Hawai'i Pacific University

Characterizing Growth Patterns to Facilitate Restoration of the seagrass *Halophila hawaiiana*

by

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SUMMARY

Since the recovery of the threatened endemic Hawaiian seagrass *Halophila hawaiiana* in anthropogenically disturbed areas could be expedited through transplantation, both potted laboratory raised *H. hawaiiana* planting units and plugs were transplanted at eight locations in Maunalua Bay and protected by combinations of caging and mesh. Although transplanted plugs protected by mesh appeared to be most successful, the disappearance of all planting units within 28 days highlighted the need to gain further insight into the survival and growth of *H. hawaiiana* fragments under different collection methods. To inform future *H. hawaiiana* transplantation efforts, experimental field trials were performed in Maunalua Bay to determine how the collection of plugs from donor patches affects the performance of collected plant material. Plugs that were extracted from donor beds using a PVC corer and replanted in the same location showed no significant difference in growth and survival compared with uncut controls, showing that the act of severing rhizomes with a corer alone does not directly result in the death of *H. hawaiiana* rhizomes. The survival of 38% of plugs planted in bare sediment demonstrated that, although moving *H. hawaiiana* plugs outside of donor patches is feasible, the loss of approximately half of planting units should be anticipated when using the plug transplantation method. The morphology of seagrass samples collected from the edges and centers of *H. hawaiiana* patches in Maunalua Bay, Kaneohe Bay, and Kahala were analyzed to determine whether characteristics suggesting a higher potential for growth tend to vary with position within patches. At all locations, mean internode length and the median number of leaf pairs per rhizome were consistently greater at patch edges compared with patch centers. Since these morphological traits allow for rapid growth and could enhance the ability of transplants to quickly occupy uncolonized areas, planting material should be collected from patch edges. Also, laboratory

trials investigating the growth of rhizomes with experimentally removed apical meristems showed no difference in growth and survival between uncut control rhizomes and rhizomes with main apical meristems removed. However, the reduced growth and survival of rhizomes with branching meristems experimentally removed highlighted the importance of avoiding damage to branching meristems when collecting material for transplantation. It was determined that *H. hawaiana* can thrive after being cut with a corer and can continue to grow following the removal of apical meristems, suggesting that transplanting plugs of *H. hawaiiana* from the edges of donor patches into areas of bare sediment fortified with mesh can be successful.

CHAPTER I: Literature Review

INTRODUCTION

Seagrasses form some of the world's most highly productive ecosystems and provide vital ecological services such as enhanced biodiversity, sediment stabilization, improved water clarity, and carbon sequestration (Hemminga and Duarte, 2000). Despite their key role in the marine environment, one in five seagrass species is now listed as Endangered, Vulnerable, or Near Threatened, and one-third of all seagrass species are in decline globally (Short *et al.,* 2011). A comprehensive global assessment of 215 studies found that over the 127-year period from 1879-2006, 29% of the known areal extent of seagrasses has disappeared worldwide, largely as a result of changes in water quality, sediment loading, and eutrophication associated with coastal development (Waycott *et al*., 2009). Although a large gap in seagrass monitoring data exists in the tropical Indo-Pacific region from East Africa to Hawai'i, the same study found that rates of seagrass decline have increased dramatically from a median of 0.9% per year before 1940 to a median of 7% per year from 1990-2006. Hawai'i's only endemic seagrass species, *Halophila hawaiiana*, is listed as a vulnerable species "facing a high risk of extinction in the wild in the medium-term future" by the International Union for Conservation of Nature (IUCN), and it is estimated that *H. hawaiiana* populations have declined over 30% in the past decade as a result of competition with invasive algal species and coastal development (IUCN, 2012). These declines will likely continue to accelerate as human activity intensifies along coastal zones (Duarte, 2002).

Because of the exceptionally high ecological and economic value of seagrasses (estimated to be approximately US$19,000 $ha^{-1}yr^{-1}$ by Costanza *et al.,* 1998), efforts have been aimed at restoring lost or damaged seagrass habitats. There are conflicting findings in the

literature regarding the rate at which planted beds acquire the attributes of natural seagrass habitats, perhaps as a result of intrinsic differences among natural reference sites and planted beds and the organisms that were chosen to monitor successful restoration of the seagrass ecosystem. However, it has been demonstrated that, over time, transplanted seagrass beds can support communities with faunal abundances and diversity similar to those present in naturally occurring seagrass beds (Fonseca *et al*., 1998, Sheridan, 2004). For example, densities of benthic organisms in transplanted *Zostera marina* beds were found to exceed those of adjacent bare substrates in approximately 200 days (Homziak *et al.*, 1982) and population densities of polychaetes in transplanted *Halodule wrightii* beds were found to equal or exceed those within natural beds after 2 to 4 years (Bell *et al.,* 1993). Also, Fonseca *et al.* (1996b) found that fish, shrimp, and crab densities as well as species compositions in transplanted *Halodule wrightii* and *Syringodium filiforme* beds were similar to those of adjacent natural beds within 1 to 2 years. Effective transplantation techniques have been developed for at least six seagrass species, including the only seagrass listed under the Endangered Species Act, *Halophila johnsonii* (Fonseca *et al*., 1996a, Fonseca *et al*., 1998, Heidelbaugh *et al.,* 2000, Paling *et al*., 2009). However, there are many understudied seagrass species for which transplantation techniques have yet to be developed, such as the endemic Hawaiian seagrass *H. hawaiiana.*

Prior to apparent displacement by the invasive green alga *Avrainvillea amadelpha* in 1987 (Peyton, 2009), Maunalua Bay on the southeastern shore of O'ahu supported large meadows of *H. hawaiiana* interspersed with coral reefs and native algae. From 2010 through 2011, the majority of *A. amadelpha* was removed from 27 acres of reef flat with the goal of restoring seagrass and coral reef habitats that previously existed in Maunalua Bay (Minton and Conklin, 2012). However, since *H. hawaiiana* propagates mainly through vegetative growth and

rarely produces seeds (Herbert, 1986b), decades to centuries would likely be required for *H. hawaiiana* to spread from surviving patches and recolonize the bay. Since active restoration of *H. hawaiiana* could potentially accelerate the recovery of Maunalua Bay by encouraging the recolonization of other species that rely on the presence of seagrass, two techniques for transplanting *H. hawaiiana* are tested in Chapter 2: (i) raising seagrass in coconut fiber pots in an aquaculture system prior to transplantation and (ii) directly transplanting seagrass and sediment plugs collected from donor patches. In addition, varying degrees of protective equipment were installed around the transplants, including combinations of caging to discourage bioturbation from fishes foraging around the seagrass and mesh secured to the sea floor to limit burial and erosion by holding sediment in place.

The ultimate failure of laboratory and plug transplantation techniques, both of which have shown promise in previous studies with the congener *Halophila johnsonii* (Heidelbaugh *et al.,* 2000, Nova Southeastern University Oceanographic Center, 2003), suggests the need to examine the growth patterns of *H. hawaiiana*. Specifically, further insight should be gained into the survival and growth of *H. hawaiiana* fragments under different collection and culture methods and the most promising areas to collect planting material within donor seagrass patches. Therefore, Chapter 3 presents growth data that can be used to improve transplantation methods for *H. hawaiiana*. First, growth trials are performed in Maunalua Bay to determine how the collection of seagrass plugs from donor patches affects the survival of the collected plant material. Second, growth characteristics of seagrass samples collected from the edges and centers of *H. hawaiiana* patches in Maunalua Bay, Kaneohe Bay, and Kahala are analyzed to determine whether morphological characteristics suggesting a higher potential for growth (such longer internodes) tend to vary with position within seagrass patches. Finally, laboratory trials

are performed to study the growth of rhizomes whose apical meristems had been experimentally removed, simulating the severing of meristems that occurs during the collection of plant material for transplantation.

This literature review is organized to address both general background and specific approaches used in this thesis. The first section of this chapter provides background on seagrass taxonomy and diversity, growth, and patch heterogeneity, with a focus on the endemic Hawaiian species *H. hawaiiana*. The section concludes with a description of seagrass ecology, including the vital ecological services seagrasses provide. The second section of this chapter focuses on the need for seagrass restoration efforts stemming from global declines in seagrass ecosystems. It describes and assesses restoration techniques that have been developed for accelerating the recovery of damaged seagrass habitats, along with potential challenges associated with restoration techniques, especially as they relate to *H. hawaiiana*. The information within this review is used to anticipate the most effective restoration methods for *H. hawaiiana* and to justify proposed methods for restoration in the field.

SEAGRASSES: MARINE ANGIOSPERMS

Taxonomy and Diversity

Although seagrasses comprise <0.02% of all flowering plants, they are remarkable in being the only group of angiosperms in the sea (Hemminga and Duarte, 2000). These unique angiosperms are found in nearly every temperate and tropical shallow coastal environment in the world and form beds off all continents except Antarctica (Green and Short, 2003). While some seagrass species such as *Zostera marina* and *Halophila decipiens* have a pandemic distribution, others such as *Halophila hawaiiana* and *Halophila johnsonii* are highly endemic with distributions limited to a few hundred square miles (Green and Short, 2003).

Although both seagrasses and algae grow fully submerged in marine environments, differences in evolutionary history, reproduction, structure, and nutrient transport distinguish seagrasses, which are vascular plants, from algae, which are protists. While algae evolved in the ocean and some are ancestors to land plants, the direct ancestors of seagrasses evolved in non-marine habitats (terrestrial or freshwater) and subsequently migrated back into the sea (Thorhaug, 1986). Seagrasses and algae can both reproduce asexually, but they differ in their reproductive organs: although many algae have specialized reproductive structures, they lack the flowers and seeds that characterize flowering plants. While algae have relatively simple morphologies and anchor to hard substratum with holdfasts, seagrasses are like terrestrial angiosperms in possessing true tissues that perform specific tasks, including roots that anchor the plants in place and extract nutrients from the sediment (Hemminga and Duarte, 2000). While all algal cells contain chloroplasts capable of producing chemical energy from sunlight, the location of chloroplasts in seagrasses confines photosynthesis primarily to the leaves. Finally, while both algae and seagrasses absorb minerals and other nutrients directly from the water column via diffusion, only seagrasses have roots and vascular systems that also allow the transport of minerals and nutrients in xylem and phloem (Hemminga and Duarte, 2000).

Approximately 50 seagrass species have been identified within 12 genera in the two plant families, Potamogetonaceae and Hydrocharitaceae. Three of the genera, *Halophila*, *Zostera*, and *Posidonia* comprise 55% of all seagrass species and are likely to have evolved from lineages that appeared relatively early in seagrass evolution, approximately 100 million years ago. *Halophila* is the most diverse and widely distributed seagrass genus, containing twelve species and growing in nearly every habitat capable of supporting seagrasses from the intertidal to depths of 60 m (den Hartog, 1970). There is no generally accepted number of species since different criteria are

used to classify seagrasses, including molecular phylogenetic techniques and analyses of morphological characteristics such as differences in the venation patterns of leaves and the structure of reproductive organs (Hemminga and Duarte, 2000). Since the degree of variation in the characteristics used for taxonomic purposes is poorly resolved, there is a great need to quantify morphological plasticity within seagrass species.

Since not all seagrass families are closely related, seagrasses form an ecological rather than a taxonomic group (Larkum *et al.,* 2006). They are defined by the ecological niche they inhabit and the adaptations that allow survival in this niche, including: (i) the ability to grow while completely submerged, (ii) the ability to survive in high, and often varying, salinities, (iii) an anchoring system to withstand water movement associated with wind-driven and tidal currents, (iv) a marine pollination mechanism, and (v) an ability to compete with other species in the marine environment (den Hartog, 1970). These adaptations have led to several morphological characteristics that are shared by most seagrass species, including flattened leaves and extensive root and rhizome systems (Green and Short, 2003). While most seagrasses have evolved long strap-like leaves, species in the genus *Halophila* have rounded, paddle-shaped leaves divided into distinct petioles supporting simple blades, or compound leaves consisting of a number of leaflets arranged around a common stalk (Lanyan, 1986).

Of Hawai'i's three seagrasses (*Halophila hawaiiana, Halophila decipiens*, and *Ruppia maritima*), only *H. hawaiiana*, in the family Hydrocharitaceae, is endemic to the Hawaiian Islands (Doty and Stone, 1966). Populations of *H. hawaiiana* have been found on the Main Hawaiian islands (O'ahu, Kaua'i, Moloka'i, and Maui) and the Northwestern Hawaiian islands (Midway Atoll and Pearl and Hermes Reef) (McDermid *et al.*, 2003) forming patches or large meadows in soft substrates at depths ranging from 0.5 m to 10 m in sheltered to semi-exposed

areas (Michael-Taxis, 1993). *H. hawaiiana* exhibits rhizomatous growth and produces erect pairs of leaves that protrude a few centimeters above the sediment. The leaf turnover rate of *H. hawaiiana* (*i.e.*, the time required for a leaf pair to form, senesce, and fall), calculated from growth data collected from growth-chamber and floating-cage experiments performed in the lagoon at the Hawai'i Institute of Marine Biology in Kaneohe Bay, was determined to be approximately 14.7 days (Herbert, 1986b). With pairs of smooth, rounded 1.8 - 6 mm wide leaves attached to the buried rhizome by petioles, *H. hawaiiana* has an appearance similar to its closest relatives, *Halophila minor* and *Halophila ovalis*. However, Doty and Stone (1966) first noted that *H. hawaiiana* is "significantly distinct" from *H. minor* and *H. ovalis* in the length and narrowness of its blades and the manner in which the basal part of the leaf tapers gradually into the petiole. In 1980, McMillan and Williams confirmed the separation of *H. hawaiiana* by demonstrating the occurrence of unique isozymes, or enzymes that differ in amino acid sequence but catalyze the same chemical reaction, based on a study of seven enzyme systems.

Vegetative Growth and Apical Dominance

Although highly diverse, all seagrasses consist of three distinct units that are replicated during clonal growth: (i) a piece of horizontal or vertical rhizome, (ii) a series of leaves attached to the rhizome, and (iii) a root system (Figure 1) (Marbá *et al*., 2004). The production of new plant tissue is entirely dependent on the activity of apical meristems, which are the only tissue regions where active cell division takes place throughout the entire lifespan of the plant (Tomlinson, 1974). Vegetative expansion originating from apical meristems is of particular importance to the persistence and expansion of seagrass species that rarely produce seeds, such as *H. hawaiiana*. Seed production is infrequent for *H. hawaiiana* because staminate and ovulate flowers occur on separate plants and male and female flowers have yet to be observed blooming

in the same meadow (Herbert, 1986a). Although the ovulate flowers persist for several days, the staminate flowers open only at night and are highly ephemeral, blooming for only six hours (Herbert, 1986a). As is the case for all marine species with water-dispersed gametes, successful sexual reproduction for *H. hawaiiana* depends on the synchrony of sexual activity and the presence of compatible mates (Levitan and Petersen, 1995). Therefore, in order for fertilization to occur, staminate and ovulate flowers must be produced simultaneously and in close enough proximity for the transfer of pollen. Since this rarely occurs, growth and expansion of *H. hawaiiana* is dependent on the presence of apical meristems that produce a new leaf pair and an unbranched root at each node, with a new node forming approximately every 4 days (Herbert, 1986b). Even in seagrass species that frequently produce seeds, the formation of meadows depends largely on the extension and branching of rhizomes from apical meristems. This condition has been termed "meristem dependence" by Tomlinson (1974) since continually active apical meristems are needed to maintain seagrass populations.

The apical meristem at the rhizome apex may divide to form branches, each possessing independent apical meristems. Although branch formation is a key component of space occupation by seagrasses (Hemminga and Duarte, 2000), it remains unclear what factors influence the frequency of branching. Lateral branch meristems of *H. hawaiiana* generally remain inactive, but occasionally become active and produce branches that assume the characteristics of the central rhizome. The exact mechanisms behind the activation of *H. hawaiiana* lateral branch meristems is unknown. However, seemingly as an afterthought in his 1986(b) publication outlining a functional growth model for *H. hawaiiana*, Herbert performed a short-term 14-day apical meristem excision experiment on 39 rhizomes collected from Kaneohe Bay. Although no statistical analyses were performed on the data and no other aspects of the

rhizomes' health or growth were reported, Herbert's results showed that the formation of lateral branches might be stimulated by the removal of the apical meristem.

As suggested by Herbert's experiment, meristematic activity in seagrasses may sometimes be controlled by a process called apical dominance, which involves a suppression of lateral branching in the proximity of an actively growing rhizome apex, or main meristem. Although apical dominance is widespread in terrestrial clonal plants, the process is poorly documented in seagrasses with most of the evidence consisting of observations of increased rates of branching following the removal of main apical meristems from rhizomes of *Cymodocea nodosa* (Terrados *et al*., 1997). Results of the same study also indicated that total plant production decreased with the removal of main apical meristems, perhaps reflecting the time needed for the seagrass to recover from the mechanical damage associated with cutting the meristem or the shunting of energy from normal growth to the formation of new main apical meristems. An experiment performed by Muñoz (1995) found that branching in *C. nodosa* increased following the manipulation of hormone levels, suggesting that the suppression of branching through apical dominance involves hormonal regulation. More specifically, apical dominance is thought to function similarly to that of angiosperms, through the release of hormones from the main apical meristems which travel through the rhizome and limit the formation of branches at other potential growth sites. Since the hormonal signal becomes diluted with distance from the main meristem, branching sites laying a further distance from the main meristem may normally be released from apical dominance. Also, the removal or destruction of the main apical meristem can stop the production of hormones and, therefore, release the branch suppression associated with apical dominance (Schwarzschild and Zieman, 2008).

Seagrass Growth Strategies

Since seagrass growth varies among species, Short and Short (2000) devised four seagrass categories that take into account a wide variety of growth strategies and patterns (Figure 2). The two main growth types include non-leaf-replacing seagrasses, which generate a fixed number of leaves in independent leaf clusters, and leaf-replacing seagrasses, which continuously produce and lose strap-like leaves within a leaf cluster. These growth types are further divided into two groups based on growth pattern: seagrasses with mono-meristematic (single) or di-meristematic (double) growth areas. Since the physiological integration between shoots is low within the mono-meristematic leaf-replacing seagrasses compared to the other three seagrass forms, the terminal shoots of species within this category form viable planting units that have a high potential for further growth (Cabaco *et al.,* 2005) while the remaining three seagrass growth forms require a minimum of three to four shoots and an apical meristem to form a complete planting unit (Perrow and Davy, 2008).

Like most other *Halophila* species, *H. hawaiiana* rhizomes follow a mono-meristematic non-leaf-replacing form and produce new leaf clusters and rhizome nodes from a single apical meristem area on the end of the rhizome. Shoots on the rhizome are fully formed as they emerge at the growing tip. As the fully-expanded shoot ages no new leaves are produced, and new rhizome sections and new shoots are produced simultaneously at the apical meristem.

In contrast, mono-meristematic leaf-replacing forms produce leaves continuously at a combined basal leaf and rhizome area and are found in the genera *Zostera, Phyllospadix, Heterozostera, Enhalus,* and *Posidonia* (Short and Duarte, 2001). Leaves are formed in the center of a leaf bundle and are held together by the protective sheaths of more mature leaves. These older leaves are shed and replaced by new leaves as the plant grows. Vegetative growth

occurs as the internode lengthens and the rhizome elongates, causing the shoot to migrate as it grows. Di-meristematic leaf-replacing forms produce both leaf and stem tissue at the basal leaf meristem area and are found within the genera *Amphibolis, Cymodocea, Thalassodendron, Syringodium,* and *Thalassia.* Sections of rhizome and new shoots are produced at separate rhizome meristem areas, and shoots form new leaves and shed old leaves by producing a vertical stem, elevating the leaf meristem above the rhizome. Finally, di-meristematic non-leaf-replacing forms, including the species *Halophila tricostata* and *Halophila spinulosa,* produce new leaf clusters and rhizome nodes at a combined meristem area on the end of the rhizome and produce new leaves at a separate leaf meristem area on a stem extending upwards out of the sediment. Although new shoots develop in the same way as they do for the mono-meristematic non-leaf-replacing seagrass forms (most *Halophila* species), additional leaves are continuously produced at the tip of the leaf cluster. Rather than being replacements for shed leaves, these new leaves are additions to the existing leaf cluster.

Seagrass Patch Spatial Heterogeneity

Many factors have been shown to affect the growth of seagrasses, such as light (Cabello-Pasini *et al.,* 2003, Lee *et al.,* 2007), water temperature (Lee *et al.,* 2007), sediment quality (Terrados *et al.,* 1999), and hydrodynamics (Fonseca *et al.* 1983). However, the way that these factors specifically influence the morphology or growth patterns within seagrass patches is poorly understood. Although within-patch variability has yet to be studied for *H. hawaiiana*, field observations suggest that colonizing rhizomes at the edges of patches tend to have longer internodes and more prominent apical meristems, but fewer branches compared to rhizome sections within patch interiors.

Differences in local conditions within patches create the potential for different growth patterns to form along a gradient of physical variables. For example, observed gradients in water velocity from the edge to the center of seagrass patches, with center regions tending to experience reduced currents (Fonseca *et al.* 1983; Worcester, 1995), could cause differences in morphology between these locations. Jensen and Bell (2001) found that rhizome morphological characteristics varied with spatial position within five *Halodule wrightii* patches in Tampa Bay, Florida, with longer internode lengths at the edges of the patches relative to patch centers. Also, a study performed on *Syringodium filiforme* in Florida Bay found that rhizome runners (which are the young, growing portion of a seagrass clone) had longer internode lengths and greater branching frequencies at the edges of meadows compared with the centers of meadows (Schwarzschild and Zieman, 2008).

Seagrass Ecology

As rooted ocean plants, seagrasses have four basic habitat requirements, beginning with a marine environment. Seagrasses generally thrive in salinities ranging from approximately 24 ppt to 35 ppt, although some species can tolerate salinity extremes from freshwater (*e.g.*, *Ruppia* spp.) to 50 ppt (*e.g.*, *Halodule wrightii*)(Hemminga and Duarte, 2000). Second, seagrasses require a suitable substrate in which to root. While some seagrasses such as *Phyllospadix* spp. have thick roots that can penetrate the crevices of rocks (den Hartog, 1970), most species grow over sandy or muddy sediment that is easily penetrated by roots. Areas of highly mobile fine sediment where currents and waves generate large sand ripples are generally unsuitable for supporting plant growth (Hemminga and Duarte, 2000). In addition to reducing the vegetative spreading of seagrass (Fonseca and Bell, 1998), high current speeds and wave exposure can cause both burial and erosion, which may result in seagrass mortality depending on

the size and frequency of these events. Third, seagrasses require immersion in water. Not all seagrasses can withstand exposure to the air, but some *Phyllospadix*, *Thalassia,* and *Halophila* species, including *H. hawaiiana,* can form large intertidal meadows and resist exposure to the air at low tide by laying their leaves flat on the sediment surface to retain water (Powell and Schaffner, 1991). Finally, seagrasses require sufficient light to sustain growth. The percentage of surface irradiance that must be received ranges from 4% to 29% (Dennison *et al.*, 1993), which is greater than the light requirements of macroalgae and microalgae (as little as 1% light). Although there are constraints on the colonization of marine environments by angiosperms, seagrass habitat on a global scale is extensive, allowing seagrasses to play a vital part in supporting biodiversity and nutrient cycling in the sea.

Seagrass meadows form valuable ecosystems that fulfill key roles in coastal zones worldwide (Duarte, 2002) and are among the most productive autotrophic communities on the planet, with an average production of 1,012 g $m^{-2}yr^{-1}$ dry weight (Duarte and Chiscano, 1999). Although seagrass meadows cover only 0.15% of the global oceans (Hemminga and Duarte, 2000) and contribute a mere 1% of net marine primary productivity, they are responsible for approximately 12% of the total carbon storage in the sea (Duarte and Cebrián, 1996). Due to the low decomposition rates of seagrass carbon, most of the biomass produced by seagrasses ends up as refractory detritus, which is organic matter that decomposes at a relatively slow rate, allowing seagrasses to act as sinks for atmospheric carbon (Duarte and Cebrián, 1996). The results of a recent global analysis of carbon stored in seagrass demonstrate that meadows store up to 83,000 metric tons of carbon per square kilometer while, in comparison, a typical terrestrial forest stores about 30,000 metric tons per square kilometer (Fourqurean *et al.*, 2012).

In addition to playing important roles in global carbon cycling, seagrasses enhance

biodiversity and habitat diversity in coastal waters. Seagrasses provide ample substrate for epibiota (Borowitzka *et al.*, 1990), and since seagrass meadows reduce current speeds, they may enhance recruitment rates of epiphytes by allowing propagules more time to settle on leaves (Michael-Taxis, 1993). By supporting epiphytic growth, seagrasses also support epiphyte-grazing invertebrates (Williams and Heck, 2001; Larkum *et al.*, 2006) and their larger predators such as fishes and crustaceans (Edgar and Shaw, 1995; Bologna and Heck, 1999). In addition to providing foraging grounds for mature organisms, seagrasses maintain high densities of fishes and invertebrates by providing nursery grounds and feeding areas for juvenile stages (Nagelkerken and van der Velde, 2004; Schaffmeister *et al.*, 2006; Nakamura and Tsuchiya, 2008). Seagrass patches have also been shown to support greater total abundances and diversity of infauna when compared to nearby areas of unvegetated sediment, perhaps because seagrasses alter the hydrodynamic environment in ways that increase organic matter and decrease sediment grain size, thereby providing food resources and shelter for infaunal species (Bostrom and Bonsdorff, 1997; Bostrom *et al.*, 2006).

Halophila hawaiiana supports a diverse community of organisms (Spielman, 2012), like other seagrasses of all sizes (Casares and Creed, 2008). The endemic, monophagous snail *Smaragdia bryanae* is a specialist on *H. hawaiiana* and relies entirely on the plant as both its source of food and habitat (Unabia, 2011) (Figure 3), and the plant is also consumed by the threatened green turtle *Chelonia mydas* (Arthur and Balazs, 2008). According to a study by Michael-Taxis (1993), the blades of *H. hawaiiana* support diverse assemblages of crustose coralline and filamentous red algae, diatoms and cyanobacteria, and it is assumed that these epiphyte communities are, in turn, important food sources for a wide range of grazers and predators, as they are in other areas (Larkum *et al.*, 2006).

Seagrasses act as ecosystem engineers by modifying their abiotic environment (van derHeide *et al.*, 2007), helping to improve water quality and stabilize sediment in the process. They reduce water flow velocities through their canopies, thereby creating a relatively still environment allowing particles to drop out of the water column (Rybicki *et al.*, 1997). Also, many seagrasses form a dense web of horizontal rhizomes that can effectively trap and hold large amounts of sediment (Dauby *et al.*, 1995). As a result of these features, both large seagrasses such as *Zostera marina* (Christiansen *et al.,* 1981) and diminutive seagrasses such as *Halophila decipiens* (Fonseca, 1989) can alter sediment dynamics by both increasing the accumulation of sediment and preventing the resuspension of particles. By stabilizing sediment in this manner under conditions of both high and low wave energy (Gacia *et al.*, 1999; Terrados and Duarte, 2000), seagrasses play a role in preventing coastal erosion and improving water clarity by reducing turbidity (Bos *et al.*, 2007).

SEAGRASS RESTORATION

Loss of Seagrass Habitat and Need for Restoration

Seagrass ecosystems are presently experiencing rapid worldwide declines, largely due to intensifying anthropogenic disturbances (Short and Wyllie-Echeverria, 1996, Duarte, 2002). Human activities such as dredging (Erftemeijer and Lewis, 2006), trawling (Gonzalez-Correa *et al.*, 2005), and boat anchoring (Milazzo *et al.*, 2004) cause mechanical damage to seagrass meadows, while coastal development disturbs seagrass meadows both through land reclamation and poor water quality associated with high turbidity and eutrophication (Short and Wyllie-Echeverria, 1996). According to a review of scientific literature from 1990-2006, invasive species can also negatively affect seagrass growth and density when introduced to meadows

through activities such as shipping and aquaculture (Williams, 2007).

The loss of seagrass cover often leads to undesirable ecological changes such as altered species composition and reduced abundance and diversity of fishes and crustaceans (Fonseca *et al.*, 1998; Orth *et al.*, 2006). Since seagrasses perform important functions such as stabilizing sediment in coastal areas, seagrass declines can also produce environmental changes such as increases in shoreline erosion and turbidity (Christiansen *et al.,* 1981). In addition, the loss of these functions may fuel the continuing disappearance of seagrasses through a series of feedback mechanisms (van derHeide *et al.*, 2007). For example, seagrasses are often light limited, and without seagrass canopies to prevent sediment resuspension, water clarity can be reduced, thereby making less light available for surviving seagrasses and causing further declines (Olesen, 1996).

Once seagrass cover is lost, natural recovery can take decades for species that lack traits that allow rapid recolonization of previously occupied areas, such as fast growth rates and frequent seed production (Bryars and Neverauskas, 2004; Gonzalez-Correa *et al.*, 2005). The widespread loss and slow recovery of seagrass meadows, coinciding with a growing awareness of the value of seagrass ecosystems, has sparked efforts to restore meadows in areas where they were damaged, or to create them in areas that appear suitable for growth to compensate for seagrass losses elsewhere (Hemminga and Duarte, 2000). Beginning in the 1960's, several transplantation techniques have been developed to accelerate the recovery of anthropogenically disturbed seagrass habitats by transplanting plant material collected from existing seagrass patches (Zarranz *et al.*, 2010). To ensure that the transplanted seagrass can expand vegetatively in addition to producing seeds, all planting methods require that the portions of rhizome that produce new shoots, or the apical meristems, be present in each individual planting unit (Fonseca

et al., 1998). This suggests that it is especially important to maximize the number of apical meristems present when transplanting seagrass species such as *H. hawaiiana* that rarely produce seeds and rely on vegetative growth as the primary form of reproduction.

Restoration Approaches

Seagrass transplantation methods can be grouped into three categories based on the type of plant material that is gathered: (i) shoots with surrounding sediment left intact, (ii) seeds, and (iii) shoots with bare roots (Davis and Short, 1997). Descriptions, benefits, and disadvantages of these transplantation methods are summarized in Table 1. Planting shoots with surrounding sediment left intact involves extracting plugs of sediment from natural seagrass patches and installing plugs in holes created at planting sites. The use of this method requires that sediment be sufficiently cohesive for plug collection, but it has been used extensively with many seagrass species with promising results (Fonseca *et. al*, 1998). The drawbacks of transplanting seagrass plugs include the creation of holes in natural donor patches and the high labor requirements associated with collecting and installing heavy planting units. The seed method involves collecting reproductive shoots from natural seagrass patches and storing the shoots in seawater until seeds are released, then sowing the seeds in areas where seagrass has been lost or damaged. Because of difficulty in harvesting seeds, NOAA's Guidelines for the Conservation and Restoration of Seagrasses in the United Sates and Adjacent Waters mentions that this method is feasible for only three species: *Zostera marina*, *Ruppia maritima*, and *Thalassia testudinum* (Fonseca *et. al*, 1998). Although seeding is promising for large-scale restoration in quiescent areas, there are important issues to consider when using the seed method, including hydrodynamic regime, seed predation, and low seedling viability (Harrison, 1991, Moore *et al.,* 1993). Planting shoots with bare roots involves removing sections of leaf-bearing rhizomes from

donor patches and replanting them at transplantation sites either singly or in clusters of multiple rhizomes, often securing plants in the sediment with metal or wooden staples. The bare root technique has been used successfully with both large species such as *Zostera marina* (Park and Lee, 2007) and diminutive species such as *Halophila johnsonii* (Nova Southeastern University, 2001). Major advantages of the bare root method include relatively minor impacts on donor beds and smaller volumes of material to transport (Fredette *et al.,* 1985), while disadvantages include root damage sustained during harvest and the potential loss of beneficial nutrients and microbes surrounding seagrass roots within donor patches.

Although there are unique challenges associated with each transplantation method, a challenge common to all planting methods is the process of site selection. Transplantation failures are often the result of poor site selection, including targeting areas where the original cause of seagrass decline has not been mitigated or where seagrasses did not historically occur (Fonseca *et al.*, 1998; Short *et al.*, 2002). One of the critical environmental factors influencing habitat suitability for seagrasses is water quality. For instance, the selection of sites with high levels of suspended sediment often results in low transplant survival due to insufficient light penetration through the water column (Zimmerman *et al.*, 1995). Also, high levels of nutrients such as dissolved phosphate and dissolved inorganic nitrogen can reduce light availability by stimulating algal growth (Stevenson *et al.*, 1993, Short and Burdick, 1995). Excessive water movement, shifting sediment, and bioturbation can result in transplant loss by erosion or burial (Meehan and West, 2002; Heidelbaugh *et al.*, 2000). The results of burial experiments performed on 15 species suggested that seagrasses can be vulnerable to both high and low burial levels, since all species studied experienced at least 50% shoot mortality (Cabaco *et al.* 2008). Small seagrasses without vertical rhizomes, such as *H. hawaiiana* may be particularly sensitive to

burial. A review by Cabaco *et al.* (2008) found that the small species *Halophila ovalis*, *Cymodocea rotundata*, and *Cymodocea serrulata* reached 50% mortality when buried under only 2 cm of sediments, while larger seagrass species with high plant shoot mass, long leaves, and vertical rhizomes tended to be more tolerant of burial. For example, *Posidonia* species with large leaves and wide vertical rhizomes showed high mortality only under burial levels exceeding 10 cm.

Finally, marine sediment with high levels of organic matter can be unsuitable for seagrass transplants since high inputs of organic matter stimulate bacterial activity and bring the anoxic layer closer to the sediment surface, encouraging the development of anaerobic bacterial communities. These bacteria have metabolic pathways that result in the accumulation of sulphide and other compounds that have toxic effects on plant growth (Hemminga, 1998). Since sediment with high concentrations of organic matter are likely to support high bacterial activity, the organic matter concentrations of sediment supporting seagrass is generally <6% of the dry weight (Hemminga and Duarte, 2000). However, similar inputs of organic matter and associated increases in sulphide concentrations have been experimentally shown to have different effects on the growth of different seagrass species (Terrados *et al.*, 1999), revealing that the negative effects of high organic matter concentrations on seagrass growth are complicated and poorly understood.

Measuring Transplantation Success

Despite many cases of success, according to a meta-analysis of seagrass rehabilitation projects in the United States, little more than 50% of efforts have met success criteria, defined as the unassisted persistence of a required acreage of seagrass coverage for a prescribed period of time (Fonseca *et al.*, 1998). The required acreage is determined by taking into account the ratio

of seagrass habitat restored and lost, as well as the policies of the agencies involved in restoration and the nature of the planting site (Fonseca *et al.*, 1998). For example, in the district court case of the United States of America versus Melvin A. Fisher (1997), it was determined that 1.55 acres of seagrass should be restored within the Florida Keys National Marine Sanctuary in order to compensate for losses of seagrasses (primarily *Thalassia testudium*) caused by boat propeller scars. Although the overall objective of the project was to restore *T. testudium* within scar sites, planting efforts were initially focused on planting a native fast-growing seagrass species, *Halodule wrightii*, to facilitate the eventual recovery of the slow-growing *T. testudium*. This approach was predicted to stabilize the sediment and create more suitable conditions for *T. testudinum* to naturally recolonize the propeller scars. Project success was quantified using three categories of performance standards: (i) the number of apical meristems, (ii) the survival rate, and (iii) the growth rate of the transplants (Fonseca *et al.,* 2000, Table 2). On the other hand, when transplanting *Posidonia australis* in Western Australia, short-term success was defined as 50% survival of transplant units and extension of 50% of the transplant units within 2 years (Campbell, 2002). Long-term success was determined by the persistence of transplant units for 5 years and coalescence of individual transplant units into patches.

Ultimately, success is achieved when transplanted seagrass beds are functionally equivalent to naturally occurring beds. While seagrass beds have many ecological functions, some of which may be more difficult to restore than others, no published study has suggested that any seagrass bed has been anything less than a highly productive habitat. Therefore, it can be inferred that if a desired acreage of seagrass is successfully reestablished through transplantation, the majority of its ecosystem functions will be restored as well. For example, it was found that transplanted *Halophila wrightii* and *Syringodium filiforme* beds in Tampa Bay

developed fish, shrimp, and crab densities indistinguishable from nearby natural seagrass habitats when the transplanted beds reached only one-third of the mean natural bed shoot density. Animal density had an asymptotic relationship with shoot density, implying that monitoring shoot density over time may be a useful and inexpensive parameter for determining transplantation success in terms of restored faunal communities (Fonseca *et al.,* 1996 a,b).

Although the relationship between the size of a seagrass bed and its functional attributes has not been thoroughly investigated, it has been demonstrated that even small patches can provide important habitats. For example, 1-2 m^2 patches were found to contain significantly greater numbers of fishes, shrimp, and crabs compared with abundances in adjacent bare sediment (Fonseca *et al.*, 1998), and in an experimental investigation of the effects of fragmentation in beds of the seagrass *Zostera marina*, it was found that even very small (0.01 m^2) seagrass patches have significant refuge value for juvenile blue crabs (Hovel and Lipcius, 2001). Also, patchy beds of *Halodule wrightii* and *Zostera marina* with 30-40 percent seagrass cover can support densities of penaeid shrimp nearly indistinguishable from beds with continuous 100 percent cover (Murphey and Fonseca, 1995). Studies such as these suggest that even small, isolated seagrass patches provide valuable habitat and enhance biodiversity. Since additional ecological services provided by seagrasses such as sediment stabilization and improved water quality require the plants to modify their environment, patch size will affect the functional attributes of both natural and restored patches (Perrow and Davy, 2002). For example, even small patches (diameter 1.8 m^2) have been shown to increase sediment accretion, thereby improving water clarity by reducing turbidity (Bos *et al.,* 2007). However, it is expected that more sediment would accumulate within larger seagrass patches since water must travel

some distance through seagrass canopies before hydrodynamic energy is reduced (Fonseca and Koehl, 2006). The effect of patch size on seagrass ecological functions is an important area of study requiring further investigation.

Recommended Management Actions for *Halophila hawaiiana*

H. hawaiiana is both a highly valuable and vulnerable Hawaiian coastal resource. Like all seagrasses, *H. hawaiiana* enhances biodiversity, improves water quality, reduces coastal erosion by stabilizing sediment, and plays a role in carbon and nutrient cycling. However, the limited ability of *H. hawaiiana* to produce seeds restricts its ability to recolonize areas where it has been displaced by invasive species or otherwise anthropogenically disturbed. The combination of its ecological value and the long timescale expected for natural recovery makes *H. hawaiiana* an excellent candidate for active restoration. Restoring *H. hawaiiana* habitat using harvested seeds is clearly infeasible, but transplanting shoots collected from donor beds holds promise. Since *H. hawaiiana* has a mono-meristematic non-leaf-replacing growth form, it requires planting units with a minimum of 3-4 shoots, either with surrounding sediment left intact or shoots with bare roots. Because less than 2,000 km^2 of *H. hawaiiana* remain and populations are declining (IUCN, 2012), the less destructive bare root technique would be preferable since no holes would be created in donor patches. However, when shoots are planted with surrounding sediment left intact, rhizomes and roots are less likely to suffer physiological damage and transplants have the benefit of added stability. Ideally, samples of *H. hawaiiana* could be collected from donor beds and raised in containers of sediment in a laboratory setting, both allowing the amount of planting material to expand so that less seagrass would need to be collected and allowing roots that were damaged during collection to reestablish in the sediment. If successful, this strategy would limit mechanical damage to donor beds without sacrificing the

stability of the new transplants. The diminutive size of *H. hawaiiana* makes it particularly vulnerable to erosion and burial, so measures should also be taken to stabilize sediment surrounding the transplants and to exclude potential bioturbators that forage near seagrass beds. Since evidence suggests that restored seagrass beds become functionally equivalent to natural beds over time, success of *H. hawaiiana* transplantation should be measured by the growth and persistence of planted patches.

Table 1. Summary of three categories of seagrass transplantation methods. Categories are based on the manner in which seagrass material is collected for transplantation.

Transplantation Method	Description	Benefits	Disadvantages
Use of shoots with surrounding sediments left intact	Involves extracting plugs of sediment from natural seagrass patches using any of a variety of devices such as PVC core tubes, sod pluggers, or shovels. Cores are then placed in holes made at the planting site.	i) Rhizome and roots system is left intact and is less likely to suffer physiological damage ii) Sediment immediately surrounding the roots may contain nutrients and microbes that are beneficial to the seagrass	i) Creation of holes in natural donor seagrass patches ii) High labor requirements
Use of seeds	Involves harvesting reproductive shoots from natural seagrass patches and storing the shoots in seawater until the mature seeds are released	After seeds have been collected, they can be quickly sown over large desired areas in a manner that mimics natural processes	i) Currents can transport seeds away from the areas in which they are sown ii) Impossible for species that produce seeds infrequently iii) Unpredictable germination time and low seedling viability a,b iv) Limits natural recruitment in species that produce seeds that do not disperse far from existing seagrass patches[c]
Use of shoots with bare roots	Involves removing sections of leaf-bearing rhizome from donor patches and replanting them at transplant sites. Rhizomes can be planted singly or in clumps of multiple rhizome sections.	i) Eliminates need to transport large quantities of sediemnt ii) Minimal disturbance to donor seagrass patches	i) Roots must reestablish at transplantation sites ii) Potential loss of beneficial nutrients and microbes surrounding seagras roots

a. Harrison, 1991, b. Moore *et al.,* 1993, c. Orth *et al.,* 1994

Table 2. Criteria for determining successful transplantation of *Halodule wrightii* in boat propeller scars within the Florida Keys National Marine Sanctuary (Fonseca *et al.,* 2000).

Performance Category	**Success Criteria**
Apical Meristems	a minimum of one apical meristem must be installed at planting
Survival	a minimum of 75% planting unit survival by the end of one year after installation
Growth	i) transplant shoot density must not be statistically different from that of nearby naturally-occuring patches ii) target acerage of bottom cover must be achieved within a 3-year monitoring period

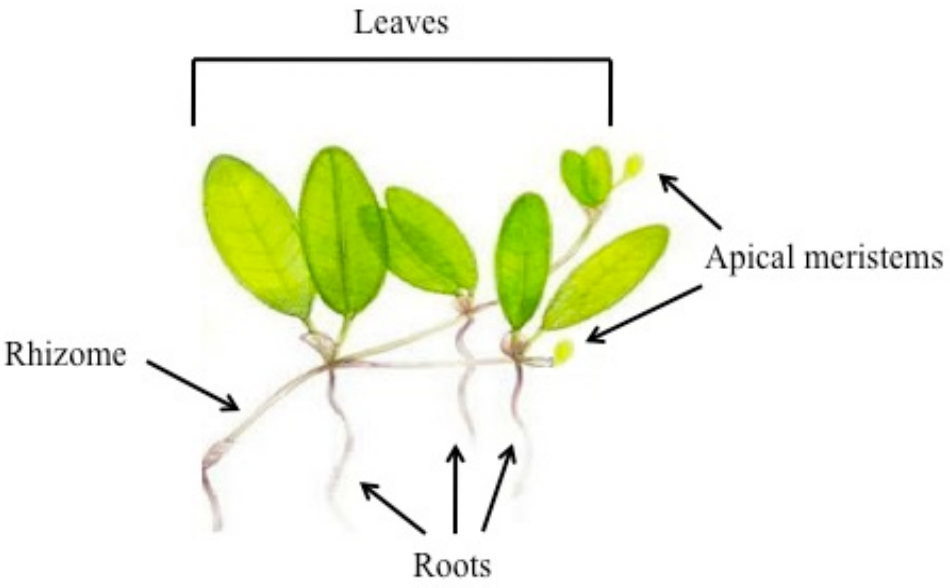

Figure 1. Schematic representation of the basic module of seagrass clones, containing a piece of rhizomes, a series of attached, and a root system. These three units are replicated during clonal growth. Pictured here is *Halophila decipiens*, which has small, rounded leaves characteristic of *Halophila* species.

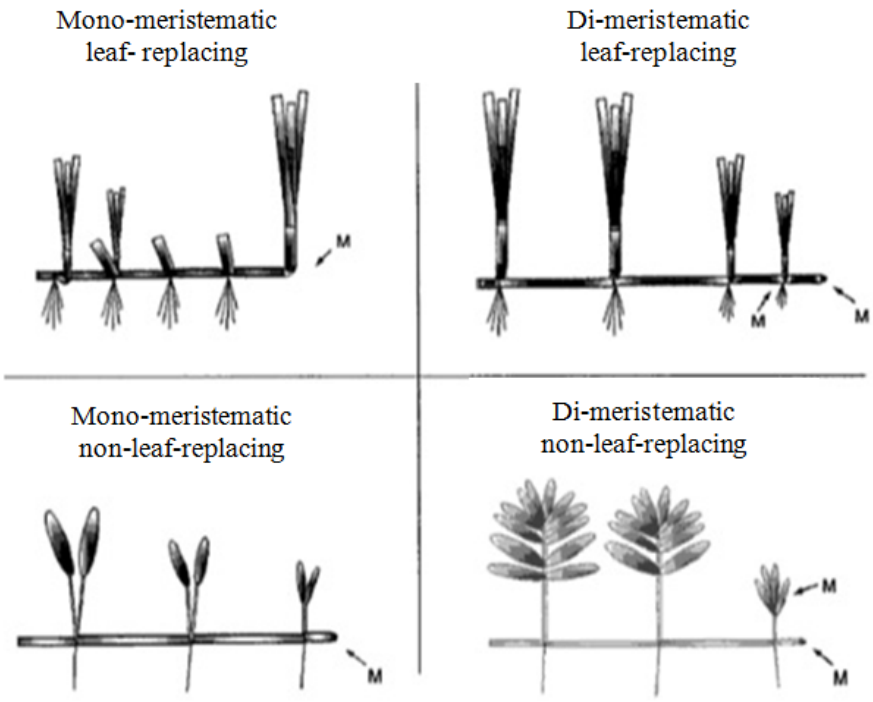

Figure 2. Rhizome fragments and shoots of four seagrass growth forms. The meristem areas of growth are indicated on each form with an M. Figure from Short and Short (2000).

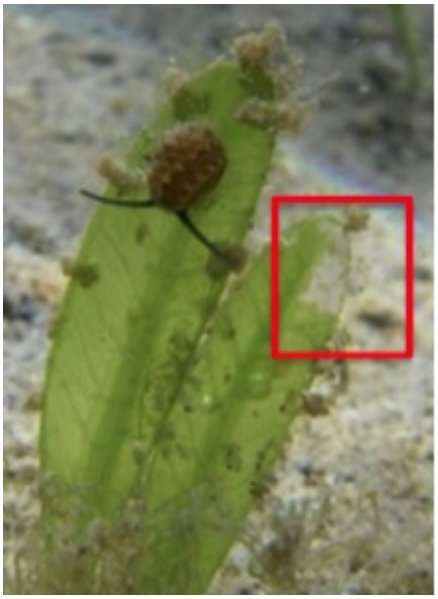

Figure 3. Endemic, monophagous marine snail *Smaragdia bryanae* grazing on a pair of *Halophila hawaiiana* leaves. The snail's distinctive grazing scar is boxed in red. Photo by F.T. Short.

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CHAPTER II: Experimental Restoration of *Halophila hawaiiana* in Maunalua Bay

INTRODUCTION

Maunalua Bay on the southeastern shore of O'ahu, Hawai'i has historically supported large meadows of the seagrass *Halophila hawaiiana,* as well as coral reefs and a wide variety of organisms that rely on these complex habitats. Recent changes, however, have dramatically altered the area. According to anecdotal knowledge and historic records, in the early 1940's and 1950's Maunalua Bay teemed with mullet, lobsters, squids, crabs, native algae, and sea turtles. However, large-scale development of the area beginning in the 1960's contributed to increased nutrients, pollutants, and terrigenous sediment from land runoff, resulting in the degradation of coastal coral reefs (Wolanski *et al.,* 2009). The bay ecosystem was further weakened during the New Year's Eve Flood of 1987 when inshore waters received a large input of muddy terrestrial sediment that buried the reef flat and reduced seagrass cover (Peyton, 2009). These conditions provided an ideal habitat for the rapid colonization of the invasive green alga *Avrainvillea amadelpha* or leather mudweed which, like other *Avrainvillea* species, has the ability to form immense monospecific mats that can displace other marine vegetation (Littler *et al.,* 2004). First discovered in Hawai'i in 1981 at Kahe Point on the western side of O'ahu (Brostoff, 1989), leather mudweed thrives in shallow habitats with sandy or muddy sediment. Once established in the Paiko area of Maunalua Bay (Figure 1), *A. avrainvillea* trapped and held the mud deposits by covering the substrate in thick mats, appearing to smother and displace seagrass meadows in the bay.

As is the case for many disturbed ecosystems, the degradation of the bay and, later, its restoration, provided important opportunities for study. From March 2010 to May 2011, *A. amadelpha* was removed from over twenty-four acres of the Paiko inshore area of Maunalua Bay

in an effort led by the community organization Malama Maunalua and The Nature Conservancy of Hawai'i as a first step towards the restoration of coral reef and seagrass habitats that previously existed in the area (Figure 2). The percent cover of *A. amadelpha* has been reduced from 57.9% to 2.6% (Minton and Conklin, 2012), and early studies suggest that trapped mud is being flushed from the bay (Macduff *et al.,* 2011.). As a result of these efforts, sandy areas that once supported the seagrass *H. hawaiiana* have been uncovered. However, since the plant's main form of propagation is vegetative growth and branching from the rhizome rather than seed production (Herbert, 1986), decades to centuries would likely be required for *H. hawaiiana* to spread from surviving patches and recolonize the bay. Since the absence of seagrass patches may prevent the recolonization of other organisms that rely on the seagrass, thereby impeding the return of the bay's rich natural communities, active restoration through the transplantation of *H. hawaiiana* could potentially accelerate the recovery of the Paiko area of Maunalua Bay. Reintroducing seagrass to cleared areas of the bay will require learning the most effective techniques for transplanting Hawai'i's endemic seagrass since little research has been conducted on *H. hawaiiana* and no successful transplantation methods have been developed for this species.

Even for seagrass species for which transplantation methods have previously been developed, studies have shown that the success of seagrass restoration projects can vary considerably. Despite efforts to select appropriate transplantation sites based on available knowledge, transplants often experience mortality as a result of inappropriate growing conditions. Three key site-specific variables that may influence seagrass growth and survival include sediment grain size, water motion, and bioturbation (Fonseca and Bell, 1998, Short *et al.,* 2002). Sediment grain size can influence the amount of porewater nutrients available to

seagrasses (Fourqurean *et al.,* 1992; Holmer *et al.,* 2001). Since finer-grained sediment adsorbs more phosphate than coarse-grained sediment, coarser sediment often maintains higher dissolved phosphate concentrations in the porewater (Erftemeijer and Middelburg, 1993). On the other hand, because of its cohesiveness, the finest-grained sediment (*i.e.* clay) is less prone to erosion and tends to shift only when current speeds are high (Boer, 2007). Wave exposure and strong currents can both uproot plants and disturb the sediment resulting in transplant burial and erosion, ultimately resulting in the loss of transplants (Paling *et al.,* 2003). Finally, at some sites, bioturbation has been shown to be one of the primary factors causing transplant failure (Davis and Short, 1997, Davis *et al.* 1998). Even when previously developed transplantation methods are used, the selection of transplantation sites with inadequate sediment grain sizes, levels of water motion, and bioturbation could result in a failed restoration effort.

In an attempt to determine the best technique for restoration, I implement two different methods for actively restoring *H. hawaiiana*: (i) using an aquaculture system to raise seagrass in biodegradable pots prior to transplantation and (ii) performing direct plug transplantation. I then evaluate the survival and growth rates of the transplanted seagrass, expressed as Mean Leaf Days, when provided with varying levels of sediment stabilization and protection from bioturbation.

METHODS

Study Location and Transplantation Site Selection

Maunalua Bay (157° 45' W, 21° 15' N) is located on the southeastern shore of O'ahu between Diamond Head and Koko Head (Figure 1). The shallow embayment is generally less than 2.5 m deep and is approximately 8 km long. The beach and nearshore waters are protected

from large waves by a fringing reef flat extending from around 0.3 to 1 km offshore. The substratum of the Paiko area of Maunalua Bay consists of soft sediment, sediment covered carbonate, and exposed carbonate, all of which form a thin layer over the underlying basalt (Peyton, 2009). Maunalua Bay receives discharges from nine small watersheds that are each approximately 10 km^2 in size. The area receives a mean annual rainfall of 52 cm, but during wet years, rainfall can surpass 100 cm (Wolanski, 2009). Salinity within the bay ranges from 28 to 34 ppt (Peyton, 2009).

Potential *H. hawaiiana* transplantation sites were selected in a step-wise process from locations free of invasive *A. amadelpha*: (i) at water depths within the depth range of existing seagrass patches in Maunalua Bay (<1 m to approximately 2.5 meters), and (ii) with appropriate sediment thickness (*i.e.,* the depth of unconsolidated sediment lying above the bedrock). The top edges of the 10.5 cm high biodegradable pots containing seagrass transplants were broken to allow the seagrass to expand into the surrounding sediment, therefore approximately 8.5 cm of sediment was needed to bury the 10.5 cm high biodegradable pots.

To determine the sediment thickness within naturally-occurring *H. hawaiiana* patches, measurements were taken haphazardly 43 times in places supporting seagrass throughout the Paiko area by inserting a marked chopstick into the sand until it contacted an impenetrable layer of bedrock. Sediment thickness ranged widely from 1.0 cm to 15.3 cm with an average thickness of 7.9 cm (SD=3.59) and a median thickness of 6.6 cm. Next, measurements of sediment thickness were taken throughout potential transplantation sites where either *A. amadelpha* had been manually removed or where *A. amadelpha* did not occur. Areas that were exposed to the air at extreme low tides were excluded to avoid unnecessary stress on the transplants. Locations with sediment thickness equal to or greater than the 8.5 cm required to bury the transplant pots

were marked with a global positioning system (GPS) unit and finer scale sediment thickness measurements were taken around these points to determine which areas were uniform enough in sediment thickness to support transplantation blocks measuring 2.5 x 3.5 m. Due to the close proximity of transplantation sites to natural seagrass patches within the sheltered bay, light intensity and salinity at the planting sites were assumed to be within the range of acceptable values for the growth of *H. hawaiiana*.

Twelve locations with adequate sediment thickness over a 2.5 x 3.5 m area were identified and divided into two categories: six sites on the western side of the Paiko study site were in an area that had been manually cleared of *A. amadelpha* while six sites on the eastern side of the study site were in areas of bare sand that had remained free of *A. amadelpha* without manual clearing. A total of eight locations were selected for transplantation (Figure 3), including four cleared and four uncleared locations, by randomly assigning numbers to the six potential locations in both site type categories and placing the numbers in random order. The first four sites within the two categories that were confirmed to have a sediment thickness of at least 8.5 cm extending over a 2.5 x 3.5 area were selected as transplantation sites and the eight selected sites were cleared of algae and rocky debris.

Prior to transplantation, relative water motion was measured at the eight chosen sites to ensure than no areas of unusually strong currents had been selected. To measure current energy, three ice cube shaped trapezoids made of water and plaster of Paris (DAP brand, UPC 7079810308), nicknamed clod cards (Doty, 1971), were deployed at each of the eight randomly selected sites. Since dissolution occurs at a faster rate in areas of stronger water motion, clods exposed to greater water flow lose mass at a greater rate than do those exposed to less rapid water flow. To make each ice cube tray of sixteen clod cards, 330 mL of water were mixed with

500 g of plaster according to the solid to liquid ratio described in Thompson and Glenn (1994). The plaster-filled tray was pounded on a countertop for two minutes to remove air bubbles and allowed to dry for twenty-four hours before being weighed. During deployment in the bay, three clods were zip-tied underwater to protective seagrass cages at each of the 8 sites for a twenty-four hour and fifty minute tidal cycle. The clods were then collected, allowed to air dry for five days to a constant weight, and re-weighed to determine the precise weight of plaster of Paris lost from each clod. Three control clods were calibrated for twenty-four hours and fifty minutes in a covered bucket filled with 10 gallons of still seawater to determine the weight of plaster of Paris lost in the same amount of time in the presence of no water flow. The calibrated value, or "diffusion factor," was calculated by dividing the mass loss of each of the field-deployed clods by the mean mass loss of the control clods. The weights of the field-deployed clod cards were used to calculate percent mass loss, which indicated no significant differences in water movement at the eight transplantation sites (Figure 4).

Laboratory-raised Seagrass Transplantation

The material to be transplanted in each experimental block was obtained from *H. hawaiiana* patches in the Paiko area of Maunalua Bay. Rhizomes were collected by hand and attached sediment was removed before placing the seagrass in Ziploc bags filled with seawater. The seagrass was then transported to the outdoor marine culture area at the Hawai'i Pacific University (HPU) Hawai'i Loa campus consisting of a shaded 110 gallon seawater holding tank and six 30 gallon tanks of seawater, which served as growing chambers for the seagrass before outplanting. A pump continuously circulated seawater collected from Maunalua Bay and Kaneohe Bay to the six seagrass growing chambers, and seawater returned from the six growing

chambers back to the 110 gallon holding tanks through individual PVC pipe drains. The water temperature and salinity were monitored several times weekly and were maintained between 25˚C to 27˚C and 34 to 36 ppt respectively.

Rhizomes were planted in commercially available biodegradable, compressed coconut coir fiber pots that were filled with beach sand collected from the shore of Maunalua Bay. The use of fiber pots was intended to stabilize the seagrass roots within the sediment, reduce the damage of roots and rhizomes during handling, and reduce the initial loss of transplants resulting from erosion after being outplanted in the bay. Each coconut fiber pot transplant unit was started with seagrass samples with between 16-18 total leaf pairs and 6-9 total apical meristems per pot. Seagrass units were raised in the 30-gallon circulating saltwater tanks for several weeks to allow for both additional rhizome and leaf growth and the reestablishment of roots that may have been damaged during collection. Approximately every five days, additional pieces of seagrass rhizome were added to pots with fewer than 10 remaining leaf pairs.

Each of the eight 2.5 x 3.5 m transplantation sites was considered an experimental block and was divided into six treatment plots, each measuring 0.5 x 0.5 m with a minimum of a 0.5 m buffer along each side (Figure 5). In the present study, six protective treatments were designed to test the effect of increased levels of protection on transplant growth rates and survival through reduced erosion, burial, and bioturbation near the transplanted seagrass. Treatments were as follows:

Treatment 1 Cage: a low mesh cage (1 x 1 cm mesh size) secured over transplants to limit bioturbation (Figure 6).

Treatment 2 Mesh: plastic mesh (1 x 1 cm hole size) placed over the transplantation plot and secured with bent pieces of 14 gauge galvanized steel wire to reduce sediment loss and limit the burial of the new transplants by shifting sand (Figure 7).

Treatment 3: Cage & Mesh: both caging and mesh secured over transplant plots to simultaneously limit bioturbation and stabilize the sediment (Figure 8).

Treatment 4: Cage Control: a cage control consisting of a PVC pipe frame and cage top with no sides to examine if reduced light (rather than altered water flow) effects the growth of transplants (Figure 9).

Treatment 5 No Equipment: an unprotected control with neither caging nor mesh to evaluate the growth of transplants with no equipment added. If transplantation was equally or more successful without the addition of protective equipment, both time and money could be saved by excluding caging and mesh from future transplantation efforts.

Treatment 6: No Seagrass: a bare sediment control plot serving as a reference to indicate what organisms colonize in the absence of seagrass.

To select an appropriate mesh size for stabilizing the sediment around the transplants and limiting the burial of the new transplants by shifting sand (used in treatments 2 and 3), a variety of meshes with hole sizes ranging from 1 mm to 3 cm were tested near potential transplantation sites by securing pieces of mesh to the sediment and manually creating strong currents. Mesh with hole sizes of approximately 1 x 1 cm were observed to be the most effective at holding the sediment in place. In treatments 2 and 3, a 15 x 15 cm space was cut from the center of each of the mesh squares to allow for the seagrass transplant units and could be enlarged as necessary to accommodate seagrass growth (Figure 7). The same mesh size was also used to construct the cages in treatments 1 and 3, which were designed to both exclude larger herbivores and limit

bioturbation by excluding fishes that forage for invertebrates in the sediment near seagrass, since mesh with a hole size of 1 x 1 cm was large enough to minimize alteration of light but small enough to exclude most fishes. Cages were cleaned at least once per week to limit fouling and ensure water flow through the cages. Treatments were randomly placed in relation to each other within each block, resulting in different combinations of treatments next to one another.

During outplanting in November and December, the pots of sediment and seagrass were transported from the lab to the Paiko area of Maunalua Bay in individual bags of seawater placed in large coolers. At each of the eight experimental blocks, individual sediment samples were collected from the centers of the six treatment plots using a PVC corer (10 cm length, 10 cm inside diameter). The holes created by the corer were enlarged with a garden trowel to accommodate the potted seagrass transplants.

Seagrass Plug Transplantation

Following poor results with the laboratory-raised seagrass transplants, a second approach using plugs of seagrass transplanted directly from seagrass beds was also investigated. A PVC hand corer (10 cm length, 10 cm inside diameter) was used to extract entire sediment and seagrass plugs from donor beds by driving the hand corer into the sediment and capping the top so that, when the corer was pulled from the sediment, the plug of seagrass was trapped inside. A stiff sheet of plastic was slipped beneath the corer to prevent the loss of the seagrass and sediment and plugs within the corer were transported and placed directly into holes created at the transplantation sites.

The plug transplantation method was previously avoided since it creates holes in donor beds and is therefore more destructive than raising seagrass planting material in the lab. Instead,

small samples of seagrass were collected and raised in biodegradable pots of sediment in the laboratory in order to minimize disturbance to donor beds. The use of fiber pots was intended to stabilize the seagrass roots, prevent damage to roots and rhizomes during handling, and reduce the likelihood of erosion after transplants were installed in the bay. However, the plug method is far less costly and time-consuming than raising seagrass in the lab prior to transplantation since the need to purchase pots and maintain a growing tank is eliminated, as is the time and labor required to transport pots of seagrass and sediment in bags of seawater from the lab to the planting sites. In addition to reducing costs and labor, previous studies performed on other small seagrass species such as *Halophila johnsonii* suggest that this 'direct' method may be effective (Heidelbaugh *et al.,* 2000, Fonseca *et al.,* 1998). Seagrass was replanted using the plug method at Site #1, #2, and #8.

Sediment Characteristics of Transplantation Sites

The methods used to characterize the sediment at the eight transplantation sites were the same as those used in 2011 to document the range of sediment grain sizes and organic content found in natural *H. hawaiiana* patches throughout the bay (Spielman, 2012). Sediment grain size was analyzed by first drying the sediment to a constant weight in a drying oven for two days at 100ºC and separating the dry sediment through 4, 0.5, 0.3, 0.125, and 0.063 mm sieves shaken in a mechanical sieve shaker for ten minutes. The sediment retained on each sieve was weighed, and size classes were converted into phi values based on a modified Udden-Wentworth grain-size scale (Udden, 1914). The program GRADISTAT was used to calculate the mean phi values and gravel/sand/mud distributions at each of the 6 treatment sites at the 8 transplant locations (Blott and Pye, 2001). Sediment size classes were then recombined and subsamples of 2.0-2.5 g of sediment were combusted at 550ºC for 6 hours per the methods recommended by Luczak *et*

al., 1997. The weight loss on ignition (LOI) was used to calculate both the percent of organic matter in each sample and the mean percent of organic matter at each of the eight transplant locations using the following equation: [(Initial mass of dry sediment) – (Mass of combusted sediment)] / (Initial mass of dry sediment) x 100.

Monitoring and Statistical Analysis

Leaf pair counts were conducted on planting units an average of every five days. The potted, laboratory-raised transplants were monitored from November 24th through December 27th, 2011 while the plug transplants were monitored from January 2nd through January 31st. Since *H. hawaiiana* sheds leaf pairs when the leaves are approximately 12 days old, all leaf pairs observed later during monitoring represented new growth. To that this into account and quantify both the survival and growth of the transplants, Mean Leaf Days were calculated for each planting unit. According to the following equation, Mean Leaf Days simultaneously describes the number of days planting units survived in the bay and the number of leaf pairs present at each time the planting units were monitored, giving the greatest weight to leaf pairs present the longest time after transplantation:

$$\frac{\text{Sum of (\# of days since transplantation) (Number of leaf pairs observed)}}{\text{Grand sum of leaf pairs observed throughout entire time planting unit was in the bay}}$$

For example, a planting unit that initially had 10 leaf pairs and lost all leaf pairs 2 days after transplantation would have a Mean Leaf Day of one: [(1 x 10) + (2 x 0)] / (10 + 0) = 10/10 = 1
A planting unit that initially had 10 leaf pairs, had 7 leaf pairs 2 days after transplantation, had 3 leaf pairs 5 days after transplantation, and lost all leaf pairs 8 days after transplantation would

have 1.95 mean leaf days: [(1 x 10) + (2 x 7) + (5 x 3) + (8 x 0)] / (10 + 7 + 3) = 39/20 = 1.95. The number of Mean Leaf Days increases if a planting unit has either survived (maintained a minimum of one living leaf pair) over an extended period of time or has a high number of leaf pairs for even a short period of time. However, a planting unit achieves the greatest number of Mean Leaf Days by surviving for an extended period of time while continuously replacing and producing additional leaf pairs.

To detect possible interactions between transplantation method (potted, laboratory-raised versus plug transplants) and protective treatment (Cage, Mesh, Cage & Mesh, Cage Control, No Equipment), a two-way ANOVA was performed with method and treatment fixed and mean leaf days as the dependent variable (Zar, 1999). Since transplants disappeared the day following installation at Site #5 using the potted method and from Site #2 using the plug method, strongly suggesting that an unusual event had occurred and interfered with the experiment, Mean Leaf Days data from those trials were excluded from further analyses.

RESULTS

Weather Conditions at Study Location

In the present study, pots of laboratory-raised seagrass were installed at transplantation sites and monitored from November 24th to December 27th, 2011 while plugs of seagrass and sediment collected directly from donor patches were installed at transplantation sites and monitored from January 2nd to January 31st, 2012. Although mean air temperatures remained relatively stable while seagrass transplanted using both methods was being monitored, wind speeds and precipitation varied dramatically as a result of winter storms in December. The period when laboratory-raised transplants were in the bay was characterized by greater precipitation,

stronger winds, and slightly warmer air temperatures than the period when plug transplants were in the bay (Table 1). While the potted laboratory-raised transplants were in the bay, November 26th through December 27th 2011, precipitation averaged 0.06 cm per day (SD=0.2) with a total of 2.2 cm over the thirty-five day period, which is low compared with an average rainfall during November and December of approximately 10 cm. Although the majority of days received less than 0.05 cm of rain, rainstorms on December 9th, 10th, and 13th accounted for 1.55 cm of precipitation, equal to 70% of the precipitation over the entire potted transplant monitoring period. Winds ranged from 0 to 77 km/h with an average of 18 km/h (SD=7), which was slightly higher than the average wind speed of 16 km/h during November and December, and mean temperature ranged from 19˚C to 29˚C with an average of 25˚C (SD=1). However, during the monitoring period for the plug transplants, January 2nd through January 31st, 2012, precipitation averaged 0.03 cm per day (SD=0.1) with a total of only 0.8 cm over the twenty-nine day period. Winds ranged from 0 to 53 km/h with an average of 12 km/h (SD=5), which was slightly lower than the average wind speed of 13 km/h during January, and mean temperature ranged from 16˚C (2˚C colder than the yearly average) to 28˚C with an average of 23˚C (SD=1)(Weather Underground, Inc.).

Survival of Laboratory-raised and Plug Transplants

Since there was no interaction between transplantation method (potted, plug) and treatment (Cage, Mesh, Cage & Mesh, Cage Control, No Equipment) (ANOVA, p=0.879), the effect of treatment on Mean Leaf Days could be generalized to planting units installed using both methods (Table 2). Experimental transplantation of *H. hawaiiana* in Maunalua Bay was unsuccessful using both potted, laboratory-raised transplants and plug transplants within all

protective treatments, with no planting units surviving longer than 28 days (Figure 10). As a result only 3 out of the 55 planting units had Mean Leaf Days of 10 or greater. However, transplants protected by the Mesh treatment had significantly greater Mean Leaf Days than did transplants protected by the Cage & Mesh treatment (Tukey test, $p<0.05$) and transplants within the Cage Control treatment (Tukey test, $p<0.05$). No significant difference in Mean Leaf Days was detected between the potted and plug methods (ANOVA, $p=0.469$). However, since transplants disappeared the day following transplantation at Site #5 using the potted method and from Site #2 using the plug method, these locations were excluded from the two-way ANOVA, leaving seven sets of potted transplantation treatments to be compared with only two sets of plug transplants. The two most successful of the fifty-five planting units monitored in the present study were both protected by mesh and both survived approximately 25 days. One was installed using the potted method and the other was installed using the plug method. Although both transplants remained in the bay approximately the same number of days, the potted transplant gradually lost leaf pairs over time while the plug transplant quickly grew, expanding from an initial 17 leaf pairs to a maximum of 27 leaf pairs, then disappeared suddenly. Since it continuously produced new leaf pairs, the plug transplant had 12.5 Mean Leaf Days while the potted transplant had only 10 Mean Leaf Days.

With the exception of planting units at Site #8, which had the thinnest layer of sediment, transplants within cage treatments were buried by approximately 10-15 cm of sand, which appeared to be trapped and held by the mesh cages themselves. Also, across all protective treatments, 17 of the 40 pots containing laboratory-raised seagrass were found only half-filled with sediment and contained no remaining leaf pairs, appearing as if the initial sediment content had been swept from the pots by water movement. Mesh secured to the sea floor did not

stabilize the sand within the pots since a hole was cut from the center of the mesh to accommodate the transplants. The transplants that were not lost through burial or erosion slowly lost leaves over the course of approximately three weeks and were eventually observed as bare rhizomes with no remaining leaf pairs.

Sediment Characteristics of Transplantation Sites

Sediment at all eight transplantation sites had mean phi values lying within the sand category as defined by Blott and Pye (2001) and contained little mud or gravel. Mean phi values ranged from 0.6 (coarse sand) at Site #8 to 1.4 (medium sand) at Site #2 with a grand mean phi of 1.3 (SD=0.4) across the eight sites (Figure 11). The mean percent organic matter ranged from 3.7% (SD=0.4) at Site #6 to 5.1% (SD=0.3) at Site #8 (Figure 12) with an average of 4.3% organic matter (SD=0.5) across the eight sites. There was no significant difference in mean percent organic matter between the sites where *A. amadelpha* did not occur (Sites #1-4) and the sites where *A. amadelpha* was manually removed (Sites #5-8). Mean phi values (ANOVA, $p=0.588$) and mean percent organic matter (ANOVA, $p=0.716$) did not differ from mean values observed in sediment cores collected within naturally occurring seagrass patches in Maunalua Bay, which had mean phi values lying within the sand category with phi values ranging from 1.0-1.9 with a grand mean of 1.4 (SD=0.4) and mean percent organic material ranging from 3.7-5.4% with a grand mean of 4.4% organic matter (SD=0.9) across three study sites (Spielman, 2012). Within the Cage & Mesh and Cage only treatments, mean phi values were negatively correlated with Mean Leaf Days and mean percent organic matter was positively correlated with Mean Leaf Days, revealing that transplants surrounded by caging were more successful in areas with slightly smaller grain sizes and higher organic matter.

DISCUSSION

The goal of the present study was to test the relative effectiveness of *H. hawaiiana* transplantation methods using both laboratory-raised seagrass and seagrass plugs protected by combinations of caging and mesh. Although both methods were unsuccessful, their development and implementation have provided several important insights with implications for the restoration of *H. hawaiiana*. Transplants protected by mesh survived longer than transplants surrounded by both cage and mesh and transplants within the cage control, suggesting that adding mesh to the sea floor could potentially enhance transplantation success. However, the short survival time of all transplants, even those protected by mesh, shows that additional data should be collected prior to future attempts at *H. hawaiiana* transplantation. A description of possible reasons for the rapid disappearance of the transplants is provided and recommendations are made for ways that transplantation might be improved.

Two observations suggest that large sand movements resulting from high wind speeds during the time the transplants were in the bay (Table 1) may have been a cause of mortality for the transplants. First, in addition to reducing light availability, excessive burial such as that observed within cage treatments can cause shoot death by killing the apical meristems required for continued growth (Duarte *et al.,* 2005). Burial can also harm seagrasses by decreasing the area available for performing photosynthesis, forcing plants to rely heavily on carbon reserves stored in belowground tissues. Seagrasses that are carbon limited in this way may suppress the production of new roots and experience reduced growth rates (Alcoverro *et al.,* 1999). The fact that transplants surrounded by the cage control or no equipment tended to be buried by only a thin (approximately 5 cm) layer of sand and transplants protected only by mesh experienced no burial might explain why, in some cases, the transplants protected by only mesh survived longer

than transplants protected by the other four treatments. It is possible that transplants surrounded by caging were more successful in areas with slightly smaller grain sizes because finer sediment could remain suspended in the water and therefore pass more easily through the cages rather than becoming trapped and burying the transplants. Second, poor survival of the laboratory-raised seagrass transplants may have resulted from the removal of sand from the fiber pots by excessive water movement, as suggested by the observation of 17 of the 40 pots only half filled with sediment.

Since the vegetative growth and proliferation of *H. hawaiiana* is dependent on the presence and continued health of active apical meristems, it is also possible that insufficient numbers of meristems were present in each of the planting units. Without sufficient numbers of active apical meristems, *H. hawaiiana* transplants would cease to produce new leaves and would die following the senescence of their initial leaf pairs. According to Herbert (1986), the leaf turnover rate of *H. hawaiiana* (*i.e.,* the time required for a leaf pair to form, senesce, and fall) is approximately 14.7 days, so this possible explanation for the death of the transplants is consistent with the short survival time of the majority of the transplants. Observations of the transplants were also consistent with this hypothesis, since many of the transplants, especially those protected only by mesh, died slowly over the course of approximately three weeks as old leaves were lost and an insufficient number of new leaves were produced. On the other hand, the rapid mortality of the plug transplants may reflect the inability of *H. hawaiiana* to resume growth following mechanical damage during collection from donor beds.

Observations of *H. hawaiiana* transplants installed using a range of techniques have revealed several possible factors that may have been responsible for death of the planting units and suggest how these factors might be avoided in the future. First, the present study suggests

that caging transplants to discourage bioturbation is unwise since sediment can easily enter cages as a result of water motion and remain trapped inside, even in relatively sheltered areas such as the Paiko area of Maunalua Bay. Also, cages are both time-consuming to construct and maintain, averaging approximately 1.5 hours each to assemble and requiring weekly scrubbing to remove fouling organisms. Second, as concluded in a previous study by van Keulen *et al.*, 2003, mesh attached to the sea floor may help to stabilize sediment, thereby minimizing the loss of transplants from burial and erosion. For example, of the five treatments that were installed on November 27th, 2011 when winds reached high speeds of 61 km/h, the Mesh treatment survived 25 days while no other treatment survived more than 12 days. Third, if transplanting either laboratory-raised seagrass or plugs of seagrass within biodegradable pots, it is important to thoroughly break off all exposed edges of the pots after installing them in the sediment. Fourth, although the seasonal growth patterns of *H. hawaiiana* are yet unknown, it would be ideal to install transplants during a time of calm ocean conditions. Finally, if at all possible, it is best to transplant seagrass in the exact location where it previously thrived. If no seagrass was historically found in a location that appears to be suitable, it is possible that the area is unsuitable for supporting seagrass for a reason that is not readily apparent. Although the historic presence of large fields of *H. hawaiiana* throughout Maunalua Bay is well established, no maps indicating the specific location of seagrass beds have been published. Following the removal of *A. amadelpha*, however, The Nature Conservancy of Hawai'i began recording the exact location of surviving seagrass patches in the bay. Since areas currently supporting *H. hawaiiana* are clearly suitable for seagrass growth, these data will be invaluable for restoration efforts in the future should the remaining seagrass patches in the bay be lost due to anthropogenic activities.

Developing successful restoration strategies will also require better knowledge of how *H. hawaiiana* grows and expands, as well as how the seagrass may respond to mechanical damage that occurs during transplantation from donor beds. For example, it is still unknown if *H. hawaiiana* can survive following mechanical damage from a corer such as that which occurs during plug transplantation or if *H. hawaiiana* rhizomes possess the ability to resume growth if the main apical meristem or branching meristems are severed from the rhizome. Also, it may be possible that seagrass material collected from specific areas of donor patches may have a higher potential for survival and further growth after transplantation. These critical but unanswered questions will be investigated in Chapter 3. The implications of these findings will provide basic but important insight into *H. hawaiana* growth patterns and will be used to generate recommendations for future *H. hawaiiana* transplantation efforts in Chapter 4.

Table 1. Weather conditions while potted and plug transplants were in Maunalua Bay.

	Dates in Bay	Mean Precipitation (cm)	Total Precipitation (cm)	Average Wind Speed (km/h)	Max. Wind Speed (km/h)	Mean Air Temperature (°C)
Pottted Transplants	November 24-December 27, 2011	0.06 +/-0.2	2.2	18+/-7	77	25 +/-1
Plug Transplants	January 2-January 31, 2012	0.03 +/0.1	0.8	12+/-5	53	23 +/-1

Table 2. Results of two-way ANOVA testing for differences in Mean Leaf Days of potted and plug transplants surrounded by five protective treatments.

Source of Variation	Sum of Squares	df	Mean Square	F	P-Value
Dependent Variable: Mean Leaf Days					
Corrected Model	111	9	12	1.7	0.12
TREATMENT	85	4	21	3	0.03
METHOD	4	1	4	0.5	0.47
TREATMENT*METHOD	8	4	2	0.3	0.88
Error	251	35	7		
Corrected Total	362	44			

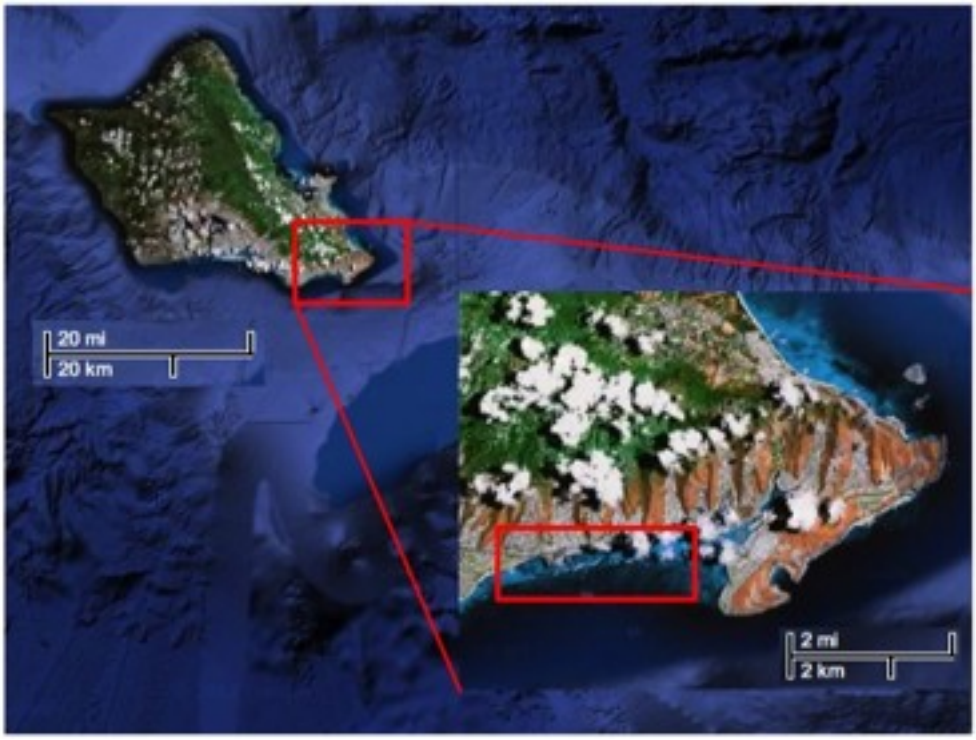

Figure 1. Location of the Paiko area of Maunalua Bay, O'ahu.

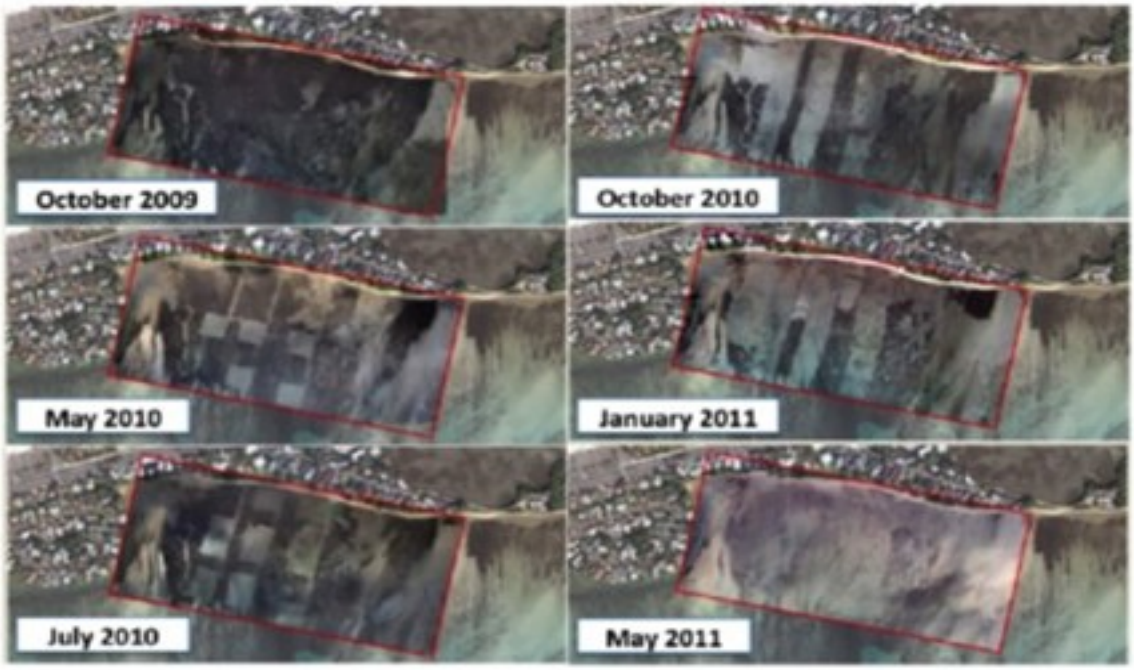

Figure 2. Aerial views of the Paiko area of Maunalua Bay from October 2009-May 2011. Dark areas of the bay indicate the presence of the invasive alga *Avrainvillea amadelpha* and trapped mud.

Figure 3. Eight seagrass transplantation sites in Maunalua Bay. Sites #1-4 are areas where invasive *Avrainvillea amadelpha* never invaded and Sites #5-8 are areas that were cleared of *A. amadelpha*.

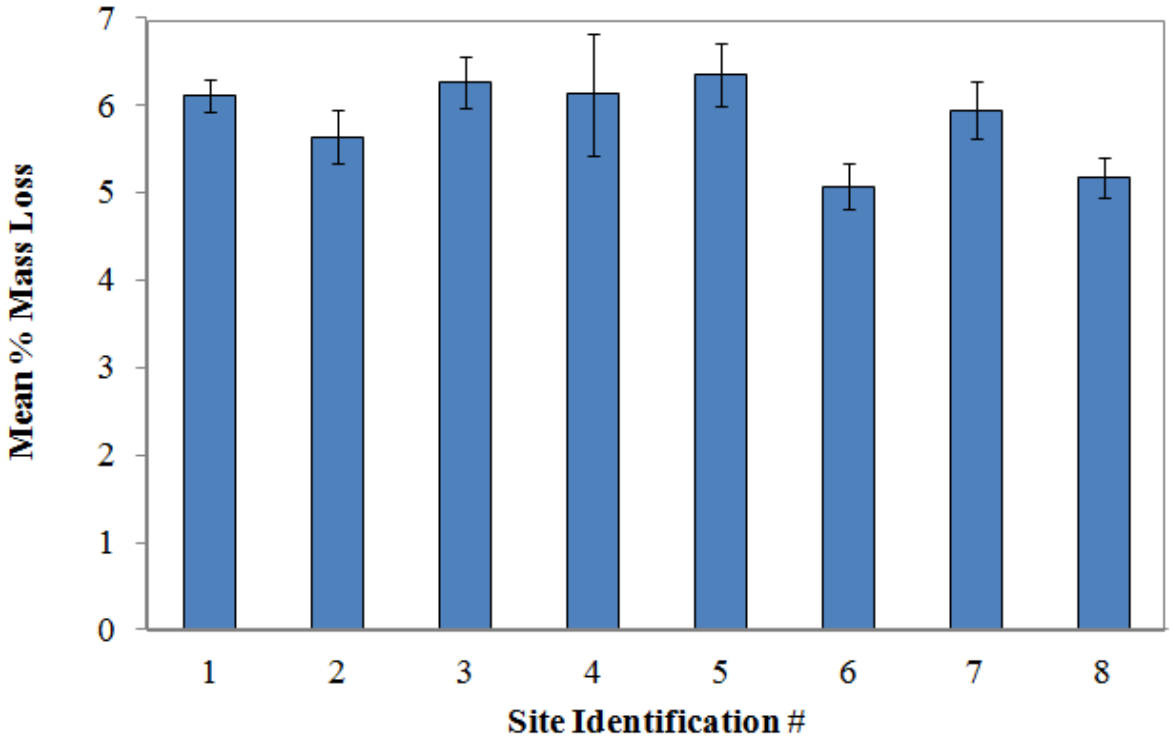

Figure 4. Mean percent mass loss of three clod cards deployed at each of eight transplantation sites over a twenty-four hour 50 minute tidal cycle. Error bars show +/- one standard deviation. Since clod card dissolution is positively correlated with the amount of water movement, a higher percent mass loss indicated a higher relative rate of water movement.

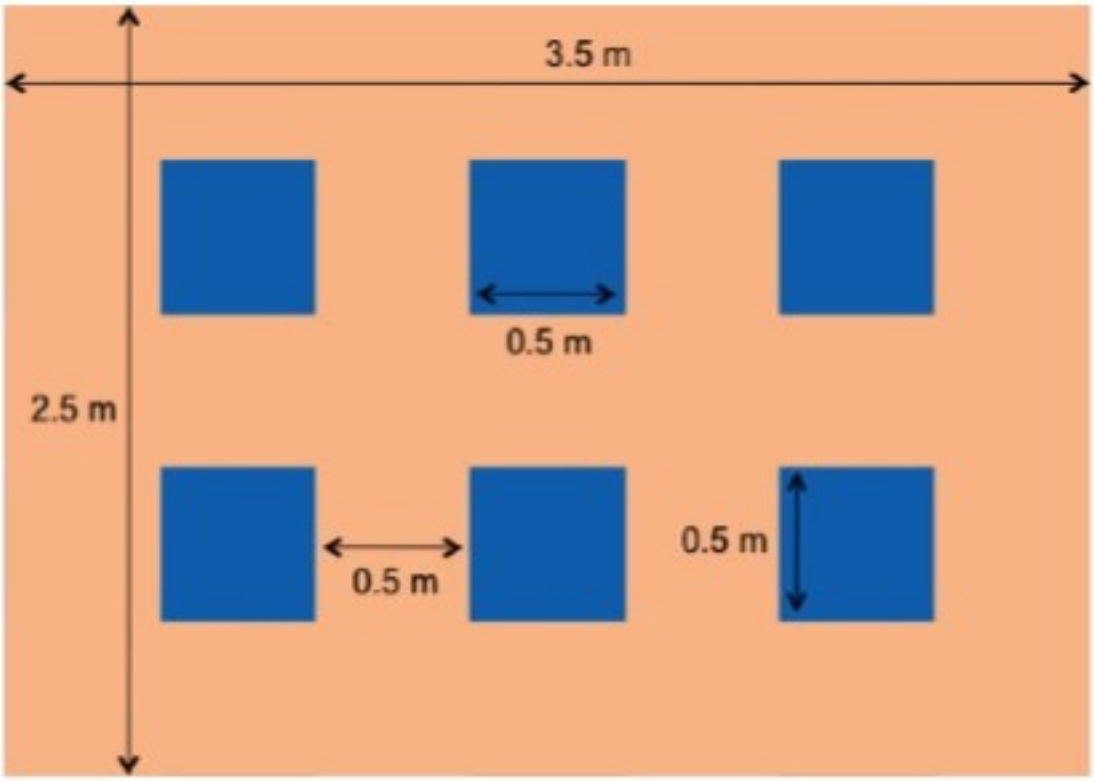

Figure 5. The 2.5 x 3.5 m experimental blocks were divided into six 0.5 x 0.5 m treatment plots with a minimum 0.5 m buffer along each side. Treatments were randomly assigned to the six plots.

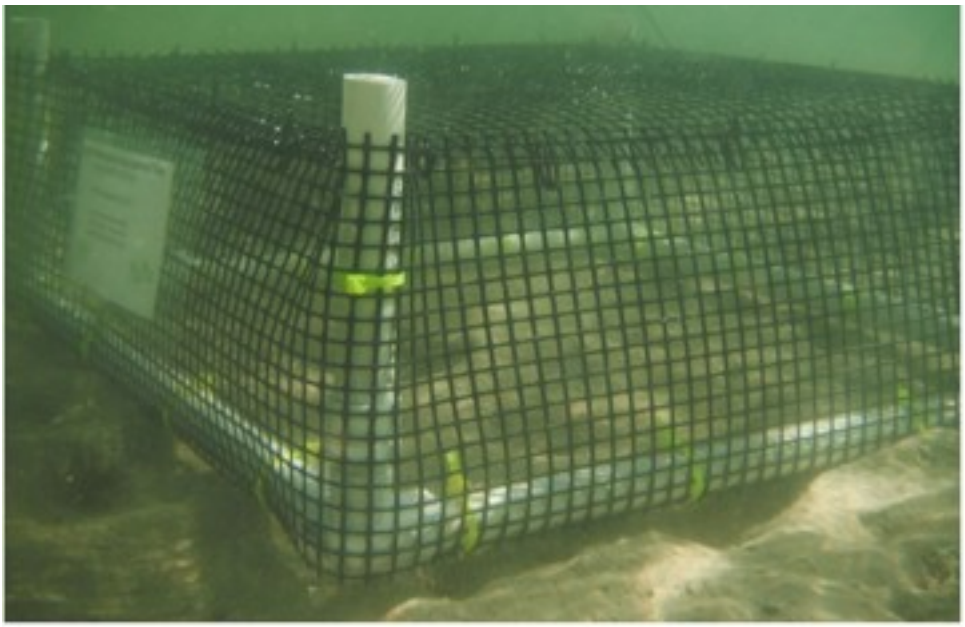

Figure 6. Transplant treatment #1 involved surrounding transplants with a low cage.

Figure 7. Transplant treatment #2 involved placing plastic mesh over the plot, secured with bent pieces of 14 gauge galvanized steel wire.

Figure 8. Transplant treatment #3 involved both caging and adding mesh to the area surrounding the transplants.

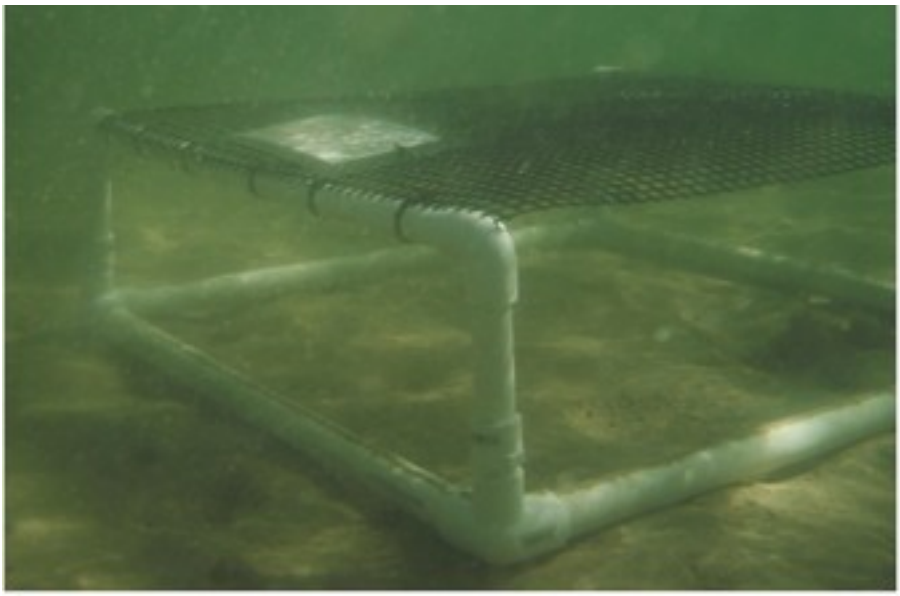

Figure 9. Transplant treatment #5 (cage control for light) was a PVC pipe frame and cage top with no sides.

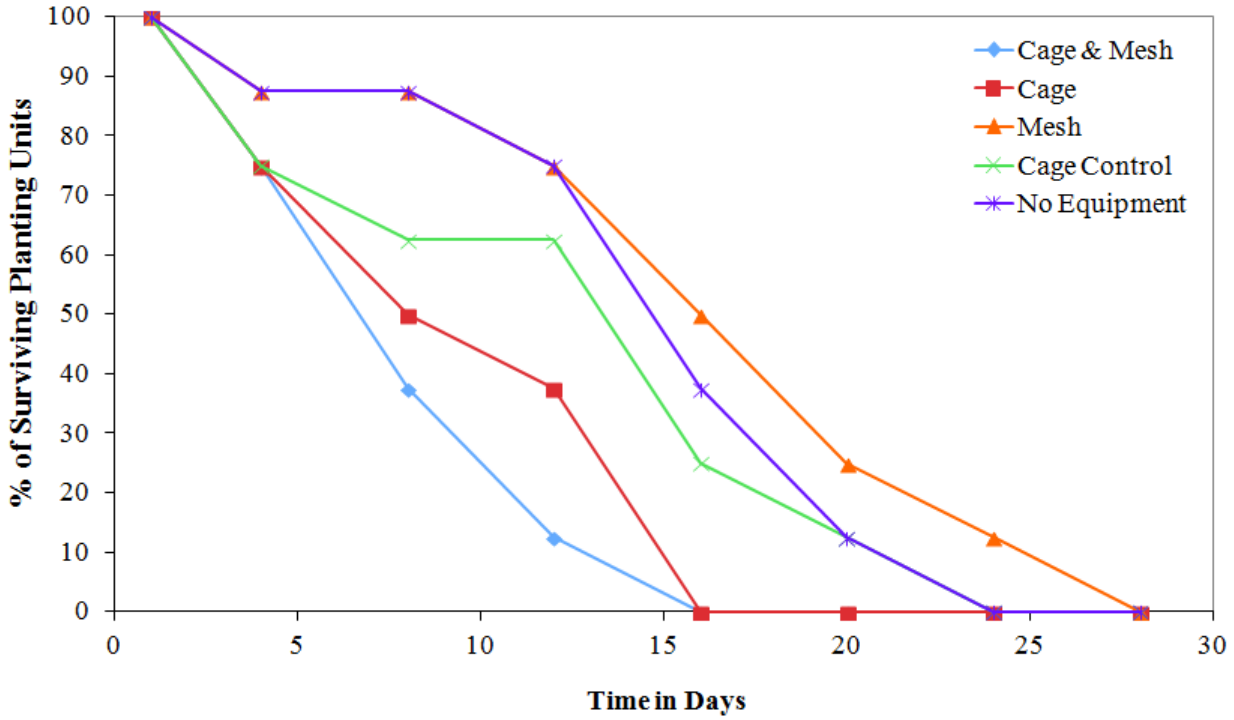

Figure 10. Percent of total potted and plug seagrass transplant units with a minimum of one leaf pair over thirty days (N=11 for each treatment). Not all transplants were installed on the same day. Initial installation of the 40 potted transplants began on November 24th and ended on December 4th, and all potted transplants were monitored through December 27th, 2011. Initial installation of the 15 plug transplants began on January 2nd and ended on January 4th, and all plug transplants were monitored through January 31st, 2012.

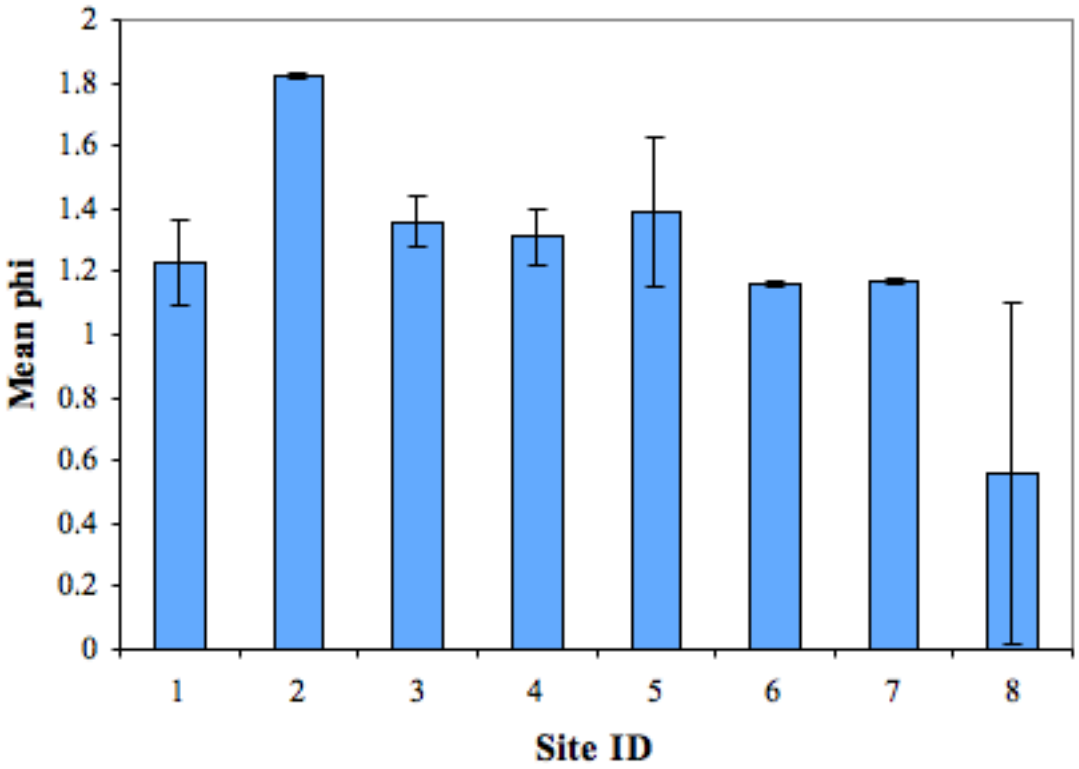

Figure 11.. Mean phi values +/- one standard deviation are shown for eight transplantation sites. Six sediment samples (one from the center of each of the six treatment plots) were collected from each of the sites.

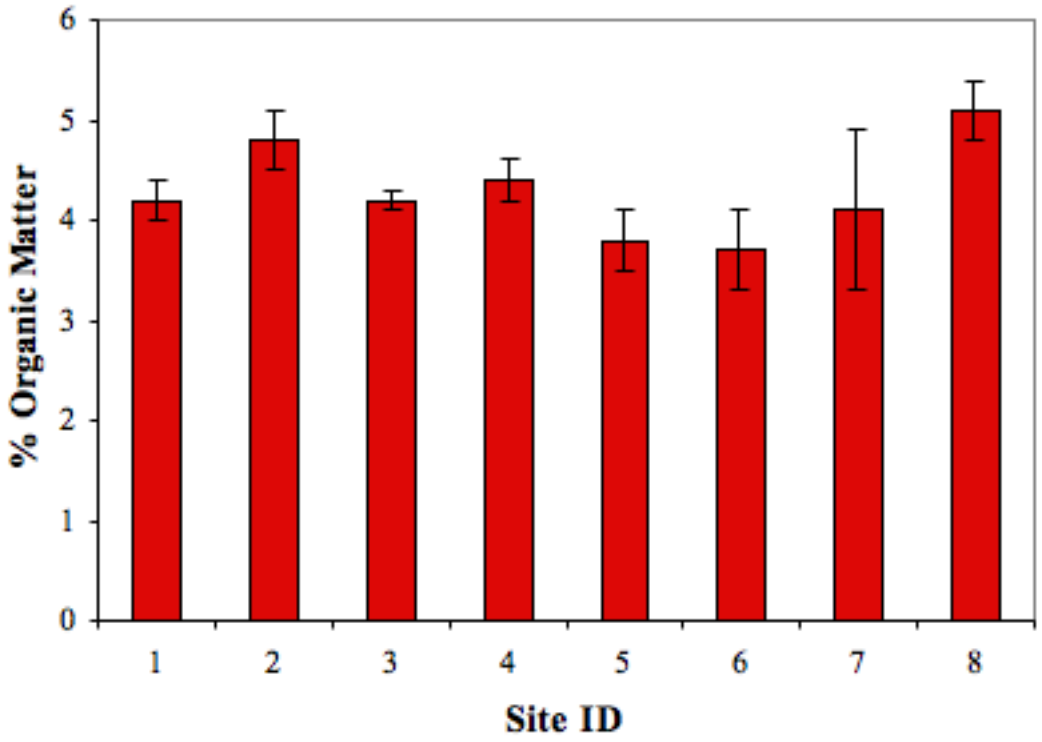

Figure 12. Mean percent organic matter +/- one standard deviation are shown for eight transplantation sites. Six sediment samples (one from the center of each of the six treatment plots) were collected from each of the sites.

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CHAPTER III: Characterizing Growth Patterns of *Halophila hawaiiana*

INTRODUCTION

Since the 1980's, seagrass loss and slow recovery times from anthropogenic disturbances have motivated the development of restoration techniques for accelerating the recovery of these vulnerable species. Although several successful techniques have been developed, seagrass restoration strategies have shown varied levels of success, including complete failure, which highlights the limitations of current techniques and underscores the challenges involved in re-creating self-sustaining habitats (Fonseca *et al*., 1998). Despite these difficulties, decades of experiments have provided insight suggesting that limits to successful seagrass restoration are based on a combination of biological constraints such as the tolerance of species to specific transplantation techniques (Fonseca *et al*., 1998) and spatial factors such as environmental variability between transplantation sites (Short *et al*., 2002). Although some of these challenges are difficult to avoid, others can be overcome by tailoring restoration techniques to the biology, life-history strategies, and dispersal potentials of target seagrass species.

The importance of understanding the growth patterns and responses of a species to manipulation as a prerequisite for successful transplantation was recently demonstrated with the poorly-understood endemic Hawaiian seagrass species *Halophila hawaiiana*, which has experienced a greater than 30% population decline over the past decade (IUCN, 2012). When the invasive alga *Avrainvillea amadelpha* colonized the inshore of the Paiko area of Maunalua Bay on the southeastern shore of O'ahu following a large storm on New Year's Eve in 1987, it altered the shallow reef flat environment by forming dense beds that trapped fine sediment, slowed the movement of water within the bay, and appeared to displace native benthic species, including the seagrass *H. hawaiiana* (Minton and Conklin, 2012). The manual removal of *A.*

amadelpha from 27 acres inshore of the reef crest exposed areas of bare sand that appeared capable of once again supporting *H. hawaiiana*, but the low reproductive capabilities of *H. hawaiiana* combined with the limited number of remaining seagrass patches near the cleared areas of Maunalua Bay suggested the need for active restoration. While pilot transplantation studies performed in the bay between November 2011- January 2012 using both potted laboratory-raised *H. hawaiiana* and direct plug methods have provided some insights (see Chapter 2), they were ultimately unsuccessful with no transplants surviving past one month. Although potted transplanted were visibly disturbed by the removal of sediment and seagrass from the pots by water motion, it was unclear what factors contributed to the death of the plug transplants, especially since plug methods have been successfully used to transplant similar small seagrass species such as *Halophila johnsonii* (Heidelbaugh *et al.*, 2000, Fonseca *et al.*, 1998).

Tailoring the more promising plug transplantation technique to the biology of *H. hawaiiana* will require better knowledge of how *H. hawaiiana* grows and responds to the mechanical damage that occurs during transplantation from donor beds. First, to determine if the plug technique is a viable option for transplanting *H. hawaiiana*, it must be verified that the species can survive following mechanical damage from a corer such as that which occurs during plug transplantation. Cutting creates open wounds, potentially making the plant more susceptible to fungal pathogens (Struck, 2006). Also, it has been repeatedly shown that there is interdependence between physiological processes of connected shoots in terrestrial clonal plants (Hartnett and Bazzaz, 1983, Alpert and Mooney, 1986, Slade and Hutchings, 1987,), thereby allowing resource translocation from neighboring plant modules as long as the physical connection between modules is maintained. Because of their clonal nature, seagrasses also have physiologically connected ramets, or potentially independent individuals, composed of a leaf pair

or bundle, a piece of rhizome, and roots (Tomlinson, 1974) and may experience shoot mortality or reduced growth as a result of the physical separation of these ramets through rhizome cutting.

Although relatively few studies have investigated the integration of seagrass shoots, rhizomes have been shown to play an important role in facilitating the exchange of nutrients, water and carbohydrates among clonal seagrass ramets. Working with 5 tropical (*Cymodocea serrulata*, *Halophila stipulacea*, *Halodule uninervis*, *Thalassodendron ciliatum*, *Thalassia hemprichii*) and 3 temperate (*Cymodocea nodosa*, *Posidonia oceanica*, *Zostera noltii*) seagrass species, Marbá *et al*. (2002) demonstrated that carbon and nitrogen incorporated by seagrass leaves from the water column is rapidly transferred to other parts of the seagrass clone, traveling distances ranging from a few centimeters (*e.g.,* in *Halophila stipulacea*) to over 80 cm (*e.g.,* in *Cymodocea nodosa*). Also, in a series of manipulative field experiments, Terrados *et al.* (1997) found that both leaf growth and the production of biomass by the apical meristem in *Cymodocea nodosa* are dependent on the transfer of resources from older to younger ramets. As a result, growth of *C. nodosa* was greatly reduced when the horizontal rhizome was severed, characterized by a reduced number of new internodes, new shoots, and growth of rhizome runners, as well as a decrease in the size of the internodes and shoots. Given these results in congeners and other related seagrasses, the severing of *H. hawaiiana* rhizomes with a corer during transplantation has the potential to negatively influence future growth and survival by impeding the flow of resources to ramets within the collected seagrass plugs.

In addition to cutting rhizomes, the collection of planting material from a donor bed also involves the severing of apical meristems, which are the dome-shaped tissue regions of active cell division responsible for the maintenance and expansion of seagrass clones. As with other colonial angiosperms, the vegetative growth and proliferation of *H. hawaiiana* is dependent on

the presence of active apical meristems. Therefore, the number and continued health of the meristems in a seagrass plug will influence the survival and growth rate of the transplanted seagrass. Along with horizontal expansion by main apical meristems, the process of forming branching meristems allows *H. hawaiiana* to spread in two dimensions and more efficiently colonize new space.

The main apical meristem located at the tip of the main horizontal rhizome axis and branching meristems that form on alternating sides of the main axis are susceptible to cutting, and the removal of the two types of meristems could have different effects on *H. hawaiiana* growth. Previous studies demonstrate that growth can be significantly altered when the main meristem is removed from clonal species that exhibit apical dominance, which is the phenomenon whereby branching is suppressed in the proximity of an actively growing main meristem as the main meristem releases hormones (auxins and cyokinins, Martin, 1987) that limit the formation of branches at other potential growth sites. The effect of meristem excision has been studied with other species, but little previous research has investigated how the removal of main and branching meristems affects the growth of diminutive *Halophila* species. In the only published experiment measuring the degree of branch suppression by the main apical meristem in *H. hawaiana*, Herbert (1986) observed that *H. hawaiana* rhizomes produced branches at 69.7% of all available nodes when main apical meristems were severed while, in contrast, uncut control rhizomes produced branches at only 10.3% of available nodes. Similarly, Terrados *et al.* (1997) reported increased rates of branching following the removal of main apical meristems from rhizomes of another, larger seagrass, *Cymodocea nodosa*, which has rhizomes that are only 1 mm in diameter but narrow leaves that may reach lengths of 40 cm. Although seagrass clonal growth simulation analyses show that increased branching rates result in accelerated space

occupation (Hemminga and Duarte, 2000), the aforementioned study by Terrados *et al.* (1997) indicated that total plant production decreased with the removal of main apical meristems, perhaps reflecting the time needed for *C. nodosa* to recover from the physiological stress associated with the cutting of the meristem or the redirection of energy from normal growth to the formation of new main apical meristems.

If plugs of *H. hawaiiana* collected from donor patches can continue to grow despite the cutting of rhizomes and meristems which occurs during this process, thereby making plug transplantation a possibility, site-specific differences in plant morphology such as degree of branching, number of meristems per rhizome, the length of internodes, and the number of leaf pairs per rhizome could affect the growth and survival of transplants. Thus, the area from which planting material is collected could affect the likelihood of transplant success. For example, there is a higher likelihood of severing branching meristems from rhizomes when collecting plugs from areas of seagrass with higher branching rates. However, even in the unlikely event that no meristems are severed during collection, seagrass material collected from specific areas of donor patches may have morphological traits conferring a higher potential for survival and optimal growth after transplantation. Along with the dispersal and recruitment of vegetative fragments, seagrasses rely on growth and the branching of apical meristems for population maintenance and growth (Tomlinson, 1974, Hemminga and Duarte, 2000), and the ability of seagrass meadows to expand vegetatively into adjacent areas is particularly influenced by two morphological traits: the frequency of branch formation and the length of internodes. Traits that may have an influence on seagrass growth after transplantation such as branching rate and internode length as well as the number of apical meristems and leaf pairs present per rhizome may vary depending on where within donor beds planting material is collected.

In terrestrial clonal plants, it has been established that growth rate and morphologic characteristics such as number of meristems and the occurrence of branching are not necessarily uniform among different positions within a single patch (Hutchings and Wijesinghe, 1997), and previous studies have demonstrated more rapid clonal expansion at edge rather than at interior positions within patches. Two likely explanations for this observed pattern include: i) release at patch edges from negative density effects or crowding effects (Antonovics and Levin, 1980) and ii) aging effects on growth or morphology near patch centers (Masuzawa and Suzuki, 1991). Research on terrestrial plants also indicates strong relationships between changes in rhizome internodal distances and branching frequency associated with small-scale differences in availability of light, nutrients, and unoccupied space (McDonald and Lieffers, 1993). Morphological plasticity such as this, whereby the expression of a genotype can be altered by environmental influences, can provide adaptive advantages in heterogeneous environments by allowing organisms to maximize resource acquisition under different conditions (Hutchings and de Kroon, 1994).

Although the spatial arrangement of resources has not been documented in seagrass patches at the level of the ramet, several studies have provided evidence of morphological plasticity and associated differences in growth rates at different positions within seagrass patches. For example, in a study of the growth and architecture of the seagrass *Posidonia oceanica*, Molenaar *et al.* (2000) describe rhizomes growing at patch edges as having a growth form with longer internodes, more rapid expansion and widely spaced shoots, while rhizomes in the center of dense beds typically have short internodes and crowded shoots, leading to vertical rather than horizontal growth. Similar growth architecture has been described for the seagrass *Posidonia australis* (Meehan and West, 2002), and an experimental *P. australis* transplantation

study by Bastyan and Cambridge (2008) reported that the source of planting material, either from edges or centers of seagrass beds, influenced the expansion of transplants in the first 2 years of monitoring, with transplants from edges expanding more rapidly than transplants extracted from the centers of beds. Jensen and Bell (2001) found differences in rhizome morphologies between the edges and centers of *Halodule wrightii* patches, specifically with longer internodal distances at seagrass patch edges relative to patch centers. In the same study, Jensen and Bell (2001) found that intermodal distance was positively correlated with increases in areal coverage of seagrass at the edges of patches.

Although no previous studies have investigated differences in growth rates at different positions within *H. hawaiiana* patches, these experiments performed on other seagrass species suggest the possibility for morphological plasticity within *H. hawaiiana* patches. If the hypothesis that growth originates from the edges of *H. hawaiiana* patches rather than patch centers is correct, plug samples collected from edges would support some or all of the following sub-hypotheses: compared to patch centers, seagrass at patch edges have (i) longer internode lengths, (ii) more branches per rhizome, (iii) more apical meristems per rhizome, (iv) more leaf pairs per rhizome, and/or (iv) contain fewer dead or dormant rhizomes compared with the centers of patches, and as a result would serve as more promising material for transplantation. If instead growth originates from the centers of *H. hawaiiana* patches, the opposite patterns would be expected and plugs collected from patch centers may show more promising growth following transplantation.

Unsuccessful pilot transplantation studies performed in Maunalua Bay highlighted the lack of fundamental biological information available on *H. hawaiiana* (Chapter 2). Since it will be difficult to develop a successful restoration technique for *H. hawaiiana* without addressing

gaps in our basic understanding, I conducted a series of experiments with the following objectives:

(1) *Determine if plugs collected from H. hawaiiana donor patches can survive and grow after being severed with a corer*, which is a pre-requisite for plug transplantation.

(2) *Determine if morphology differs with spatial position* by examining growth characteristics of seagrass samples taken from both the centers and edges of existing seagrass beds.

(3) *Determine how the removal of both main and branching apical meristems affects the growth* of *H. hawaiiana.*

Beyond helping to develop successful transplantation techniques for *H. hawaiiana*, answering these questions provided critical ecological insights into the morphology and growth patterns of this threatened Hawaiian seagrass species.

METHODS

Throughout this paper, survival is defined as having a minimum of one living leaf pair and growth is tracked as Mean Leaf Days which, as later described, is calculated based on the number of leaf pairs present over time.

Objective #1: *Determine if plugs collected from H. hawaiiana donor patches can survive and grow after being severed with a corer.*

To determine the impacts of cutting *H. hawaiiana* with a corer, three treatments (Figure 1) were established in 8 stations spanning two adjacent seagrass beds on the western side of the Paiko area of Maunalua Bay. Treatments were as follows, replicated at each of the 8 sites:

Treatment 1 Replacement: plugs were extracted from donor beds using a PVC corer (10 cm

length, 10 cm inside diameter). The corer was hand-driven into the sediment, suction was created by placing one hand over the narrow top of the corer, and the seagrass plug was lifted from the bed. A rigid plastic tablet was slipped beneath the plug to retain sediment and minimize disturbance to the roots and rhizomes. After being lifted above water to simulate movement during transplantation, the plug was replaced with the same orientation into the hole that was created during collection.

Treatment 2 Outplanting: plugs were extracted in the same manner as in Treatment 1, but instead of being replaced in the donor bed, the plugs were planted in holes dug in bare sediment spaced 1 meter from the edge of the donor bed.

Treatment 3 Control: circular areas with 10 cm diameters (the inside diameter of the seagrass corer) were marked within the seagrass beds to serve as controls, allowing the monitoring of seagrass growth under natural, unaltered conditions. In all 8 replicates, the three treatments were spaced no greater than 1 meter from each other. Plugs were extracted and control areas were selected from locations within the donor beds that appeared representative of the surrounding area with regards to leaf density. The numbers of leaf pairs present in each treatment was monitored approximately every 6 days over the course of five weeks.

Monitoring and Statistical Analysis

Leaf pair counts were conducted on the treatments an average of every six days during the 37-day monitoring period from March 29th, when treatments were established, through May 5th, 2012. To quantify survival and growth, Mean Leaf Days were calculated for each treatment and control area. According to the following equation, Mean Leaf Days simultaneously describes the number of days treatments survived in the bay and the number of leaf pairs present

at each time the treatments were monitored, giving the greatest weight to leaf pairs present the longest amount of time:

$$\frac{\text{Sum of (\# of days since transplantation) (Number of leaf pairs observed)}}{\text{Grand sum of leaf pairs observed throughout entire time planting unit was in the bay}}$$

To determine if Mean Leaf Days differed significantly between the three treatments, treatments were compared with a nonparametric one-way ANOVA (Kruskal-Wallis test).

Objective #2: *Determine if morphology differs with spatial position* by examining growth characteristics of seagrass samples taken from both the centers and edges of existing seagrass beds.

To determine if differences in morphology related to higher growth potential (*e.g.,* greater numbers of apical meristems, greater occurrence of branching, and/or longer internodes) tend to be found at the edges or centers of *H. hawaiiana* patches, samples were collected from both positions within existing *H. hawaiiana* beds in three locations: Kaneohe Bay near Lilipuna Pier, the Paiko area of Maunalua Bay, and Kahala. Three large plugs with a diameter of 15.4 cm were collected from both the edges (defined as the interface between seagrass and bare sediment) and from the centers of three patches at each of the three locations for a total of 6 plugs per sampling location. Sampled seagrass patches measured no less than 5 meters along their two longest axes to ensure a buffer between the collection of edge and center samples. To select locations within patches from which to collect samples, patches were first mapped by marking GPS points around the patch perimeters. Then, a random number corresponding to one of the points was chosen and the "edge" sample was taken at that selected point. The location of the

"center" sample was determined by moving 2 m inward from the selected edge point towards the center of the patch. Samples were only collected in locations containing a minimum of 60% seagrass cover. If less than 60% seagrass cover was present at the randomly selected point on the patch perimeter, I moved in a clockwise direction from that point until an area that met the criterion was located. If less than 60% seagrass cover was present 2 m inward from the selected point on the patch perimeter, I moved perpendicularly towards the center of the patch until an area that met the criterion was located.

Samples were gently washed free of sediment, separated into individual rhizome pieces, and placed in a shallow tray of seawater. Photographs of each rhizome piece were taken at a resolution of 16M (Figure 2) and the number of apical meristems, leaf width (measured for one leaf at the center of each rhizome from the widest point on the leaf), internode lengths (the distances between adjacent leaf pairs), number of branches off the central rhizome, and the number of internodes with and without leaves were measured for each individual rhizome. Internode length was measured using the image processing program ImageJ (Abramoff *et al.,* 2004).

Statistical Analysis

Differences in morphology between patch centers and edges at Kaneohe Bay, Maunalua Bay, and Kahala were tested using permutational analysis of variance (PERMANOVA) with Relative Sorensen, to ensure that each morphological trait was weighted equally in the analysis, and 999 permutations. Since PERMANOVA requires a balanced design with an equal number of observations in each cell and different numbers of rhizomes were collected within each of the samples, the average mean internode length, number of leaf pairs

and missing leaf pairs, number of branches, number of apical meristems, and number of internodes were calculated prior to analysis for each of the 18 samples (center and edge samples at three individual patches in Kaneohe Bay, Maunalua Bay, and Kahala).

Preliminary tests for differences in morphological traits at the centers and edges of seagrass patches within Kaneohe Bay, Maunalua Bay, and Kahala with location as a factor in the analyses showed that these locations were distinct and could not be pooled in analyses comparing morphological traits between patch positions. The number of apical meristems and number of branches per rhizome, mean internode lengths, the number of leaf pairs per rhizome, and the mean percent of dead or dormant rhizomes present within samples collected from the centers and edges of patches were compared separately at each location using Wilcoxon signed-rank tests.

Objective #3: *Determine how the removal of both main and branching apical meristems affects the growth of H. hawaiiana.*

Rhizomes collected from the centers and edges of the same 9 seagrass patches sampled during the aforementioned morphology study (3 in Kaneohe Bay, 3 in Maunalua Bay, 3 in Kahala) were labeled and planted in 30 gallon re-circulating saltwater tanks draining into a single 110 gallon tank (Figure 3a), each containing a 7 cm thick layer of beach sand collected from Maunalua Bay (Figure 3b). Experimental manipulations were performed to test the effect of severing main and branching meristems on the growth of *H. hawaiiana* rhizomes collected from both patch centers and edges. Treatments were as follows:

Treatment 1 Cut Main Meristem: only *main* apical meristems were removed from rhizomes and branching meristems were left intact

Treatment 2 Cut Branching Meristems: only *branching* apical meristems were removed from rhizomes and main meristems were left intact

Treatment 3 Control: no meristems were removed from rhizomes

Each treatment contained six individual rhizomes from each of the 9 sampled seagrass patches: 3 rhizomes from the center and 3 rhizomes from the edge of each patch (Figure 4). Rhizomes used in the growth trials were selected to ensure that all treatments contained equivalent material. Although it would have been ideal to work exclusively with rhizomes with the same initial number of meristems, such pieces were prohibitively difficult to locate in natural seagrass beds without destructively sorting through large amounts of seagrass. Instead, healthy-looking rhizomes collected from the center and edge of each of the 9 sampled seagrass patches were separated into sets of three pieces with the same initial number of meristems and approximately the same initial rhizome length and number of leaf pairs. From within these six sets of three rhizomes (three sets from the patch center and three sets from the edge), pieces were randomly assigned to Treatment 1, Treatment 2, or Treatment 3 (control). Since one rhizome from each set was randomly assigned to each of the three experimental treatments, a total of 18 rhizomes from each of the 9 seagrass patches were monitored.

Although plant material from the 9 patches was processed and planted over the course of several months (April-August 2012), all material from each individual patch was planted on the same day to ensure a uniform initial monitoring date. Since *H. hawaiiana* produces a new leaf pair approximately every four days (Herbert, 1986), leaf pair counts were conducted every four days over a twenty-eight day period. Since *H. hawaiiana* has a rapid leaf turnover rate ranging from approximately 4 to 12 days, all leaf pairs present at the beginning of the experiment were expected to senesce before the conclusion of the twenty-eight day period.

Statistical Analysis

To quantify survival and growth, Mean Leaf Days were calculated for each treatment using the following equation:

$$\frac{\sum (\text{\# of days since treatment installation}) (\text{Sum of the leaf pairs observed})}{\text{Grand sum of leaf pairs observed over twenty-eight day monitoring period}}$$

To detect possible interactions between the three treatments (Cut Main Meristem, Cut Branching Meristems, Control) and the position within the donor patches from which the rhizomes were collected (center, edge), a two-way ANOVA was performed with treatment and position fixed and Mean Leaf Days as the dependent variable (Zar, 1999).

RESULTS

Field Experiments

Objective #1: *Determine if plugs collected from H. hawaiiana donor patches can survive and grow after being severed with a corer.*

Despite high variability within all treatments at the 8 replicate sites (Figure 5), Mean Leaf Days differed significantly with treatment (Kruskal-Wallis, $p=0.02$) since the Outplanting treatment, consisting of seagrass plugs moved a distance out from donor beds, had fewer Mean Leaf Days than did uncut Control areas within donor patches (Mann-Whitney, $p=0.01$). However, no significant difference in Mean Leaf Days was detected between the Replacement treatment and the uncut Control (Mann-Whitney, $p=0.08$) nor between the Replacement and Outplanting treatments (Mann-Whitney, $p=0.17$). Within the Replacement treatment, 63% of the plugs survived the 37-day trial and two of the five surviving plugs

experienced net growth with one plug increasing from an initial 10 leaf pairs to a final 12 leaf pairs and the other showing substantial growth by increasing from an initial 5 leaf pairs to a final 9 leaf pairs. Within the Outplanting treatment, 38% of the plugs survived the trial. Of the three surviving plugs, one plug replaced senescent leaf pairs and maintained the same number of leaf pairs from the beginning to the end of the trial, one plug decreased in number of leaf pairs, and one plug experienced rapid growth, increasing from an initial 12 leaf pairs to a final 34 leaf pairs over the course of the experiment. Within the uncut controls, four areas experienced net growth while four areas had fewer leaf pairs at the conclusion of the trial.

Objective #2: *Determine if morphology differs with spatial position* by examining growth characteristics of seagrass samples taken from both the centers and edges of existing seagrass beds.

Significant morphological differences between seagrass from the edges and centers of seagrass patches were consistently observed in Kaneohe Bay, Maunalua Bay, and Kahala. There was no significant interaction between patch position and location (Table 1). A wide range of variation in number of apical meristems, number of branches, number of leaf pairs per rhizome and internode lengths (Figure 6a-d) was observed both at the centers and edges of seagrass patches at all locations, but little variation in leaf width was observed between patch edges and centers or between locations (Figure 6e). Since the morphology data were not normally distributed, median values were calculated for each measured morphological trait and, since multiple internode lengths were measured on each rhizome, mean internode length was calculated for material within each core (Figure 7). A greater mean number of individual rhizomes were collected from patch centers compared with edges (Table 2).

Five sub-hypotheses were developed to express expected relationships between these morphological traits and differences in growth at patch edges and centers. Overall, the five sub-hypotheses supported the main hypothesis that *H. hawaiiana* growth originates primarily from patch edges rather than from patch centers, tested for significance using Wilcoxon signed-rank tests. The sub-hypotheses and corresponding results were as follows:

(i) *Rhizomes collected from patch edges will have longer internode lengths than those collected from patch centers.* As predicted, there were significantly longer mean internodes and more leaf pairs per rhizome at the patch edges compared with patch centers at all three locations.

(ii) *Rhizomes collected from patch edges will have more branches than those collected from patch centers.* The median number of branches per rhizome was significantly greater at the patch edges at Kahala, but not in Kaneohe Bay or Maunalua Bay.

(iii) *Rhizomes collected from patch edges will have more apical meristems than those collected from patch centers.* The median number of apical meristems was greater at the patch edges within Kaneohe Bay, Maunalua Bay, and Kahala, but this difference in number of apical meristems per rhizome was only statistically significant in Kaneohe Bay and nearly significant in Maunalua Bay ($p=0.07$).

(iv) *Rhizomes collected from patch edges will have more leaf pairs than those collected from patch centers.* As predicted, there were more leaf pairs per rhizome at the patch edges compared with patch centers at all three locations.

(v) *Samples collected at patch edges will contain fewer dead or dormant rhizomes compared with the interiors of patches.* Although there was a greater mean percentage of dead or dormant rhizomes within patch centers compared with patch edges at all three locations (Figure 8), this difference was only statistically significant in Maunalua Bay.

Laboratory Growth Trial

Objective #3: *Determine how the removal of both main and branching apical meristems affects the growth of H. hawaiiana.*

Rhizomes collected for the laboratory growth trials had initial numbers of meristems ranging from 2 to 7 with a mean of 5 meristems (SD=1.1), and rhizomes collected from the edges of patches had significantly greater numbers of apical meristems compared with rhizomes collected from the centers of patches at all locations (Wilcoxon signed-rank test, $Z=-11.144$, $p<0.01$). There was a difference in Mean Leaf Days between the three treatments: Cut Main Meristem, Cut Branching Meristems, and Control (two-way ANOVA, $p<0.01$) (Figure 9). The Cut Main Meristem treatment showed a greater number of Mean Leaf Days than did the rhizomes from the Cut Branching Meristem treatment (Tukey, $p=0.01$), and the Control with no cuts had greater Mean Leaf Days than did the Cut Branching treatment (Tukey, $p<0.01$). However, there was no difference in Mean Leaf Days between the Cut Main treatment and the Control ($p=0.94$), showing that rhizomes can survive and grow after meristems are cut . There was neither a difference in Mean Leaf Days between the two positions from which rhizomes were collected: the center and edge (two-way ANOVA, $p=0.33$), nor was there an interaction between treatment and position (two-way ANOVA, $p=0.21$).

DISCUSSION

In this study aimed at understanding the growth patterns of *H. hawaiiana* and informing future transplantation efforts, I determined the impacts of cutting *H. hawaiiana* with a corer,

compared morphology at the centers and edges of existing patches, and explored how the removal of both main and branching apical meristems affects the growth of *H. hawaiiana.*

Effects of Coring on Growth

While investigating the impacts of cutting *H. hawaiiana* with a corer, the Replacement treatment did not differ from the uncut Control, showing that the act of severing rhizomes with a corer alone does not directly result in the death of rhizomes. The fact that 2 of the 5 surviving plugs within the Replacement treatment experienced a net increase in leaf pairs over the 37-day trial, one increasing in number of leaf pairs by 20% and the other by 80%, shows that severed rhizomes can not only survive by replacing senescent leaf pairs but can also thrive. This observation is consistent with several studies performed on other clonal plants showing that severed rhizomes can continue to grow since rhizomes function as stores of carbohydrate and are capable of supplying local ramets (Suzuki and Stuefer, 1999). In some cases, rhizome severing can even enhance growth by stimulating the production of new branching meristems (Charpentier *et al.*, 1998). However, the fact that only 63% of the plugs survived in the Replacement treatment compared with 100% survival within the uncut Control demonstrates that the severing of rhizomes also has the potential to reduce growth and survival, possibly by disturbing root systems, inflicting physical damage and interrupting the physiological integration among clonal ramets responsible for distributing resources between plant modules (Hartnett and Bazzaz, 1983). Similarly, the Replacement treatment did not differ from the Outplanting treatment, showing that the act of moving seagrass out of the donor patch alone does not directly result in the death of rhizomes. The fact that 1 of the 3 surviving plugs experienced rapid growth, increasing from an initial 12 leaf pairs to a final 34 leaf pairs, shows that outplanted

rhizomes have the potential to thrive. However, the fact that only 38% of the plugs survived in the Outplanting treatment compared with 63% of the plugs within the Replacement treatment demonstrates that, in addition to the risks associated with the severing of rhizomes, the act of moving seagrass to bare sediment outside of a donor patch can reduce survival, perhaps because the surrounding patch protects replaced plugs by trapping the surrounding sediment and slowing water movement (van Keulen *et al.,* 2003). Despite no detectable differences between other combinations of treatments, the Outplanting treatment differed from the Control, showing that the cumulative effects of cutting rhizomes and removing plugs from the safety of donor patches can significantly reduce growth. Although transplantation involves both types of disturbance, the survival of 3 out of 8 Outplanting treatment plugs shows that plug transplantation may still be a viable option for transplanting *H. hawaiiana*.

Effects of Patch Position on Morphology

There was an overall difference in morphology between patch centers and edges, with longer mean internode lengths and more leaf pairs per rhizome at patch edges within Kaneohe Bay, Maunalua Bay, and Kahala. A greater number of apical meristems was found at patch edges in Kaneohe Bay and the same pattern was nearly-significant in Maunalua Bay ($p=0.07$), but not in Kahala, which might be influenced by different patch dynamics observed in the latter. While seagrass in Kaneohe Bay and Maunalua Bay appears to form discrete patches, seagrass in Kahala tends to form large, indistinct meadows that sometimes become fragmented and later coalesce, blurring the distinction between patch edges and centers. Similar morphological differences between rhizomes within patch centers and at patch edges where seagrass transitions into adjacent unvegetated sediment have been detected in other species such as *Syringodium*

filiforme (Schwarzschild and Zieman, 2008) and *Halodule wrightii* (Jensen and Bell, 2001), but there is still insufficient empirical evidence available to assess whether these differences reflect general trends or responses unique to the species investigated so far. While the results of the present study cannot explain which environmental variables are responsible, morphological variation in seagrass can result from small-scale differences in nutrient availability (Duarte and Sand-Jensen, 1996), sediment characteristics (van Tussenbroek *et al.,* 2000), or tidal currents (Schanz and Asmus, 2003).

The observed morphological differences between patch positions may have important consequences for the occupation of space, allowing for more rapid growth at patch edges. For example, horizontal rhizomes have an exploratory ability since they are responsible for the extension of seagrass into new areas, and longer internodes at the edges of patches allow plants to use a 'guerilla' strategy to exploit bare sediment by expanding rapidly into bare sediment before other, slower-growing species (Lovett Doust, 1981). Similarly, a greater frequency of branching and shoot production at patch edges allows for a greater degree of space exploitation in two dimensions (Marbá *et al.,* 2004). During transplantation, selecting planting material from the edges of *H. hawaiiana* patches with morphological traits associated with higher rates of growth may increase the growth rate of the transplants, allowing the planting units to establish and expand more quickly. This phenomenon was examined in a field study performed on a larger seagrass species, *Posidonia australis*, in which planting material with differing morphological traits was collected from the edges and centers of patches and transplanted in bare sediment. The rate at which planting units expanded was greatly influenced by the source of the planting units, with seagrass collected from patch edges spreading more rapidly. Approximately one year was required for the planting units collected from patch centers to assume the

morphology of patch edges, developing longer internodes and growing more rapidly (Bastyan and Cambridge, 2008).

There was also an overall difference in morphology between patches in Kaneohe, Maunalua, and Kahala, suggesting that *H. hawaiiana* responds to environmental factors that may differ with location, such as nutrient and fresh water input, relative current speeds, sediment conditions, and the presence of other species such as beneficial bacteria or invasive algae. The high degree of morphological variation observed both within and between geographically distinct areas demonstrates intrinsic differences among *H. hawaiiana* populations, their capacity to colonize different environments, and may reflect their ability to survive environmental changes such as resource availability and plant density.

Effects of Main and Branching Meristem Removal on Growth

Between the three manipulative treatments (Cut Main Meristem, Cut Branching Meristems, Control), the rhizomes with severed branches had fewer Mean Leaf Days than did the Cut Main or Control treatments, thereby highlighting the harm caused by damaging branching meristems. Removing branching meristems and leaving only the main meristem in tact greatly reduces growth potential since all seagrass maintenance and expansion originates from fairly continuous cell division at the apical meristems (Tomlinson, 1974) and, over the course of the growth experiment, no rhizomes were observed to regenerate or form additional branching meristems. Also, since multiple branching meristems were generally cut from each rhizome (Figure 1b), as opposed to one meristem cut from the Main Meristem treatment, the Cut Branching treatment sustained more mechanical damage and the wounds caused by cutting may have increased susceptibility to pathogens. Rhizomes within the Cut Main Meristem treatment

did not differ in Mean Leaf Days from the uncut Control showing that, in addition to the cutting that occurred during the collection of material for the laboratory study, *H. hawaiiana* rhizomes can also sustain damage to their largest, most prominent apical meristems and continue to thrive.

The ability of plugs to survive collection with a corer demonstrated that the plug method is a viable option for transplanting *H. hawaiiana*, and a close examination of the morphology of *H. hawaiiana* revealed that morphological traits contributing to rapid expansion are most prominent at patch edges, thereby suggesting that the patch position from which plugs are collected may affect the growth of the transplants. Finally, the ability of rhizomes to thrive despite the removal of main apical meristems shows that *H. hawaiiana* is capable of sustaining a low degree of meristem damage, but the shorter lifespan of rhizomes with all branching meristems removed suggests that the severing of meristems should be reduced as much as possible during the collection of planting units. Based on these findings and observations made during the experimental transplantation of *H. hawaiiana* in Maunalua Bay (Chapter 2), Chapter 4 of this thesis focuses on recommendations for managers and insight into how *H. hawaiiana* transplantation techniques may be improved.

Table 1. Effects of Patch Position on Morphology: Summary of morphological differences between two patch positions (center and edge) at three sampling locations (Kaneohe Bay, Maunalua Bay, Kahala), tested for significance with PERMANOVA (degrees of freedom (df), sum of squares (SS), mean squares (MS)). Tests were based on 999 data permutations using the Relative Sorensen distance measure. Significant *P*-values for PERMANOVA ($p<0.05$) are in bold.

Source	df	SS	MS	*F*	*P*
Position	1	0.0799	0.0799	4.5178	**0.025**
Location	2	0.1264	0.0632	3.5718	**0.029**
Interaction	2	0.0236	0.0118	0.6663	0.644
Residual	12	0.2123	0.0177		
Total	17	0.44217			

Table 2. Effects of Patch Position on Morphology: Total number of rhizomes collected at both positions at each location is shown with the number of individual rhizomes in each core sample shown in parentheses beneath the totals.

	Kaneohe		**Maunalua Bay**		**Kahala**	
	Center	Edge	Center	Edge	Center	Edge
Total Number of Rhizomes (within 3 core samples)	**76** (37, 22, 17)	**77** (37, 22, 17)	**78** (37, 22, 17)	**79** (37, 22, 17)	**80** (37, 22, 17)	**81** (37, 22, 17)

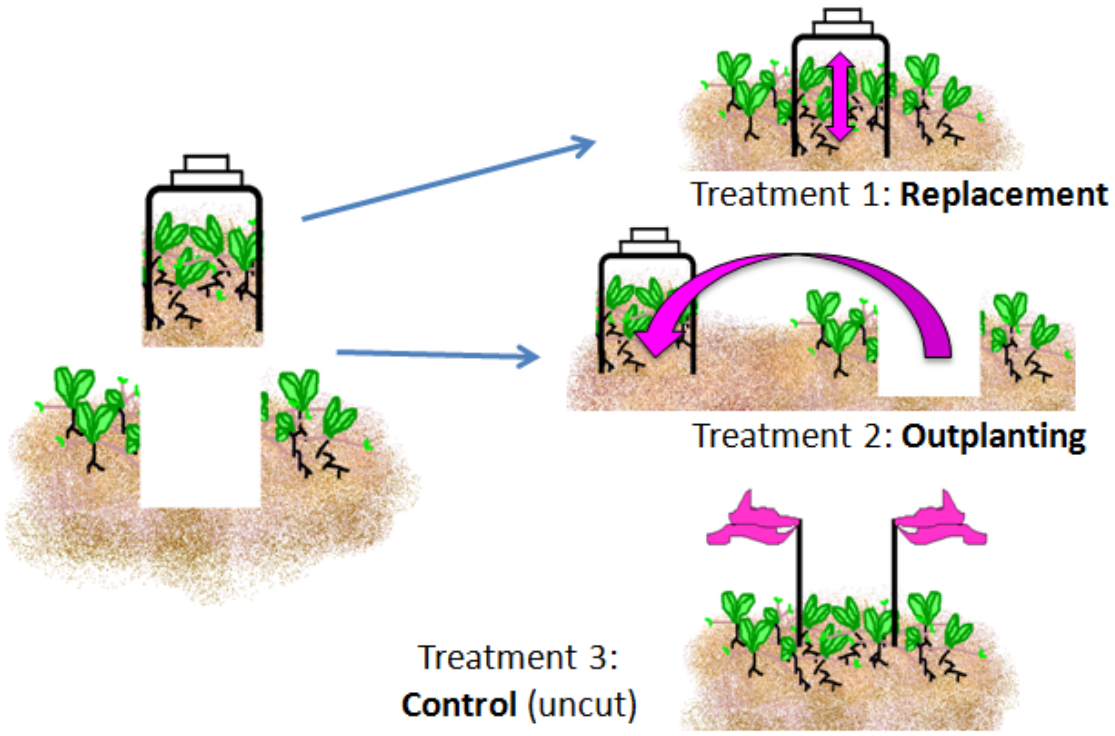


Figure 1. Effects of Coring on Growth: Three treatments designed to test the growth and survivorship of *H. hawaiiana* transplants cut with a corer. Treatment 1 involves taking a seagrass plug from a donor bed and planting the plug back into the same hole with the same orientation. Treatment 2 involves taking another seagrass plug and planting it in bare sand near the edge of the donor bed. Treatment 3 involves marking a circular area with the same diameter as the corer (10 cm), serving as a control.

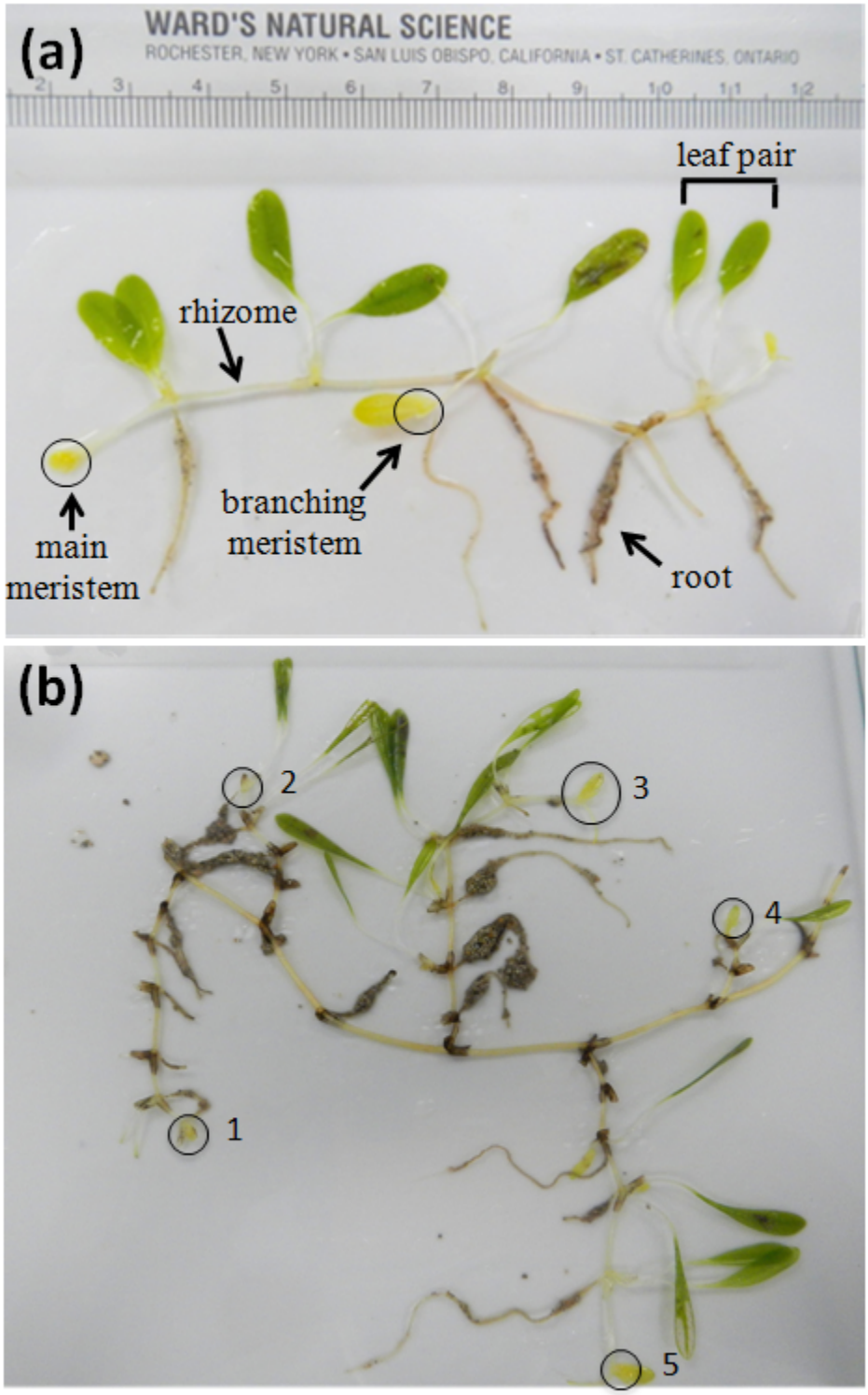


Figure 2. Effects of Patch Position on Morphology: (a) A single *H. hawaiiana* rhizome collected within a core sample in Kaneohe Bay and rinsed free of sediment. **Effects of Meristem Removal on Growth:** (b) Most rhizomes have multiple branches. The pictured rhizome has 5 branching meristems, which are circled.

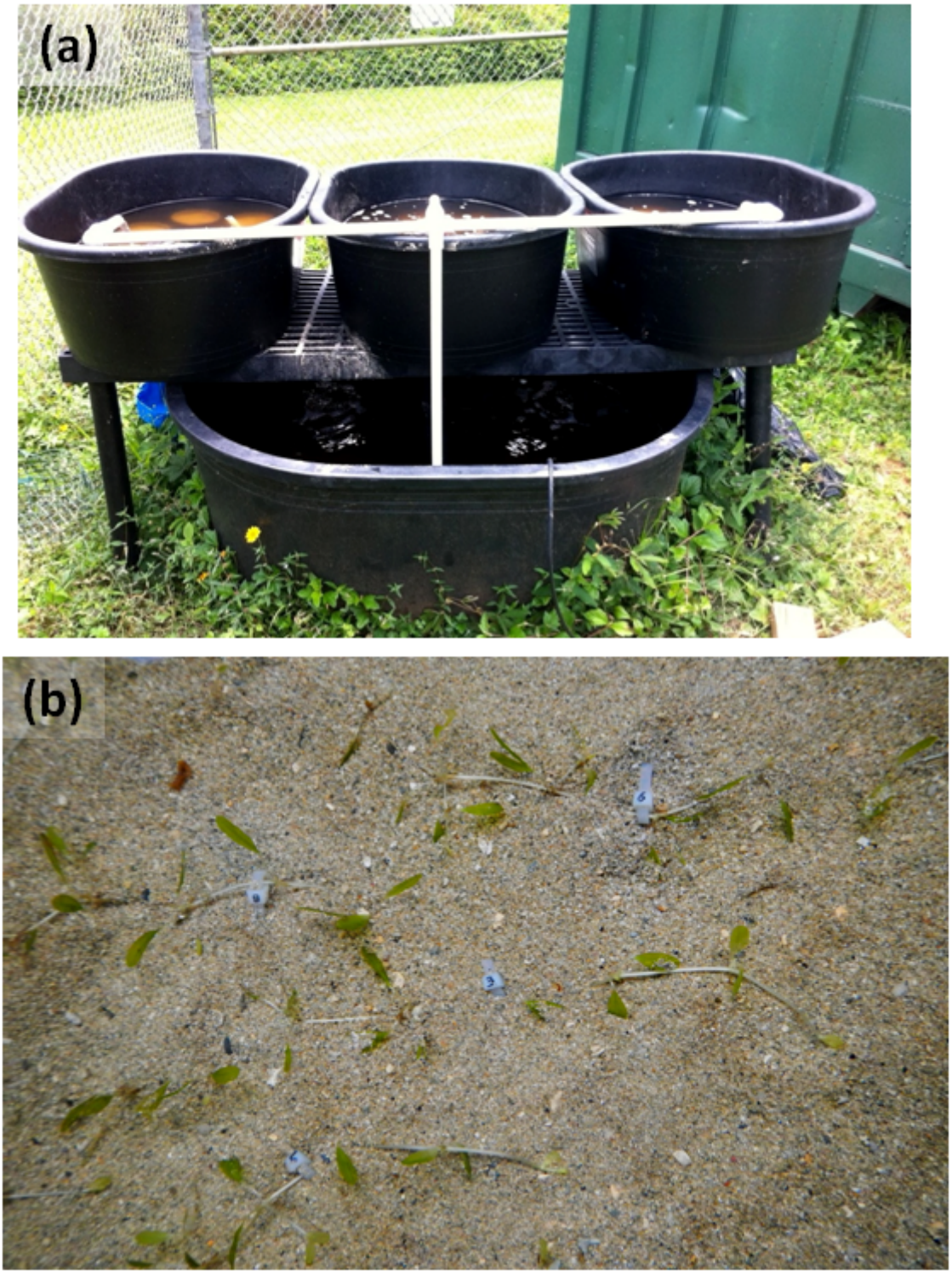


Figure 3. Effects of Meristem Removal on Growth: (a) Growth trials were conducted in 30 gallon re-circulating saltwater tanks draining into a single 110 gallon tank. (b) Rhizomes were labeled and planted in 7 cm of beach sand collected from Maunalua Bay.

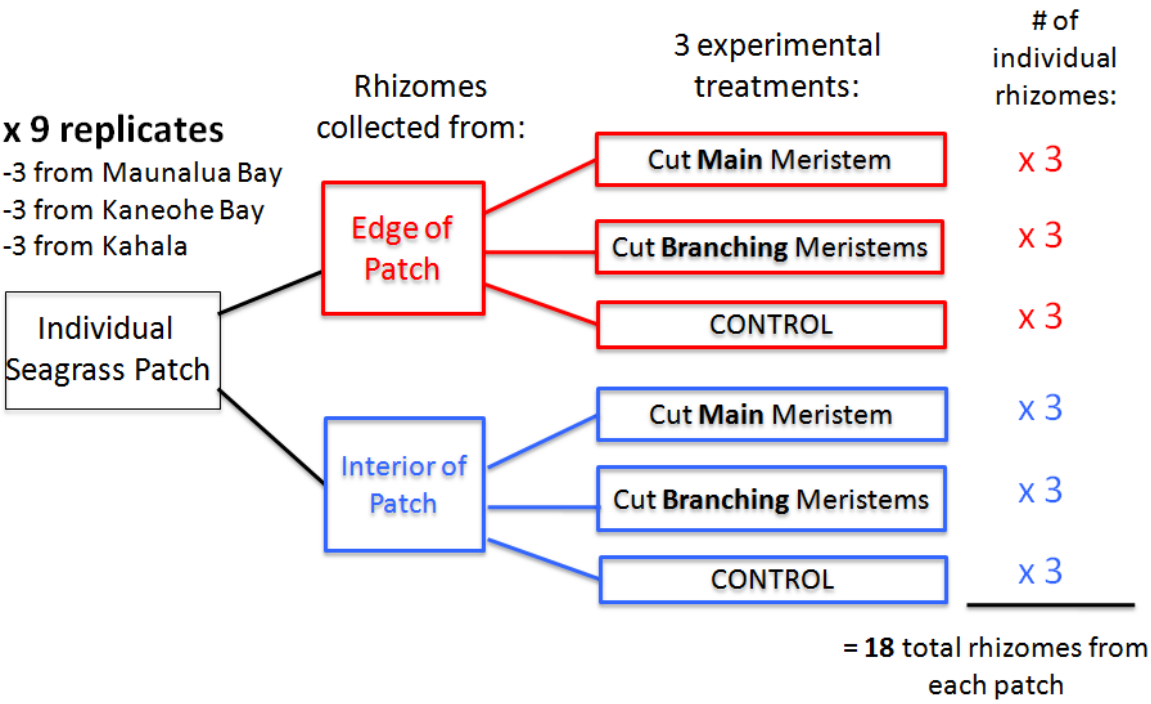


Figure 4. Effects of Meristem Removal on Growth: Schematic diagram depicting assignment of rhizomes to experimental growth treatments. From each of the nine replicate seagrass patches, rhizomes were collected from the edge and interior of the patch. From both locations in the patch, three rhizomes were assigned to each of the three experimental treatments for a total of 18 rhizomes per patch.

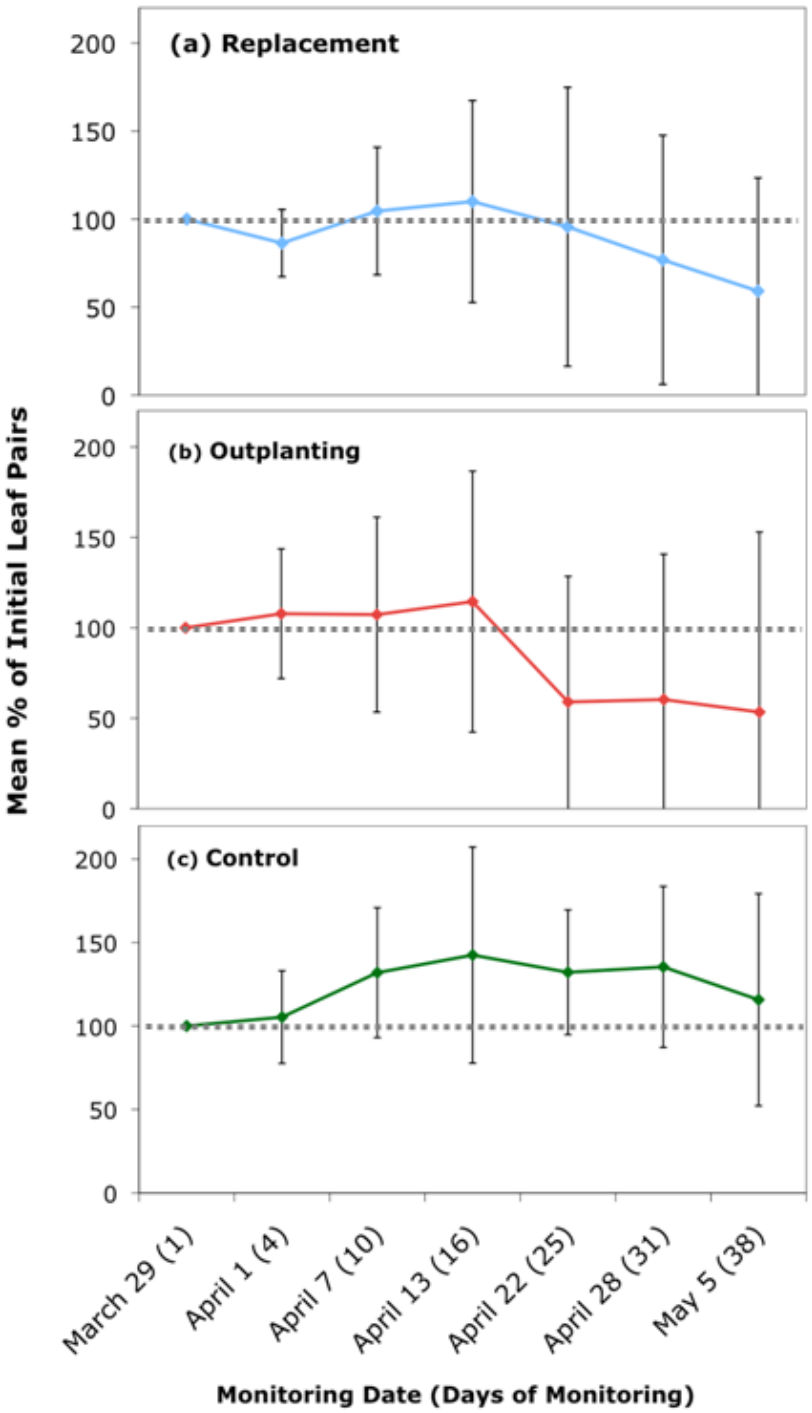
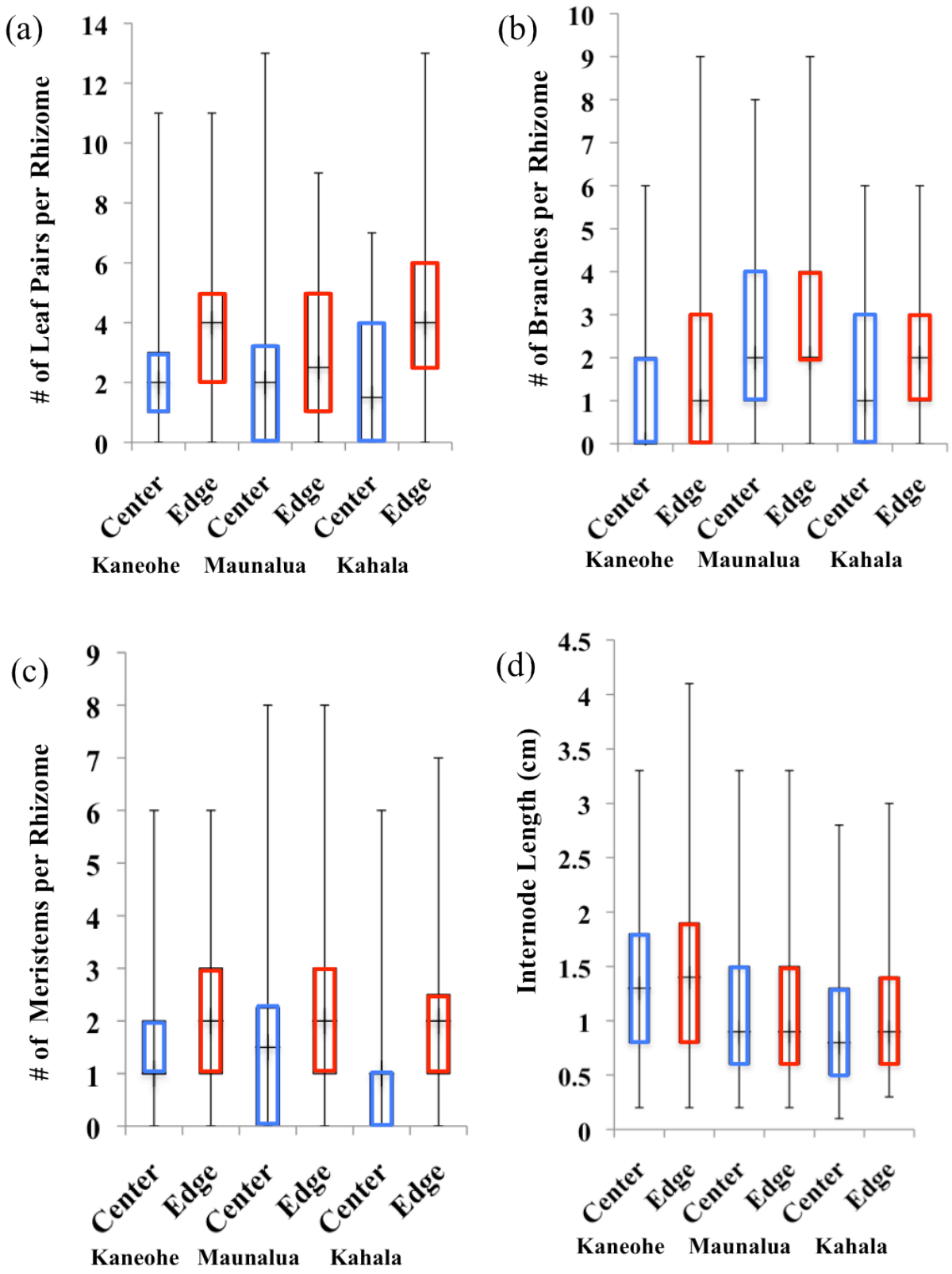


Figure 5. Effects of Coring on Growth: Mean percent of initial leaf pairs remaining over the course of 38 days within (a) Treatment 1, which involved collecting and lifting a seagrass plug, then replacing the plug in the same location within the donor patches, (b) Treatment 2, which involved collecting and lifting a seagrass plug, then planting the plug in bare sediment outside of donor patches, and (c) Treatment 3, which was an uncut control area within the donor patches. Error bars represent SD.


(a)
# of Leaf Pairs per Rhizome
14
12
10
8
6
4
2
0
Center
Edge
Center
Edge
Center
Edge
Kaneohe
Maunalua
Kahala
(b)
# of Branches per Rhizome
10
9
8
7
6
5
4
3
2
1
0
Center
Edge
Center
Edge
Center
Edge
Kaneohe
Maunalua
Kahala
(c)
# of Meristems per Rhizome
9
8
7
6
5
4
3
2
1
0
Center
Edge
Center
Edge
Center
Edge
Kaneohe
Maunalua
Kahala
(d)
Internode Length (cm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Center
Edge
Center
Edge
Center
Edge
Kaneohe
Maunalua
Kahala

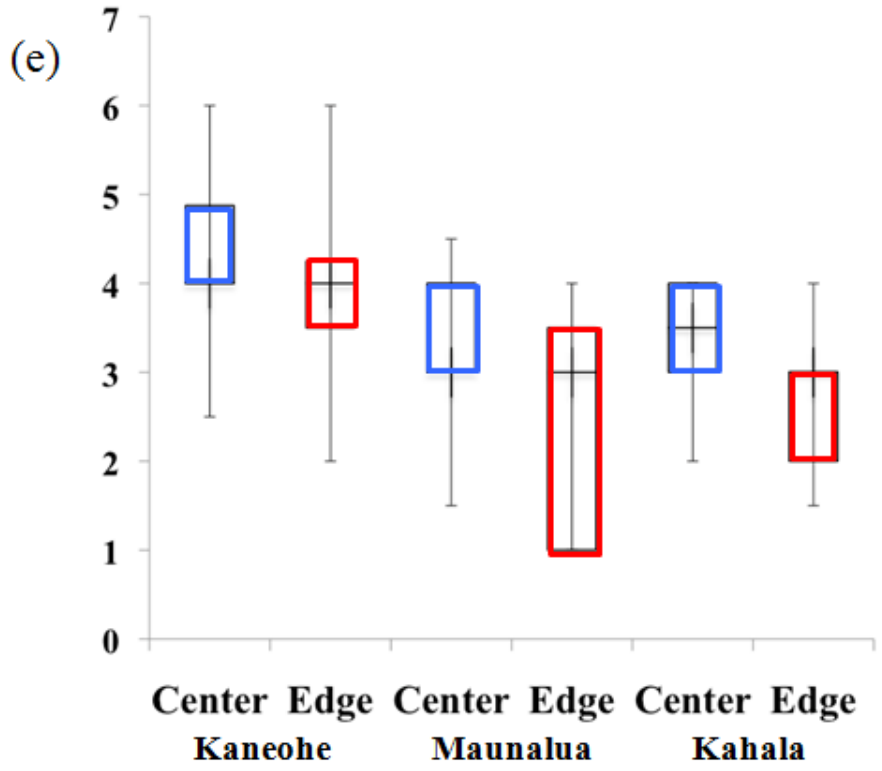


Figure 6. Effects of Patch Position on Morphology: Box-and-whisker plots of (a) number of leaf pairs per rhizome, (b) number of branches per rhizome, (c) number of meristems per rhizome, (d) internode length, and (c) leaf widths at patch centers and edges, with three core samples collected at each of three sampling locations. Samples collected from patch centers are highlighted in blue and edge samples in red. Explanation of plots: medians are represented by a thick black bar in each box; the lower and upper ends of each box represent the 25% and 75% quartiles, respectively. Skewness is reflected by the position of the median relative to the ends of each box. Sample sizes for each patch position and location are provided in Table 2.

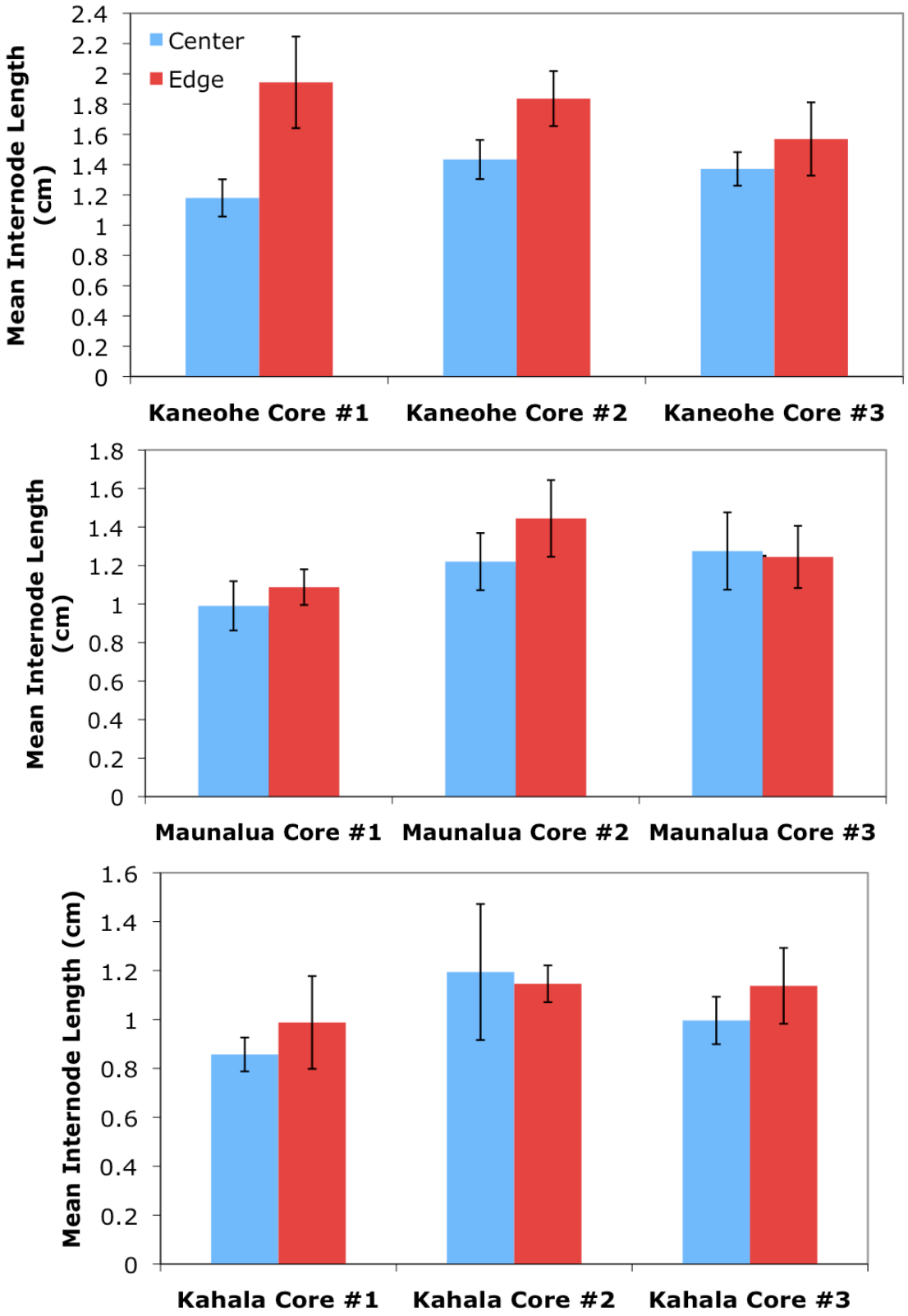


Figure 7. Effects of Patch Position on Morphology: Mean internode lengths for rhizomes within three core samples collected from three locations. Sample sizes for each patch position and location are given in Table 2. Error bars represent 95% CI.

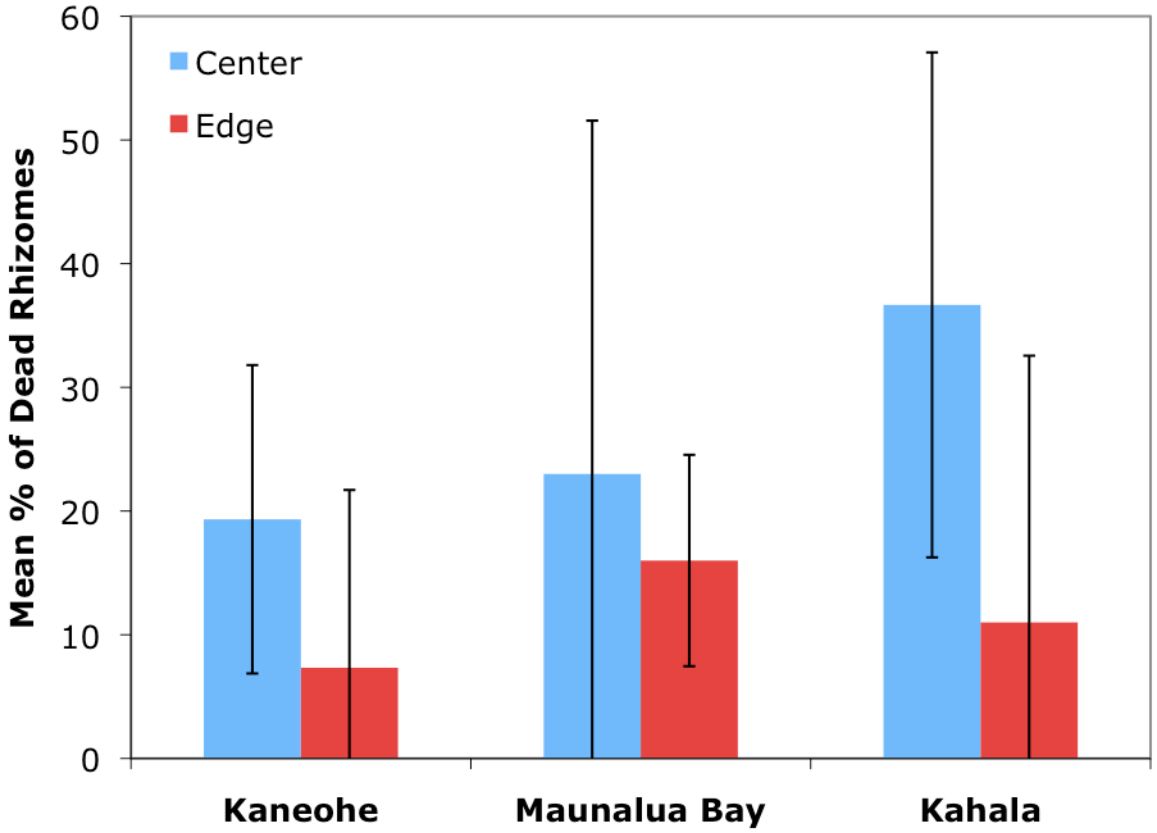


Figure 8. Effects of Patch Position on Morphology: Mean percent of dead rhizomes present in core samples collected in Kaneohe Bay, Maunalua Bay, and Kahala. Error bars represent 95% CI (n=3).

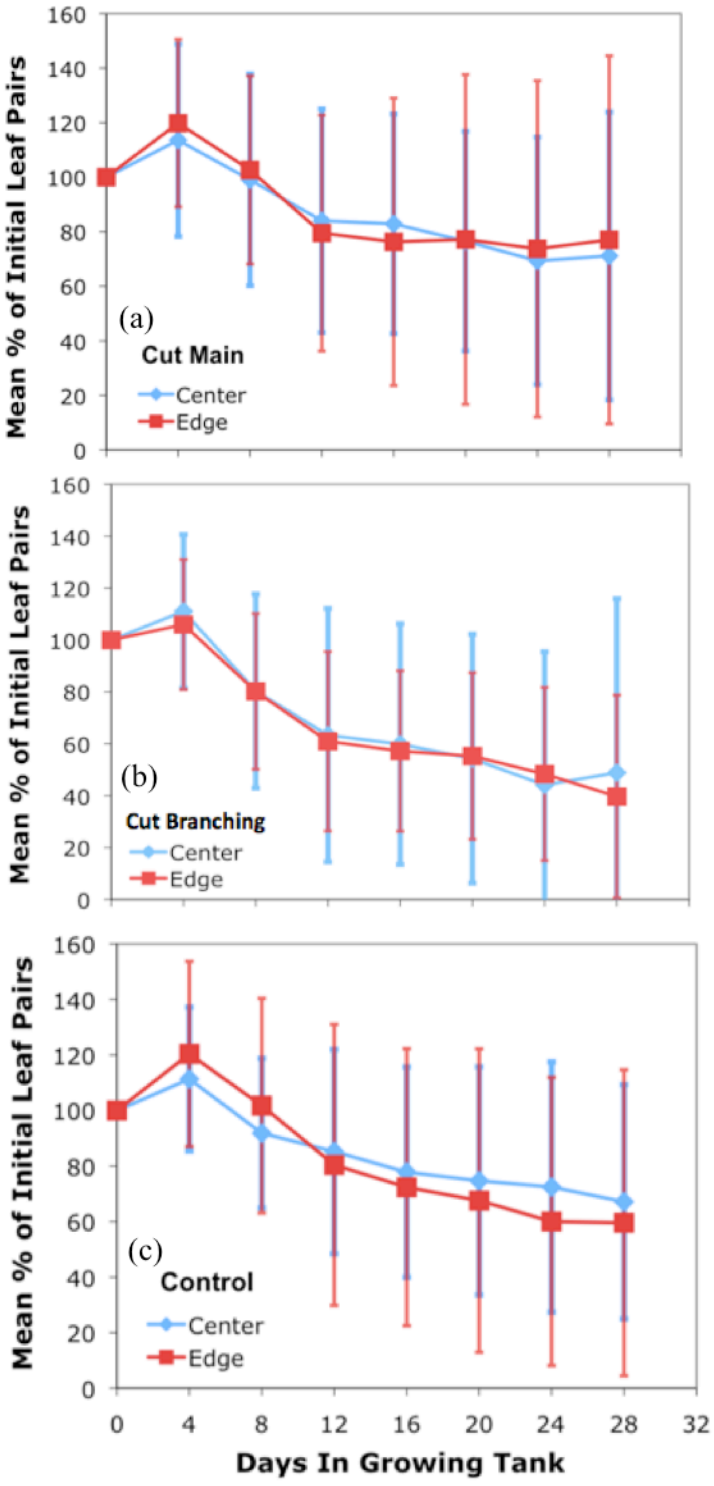


Figure 9. Effects of Meristem Removal on Growth: Mean percent of initial leaf pairs remaining over the course of 28 days within (a) Treatment 1, which involved severing the main apical meristem from rhizomes, (b) Treatment 2, which involved severing all branching apical meristems from rhizomes, and (c) Treatment 3, which was an uncut control. Error bars represent SD.

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CHAPTER IV: Management Recommendations

After several decades of documented successes, seagrass transplantation is now a proven management tool (Fonseca *et al.,* 1998) and can be particularly useful when restoring a species that rarely produces seeds, such as the Hawaiian endemic *Halophila hawaiiana*. For example, there have been successful transplantation projects with mixed seagrass species in areas measuring up to 6 ha in size in Florida (Lewis III, 1987) and bare root transplantations of *Zostera noltii* performed in 1993 in the Wadden Sea were still observed to be expanding 13 years after installation (van Katwijk *et al.,* 2006). According to a review of the status of international seagrass restoration, there were many successes in restoring small areas of lost or damaged seagrasses in 1996, particularly with faster growing species such as *Zostera marina*, *Halodule wrightii*, and *Syringodium filiforme* (Gordon, 1996). More recently, mechanical techniques have also been developed to enable the transplantation of large sods of seagrass which, because of their greater mass, have better anchorage in high energy environments compared to plugs and springs. For example, an underwater seagrass harvesting and planting machine called ECOSUB1 was used in Western Australia to transplant over 2000 sods of *Amphibolis griffithii* and *Posidonia coriacea* (each 0.25 m^2 in surface area and 0.5 m deep), with an average survival of approximately 70% over a 3-year period, and new machines (ECOSUB2) have since been constructed to improve efficiency by planting sods that are twice as large in size (Paling *et al.,* 2001).

However, as a result of poor site selection or inappropriate planting procedures, seagrass transplantation is not always successful. Data compiled from 53 scientific reports published in the United States show a median percentage planting unit survival of 35% and a mean

percentage of 42%, with only 5% of the studies reporting 100% sprig or plug planting unit survival (Fonseca *et al.,* 1998). Transplanting any plant, marine or terrestrial, has a degree of risk involved, and not all planting units should be expected to survive. In particular, working with seagrasses underwater involves unique logistical considerations. Water movement and wave action are constant forces, and site selection is a complex problem since areas that appear to be ideal potential seagrass habitats may be unsuitable for reasons that are not readily apparent. For example, if shallow-water areas of soft substrate do not already support seagrass, the situation begs the question, "If seagrass does not currently exist at the site, what suggests that it can be successfully established here?" and transplantation should be avoided unless the site historically supported seagrass and an anthropogenic disturbance that is no longer present resulted in its loss (Fonseca, 1989). In addition, work may only be performed in the water for limited amounts of time since the window of opportunity for working in intertidal environments is restricted by the tides, and tasks performed underwater seem to require far more time than they do on land. Seagrass transplantation is also particularly challenging because relatively little is known regarding the basic biology of many species, and a lack of knowledge about growth and reproduction makes it difficult to design appropriate methods for maximizing success. However, when techniques can be tailored to best accommodate the biology and growth patterns of a specific species, restoration efforts are more likely to be effective. In chapters 1 and 2 of this thesis, the results of field and laboratory studies informed the best methods and practices for transplanting *H. hawaiiana*.

These results suggested that the plug transplantation technique is more promising than raising seagrass in a lab setting prior to outplanting because of its efficacy and cost-effectiveness. There was not a substantial difference in performance between transplants installed using the

plug technique compared to the potted, laboratory-raised transplants, perhaps as a result of the small sample size (ANOVA, p=0.469). Yet, the most successful of the fifty-five planting units was a plug transplant with 12.5 Mean Leaf Days, highlighting that the plug technique is no less effective than laboratory methods and has several potential advantages. Plug transplantation is a more streamlined process than working with potted transplants, allowing fewer opportunities to damage seagrass during transportation and no possibility of complications while growing planting material in the lab. Similarly, since some types of sediment bacteria associated with seagrass roots can have beneficial effects by metabolizing toxic substances surrounding the roots (Lee and Dunton, 2000, Küsel *et al.*, 2006) using plugs collected directly from donor patches may enhance the success of the transplants. Also, directly outplanted material is most likely to be healthy at the time of installation while the health of laboratory-raised material may be more uncertain.

In addition to being equally if not more effective, plug transplantation involves fewer labor and material costs. Purchasing materials to construct growing tanks and obtaining biodegradable pots for accommodating planting material is costly, and collecting and transporting sediment to fill the pots and seawater to supply the tanks required several days of hard labor for even our modest 110 gallon setup with six individual 30 gallon growing tanks. In order to supply sufficient planting material for a full-scale restoration project, tanks would need to be constructed on a larger scale, ideally in a facility with flow-through seawater. Once tanks are supplied with seawater, the water must be checked almost daily for changes in temperature since algal blooms appeared to be more common when temperatures rose above 27˚C. Salinity must also be monitored frequently and adjusted to compensate for changes from evaporation, and fresh seawater must be added to the growing tanks several times per month to ensure that

nutrients are available to support plant growth. Finally, time must be spent almost daily cleaning tanks and filters and monitoring the health of the seagrass. While these challenges emphasize the benefits of raising *H. hawaiiana* in circulating tanks that are constantly receiving fresh seawater to maintain optimal temperature, salinity, and nutrient supply and discourage tank fouling, gaining access to such facilities may be prohibitively difficult.

Results of the present study provided insights into both the appropriate numbers of *H. hawaiiana* plugs to install at transplantation sites and the length of time over which transplants should be monitored. The survival of 38% of outplanted plugs over a 32-day period (Chapter 3) demonstrated the likelihood that fewer than half of installed planting units should be expected to survive and that multiple plugs should be installed at each planting sight to help ensure that plugs achieve coalescence. Although the relationship between *H. hawaiiana* patch size and functional attributes has yet to be investigated, studies have shown that even very small seagrass patches provide valuable habitat (Murphey and Fonseca, 1995, Hovel and Lipcius, 2001). Since other ecological services provided by seagrasses such as sediment stabilization and nutrient filtration require them to modify their environment, size and shape will affect the functional attributes of both natural and restored patches (Perrow and Davy, 2002), but this is an area of study needing much additional work (Fonseca *et al.,* 1998). The disappearance of apparently healthy plug transplants over a 28-day period during pilot transplantation studies in Chapter 2 highlighted the necessity of monitoring transplants over an extended period of time, ideally for three years (Perrow and Davy, 2002), to accurately assess planting success and perform remedial plantings if project performance standards are not being met.

There are several different ways that the success of the plug technique might be optimized. Since rhizomes that had all branching meristems severed had lower survival and

growth rates than did rhizomes with only main meristems or no meristems removed (Chapter 3), a large corer should be used to minimize the chances of severing branching meristems. During the experimental plug transplantation of *H. hawaiiana* in the present study (Chapter 2), a PVC corer with a 10 cm diameter was used, but larger corers with 20 cm diameters have also been used to successfully extract plugs from *H. hawaiiana* patches (Angelica Chan, Hawaii Pacific University, personal communication). Although the growth and survival of transplants may be enhanced by limiting the cutting of meristems, larger plugs are much more difficult to extract from donor patches and require more effort to move to transplantation sites. Also, the potential benefit of using a larger corer must be balanced with the damage that plug collection inflicts on donor patch, since holes created in the sediment of the donor site are susceptible to erosion.

In addition to the size of the corer used during collection, the location within a donor patch from which planting material is collected may also influence the growth rate of the transplants. Selecting planting material with morphological traits that allow for rapid growth may enhance the ability of transplants to expand and quickly occupy uncolonized areas. Although *H. hawaiiana* morphology differed between Kaneohe Bay, Maunalua Bay, and Kahala, mean internode length and the median number of leaf pairs per rhizome were consistently greater at patch edges compared with patch centers across all locations, suggesting that plugs should be collected from areas where donor patches contact bare sediment. This practice may also be less disruptive to the integrity of donor patches.

The survival of *H. hawaiiana* transplants can also be enhanced by taking steps to limit erosion and the burial of planting units by the surrounding sediment. Since planting units can be collected and installed on the same day using the plug technique, transplantation should take place on still days during a period of calm ocean conditions. The use of caging to discourage

bioturbation was found to shorten the lifespan of the transplants by trapping sediment and is not recommended for use in the future, but the use of plastic mesh (1 x 1 cm hole size) secured to the sea floor appeared to effectively stabilize the sediment surrounding the transplants. For example, of the five protective treatments (Cage & Mesh, Cage, Mesh, Cage Control, No Equipment) that were installed on November 27th, 2011 when winds reached high speeds of 61 km/h, the rhizomes in the Mesh treatment survived 25 days while no other treatment survived more than 12 days.

Although challenging and time-consuming, transplanting plugs of *H. hawaiiana* from the edges of donor patches into areas of bare sediment fortified with mesh proved feasible since *H. hawaiana* can thrive after being cut with a corer and can continue to grow following the removal of apical meristems. Under favorable weather conditions, plug transplants can expand relative quickly from multiple apical meristems and occupy space in several directions. After coalescing to form a new seagrass patch at a transplantation site, planting units would gradually enjoy the protective benefits that a dense patch can provide, such as more stable sediment and reduced current speeds. Since *H. hawaiiana* rarely produces seeds, transplantation may be the only way to restore this species in areas where it has been extirpated.

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