalifolia* establishes on soils that appear relatively dry and on soils slightly upland from partially submerged vegetation.

Sida fallax is commonly known as I'lima or I'lima-ku-kahakai in Hawaiian and is a common beach plant of Hawai'i. *Sida fallax* belongs to the Malvaceae family and can be a small, low lying plant with trailing branches or a tall erect shrub. It is found growing along many of the drier coastal areas in small prostrate clumps or perched on cliffs above the ocean (Merlin 1986, Wagner et al. 1999). A large stand of I'lima is intermixed with Pā'ūohi'iaka at Kalaeloa in what appears to be relatively dry soil (personal observation).

Bolboschoenus maritimus, a synonym for *Scirpus maritimus*, is commonly known as alkuli bulrush, Kaluhā, or Makai in Hawaiian and belongs to the Cyperaceae family. It is a robust perennial with long, horizontal, creeping rhizomes. It is widely distributed in saline water or freshwater, on mudflats, and in marshes at low elevations in temperate and tropical areas worldwide (Merlin 1986).

The principal natural habitat of *Bolboschoenus maritimus* is estuarine intertidal emergent wetland as classified by Cowardin et al 1979. It occurs in relatively shallow waters (less than 1 meter deep) (USGS 2005) and is usually found in monotypic stands in wetlands with salinities ranging from 7 ppt to 46 ppt (Ungar 1974). In Hawai'i, it is primarily found in coastal sites on Ni'ihau, Kaua'i, O'ahu, Moloka'i, and Maui (Wagner et al. 1999). It has been seen growing in small stands along the pond edge at Honouliuli NWR and throughout the marsh at Pouhala (personal observation).

Cyperus javanicus is a synonym for *Mariscus javanicus* and is commonly known as Ahu'awa. Ahu'awa is perennial sedge with short rhizomes, known from tropical Africa to Asia occurring throughout the Pacific Islands. *Cyperus javanicus* belongs to the Cyperaceae family and occurs on all of the main Hawaiian Islands except Kaho'olawe. It is found in coastal marshes exposed to salt or brackish water at the mangrove coastal strand interface, and as an occasional weed in lowland taro patches (Merlin 1986, Wagner et al. 1999). It is prevalent at and just inside the pond edge at Honouliuli NWR (personal observation).

Sesuvium portulacastrum is commonly known as sea purslane or 'Akulikuli in Hawaiian. *Sesuvium portulacastrum* belongs to the Aizoaceae family; it is a succulent, low lying or prostrate herbaceous halophyte that grows in coastal wetlands and other coastal areas in mud flats, alongside brackish marshlands, or surrounding the shores of sandy lagoons. It is distributed near the coast throughout the Pacific. (Stemmermann 1981, Merlin 1986)

Salinity

Halophytes are plants adapted to live in salty environments and occupy areas ranging from predominantly wet maritime marshes to arid salt deserts (Flowers et al. 1986). Many halophytes are able to acquire adequate amounts of water under saline conditions, because they respond to the high external osmotic pressure by increasing the internal pressure to a level higher than that of the external medium by accumulating additional salt from the external medium (Bernstein 1961, Queen 1974).

In coastal wetlands, organisms must cope with high and variable external salt concentrations (Mitsch and Gosslink 1993). Under many conditions the concentration of

the soil is about that of seawater or around 500 mM (34 ppt) (Flowers et al. 1986).

Halophytes are salt tolerant by definition, but salinity still affects their establishment, growth, survival, and reproduction (Waisel 1972, Zedler 1984). The dangers of salt are two-fold: osmotic and direct toxicity (Mitsch and Gosslink 1993). High salt concentrations can reduce seed germination and initial seedling growth as well as the growth and reproduction of established plants (Ungar 1978, Kuhn and Zedler 1997).

It is sometimes difficult to predict the salt tolerance of halophyte seeds based on the location of mature plants in the field (Workman and West 1967). Previous studies imply that increasing soil salinity has a negative effect on plant development, but the strength of this effect varies dramatically between different species (Flowers et al. 1986).

Soil Moisture and Flooding

Water is essential to the establishment of plants; too much water can place as much stress on plants as too little water. The responses of flooding may be due in part to effects on soil E_H (soil redox). Flooding can result in strongly reduced conditions due to a decrease in the oxygen diffusion rate and use of oxygen by plant roots and microorganisms (Baldwin and Mendelssohn 1998). Water logging of soil is not restricted to areas of heavy rainfall, but also occurs periodically due to irrigation of arid areas and tidal inundation in coastal areas (Kozlowski 1984).

Differences among species in their ability to tolerate flooding is evident in species such as rushes (Carlquist 1980), cattails, rice, mangroves, *Taxodium disticum* (bald cypress), and *Nyssa sylvatica* (tupelo gum). These species are seen thriving in moist or saturated areas (Kramer and Boyer 1995). Adaptations that enable plants to tolerate or avoid anoxia are fairly common among vascular plants and include changes in

morphology, physiological adjustment, or biochemical solutions (Kozlowski 1984, Baldwin and Mendelssohn 1998). Some of these adaptations will be at a cost to overall dry-matter accumulation, but in the long term these changes may benefit plant performance and the prospect of survival (Kozlowski 1984). In this experiment, the terms flooding stress and flooded conditions are used interchangeably and are defined as soil (4 cm below the surface) completely inundated with water. Moist conditions are defined as the soil having contact with water, but the top 4 cm of surface soil was not saturated with water.

Competition

Competition in the most general terms refers to the interaction of two organisms attempting to utilize the same limiting resource. This interaction may change under different abiotic or biotic conditions, environments, or even during different life stages such as the seedling or reproductive stage. Inter-specific competition can result in one species becoming dominant, forcing the other species to occupy a different habitat or use another food source, depending on the basis of competitive action (Odum 1971).

The competitive ability of plants can be difficult to define, because plants can compete for a variety of different things. There can be the competition for resources such as light, nutrients, or water or competition for “other aspects for the struggle for existence” (Darwin 1859) such as mates or pollinators (Grime 2002).

Proposed Research

Purpose of Project

Interaction of managers with scientists early in the restoration process can improve chances of project success and make future restoration efforts more effective

(Zedler 1984). Identification of potential problems important to the wetland restoration planning process, such as salinity tolerances, the ability of plants to withstand flooding conditions due to tidal inundations, and limiting the potential for invasion of alien species, are especially important at an early stage to assess the site's potential for enhancement or restoration. Because so little is known about marsh restoration, each site has potential for advancing our knowledge (Zedler 1984).

This research attempts to answer four main questions that would increase knowledge of the Waiawa unit wetland restoration site and improve knowledge of the basic requirements for germination and establishment of five native coastal species: *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum*, and one invasive species, *Batis maritima*. In addition, I hope to provide insight on whether abiotic stresses such as salinity and flooding affect the competitive ability of a native species, *Sesuvium portulacastrum*, and its invasive competitor, *Batis maritima*.

Research Questions

1. How does salinity and flooding affect germination of selected species?

- A. What are the salinity tolerances of the different species during germination?
- B. Are species able to germinate under different levels of water inundation?

2. How does salinity and flooding affect early growth of the selected species?

- A. Under what salinity levels can these plant species can grow?
- B. Under what water inundation levels can these plant species can grow?

3. Is the relative competitiveness of *Batis maritima* and *Sesuvium portulacastrum* affected by different salinity or flooding regimes?

4. What are Waiawa unit physical site characteristics and based on salinity, which areas are suitable for replanting of selected native species?

This project will help to determine the feasibility of using these native species for successful wetland restoration projects, provide insight into the optimal time for replanting, promote possible techniques such as irrigation to aid in promoting establishment, as well as assist in understanding why some species have a greater competitive ability than other species.

Hypotheses

Based on information gathered from previous studies, I have generated hypotheses addressing the four research questions. Based on the general trend of a decrease in germination as salinity increases, as reported by Ungar (1978, 1982, 1991) and Kuhn and Zedler (1997), I hypothesize that:

H(1a): Germination of *Batis maritima* and the native species will be negatively affected by increasing soil salinities.

The majority of land plants will not germinate underwater (Crocker and Davis 1914, Kozlowski 1984). However, species that are located in areas susceptible to flooding may have seeds that have adapted to germinate in flooded areas. *Cyperus* and *Bolboschoenus* are traditionally considered wetland plant species, growing at the edge or just inside of edge of the pond. Because of their location in saturated or flooded areas, I hypothesize that:

H(1b): *Cyperus javanicus*, & *Bolboschoenus maritimus* will germinate more successfully in flooded conditions than in moist conditions.

Sida fallax and *Jacquemontia ovalifolia* are traditionally considered coastal species, found growing in drier soils and seeds may not have adapted to being able to germinate in flooded conditions if the adult plant is not situated in those areas. *Batis* and *Sesuvium* both grow and spread primarily through clonal reproduction rather than by seed and this may indicate a need for specialized conditions to successfully germinate. Based on this location information, data from Brimacombe's 2002 study, previous studies that indicate excessive water negatively affects germination in many species (Brimacombe 2000, Baskin and Baskin 2001) and inferences from vegetative spread, I hypothesize that:

H(1c): *Batis maritima*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* will have higher germination in moist than flooded conditions.

Halophytes, which are salt tolerant by definition are still affected by salinity during establishment, growth, and reproduction (Waisel 1972, Zedler 1984). High salt concentrations can reduce initial seedling growth as well as the growth and reproduction of established plants (Ungar 1978, Kuhn and Zedler 1997). Since little is known about the salt tolerance of these specific species, I hypothesize:

H(2a): Early growth of all species will be negatively related to increasing salinity.

In addition, since a large percentage of halophytes exhibit succulence (Daubenmire 1974) and leaves develop more pronounced succulence under saline conditions (Ottow et al. 2005), I hypothesize:

H(2b): The succulent species, *Batis maritima* and *Sesuvium portulacastrum*, will more effectively tolerate high levels of salinity during early growth.

Based on information of plant characteristics associated with an unfavorable water balance (Daubenmire 1974), I hypothesize that

H(2c): Species, exposed to flooding conditions, will show a decrease in root and shoot length relative to plants exposed to moist conditions.

In the natural environment, *Batis maritima* forms large dense mats of plants that do not allow other species to grow in or around it (Personal observation). *Sesuvium portulacastrum* grows more sparsely and can be seen growing in and around other coastal wetland plant species (Personal observation). Therefore, the growth rate of *Batis maritima* was perceived to be extremely fast and the growth rate of *Sesuvium portulacastrum* was perceived to be slower than that of *Batis*. Following Grime's theory (Grime 1979, 2002), there are tradeoffs in the adaptations of plants to certain environments and situations; typically plants that grow quickly are more susceptible to stress. Therefore, I hypothesize:

H(3A): The ability of *Sesuvium portulacastrum* to compete with *Batis maritima* will be greater at higher levels of salinity stress and the ability of *Batis* to compete with *Sesuvium* will be greater under lower levels of salinity stress.

From brief investigations at Waiawa (Englund and Preston 2000) (Personal communication with Dr. Amanda Demopolus, Scripps Institution of Oceanography) showing that salinity decreases from the ocean inland and because of the consistency of tidal inundation, I hypothesize:

H(4A): Intertidal soil salinity will remain constant throughout time.

H(4B): Soil salinity & moisture will decrease as elevation increases.

H(4C): Salinity will vary at the Waiawa unit, but soil salinity will not exceed the tolerance level of the selected species.

Chapter 2. Germination of Five Native and One Invasive Coastal Species Exposed to Different Salinity and Flooding Treatments

Abstract

Coastal wetlands are harsh environments for plant species to establish in due to tidal inundation and high soil salinities. This study examined the effect of salinity and flooding on the germination of five native species to evaluate their potential use in restoring a Hawaiian coastal marsh. Germination of an introduced invasive species, *Batis maritima*, was also assessed due its abundance along the coastline. Plants were germinated in four salinities (0, 10, 20, and 30 ppt) and in two water levels: moist (saturated filter paper) and flooded (in ½ cm of water). Final germination percentages of all five native species were significantly reduced when exposed to salinities greater than freshwater. *Sesuvium portulacastrum* was the most salt tolerant, with a significant reduction in germination first occurring at 20 ppt. *Cyperus javanicus*, *Sida fallax*, and *Jacquemontia ovalifolia* showed significant decreases in germination at 10 ppt and *Bolboschoenus maritimus* only germinated in freshwater conditions. The flooding treatment also significantly reduced germination of *Cyperus javanicus* and *Sida fallax*, whereas the other natives were not significantly affected by water level. Germination of *Batis maritima* was not significantly affected by salinity or water level. Results of this study indicate that none of the native species, with the exception of *Sesuvium portulacastrum*, should be sown in field conditions exceeding 10 ppt for successful

germination. In contrast, the exotic species *Batis maritima* will germinate in near seawater conditions and can be expected to establish in coastal habitats.

Introduction

High salt concentrations have been shown to reduce seed germination (Ungar 1978, Kuhn and Zedler 1997). In general, researchers have concluded that salinity is inhibitory to the germination of seeds in two ways: (1) causing a complete inhibition of the germination process at salinities beyond the tolerance limits of a species and (2) delaying the germination of seeds at salinities that cause some stress to seeds but do not prevent germination. Many young plants suffer mortality under saline conditions, so a lack of germination or delayed germination until salinity is reduced could improve a plant's chance for survival to maturity (Ungar 1978, 1982, 1991, Martinez and Valverde 1992).

There is great variability among plants in terms of the level of salinity that will completely inhibit or delay germination, and even in the case of halophytes, many seeds will not germinate at salinities of full seawater strength (Martinez and Valverde 1992, Collinge and Wise 2003). A delay in germination and final reduction in the percentage of seeds germinated can occur in salt concentrations as low as 1 ppt NaCl for the least salt tolerant species (Ungar 1978).

Depending on the species, water stress during the time of seed development can also cause an increase or decrease in the percentage of seeds germinating. Soaking injury in seeds has been the subject of scientific controversy. Many scientists have assumed that a flooded environment equates to an anaerobic environment, leading to an inability for plants to germinate. Other researchers hypothesize that products of anaerobiosis, such as

ethanol are toxic to the seed (McKee and Mendelssohn 1989). Alternatively, it has been hypothesized that sucrose, which is used during germination, is depleted through leakage or conversion to alcohol, preventing germination of the seed (Norton 1986).

Similar to salinity, the response of plants to water availability varies among species. Seeds of some plant species (e.g. *Salix* and *Populus*) must make contact with moist soil within a few days after maturation or perish, while seeds of other species become dehydrated when dormant and may not germinate readily unless soil moisture exceeds soil field capacity for a time (Doneen and MacGillivray 1943, Daubenmire 1974). The maximum moisture content tolerated by seeds differs widely among species (Daubenmire 1974).

The objective of this germination experiment was to provide knowledge about the germination ability of five native Hawaiian wetland species, and the invasive wetland species *Batis maritima* under various salinity and inundation (water) levels. Specific information about native seed germination will be beneficial in determining if the native species are appropriate for use in restoration at the Waiawa unit of Pearl Harbor NWR, while also suggesting the best possible placement of these species for replanting and the proper timing for revegetation. It will also determine whether *Batis maritima* performs equally well at all salinity or soil moisture levels and whether changes to soil salinity or moisture levels can reduce seed establishment of this exotic species.

Batis maritima is abundant along the mangrove edge at the Waiawa unit of Pearl Harbor NWR (personal observation) and the removal of mangroves may initially promote the colonization of opportunistic invasive species such as *Batis* (Allen 1998, Smith 2001). The native species, *Sesuvium portulacastrum*, is also known to occur on estuarine

mudflats adjacent to mangrove swamps and could be restored along the intertidal area of the Waiawa unit. These two species could potentially be in competition for resources in this area and therefore I directly compared the germination abilities of these two species.

The experiment tested the hypotheses that 1) germination of all native species and *Batis maritima* will be negatively affected by increasing soil salinities, 2) the native groundcover species (*Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum*) and the invasive *Batis maritima* will have higher germination under moist than flooded conditions, and 3) *Cyperus javanicus* and *Bolboschoenus maritimus*, which typically grow in flooded environments, will germinate more successfully under flooded conditions than under moist conditions.

Materials and Methods

Seed Collection

Seeds of *Jacquemontia ovalifolia* and *Sida fallax* were collected from Barber's Point between March and May of 2005. Because they are mat forming plants, it is difficult to distinguish one plant from another, but approximately 50 shoots, two meters apart, were sampled on three separate collection trips. *Bolboschoenus maritimus* and *Cyperus javanicus* seeds were collected from Honouliuli NWR between February and May of 2005. Approximately ten *Bolboschoenus maritimus* and fifteen *Cyperus javanicus* plants were sampled on three separate collection trips.

Batis maritima seeds were collected from the Waiawa unit of Pearl Harbor NWR in April and May of 2005. Approximately 50 plants were sampled on two separate collection trips. *Sesuvium portulacastrum* seeds were collected from Flat Island in Kailua in early September and approximately 50 plants were sampled on one collection trip.

All seeds were collected from mature fruits or inflorescences, placed in brown paper bags and stored in a refrigerator to maintain viability prior to use (Baskin and Baskin 2001). The storage of seeds varied from 1 month to 6 months and seeds from different collection trips were pooled together for use in the experiments.

Seed Pre-Treatment

From pretrial experiments, it became apparent that a few of the species needed special pre-treatment before they would germinate. Seeds of *Cyperus javanicus*, *Jacquemontia ovalifolia*, and *Sida fallax* were first placed in water to help determine viability of the seeds. Seeds that sunk were considered viable and seeds that floated were discarded (Personal Communication with Greg Koob, Horticulturist). However, all seeds from *Batis maritima*, *Bolboschoenus maritimus*, and *Sesuvium portulacastrum* floated and therefore viability was not determined by this method.

Batis maritima, *Cyperus javanicus*, and *Sesuvium portulacastrum* seeds did not receive any pre-treatment. *Bolboschoenus maritimus* and *Jacquemontia ovalifolia* seeds were vulnerable to fungal invasion in pre-trial germination experiments. Therefore, *Jacquemontia* seeds were soaked a 3% hypochlorite bleach solution for five minutes. *Bolboschoenus* also had an extremely hard seed coat; to kill any fungus and allow the seeds to imbibe water, seeds were soaked in a 3% hypochlorite bleach solution for five days (Clevering 1998, Brimacombe 2000). For both species, seeds were thoroughly rinsed five times to remove any hypochlorite bleach solution from the seed coat before the experiment began.

Sida fallax seeds required a more complicated pre-treatment. Individual seeds were soaked in water for twenty minutes to soften and remove the mericarp. Extracted

seeds were screened for viability using the sink or float method. Subsequently, the seeds were placed under a dissecting scope and seed coats were nicked with a razor blade to allow the seed to imbibe water (Yorkston 2005).

Experimental Germination

The germination study comparing salinity and water treatments was conducted in Percival I30-VL incubators in the St. John Building at the University of Hawai'i at Manoa. Seeds were placed onto 100 x 15 mm sterile plastic Petri dishes lined with Whatman #1 filter paper. The experiment employed a two by four factorial design; germination of the six species was compared at four salinity levels (freshwater= 0 ppt, low salinity = 10 ppt, medium salinity =20 ppt, and high salinity = 30 ppt) and two inundation levels (moist = filter paper saturated with 2 ml of water and flooded = filter paper flooded with 30 ml of water, resulting in standing water 0.5 cm deep). Salt solutions were prepared by mixing distilled water with Instant Ocean (a commercial seawater mix) to create the salinity levels indicated above and salinity levels were determined by using a refractometer.

Seeds of each species were placed in separate Petri dishes to avoid potential allelopathic interaction. Each Petri dish contained ten seeds and dishes were randomly assigned one of the eight salinity and inundation treatment combinations (freshwater moist (FWM), freshwater flooded (FWF), low salinity moist (LSM), low salinity flooded (LSF), medium salinity moist (MSM), medium salinity flooded (MSF), high salinity moist (HSM) and high salinity flooded (HSF)). Treatments are abbreviated to save space in figures and tables.

Each species had six replicate Petri dishes per treatment yielding a total of 288 Petri dishes. Petri dishes were stacked on top of one another to fit in one incubator and the position of all the Petri dishes was randomized every week. Seeds were exposed to an alternating twelve hour regime of 30 degrees Celsius exposed to 80 $\mu\text{mol}/\text{m}^2/\text{s}$ light and a twelve hour regime of 20 degrees Celsius exposed to darkness. Lids were used to reduce fungal contamination and water evaporation. Lids were sealed on the dishes using Parafilm and seeds were monitored for twelve weeks. In the beginning of the experiment, germination was assessed every 3–4 days, but as the experiment continued, dishes were checked weekly.

The emergence of the radicle from the seed constituted germination and when seeds germinated they were removed from the Petri dish. If fungus appeared on a seed, the seed was removed, rinsed with tap water, and squeezed gently to determine if the seed was rotten. Rotten seeds were discarded and intact seeds were returned to the dish (Baskin and Baskin 2001).

Freshwater Transfer Germination

As shown in previous studies, some seeds, when exposed to salinity or water levels in which they cannot germinate, remain viable until a period of reduced salinity or water stress (Ungar 1978, 1982, 1991, Martinez and Valverde 1992). To determine whether seeds remained viable after 12 weeks of exposure to the salinity and water treatments, all remaining ungerminated seeds at the end of the experiment were placed in dishes containing 2ml of freshwater (enough water to saturate the filter paper) and additional germination was assessed over one month. Seeds germinating during the treatment and after freshwater transfer were totaled. Uniformity of germination

percentage of a species across treatments was examined to determine whether seeds were quiescent or inhibited during periods unsuitable for germination, but remained viable after exposure to salinity and flooding stress.

Germination Experiment Timeline

The germination experiment for *Cyperus javanicus*, *Bolboschoenus maritimus*, and *Jacquemontia ovalifolia* began on October 11th. After 12 weeks, the ungerminated seeds were transferred to the moist freshwater treatment to assess viability. Due to difficulties with germination and/or availability of seeds, the germination experiment for *Sesuvium portulacastrum*, *Batis maritima*, and *Sida fallax* began on November 15th. After 12 weeks, the seeds were transferred to the moist freshwater treatment to assess viability.

Data Analysis

ANOVA Analysis

A two-way ANOVA (Systat Version 10.2, Systat Software Inc.), with salinity and water as fixed factors, was used to analyze differences in final germination percentages among treatments for *Batis maritima* and *Cyperus javanicus*. However, *Cyperus javanicus* had zero germination in the medium and high salinity treatments. Therefore, only freshwater and low salinity treatments were used for the *Cyperus javanicus* ANOVA. Germination percentages were arc sine transformed (Zar 1984) and probability plots were completed to ensure normality of the data prior to use in the ANOVA.

Chi-Square Exact Test

Bolboschoenus maritimus, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* had many Petri dishes with zero germination, precluding the use of ANOVA. Chi-Square exact tests (Stat Exact, Version 4, Cytel Software) were performed

to examine differences in germination percentages among salinity and water treatments. For *Bolboschoenus*, *Jacquemontia*, and *Sesuvium*, percent germination within each treatment was determined by pooling results from replicate Petri dishes, as there were no statistical differences in germination percentages within treatments (Chi-square test).

Sida fallax had significant variation among Petri dishes in the low salinity flooded treatment (one of the Petri dishes had 90% germination, while the other Petri dishes in the low salinity flooded treatment had between 40% and 70% germination). Chi-square analysis showed that germination in the low salinity flooded treatment was significantly lower than germination in the freshwater flooded treatment and the low salinity moist treatment; therefore, the Petri dish that reached 90% germination was omitted in order to provide a more conservative estimate of germination percentage in the low salinity flooded treatment.

Initially, a Chi-Square exact test was used to compare germination in the moist treatment and flooded treatment within individual salinity treatments. If there was not a significant difference between moist and flooded treatments within salinity treatments, the two water treatments were combined and the Chi-Square exact test was used to compare germination between salinity treatments.

My primary interest was to determine the salinity level at which germination significantly declined, relative to freshwater. Pairwise tests compared freshwater to each of the higher salinity treatments until a significant difference was indicated. These tests were corrected for multiple comparisons using the sequential Bonferroni adjustment. If germination was significantly different between moist and flooded treatments, pairwise tests compared germination in the freshwater moist treatment to germination in each of

the higher salinity moist treatments and germination in freshwater flooded treatments were compared to germination in each of the higher salinity flooded treatments.

Mann Whitney U Test

A Mann-Whitney U-Test was completed with species and salinity as fixed factors to determine whether germination percentage for *Batis* and *Sesuvium* differed significantly from one another.

Germination Velocity

Germination of all six species was observed for twelve weeks and germinated seeds were counted weekly (12 observations). Germination velocities of the various species were calculated by using a modified version of Timson's index of germination velocity (Timson 1965).

$$\text{Germination Velocity} = (\sum G)/t$$

(G is the percentage of the total germinated seeds at any weekly interval among all the seeds that germinated and t is the entire germination period)

Using this index, my data created a maximum value of 100, if there was 100% germination occurring at the first observation ($\sum G = 100\% \times 12 \text{ observations} = 1200/12$)

Mean germination velocities for each species exposed to each of the eight treatments were calculated. Germination velocities were analyzed using a two way ANOVA for each species (except for *Jacquemontia ovalifolia*) with salinity and water as fixed factors. The fully factorial ANOVA could not be used for *Jacquemontia* because germination did not occur at all in the medium salinity flooded treatment. Therefore, two one way ANOVAs (with water as a factor in one ANOVA and salinity as a factor in the other ANOVA) were completed for the analysis of *Jacquemontia*'s germination velocity.

Results

Species Comparison

Species differed tremendously in their ability to germinate under various salinity and water treatments. Overall, *Sida fallax* had the highest mean germination percentage of all species studied in the freshwater moist treatment (98.3%), freshwater flooded treatment (96.6%), low salinity moist (81.7%), and low salinity flooded (55.0%) treatments (Table 2.1).

Several species had zero germination in one or more treatments. Neither *Cyperus javanicus* nor *Jacquemontia ovalifolia* germinated in either the moist or flooded treatments in the medium or high salinity treatments. *Bolboschoenus maritimus* did not germinate in either water level when subjected to salinities greater than freshwater. In addition, most species showed considerable variation in the range of germination percentages across treatments.

All native plant species showed a decrease in mean percent germination as salinity levels increased. In contrast, *Batis maritima* had relatively uniform germination percentages under all treatments and demonstrated the highest germination of all species under the medium and high salinity levels at both water levels (Table 2.1).

Table 2.1. Final percent germination (mean \pm 1 SD, n=6) for five native and one invasive species under eight different treatments, consisting of four salinity and two water levels.

Treatment/ Species	FWM	FWF	LSM	LSF	MSM	MSF	HSM	HSF
<i>Jacquemontia</i>	18.3 \pm	25.0 \pm	8.3 \pm	5.0	0.0 \pm	1.7 \pm	0.0 \pm	0.0 \pm
<i>Ovalifolia</i>	7.5	13.8	9.8	\pm 8.4	0.0	4.1	0.0	0.0
<i>Sida fallax</i>	98.3 \pm	96.7 \pm	81.7 \pm	55.0 \pm	3.3 \pm	1.7 \pm	0.0 \pm	0.0 \pm
	4.1	5.2	9.8	20.7	5.2	4.1	0.0	0.0
<i>Cyperus javanicus</i>	88.3 \pm	73.3 \pm	33.3 \pm	3.3 \pm	0.0 \pm	0.0 \pm	0.0 \pm	0.0 \pm
	11.7	24.2	8.2	5.2	0.0	0.0	0.0	0.0
<i>Bolboschoenus maritimus</i>	38.3 \pm	45.0	0.0 \pm	0.0 \pm	0.0 \pm	0.0 \pm	0.0 \pm	0.0 \pm
	9.8	\pm 17.6	0.0	0.0	0.0	0.0	0.0	0.0
<i>Sesuvium portulacastrum</i>	23.3 \pm	21.7 \pm	10.0 \pm	16.7 \pm	1.7 \pm	3.3 \pm	1.7 \pm	1.7 \pm
	12.1	7.5	8.9	13.7	4.1	5.2	4.1	4.1
<i>Batis maritima</i>	43.3 \pm	41.7 \pm	46.7 \pm	46.7 \pm	51.7 \pm	45.0 \pm	26.7 \pm	41.7 \pm
	26.6	18.3	21.6	15.1	22.3	31.5	10.3	18.3

Jacquemontia ovalifolia

Jacquemontia ovalifolia had a relatively low germination percentage across all treatments, with the highest percent germination occurring in the freshwater treatments (18-25%) (Table 2.1). There were no significant differences between *Jacquemontia* seed germination in moist and flooded treatments under any salinity treatment (Table 2.2).

Table 2.2. Chi-Square Exact Test of *Jacquemontia ovalifolia* germination comparing moist and flooded treatments under all salinity levels.

Comparison	Chi-Square Value	Raw P-Value
Freshwater moist vs. Freshwater flooded	0.786	0.507
Low salinity moist vs. Low salinity flooded	0.536	0.717
Medium salinity moist vs. Medium salinity flooded	1.008	1.000
High salinity moist vs. High salinity flooded	0.000	1.000

The percentage of seeds germinating in freshwater was significantly higher than the percentage of seeds germinating in the low salinity solutions at both water levels

(Chi-Square = 11.102, $p = 0.001$). Higher salinities had an even greater inhibitory effect on germination (Figure 2.1).

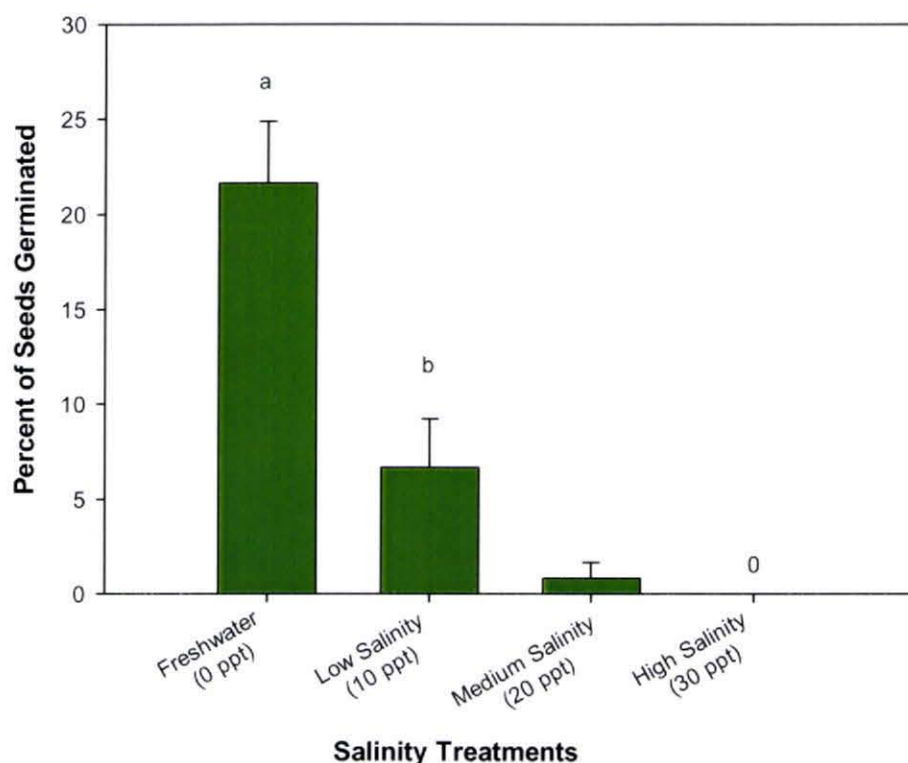


Figure 2.1. Mean germination percentage (\pm SEM, $n = 12$) of *Jacquemontia ovalifolia* seeds exposed to four salinity treatments. Water treatments (moist and flooded) were merged within the different salinity treatments. Different letters represent a significant difference by Chi-Square Exact Test ($p = 0.001$). No letter indicates that the statistical analysis on the treatment was not performed.

Sida fallax

Sida fallax had nearly 100% germination when exposed to freshwater, but only a few *Sida* seeds germinated when exposed to the medium salinity treatments (3.3%) and no seeds germinated in the high salinity treatments (Table 2.1). *Sida fallax* germination was significantly lower under flooded conditions, relative to moist conditions in the low

salinity treatment (Figure 2.2, Chi-Square = 9.859, $p = 0.003$). Water level did not significantly affect *Sida* germination in any other salinity treatment.

Sida fallax had significantly higher germination percentages in the freshwater treatments compared to the low salinity treatments when exposed to both moist (Chi-Square = 9.259, $p = 0.004$) and flooded (Chi-Square = 34.041, $p = 0.001$) conditions.

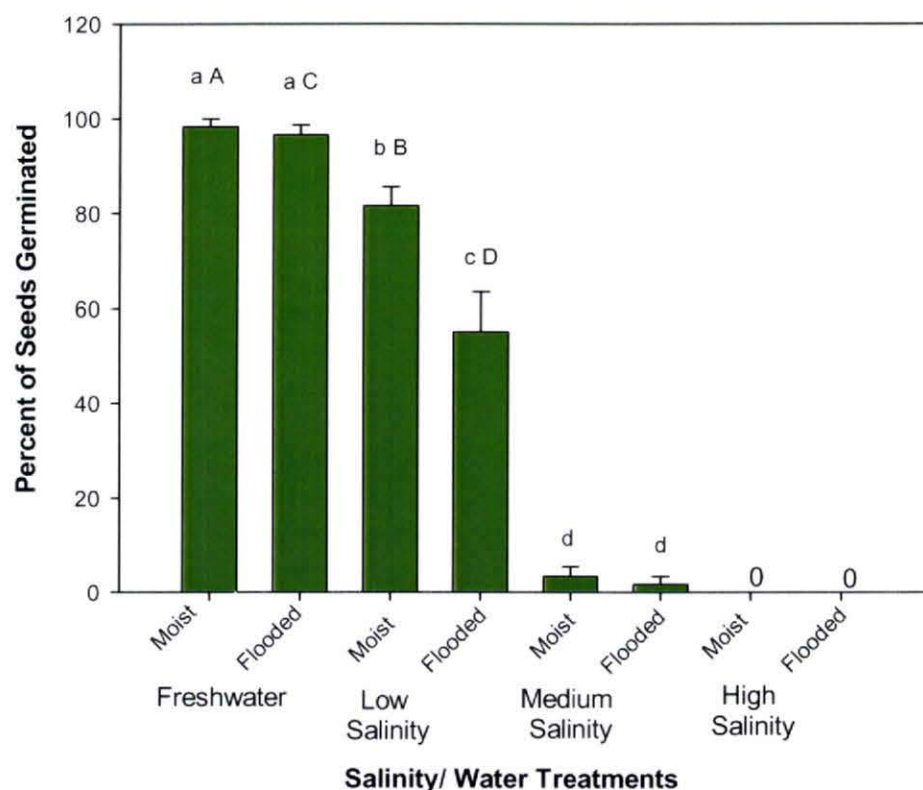


Figure 2.2. Mean germination percentage (\pm SEM, $n = 6$) of *Sida fallax* seeds exposed to eight treatment combinations of four salinity levels and two water levels. Lower case letters signify significant differences by Chi Square Exact Test ($p < 0.05$ after a sequential Bonferroni adjustment) between water treatment levels, within salinity treatments. Upper case letters signify significant differences between salinity treatments. If no letter is denoted, this indicates that no analysis on the treatment was performed.

Cyperus javanicus

The highest *Cyperus javanicus* germination percentage occurred in the freshwater moist treatment (88%) (Table 2.1). Germination of *Cyperus* seeds was significantly lower as salinity and water levels increased (Figure 2.3 Water: F-ratio =6.323, $p = 0.021$; Salinity: F-ratio =58.42, $p < 0.001$). Germination, in the low salinity treatment was significantly lower than germination in the freshwater treatments and germination in the flooded treatments was significantly lower than germination in the moist treatments (Figure 2.3). Germination did not occur when seeds were exposed to the medium or high salinity treatments.

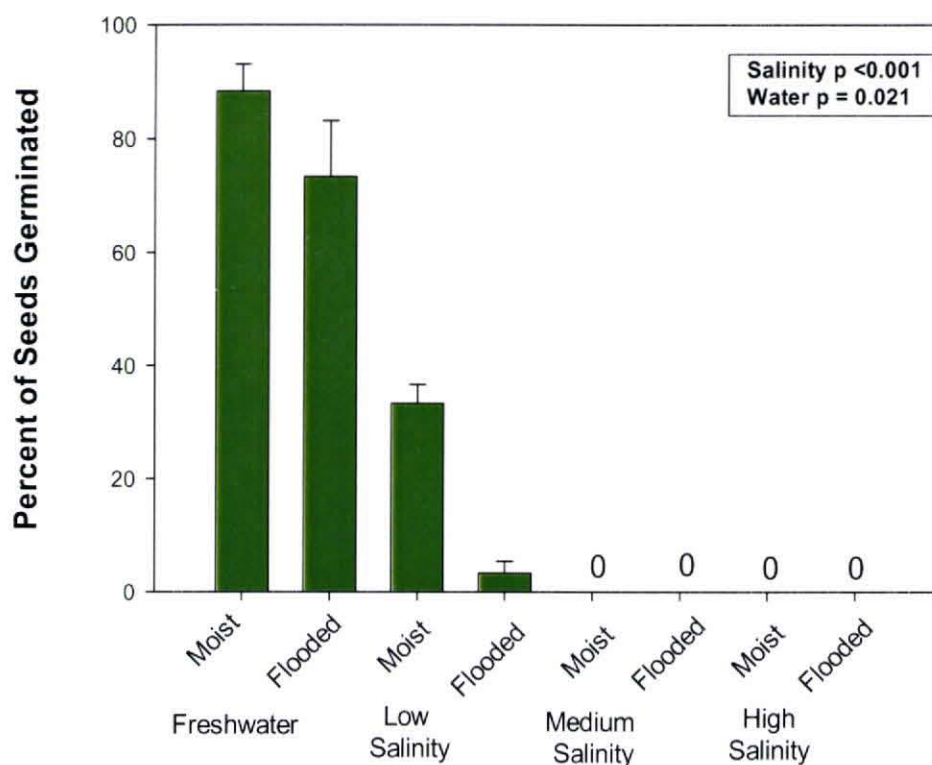


Figure 2.3. Mean germination percentage (\pm SEM, $n=6$) of *Cyperus javanicus* seeds exposed to eight treatment combinations of four salinity levels and two water levels. This analysis did not include medium or high salinity treatments. Salinity and water significantly affected germination of *Cyperus javanicus*.

Bolboschoenus maritimus

In freshwater, *Bolboschoenus maritimus* had the second lowest germination, (behind *Sesuvium portulacastrum*) and its seed germination indicated the highest sensitivity to increasing salinity. *Bolboschoenus* germinated only under freshwater conditions. Germination was slightly higher in the freshwater flooded treatment (45%) than the freshwater moist treatment (38%) (Table 2.1), but water level did not have a significant effect on the germination (Chi-Square =0.549, $p = 0.576$). Germination in freshwater was significantly greater than germination in the low salinity treatment (Chi Square = 63.158, $p < 0.001$).

Sesuvium portulacastrum

Sesuvium portulacastrum seeds germinated in all salinity treatments and water treatments (Figure 2.4). The highest germination percentages occurred in the freshwater moist and flooded treatments (21-23%) (Table 2.1). There was no statistical difference in germination percentages between water treatments (Figure 2.4, Table 2.3)

Table 2.3. Chi-Square Exact Test of *Sesuvium portulacastrum* germination comparing moist and flooded treatments under all salinity levels.

Comparison	Chi-Square Value	Raw P-Value
Freshwater moist vs. Freshwater flooded	0.048	1.000
Low salinity moist vs. Low salinity flooded	1.154	0.421
Medium salinity moist vs. Medium salinity flooded	0.342	1.000
High salinity moist vs. High salinity flooded	0.000	1.000

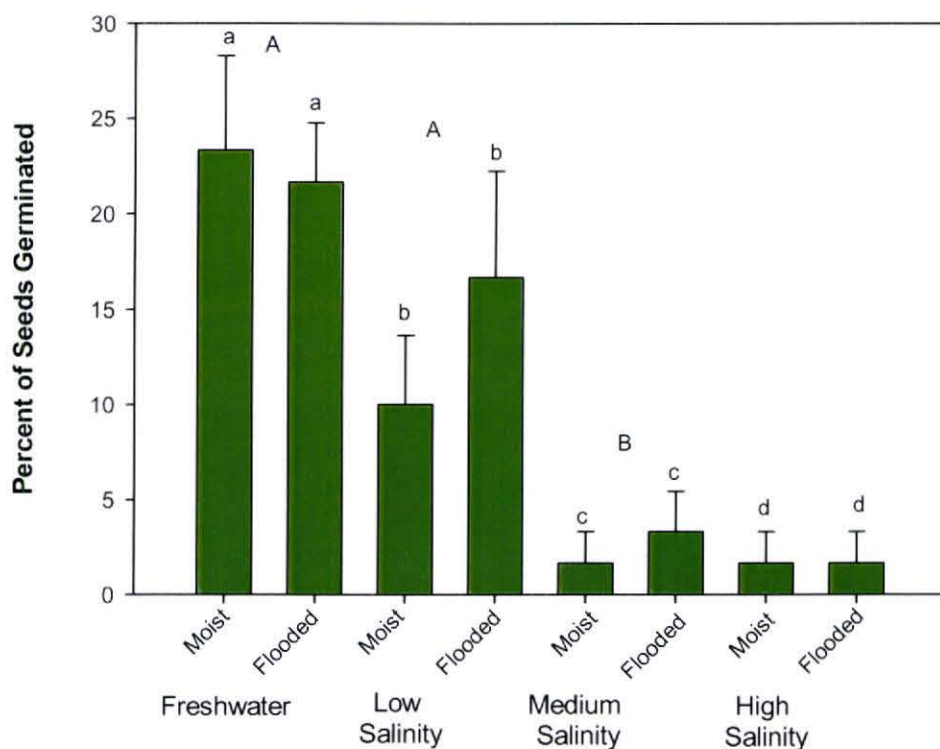


Figure 2.4. Mean germination percentage (\pm SEM, $n=6$) of *Sesuvium portulacastrum* seeds exposed to eight treatment combinations of four salinity levels and two water levels. Lower case letters signify significant differences between water levels, within salinity treatments. Upper case letters signify significant differences by Chi-Square Exact Test ($p < 0.05$ after a sequential Bonferroni adjustment) between salinity treatments. If no letter is denoted, this indicates that no analysis on the treatment was performed.

There was a small and non-significant reduction in germination as salinity levels increased from freshwater to the low salinity treatment (Chi-Square = 3.428, $p = 0.092$). As salinity increased to the medium salinity treatment, there was a significant reduction in percent germination, relative to freshwater (Figure 2.4, Chi-Square = 21.943, $p = 0.001$). These results indicate that *Sesuvium portulacastrum* has a higher tolerance to salinity than most other native species analyzed (*Sida fallax* had a relatively high tolerance to salinity), although germination rates were below 5% at medium and high salinities (Figure 2.4).

Batis maritima

Batis maritima had the most uniform germination percentages when exposed to different salinity and water treatments, averaging approximately 43% (Table 2.1). There were no statistical differences among treatments (Figure 2.5, Table 2.4).

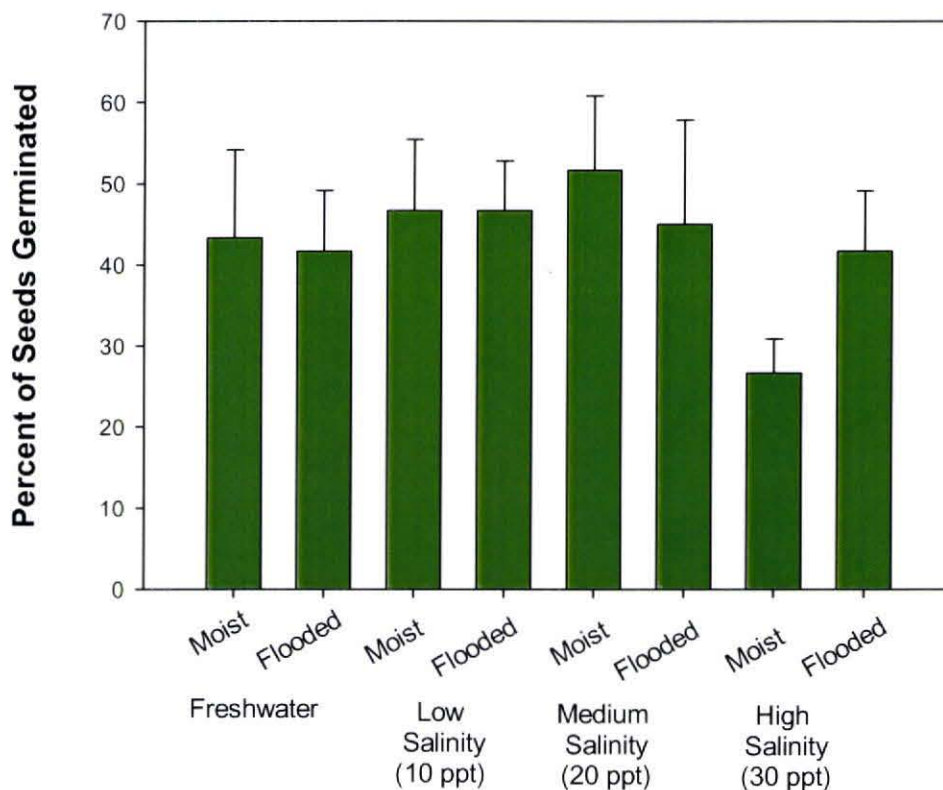


Figure 2.5. Mean germination percentage (\pm SEM, $n=6$) of *Batis maritima* seeds when exposed to eight treatment combinations of four salinity treatments and two water levels. Germination percentage was not significantly affected by water or salinity.

With high germination rates even at 30 ppt salinity, *Batis* had the highest tolerance to salinity and flooding of all species studied (Table 2.1).

Table 2.4. ANOVA analysis of *Batis maritima*'s arc sine transformed germination percentages with salinity and water as fixed factors.

Comparison	F-ratio	P-Value
Salinity	1.127	0.350
Water	0.127	0.723
Salinity x Water	0.307	0.820

***Batis maritima* and *Sesuvium portulacastrum* comparison**

Since *Sesuvium portulacastrum* and *Batis maritima* are often found growing in similar areas (personal observation), germination was analyzed to determine which species was more suited to different salinity and water levels. *Batis* had a higher mean germination percentage than *Sesuvium* under all treatment conditions (Table 2.1). Given that water level did not significantly affect either species (Table 2.3, Table 2.4), moist and flooded treatments were pooled. In addition, since salinity only significantly affected *Sesuvium* when salinities were at the medium or high treatments and *Batis* was not significantly affected by salinity (Table 2.3, Table 2.4), freshwater and low salinity germination percentages were pooled and medium salinity and high salinity germination percentages were pooled. This allowed the comparison of *Batis* and *Sesuvium*'s germination percentages in low (0-10 ppt) and high salinity treatments (20-30 ppt).

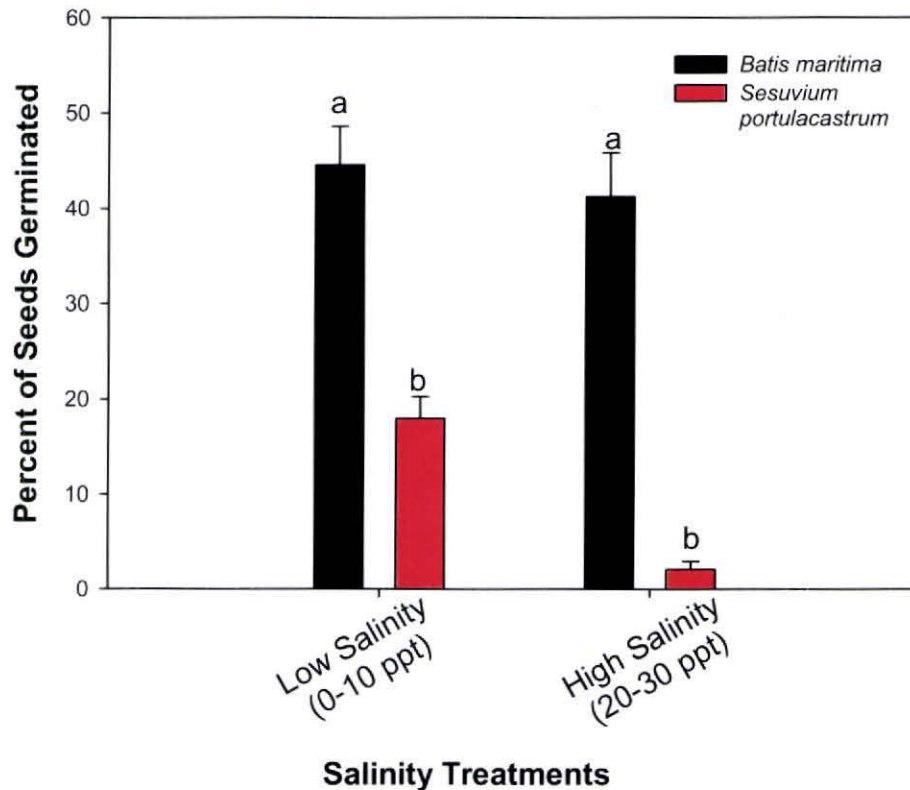


Figure 2.6. Mean germination percentage (\pm SEM, $n=24$) of *Batis maritima* and *Sesuvium portulacastrum*. The two water treatments were merged; the freshwater and low salinity treatments were merged to form the lower salinity treatment and the medium and high salinity treatments were merged to form the higher salinity treatment. Different letters represent a significant difference by Mann Whitney U Test ($p < 0.001$) between the two species at each treatment level.

Analysis of *Sesuvium portulacastrum* and *Batis maritima* showed that the germination percentage of *Batis* was significantly greater than the germination percentage of *Sesuvium* when seeds were subjected to both lower salinity treatments (Mann-Whitney U Test Statistic = 65.50, $p < 0.001$) and higher salinity treatments (Mann-Whitney U Test Statistic = 5.000, $p < 0.001$).

Germination Velocity

Typically, halophytes experience a delay in germination as salinity levels are increased (Chapman 1974); therefore all species germination velocities were expected to decline with increasing salinity. Germination velocity had a minimum value of zero and a maximum value of one hundred. Overall, *Sida fallax* had the highest germination velocity of any of the species, which occurred in the freshwater treatments (95.5 - 97.4). *Batis maritima*, *Bolboschoenus maritimus*, *Cyperus javanicus*, and *Sida fallax* all had their highest germination velocities when exposed to one of the freshwater treatments (Table 2.5).

Jacquemontia ovalifolia and *Sesuvium portulacastrum* had their highest germination velocities in the medium and high salinity treatments. However, it is important to note that *Jacquemontia*'s germination velocity in the medium salinity flooded treatment is based on only one Petri dish (< 2% germination) (Table 2.1). Similarly, *Sesuvium*'s high germination velocity in the medium and high salinity treatments could be an artifact of small sample size (one or two Petri dishes per treatment).

Table 2.5. Mean germination velocity (GV) of all species for each of the eight treatments over the entire twelve week experiment. GV values can range from zero (no germination) to 100 (100% germination occurring by week one) Analysis did not include treatments with a GV of zero. Significant differences between water levels, within salinity treatments, are indicated by different lower case letters determined by ANOVA ($p < 0.05$ after a sequential Bonferroni adjustment) and significant differences between salinity treatments are indicated by different upper case letters. If no letters are present no analysis on the treatment was performed.

Treatment/ Species	FWM	FWF	LSM	LSF	MSM	MSF	HSM	HSF
<i>Batis maritima</i>	66.70 aA	57.28 aA	55.21 bA	55.87 bA	65.15 cA	61.16 cA	63.77 dA	56.88 dA
<i>Bolboschoenus maritimus</i>	88.26 a	85.08 a	0	0	0	0	0	0
<i>Cyperus javanicus</i>	59.04 aA	46.77 aA	22.22 bB	16.67 bB	0	0	0	0
<i>Jacquemontia ovalifolia</i>	64.12 aA	64.11 aB	40.28 bA	64.58 bB	0	91.67	0	0
<i>Sida fallax</i>	95.53 aA	97.42 aA	81.24 aB	59.20 bB	75 a	33.33 b	0	0
<i>Sesuvium portulacastrum</i>	73.96 aA	54.40 aA	57.29 bA	67.92 bA	91.67 cA	79.17 cA	83.33 dA	91.67 dA

For *Jacquemontia ovalifolia*, ANOVA analysis demonstrated that neither water (F-ratio = 1.414, $p = 0.253$) nor salinity (F-ratio = 3.709, $p = 0.051$) were significant factors in germination velocity (Table 2.5)

Sida fallax experienced its highest germination velocities in the freshwater treatments (Table 2.5) and ANOVA analysis indicated that salinity and water both significantly decreased the germination velocity of *Sida* (F-ratio = 111.83 and 63.81 respectively, $p < 0.001$). However, the ANOVA showed a significant interaction between water and salinity (F-ratio = 26.39 $p < 0.001$), indicating that the effect of salinity on germination velocity was influenced by the water treatments.

Table 2.6. Hypothesis tests (ANOVA) comparing *Sida fallax* germination velocities in different treatments. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

Hypothesis Test Comparisons	F-Ratio	Raw P-value
Freshwater moist vs. Freshwater flooded	0.391	0.538
Low salinity moist vs. Low salinity flooded *	52.37	< 0.001
Medium salinity moist vs. Medium salinity flooded *	41.89	< 0.001
Freshwater moist vs. Low salinity moist *	22.17	< 0.001
Freshwater flooded vs. Low salinity flooded *	158.64	< 0.001

The germination velocity of *Sida fallax* was significantly affected by water at the low and medium salinity treatments (at the 0.05 level after a sequential Bonferroni adjustment), but not in freshwater treatment (Table 2.5, Table 2.6). In addition, the germination velocities of *Sida fallax* seeds exposed to the low salinity moist and flooded treatments were significantly lower (at the 0.05 level after a sequential Bonferroni adjustment) than germination velocities in the freshwater moist and flooded treatments (Table 2.5, Table 2.6).

Cyperus javanicus had a significant decrease in germination velocity as salinity increased (Table 2.6, F-ratio = 38.12, $p < 0.001$). It also appeared that *Cyperus*' germination velocity decreased when exposed to a higher water level (Table 2.5), but ANOVA analysis showed that this decrease was not significant at the $p < 0.05$ level (F-ratio = 2.705, $p = 0.120$).

Bolboschoenus maritimus had germination only in the freshwater treatments. Water level did not effect germination velocity (F-ratio = 0.787, $p = 0.396$)

The germination velocities of *Sesuvium portulacastrum* and *Batis maritima* were not statistically different among water or salinity treatments (Table 2.5, Table 2.7).

Table 2.7. ANOVA analysis of the germination velocity of *Sesuvium portulacastrum* and *Batis maritima*.

	<i>Sesuvium portulacastrum</i>		<i>Batis maritima</i>	
	F-Ratio	P-Value	F-Ratio	P-Value
Salinity	1.226	0.329	1.529	0.222
Water	0.074	0.789	3.288	0.077
Salinity x Water	0.795	0.513	0.642	0.593

Freshwater Transfer Germination

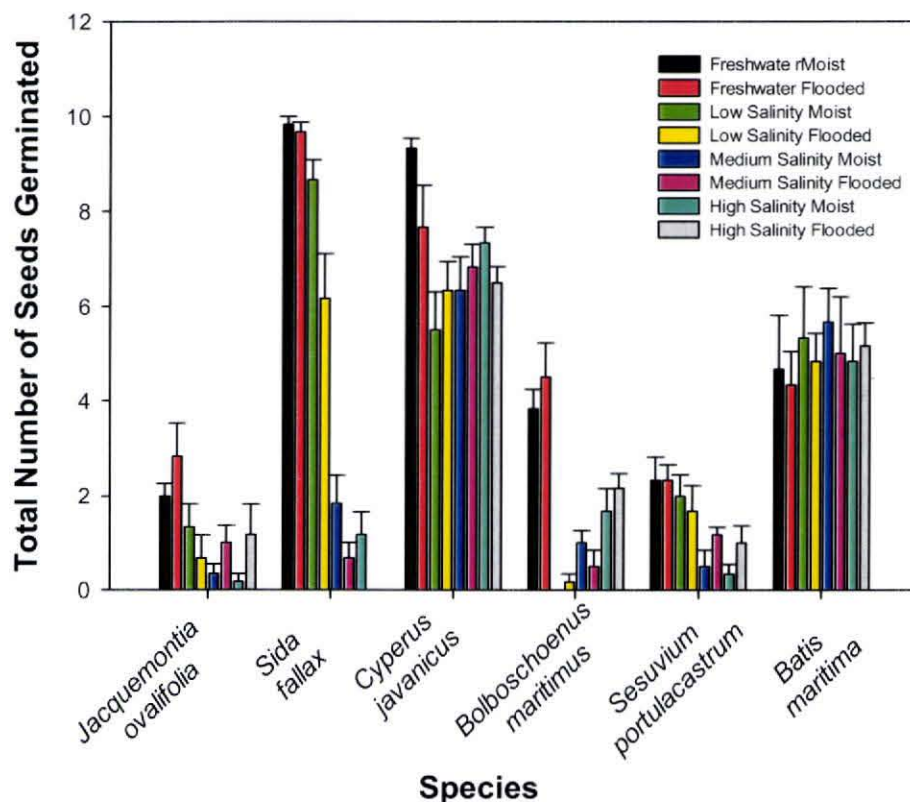


Figure 2.7. Mean total number of seeds germinated (\pm SEM, $n=6$) of all species studied (maximum = 10) in each of the eight salinity/water treatments including the twelve week experimental germination period and the four week period when seeds were transferred to freshwater to germinate. Seed mortality due to salinity is implied for species that retained lower germination rates in the salinity, relative to freshwater treatments.

Species varied in their ability to germinate in freshwater after exposure to the various salinity/water treatments (Figure 2.7). Overall, *Jacquemontia ovalifolia* had very low levels of germination. Some seeds, originally exposed to the low, medium, and high salinity levels germinated when seeds were transferred to freshwater, but germination levels were not as high as in the freshwater treatments (Figure 2.7). This indicates that some seeds exposed to higher levels of salinity may retain viability, but the majority of seeds were probably killed by the salinity.

A few *Sida fallax* seeds germinated after exposure to the medium and high salinity treatments. However, the total number of germinated seeds was not nearly as high as in the freshwater and low salinity treatments (Figure 2.7). Again, this indicates that while a few seeds may maintain viability, many seeds exposed to medium or high levels of salinity were probably killed.

Although *Cyperus* seeds did not germinate when exposed to high salinity levels, they retained high viability following the salinity treatments and germinated when transferred to freshwater (Figure 2.7).

A small portion of *Bolboschoenus maritimus* seeds exposed to the low, medium, and high salinity treatments germinated after transfer to freshwater conditions, but germination after freshwater transfer was not nearly as high as germination of seeds that started in freshwater conditions (Figure 2.7). Interestingly, the number of seeds able to germinate after exposure to salinity increased as salinity levels increased.

Sesuvium portulacastrum seeds exposed to the low salinity treatment germinated very well after freshwater transfer, with germination rates similar to those observed in freshwater. In addition, some seeds exposed to the medium and high salinity levels were

able to germinate after freshwater transfer, but total germination was considerably less in these treatments (Figure 2.7). Salinity did not affect additional germination by *Batis maritima* seeds after freshwater transfer (Figure 2.7).

Discussion

Direct Salinity Effects on Germination

Batis maritima, *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* are considered coastal species and therefore could be naturally subject to various stresses such as tidal inundation and salinity (Ungar 1991). The establishment potential of many plant populations is dependent upon the germination response of their seeds. The germination response of these six plants to salinity and flooding treatments was variable and species specific.

All five native plant species experienced their highest germination levels in either the freshwater treatments. This supports a previous review of literature (Ungar 1978), which concluded that the majority of halophytic species achieved optimal germination in distilled water. Furthermore, all five native species experienced decreased germination as salinity levels were increased; which supports previous investigations that salinity decreases germination (Williams and Ungar 1972, Ungar 1978, Baskin and Baskin 2001).

Bolboschoenus maritimus' low germination in freshwater and inability to germinate at high salinities supports previous research. Brimacombe (2003) showed that *Bolboschoenus* had a mean germination of 16% under freshwater and did not germinate at all at a salinity of 8.5 ppt. Seed dormancy mechanisms may have evolved in response to stressful conditions to enhance survival and fecundity at later stages in the life cycle of halophytes (Baskin and Baskin 2001). The low germination of *Bolboschoenus maritimus*

seeds in the freshwater treatment may be caused by physiological dormancy. Previous research has shown that species belonging to the Cyperaceae family achieved higher germination when exposed to cold stratification (Baskin and Baskin 2001). Increased germination, produced by soaking seeds in 3% bleach solution (Clevering 1998, Brimacombe 2003), is also an indication of non deep physiological dormancy (Baskin and Baskin 2001). Alternatively, it is possible that *Bolboschoenus* seeds were killed by the concentration or lengthy exposure of hypochlorite bleach.

Assuming that *Bolboschoenus* has some physiological dormancy that was not overcome by the seed treatments, it is possible that *Bolboschoenus* could have at least some ability to germinate at higher salinities (Baskin and Baskin 2001), even though no germination occurred in these experiments. For example, nonstratified seeds of *Salicornia europaea* germinated to 4% and 0% at 30 ppt and 50 ppt respectively whereas after cold stratified for four weeks, the seeds germinated to 82% and 43% at the same salinities (Philipupillai and Ungar 1984). Seeds of other species such as *Spergularia marina*, *Spergularia patula*, and *Puccinellia festucaeformis* also germinated to higher percentages at higher salinities when cold stratified compared to untreated seeds (Onnis and Miceli 1975, Ungar 1984, Berger 1985).

Furthermore, *Bolboschoenus maritimus* seedlings are rare in established vegetation (USGS 2005). Sexual reproduction perhaps serves primarily as means of creating seed banks of dormant seeds that can germinate when optimal conditions exist, or to facilitate long distance dispersal of seeds. (McNaughton 1975, Clevering 1998).

Jacquemontia ovalifolia's low germination percentages, when exposed to the freshwater treatments, could indicate either that a large number of the seeds were not

viable or that seeds were dormant. Other genera in the Convolvulaceae family have physically dormant seeds (Koller and Cohen 1959, Baskin and Baskin 2001); the primary reason for the lack of germination is the impermeability of the seed coat. It is possible that *Jacquemontia*'s low germination could be due to physical dormancy (Baskin and Baskin 2001). Alternatively, it is possible that a large number of *Jacquemontia* seeds were killed by the hypochlorite bleach.

Sida fallax and *Cyperus javanicus* seed viability was high, with germination percentages reaching 98% and 88% respectively in the freshwater moist treatment. These species are mildly salt tolerant. Both germinate when exposed to 10 ppt salinity, but germination at this level is significantly reduced. *Sida* appeared to be more salt tolerant, achieving more substantial germination percentages (81% and 55%) than *Cyperus* (33% and 3%) in the low salinity treatments. However, *Sida* seeds were nicked to allow water to imbibe the seed, which may have enhanced germination percentages at all salinities, relative to the other species.

Species in the Malvaceae family, to which *Sida fallax* belongs, have demonstrated physical dormancy (Baskin and Baskin 2001). This dormancy requirement was overcome by removing the mericarp and nicking the seeds (Yorkston 2005). Results from this study should be used with caution, because under natural field conditions, *Sida* seeds must naturally overcome physical dormancy before germination occurs. On the other hand, *Cyperus javanicus* is well suited for germination under low salinity conditions without pre-treatment.

Cyperus javanicus' salinity tolerance at the germination stage supports previous research by Brimacombe (2003), which found that *Cyperus* had zero germination in

salinities above 17.5 ppt. However, Brimacombe's 2003 study indicated a maximum germination of 74% under 8.5 ppt, in contrast with a mean germination of 33% in at 10 ppt observed in the present study. Brimacombe's higher germination rate occurred under a fluctuating temperature regime of 24.3/18.2 degrees Celsius whereas the germination percentage of *Cyperus* at 8.5 ppt was only 1% under a fluctuating temperature regime of 29/23 degrees Celsius (Brimacombe 2003), which is closer to conditions in this experiment. Temperature seems to influence the ability of *Cyperus* to germinate at higher salinities. Germination percentages of *Sesuvium portulacastrum* were relatively low, even in the freshwater treatments. However, based on the salinity levels at which germination significantly declines, it appears to be the most salt tolerant native species in this experiment. Germination percentages did not significantly decline until salinity levels of approximately 20 ppt were reached. Germination of *Sesuvium* in this experiment, was considerably lower than germination of *Sesuvium* in previous research, but its observed tolerance to salinity was consistent (Martinez and Valverde 1992). *Sesuvium* seeds exposed to 25% and 50% full seawater (8.5 ppt and 17.5 ppt, respectively) germinated at a rate around 75% (Martinez and Valverde 1992), but the germination percentage of *Sesuvium* seeds exposed to full seawater (35 ppt) was still 20%. In the present experiment, seeds had less than 5% germination in the 20 ppt and 30 ppt treatments. Low germination could be an effect of the population from which seeds were sampled. *Sesuvium* roots easily from shoot fragments (Lonard and Judd 1995) and it is possible that the maintenance of this species is typically dependent on growth and spread of clonal populations rather than by sowing seeds.

A reduction in germination as salinity levels increase is common among terrestrial plant species (Ungar 1982, Pujol et al. 2001) and previous studies have revealed that most halophytic species experience a reduction in germination when salinity levels surpass 10 ppt (Ungar 1978). *Batis maritima* appears to be an exception to this rule. In contrast with native species, *Batis* had no significant decline in germination rate from freshwater to 30 ppt salinity. If higher salinity levels had been examined, *Batis* may have indicated a decrease in germination. Previous research examined the growth of *Batis maritima* under higher salinity levels (Miyamoto et al. 1996, El-Sayed and Noaman 2001), but germination responses were not studied. Results from this study show that *Batis* can germinate in near sea water conditions (34 ppt), allowing the species to germinate in the lower tidal portions of saline wetlands, where few other plant species can germinate and establish.

Water Effects on Germination

Although research has shown that germination can be inhibited if too much water is present (Baskin and Baskin 2001), seed germination of *Batis maritima*, *Bolboschoenus maritimus*, *Jacquemontia ovalifolia*, and *Sesuvium portulacastrum* were not significantly different in moist and flooded water levels. The technique of exposing these seeds to flooded conditions may have affected germination. Thirty ml of water was added to Petri dishes lined with filter paper, and seeds were placed on top of the paper. Many *Batis*, *Bolboschoenus*, and *Cyperus* seeds remained floating in the treatment; therefore, all of the species may not have actually been submerged in water. Seeds should have been placed under the filter paper to ensure submersion of the seed.

Cyperus javanicus germination percentages significantly decreased as water levels increased and this supports previous research by Brimacombe (2003), which showed that *Cyperus* percent germination was significantly greater when exposed to saturated filter paper than in one cm of standing water.

The significant decrease in *Sida fallax* germination only in the low salinity (10 ppt) flooded treatment, could be the result of additional stress. Seed germination was significantly reduced in the low salinity treatment and the additional stress of increasing water level could have caused further reduction in germination.

Indirect Effects of Salinity on Germination

Various biological and environmental conditions and their interactions can signal a plant to induce germination (Khan and Ungar 1984). A distinguishing trait of many halophytic species is the ability of seeds to remain dormant when exposed to high salinities. The seeds can then germinate when more favorable salinity levels occur (Ungar 1978, Baskin and Baskin 2001). Determining whether ungerminated seeds remain viable after prolonged salinity exposure is important because it determines whether a species can take advantage can build up a seed bank in the lower tidal portions of saline wetlands, allowing them to potentially germinate during brief windows of decreased salinity, such as following heavy rain (Ungar 1982).

A small portion of *Bolboschoenus maritimus* seeds were able to germinate in freshwater after exposure to each of the higher salinity levels. Previous research has shown that *Bolboschoenus* achenes are viable for at least two years after sinking into sediments of saline wetlands (Grillas et al. 1993). Interestingly, in this study more *Bolboschoenus* seeds seemed to germinate after exposure to the high salinity than seeds

exposed to the low and medium salinities. This may be an adaptation of the species to establish in areas that have high levels of salinity, where less competition from non-salt tolerant species occurs.

The ability of *Cyperus javanicus* seeds to remain viable during periods of high salinity exposure, and enhancement of germination when salinity is decreased is reflective of findings by (Brimacombe 2003). Although *Cyperus* seeds did not germinate at high salinity levels (17 ppt and 34 ppt), seeds rapidly germinated when transferred to freshwater (Brimacombe 2003).

Both *Bolboschoenus maritimus* and *Cyperus javanicus* will have an advantage in areas where saline conditions remain high for a period, killing non-tolerant species, and allowing viable *Cyperus* and *Bolboschoenus* to germinate and establish when low salinities return. *Batis maritima* and *Sesuvium portulacastrum* would also have an advantage because they can establish in the higher salinities sooner than other species that must wait for a decrease in salinity.

Jacquemontia ovalifolia and *Sida fallax* seeds had a more limited ability to recover from high salt exposure. The reduced ability of seeds to germinate after exposure to salinity, indicates that prolonged saline conditions will substantially decrease seed viability, even when freshwater conditions are restored. This is important information for managers because even if mature plants of *Jacquemontia* and *Sida* can withstand high saline conditions, recruitment will not occur in large numbers in areas exposed to high salinities.

A small percentage of *Sesuvium portulacastrum* seeds remained viable after exposure to the medium or high salinity levels, but germination was reduced by exposure

to the higher salinity levels. In contrast, Martinez et al (1992) found that the germination of *Sesuvium* seeds, exposed to 35 ppt, had only 20% germination, but when seeds were transferred to freshwater, germination increased to over 60%, which was not significantly different than the germination of seeds constantly exposed to freshwater conditions.

Seed source may influence germination percentages of *Sesuvium portulacastrum* at increased salinities (Baskin and Baskin 2001, Collinge and Wise 2003). For example, seeds of *Ceratoides lanata*, from Rush Valley, Utah, showed approximately 15% germination under salinities of 20 ppt, whereas seeds from Cisco, Utah showed approximately 40% germination under salinities of 20 ppt (Workman and West 1967, Baskin and Baskin 2001). *Sesuvium* seeds were collected on rocky substrate along the edge of a small off-shore island; soils were not inundated with tidal water, but may have increased salinities due to salt spray.

It is ecologically significant that many halophytic species can successfully germinate after exposure to high salt stress because it is a sign that these species have developed a physiological response to successfully establish in an area that is not habitable to many other plant species (Ungar 1982). Delaying germination until salinities are reduced could be extremely beneficial for a species by ensuring that its future seedlings will establish, grow to maturity and reproduce (Ungar 1982).

Germination Velocity

Cyperus javanicus and *Sida fallax* experience a delay in germination as salinities increase, which is supportive of previous research on other species (Baskin and Baskin 2001). However, germination was not significantly delayed in *Batis maritima*, *Jacquemontia ovalifolia*, or *Sesuvium portulacastrum* when seeds of these species were

exposed to high saline conditions. Therefore, *Jacquemontia*, *Batis*, and *Sesuvium*'s germination velocities do not support previous research which showed that even halophytes had slower rates of germination at salinities above 10 ppt (Ungar 1978, Baskin and Baskin 2001).

Experimental Techniques and Low Germination

Bolboschoenus maritimus, *Jacquemontia ovalifolia*, and *Sesuvium portulacastrum*'s germination did not exceed 45% in any of the treatments and many factors may have affected these seeds' ability to germinate. Seeds of the selected species were not used immediately after collection. Seeds were refrigerated to minimize physiological changes prior to use, but because seeds were not tested before and after storage, so it is not known whether the observed germination percentages are representative of fresh seeds (Baskin and Baskin 2001).

Bolboschoenus maritimus and *Jacquemontia ovalifolia* seeds were exposed to strong and prolonged hypochlorite bleach pre-treatment to decrease the chance of fungal invasion and increase the probability of germination, however, this hypochlorite bleach solution may have been lethal to some of the seeds.

While, non-dormant seeds germinate equally well in light and darkness, some species germinate better under constant light conditions (Grime 1979, Baskin and Baskin 2001), while other species germinate better under dark conditions (Baskin and Baskin 2001). Since little was known about the light requirements for these species and in order to simulate soil surface conditions in the field, I exposed seeds to twelve hours of light and 12 hours of darkness. Nevertheless, species may have germinated more successfully under constant light or dark conditions.

The germination success of seeds can be affected by the orientation of the seed on the substrate (Bosy and Aarssen 1995, Baskin and Baskin 2001). The flat filter paper onto which the seeds were placed in this experiment did not allow the same range of seed orientations as seeds in soil. Since, seeds did not have the same degree of contact with the substrate, germination could have been affected.

Conclusions

Results from this experiment show that all the native coastal Hawaiian species were negatively affected by increasing salinity. However, *Batis maritima*'s germination was not significantly decreased with increasing salinity. Therefore, my hypothesis that all species would be negatively affected by increasing salinities was supported for the native species, but was not supported for *Batis maritima*. My hypothesis that the native groundcover species (*Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum*) and the invasive *Batis* would have higher germination under moist than flooded conditions was not generally supported. *Batis*, *Jacquemontia*, and *Sesuvium* were not significantly affected by water level. However, *Sida* did show a significant decrease in germination as water level increased, but only in the low salinity treatment.

My final hypothesis that *Bolboschoenus maritimus* and *Cyperus javanicus* would germinate more successfully under flooded conditions than other species was also was not supported. *Bolboschoenus* was not significantly affected by water level and *Cyperus* showed a significant reduction in germination under flooded conditions.

Future Studies

Seeds are not always available when they are needed for restoration projects. Therefore, germination studies are needed for both fresh seeds and seeds that have been

stored with different techniques for a variety of time periods (Baskin and Baskin 2001).

This will allow managers to determine individual species' ability to germinate after storage. In addition, more research is needed to investigate the effects of salinity on dormancy loss during cold stratification in order to more fully understand the germination ecology of halophytes (Baskin and Baskin 2001).

Finally, because of the extremely low germination percentages in *Bolboschoenus maritimus*, *Jacquemontia ovalifolia*, and *Sesuvium portulacastrum*, dormancy mechanisms and techniques for inducing germination should be investigated further to maximize success in using seeds of these species to restore for coastal restoration projects.

Chapter 3. Early Growth of Four Native Hawaiian Coastal Species and an Invasive Species in Response to Salinity and Flooding

Abstract

This study examined the ability of four native Hawaiian plant species, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum*, and one exotic species, *Batis maritima*, to tolerate flooding and salinity during early seedling growth. Species were grown in a greenhouse under a combination of two salinities (10 ppt and 30 ppt) and two water levels (moist = water contact at 17 cm below the soil surface and flooded = water contact at 4 cm below the soil surface). Total dry biomass, relative growth rate of shoot length (RGR_{SH}), specific leaf area, and leaf area ratio of all species were significantly reduced in the high salinity treatment. For *Jacquemontia* and *Cyperus*, root and shoot lengths were significantly reduced under the high salinity treatment. Leaf mass fraction was not significantly affected by water or salinity, but root mass fraction of *Cyperus* and *Sesuvium* was significantly smaller under increasing water levels.

Introduction

Coastal wetlands are unique because of their proximity to salt water. They can be classified into three major groups: tidal salt marshes, tidal freshwater marshes, and mangrove swamps (Adam 1990, Mitsch and Gosslink 1993). The two primary factors which often control the vegetational composition of these ecosystems are 1) tidal inundation and 2) salt content of the water (Kozlowski 1984, Eleuterius 1990).

High salt concentrations can reduce initial seedling growth as well as the growth and reproduction of established plants (Ungar 1978, Kuhn and Zedler 1997). Determining the salinity tolerance of a species can be complicated because salt tolerance at the germination stage is not always correlated with tolerance at later stages of development (Waisel 1972). Furthermore, establishment in hypersaline sites might be difficult for a particular species, since evidence shows that some halophytes germinate at higher rates and grow better at salinities well below what they can tolerate at maturity. (Naidoo 1992, Khan 2001).

While many plants show growth inhibition at increasing salinities, adaptations such as adventitious root development, succulence, and the ability to adjust osmotically, allow some plants to grow and survive in saline habitats (Ungar 1991). Cellular ion concentrations may be reduced by increased water uptake, ion excretion via salt glands, or exclusion of salt by root membranes (Caldwell 1974).

Many halophytes are succulent and succulent plants exhibit different anatomical and ultrastructural features than non-succulent plants (Daubenmire 1974). The most obvious differences are increased leaf thickness, decreased number of leaves per plant, increased leaf area, decreased stomatal density, and increased cell size (Flowers et al.

1986). Researchers have also found evidence of increased succulence with increased salinization (Caldwell 1974, Ottow et al. 2005). Generally, increasing soil salinity has a negative effect on plant growth and development, but the strength of this effect varies dramatically between species (Flowers et al. 1986).

Growth responses to salinity can differ at different stages of plant development (Ungar 1991). Development of physiological adaptations may not have occurred in young seedlings and could be a major cause of seedling mortality (Adam 1990). Vascular plants may differ in their tolerance to salinity, but salinity levels beyond which no growth is possible does exist (Adam 1990). However, it can be difficult to predict the natural distribution of plants in the field based on physiological limits of tolerance produced from laboratory and greenhouse experiments because of other biotic and abiotic influences in the natural environment (Ungar 1991).

Growth and distribution of plants can be affected by drought or excess amounts of water, and while water itself is not damaging to a plant, excess water in the soil displaces air from the non-capillary pore space, producing an oxygen deficiency that may reduce the growth and functioning of the plant (Kramer and Boyer 1995).

Although many researchers have found that when flooding occurs for a long time, plants are often killed (Whitlow and Harris 1979, Kozlowski 1984), the occurrence and extent of damage caused by flooding depends on many factors including species, plant age, the duration, depth and timing of flooding, soil types, and conditions of temperature and light, before and during flooding (Kozlowski 1984). The structure of plants is also influenced by water balance; plants grown under excess water balances may have smaller shoots, a smaller root system, and greater longevity (Daubenmire 1974). Flooding for

prolonged periods reduces growth, causes epinasty, leaf chlorosis, and sometimes mortality (Kramer and Boyer 1995).

Higher water tables can also cause plants to root more superficially and water logging inhibits leaf growth, stem extension, and photosynthesis (Daubenmire 1974, Kozlowski 1984). Nevertheless, some aquatic species and marsh dwelling plants elongate their stems or petioles more quickly under flooded conditions (Kozlowski 1984). In Hawai'i, two native sedges, *Bolboschoenus maritimus* and *Cyperus javanicus* are often seen growing in shallow water at Honouliuli NWR and Pouhala NWR. The introduced species, *Batis maritima*, has a significant presence at these sites as well as at the Waiawa unit of Pearl Harbor NWR, where it grows in both dry and flooded soils (Personal observation).

The objective of this experiment was to investigate salinity and flooding effects on the early growth of four native species *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* as well as the invasive species, *Batis maritima*. The experiment tested the hypotheses that 1) early growth of all species will decline with increasing salinity 2) the succulent species, *Batis maritima* and *Sesuvium portulacastrum* will more effectively tolerate high levels of salinity during early growth, and 3) species grown under flooded conditions will show a decrease in root and shoot length.

Materials and Methods

Methods for seed collection and seed pre-treatment were the same as for the germination experiment (Chapter 2) ; please refer to Chapter 2: Germination Methods Section for details.

Seedling establishment

In late December 2005, after pretreatment, seeds were placed in Petri dishes filled with 4 ml of deionized water and put in the Percival I30-VL incubator to germinate. Seeds were exposed to an alternating twelve hour regime of a temperature of 30 degrees Celsius under $80\mu\text{mol}/\text{m}^2/\text{s}$ light and a twelve hour regime of 20 degrees Celsius under darkness.

Germinated seeds were transferred to 24 x 54cm plastic flats containing one part perlite and one part sphagnum moss. These flats were kept on the fifth floor lanai of the St. John Building until seedlings established roots and grew large enough to transfer to dibble tubes in the greenhouse. On the lanai, seedlings received ambient light, but were not exposed to rain or direct sunlight. Average minimum and maximum temperatures during December and January in Honolulu, Hawai'i are 18° and 27° Celsius, respectively (WRCC 2006).

The dibble tubes were placed in 32 x 32 cm bins filled with 7 cm of freshwater for approximately one week (freshwater moist conditions). This allowed seedlings to overcome the initial transplant shock before beginning the experimental treatments.

Experimental Early Growth

The early growth experiment occurred in the University of Hawai'i at Manoa's Botany greenhouse. Individual plants were grown in dibble tubes (4 cm wide at the top and 21 cm in length), which were placed in 30 x 30 cm dibble tube racks, immersed in 32 x 32 cm bins containing water, and placed on benches. This experiment was a two by two factorial design with two levels of salinity (low salinity = 10 ppt and high salinity = 30 ppt) and two levels of inundation (moist = water level 17 cm from the soil surface and flooded = water level 4 cm from the soil surface).

Each bin represented a treatment and there were five replicate bins per treatment. Each species had between one and three replicate plants per bin, depending on the success of germination and transplant survival of seedlings. Plants were grown in dibble tubes containing 3-5 stones of gravel at the bottom (to prevent soil from washing out of the bottom) and a soil mixture containing two parts local top soil, one part Menehune Soil Compost (Hawaiian Earth Products, Ltd. Kailua, HI), and two parts perlite. The soil mixture is not representative of local wetland soils, but was used to prevent soil compaction and allowed water to wick from the water bin through the tube to the soil surface.

Approximately 4 grams of 19-6-12 Osmocote (Scotts Miracle Grow Company, Maryville, Ohio) were added to each of the dibble tubes at the beginning of the experiment to provide nutrients for the plants. Salinity and water levels were checked weekly by refractometer measurements and reference to the experimental fill line, respectively, and then adjusted as necessary. In order to prevent the accumulation of salts at the soil surface, once per week, plants were flushed twice with tap water until water poured out the bottom holes. (McKee and Mendelssohn 1989).

For *Cyperus javanicus*, *Jacquemontia ovalifolia*, and *Sida fallax*, experimental treatments began on February 7th 2006; final harvest occurred after eight weeks. For *Sesuvium portulacastrum* and *Batis maritima*, treatments began on March 1, 2006; final harvest occurred seven weeks later.

Measurements were recorded weekly, including shoot number, shoot length, leaf number, and number of flowers. At the end of the experiment, plants were removed from the soil, roots were washed to remove excess particles, and above and belowground parts

were separated. Final measurements were recorded including root length, root fresh weight, shoot length, shoot fresh weight, shoot number, leaf number, and leaf surface area. Leaf surface area was measured with a Licor 3100 leaf area meter (LICOR Lincoln, Nebraska). Separated parts were dried at 55 degrees Celsius for three days and weighed.

Data Analysis

A three way ANOVA (Systat Version 10.2, Systat Software Inc.) was used to compare differences in the final harvest measurements with species, salinity, and water as fixed factors. *Batis maritima* was excluded from the root length ANOVA due to non-normality of the data. For *Batis*, to test my hypothesis that root length would decline in flooded conditions, I pooled my salinity treatments and used a Mann-Whitney non-parametric U test on the log of root length with water as the grouping variable.

Normal probability plots were visually inspected to assess normality of the data, and a log transformation was performed to normalize data. Hypothesis tests were performed to clarify how specific treatments affected individual species, and a sequential Bonferroni adjustment was applied to all analyses.

All recorded measurements, including root length, root dry weight, shoot length, shoot dry weight, additional shoot number, leaf number, leaf dry weight, total dry weight biomass, leaf surface area, specific leaf area (SLA), root mass fraction (RMF), shoot mass fraction (SMF), leaf mass fraction (LMF), and leaf area ratio (LAR), were analyzed using the three way ANOVA. However, many of these variables were correlated (Appendix A). Therefore, only total dry weight, root length, shoot length, RMF, LMF, SLA, and LAR are presented and discussed.

RMF was calculated as the fraction of total dry weight allocated to the roots and LMF was calculated as the total dry weight allocated to leaves (Lambers et al. 1998). SLA was calculated as the mean area of leaf displayed per unit of leaf weight (Include Bayuelo-Jiminez 2003 p. 211). Leaf area ratio (LAR) is the product of specific leaf area and the leaf mass ratio (Lambers et al. 1998). Appendix B contains the ANOVA analyses of all remaining variables.

In addition to these seven variables, the relative growth rate of shoot length (RGR_{SH}) was also calculated in order to assess whether growth occurred during the experiment. The calculation for RGR_{SH} is as follows:

$$RGR_{SH} = \frac{\text{Log(Final Shoot Length)} - \text{Log(Initial Shoot Length)}}{\text{Time}}$$

A three way ANOVA was performed on RGR_{SH} with species, salinity, and water as fixed factors.

Results

Relative Growth Rate of Shoot Length (RGR_{SH})

Sesuvium portulacastrum had the highest RGR_{SH} of all species studied in all salinity and water treatments (Figure 3.1). *Batis maritima* and *Jacquemontia ovalifolia* also had a high RGR_{SH} in the low salinity treatments (Figure 3.1).

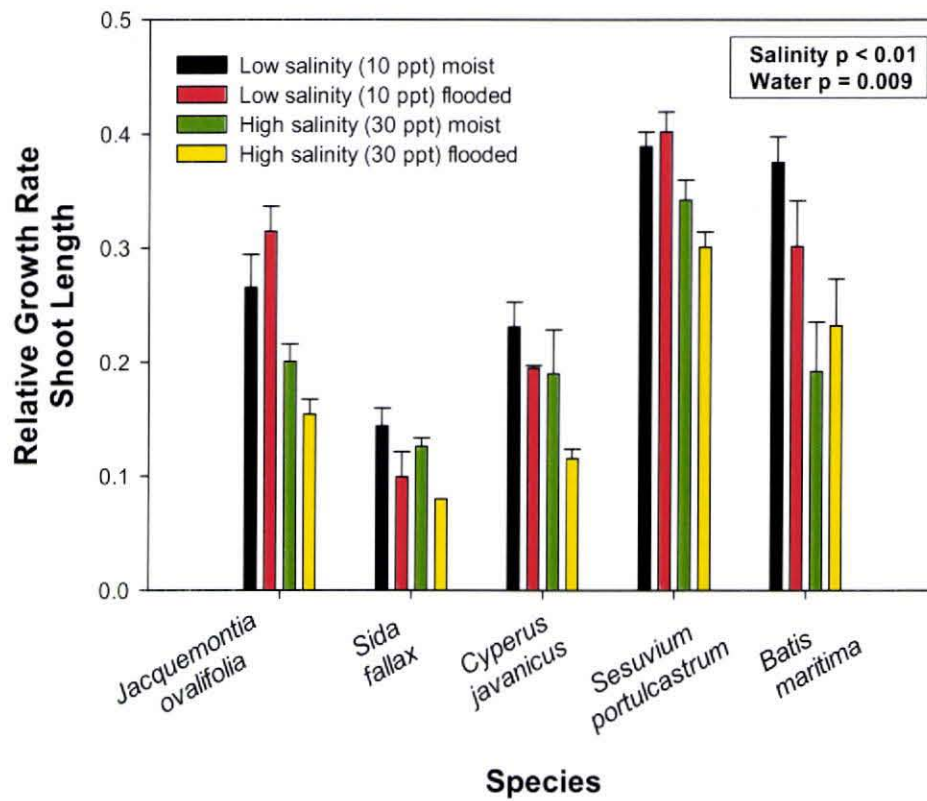


Figure 3.1. Relative growth rate of shoot length (RGR_{SH}) (mean \pm SEM, $n=1-15$) of five species under four treatment combinations of two salinity levels and two water levels. Across all species, RGR_{SH} was significantly different between salinity treatments ($p < 0.01$). RGR_{SH} is significantly different between water levels ($p = 0.009$).

Salinity and water level significantly affected the RGR_{SH} of all species studied (Table 3.1). All species' RGR_{SH} were significantly higher in the low salinity treatments compared to the high salinity treatments (Figure 3.1, Table 3.1). *Sida fallax* and *Sesuvium portulacastrum* had a 13% decrease in their RGR_{SH} when salinity was increased in the moist treatments; *Cyperus javanicus* and *Jacquemontia ovalifolia* had a 18% and 25% decrease in their RGR_{SH} when salinity was increased in the moist treatments, respectively. *Batis maritima* showed a 50% decrease in RGR_{SH} when salinity was increased in the moist treatments (Figure 3.1).

Table 3.1. ANOVA results for the log RGR_{SH} with species, salinity, and water as fixed factors. An asterisk indicates significance at p<0.05 level.

ANOVA Log Shoot Length	F-Ratio	P-Value
Species *	43.256	<0.001
Salinity *	31.505	<0.001
Water *	7.228	0.009
Species x Salinity	1.692	0.159
Species x Water	1.460	0.221
Salinity x Water	1.045	0.309
Species x Salinity x Water	1.735	0.149

ANOVA showed that RGR_{SH} was significantly affected by water level (Table 3.1). RGR_{SH} was significantly higher in the moist treatments compared to the flooded treatments for *Cyperus javanicus* and *Sida fallax* (Figure 3.1) Significant interactions were not apparent, probably due to the large variation within treatments.

Total Dry Weight

Total dry weight was used as a measure of overall growth. The total dry weight of all species was predicted to decline with increasing salinity, but *Batis maritima* and *Sesuvium portulacastrum* were predicted to be more tolerant of high levels of salinity stress.

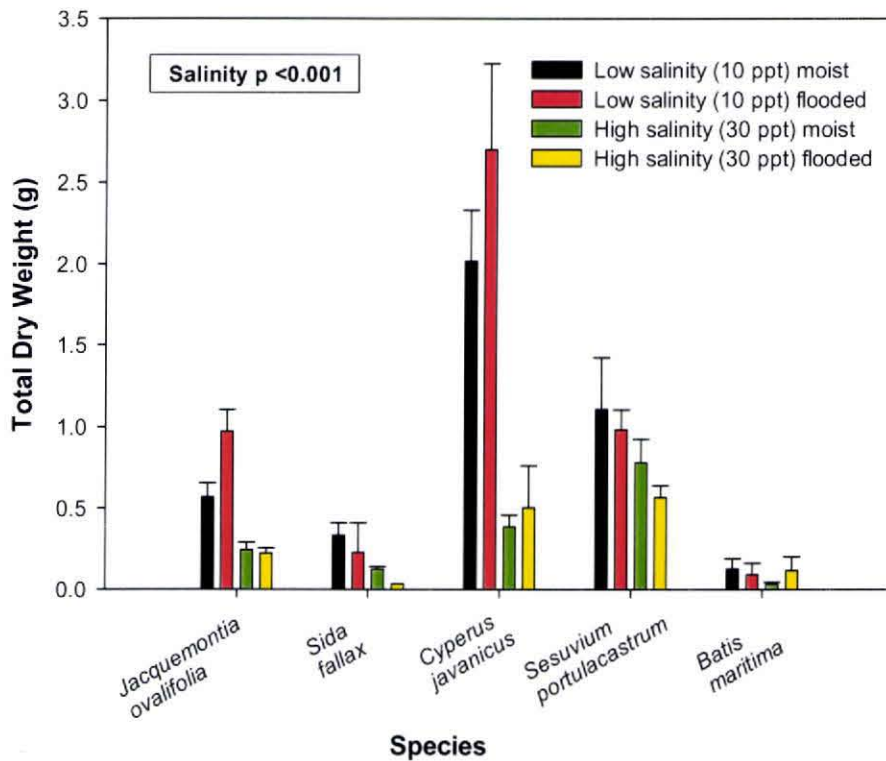


Figure 3.2. Mean final total dry weight (\pm SEM, $n=1-15$) of five species exposed to four treatment combinations of two salinity levels and two water levels. For each species, total dry weight was significantly different by ANOVA ($p < 0.001$) between salinity treatments. Water level did not significantly affect total dry weight.

Total dry weights of all species exposed to the low salinity treatment were significantly higher than total dry weights of plants exposed to the high salinity treatment (Figure 3.2, Table 3.2). *Batis maritima* showed the smallest absolute difference in total dry weight between salinity levels (Table 3.2, Figure 3.2).

For all species, total dry weights were not significantly different in the moist and flooded treatment for either salinity level (Figure 3.2, Table 3.2).

Table 3.2. ANOVA results for the log of total dry weight with species, salinity, and water as fixed factors. An asterisk indicates significance at the $p < 0.05$ level.

ANOVA Log of DW Biomass	F-Ratio	P-Value
Species *	34.377	<0.001
Salinity*	26.085	<0.001
Water	0.614	0.437
Species x Salinity	1.435	0.235
Species x Water	1.001	0.415
Salinity x Water	0.306	0.582
Species x Salinity x Water	0.259	0.903

Root Length

All species root lengths were expected to be smaller when exposed to flooded conditions. A significant interaction between species and salinity and species and water indicates that the effect of these factors on root length depends on the species (Table 3.3).

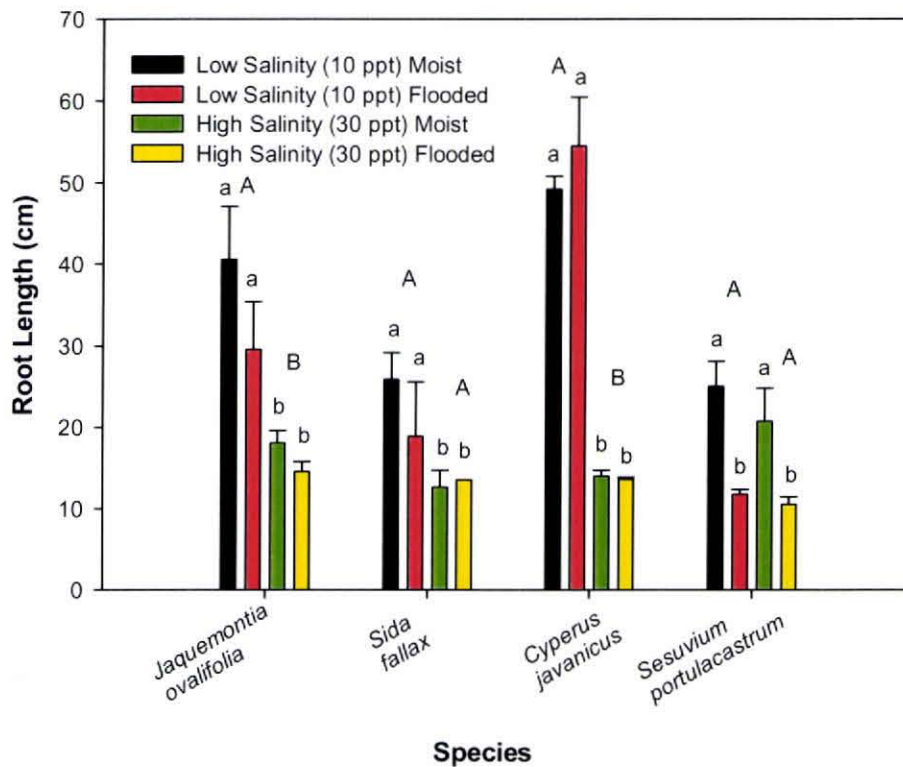


Figure 3.3. Final root lengths (mean \pm SEM, $n=1-15$) of four species under four treatment combinations of two salinity levels and two water levels. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between salinity levels are indicated by different uppercase letters.

Cyperus javanicus, *Jacquemontia ovalifolia*, and *Sida fallax* root lengths were smaller in the high salinity treatments (Figure 3.3), although the difference in *Sida fallax* root length was no longer significant at the $p < 0.05$ level after a sequential Bonferroni adjustment (Figure 3.3, Table 3.4). *Sesuvium portulacastrum*'s root length was also not significantly affected by salinity (Table 3.4)

Table 3.3. ANOVA results for the log of root length with species, salinity, and water level as factors. *Batis maritima* was not included in analysis. An asterisk indicates significance at the $p < 0.05$ level.

ANOVA Log of Root Length	F-Ratio	P-Value
Species *	10.996	<0.001
Salinity *	65.419	<0.001
Water *	9.243	0.004
Species x Salinity*	8.611	<0.001
Species x Water*	3.851	0.015
Salinity x Water	0.817	0.371
Species x Salinity x Water	0.387	0.763

Jacquemontia ovalifolia and *Sesuvium portulacastrum* both had smaller root lengths in the flooded treatments, but the effect of water was only significant for *Sesuvium portulacastrum* at the $p < 0.05$ level after sequential Bonferroni adjustment (Figure 3.3, Table 3.4). Therefore, *Jacquemontia ovalifolia*, *Sida fallax*, and *Cyperus javanicus*’ root lengths were not significantly affected by water level (Table 3.4).

Table 3.4. Hypothesis tests comparing individual species’ final root length in the low salinity treatment to its final root length in the high salinity treatment and comparing final root length in the moist treatment to its root length in the flooded treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

Species Comparison	F-Ratio	Raw P-Value
<i>Jacquemontia ovalifolia</i> * Low vs. High Salinity	28.698	<0.001
<i>Sida fallax</i> Low vs. High Salinity	5.661	0.021
<i>Cyperus javanicus</i> * Low vs. High Salinity	51.593	<0.001
<i>Sesuvium portulacastrum</i> Low vs. High Salinity	2.031	0.161
<i>Jacquemontia ovalifolia</i> Moist vs. Flooded	4.157	0.047
<i>Sida fallax</i> Moist vs. Flooded	0.352	0.556
<i>Cyperus javanicus</i> Moist vs. Flooded	0.043	0.836
<i>Sesuvium portulacastrum</i> * Moist vs. Flooded	25.121	<0.001

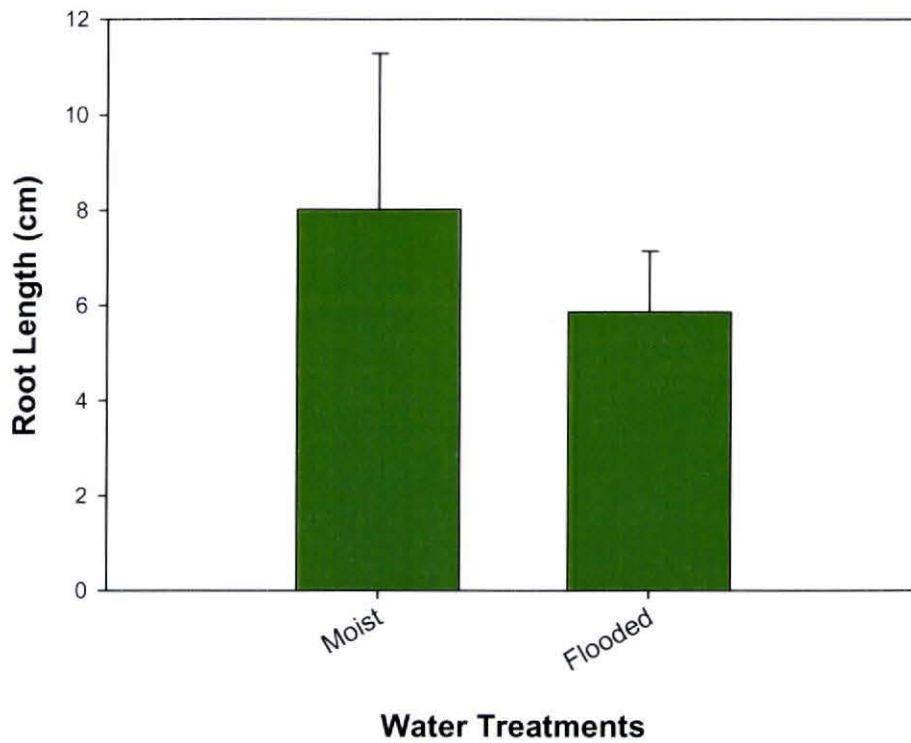


Figure 3.4. Mean root length (\pm SEM, $n = 6-7$) of *Batis maritima* under two different water treatments. Root length was not significantly affected by water level.

A Mann-Whitney U test showed that the root length of *Batis* was not significantly affected by water level (Mann-Whitney U test statistic = 23.5, p-value = 0.721).

Shoot Length

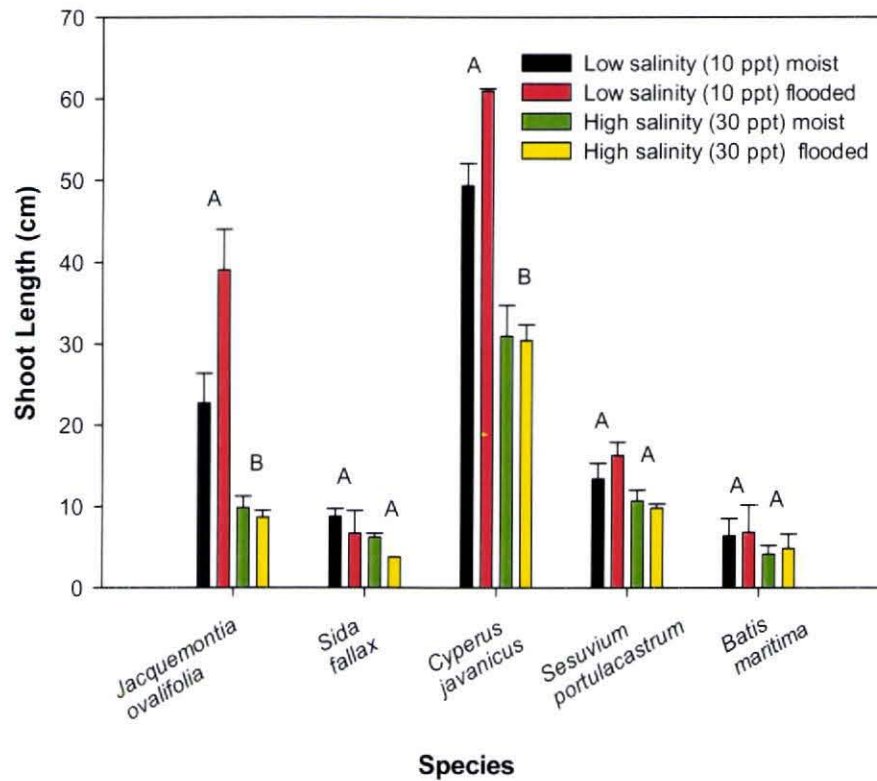


Figure 3.5. Final shoot length (mean \pm SEM, $n=1-15$) of four species under four treatment combinations of two salinity levels and two water levels. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between salinity levels are indicated by different uppercase letters. Water level did not significantly affect the shoot length of any species.

Shoot length of all species was predicted to be smaller when exposed to flooded treatments as opposed to moist treatments, but water level did not significantly affect shoot length of any of the species (Figure 3.5, Table 3.5). The effects of salinity on shoot lengths differed across species, as indicated by the significant species-by-salinity interaction (Table 3.5).

Table 3.5. ANOVA results for the log of shoot length with species, salinity, and water as fixed factors. An asterisk indicates significance at the $p < 0.05$ level.

ANOVA Log Shoot Length	F-Ratio	P-Value
Species *	52.028	<0.001
Salinity *	32.117	<0.001
Water	0.008	0.931
Species x Salinity *	3.314	0.017
Species x Water	1.008	0.411
Salinity x Water	1.864	0.178
Species x Salinity x Water	0.384	0.819

For *Jacquemontia ovalifolia* and *Cyperus javanicus*, shoot lengths were significantly larger ($p < 0.05$ after a sequential Bonferroni adjustment) at low salinities than at high salinities (Figure 3.5, Table 3.6). *Sida fallax*, *Sesuvium portulacastrum*, and *Batis maritima*'s shoot lengths also showed the same trend, but the difference was not significant at the p -value < 0.05 after a sequential Bonferroni adjustment (Figure 3.5, Table 3.6).

Table 3.6. Hypothesis tests investigating salinity's affect on individual species' shoot length. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

Species Comparison	F-Ratio	Raw P-Value
<i>Jacquemontia ovalifolia</i> * Low vs. High Salinity	42.340	<0.001
<i>Sida fallax</i> Low vs. High Salinity	2.123	0.151
<i>Cyperus javanicus</i> * Low vs. High Salinity	5.742	0.020
<i>Sesuvium portulacastrum</i> Low vs. High Salinity	4.013	0.050
<i>Batis maritima</i> Low vs. High Salinity	2.355	0.131

Specific Leaf Area (SLA)

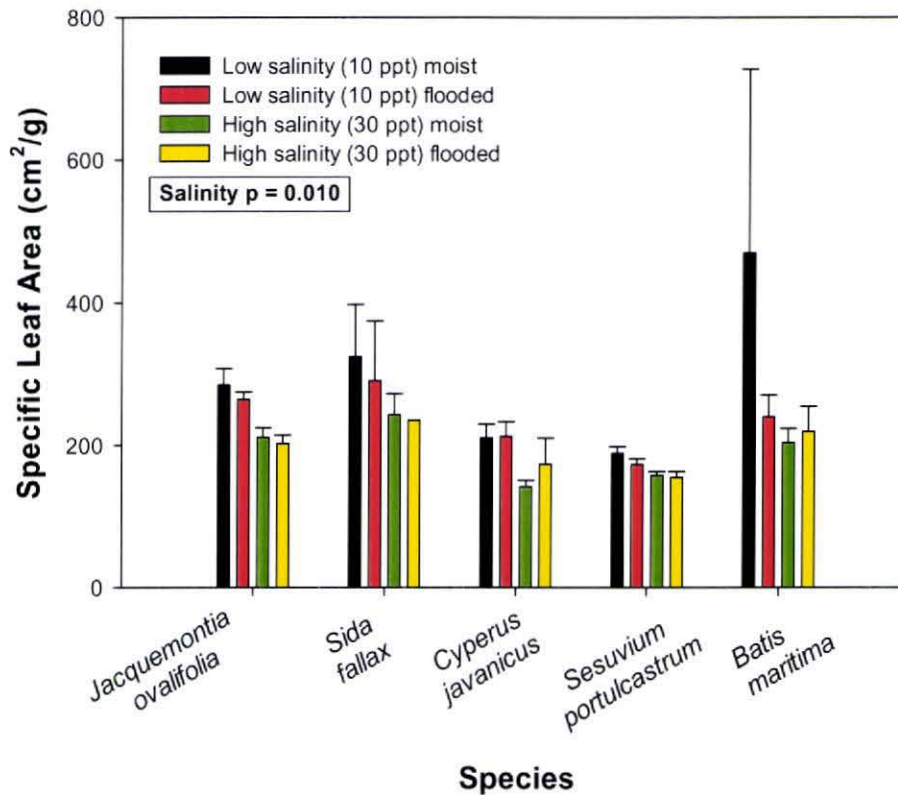


Figure 3.6. Specific leaf area (SLA) (mean \pm SEM, $n=1-15$) of four species under four treatment combinations of two salinity levels and two water levels. SLA was significantly different between salinity treatments ($p = 0.010$). Water level did not significantly affect SLA.

Specific leaf area (SLA) is the amount of leaf area per unit leaf mass (Lambers et al. 1998). All species SLA values were significantly smaller in the high salinity treatment compared to the low salinity treatment (Figure 3.6, Table 3.7). *Sesuvium portulacastrum* had the smallest absolute difference in SLA between salinity treatments (Figure 3.6). SLA was not significantly different between the water treatments for any of the species studied (Figure 3.6, Table 3.7)

Table 3.7. ANOVA results for specific leaf area with species, salinity, and water as fixed factors. An asterisk indicates significance.

ANOVA Log Leaf Surface Area	F-Ratio	P-Value
Species *	3.326	0.014
Salinity *	6.893	0.010
Water	0.958	0.330
Species x Salinity	0.560	0.692
Species x Water	0.514	0.726
Salinity x Water	1.414	0.237
Species x Salinity x Water	0.661	0.620

Leaf Area Ratio (LAR)

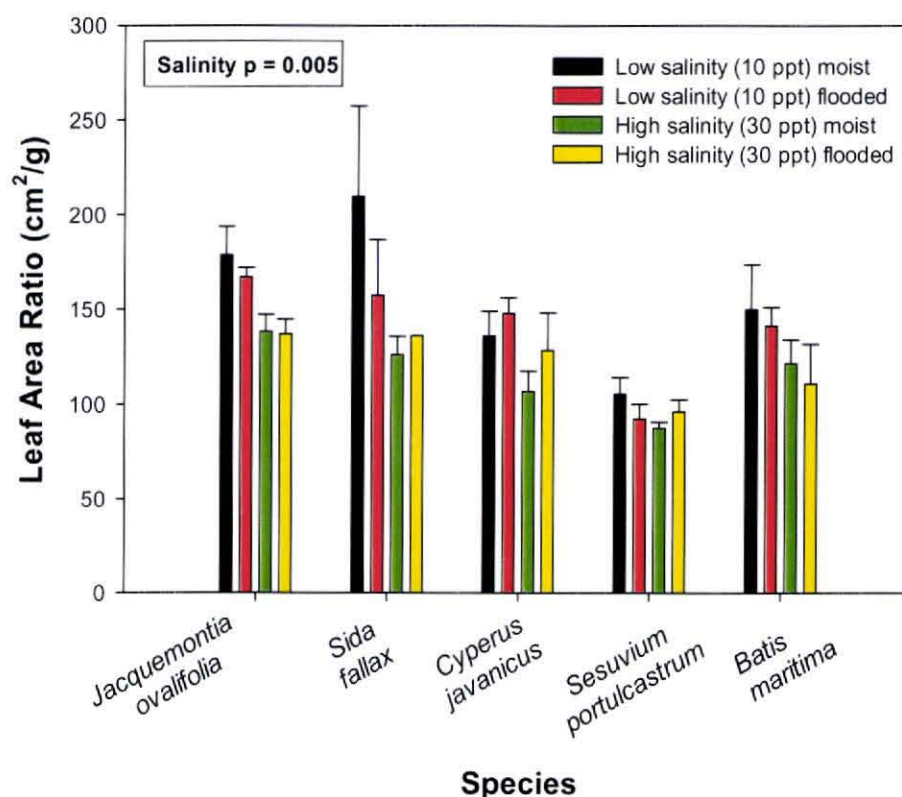


Figure 3.7. Leaf area ratio (mean \pm SEM, $n = 1-15$) of four species under four treatment combinations of two salinity levels and two water levels. LAR was significantly different between salinity treatments ($p < 0.001$). LAR was significantly different between water levels ($p = 0.005$).

All species LAR values were significantly larger in the low salinity treatment compared to the high salinity treatment. LAR was not significantly different between the two water treatments (Figure 3.7, Table 3.8).

Table 3.8. ANOVA results for leaf area ratio (LAR) with species, salinity, and water as fixed factors. An asterisk indicates significance.

ANOVA Log Leaf Surface Area	F-Ratio	P-Value
Species *	8.799	<0.001
Salinity *	8.403	0.005
Water	0.203	0.653
Species x Salinity	0.663	0.619
Species x Water	0.264	0.900
Salinity x Water	1.015	0.316
Species x Salinity x Water	0.217	0.928

Root Mass Fraction

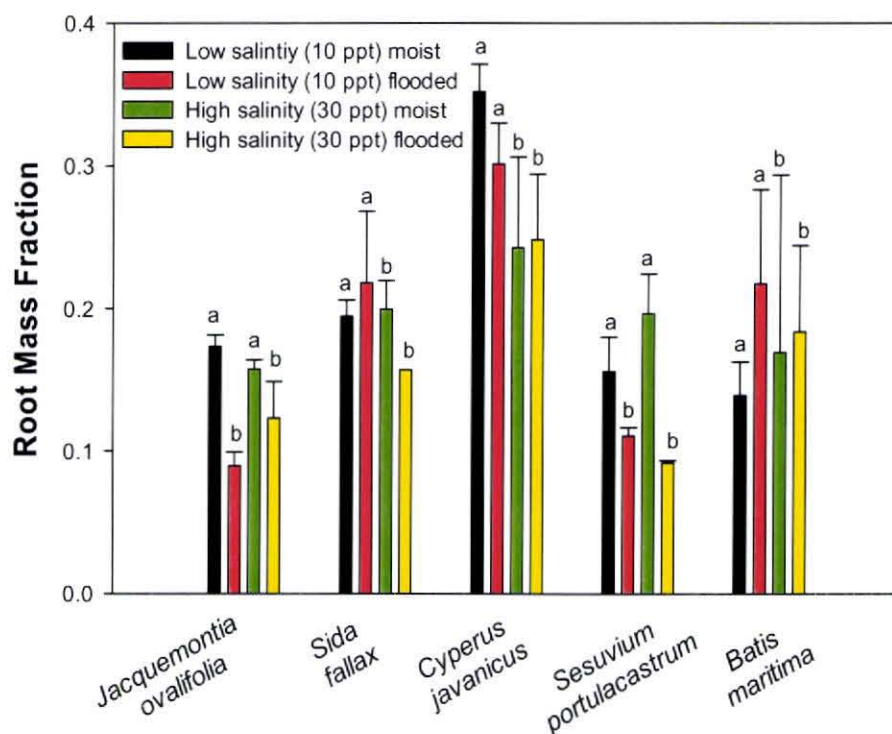


Figure 3.8. Root mass fraction (RMF) (mean \pm SEM, $n=1-15$) of four species under four treatment combinations of two salinity levels and two water levels. RMF was not significantly different between salinity treatments. Significant differences by ANOVA (p -value <0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters.

Root mass fraction (RMF) was significantly different among species, but RMF was not significantly different between the two salinity treatments (Table 3.9, Figure 3.8). The significant interaction between species and water indicates that the effects of water level on RMF differed across species (Table 3.9).

Table 3.9. ANOVA results for root mass fraction (RMF) with species, salinity, and water as fixed factors. An asterisk indicates significance.

ANOVA Log Leaf Surface Area	F-Ratio	P-Value
Species *	11.030	<0.001
Salinity	1.485	0.228
Water	2.789	0.101
Species x Salinity	0.824	0.516
Species x Water *	4.207	0.005
Salinity x Water	0.053	0.818
Species x Salinity x Water	1.001	0.415

Pairwise comparisons showed that for *Jacquemontia ovalifolia* and *Sesuvium portulacastrum*, RMF was significantly affected by water level (Table 3.10). The RMF of both of these species is significantly smaller in the flooded treatments compared to the moist treatments (Figure 3.8, Table 3.10).

Table 3.10. Hypothesis tests comparing individual species' RMF in the moist treatment to their RMF in the flooded treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

Species Comparison	F-Ratio	Raw P-Value
<i>Jacquemontia ovalifolia</i> * Moist vs. Flooded	10.407	0.002
<i>Sida fallax</i> Moist vs. Flooded	0.075	0.785
<i>Cyperus javanicus</i> Moist vs. Flooded	0.036	0.850
<i>Sesuvium portulacastrum</i> * Moist vs. Flooded	10.975	0.002
<i>Batis maritima</i> Moist vs. Flooded	3.630	0.062

Leaf Mass Fraction

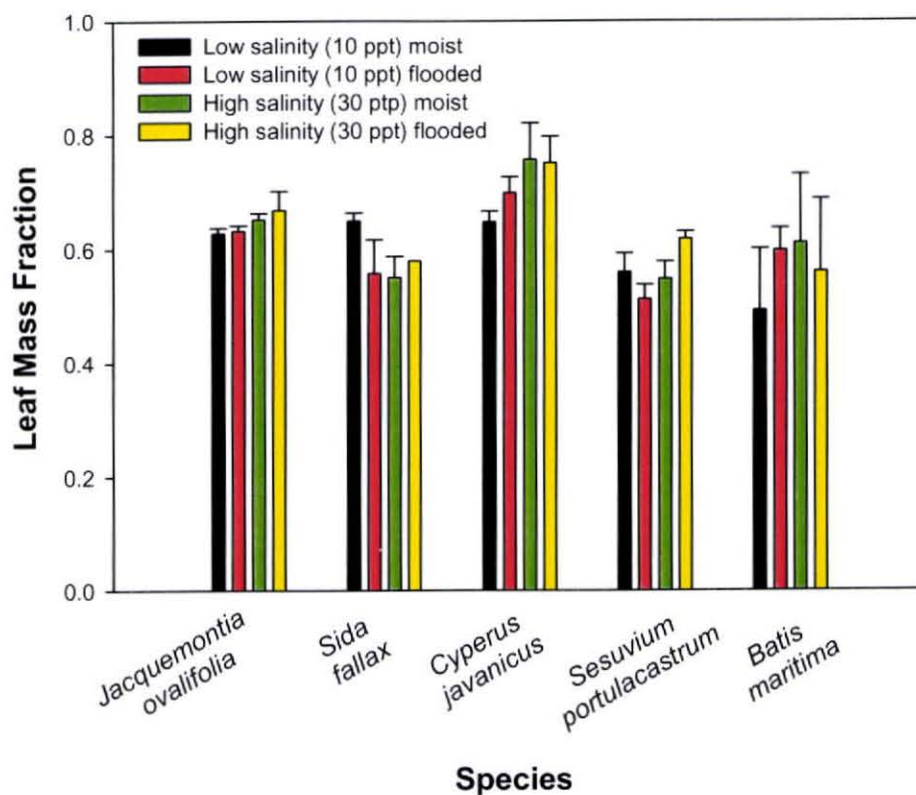


Figure 3.9. Leaf mass fraction (mean \pm SEM, $n=1-15$) of four species under four treatment combinations of two salinity levels and two water levels. LMF was not significantly affected by salinity or water level.

LMF varied significantly among species, but neither salinity nor water level significantly affected the LMF (Figure 3.9, Table 3.11).

Table 3.11. ANOVA results for leaf mass fraction (LMF) with species, salinity, and water as fixed factors. An asterisk indicates significance.

ANOVA Log Leaf Surface Area	F-Ratio	P-Value
Species *	5.832	0.001
Salinity	1.708	0.197
Water	0.108	0.743
Species x Salinity	0.468	0.759
Species x Water	0.128	0.972
Salinity x Water	0.026	0.873
Species x Salinity x Water	1.164	0.336

Mortality

Table 3.12. Mean mortality percentage (\pm SEM, n= 5-15) for five species under four treatment combinations of two salinity levels and two water levels.

Species/ Treatment	<i>Jacquemontia ovalifolia</i>	<i>Sida fallax</i>	<i>Cyperus javanicus</i>	<i>Sesuvium portulacastrum</i>	<i>Batis maritima</i>
Low salinity (10 ppt) moist	26.7 \pm 0.12	60.0 \pm 0.13	73.3 \pm 0.13	0 \pm 0	20.0 \pm 0.20
Low salinity (10 ppt) flooded	20.0 \pm 0.11	80.0 \pm 0.11	86.7 \pm 0.09	0 \pm 0	40.0 \pm 0.25
High salinity (30 ppt) moist	33.3 \pm 0.13	53.3 \pm 0.13	73.3 \pm 0.12	0 \pm 0	60.0 \pm 0.25
High salinity (30 ppt) flooded	40.0 \pm 0.13	93.3 \pm 0.07	86.7 \pm 0.12	0 \pm 0	20.0 \pm 0.20

Sesuvium portulacastrum had zero mortality in all treatments. The remaining species had relatively high mortality, even under the low salinity moist conditions. *Jacquemontia ovalifolia* had higher mortality in the high salinity treatments, but the mortality percentage was lower under flooded conditions in low salinity and higher under flooded conditions in the high salinity. *Sida fallax* had its highest mortality in the low salinity flooded and high salinity flooded treatments. For *Cyperus javanicus*, mortality was higher in the flooded treatments than in the moist treatments under both salinities, but mortality was not affected by salinity.

Discussion

Total Dry Weight

Plant growth can be analyzed in terms of an increase in total plant dry mass and its allocation among organs involved in acquisition of above-ground or below-ground resources (Lambers et al. 1998). All species in this study had significantly higher total dry weights in the low salinity treatments compared to the high salinity treatments. The growth of halophytes in several genera is stimulated by some level of salinity (Ungar 1991). However, for most species, including halophytic species, productivity is inhibited by high salinity, and this decrease in growth may be caused by damage to plant tissue (Ungar 1991, Broome et al. 1995, Kuhn and Zedler 1997).

It was not surprising that *Batis maritima* showed the smallest absolute difference in total dry weight between the low and high salinity treatments, since research has shown that *Batis maritima* plants receiving salt water containing up to twice the concentration of seawater still maintained positive growth (USDA and Francis, Miyamoto et al. 1996). However, *Batis maritima* had the lowest total dry weight of all species at the end of the study, regardless of treatment. The observed slow seedling growth is supportive of claims that the most effective reproduction of the species is by clonal growth (USDA and Francis) and implies that the time required for establishment of *Batis maritima* from seed is lengthy.

Being one of the most widely distributed seashore plants (Lonard and Judd 1995), it was not unexpected that *Sesuvium portulacastrum* exhibited the highest total dry weight of all species in the high salinity treatment and the reduction of total dry weight as salinity increased was relatively small compared to other species. Previous research

shows that high salinity affected the overall growth of *Sesuvium*, but dry matter production increased with increasing concentrations of NaCl up to 600 mM (~40 ppt) and declined at concentrations above 600 mM (Venkatesalu and Chellappan 1993)

Coastal wetlands soils are frequently waterlogged and anaerobic (Adam 1990); regular flooding of salt water can cause gas exchange between the soil and the air to be reduced or virtually non-existent (Kozlowski 1984). Water level did not significantly affect the total dry weight of any of the species in this study and this is surprising, since a reduction in soil aeration usually negatively affects growth (Kozlowski 1984). However, plants were flushed weekly with tap water to ameliorate evaporative salt build-up and this process provided the opportunity for oxygen to be introduced. Therefore, a reduction in soil aeration may not have been pronounced due to this technique.

Root Length

The transfer of water from the soil to plant takes place in the root systems. Roots experience the flooded environment first hand and the response of roots to salinity and flooding will have consequences for shoot and leaf development (Winter 1974, Kramer and Boyer 1995). Root length of *Cyperus javanicus* and *Jacquemontia ovalifolia* was significantly reduced as salinity levels increased. No other species root lengths were significantly different between salinity treatments. The non-significant effect of salinity on *Sida fallax* roots appears to be the result of high within-treatment variation in lengths.

Research has demonstrated that mangroves and other halophytes regulate internal ion concentrations through the exclusion of ions in root membranes or through a non-metabolic ultra filtration process for removal of salt in the root membrane (Caldwell 1974). Halophytes can also effectively compartmentalize salt into the vacuole of the cell,

allowing them to grow for long periods of time in saline soil (Munns 2002). This process has been noted in *Batis maritima* (Company 2002) and could be a reason why *Sesuvium portulacastrum* can withstand such high levels of salinity.

The root length of *Sesuvium portulacastrum* was significantly reduced in the flooded treatment compared to the moist treatment. Negative effects of flooding have been attributed to increases in the concentrations of ethylene in the root, due to gas diffusing more slowly in flooded soil than in well-aerated soil. (Smith and Robertson 1971, Kozlowski 1984, Kramer and Boyer 1995) Other researchers suggest that the absence of oxygen triggers a sequence of chemical and biochemical reductions in the soils and these reduced components are injurious to root metabolism (Kozlowski 1984).

Roots of some species may be little-affected by flooded conditions as a result of several factors. Roots vary in their tolerance to anaerobic conditions, with some plants dying after a few hours of exposure, and others existing for months under these conditions (Kozlowski 1984). Many wetland species can develop aerenchyma tissues, which increase the area available for oxygen, and this is thought to contribute to the survival of many wetland species (Kawase 1980, Kozlowski 1984). The cellular structure of the roots and shoots was not investigated in the present study; therefore I am unable to attribute maintenance of root length under flooded conditions to aerenchyma development and I am not aware of research that has investigated the presence of aerenchyma in these species. In addition, plants can alleviate the stress of low concentrations of oxygen and subsequent reduction of root length of the main root, through growth and extension of lateral roots (Kozlowski 1984). The occurrence and measurement of lateral roots was also not examined in this experiment.

It is relevant to note that these species were exposed to permanently flooded soils, rather than a periodic or episodic flooding of the soil. If roots have penetrated more than a few centimeters below the soil and are suddenly exposed to anoxic conditions, this could lead to rapid death of those roots. However, plants could develop a tolerance to prolonged soil wetness if they develop roots which utilize oxygen present near the surface (Kramer and Boyer 1995).

Shoot Length

Stems are often referred to as the center of higher plant organization; many plants can utilize more space through vegetative growth or colonize new areas through vegetative fragmentation (Lonard and Judd 1995) and shoot length will directly affect the ability of a species to occupy space.

Shoot length was reduced as salinity increased in *Jacquemontia ovalifolia* and *Cyperus javanicus*, but shoot length of *Sida fallax*, *Sesuvium portulacastrum*, and *Batis maritima* were not affected by increased salinity (Figure 3.8). Inhibition of stem extension is almost always observed when soil is flooded (Kozlowski 1984). Although some species are stimulated to extend their stems or petioles more quickly under these flooded conditions, most of these plants are common marsh dwellers (Kozlowski 1984). A number of rhizomatous species have been shown to produce healthy shoots in a oxygen free zone in the dark for 7 days or more (Crawford 1982, Kozlowski 1984). However, water level did not significantly affect shoot length of any species in this study (Table 3.4).

Adventitious rooting, which is rooting from the submerged part of the stem or hypocotyle, has been reported in many plants in the field and in laboratory conditions.

Bergman 1920 observed adventitious rooting in non-wetland mesophytes after ten days of flooding and noted its association with the death of original root system (Kozlowski 1984). Numerous smaller adventitious roots have developed from the nodes of *Sesuvium portulacastrum* and typically only extend 2-6 cm into the soil (Lacerda and Pfeiffer 1983, Lonard and Judd 1995). In the present study, adventitious roots were observed on *Cyperus javanicus* and *Sesuvium portulacastrum*, but no records were kept to allow comparison of prevalence among treatments. Flood induced rooting from the stem base is probably best considered as regenerative, occurring when original roots succumb to anoxia (Kramer 1951, Kozlowski 1984). A significant reduction in the root length of *Cyperus javanicus* and *Sesuvium portulacastrum* may have induced the growth of adventitious roots to aid in survival. Circumstantial evidence has shown that shoot regrowth and leaf reemergence was apparent after adventitious roots formed at the stem base (Kramer 1951, Kozlowski 1984).

Relative Growth Rate of Shoot Length (RGR_{SH})

Relative growth rate (RGR) is defined as the rate of increase in biomass per unit plant mass. Plant species differ in the rate of growth they can achieve, even when grown under optimal conditions (Poorter 2002). Plants generally respond to suboptimal conditions through reductions in growth rate and changes in allocation to minimize the limitation of growth by these factors (Lambers et al. 1998).

The RGR_{SH} of all species was significantly greater under low salinity conditions than under high salinity conditions. These results are supportive of previous research, which has showed that the RGR of many species is decreased as salinity levels increase

(Morris and Ganf 2001, Bayuelo-Jimenez et al. 2002, Bell 2005, Ewe and Silveira Lobo Sternberg 2005).

It is interesting that *Batis maritima* showed the largest decrease in RGR_{SH} with increasing salinity level, since seed germination in *Batis* was not affected by high salinity (Chapter 2). This indicates that although *Batis* seeds can germinate in high saline areas, seedlings would grow relatively slowly. However, *Batis* is known to clonally propagate (Trillmich 2002) and it is possible that fragments deposited in soft, saline sediment may have faster growth rates than seedlings. Other plants, such as *Aster tripolium* are also capable of establishing in a similar manner (R.L. Jefferies, unpublished data, 1969). Vegetative propagules may be less susceptible to high salinities than germinating seeds or young seedlings (Jefferies and Rudmik 1991).

Relative to studies on salinity effects, fewer studies have examined the how flooding affects relative growth rate. RGR of *Batis maritima*, *Cyperus javanicus*, and *Sida fallax* was significantly larger in moist treatments than in flooded treatments and this is supportive of previous studies, which have shown a decrease in RGR in flooded conditions (Nash and Graves 1993, Muthuchelian et al. 1995, Matsura et al. 2002, Busch et al. 2004). It is possible that the relative growth rates of these species may have improved if studied over a longer period of time, due to adaptations to alleviate flooding stress. For example, *Pinus serotina* was shown to have an initial decrease in RGR due to flooding during the first six weeks of growth, but RGR increased due to the production of adventitious roots (Topa and Cheeseman 1992). On the other hand, RGR may have decreased over time if root growth was largely limited to the top 4 cm of unflooded soil in the flooded treatment.

Specific Leaf Area (SLA) & Leaf Area Ratio (LAR)

It has been suggested that specific leaf area is a key trait in relative growth because it enables the plant to expose a large leaf area to light and CO₂ per given biomass invested in leaves (Lambers et al. 1998). Specific leaf area (SLA) declined with increasing salinity levels, indicating that the growth of these species was reducing leaf area expansion by forming thicker or more dense leaves or a combination of both (Whitowski and Lamont 1991). This supports research that plants, exposed to saline conditions often have fewer and smaller leaves. (Aspinall 1986, Marcelis and van Hooijdonk 1999, Ewe and Silveira Lobo Sternberg 2005)

Leaf area ratio (LAR) is an index of leafiness of the plant (Hunt 1990) and salinity may affect leaf elongation (Rozema 1991). Therefore, salinity may also affect the development of photosynthetic surface area (LAR) in some species (Curtis and Lauchli 1986). LAR of all species studied was significantly smaller under higher salinity treatments and this could be a result of a reduction in SLA and/or a reduction in the proportion of dry matter allocated to leaf tissue (LMF) (Lambers et al. 1998)..

Several extensive surveys and literature reviews indicate that leaf area ratio (LAR), and more specifically SLA, is the most important factor explaining variation in RGR (Lambers et al. 1998, Poorter and Van Der Werf 1998, Poorter 2002). Low specific leaf area (SLA) decreases the amount of leaf area available for light interception and therefore photosynthetic carbon gain. This research is supportive of previous research on other species, including field crops (Bayuelo-Jimenez et al. 2002), invasive upland and coastal trees (Ewe and Silveira Lobo Sternberg 2005), and perennial legumes (Bell 2005) which have shown that RGR is strongly associated with a SLA and hence LAR.

Biomass Allocation

Many studies only assess the above ground portion of the plants when investigating salinity tolerance of plants. This can be misleading because changes in the dry weight of above ground parts may be associated with reallocation of resources towards below ground plant parts, rather than indicate changes in the growth of the whole plant (Adam 1990). Some researchers suggest that plants can minimize the cost of growth (maximizing the growth rate) if allocation of resources is adjusted such that all resources are equally limiting to growth (Lambers et al. 1998). One theory is that plants respond to a decrease in above-ground resources with increased allocation to shoots (leaves), whereas they respond to a decrease in below ground resources with increased allocation to roots (Poorter and Nagel 2000).

Root Mass Fraction (RMF)

Root mass fraction (RMF) is the fraction of the total plant biomass allocated to roots (Lambers et al. 1998). Research conducted on cell water relations has shown that the early response to water limitation and salt stress are essentially identical (Munns 2002). Therefore, we could expect a greater allocation to roots in response to water limitation caused by salinity stress.

Research has shown great variability in the effect of salinity on root to shoot ratios. Some researchers found a decrease in root to shoot ratio (a decrease in RMF) as salinities levels increased (Gallagher 1979, Osmond and Bjorkman 1980, Pearcy and Ustin 1984, Adam 1990), while others reported a considerable increase in root to shoot ratio (increase in RMF) as salinities increase (Clarke and Hannon 1970, Parrondo and

Gosselink 1978, Adam 1990). In the present study, RMF was not significantly affected by increasing salinities.

RMF of *Jacquemontia ovalifolia* and *Sesuvium portulacastrum* was significantly smaller in flooded treatments than in moist treatments and this is supportive of research showing shifts in biomass allocation from roots to shoots (Rubio et al. 1995, Kercher and Zedler 2004). However, the other species showed any significant effect of flooding on RMF.

Leaf Mass Fraction (LMF)

Leaf mass fraction (LMF) is the fraction of the total plant biomass allocated to leaves (Lambers et al. 1998). In this study, LMF was not significantly affected by salinity or water level in any of the species studied. This is supportive of a comparative assessment of biomass accumulation of fourteen wetland plant species which also showed that root to shoot ratios were not significantly different under different water depths (Fraser 2005). Because the $LAR = SLA * LMF$ (Lambers et al. 1998) and LMF was not significantly affected by salinity or water treatments, the decrease in LAR as salinity levels increased was primarily due to the decrease in SLA.

Mortality

Although salt tolerance is usually assessed as the percent biomass production in saline areas versus control conditions, salt tolerance can also be assessed in terms of survival (Munns 2002). Results from this analysis show that *Sesuvium portulacastrum* had the highest RGR_{SH} as well as no mortality even at high salinity, indicating that seedlings are very tolerant to increases in salinity. *Sida fallax* and *Cyperus javanicus* both had the lowest RGR_{SH} and the highest mortality of all species studied, especially in the

low salinity flooded and high salinity flooded treatments, indicating that these species are more vulnerable to flooding. *Jacquemontia ovalifolia* also had a relatively low RGR_{SH} and higher mortality in the higher salinity treatments, indicating that it is more vulnerable to increasing salinity. *Batis maritima*'s seedling mortality did not follow a particular trend of increase or decrease in response to salinity or flooding.

Conclusions

Greenhouse results should be used with caution for restoration projects, because this study examined only the effects of root exposure to salinity. While roots are exposed to high salt levels in the field, other plant parts may also have to endure periods of partial or total immersion in salt water(Adam 1990) and the effects of this immersion on the ionic balance of plants has not been given consideration in this study. Salt spray, which can affect the growth of coastal plants in the field, was also not studied.

Results from this study confirm the hypothesis that early growth of all species declined with increasing salinity when total dry weight or RGR_{SH} was considered the indicator of growth. The hypothesis that *Batis maritima* and *Sesuvium portulacastrum* would more effectively tolerate higher levels of salinity was partially confirmed. The absolute and relative difference between total dry weight and RGR_{SH} of *Sesuvium* grown under low salinity and high salinity was smaller than other species (excluding *Sida fallax*) and *Sesuvium* experienced no mortality even in the high salinity treatments. Because of *Batis*' slow growth, the absolute decrease in total dry weights as salinity increased was smaller than most other species, but the relative decrease in RGR_{SH} was decreased more than any other species when exposed to increased salinity. This indicates that higher salinities had less of an effect on the relative plant growth of *Sesuvium* than *Batis*.

In addition, salinity did not significantly affect the root or shoot length of *Sesuvium* and did not significantly affect the shoot length of *Batis*. However, salinity also did not significantly affect the root or shoot length of *Sida*. Therefore, these results indicate that the early growth of *Batis* and *Sesuvium* are less affected by high salinity levels than *Jacquemontia ovalifolia* and *Cyperus javanicus*, but not necessarily *Sida*. Results do not support the hypothesis that flooding significantly decreases root and shoot length; *Sesuvium portulacastrum* was the only species whose root length was significantly decreased by water, and water was not shown to significantly decrease the shoot length of any species.

Future Studies

This study investigated species' responses to flooded soils. While tidal flooding may not completely cover adult plants, it can submerge tiny seedlings. Seedlings, in this experiment, were not covered with water and this flooding could affect the establishment of these species in the field.

It would be interesting to investigate the adaptations of species such as *Sesuvium portulacastrum* and *Batis maritima*, which have shown less vulnerability to salt stress. It would also be interesting to investigate whether their ability to tolerate salt is due to ion exclusion, growth or succulence, ion secretion, or another physiological mechanism.

Chapter 4. Competition Between a Native Hawaiian Coastal Species, *Sesuvium portulacastrum*, and the Invasive Species, *Batis maritima*, Under Different Salinity and Water Levels.

Abstract

Coastal environments are often invaded by non-native species and research has demonstrated that the amount of unused resources is correlated with invasion by non-native species. However, there is disagreement as to whether increased salinity promotes or limits the invasion of non-native species. This study examined the relative competitive abilities of a native species, *Sesuvium portulacastrum* and an invasive species, *Batis maritima*, under low and high salinity and moist and flooded water conditions in a greenhouse. Relative competitive abilities were not consistently influenced by salinity or flooding treatments, and few variables even indicated that competition was occurring.

Introduction

Theoretically, the interaction of two species can be positive, negative or neutral (Odum 1971). Certain plants are viewed as undesirable because they are aggressive competitors that reduce diversity and impact wildlife (Mitsch and Gosslink 1993). Invasion by non-native species is influenced by three major factors 1) propagule number entering the system, 2) characteristics of the invading species, and 3) environmental susceptibility to invasion (Lonsdale 1999, Davis and Grime 2000). Therefore, knowledge about characteristics of invasive species and investigation of a community's susceptibility

to invasion is instrumental in understanding or predicting changes in vegetation composition and structure (Rejmanek 1996, White and Campbell B.D. 1997).

Plant responses to abiotic stress, competitive interactions, and site disturbance are all important factors affecting the vegetation composition of an area (Hobbs and Mooney 1991, Nernberg and Dale 1997, Greiner La Peyre and Grace 2001). Researchers have theorized that plant communities become more vulnerable to invasion when the amount of unused resources increases (Davis and Grime 2000). This implies that vulnerability is not a permanent trait. Rather than existing as an intrinsic characteristic, the invasion potential of any community can change over time in response to resource fluctuation (Davis and Grime 2000).

Investigations comparing the interaction of invasive and native species under various growing conditions are extremely valuable, because site specific management strategies can be tailored to promote the growth of native plants and suppression of invasive plants (Daehler 2003). Previous research demonstrated that an increase in water availability is associated with an increase in the relative abundance of non-natives (Hobbs and Mooney 1991, White and Campbell B.D. 1997, Callaway and Zedler 1998). Increased resource availability has also been shown to decrease the ability of native species to compete with non-native species under lower salinity levels (Barbour 1978, Nernberg and Dale 1997, Callaway and Zedler 1998).

Salinity can affect the distribution and abundance of non-native species if natives and non-natives differ in salt tolerance (Kuhn and Zedler 1997, Callaway and Zedler 1998, Weber and D'Antonio 1999, Hoopes and Hall 2002, Kolb and Alpert 2003). Daehler (2003) showed that invaders performed better than natives in approximately 60%

of salt tolerance studies and invasion has been associated with decreased salinity levels in at least two communities: a coastal salt marsh (Noe and Zedler 2001) and an inland riparian grassland (Hoopes and Hall 2002, Kolb and Alpert 2003). However, salinity has an effect on water available to plants, so low salinity could be associated with invasion by effectively increasing availability of water or nutrients (Kolb and Alpert 2003).

The ability of plants to be successful invaders has been of interest to many researchers. Grime (2002) suggested that plants have evolved different strategies in response to the habitats in which they live, and his C-S-R model (Grime 1979) provides one theory that can explain changes in species composition. This C-S-R theory suggests that adaptive responses of plant species are heavily influenced by habitat productivity, frequency of disturbance, and severity of disturbance; Grime identifies three primary plant strategies: competitors (C), which dominate communities whose conditions include low stress and low disturbance, stress tolerators (S), characteristic of communities with high stress and high disturbance, and ruderals (R), which are associated with low stress and high disturbance (Grime 2002). Grime's theory implies that the species with the highest maximal growth rate of vegetative tissue will be the superior competitor. (Grace 1990a). An alternative theory presented by David Tilman predicts that the species with the minimum resource requirement will be the superior competitor (Grace 1990a).

Stresses can be defined as "the external constraints which limit the rate of dry matter production of all or of part of the vegetation" (Grime 2002). Competitive ability depends on a combination of plant characteristics, such as storage organs, specific leaf area, leaf and root longevity, growth rate, leaf nutrients, and the ability to reach sufficient height or spread laterally for capture of light or other resources (Grime 2002).

The C-S-R theory identifies three extreme plant specializations, but Grime (2002) recognizes intermediate plant strategies arising from combinations of disturbance, competition, and stress. Nevertheless, if a plant evolves traits that allow it to succeed in a specific environment, it may sacrifice fitness in another environment.

Batis maritima and *Sesuvium portulacastrum* have both been shown in greenhouse and field experiments to have the ability to withstand a high level of salt stress (Lonard and Judd 1995, Miyamoto et al. 1996, El-Sayed and Noaman 2001), but anecdotal evidence at James Campbell National Wildlife Refuge (NWR) suggests that *Batis maritima* outperforms *Sesuvium portulacastrum* in when grown in similar areas.

According to Grime, there are strong tradeoffs between the ability to tolerate low resource supply and the ability to grow rapidly and to exploit resources (Grace 1990a). If *Sesuvium portulacastrum* is a true stress tolerator, it would out perform *Batis maritima* under higher levels of stress, whereas *Batis*, which seems to have a mixed competitor (C) and stress tolerator (S) strategy, would outperform *Sesuvium* under moderate to low stress conditions. The objective of this experiment was to determine the relative competitive abilities of the native species, *Sesuvium portulacastrum* and the invasive species, *Batis maritima*, under low and high salinity and moist and flooded water conditions. This experiment tested the hypothesis that the ability of *Sesuvium portulacastrum* to compete with *Batis maritima* will be greater at higher levels of salinity stress and *Batis maritima*'s ability to compete with *Sesuvium portulacastrum* will be greater under lower levels of salinity stress.

Methods

Plant Material Collection

Vegetative cuttings of *Batis maritima* and *Sesuvium portulacastrum* were collected from the Waiawa unit of the Pearl Harbor NWR and Barbers Point, respectively, during the first week of August 2005. Cuttings were approximately 6 to 8 inches long and were removed with sharp scissors at the site. Approximately 100 cuttings were sampled from different individuals. Cuttings were placed in Ziploc bags containing moistened paper towels to achieve 100% humidity. All cuttings were brought back to the laboratory and any flowers or flower buds were removed to allow the cutting to use all available energy and stored carbohydrates for root formation.

Establishment of Cuttings

Tip cuttings were cut again with a sharp razor blade just below a node to a length of approximately four inches. To accelerate rooting, “Dip’n’Grow” (Dip ‘N Grow, Inc., Clackamas, OR), containing the rooting hormone Indole-3-butyric acid, was applied to the cuttings at a combination of one part solution to twenty parts water. Severed ends of the tip cuttings were dipped in the rooting hormone for five seconds. Cuttings were then placed in 28 x 54 cm plastic trays containing moistened sterile planting medium, consisting of one part vermiculite, one part perlite, and one part sphagnum moss. Clear plastic wrap was sprayed with water and placed over the trays to obtain a near 100% humidity environment and all trays were placed under growing lamps.

Plants were checked daily and the plastic wrap was rewetted to maintain the moist environment. Plastic wrap was secured on the trays until the plants showed signs of rooting. In order to determine rooting, I gently pulled on the stem of the cuttings;

resistance indicated that roots had formed. After three weeks the majority of the cuttings rooted, and the plastic wrap was removed. Plants were kept in the trays under the growing lamps and watered daily for an additional two weeks to promote further root growth.

Experimental Competition

During the second week of September, eighty cuttings of both species were transferred to 15 cm diameter pots. Forty pots contained one *Batis maritima* cutting, forty pots contained one *Sesuvium portulacastrum* cutting, and forty pots contained one *Batis maritima* cutting and one *Sesuvium portulacastrum* cutting. Plants were randomly selected for placement in a pot with or without the other species by flipping a coin.

All cuttings were planted in a soil mixture containing one part screened top soil and one part Menhune Soil Conditioner (Hawaiian Earth Products Ltd., Kailua, HI). Initially, approximately 10 grams of 19-6-12 Osmocote fertilizer (Scotts Miracle Grow Company, Maryville, Ohio) was applied to each of the pots. These plants were allowed to grow for an additional two weeks in the greenhouse to allow the transplants to stabilize.

The competition experiment was set up in the greenhouse with a 2 x 2 x3 factorial design. There were two salinity treatments, two water treatments and three competition treatments. On October 1st, thirty-six pots of each of the three planting treatments (*Sesuvium*, *Batis*, or mixed) were randomly selected for the experiment. Extra plants were kept in case plants died immediately from transplant shock, but no plants were substituted during the experiment. One pot of each competition treatment was placed in a 30x36x36 cm bin, which was randomly assigned a salinity/water treatment (combination of salinity and flooding level). Low salinity (10 ppt) and high salinity (30 ppt) treatments

were established under moist and flooded conditions by filling the bins to 10 cm and 2 cm below the soil surface, respectively. There were nine replicate bins for each of the four salinity/water treatments, for a total of 36 bins containing pots with all three planting treatments.

To avoid immediate shock to the plants, salinity and water levels were gradually increased during the first week. Measurements including shoot height, leaf number, shoot number, and flower number, were recorded weekly. Salinity and water levels were checked every three days by a refractometer and water level mark and altered if necessary. In order to avoid salt accumulation at the surface of each of the pots, pots were removed from bins once every two weeks and flushed twice with fresh water until water flowed out of the bottom holes.

Plants grew for a total of eight weeks; final measurements of all plants occurred during the first week of December 2005. After eight weeks, plant shoots began to sprawl outside the area of the pot and treatment bin; to reduce interaction between plants in different treatments, the experiment was ended. At final harvest, plants were removed from the soil and roots were washed to remove any excess soil particles; plants were separated into roots, stems and leaves and measured. Separated parts were dried at 55 degrees Celsius for three days and weighed.

Final measurements included root length, root fresh weight, root dry weight, stem length, stem fresh weight, stem dry weight, leaf number, leaf fresh weight, and leaf dry weight. Due to strong correlations between many of the measured variables (Appendix C), relative growth rate of shoot length (RGR_{SH}), total dry weight, root length, number of additional shoots (final shoot number- initial shoot number), number of additional leaves

(final leaf number – initial leaf number), Root Mass Fraction (RMF), and Leaf Mass Fraction (LMF) will be the only variable discussed, as they provide an adequate understanding of salinity and water's effect on both above and below ground growth.

Data Analysis

Competitive Effects

In order to compare the relative effect of competition between species and treatments, the measured variable (e.g. *Batis maritima* total dry weight) when exposed to competition was divided by the measured variable when exposed to no competition. This ratio is a measure of the effect of competition; ratios closer to one indicate a smaller effect of competition (Nernberg and Dale 1997).

$$\text{Competitive Effect} = \frac{\text{competition Total Dry Weight}}{\text{no competition Total Dry Weight}}$$

Total dry weight, root length, number of additional shoots, and number of additional leaves were the variables used to measure competitive effects. These ratios were log transformed to normalize data and a three way ANOVA (Systat Version 10.2, Systat Software Inc.) was completed with species, salinity, and water as fixed factors. Hypothesis tests were performed to clarify interactions between species salinity, and water, and results were subjected to a sequential Bonferroni adjustment.

ANOVA Analysis

In addition, four way ANOVAs (Systat Version 10.2, Systat Software Inc.), with species, salinity, water, and competition as fixed factors, were used to analyze total dry weight, RGR_{SH} , RMF, and LMF.

Results

Total Dry Weight Competitive Effects

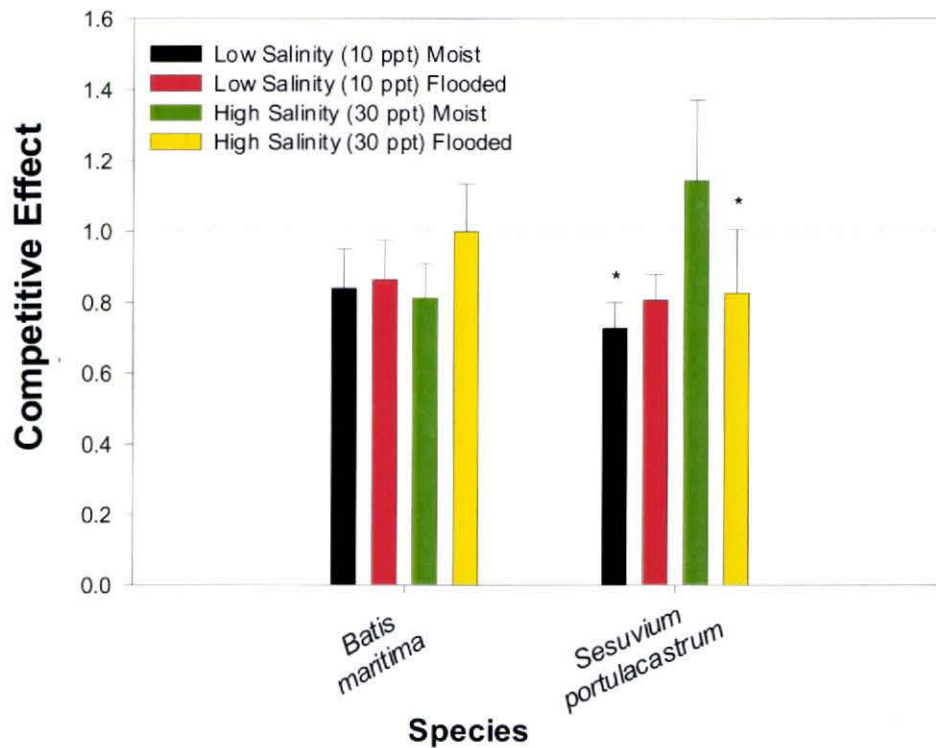


Figure 4.1. Mean competitive effects on total dry weight (\pm SEM, $n=9$) of *Batis maritima* and *Sesuvium portulacastrum*, when subjected to a combination of two salinity levels and two water levels after an eight week growth period. Salinity, water, and species did not significantly affect competitive effect. An asterisk indicates that a competitive effect was significantly less than one (indicating competition) at the $p < 0.05$ after a sequential Bonferroni adjustment. Smaller values indicate greater competition.

The competitive effect on total dry weight was not significantly different between species or salinity/water treatments (Figure 4.1, Table 4.1). However, hypothesis tests showed that the competitive effect on *Sesuvium portulacastrum*'s total dry weight was significantly less than one when *Sesuvium* plants were subjected to the low salinity moist and high salinity flooded treatments (Table 4.2, Figure 4.1).

Table 4.1. ANOVA results for the log of the competitive effect on total dry weight biomass, with species, salinity and water as fixed factors.

Comparison	F-ratio	P-value
Species	0.632	0.430
Salinity	0.530	0.469
Water	0.000	0.998
Species x Salinity	0.140	0.710
Species x Water	1.637	0.205
Salinity x Water	0.410	0.524
Species x Salinity x Water	1.309	0.257

The competitive effect on the total dry weight of *Sesuvium portulacastrum* was very close to being significantly different than one under the low salinity flooded treatment ($p=0.051$), but was not significant after a sequential Bonferroni adjustment (Table 4.2). The competitive effect on the total dry weight of *Batis maritima* was not significantly different from one under any treatment conditions, indicating a lack of evidence for competitive effects of *Sesuvium* on *Batis* (Table 4.2).

Table 4.2. Hypothesis tests investigating whether the competitive effect on the total dry weight of each species, in a particular treatment, is significantly less than one. An asterisk indicates when a competitive effect was significantly less than one after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Low Salinity Moist	2.607	0.111
<i>Batis maritima</i> Low Salinity Flooded	1.133	0.291
<i>Batis maritima</i> High Salinity Moist	2.961	0.090
<i>Batis maritima</i> High Salinity Flooded	0.210	0.648
<i>Sesuvium portulacastrum</i> * Low Salinity Moist	5.502	0.022
<i>Sesuvium portulacastrum</i> Low Salinity Flooded	3.946	0.051
<i>Sesuvium portulacastrum</i> High Salinity Moist	0.093	0.762
<i>Sesuvium portulacastrum</i> * High Salinity Flooded	6.098	0.016

Root Length Competitive Effects

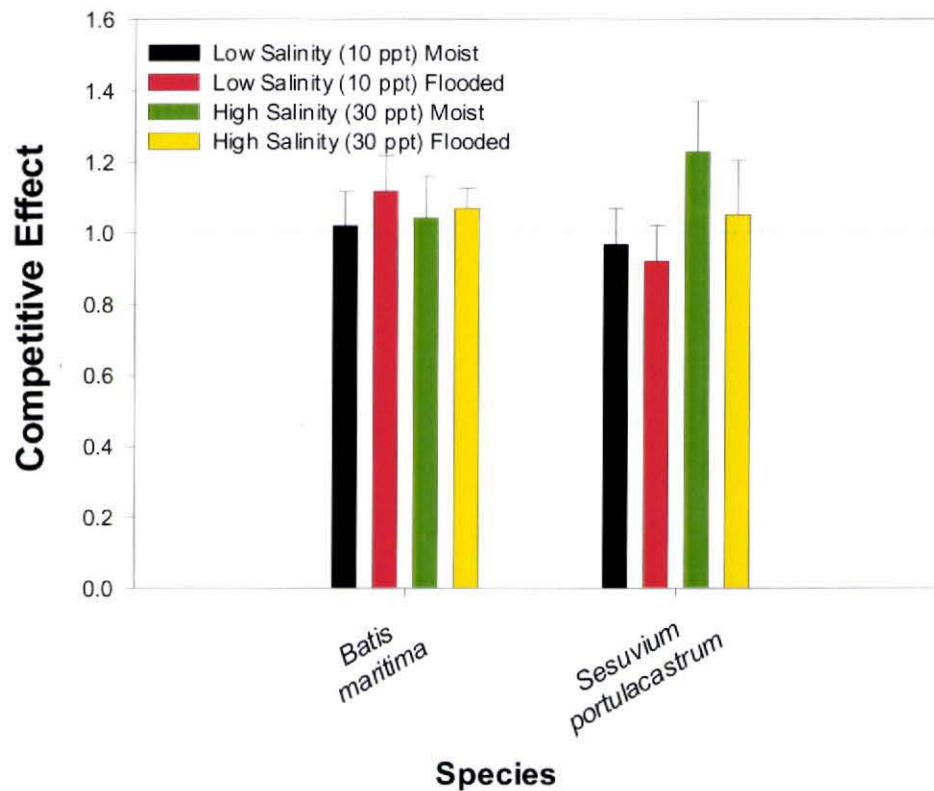


Figure 4.2 Mean competitive effect (\pm SEM, $n=9$) on the final root length of *Batis maritima* and *Sesuvium portulacastrum* when exposed to a combination of two salinity levels and two water levels after an eight week growth period. Salinity, water, and species did not significantly affect competitive effect. No competitive effects were significantly different than one at the $p < 0.05$ after a sequential Bonferroni adjustment. Smaller values indicate greater competition.

The competitive effect on the final root length was not significantly different between species and was not significantly affected by salinity/water treatments (Figure 4.2, Table 4.3). In addition, hypothesis tests showed that competitive effects on both species were not significantly different than one, so there was no evidence of competition (Figure 4.2, Table 4.4).

Table 4.3. ANOVA results for of the log of the competitive effect on root length, with species, salinity and water as fixed factors.

Comparison	F-ratio	P-value
Species	0.471	0.495
Salinity	0.832	0.365
Water	0.087	0.769
Species x Salinity	1.076	0.304
Species x Water	1.731	0.193
Salinity x Water	0.306	0.582
Species x Salinity x Water	0.104	0.748

Table 4.4. Hypothesis tests investigating whether the competitive effect on the root length of each species, in a particular treatment, was significantly less than one. No competitive effects were significantly less than one after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Low Salinity Moist	0.018	0.893
<i>Batis maritima</i> Low Salinity Flooded	0.562	0.456
<i>Batis maritima</i> High Salinity Moist	0.004	0.952
<i>Batis maritima</i> High Salinity Flooded	0.248	0.620
<i>Sesuvium portulacastrum</i> Low Salinity Moist	0.424	0.517
<i>Sesuvium portulacastrum</i> Low Salinity Flooded	1.371	0.246
<i>Sesuvium portulacastrum</i> High Salinity Moist	1.814	0.183
<i>Sesuvium portulacastrum</i> High Salinity Flooded	0.170	0.682

Additional Shoot Number Competitive Effects

Competitive effects on the number of additional shoots were not significantly different between species, but increased salinity significantly decreased the competitive effects for both species. (Figure 4.3, Table 4.5).

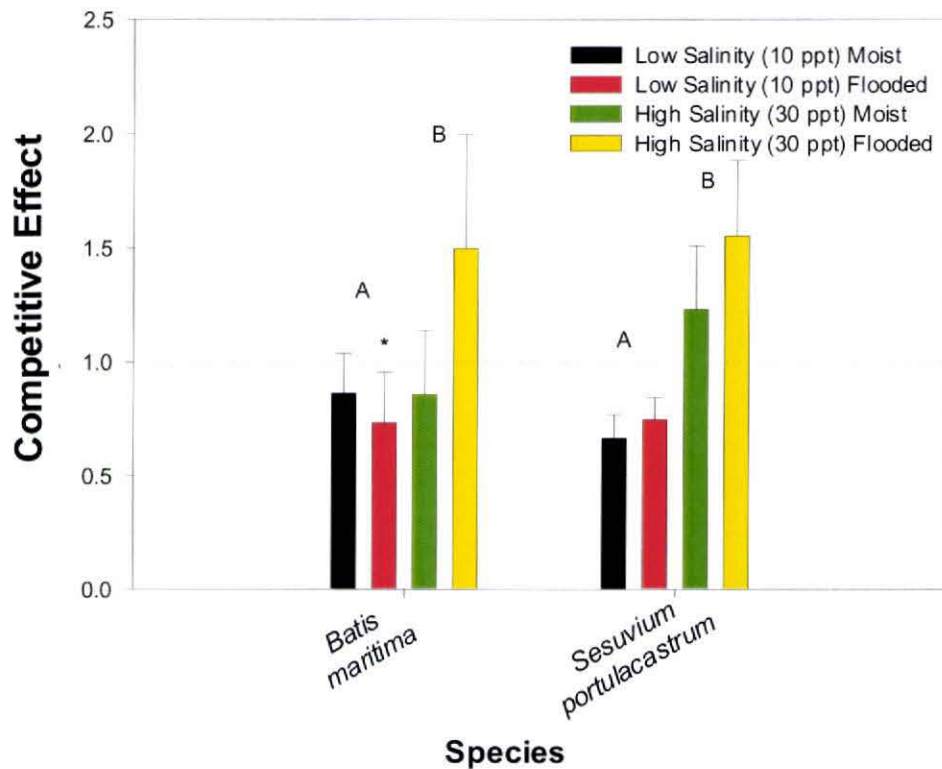


Figure 4.3. Mean competitive effects (\pm SEM, $n=9$) on the number of additional shoots of *Batis maritima* and *Sesuvium portulacastrum* when exposed to a combination of two salinity levels and two water levels after an eight week growth period. Significant differences (at the p -value < 0.05 after a sequential Bonferroni adjustment) between salinity levels are indicated by different upper case letters. An asterisk indicates that a competitive effect was significantly less than one (indicating competition) at the $p < 0.05$ after a sequential Bonferroni adjustment. Smaller values indicate greater competition.

Hypothesis tests showed that the competitive effect of *Sesuvium* on *Batis maritima*'s additional shoot number was significantly less than one when plants were exposed to the low salinity flooded treatment. The competitive effect of *Batis* on *Sesuvium portulacastrum*'s additional shoots was also less than one under the low salinity treatments, but this difference was not significant at the $p < 0.05$ level after a sequential Bonferroni adjustment. The competitive effects in other treatments were not significantly less than one (Figure 4.3, Table 4.6).

Table 4.5. ANOVA results for the log of the competitive effect on additional shoots, with species, salinity and water as fixed factors. An asterisk indicates a significant factor at the p-value < 0.05 level.

Comparison	F-ratio	P-value
Species	0.823	0.368
Salinity *	4.976	0.029
Water	0.642	0.426
Species x Salinity	0.907	0.345
Species x Water	0.020	0.887
Salinity x Water	1.315	0.256
Species x Salinity x Water	0.556	0.458

Table 4.6. Hypothesis tests investigating whether the competitive effect on the number of additional shoots of each species, in a particular treatment, was significantly less than one. An asterisk indicates when a competition effect was significantly less than one after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Low Salinity Moist	1.994	0.163
<i>Batis maritima</i> * Low Salinity Flooded	5.220	0.026
<i>Batis maritima</i> High Salinity Moist	3.410	0.069
<i>Batis maritima</i> High Salinity Flooded	0.002	0.966
<i>Sesuvium portulacastrum</i> Low Salinity Moist	4.294	0.042
<i>Sesuvium portulacastrum</i> Low Salinity Flooded	2.851	0.096
<i>Sesuvium portulacastrum</i> High Salinity Moist	0.011	0.917
<i>Sesuvium portulacastrum</i> High Salinity Flooded	0.715	0.401

Additional Leaf Number Competitive Effects

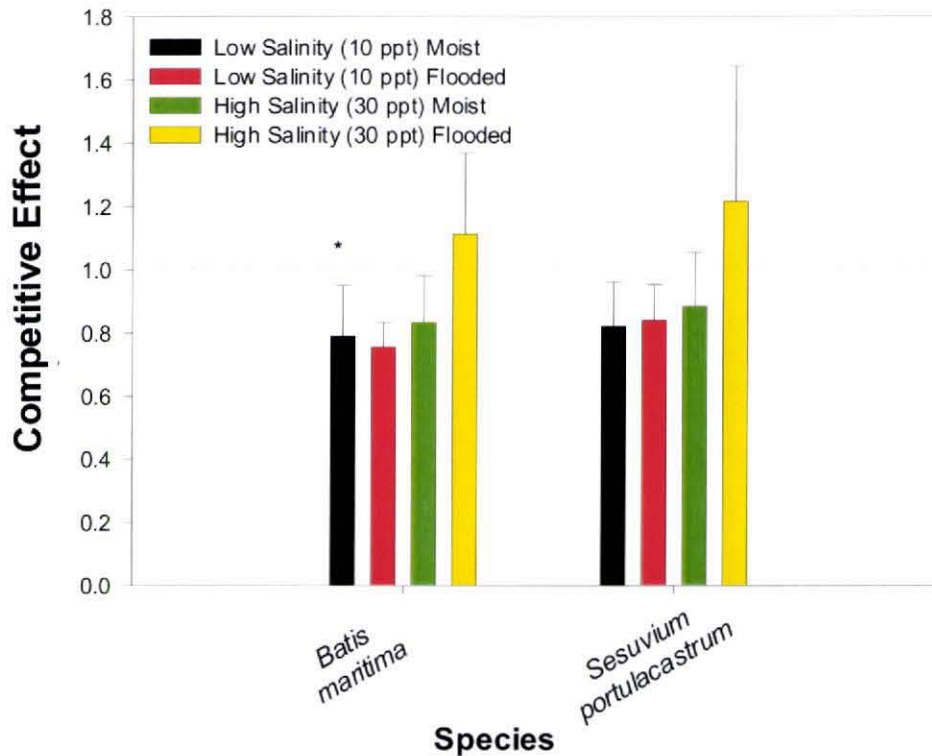


Figure 4.4. Mean competitive effect (\pm SEM, $n=9$) on the number of additional leaves of *Batis maritima* and *Sesuvium portulacastrum* when exposed to a combination of two salinity levels and two water levels after an eight week growth period. Salinity, water, and species did not significantly affect competitive effect. An asterisk indicates that a competitive effect was significantly less than one (indicating competition) at the $p < 0.05$ after a sequential Bonferroni adjustment. Smaller values indicate greater competition.

The competitive effect on the number of additional leaves was not significantly different between species and was not significantly affected by salinity/water treatment (Figure 4.4, Table 4.7). The competitive effect of *Sesuvium* on *Batis maritima*'s additional leaf number was significantly increased under the low salinity moist treatment (Figure 4.4, Table 4.8).

Table 4.7. ANOVA results for the log of the competition effect on additional leaves, with species, salinity and water as fixed factors.

Comparison	F-ratio	P-value
Species	0.028	0.867
Salinity	0.855	0.358
Water	0.539	0.466
Species x Salinity	0.200	0.656
Species x Water	0.392	0.533
Salinity x Water	0.033	0.857
Species x Salinity x Water	0.857	0.684

Table 4.8. Hypothesis tests investigating whether the competitive effect on the number of additional leaves of each species, in a particular treatment, was significantly less than one. An asterisk indicates when a competitive effect was significantly less than one after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> * Low Salinity Moist	4.047	0.048
<i>Batis maritima</i> Low Salinity Flooded	2.151	0.147
<i>Batis maritima</i> High Salinity Moist	2.127	0.150
<i>Batis maritima</i> High Salinity Flooded	0.006	0.937
<i>Sesuvium portulacastrum</i> Low Salinity Moist	2.759	0.102
<i>Sesuvium portulacastrum</i> Low Salinity Flooded	2.026	0.160
<i>Sesuvium portulacastrum</i> High Salinity Moist	1.349	0.250
<i>Sesuvium portulacastrum</i> High Salinity Flooded	1.554	0.217

Total Dry Weight

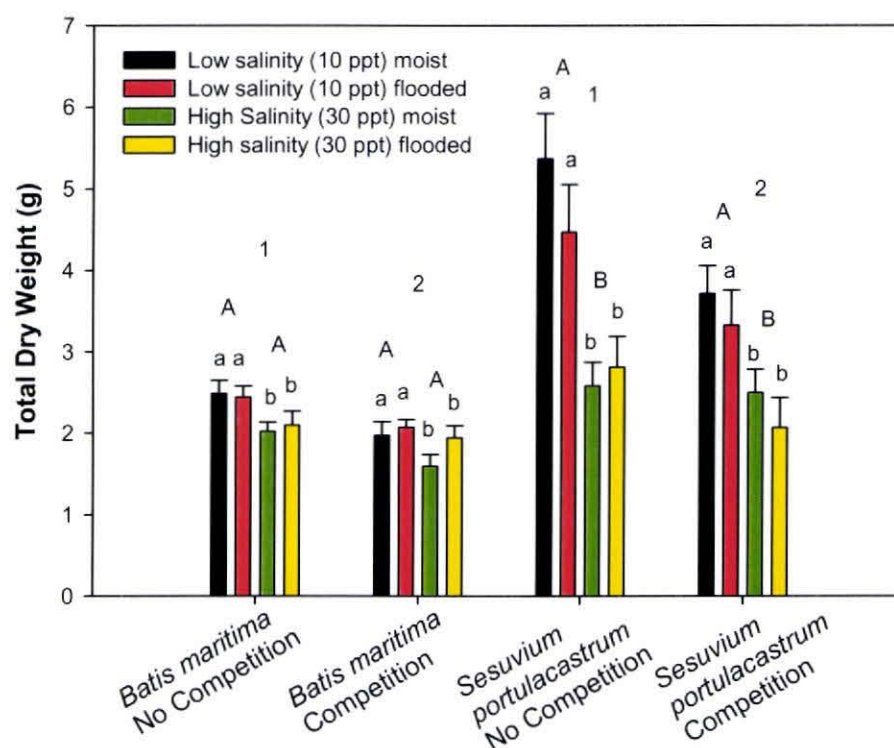


Figure 4.5. Mean final total dry weight (\pm SEM, $n=9$) of *Batis maritima* and *Sesuvium portulacastrum* exposed to six treatment combinations of two salinity, two water, and two competition treatments. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between salinity treatments are indicated by different uppercase letters. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between competition treatments are indicated by different numbers.

Sesuvium portulacastrum's total dry weight was significantly higher than *Batis maritima*'s total dry weight, and total dry weight for both species under no competition was significantly higher than total dry weight under competition (Table 4.9 and Figure 4.5). Total dry weight was also significantly affected by salinity and water, but the significant interaction between species and salinity and species and water indicated that each species were affected differently by these two factors (Table 4.9)

Table 4.9. ANOVA results for the log total dry weight with species, salinity, water and competition as fixed factors. An asterisk indicates a significant factor at the p-value < 0.05 level.

Comparison	F-ratio	P-value
Species *	49.154	<0.001
Competition *	16.673	<0.001
Salinity *	40.183	<0.001
Water	0.443	0.507
Species x Competition	0.589	0.444
Species x Salinity *	11.317	0.001
Species x Water *	3.830	0.053
Competition x Salinity	0.493	0.484
Competition x Water	0.000	0.998
Salinity x Water	0.560	0.455
Species x Competition x Salinity	0.130	0.719
Species x Competition x Water	1.526	0.219
Species x Salinity x Water	0.000	0.991
Competition x Salinity x Water	0.382	0.537
Species x Competition x Salinity x Water	1.220	0.271

Pairwise comparisons determined that *Sesuvium portulacastrum*'s total dry weight was significantly higher in the low salinity treatments than the high salinity treatments when exposed to moist or flooded conditions grown in competition or grown alone (Table 4.10, Figure 4.5). It also appears as if *Batis maritima*'s total dry weight was higher in the low salinity treatments than the high salinity treatments, but this difference is not significant at the $p < 0.05$ level, after a sequential Bonferroni adjustment (Figure 4.5, Table 4.9) Water level did not significantly affect the total dry weight of either species at the $p < 0.05$ level (Table 4.10).

Table 4.10. Hypothesis tests comparing species' total dry weight in the moist treatment to their total dry weight in the flooded treatment and comparisons of species' total dry weights in the low salinity treatment to their total dry weight in the high salinity treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Moist vs. Flooded	0.834	0.363
<i>Sesuvium portulacastrum</i> Moist vs. Flooded	3.438	0.066
<i>Batis maritima</i> Low salinity vs. High Salinity	4.425	0.037
<i>Sesuvium portulacastrum</i> * Low Salinity vs. High Salinity	47.074	<0.001

Relative Growth Rate of Shoot Length (RGR_{SH})

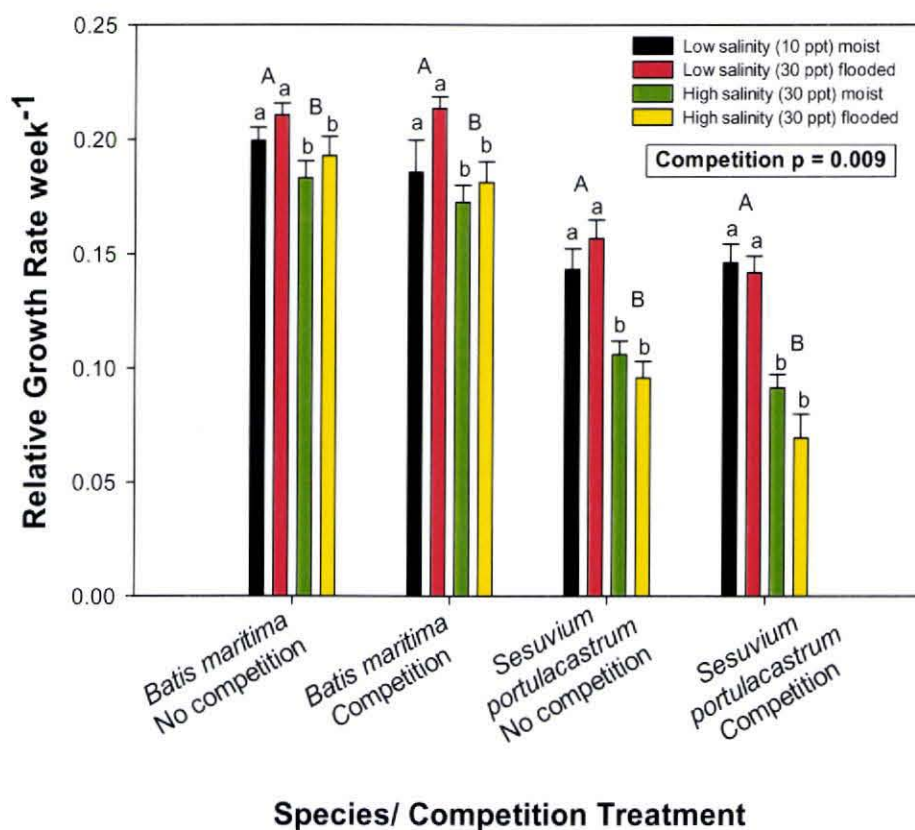


Figure 4.6. Mean relative growth rate of shoot length (RGR_{SH}) (mean \pm SEM, n= 9) of *Batis maritima* and *Sesuvium portulacastrum* exposed to six treatment combinations of two salinity, two water, and two competition treatments. Significant differences by ANOVA (p-value<0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters. Significant differences by ANOVA (p-value <0.05 after a sequential Bonferroni adjustment) between salinity treatments are indicated by different uppercase letters. RGR_{SH} is significantly affected by competition.

Batis maritima's RGR_{SH} was significantly higher than *Sesuvium portulacastrum*'s RGR_{SH} in all treatments and RGR_{SH} was significantly different when plants were grown alone and in competition (Table 4.11 and Figure 4.6). RGR_{SH} was also significantly affected by salinity; the significant interaction between species and salinity and species

and water indicated that each species was affected differently by these two factors (Table 4.11)

Table 4.11. ANOVA results for the RGR_{SH} with species, salinity, water and competition as fixed factors. An asterisk indicates a significant factor at the p -value < 0.05 level.

Comparison	F-ratio	P-value
Species *	330.112	<0.001
Competition *	7.026	0.009
Salinity *	88.513	<0.001
Water	1.124	0.291
Species x Competition	0.367	0.546
Species x Salinity *	20.392	<0.001
Species x Water *	6.160	0.014
Competition x Salinity	1.508	0.222
Competition x Water	0.202	0.654
Salinity x Water	3.648	0.058
Species x Competition x Salinity	0.303	0.583
Species x Competition x Water	2.012	0.158
Species x Salinity x Water	0.396	0.530
Competition x Salinity x Water	0.121	0.729
Species x Competition x Salinity x Water	0.540	0.464

Pairwise comparisons determined that RGR_{SH} of *Sesuvium portulacastrum* and *Batis maritima* was significantly higher in the low salinity treatments than the high salinity treatments when exposed to moist or flooded conditions grown in competition or grown alone (Table 4.12, Figure 4.6). Water level did not significantly affect the RGR_{SH} of either species at the $p < 0.05$ level, after Bonferroni adjustment (Table 4.10).

Table 4.12. Hypothesis tests comparing species' RGR_{SH} in the moist treatment to the flooded treatment and comparisons of species' RGR_{SH} in the low salinity treatment to the high salinity treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Moist vs. Flooded	6.237	0.014
<i>Sesuvium portulacastrum</i> Moist vs. Flooded	1.011	0.317
<i>Batis maritima</i> Low salinity vs. High Salinity *	11.968	0.001
<i>Sesuvium portulacastrum</i> * Low Salinity vs. High Salinity	96.937	<0.001

Root Mass Fraction

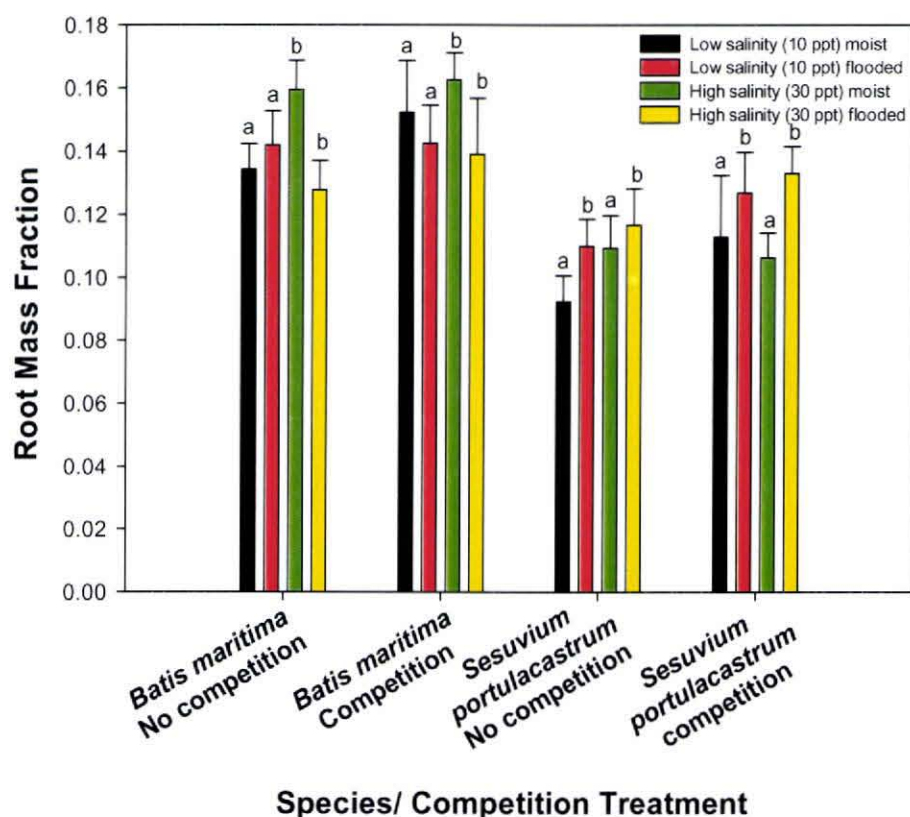


Figure 4.7. Mean RMF (\pm SEM, $n=9$) of *Batis* and *Sesuvium* exposed to six treatment combinations of two salinity, two water, and two competition treatments. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters.

Batis maritima's RMF was significantly higher than *Sesuvium portulacastrum*'s RMF in all treatments, but neither species' RMF were significantly affected by competition or salinity (Table 4.13 and Figure 4.7). The significant interaction between species and water indicated that RMF for each species was affected differently by water level (Table 4.13).

Table 4.13. ANOVA results for the log of RMF with species, salinity, water and competition as fixed factors. An asterisk indicates a significant factor at the p-value < 0.05 level.

Comparison	F-ratio	P-value
Species *	34.956	<0.001
Competition	2.745	0.100
Salinity	1.263	0.263
Water	0.372	0.543
Species x Competition	0.459	0.499
Species x Salinity	0.214	0.645
Species x Water *	9.060	0.003
Competition x Salinity	0.180	0.672
Competition x Water	0.068	0.795
Salinity x Water	1.852	0.176
Species x Competition x Salinity	0.088	0.767
Species x Competition x Water	0.415	0.521
Species x Salinity x Water	1.047	0.308
Competition x Salinity x Water	0.814	0.369
Species x Competition x Salinity x Water	0.038	0.847

Pairwise comparisons determined that RMF of *Sesuvium portulacastrum* was significantly higher in the flooded treatments than the moist treatments when exposed to low salinity or high salinity grown in competition or grown alone (Table 4.14, Figure 4.7). Water level did not significantly affect the RMF of *Batis maritima* at the p<0.05 level (Table 4.14).

Table 4.14. Hypothesis tests comparing species' root mass fractions (RMF) in the moist treatment to their RMF in the flooded treatment and comparisons of species' RMF in the low salinity treatment to their RMF in the high salinity treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Moist vs. Flooded	2.881	0.092
<i>Sesuvium portulacastrum</i> * Moist vs. Flooded	6.550	0.012

Leaf Mass Fraction

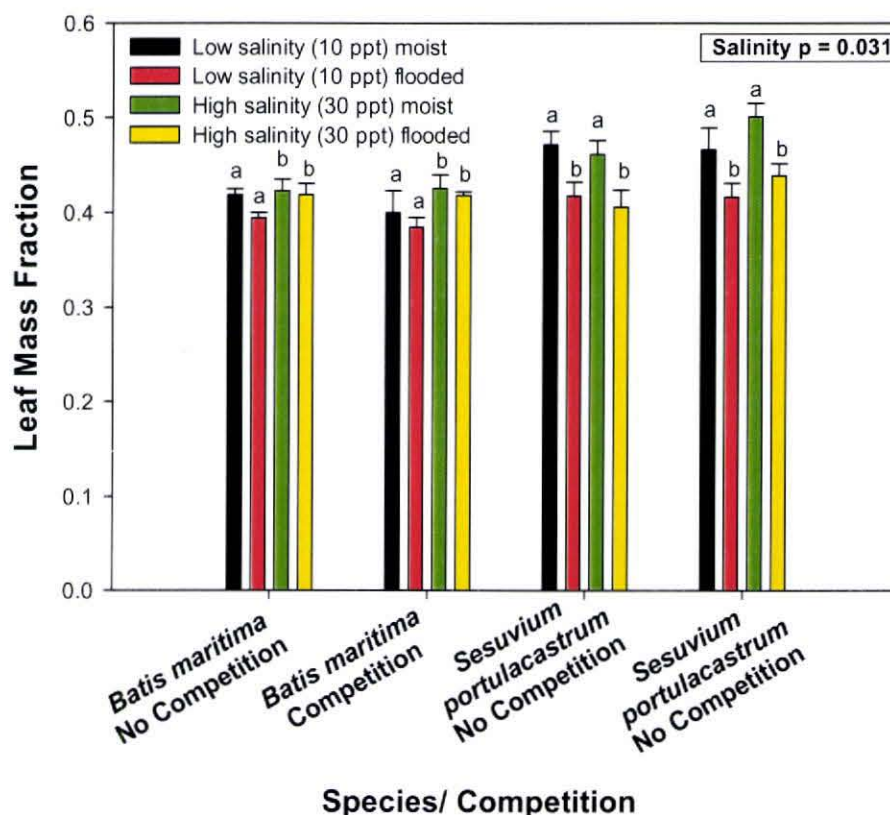


Figure 4.8. Mean LMF (\pm SEM, $n=9$) of *Batis maritima* and *Sesuvium portulacastrum* exposed to six treatment combinations of two salinity, two water, and two competition treatments. Significant differences by ANOVA (p -value < 0.05 after a sequential Bonferroni adjustment) between water levels, within salinity treatments, are indicated by different lower case letters. Salinity significantly affected LMF ($p = 0.031$) by ANOVA. LMF is not significantly affected by competition at the $p < 0.05$ level.

Sesuvium portulacastrum's LMF was significantly different than *Batis maritima*'s LMF, but neither species' LMF were significantly affected by competition (Figure 4.8, Table 4.15). LMF was significantly affected by salinity level, but no pattern was apparent (Figure 4.8, Table 4.15). The significant interaction between species and water indicated that LMF for each species was affected differently by water level (Table 4.15).

Table 4.15. ANOVA results for the log of LMF with species, salinity, water and competition as fixed factors. An asterisk indicates a significant factor at the p-value < 0.05 level.

Comparison	F-ratio	P-value
Species *	22.900	<0.001
Competition	0.202	0.654
Salinity *	4.768	0.031
Water *	18.278	<0.001
Species x Competition	2.583	0.110
Species x Salinity	1.061	0.305
Species x Water *	7.576	0.007
Competition x Salinity	3.915	0.050
Competition x Water	0.104	0.748
Salinity x Water	0.055	0.814
Species x Competition x Salinity	0.396	0.530
Species x Competition x Water	0.010	0.919
Species x Salinity x Water	0.453	0.502
Competition x Salinity x Water	0.148	0.701
Species x Competition x Salinity x Water	0.027	0.869

Pairwise comparisons determined that LMF of *Sesuvium portulacastrum* was significantly lower in the flooded treatments than the moist treatments when exposed to low salinity or high salinity grown in competition or grown alone (Table 4.14, Figure 4.7). Water level did not significantly affect the LMF of *Batis maritima* at the $p < 0.05$ level (Table 4.14).

Table 4.16. Hypothesis tests comparing species' leaf mass fraction (LMF) in the moist treatment to their LMF in the flooded treatment and comparisons of species' RGR_{SH} in the low salinity treatment to their LMF in the high salinity treatment. An asterisk indicates significance at the $p < 0.05$ level after a sequential Bonferroni adjustment.

	F-Ratio	Raw P-Value
<i>Batis maritima</i> Moist vs. Flooded	1.241	0.267
<i>Sesuvium portulacastrum</i> * Moist vs. Flooded	25.063	<0.001

Discussion

Interspecific competition can be defined as “an interaction between individuals, brought about by a shared requirement for a resource in limited supply, leading to a reduction in the survivorship, growth, and/ reproduction of the competing individuals concerned” (Begon et al. 1986, Grace 1990b).

Salinity and water level had very little effect on competition between *Batis maritima* and *Sesuvium portulacastrum*. Competitive effects were only significantly different between salinity treatments when examining additional shoot number and in this instance competition was lessened for both species as salinities rose. The general lack of growth differences between the competition and no competition treatments indicates that plants were often not limited by the same resource and therefore were not in competition with one another.

When examining total dry weight with species, salinity, water, and competition as fixed factors, total dry weight was significantly smaller when plants were grown in competition with one another in all salinity and water treatments. These results indicate competition was occurring at all water and salinity levels. However, this conclusion is less reliable than the competitive effects analysis because the analysis of raw dry weights does not take into account the within-bin pairing of competition-no competition pots and

instead treats each pot as statistically independent. This results in over-inflated degrees of freedom in the ANOVA based on raw dry weights.

RGR_{SH} was significantly higher in the low salinity treatments than the high salinity treatments, which is supportive of my early growth results (Chapter 3). RGR_{SH} was also significantly affected by competition and generally RGR_{SH} was reduced when plants were grown in competition compared to plants grown alone. Water did not significantly affect RGR_{SH} of either species.

Sesuvium portulacastrum's root mass fraction (RMF) and leaf mass fraction (LMF) were significantly affected by water and salinity level, but competition did not significantly affect the biomass allocation of either of these species. These findings are interesting because results, from my early growth experiment (Chapter3), showed that *Sesuvium*'s RMF was significantly lower in flooded treatments than in moist treatments, but in this competition experiment, the opposite effect was seen. The primary difference between these two experiments was plant age; seedlings may not have the ability to allocate more resources to its root system because of the smaller amount of resources present in the plant. Cuttings do not need to allocate as many resources to the growth structure of the plant and therefore have more resources available to allocate to the root system. Allocation of resources to the root system could be a cause of the significantly smaller LMF of *Sesuvium portulacastrum* in flooded conditions.

The lack of competition could be the result of several different factors in the experimental design. First, pot size may have been too large to induce competition during early growth; smaller pots would have decreased the available space for root growth and would likely have increased competition between the two species. The addition of

fertilizer may also have been an important factor in preventing below ground competition. There were most likely sufficient nutrients, from the Osmocote application and use of the Menehune Soil Conditioner, for both species to flourish.

Both *Batis maritima* and *Sesuvium portulacastrum* spread by clonal expansion. *Batis* has been shown to grow 65 cm over thirteen weeks when irrigated with fresh and brackish water (Miyamoto et al. 1996); this ability to occupy a large area of space (resource) in a relatively short period of time can lead to dominance in a community (USDA and Francis, Lonard and Judd 1995, Grime 2002). There were no barriers placed on the side of pots to restrict growth of the plant. Therefore, many plant shoots were hanging over the edge of the pots and water bins. Plants were essentially unlimited in terms of growth space and this may have reduced competition for light. The intensity of light that a plant receives can be largely dependent upon the layers of vegetation above the plant (Daubenmire 1974). Since plants were not restricted in space, plants may not have been in competition for light.

Lastly, the length of the experiment could have had an effect on whether competition occurred between species. Perhaps, the resources were not yet limiting to the species, but if the experiment was prolonged, the effects of competition on species' growth may have been more apparent.

Conclusions

There is evidence of significant competition occurring when examining the competitive effect on *Sesuvium portulacastrum*'s total dry weight under the low salinity moist and high salinity flooded condition. Because both low and high salinity increased the competitive effect on *Sesuvium*, my hypothesis that the ability of *Sesuvium*

portulacastrum to compete with *Batis maritima* will be greater at higher levels of salinity stress is not supported.

The competitive effect on *Batis maritima*'s shoot and leaf number was significantly increased in the low salinity flooded and low salinity moist treatments, respectively. Therefore, my hypothesis that *Batis maritima* will grow faster and out-compete *Sesuvium portulacastrum* under lower levels of salinity stress is not supported.

Overall, it appears that resources (e.g. light, space, nutrients) were not limiting enough to induce significant competition between *Batis maritima* and *Sesuvium portulacastrum*. It is possible that since competition was weak in this study, the ability of plant growth was determined primarily by tolerance to flooded and saline conditions.

Future Studies

Restoration projects typically remove invasive species from an area before planting natives. Because the outcome of competition between pairs of species is often dependent on relative sizes (Huston 1994), and could potentially be affected by emergence time (Huston 1994), it would be interesting to investigate competition between already established *Sesuvium portulacastrum* plants and small fragments of introduced *Batis maritima* plants to determine the degree to which established *Sesuvium* can resist invasion by *Batis*.

Chapter 5. Site Characteristics of the Waiawa Unit of Pearl Harbor National Wildlife Refuge (NWR) and Suitability of Five Native Hawaiian Coastal Species for Restoration

Abstract

In order to assist the U.S. Fish and Wildlife Service with a restoration project at the Waiawa unit of the Pearl Harbor National Wildlife Refuge, salinity and flooding tolerances of five native Hawaiian coastal species were investigated. In addition, soil samples were analyzed for soil salinity and soil moisture to determine whether salinity or moisture gradients existed at the site and to determine if the species were appropriate candidates for restoration, based on salinity. Soil salinity and soil moisture were significantly lower in the upper diked portion of the site than soils in the lower intertidal region. Based on the salinity tolerances of the five native coastal species (Chapters 2 and 3) and the soil salinity conditions, *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, and *Sida fallax*, and would all be appropriate to use in replanting efforts on the upper dike and *Sesuvium portulacastrum* is appropriate to use in replanting efforts in the lower diked area. With the exception of *Sesuvium*, all native species had decreased germination when exposed to salinities higher than 10 ppt, suggesting that replanting efforts should focus on outplanting seedlings. Further investigation of salinity conditions during low and high tide, salinity conditions after significant rainfall, and tolerance of seedlings to submersion in saline water are suggested to ensure successful restoration using these species.

Introduction

Hawaiian coastal wetlands have received minimal attention in the past (Cuddihy and Stone 1990, Rauzon and Drigot 2002), but recently state and federal agencies have become more actively involved in the restoration of these unique and rapidly degrading ecosystems. Restoration efforts, including non-native plant removal and supplemental native Hawaiian plantings, are helping native plants to spread and colonize in areas historically known to be wetlands.

The U. S. Fish and Wildlife Service (U.S. FWS) plans to restore native Hawaiian coastal plants to the Waiawa Unit of Pearl Harbor National Wildlife Refuge (NWR), located in the northwestern corner of Pearl City Peninsula (Appendix D, Figure D.1). The wildlife refuge is separated into two major diked pond areas and was previously connected to the Waiawa Springs complex. Four acres of introduced *Rhizophora mangle* were removed to restore natural wetland habitat and provide habitat for the endangered Hawaiian water birds. Restoration planning, including species selection, species placement, and replanting time, is necessary for successful reestablishment of native plants.

Estuarine marshes typically lie on gradients of decreasing salinity from the ocean inland (Mitsch and Gosslink 1993) and this gradient of salinity dictates which species can germinate and grow successfully in an area. There is very little information about the Pearl Harbor NWR regarding potential gradients of salinity, fluctuations of salinity seasonally, moisture, or nutrient availability.

Soil salinity at the Waiawa site is variable, with more inland soils containing almost no salt to intertidal soils with salinity concentrations similar to sea water (Map

Appendix D). Limited soil sampling, performed in 1997 and 1998, indicates that surface salinities were 1.9 ppt at the piped water outlet on the more inshore side of the refuge, while only 3.1 meters away from the pipe outlet, salinities increased to 5 ppt. Salinities averaged 9 ppt in the upper half of the diked areas of the refuge and averaged 24 ppt in the diked portion of the refuge closest to the ocean (Englund and Preston 2000). More recently, soil samples indicated that the salinity levels in the high, middle, and low intertidal zone were 34 ppt, 32 ppt, and 36 ppt respectively (Dr. Amanda Demopolus, unpublished data).

To assist the U.S. FWS with selecting appropriate species for replanting, salinity and flooding tolerances of five native species were evaluated at the germination and early growth stage in the greenhouse and laboratory (Chapters 2 and 3). *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* are all indigenous to Hawai'i and occur in coastal wetland areas on the leeward side of O'ahu.

The objective of this study was to determine if salinity or moisture gradients existed at the Waiawa unit and to determine the suitability of selected plant species for restoration at the site, as determined by salinity tolerances. In addition, soil samples were analyzed for nutrients to further assist the U.S. FWS in restoring the site. This study tested the following hypotheses: 1) soil salinity and moisture will decrease with increasing elevation 2) intertidal soil salinity will remain constant; and 3) salinity will vary at the Waiawa unit, but soil salinity will not exceed the tolerance level of the native species selected for salinity tolerance testing (Chapters 2 and 3).

Methods

Waiawa Site Investigations

Beginning in May of 2005, monthly soil samples were collected from fourteen locations at the Waiawa unit. Soil samples were collected along three transect lines that split the length of the dike equally into thirds. Transect one and transect two contained five sample locations, two positioned on the top and slope of the dike and three points positioned equally from the tow of the dike into the mangrove stand (Appendix D, Figure D.2).

Transect three had a much steeper slope and the distance from the top of the dike to seawater was much smaller than transect one and two. The potential fifth sample in this transect was consistently under ½ meter of water and therefore, this transect only contained four sample locations. Sample locations were marked with flags to help quickly determine locations on subsequent visits

Surface litter and plant growth were cleared from each flagged area. A clean steel spade was used to dig a hole approximately as wide as the spade (10 cm) and 15-20 cm deep. The spade tip removed a slice of one side of the hole wall and the middle portion of the slice was removed with another clean trowel, producing a 7.5 by 7.5 cm section of soil. Using this method, four sub samples were collected, no further than one foot away from the flag, and mixed together in a plastic bin to obtain a final composite sample for each location. Approximately ½ liter of the final soil sample was placed in a small Ziploc baggie and labeled with a waterproof marker to identify the sample (UHManoa 1997). Tools were cleaned and rinsed between each sample location to prevent contamination from one point to the next.

Two sets of samples were collected in May 2005; one set of samples was analyzed by the Agricultural Diagnostic Service Center of the College of Tropical Agriculture and Human Resources (CTAHR) at the University of Hawai'i at Manoa, for nutrients. Analyses included pH, Phosphorus, Potassium, Calcium, Magnesium, and Total Nitrogen.

Soils were air dried and sieved through a 2 mm screen to prepare the samples. Soil pH is determined by weighing 30-50 g of soil and adding deionized water to make a saturated paste. This paste was equilibrated for one hour and pH was measured with a pH meter. Extractable soil nutrients (Phosphorus) were measured using the Modified Truog procedure (Ayres and Hagihara 1952, Silva and Uchida 2000). Ammonium acetate was used to extract soil cations (Calcium, Potassium, and Magnesium) with a soil to solution ratio of 1:20, with 2.5 g of soil shaken for 10 minutes (Silva and Uchida 2000). Total Nitrogen was measured using the approved Association of Official Analytical Chemists (AOAC) method of thermal analysis using LICOR 2000.

The second set of May soil samples was tested to determine soil salinity and soil moisture. Samples, for salinity and moisture analysis, were collected during the last week of every month from May continuing through November 2005.

Soil samples were brought back to the laboratory and tested the same day for salinity and moisture analysis. To determine percent moisture of the sample, approximately 100 g of soil were removed from each location sample and weighed to determine the "wet weight" of the sample. Soil was then wrapped in paper, labeled, and dried at 55 degrees for five days. Samples were weighed again to determine "dry weight".

The ratio of wet weight to dry weight was subtracted from one and multiplied by 100; this was considered the percent moisture of the sample (CSU 2006).

Salinity of the soil was measured from water extracted from a saturated soil (called saturation extract) (Brouwer et al. 1985). To determine salinity content of the sample, approximately 45 cubic cm of soil were placed in a weighing cup and distilled water was mixed into the sample until a saturation point was reached. Soil was placed onto Whatman no.2 filter paper and the sample was squeezed over a refractometer until two or three drops of soil water were produced (Personal Communication John Fong, Soil Scientist, University of Hawai'i, NREM). Three replicate salinity measurements were completed to ensure that salinity measurements were consistent for sample locations and the mean of these three replicate was recorded.

Native Plant Selection

Five native Hawaiian coastal species were chosen for potential restoration at the Waiawa Unit of Pearl Harbor NWR. Resources used to select the species included the Riparian Restoration Database (Bishop Museum, Honolulu, HI; Authors: Laura Crago, C.F. Puttock, and S.A. James), Brimacombe (2002), Merlin (1986), Wagner (1999), Stemmerman (1981). Species were chosen based on the following characteristics:

- Potential ability to tolerate salty conditions;
- Limited information on salinity tolerance at germination/early growth stage.
- Ability to collect wild seeds in the vicinity of Pearl Harbor NWR;

Bolboschoenus maritimus, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* were germinated under eight different treatments, consisting of four salinities (freshwater = 0 ppt, low salinity = 10 ppt, medium salinity =

20 ppt, and high salinity = 30 ppt) and two water levels (moist = saturated filter paper and flooded = submerged seeds). Refer to Chapter 2: Germination Methods Section for more specific details of this experiment.

Seedlings of all of these species, except *Bolboschoenus maritimus*, were then grown under four treatments, consisting of two salinity levels (low = 10 ppt and high = 30 ppt) and two water levels (moist = water contact with lower soil and flooded = roots were submerged in flooded soil). Refer to Chapter 3: Early Growth Methods Section for more specific details of this experiment. Salinity tolerances at the germination and early growth stage will be summarized in order to evaluate the suitability of using these plants for restoration at the Waiawa unit of Pearl Harbor National Wildlife Refuge.

Data Analysis

Mean salinity and moisture measurements were recorded monthly for each sample location. Samples were reclassified according to whether the sample was located in the upper portion of the diked area (on the top of the dike or on the slope) or the lower portion of the diked area (the intertidal zone). Since the upper portion of the dike contained no traces of salinity, only the lower intertidal zone was analyzed to test for differences over time in soil salinity. Salinity data appeared normal and no transformations were performed. A one way ANOVA (Systat 10.2, Systat Software Inc.) was performed with month as a fixed factor. Normally, a repeated measure ANOVA would be used in this situation. However, monthly data are represented by the collection of only one sample and any temporal fluctuations in salinity due to rainfall are expected to occur on timescales that are much shorter than the sampling interval (one month), so a

null model of independence among samples over time is appropriate, as is assumed by treating time as a fixed factor.

A log transformation was performed to normalize the moisture data. A two way ANOVA was performed on soil moisture with location and month as random factors. A summary of nutrient analysis is included in results, but no analysis was performed to investigate differences among sample locations.

Results

Soil Salinity

Soil samples collected from the upper portion of the dike contained no salt (0 ppt). Soil salinities in the lower intertidal region were variable, ranging from 5 ppt to 50 ppt. Salinities appeared to fluctuate over time but the differences over time were not significant ($p=0.089$, Table 5.1). The highest salinity levels appeared to occur during the months of August and September while the lowest salinity measurement appeared to occur during the month of November (Figure 5.1).

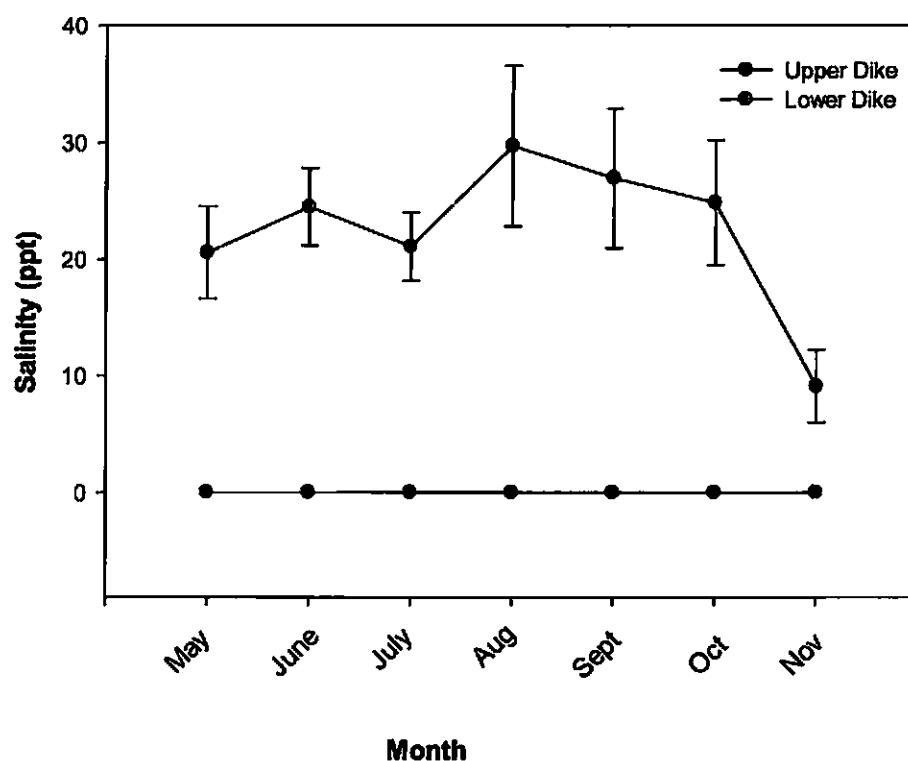


Figure 5.1. Pearl Harbor National Wildlife Refuge, Waiawa unit, soil salinities (ppt) from fourteen different samples collected from the upper dike and the lower intertidal zone over a seven month period.

Table 5.1. ANOVA of soil salinity with month as a fixed factor

	F-Ratio	P-Value
Month	1.991	0.089

Percent Soil Moisture

Percent soil moisture was also significantly higher in the lower intertidal region than in the upper diked portion of the site. (Figure 5.2, Table 5.2). ANOVA analysis showed that sample location as well as the sample location-by-time interaction were significant (Table 5.2), indicating that moisture differences between these two soil sample locations differed over time. Percent soil moisture stayed fairly constant in the

lower portion of the site, but the upper portion of the site showed an increase in percent soil moisture during November.

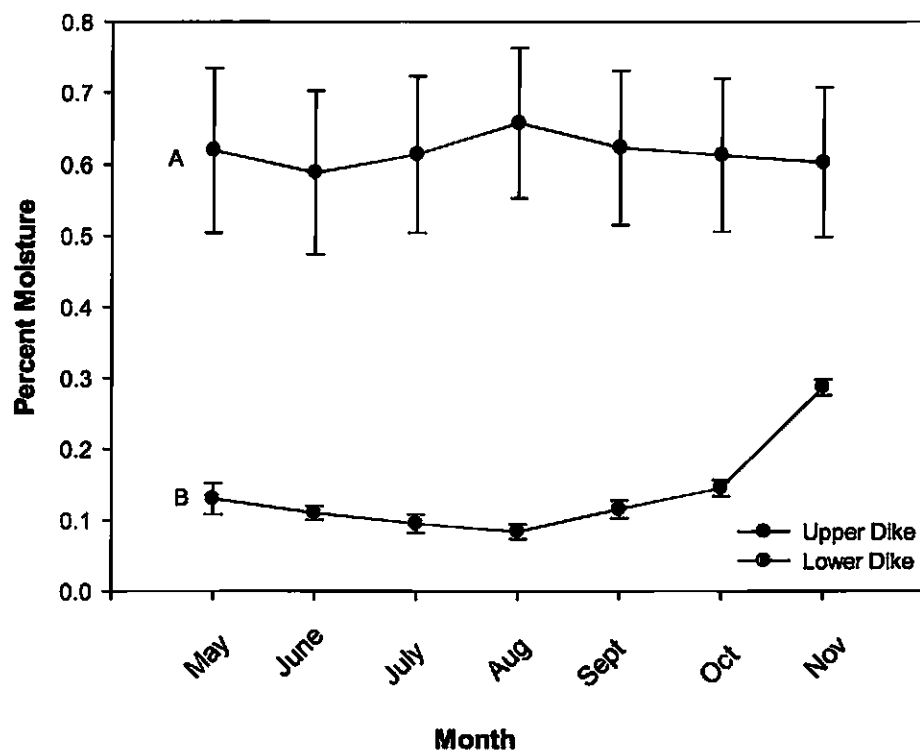


Figure 5.2. Pearl Harbor National Wildlife Refuge, Waiawa unit, percent soil moisture from fourteen sample locations located either in the upper portion of the dike or in the lower dike (intertidal zone) over a seven month period.

Table 5.2. ANOVA of the log percent soil moisture with location and month as random factors.

	F-Ratio	P-Value
Location	194.771	<0.001
Month	2.185	0.182
Location x Month	29.697	<0.001

Nutrient Analysis

The CTAHR Soil Analysis Report indicated that all the sample locations contained heavy soil. Heavy soil is a term used by CTAHR soil analysis lab and is

defined as soils that will not contain up to 300% moisture; O'ahu soils are typically heavy soils (Personal communication with Ray Uchida, Soil Scientist, CTAHR Lab). The pH of both upper dike and the lower intertidal zone was between six and seven and average Phosphorus, Potassium, Calcium, and Magnesium levels were considered to be very high in comparison to CTAHR's expected value for heavy soils. An "expected value" is the point at which additional amounts of a nutrient causes no additional response. The total percent nitrogen was sufficient to high for all samples, but total percent nitrogen was higher in the lower tidal zone than the upper diked area. All of the analyses showed considerable variation among samples within the upper dike and lower intertidal.

Table 5.3. Soil analysis of the Waiawa Unit of Pearl Harbor National Wildlife Refuge. Upper dike values are the average of eight soil samples with one standard deviation and the lower intertidal zone values are the average of seven soil samples with one standard deviation.

Soil Analysis	Upper Dike	Lower Dike (Intertidal Zone)	CTAHR Expected Value
pH	6.13 ± 0.53	6.80 ± 0.64	6
Phosphorus (ppm)	75.43 ± 87.71	62.711 ± 34.02	37.5
Potassium (ppm)	1101.29 ± 480.46	1982.57 ± 1313.54	250
Calcium (ppm)	3002.14 ± 1380.12	5810 ± 1899.42	1750
Magnesium (ppm)	1743.86 ± 342.12	4893.14 ± 1979.10	350
Total Percent N	0.20 ± 0.17	0.91 ± 0.47	No Criteria

Plant Suitability for Restoration

Species were extremely variable in their ability to tolerate salinity at the germination and early growth developmental stages. *Sesuvium portulacastrum* seeds demonstrated the highest salinity tolerance (20 ppt) at the germination stage of all species studied (Table 5.4). *Jacquemontia ovalifolia*, *Sida fallax*, and *Cyperus javanicus* were all

able to germinate in low salinity conditions (10 ppt) and *Bolboschoenus maritimus* only germinated in the freshwater conditions.

Table 5.4. Summary of species' salinity tolerance at the germination stage. Salinity tolerance was defined as the salinity level at which germination was significantly reduced and viability of seeds after salinity exposure indicates species capability and effect on germination rates.

	Salinity Tolerance	Seed Viability After Salt Exposure?
<i>Jacquemontia ovalifolia</i>	10 ppt	Yes, Reduced Germination
<i>Sida fallax</i>	10 ppt	Yes, Reduced Germination
<i>Cyperus javanicus</i>	10 ppt	Yes, Similar Germination
<i>Bolboschoenus maritimus</i>	No Salinity Tolerance Under Levels Tested	Yes, Reduced Germination
<i>Sesuvium portulacastrum</i>	20 ppt	Yes, Similar Germination

Many different growth variables were analyzed (Table 5.5), but for simplicity, I will discuss suitability for restoration based on the total dry weight of species. All native Hawaiian plant species experienced better growth when exposed to the low salinity conditions (10 ppt) compared to the high salinity conditions (30 ppt) (Table 5.5). However, *Sesuvium portulacastrum* showed the smallest relative decrease in total dry weight between the two salinity levels. This indicates that *Sesuvium portulacastrum* has a greater ability to tolerate high salt conditions than the other species tested.

Table 5.5. Summary of plant responses when plants were exposed to 30 ppt of salinity as opposed to 10 ppt of salinity.

	Total Dry Weight	Root Length	Shoot Length	Specific Leaf Area
<i>Jacquemontia ovalifolia</i>	Significant Reduction	Significant Reduction	Significant Reduction	Significant Reduction
<i>Sida fallax</i>	Significant Reduction	No Significant Change	No Significant Change	Significant Reduction
<i>Cyperus javanicus</i>	Significant Reduction	Significant Reduction	Significant Reduction	Significant Reduction
<i>Sesuvium portulacastrum</i>	Significant Reduction	No Significant Change	No Significant Change	Significant Reduction

Discussion

All native species studied appear to be suitable for restoration in the upper dike region at the Waiawa unit of Pearl Harbor NWR, due to the absence of salt in the soil. I suggest that *Bolboschoenus maritimus* be planted along the top edge of the dike, where salinity levels are zero, since *Bolboschoenus maritimus* was unable to germinate in 10 ppt.

Jacquemontia ovalifolia and *Sida fallax* should also be planted along the upper dike. Although seeds may be able to germinate at a low salinity level (10 ppt), the intertidal region sustained a higher salinity level (~20 ppt) for the majority of the months investigated. During the rainy season (as indicated by November's soil salinity), it is possible for these species to germinate at lower elevations, but even if salinities were lowered for long enough to allow germination, it is unlikely that the young seedlings would be able to tolerate a sudden increase in salinity. In addition, *Jacquemontia* and *Sida* seeds were unable to germinate after exposure to higher salinity levels.

Cyperus javanicus showed a significant decrease in germination when salinity levels reached 10 ppt. However, *Cyperus javanicus* seeds remained viable and

germination was not reduced after exposure to higher salinity levels. Therefore, I suggest that the majority of these plants be placed in the upper dike region, but they could be planted at lower elevations than the other species.

Sesuvium portulacastrum would be suitable for replanting in most areas of the proposed restoration site at the Waiawa unit based on salinity tolerance, but *Sesuvium* could be overtopped by other plants in the upper dike where no salt is present. Therefore, it may be most suitable for replanting in the lower diked area. It has shown the ability to germinate under 10-20 ppt salinity and is capable of successful germination after exposure to high saline conditions. Since this species commonly colonizes new areas through clonal growth, it may be advantageous to plant the species along the lower portion of the dike and allow it to spread to the higher saline areas. However, it remains to be tested whether *Sesuvium* would spread from non saline to saline areas and how long this would take.

In order to increase the probability of successful restoration of these species, I suggest outplanting seedlings or vegetative cuttings at the site as opposed to seeding the site, due to low germination of some of the species. Many of the native species will also spread vegetatively and root at nodes (Stemmermann 1981, Merlin 1986, Lonard and Judd 1995); a considerable area could be covered through vegetative growth rather than seeding a new plant.

Nutrient availability is often high in intertidal zones, as a result of both terrestrial runoff and nutrient rich sea water (Huston 1994) and the Waiawa unit of the Pearl Harbor NWR is no different. Nutrients should not need to be added to the soil to promote plant growth, but careful attention should be paid to species diversity in the area, since an

increase in nutrients have been consistently shown to increase the abundance of non-native species (Maron and Connors 1996, Wedin and Tilman 1996, Nernberg and Dale 1997, Kolb 1999, Davis and Grime 2000, Kolb and Alpert 2002).

Conclusions

Results from this study show that soils on the upper dike contain no salts, but soils in the intertidal region range from 10 to 40 ppt salinity. *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, and *Sida fallax*, should be suitable for replanting in the upper dike at the Waiawa unit and *Sesuvium portulacastrum* would be most suitable for planting in the lower dike. Analysis showed that salinity did not vary significantly between the sample times. However, because of limited sampling (one day representative of an entire month) it is not certain whether intertidal soil salinity remains constant with time. The collection time of the sample could have a major affect on the soil salinity due to rainfall and/or tidal action. Rainfall not only reduces osmotic stress, but dilutes the concentration of ions in the surface soil. These conditions often occur after winter rains and when temperatures increase enough to become non limiting and these are typically the most suitable times for germination (Baskin and Baskin 2001). Nevertheless, these results do support the hypothesis that soil salinities and soil moisture decrease as elevation increased.

In the naturally saline environments of the world, species have evolved that are well adapted to life in high saline environments; they are able to complete their life cycles in saline conditions (Kozlowski 1984). However, plants from different populations have been shown to respond differently to soil moisture or salinity (Collinge and Wise

2003). This should be an important consideration when investigating the salt tolerance of species and in the selection of plants and plant sources for restoration projects.

Results from this study should be used with caution, because laboratory conditions often cannot mimic exact site conditions. Other factors such as light, herbivory, and soil texture may influence the ability of these plant species to grow successfully at any restoration site. Heavy, packed clay soils affect plant growth by resisting root incursion; soil texture can also affect the aeration and water content of the soil surrounding the roots (Kozlowski 1984, Mitsch and Gosslink 1993). Flooding affects the aeration of soils differently; aeration is seldom a problem in sandy soils, but can be a serious limitation in fine textured soils (Kozlowski 1984). It is important to understand the physical properties of the soil of a particular field site to understand the ability of different plant species to establish and grow successfully.

A conservative restoration approach would most likely be the best option for the Waiawa unit of Pearl Harbor NWR. Begin by restoring a small number of plants to the area and monitor growth to determine whether species are appropriate for large scale planting.

Future Studies

This study has shed some light on site conditions of the Waiawa unit. However, the ability of the native plants to tolerate tidal inundation was not studied and could be helpful in determining specific placement within the Waiawa unit.

It would also be interesting to determine how salinity fluctuates with rainfall and how long it takes for salinities to return to their “normal level”. This information could be coupled with germination and early growth information to provide managers with a better

understanding of the appropriate planting times for seeds and seedlings, depending upon how long salinities will be reduced.

Chapter 6. Hypotheses Revisited and Conclusions

This project had three main purposes: 1) to investigate how salinity and flooding affected germination of five native Hawaiian coastal species, *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum*, and one invasive species, *Batis maritima*, 2) to investigate the early growth of these same species, and 3) to investigate whether salinity or flooding influenced the relative competitive abilities of *Sesuvium portulacastrum* and *Batis maritima*. In addition, I measured soil salinity and moisture conditions at the Waiawa Site of Pearl Harbor National Wildlife Refuge (NWR) to determine whether the selected species were appropriate for restoration at the site, based on salinity levels. Conclusions from this study partially support my original hypotheses.

Germination Experiment

Germination Hypotheses:

Hypothesis 1: germination of all species will be negatively affected by increasing soil salinities. Hypothesis 2: *Bolboschoenus maritimus* and *Cyperus javanicus* will germinate more successfully under flooded conditions than moist conditions. Hypothesis 3: *Batis maritima*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* and will have higher germination under moist than flooded conditions.

Germination Conclusions:

This study demonstrated that the five native coastal species, *Bolboschoenus maritimus*, *Cyperus javanicus*, *Jacquemontia ovalifolia*, *Sida fallax*, and *Sesuvium portulacastrum* germination decreased with increased salinity. However, the invasive

species, *Batis maritima* did not experience a significant decline in germination percentage from freshwater up to 30 ppt salinity.

Flooding did not significantly affect the germination of *Bolboschoenus maritimus*, *Jacquemontia ovalifolia*, or *Sesuvium portulacastrum*. Flooding significantly reduced germination of *Cyperus javanicus* at all salinities, while flooding significantly reduced *Sida fallax* germination when subjected to 10 ppt salinity.

Early Growth Experiment

Early Growth Hypotheses:

Hypothesis 1: early growth of all species will be negatively related to increasing salinity. Hypothesis 2: *Batis maritima* and *Sesuvium portulacastrum* will tolerate higher levels of salinity during early growth than other species. Hypothesis 3: species, under more severe flooding stress, will show a decrease in root and shoot length.

Early Growth Conclusions

Using relative growth rates of shoots (RGR_{SH}) as an indicator of growth, *Batis maritima*, *Jacquemontia ovalifolia*, *Cyperus javanicus*, *Sida fallax*, and *Sesuvium portulacastrum* experienced significantly reduced RGR_{SH} when plants were exposed to 30 ppt salinity as compared to 10 ppt salinity. *Sida fallax* and *Sesuvium portulacastrum* showed the smallest relative reduction in RGR_{SH} , suggesting that they were more capable of tolerating higher salinity levels. *Sesuvium portulacastrum*' root length was significantly reduced by flooding, but no other species' root lengths were significantly affected by water level.

Competition Experiment

Competition Hypotheses

Hypothesis 1: the competitive ability of *Sesuvium portulacastrum* will be greater at higher levels of salinity stress and the competitive ability of *Batis maritima* will be greater under lower levels of salinity stress.

Competition Conclusions

The competition treatment did not have a strong effect on most aspects of plant growth in *Batis maritima* and *Sesuvium portulacastrum*, so my hypotheses could not be addressed. Very few measured variables indicated that competition even occurred. The competitive effect of *Sesuvium* on *Batis maritima* leaf and shoot production was increased in the low salinity treatment. The competitive effect of *Batis* on *Sesuvium portulacastrum*'s total dry weight was increased in the high salinity treatments and root growth for both species was not affected by competition.

Waiawa Site Study

Waiawa Site Conditions Hypotheses

Hypothesis 1: intertidal soil salinity will remain constant over time. Hypothesis 2: soil salinities and soil moisture will decrease as elevation increases. Hypothesis 3: salinities at the Waiawa site will vary, but soil salinity will not exceed the tolerance level of the selected species.

Waiawa Site Conditions Conclusions

Results from this study confirm my hypothesis that soil salinity and soil moisture decreased as elevation increased. Soils on the upper dike did not contain salt; soil salinity

in the intertidal region was between 5 ppt and 30 ppt and varied depending on the location of the sample, but salinity did not significantly change between the sample times. Due to the wide range in soil salinity at the site, all species studied will be suitable for replanting at the Waiawa unit with proper site placement.

Further Investigation

Wetland restoration is still in the experimental phase, and as the loss of wetlands continues and restoration efforts increase, additional scientific study of wetland organisms and habitats will be essential for providing resource managers with the knowledge to successfully design restoration projects (Zedler 1984).

Tidal inundation, elevation, slope, soil salinity, wave force, and nutrients will all interact to determine the type of vegetation that can occur in a coastal wetland (Zedler 1984). Knowledge about how native Hawaiian coastal species germinate and establish when exposed to these different abiotic conditions will be essential in selecting appropriate species for a specific project. More research about how various species perform under certain conditions is needed and this information could be used to compile lists of native Hawaiian plants appropriate for particular wetland conditions.

Restoration projects not only replant native vegetation, but typically remove invasive species, and knowledge about characteristics of invasive species is instrumental in understanding their role in effecting changes in vegetation composition and structure (Rejmanek 1996, White and Campbell B.D. 1997). Investigations comparing the interaction of invasive and native species under various growing conditions are essential to understand why a species proliferates in a certain area and can provide site specific management strategies for the management of these undesired species (Daehler 2003).

Therefore, more competition studies between native Hawaiian species and invasive species will help to formulate better theories of why invasive species dominate certain areas, and this will improve our chances of future restoration success.

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Appendix A: Early Growth Experiment Correlations

Jacquemontia ovalifolia

Pearson correlation matrix

	ROOT LENGTH	ROOT FW	ROOT DW	SHT LENGTH	SHT FW	SHT DW	# SHTS	LEAVES FW	LEAVES DW
ROOT LENGTH	1.000								
ROOT FW	0.728	1.000							
ROOT DW	0.850	0.801	1.000						
SHT LENGTH	0.539	0.623	0.808	1.000					
SHT FW	0.602	0.671	0.829	0.988	1.000				
SHT DW	0.470	0.573	0.748	0.987	0.984	1.000			
# SHTS	0.752	0.645	0.712	0.499	0.529	0.404	1.000		
LEAVES FW	0.675	0.731	0.863	0.934	0.966	0.924	0.598	1.000	
LEAVES DW	0.564	0.653	0.832	0.976	0.983	0.974	0.504	0.974	1.000
# LEAVES	0.849	0.781	0.915	0.825	0.848	0.757	0.795	0.899	0.841
WET BIOMASS	0.694	0.783	0.881	0.937	0.969	0.922	0.615	0.996	0.969
DRY BIOMASS	0.577	0.658	0.842	0.985	0.990	0.981	0.506	0.970	0.998
RMF	0.171	0.046	0.007	-0.440	-0.436	-0.504	0.064	-0.419	-0.481
SMF	0.158	0.153	0.378	0.795	0.758	0.827	0.118	0.642	0.726
LMF	-0.343	-0.206	-0.394	-0.347	-0.313	-0.312	-0.188	-0.212	-0.232
LEAF SA	0.700	0.746	0.896	0.957	0.976	0.932	0.669	0.986	0.972
LAR	0.693	0.746	0.889	0.949	0.969	0.923	0.671	0.990	0.972
LEAF CHANGE	0.831	0.791	0.901	0.815	0.838	0.746	0.798	0.888	0.831
SHTHT	0.539	0.631	0.807	0.999	0.986	0.982	0.518	0.935	0.974
SHTNUM	0.752	0.645	0.712	0.499	0.529	0.404	1.000	0.598	0.504

	# LEAVES	WET BIOMAS	DRY BIOMAS	RMF	SMF	LMF	LEAF SA	LAR	LEAF CHANG E
# LEAVES	1.000								
WET BIOMASS	0.908	1.000							
DRY BIOMASS	0.842	0.968	1.000						
RMF	-0.210	-0.376	-0.450	1.000					
SMF	0.437	0.622	0.739	-0.541	1.000				
LMF	-0.224	-0.237	-0.278	-0.509	-0.449	1.000			
LEAF SA	0.926	0.989	0.974	-0.361	0.647	-0.279	1.000		
LAR	0.927	0.991	0.971	-0.380	0.633	-0.244	0.998	1.000	
LEAF CHANGE	0.996	0.900	0.831	-0.224	0.410	-0.182	0.918	0.919	1.000
SHTHT CHANGE	0.834	0.938	0.982	-0.440	0.789	-0.340	0.960	0.952	0.827
SHTNUM CHANGE	0.795	0.615	0.506	0.064	0.118	-0.188	0.669	0.671	0.798
	SHTHT CHANGE	SHTNUM CHANGE							
SHT HT CHANGE	1.000								
SHTNUM CHANGE	0.518	1.000							

Sida fallax

Pearson correlation matrix

	ROOT LENGTH	ROOT FW	ROOT DW	SHT LENGTH	SHT FW	SHT DW	# SHTS	LEAVES FW	LEAVES DW
ROOT LENGTH	1.000								
ROOT FW	0.771	1.000							
ROOT DW	0.841	0.973	1.000						
SHT LENGTH	0.811	0.896	0.941	1.000					
SHT FW	0.785	0.875	0.874	0.911	1.000				
SHT DW	0.795	0.890	0.896	0.934	0.984	1.000			
# SHTS		
LEAVES FW	0.819	0.951	0.976	0.959	0.924	0.930	.	1.000	
LEAVES DW	0.830	0.968	0.978	0.959	0.933	0.943	.	0.993	1.000
# LEAVES	0.820	0.796	0.775	0.768	0.830	0.791	.	0.821	0.843
WET BIOMASS	0.815	0.976	0.982	0.951	0.933	0.940	.	0.994	0.997
DRY BIOMASS	0.837	0.968	0.981	0.963	0.940	0.955	.	0.991	0.998
RMF	-0.177	0.094	0.078	-0.093	-0.079	-0.121	.	-0.030	-0.054
SMF	-0.291	-0.539	-0.498	-0.405	-0.371	-0.271	.	-0.511	-0.483
LMF	0.404	0.519	0.484	0.478	0.435	0.352	.	0.557	0.540
LEAF SA	0.828	0.929	0.964	0.925	0.849	0.852	.	0.973	0.971
LAR	0.820	0.919	0.952	0.917	0.835	0.836	.	0.966	0.963
LEAF CHANGE	0.821	0.808	0.800	0.797	0.814	0.784	.	0.847	0.861
SHTHT	0.763	0.849	0.907	0.983	0.849	0.877	.	0.935	0.926
CHANGE SHTNUM	0.241	0.462	0.451	0.498	0.451	0.396	.	0.461	0.437

	# LEAVES	WET BIOMAS	DRY BIOMAS	RMF	SMF	LMF	LEAF SA	LAR	LEAF CHANG E
# LEAVES	1.000								
WET BIOMASS	0.829	1.000							
DRY BIOMASS	0.830	0.997	1.000						
RMF	-0.181	0.004	-0.038	1.000					
SMF	-0.538	-0.514	-0.457	-0.359	1.000				
LMF	0.667	0.541	0.503	-0.165	-0.861	1.000			
LEAF SA	0.843	0.963	0.962	-0.015	-0.563	0.603	1.000		
LAR	0.848	0.954	0.952	-0.034	-0.578	0.630	0.999	1.000	
LEAF CHANGE	0.992	0.846	0.846	-0.189	-0.562	0.698	0.878	0.885	1.000
SHTHT CHANGE	0.754	0.915	0.926	-0.120	-0.431	0.521	0.920	0.917	0.801
SHTNUM CHANGE	0.420	0.469	0.438	0.544	-0.591	0.328	0.446	0.442	0.437
		SHTHT CHANGE	SHTNUM CHANGE						
SHTHT CHANGE		1.000							
SHTNUM CHANGE		0.515	1.000						

Cyperus javanicus

Pearson correlation matrix

	ROOT LENGTH	ROOT FW	ROOT DW	SHT LENGTH	SHT FW	SHT DW	# SHTS	LEAVES FW	LEAVES DW
ROOT LENGTH	1.000								
ROOT FW	0.962	1.000							
ROOT DW	0.932	0.982	1.000						
SHT LENGTH	0.917	0.942	0.905	1.000					
SHT FW				
SHT DW			
# SHTS	0.955	0.966	0.955	0.895	.	.	1.000		
LEAVES FW	0.923	0.952	0.915	0.937	.	.	0.954	1.000	
LEAVES DW	0.903	0.940	0.927	0.919	.	.	0.959	0.988	1.000
VLEAVES	0.902	0.960	0.980	0.851	.	.	0.966	0.929	0.949
WET BIOMASS	0.953	0.987	0.958	0.951	.	.	0.972	0.989	0.976
DRY BIOMASS	0.929	0.970	0.967	0.930	.	.	0.973	0.979	0.992
RMF	0.586	0.576	0.619	0.547	.	.	0.501	0.382	0.367
SMF
LMF	-0.586	-0.576	-0.619	-0.547	.	.	-0.501	-0.382	-0.367
LEAF SA	0.946	0.958	0.915	0.931	.	.	0.972	0.987	0.969
LAR	0.916	0.925	0.874	0.916	.	.	0.950	0.988	0.971
LEAF CHANGE	0.906	0.950	0.968	0.833	.	.	0.957	0.916	0.935
SHTHT	0.934	0.915	0.876	0.952	.	.	0.868	0.893	0.870
SHTNUM	0.439	0.440	0.400	0.463	.	.	0.506	0.662	0.640

	# LEAVES	WET BIOMAS	DRY BIOMAS	RMF	SMF	LMF	LEAF SA	LAR	LEAF CHANG E
# LEAVES	1.000								
WET BIOMASS	0.956	1.000							
DRY BIOMASS	0.975	0.987	1.000						
RMF	0.497	0.481	0.460	1.000					
SMF				
LMF	-0.497	-0.481	-0.460	-1.000	.	1.000			
LEAF SA	0.926	0.985	0.967	0.438	.	-0.438	1.000		
LAR	0.897	0.969	0.954	0.353	.	-0.353	0.993	1.000	
LEAF CHANGE	0.989	0.944	0.962	0.449	.	-0.449	0.914	0.884	1.000
SHTHT CHANGE	0.816	0.914	0.887	0.473	.	-0.473	0.886	0.866	0.837
SHTNUM CHANGE	0.494	0.563	0.568	-0.088	.	0.088	0.595	0.659	0.461

	SHTHT CHANGE	SHT NUM CHANGE
SHTHT CHANGE	1.000	
SHTNUM CHANGE	0.382	1.000

Sesuvium portulacastrum

Pearson correlation matrix

	ROOT LENGTH	ROOT FW	ROOT DW	SHT LENGTH	SHT FW	SHT DW	# SHTS	LEAVES FW	LEAVES DW
ROOT LENGTH	1.000								
ROOT FW	0.700	1.000							
ROOT DW	0.731	0.973	1.000						
SHT LENGTH	0.334	0.678	0.558	1.000					
SHT FW	0.524	0.914	0.843	0.842	1.000				
SHT DW	0.421	0.847	0.753	0.903	0.969	1.000			
# SHTS	0.719	0.879	0.837	0.599	0.837	0.785	1.000		
LEAVES FW	0.663	0.932	0.879	0.731	0.957	0.877	0.871	1.000	
LEAVES DW	0.652	0.927	0.851	0.757	0.934	0.904	0.925	0.955	1.000
# LEAVES WET	0.739	0.829	0.755	0.663	0.815	0.771	0.936	0.867	0.919
BIOMASS DRY	0.651	0.962	0.910	0.759	0.976	0.909	0.881	0.992	0.961
BIOMASS	0.620	0.957	0.896	0.802	0.973	0.950	0.900	0.958	0.981
RMF	0.633	0.569	0.713	0.070	0.323	0.227	0.472	0.373	0.341
SMF	-0.180	0.214	0.075	0.774	0.501	0.647	0.141	0.296	0.314
LMF	-0.379	-0.671	-0.671	-0.738	-0.713	-0.760	-0.525	-0.576	-0.565
LEAF SA	0.681	0.911	0.838	0.751	0.938	0.872	0.905	0.979	0.967
LAR	0.681	0.798	0.712	0.631	0.820	0.738	0.864	0.915	0.911
LEAF CHANGE	0.741	0.828	0.756	0.660	0.811	0.768	0.925	0.867	0.918
SHTHT	0.341	0.671	0.554	0.998	0.841	0.897	0.599	0.733	0.751
SHTNUM	0.711	0.876	0.837	0.603	0.849	0.795	0.994	0.885	0.933

	# LEAVES	WET BIOMAS	DRY BIOMAS	RMF	SMF	LMF	LEAF SA	LAR	LEAF CHANG E
# LEAVES	1.000								
WET BIOMASS	0.862	1.000							
DRY BIOMASS	0.872	0.980	1.000						
RMF	0.343	0.416	0.407	1.000					
SMF	0.186	0.332	0.395	-0.328	1.000				
LMF	-0.454	-0.644	-0.692	-0.561	-0.598	1.000			
LEAFSA	0.937	0.971	0.951	0.343	0.289	-0.544	1.000		
LAR	0.939	0.880	0.846	0.220	0.151	-0.319	0.962	1.000	
LEAF CHANGE	0.997	0.861	0.871	0.349	0.177	-0.452	0.938	0.941	1.000
SHTHT CHANGE	0.666	0.758	0.796	0.074	0.769	-0.737	0.753	0.636	0.664
SHTNUM CHANGE	0.935	0.891	0.908	0.467	0.142	-0.521	0.916	0.877	0.931
	SHTHT CHANGE	SHTNUM CHANGE							
SHTHT CHANGE	1.000								
SHTNUM CHANGE	0.605	1.000							

Batis maritima

Pearson correlation matrix

	ROOT LENGTH	ROOT FW	ROOT DW	SHT LENGTH	SHT FW	SHT DW	# SHTS	LEAVES FW	LEAVES DW
ROOT LENGTH	1.000								
ROOT FW	0.866	1.000							
ROOT DW	0.818	0.982	1.000						
SHT LENGTH	0.750	0.908	0.856	1.000					
SHTFW	0.738	0.962	0.953	0.933	1.000				
SHT DW	0.536	0.660	0.690	0.665	0.608	1.000			
# SHTS	0.563	0.853	0.901	0.778	0.924	0.563	1.000		
LEAVES FW	0.767	0.964	0.977	0.880	0.981	0.615	0.955	1.000	
LEAVES DW	0.754	0.942	0.970	0.831	0.957	0.594	0.964	0.994	1.000
# LEAVES	0.763	0.955	0.966	0.893	0.978	0.615	0.956	0.997	0.991
WET BIOMASS	0.782	0.976	0.980	0.901	0.989	0.625	0.940	0.998	0.986
DRY BIOMASS	0.761	0.943	0.973	0.859	0.930	0.806	0.912	0.959	0.954
RMF	-0.259	-0.253	-0.242	-0.467	-0.323	-0.440	-0.345	-0.336	-0.333
SMF	-0.028	0.008	0.035	0.058	-0.032	0.669	-0.054	-0.053	-0.071
LMF	0.167	0.132	0.101	0.204	0.206	-0.361	0.238	0.232	0.247
LEAFSA	0.636	0.818	0.847	0.803	0.794	0.961	0.762	0.804	0.786
LAR	0.739	0.952	0.971	0.885	0.974	0.674	0.964	0.994	0.989
LEAF CHANGE	0.763	0.954	0.965	0.888	0.975	0.612	0.955	0.996	0.991
SHTHT CHANGE	0.754	0.908	0.851	0.997	0.927	0.664	0.761	0.872	0.820
SHTNUM CHANGE	0.563	0.853	0.901	0.778	0.924	0.563	1.000	0.955	0.964

	# LEAVES	WET BIOMAS	DRY BIOMAS	RMF	SMF	LMF	LEAF SA	LAR	LEAF CHANGE
# LEAVES	1.000								
WET BIOMASS	0.994	1.000							
DRY BIOMASS	0.956	0.958	1.000						
RMF	-0.378	-0.323	-0.391	1.000					
SMF	-0.055	-0.040	0.193	-0.113	1.000				
LMF	0.257	0.213	0.041	-0.446	-0.839	1.000			
LEAFSA	0.804	0.810	0.933	-0.460	0.473	-0.173	1.000		
LAR	0.994	0.991	0.975	-0.392	0.012	0.204	0.850	1.000	
LEAF CHANGE	0.999	0.993	0.955	-0.378	-0.058	0.260	0.802	0.993	1.000
SHTHT CHANGE	0.882	0.894	0.851	-0.461	0.062	0.197	0.799	0.875	0.878
SHTNUM CHANGE	0.956	0.940	0.912	-0.345	-0.054	0.238	0.762	0.964	0.955
	SHTHTC HANGE	SHTNUM CHANGE							
SHTHTCH ANGE	1.000								
SHTNUMC HANGE	0.761	1.000							

Appendix B: Early Growth Experiment: ANOVA of Remaining Variables

Log Root Dry Weight

Dep Var: RTDWLOG N: 76 Multiple R: 0.899 Squared multiple R: 0.809
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	94.685	4	23.671	42.671	0.000
SALINITY	19.852	1	19.852	35.787	0.000
WATER	1.415	1	1.415	2.551	0.116
SPECIES*SALINITY	4.852	4	1.213	2.187	0.082
SPECIES*WATER	3.283	4	0.821	1.479	0.221
SALINITY*WATER	0.234	1	0.234	0.422	0.519
SPECIES*SALINITY*WATER	0.813	4	0.203	0.367	0.831
Error	31.065	56	0.555		

Jacquemontia ovalifolia

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	5.583	1	5.583	10.065	0.002
Error	31.065	56	0.555		

Sida fallax

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	3.414	1	3.414	6.155	0.016
Error	31.065	56	0.555		

Cyperus javanicus

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	10.923	1	10.923	19.691	0.000
Error	31.065	56	0.555		

Sesuvium portulacastrum
Low Salinity vs. High Salinity
Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.855	1	0.855	1.541	0.220
Error	31.065	56	0.555		

Batis maritima
Low Salinity vs. High Salinity
Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	2.128	1	2.128	3.836	0.055
Error	31.065	56	0.555		

Log Shoot Dry Weight

Dep Var: SHTDWLOG N: 64 Multiple R: 0.852 Squared multiple R: 0.726
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	83.557	3	27.852	35.004	0.000
SALINITY	10.275	1	10.275	12.913	0.001
WATER	0.307	1	0.307	0.385	0.538
SPECIES*SALINITY	1.709	3	0.570	0.716	0.547
SPECIES*WATER	2.495	3	0.832	1.045	0.381
SALINITY*WATER	0.151	1	0.151	0.190	0.665
SPECIES*SALINITY*WATER	2.166	3	0.722	0.907	0.445
Error	38.193	48	0.796		

Additional Shoot Number

Dep Var: SHHTCHGLOG N: 76 Multiple R: 0.908 Squared multiple R: 0.825
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	35.209	4	8.802	43.351	0.000
SALINITY	7.818	1	7.818	38.503	0.000
WATER	0.144	1	0.144	0.710	0.403
SPECIES*SALINITY	2.667	4	0.667	3.283	0.017
SPECIES*WATER	1.101	4	0.275	1.356	0.261
SALINITY*WATER	0.460	1	0.460	2.265	0.138
SPECIES*SALINITY*WATER	0.459	4	0.115	0.566	0.689
Error	11.371	56	0.203		

Jacquemontia ovalifolia

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	9.085	1	9.085	44.743	0.000
Error	11.371	56	0.203		

Sida fallax

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.484	1	0.484	2.386	0.128
Error	11.371	56	0.203		

Cyperus javanicus

Low Salinity vs. High Salinity

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	1.570	1	1.570	7.731	0.007
Error	11.371	56	0.203		

Sesuvium portulacastrum**Low Salinity vs. High Salinity**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.800	1	0.800	3.940	0.052
Error	11.371	56	0.203		

Batis maritima**Low Salinity vs. High Salinity**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.873	1	0.873	4.297	0.043
Error	11.371	56	0.203		

Log Leaf Number

Dep Var: VLEAFLOG N: 76 Multiple R: 0.871 Squared multiple R: 0.758

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	40.797	4	10.199	32.065	0.000
SALINITY	8.819	1	8.819	27.724	0.000
WATER	0.128	1	0.128	0.402	0.529
SPECIES*SALINITY	2.426	4	0.607	1.907	0.122
SPECIES*WATER	0.576	4	0.144	0.452	0.770
SALINITY*WATER	0.000	1	0.000	0.001	0.982
SPECIES*SALINITY*WATER	0.141	4	0.035	0.111	0.978
Error	17.812	56	0.318		

Log Leaf Dry Weight

Dep Var: LEAVESDWLOG N: 76 Multiple R: 0.862 Squared multiple R: 0.743
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	94.568	4	23.642	31.555	0.000
SALINITY	14.500	1	14.500	19.353	0.000
WATER	0.334	1	0.334	0.446	0.507
SPECIES*SALINITY	3.652	4	0.913	1.219	0.313
SPECIES*WATER	2.854	4	0.714	0.952	0.441
SALINITY*WATER	0.269	1	0.269	0.359	0.551
SPECIES*SALINITY*WATER	0.230	4	0.057	0.077	0.989
Error	41.956	56	0.749		

Log Root Mass Fraction

Dep Var: RMFLOG N: 76 Multiple R: 0.782 Squared multiple R: 0.612
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	5.190	4	1.298	11.030	0.000
SALINITY	0.175	1	0.175	1.485	0.228
WATER	0.328	1	0.328	2.789	0.101
SPECIES*SALINITY	0.388	4	0.097	0.824	0.516
SPECIES*WATER	1.980	4	0.495	4.207	0.005
SALINITY*WATER	0.006	1	0.006	0.053	0.818
SPECIES*SALINITY*WATER	0.471	4	0.118	1.001	0.415
Error	6.588	56	0.118		

Jacquemontia ovalifolia

Moist vs. Flooded

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	1.224	1	1.224	10.407	0.002
Error	6.588	56	0.118		

Sida fallax**Moist vs. Flooded**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.009	1	0.009	0.075	0.785
Error	6.588	56	0.118		

Cyperus javanicus**Moist vs. Flooded**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.004	1	0.004	0.036	0.850
Error	6.588	56	0.118		

Sesuvium portulacastrum**Moist vs. Flooded**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	1.270	1	1.270	10.795	0.002
Error	6.588	56	0.118		

Batis maritima**Moist vs. Flooded**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.427	1	0.427	3.630	0.062
Error	6.588	56	0.118		

Log Shoot Mass Fraction

Dep Var: SMFLOG N: 64 Multiple R: 0.735 Squared multiple R: 0.540
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	1.230	3	0.410	6.818	0.001
SALINITY	0.009	1	0.009	0.144	0.706
WATER	0.114	1	0.114	1.903	0.174
SPECIES*SALINITY	0.362	3	0.121	2.008	0.125
SPECIES*WATER	0.569	3	0.190	3.156	0.033
SALINITY*WATER	0.000	1	0.000	0.001	0.972
SPECIES*SALINITY*WATER	0.458	3	0.153	2.538	0.068
Error	2.886	48	0.060		

Jacquemontia ovalifolia

Moist vs. Flooded

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.225	1	0.225	3.748	0.059
Error	2.886	48	0.060		

Sida fallax

Moist vs. Wet

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.114	1	0.114	1.897	0.175
Error	2.886	48	0.060		

Sesuvium portulacastrum

Moist vs. Wet

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.199	1	0.199	3.304	0.075
Error	2.886	48	0.060		

Batis maritima**Moist vs. Wet**

Test of Hypothesis

Source	SS	df	MS	F	P
Hypothesis	0.227	1	0.227	3.767	0.058
Error	2.886	48	0.060		

Leaf Mass Fraction

Dep Var: LMF N: 76 Multiple R: 0.622 Squared multiple R: 0.387
 Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	0.216	4	0.054	5.832	0.001
SALINITY	0.016	1	0.016	1.708	0.197
WATER	0.001	1	0.001	0.108	0.743
SPECIES*SALINITY	0.017	4	0.004	0.468	0.759
SPECIES*WATER	0.005	4	0.001	0.128	0.972
SALINITY*WATER	0.000	1	0.000	0.026	0.873
SPECIES*SALINITY*WATER	0.043	4	0.011	1.164	0.336
Error	0.518	56	0.009		

Leaf Area Ratio

Dep Var: LARLOG N: 76 Multiple R: 0.857 Squared multiple R: 0.734
 Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
SPECIES	87.138	4	21.785	27.349	0.000
SALINITY	21.566	1	21.566	27.074	0.000
WATER	0.427	1	0.427	0.536	0.467
SPECIES*SALINITY	4.581	4	1.145	1.438	0.234
SPECIES*WATER	3.166	4	0.791	0.994	0.419
SALINITY*WATER	0.070	1	0.070	0.088	0.768
SPECIES*SALINITY*WATER	0.298	4	0.074	0.093	0.984
Error	44.606	56	0.797		

Appendix C: Competition Experiment Correlations

Batis maritima

Pearson correlation matrix

	RT FW	RT LENGTH	RT DW	SHT FW	SHT LENGTH	# SHTS	SHT DW	LEAF FW	# LEAF
RT FW	1.000								
RT LENGTH	-0.571	1.000							
RT DW	0.749	-0.028	1.000						
SHT FW	0.845	-0.541	0.702	1.000					
SHT LENGTH	0.816	-0.563	0.699	0.968	1.000				
# SHTS	0.875	-0.490	0.823	0.851	0.866	1.000			
SHT DW	0.839	-0.461	0.747	0.986	0.947	0.851	1.000		
LEAF FW	0.776	-0.331	0.753	0.955	0.904	0.812	0.987	1.000	
# LEAF	0.900	-0.438	0.757	0.946	0.923	0.860	0.968	0.954	1.000
LEAF DW	0.852	-0.205	0.773	0.840	0.768	0.702	0.882	0.891	0.932
TOTAL DWBIO	0.876	-0.332	0.824	0.945	0.895	0.832	0.974	0.971	0.978
RMF	-0.557	0.622	-0.082	-0.707	-0.638	-0.327	-0.677	-0.637	-0.672
SMF	0.493	-0.725	0.272	0.813	0.816	0.598	0.763	0.708	0.635
LMF	-0.236	0.502	-0.309	-0.556	-0.615	-0.561	-0.511	-0.468	-0.339
LEAF CHNG	0.890	-0.416	0.786	0.948	0.922	0.890	0.975	0.967	0.995
SHTHT CHNG	0.798	-0.569	0.697	0.974	0.995	0.875	0.947	0.904	0.902
SHTNUM CHNG	0.867	-0.435	0.842	0.835	0.834	0.992	0.853	0.828	0.864

	LEAF DW	TOTAL DWBIO	RMF	SMF	LMF	LEAF CHNG	SHTHT CHNG	SHTNUM CHNG
LEAF DW	1.000							
TOTAL DWBIO	0.960	1.000						
RMF	-0.624	-0.620	1.000					
SMF	0.404	0.602	-0.695	1.000				
LMF	-0.060	-0.335	0.162	-0.822	1.000			
LEAF CHNG	0.917	0.979	-0.622	0.642	-0.388	1.000		
SHTHT CHNG	0.739	0.883	-0.617	0.846	-0.672	0.908	1.000	
SHTNUM CHNG	0.726	0.845	-0.307	0.557	-0.522	0.899	0.842	1.000

Sesuvium portulacastrum

Pearson correlation matrix

	RT FW	RT LENGTH	RT DW	SHT FW	SHT LENGTH	# SHTS	SHT DW	LEAF FW	# LEAF
RT FW	1.000								
RT LENGTH	0.160	1.000							
RT DW	0.893	0.537	1.000						
SHT FW	0.799	0.605	0.966	1.000					
SHT LENGTH	0.801	0.643	0.959	0.905	1.000				
# SHTS	0.572	0.802	0.857	0.934	0.838	1.000			
SHT DW	0.808	0.634	0.968	0.954	0.934	0.923	1.000		
LEAF FW	0.735	0.760	0.941	0.959	0.925	0.962	0.974	1.000	
# LEAF	0.536	0.836	0.851	0.909	0.887	0.974	0.897	0.941	1.000
LEAF DW	0.623	0.817	0.889	0.929	0.886	0.987	0.955	0.984	0.968
TOTAL DWBIO	0.743	0.720	0.949	0.958	0.928	0.961	0.992	0.991	0.939
RMF	-0.089	-0.809	-0.403	-0.449	-0.456	-0.703	-0.605	-0.641	-0.663
SMF	0.733	-0.402	0.481	0.320	0.383	0.066	0.410	0.212	0.041
LMF	-0.594	0.649	-0.259	-0.104	-0.156	0.209	-0.122	0.060	0.215
LEAF CHNG	0.559	0.838	0.862	0.910	0.906	0.966	0.901	0.948	0.998
SHTHT CHNG	0.803	0.641	0.943	0.879	0.993	0.801	0.906	0.909	0.856
SHTNUM CHNG	0.585	0.803	0.866	0.945	0.848	0.997	0.914	0.965	0.977

	LEAF DW	TOTAL DWBIO	RMF	SMF	LMF	LEAF CHNG	SHTHT CHNG	SHTNUM CHNG
LEAFW	1.000							
TOTAL DWBIO	0.985	1.000						
RMF	-0.738	-0.657	1.000					
SMF	0.128	0.292	0.195	1.000				
LMF	0.169	-0.002	-0.544	-0.929	1.000			
LEAFHN G	0.967	0.942	-0.649	0.052	0.200	1.000		
SHTHTH NG	0.857	0.901	-0.427	0.360	-0.147	0.880	1.000	
SHTNUM HNG	0.980	0.955	-0.657	0.048	0.207	0.972	0.817	1.000

Appendix D: Waiawa Unit of Pearl Harbor National Wildlife Refuge



Figure D.1. The Waiawa unit of the Pearl Harbor National Wildlife Refuge showing the two major diked pond areas and *Batis maritima* and *Rhizophora mangle* stands. The upper dike refers to the area on top and on the slope of the dike and lower dike refers to the area protruding into the *Batis* and *Rhizophora* stands.



Figure D.2. Fourteen soil sample locations located along three transects at the Waiawa unit of the Pearl Harbor National Wildlife Refuge.